lower VEGF concentration. The result therefore supports the conclusion that the affinity-improved variant is at least 30-fold improved in affinity for VEGF, and that it effectively blocks VEGF activity *in vitro*. Since the variant Y0317 differs from Y0313-1 only in the reversion of the VL1 sequence to wild-type (Fig. 10A), it is predicted that Y0317 will have similar activity to Y0313-1.

Variant Y0317 (Fab) and humanized variant F(ab)-12 from Example 1 (full length and Fab) were compared for their ability to inhibit bovine capillary endothelial cell proliferation in response to a near maximally effective concentration of VEGF using the assay described in Example 1. As illustrated in Figure 12, Y0317 was markedly more effective at inhibiting bovine capillary endothelial cell proliferation than the full length and Fab forms of F(ab)-12 in this assay. The Y0317 affinity matured Fab demonstrated an ED50 value in this assay which was at least about 20 fold lower than F(ab)-12 Fab.

WHAT IS CLAIMED IS:

1. A humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M.

2. A humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 5 x 10^{-9}M.

3. A humanized anti-VEGF antibody which has an ED50 value of no more than about 5nM for inhibiting VEGF-induced proliferation of endothelial cells *in vitro*.

4. A humanized anti-VEGF antibody which inhibits VEGF-induced angiogenesis *in vivo*.

5. The humanized anti-VEGF antibody of claim 4 wherein 5mg/kg of the antibody inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model.

6. The humanized anti-VEGF antibody of claim 1 having a heavy chain variable domain comprising the following hypervariable region amino acid sequences: CDRH1 (GYX_1FTX_2YGMN, wherein X_1 is T or D and X_2 is N or H; SEQ ID NO:128), CDRH2 (WINTYTGEPTYAADFKR; SEQ ID NO:2) and CDRH3 ($YPX_1YYGX_2SHWYFDV$, wherein X_1 is Y or H and X_2 is S or T; SEQ ID NO:129).

7. The humanized anti-VEGF antibody of claim 6 comprising the amino acid sequence of SEQ ID NO:7.

8. The humanized anti-VEGF antibody of claim 6 having a heavy chain variable domain comprising the following hypervariable region amino acid sequences: CDRH1 (GYTFTNYGMN; SEQ ID NO:1), CDRH2 (WINTYTGEPTYAADFKR; SEQ ID NO:2) and CDRH3 (YPHYYGSSHWYFDV; SEQ ID NO:3).

9. The humanized anti-VEGF antibody of claim 1 having a light chain variable domain comprising the following hypervariable region amino acid sequences: CDRL1 (SASQDISNYLN; SEQ ID NO:4), CDRL2 (FTSSLHS; SEQ ID NO:5) and CDRL3 (QQYSTVPWT; SEQ ID NO:6).

10. The humanized anti-VEGF antibody of claim 9 comprising the amino acid sequence of SEQ ID NO:8.

11. The humanized anti-VEGF antibody of claim 1 having a heavy chain variable domain comprising the amino acid sequence of SEQ ID NO:7 and a light chain variable domain comprising the amino acid sequence of SEQ ID NO:8.

12. An anti-VEGF antibody light chain variable domain comprising the amino acid sequence:
DIQX$_1$TQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLH SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR (SEQ ID NO:124), wherein X$_1$ is M or L.

13. An anti-VEGF antibody heavy chain variable domain comprising the amino acid sequence:
EVQLVESGGGLVQPGGSLRLSCAASGYX$_1$FTX$_2$YGMNWVRQAPGKGLEWVGWI NTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPX$_3$YY GX$_4$SHWYFDVWGQGTLVTVSS (SEQ ID NO:125), wherein X$_1$ is T or D; X$_2$ is N or H; X$_3$ is Y or H and X$_4$ is S or T.

14. A variant of a parent anti-VEGF antibody, wherein said variant binds human VEGF and comprises an amino acid substitution in a hypervariable region of a heavy chain variable domain of said parent antibody.

15. The variant of claim 14 wherein said parent antibody is a human or humanized antibody.

16. The variant of claim 14 which binds human VEGF with a K_d value of no more than about 1×10^{-8}M.

17. The variant of claim 14 which binds human VEGF with a K_d value of no more than about 5×10^{-9}M.

18. The variant of claim 14 wherein the substitution is in CDRH1 of the heavy chain variable domain.

19. The variant of claim 14 wherein the substitution is in CDRH3 of the heavy chain variable domain.

20. The variant of claim 14 which has amino acid substitutions in both CDRH1 and CDRH3.

21. The variant of claim 14 which binds human VEGF with a K_d value less than that of said parent antibody.

22. The variant of claim 14 which has an ED50 value for inhibiting VEGF-induced proliferation of endothelial cells *in vitro* which is at least about 10 fold lower than that of said parent antibody.

23. The variant of claim 18 wherein the CDRH1 comprises the amino acid sequence: GYDFTHYGMN (SEQ ID NO:126)

24. The variant of claim 19 wherein the CDRH3 comprises the amino acid sequence: YPYYYGTSHWYFDV (SEQ ID NO:127).

25. The variant of claim 14 wherein the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO:116.

26. The variant of claim 25 further comprising the light chain variable domain amino acid sequence of SEQ ID NO:124.

27. The variant of claim 26 comprising the light chain variable domain amino acid sequence of SEQ ID NO:115.

28. The humanized anti-VEGF antibody of claim 1 which is a full length antibody.

29. The humanized anti-VEGF antibody of claim 28 which is a human IgG.

30. The humanized anti-VEGF antibody of claim 1 which is an antibody fragment.

31. The antibody fragment of claim 30 which is a Fab.

32. A composition comprising the humanized anti-VEGF antibody of claim 1 and a pharmaceutically acceptable carrier.

33. A composition comprising the variant anti-VEGF antibody of claim 14 and a pharmaceutically acceptable carrier.

34. Isolated nucleic acid encoding the antibody of claim 1.

35. A vector comprising the nucleic acid of claim 34.

36. A host cell comprising the vector of claim 35.

37. A process of producing a humanized anti-VEGF antibody comprising culturing the host cell of claim 36 so that the nucleic acid is expressed.

38. The process of claim 37 further comprising recovering the humanized anti-VEGF antibody from the host cell culture.

39. A method for inhibiting VEGF-induced angiogenesis in a mammal comprising administering a therapeutically effective amount of the humanized anti-VEGF antibody of claim 1 to the mammal.

40. The method of claim 39 wherein the mammal is a human.

41. The method of claim 39 wherein the mammal has a tumor.

42. The method of claim 39 wherein the mammal has a retinal disorder.

```
        1   .    10         20         30         40
A4.6.1  EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQA
         *    *   ** *    *** *  *              *
F(ab)-12 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQA
                                    *  ** * *
humIII  EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQA

                50  a      60         70         80
A4.6.1  PGKGLKWMGWINTYTGEPTYAADFKRRFTFSLETSASTAYL
              * *                          *  *
F(ab)-12 PGKGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYL
                ** **** ***  *** *   * * *  * *
humIII  PGKGLEWVSVISGDGGSTTYADSVKGRFTISRDNSKNTLYL

          abc    90                          110
A4.6.1  QISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGAGTTVTVSS  (SEQ.ID NO:9)
         *** ***    * *                      *  *
F(ab)-12 QMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS  (SEQ.ID NO:7)
                       *                    *
humIII  QMNSLRAEDTAVYYCARG----------FDYWGQGTLVTVSS  (SEQ.ID NO:11)
```

FIG._1A

```
        1        10         20         30         40
A4.6.1  DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKP
              **       *    * *
F(ab)-12 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKP
                                 *   *     *
humKI   DIQMTQSPSSLSASVGDRVTITCRASQSISNYLAWYQQKP

                50         60         70         80
A4.6.1  DGTVKVLIYFTSSLHSGVPSRFSGSGSGTDYSLTISNLEP
        ****                              **    * *
F(ab)-12 GKAPKVLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQP
              *   **   *
humKI   GKAPKLLIYAASSLESGVPSRFSGSGSGTDFTLTISSLQP

                90        100
A4.6.1  EDIATYYCQQYSTVPWTFGGGTKLEIKR   (SEQ.ID NO:10)
          *                  *   *
F(ab)-12 EDFATYYCQQYSTVPWTFGQGTKVEIKR   (SEQ.ID NO:8)
                   ***
humKI   EDFATYYCQQYNSLPWTFGQGTKVEIKR   (SEQ.ID NO:12)
```

FIG._1B

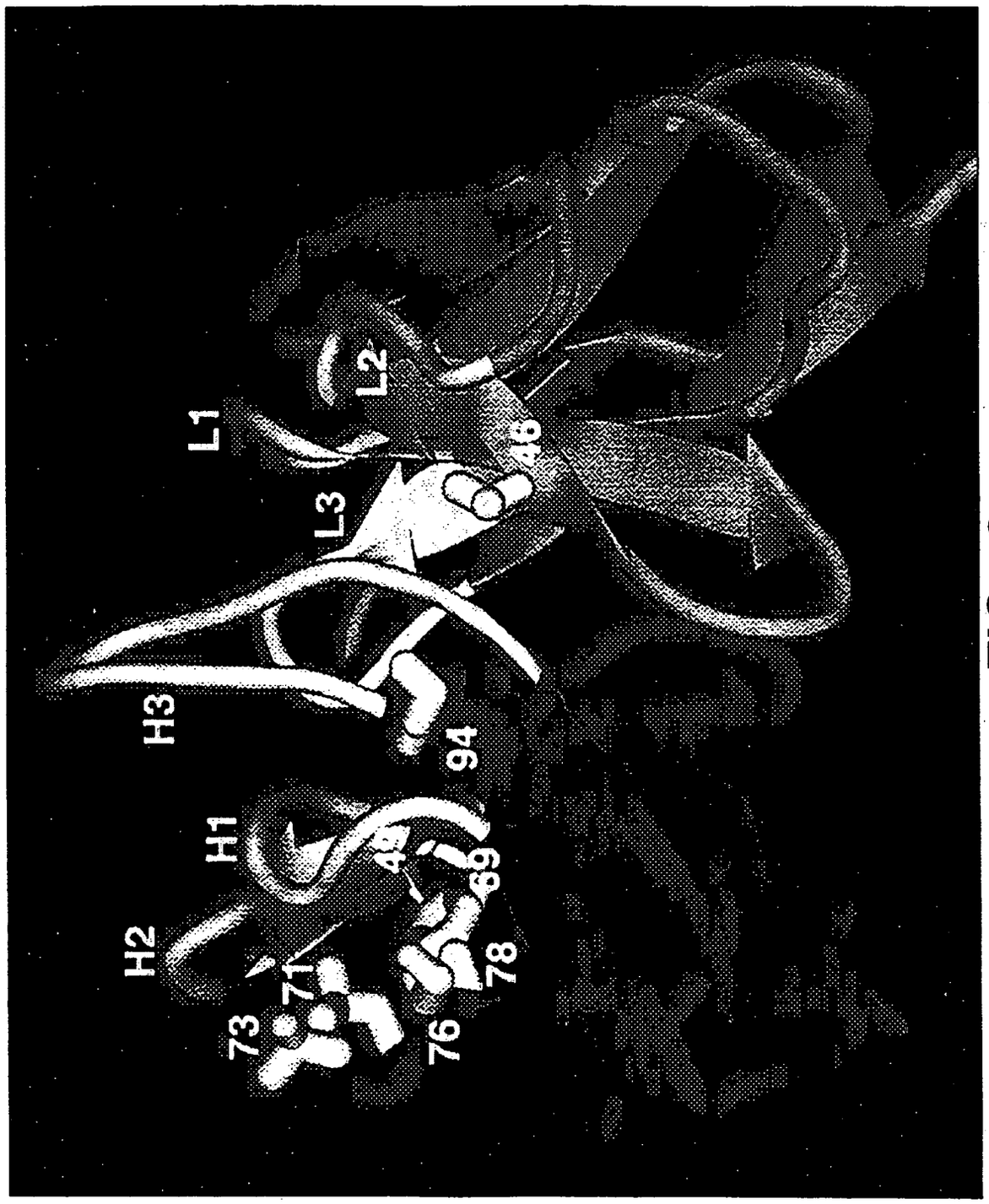


FIG._2

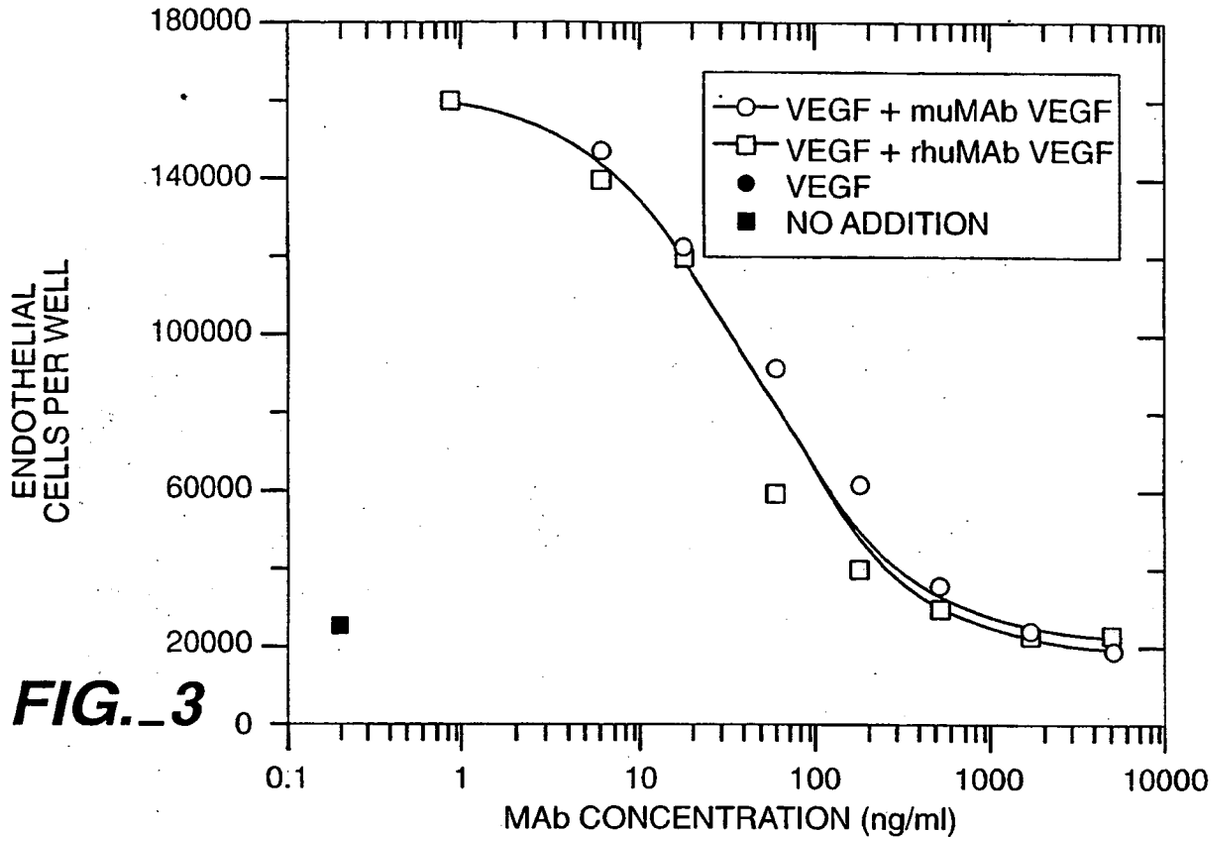


FIG._3

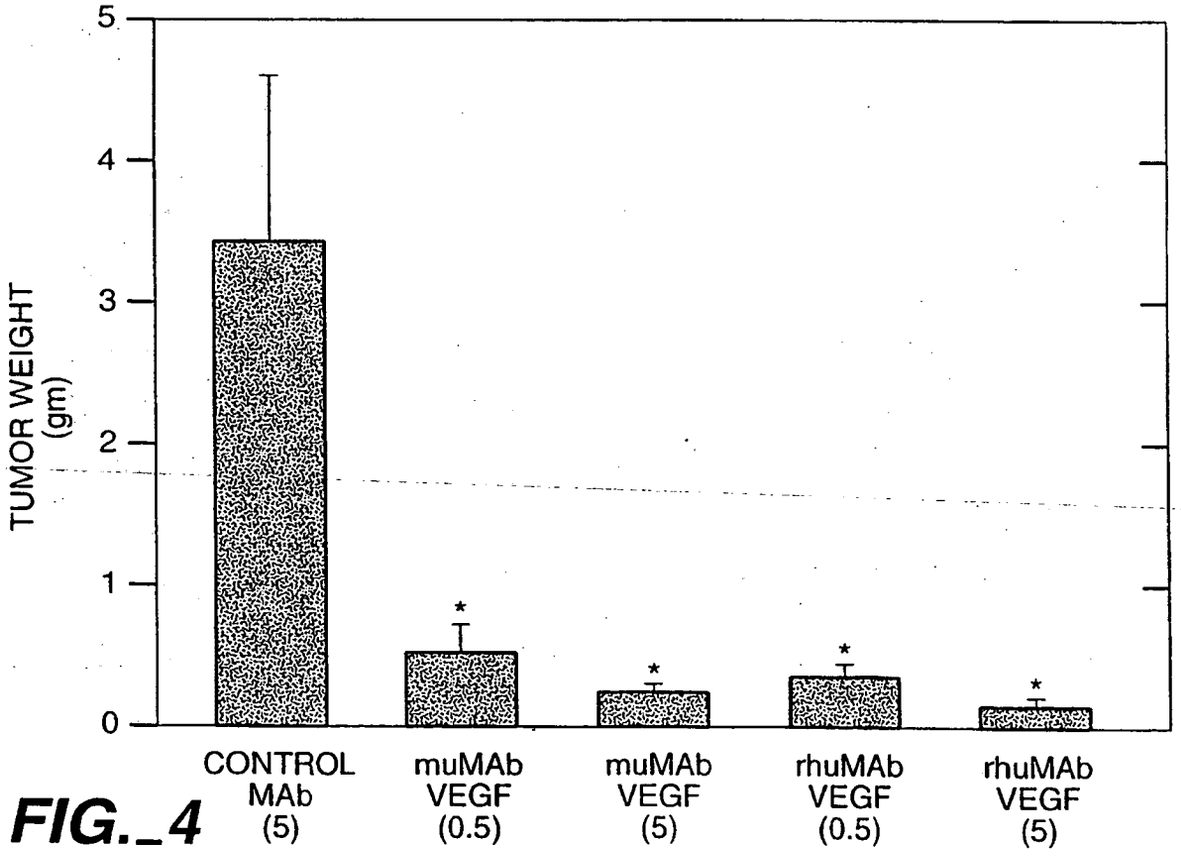


FIG._4

```
                10        20        30        40
A4.6.1  DIQMTQTTSSLSASLGDRVIISCSASQDISNYLNWYQQKP
          **      *    * *
hu2.0   DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKP

hu2.10  DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKP


                50        60        70        80
A4.6.1  DGTVKVLIYFTSSLHSGVPSRFSGSGSGTDYSLTISNLEP
        **** *                           **    * *
hu2.0   GKAPKLLIYFTSSLHSGVPSRFSGSGSGTDFTLTISSLQP
                                         •
hu2.10  GKAPKLLIYFTSSLHSGVPSRFSGSGSGTDYTLTISSLQP


                90       100
A4.6.1  EDIATYYCQQYSTVPWTFGGGTKLEIK    (SEQ.ID NO:10)
          *                  *   *
hu2.0   EDFATYYCQQYSTVPWTFGQGTKVEIK    (SEQ.ID NO:13)

hu2.10  EDFATYYCQQYSTVPWTFGQGTKVEIK    (SEQ.ID NO:15)
```

FIG._5A

```
                10        20        30        40
A4.6.1  EIQLVQSGPELKQPGETVRISCKASGYTFTNYGMNWVKQA
         *   *  ** *   *** *  *                *
hu2.0   EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQA
                                              •
hu2.10  EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWIRQA


                50 a      60        70        80
A4.6.1  PGKGLKWMGWINTYTGEPTYAADFKRRFTFSLETSASTAYL
          * *                        * *** ** *
hu2.0   PGKGLEWVGWINTYTGEPTYAADFKRRFTISRDNSKNTLYL
                                       • • •• •
hu2.10  PGKGLEWVGWINTYTGEPTYAADFKRRFTISLDTSASTVYL


          abc       90       100abcdef       110
A4.6.1  QISNLKNDDTATYFCAKYPHYYGSSHWYFDVWGAGTTVTVSS  (SEQ.ID NO:9)
         *** ***   * *  *                    *  *
hu2.0   QMNSLRAEDTAVYYCARYPHYYGSSHWYFDVWGQGTLVTVSS  (SEQ.ID NO:14)
                          •
hu2.10  QMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTLVTVSS  (SEQ.ID NO:16)
```

FIG._5B

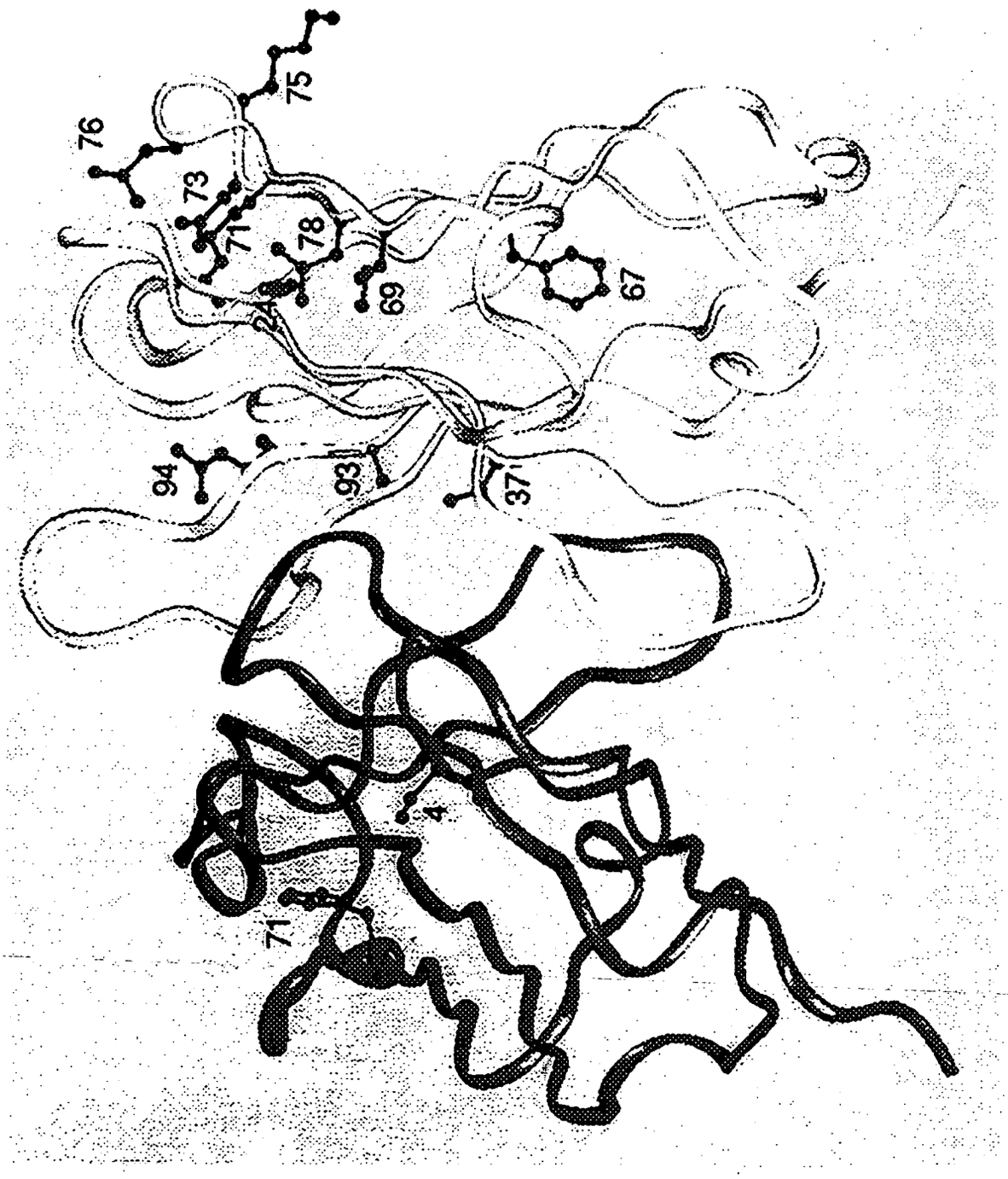


FIG._6

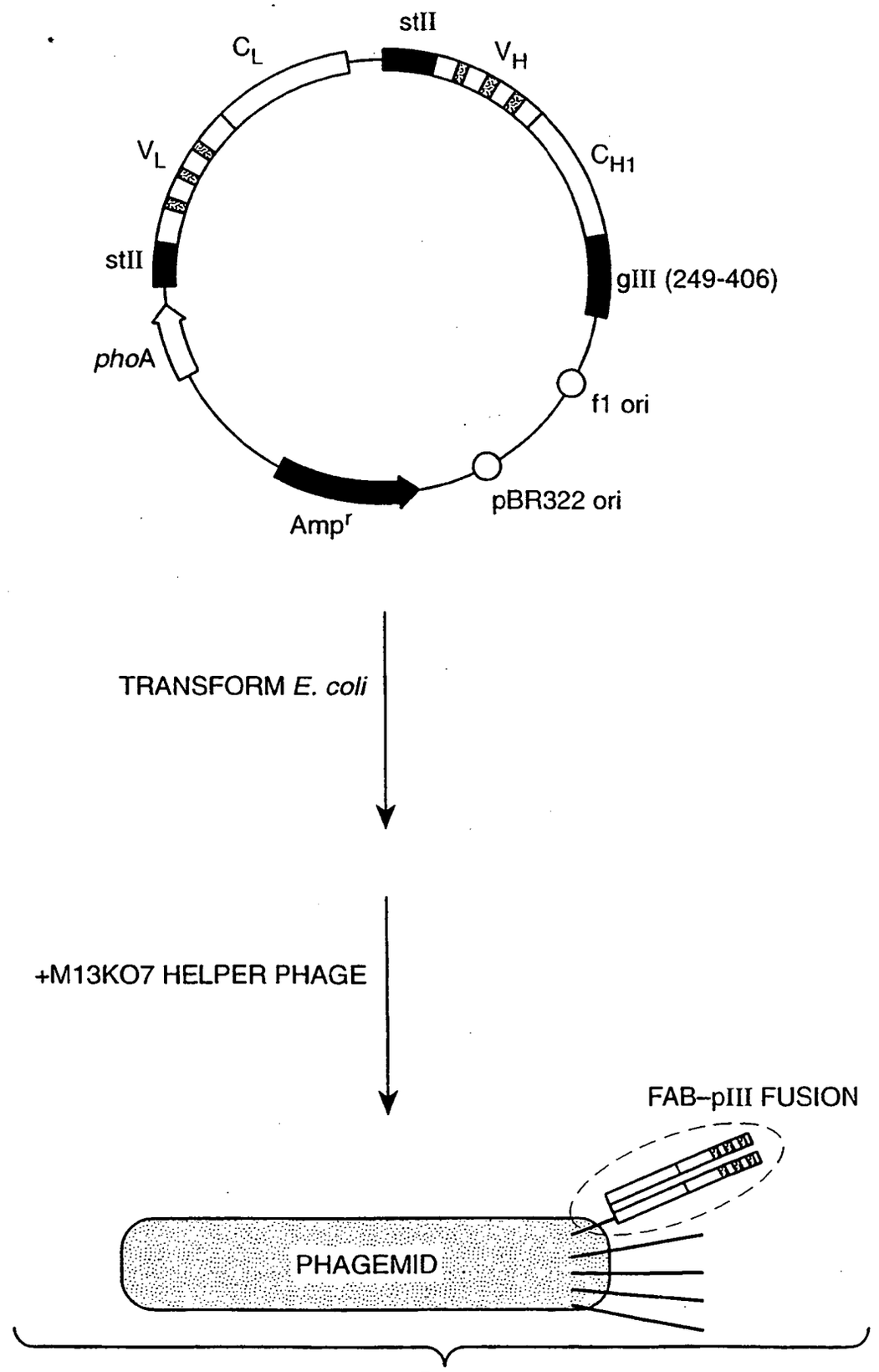


FIG._7

```
GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA     60

GTTGTTATTT AAGCTTTGGA GATTATCGTC ACTGCAATGC TTCGCAATAT GGCGCAAAAT    120

GACCAACAGC GGTTGATTGA TCAGGTAGAG GGGGCGCTGT ACGAGGTAAA GCCCGATGCC    180

AGCATTCCTG ACGACGATAC GGAGCTGCTG CGCGATTACG TAAAGAAGTT ATTGAAGCAT    240

CCTCGTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGTCATA AAGTTGTCAC GGCCGAGACT    300

TATAGTCGCT TTGTTTTTAT TTTTTAATGT ATTTGTAACT AGAATTCGAG CTCGGTACCC    360

GGGGATCCTC TAGAGGTTGA GGTGATTTT ATG AAA AAG AAT ATC GCA TTT CTT       413
                                Met Lys Lys Asn Ile Ala Phe Leu
                                -23         -20

CTT GCA TCT ATG TTC GTT TTT TCT ATT GCT ACA AAC GCG TAC GCT GAT       461
Leu Ala Ser Met Phe Val Phe Ser Ile Ala Thr Asn Ala Tyr Ala Asp
-15                 -10                 -5                   1
                    BEGIN stII SIGNAL SEQUENCE
ATC CAG TTG ACC CAG TCC CCG AGC TCC CTG TCC GCC TCT GTG GGC GAT       509
Ile Gln Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp
            5                  10                  15

AGG GTC ACC ATC ACC TGC AGC GCA AGT CAG GAT ATT AGC AAC TAT TTA       557
Arg Val Thr Ile Thr Cys Ser Ala Ser Gln Asp Ile Ser Asn Tyr Leu
        20                  25                  30
 BEGIN LIGHT CHAIN
AAC TGG TAT CAA CAG AAA CCA GGA AAA GCT CCG AAA CTA CTG ATT TAC       605
Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr
     35                  40                  45

TTC ACC TCC TCT CTC CAC TCT GGA GTC CCT TCT CGC TTC TCT GGA TCC       653
Phe Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
 50                  55                  60                  65

GGT TCT GGG ACG GAT TAC ACT CTG ACC ATC AGC AGT CTG CAG CCA GAA       701
Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
                70                  75                  80

GAC TTC GCA ACT TAT TAC TGT CAA CAG TAT AGC ACC GTG CCG TGG ACG       749
Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Ser Thr Val Pro Trp Thr
            85                  90                  95

TTT GGA CAG GGT ACC AAG GTG GAG ATC AAA CGA ACT GTG GCT GCA CCA       797
Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro
        100                 105                 110

TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT       845
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
    115                 120                 125

GCT TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA       893
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130                 135                 140                 145
```

FIG._8A

```
GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG      941
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
                150                 155                 160

AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC      989
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
            165                 170                 175

ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC     1037
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
        180                 185                 190

TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC     1085
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
    195                 200                 205

AAC AGG GGA GAG TGT T AAGCTGATCC TCTACGCCGG ACGCATCGTG              1131
Asn Arg Gly Glu Cys O C*
210

GCCCTAGTAC GCAACTAGTC GTAAAAAGGG TATCTAGAGG TTGAGGTGAT TTT ATG      1187
                                                            Met
                                                            -23
                                BEGIN stII SIGNAL SEQUENCE
AAA AAG AAT ATC GCA TTT CTT CTT GCA TCT ATG TTC GTT TTT TCT ATT     1235
Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe Ser Ile
        -20                 -15                 -10

GCT ACA AAC GCG TAC GCT GAG GTT CAG CTG GTG GAG TCT GGC GGT GGC     1283
Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly
    -5                  1                   5                  10

CTG GTG CAG CCA GGG GGC TCA CTC CGT TTG TCC TGT GCA GCT TCT GGC     1331
Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
                15                  20                  25
                            BEGIN HEAVY CHAIN
TAT ACC TTC ACC AAC TAT GGT ATG AAC TGG ATC CGT CAG GCC CCG GGT     1379
Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Ile Arg Gln Ala Pro Gly
            30                  35                  40

AAG GGC CTG GAA TGG GTT GGA TGG ATT AAC ACC TAT ACC GGT GAA CCG     1427
Lys Gly Leu Glu Trp Val Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro
        45                  50                  55

ACC TAT GCT GCG GAT TTC AAA CGT CGT TTT ACT ATA TCT GCA GAC ACC     1475
Thr Tyr Ala Ala Asp Phe Lys Arg Arg Phe Thr Ile Ser Ala Asp Thr
    60                  65                  70

TCC AGC AAC ACA GTT TAC CTG CAG ATG AAC AGC CTG CGC GCT GAG GAC     1523
Ser Ser Asn Thr Val Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
75                  80                  85                  90

ACT GCC GTC TAT TAC TGT GCA AAG TAC CCG CAC TAT TAT GGG AGC AGC     1571
Thr Ala Val Tyr Tyr Cys Ala Lys Tyr Pro His Tyr Tyr Gly Ser Ser
                95                  100                 105
```

FIG._8B

```
CAC TGG TAT TTC GAC GTC TGG GGT CAA GGA ACC CTG GTC ACC GTC TCC     1619
His Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser
            110                 115                 120

TCG GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC     1667
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
        125                 130                 135

AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC     1715
Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
    140                 145                 150

TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC     1763
Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
155                 160                 165                 170

AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC     1811
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
                175                 180                 185

TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG     1859
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
            190                 195                 200

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTC GAC     1907
Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
        205                 210                 215

AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC CTC TAG AGT GGC     1955
Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Leu  *  Ser Gly
    220                 225                 230

GGT GGC TCT GGT TCC GGT GAT TTT GAT TAT GAA AAG ATG GCA AAC GCT     2003
Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala
235                 240                 245                 250
                                    END LIGHT CHAIN         BE
AAT AAG GGG GCT ATG ACC GAA AAT GCC GAT GAA AAC GCG CTA CAG TCT     2051
Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser
                255                 260                 265
GIN g3p DOMAIN
GAC GCT AAA GGC AAA CTT GAT TCT GTC GCT ACT GAT TAC GGT GCT GCT     2099
Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala
            270                 275                 280

ATC GAT GGT TTC ATT GGT GAC GTT TCC GGC CTT GCT AAT GGT AAT GGT     2147
Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn Gly
        285                 290                 295

GCT ACT GGT GAT TTT GCT GGC TCT AAT TCC CAA ATG GCT CAA GTC GGT     2195
Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val Gly
    300                 305                 310

GAC GGT GAT AAT TCA CCT TTA ATG AAT AAT TTC CGT CAA TAT TTA CCT     2243
Asp Gly Asp Asn Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro
315                 320                 325                 330
```

FIG._8C

```
TCC CTC CCT CAA TCG GTT GAA TGT CGC CCT TTT GTC TTT AGC GCT GGT     2291
Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Phe Val Phe Ser Ala Gly
                335                 340                 345

AAA CCA TAT GAA TTT TCT ATT GAT TGT GAC AAA ATA AAC TTA TTC CGT     2339
Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg
            350                 355                 360

GGT GTC TTT GCG TTT CTT TTA TAT GTT GCC ACC TTT ATG TAT GTA TTT     2387
Gly Val Phe Ala Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe
        365                 370                 375

TCT ACG TTT GCT AAC ATA CTG CGT AAT AAG GAG TCT TAATCATGCC          2433
Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys Glu Ser OC* (SEQ.ID NO:100)
    380                 385                 390
AGTTCTTTTG GCTAGCGCCG CCCTATACCT TGTCTGCCTC CCCGCGTTGC GTCGCGGTGC   2493
                  END G3 PROTEIN
ATGGAGCCGG GCCACCTCGA CCTGAATGGA AGCCGGCGGC ACCTCGCTAA CGGATTCACC   2553

ACTCCAAGAA TTGGAGCCAA TCAATTCTTG CGGAGAACTG TGAATGCGCA AACCAACCCT   2613

TGGCAGAACA TATCCATCGC GTCCGCCATC TCCAGCAGCC GCACGCGGCG CATCTCGGGC   2673

AGCGTTGGGT CCTGGCCACG GGTGCGCATG ATCGTGCTCC TGTCGTTGAG GACCCGGCTA   2733

GGCTGGCGGG GTTGCCTTAC TGGTTAGCAG AATGAATCAC CGATACGCGA GCGAACGTGA   2793

AGCGACTGCT GCTGCAAAAC GTCTGCGACC TGAGCAACAA CATGAATGGT CTTCGGTTTC   2853

CGTGTTTCGT AAAGTCTGGA AACGCGGAAG TCAGCGCCCT GCACCATTAT GTTCCGGATC   2913

TGCATCGCAG GATGCTGCTG GCTACCCTGT GGAACACCTA CATCTGTATT AACGAAGCGC   2973

TGGCATTGAC CCTGAGTGAT TTTTCTCTGG TCCCGCCGCA TCCATACCGC CAGTTGTTTA   3033

CCCTCACAAC GTTCCAGTAA CCGGGCATGT TCATCATCAG TAACCCGTAT CGTGAGCATC   3093

CTCTCTCGTT TCATCGGTAT CATTACCCCC ATGAACAGAA ATTCCCCCTT ACACGGAGGC   3153

ATCAAGTGAC CAAACAGGAA AAAACCGCCC TTAACATGGC CCGCTTTATC AGAAGCCAGA   3213

CATTAACGCT TCTGGAGAAA CTCAACGAGC TGGACGCGGA TGAACAGGCA GACATCTGTG   3273

AATCGCTTCA CGACCACGCT GATGAGCTTT ACCGCAGGAT CCGGAAATTG TAAACGTTAA   3333

TATTTTGTTA AAATTCGCGT TAAATTTTTG TTAAATCAGC TCATTTTTTA ACCAATAGGC   3393

CGAAATCGGC AAAATCCCTT ATAAATCAAA AGAATAGACC GAGATAGGGT TGAGTGTTGT   3453

TCCAGTTTGG AACAAGAGTC CACTATTAAA GAACGTGGAC TCCAACGTCA AAGGGCGAAA   3513

AACCGTCTAT CAGGGCTATG GCCCACTACG TGAACCATCA CCCTAATCAA GTTTTTTGGG   3573

GTCGAGGTGC CGTAAAGCAC TAAATCGGAA CCCTAAAGGG AGCCCCCGAT TTAGAGCTTG   3633
```

FIG._8D

ACGGGGAAAG CCGGCGAACG TGGCGAGAAA GGAAGGGAAG AAAGCGAAAG GAGCGGGCGC 3693

TAGGGCGCTG GCAAGTGTAG CGGTCACGCT GCGCGTAACC ACCACACCCG CCGCGCTTAA 3753

TGCGCCGCTA CAGGGCGCGT CCGGATCCTG CCTCGCGCGT TTCGGTGATG ACGGTGAAAA 3813

CCTCTGACAC ATGCAGCTCC CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG 3873

CAGACAAGCC CGTCAGGGCG CGTCAGCGGG TGTTGGCGGG TGTCGGGGCG CAGCCATGAC 3933

CCAGTCACGT AGCGATAGCG GAGTGTATAC TGGCTTAACT ATGCGGCATC AGAGCAGATT 3993

GTACTGAGAG TGCACCATAT GCGGTGTGAA ATACCGCACA GATGCGTAAG GAGAAAATAC 4053

CGCATCAGGC GCTCTTCCGC TTCCTCGCTC ACTGACTCGC TGCGCTCGGT CGTTCGGCTG 4113

CGGCGAGCGG TATCAGCTCA CTCAAAGGCG GTAATACGGT TATCCACAGA ATCAGGGGAT 4173

AACGCAGGAA AGAACATGTG AGCAAAAGGC CAGCAAAAGG CCAGGAACCG TAAAAAGGCC 4233

GCGTTGCTGG CGTTTTTCCA TAGGCTCCGC CCCCCTGACG AGCATCACAA AAATCGACGC 4293

TCAAGTCAGA GGTGGCGAAA CCCGACAGGA CTATAAAGAT ACCAGGCGTT TCCCCCTGGA 4353

AGCTCCCTCG TGCGCTCTCC TGTTCCGACC CTGCCGCTTA CCGGATACCT GTCCGCCTTT 4413

CTCCCTTCGG GAAGCGTGGC GCTTTCTCAT AGCTCACGCT GTAGGTATCT CAGTTCGGTG 4473

TAGGTCGTTC GCTCCAAGCT GGGCTGTGTG CACGAACCCC CCGTTCAGCC CGACCGCTGC 4533

GCCTTATCCG GTAACTATCG TCTTGAGTCC AACCCGGTAA GACACGACTT ATCGCCACTG 4593

GCAGCAGCCA CTGGTAACAG GATTAGCAGA GCGAGGTATG TAGGCGGTGC TACAGAGTTC 4653

TTGAAGTGGT GGCCTAACTA CGGCTACACT AGAAGGACAG TATTTGGTAT CTGCGCTCTG 4713

CTGAAGCCAG TTACCTTCGG AAAAAGAGTT GGTAGCTCTT GATCCGGCAA ACAAACCACC 4773

GCTGGTAGCG GTGGTTTTTT TGTTTGCAAG CAGCAGATTA CGCGCAGAAA AAAAGGATCT 4833

CAAGAAGATC CTTTGATCTT TTCTACGGGG TCTGACGCTC AGTGGAACGA AAACTCACGT 4893

TAAGGGATTT TGGTCATGAG ATTATCAAAA AGGATCTTCA CCTAGATCCT TTTAAATTAA 4953

AAATGAAGTT TTAAATCAAT CTAAAGTATA TATGAGTAAA CTTGGTCTGA CAGTTACCAA 5013

TGCTTAATCA GTGAGGCACC TATCTCAGCG ATCTGTCTAT TTCGTTCATC CATAGTTGCC 5073

TGACTCCCCG TCGTGTAGAT AACTACGATA CGGGAGGGCT TACCATCTGG CCCCAGTGCT 5133

GCAATGATAC CGCGAGACCC ACGCTCACCG GCTCCAGATT TATCAGCAAT AAACCAGCCA 5193

GCCGGAAGGG CCGAGCGCAG AAGTGGTCCT GCAACTTTAT CCGCCTCCAT CCAGTCTATT 5253

FIG._8E

```
AATTGTTGCC GGGAAGCTAG AGTAAGTAGT TCGCCAGTTA ATAGTTTGCG CAACGTTGTT  5313
GCCATTGCTG CAGGCATCGT GGTGTCACGC TCGTCGTTTG GTATGGCTTC ATTCAGCTCC  5373
GGTTCCCAAC GATCAAGGCG AGTTACATGA TCCCCCATGT TGTGCAAAAA AGCGGTTAGC  5433
TCCTTCGGTC CTCCGATCGT TGTCAGAAGT AAGTTGGCCG CAGTGTTATC ACTCATGGTT  5493
ATGGCAGCAC TGCATAATTC TCTTACTGTC ATGCCATCCG TAAGATGCTT TTCTGTGACT  5553
GGTGAGTACT CAACCAAGTC ATTCTGAGAA TAGTGTATGC GGCGACCGAG TTGCTCTTGC  5613
CCGGCGTCAA CACGGGATAA TACCGCGCCA CATAGCAGAA CTTTAAAAGT GCTCATCATT  5673
GGAAAACGTT CTTCGGGGCG AAAACTCTCA AGGATCTTAC CGCTGTTGAG ATCCAGTTCG  5733
ATGTAACCCA CTCGTGCACC CAACTGATCT TCAGCATCTT TTACTTTCAC CAGCGTTTCT  5793
GGGTGAGCAA AAACAGGAAG GCAAAATGCC GCAAAAAAGG GAATAAGGGC GACACGGAAA  5853
TGTTGAATAC TCATACTCTT CCTTTTTCAA TATTATTGAA GCATTTATCA GGGTTATTGT  5913
CTCATGAGCG GATACATATT TGAATGTATT TAGAAAAATA AACAAATAGG GGTTCCGCGC  5973
ACATTTCCCC GAAAAGTGCC ACCTGACGTC TAAGAAACCA TTATTATCAT GACATTAACC  6033
TATAAAAATA GGCGTATCAC GAGGCCCTTT CGTCTTCAA                         6072
```

FIG._8F

```
                10          20          30          40
F(ab)-12    DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
MB1.6       DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
H2305.6     DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
Y0101       DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
Y0192       DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
                                          CDR-L1
                50          60          70          80
F(ab)-12    GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
MB1.6       GKAPKLLIYF TSSLHSGVPS RFSGSGSGTD YTLTISSLQP
H2305.6     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD YTLTISSLQP
Y0101       GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0192       GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
                      CDR-L2
                90          100         110
F(ab)-12    EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:8)
MB1.6       EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:101)
H2305.6     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:103)
Y0101       EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:105)
Y0192       EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:107)
                      CDR-L3
```

FIG._9A

```
                10          20          30          40
F(ab)-12    EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA
MB1.6       EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWIRQA
H2305.6     EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWIRQA
Y0101       EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA
Y0192       EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGINWVRQA
                                      CDR-H1
                50          60          70          80
F(ab)-12    PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
MB1.6       PGKGLEWVGW INTYTGEPTY AADFKRRFTI SADTSSNIVY
H2305.6     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SADTSSNIVY
Y0101       PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0192       PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
                       CDR-H2                CDR-7
                90          100         110
F(ab)-12    LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:7)
MB1.6       LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:102)
H2305.6     LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:104)
Y0101       LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:106)
Y0192       LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:108)
                                  CDR-H3
```

FIG._9B

```
                10          20          30          40
F(ab)-12    DIQMTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
Y0243-1     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0238-3     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0313-1     DIQLTQSPSS LSASVGDRVT ITCRANEQLS NYLNWYQQKP
Y0317       DIQLTQSPSS LSASVGDRVT ITCSASQDIS NYLNWYQQKP
                                              CDR-L1
                50          60          70          80
F(ab)-12    GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0243-1     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0238-3     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0313-1     GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
Y0317       GKAPKVLIYF TSSLHSGVPS RFSGSGSGTD FTLTISSLQP
                       CDR-L2
                90          100         110
F(ab)-12    EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:8)
Y0243-1     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:109)
Y0238-3     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:111)
Y0313-1     EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:113)
Y0317       EDFATYYCQQ YSTVPWTFGQ GTKVEIKRTV    (SEQ.ID NO:115)
                       CDR-L3
```

FIG._10A

```
                10          20          30          40
F(ab)-12    EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA
Y0243-1     EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
Y0238-3     EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGINWVRQA
Y0313-1     EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
Y0317       EVQLVESGGG LVQPGGSLRL SCAASGYDFT HYGMNWVRQA
                                   CDR-H1
                50          60          70          80
F(ab)-12    PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0243-1     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0238-3     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0313-1     PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
Y0317       PGKGLEWVGW INTYTGEPTY AADFKRRFTF SLDTSKSTAY
                       CDR-H2                CDR-7
                90          100         110
F(ab)-12    LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:7)
Y0243-1     LQMNSLRAED TAVYYCAKYP HYYGSSHWYF DVWGQGTL   (SEQ.ID NO:110)
Y0238-3     LQMNSLRAED TAVYYCAKYP YYYGISHWYF DVWGQGTL   (SEQ.ID NO:112)
Y0313-1     LQMNSLRAED TAVYYCAKYP YYYGISHWYF DVWGQGTL   (SEQ.ID NO:114)
Y0317       LQMNSLRAED TAVYYCAKYP YYYGISHWYF DVWGQGTL   (SEQ.ID NO:116)
                                   CDR-H3
```

FIG._10B

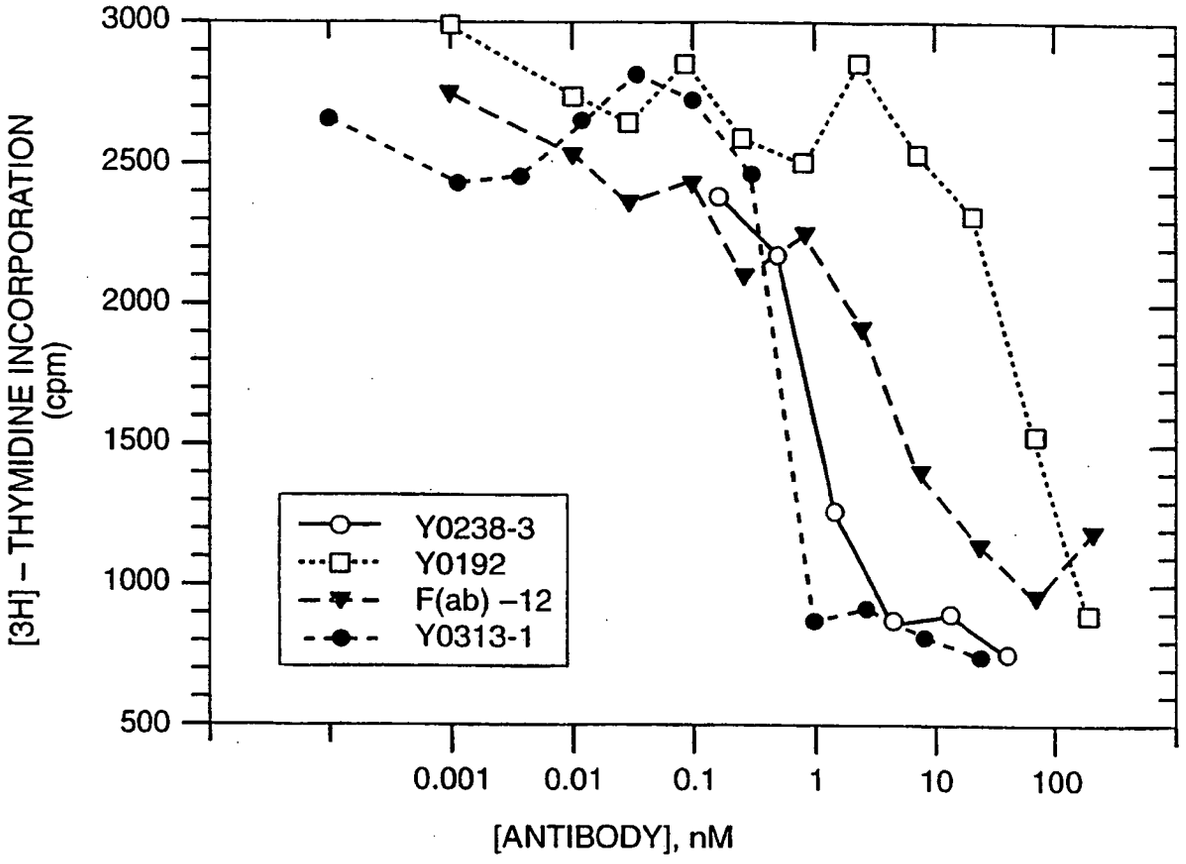

3000
2500
2000
1500
1000
500
[3H] – THYMIDINE INCORPORATION (cpm)
0.001
0.01
0.1
1
10
100
[ANTIBODY], nM
Y0238-3
Y0192
F(ab) –12
Y0313-1

FIG._11

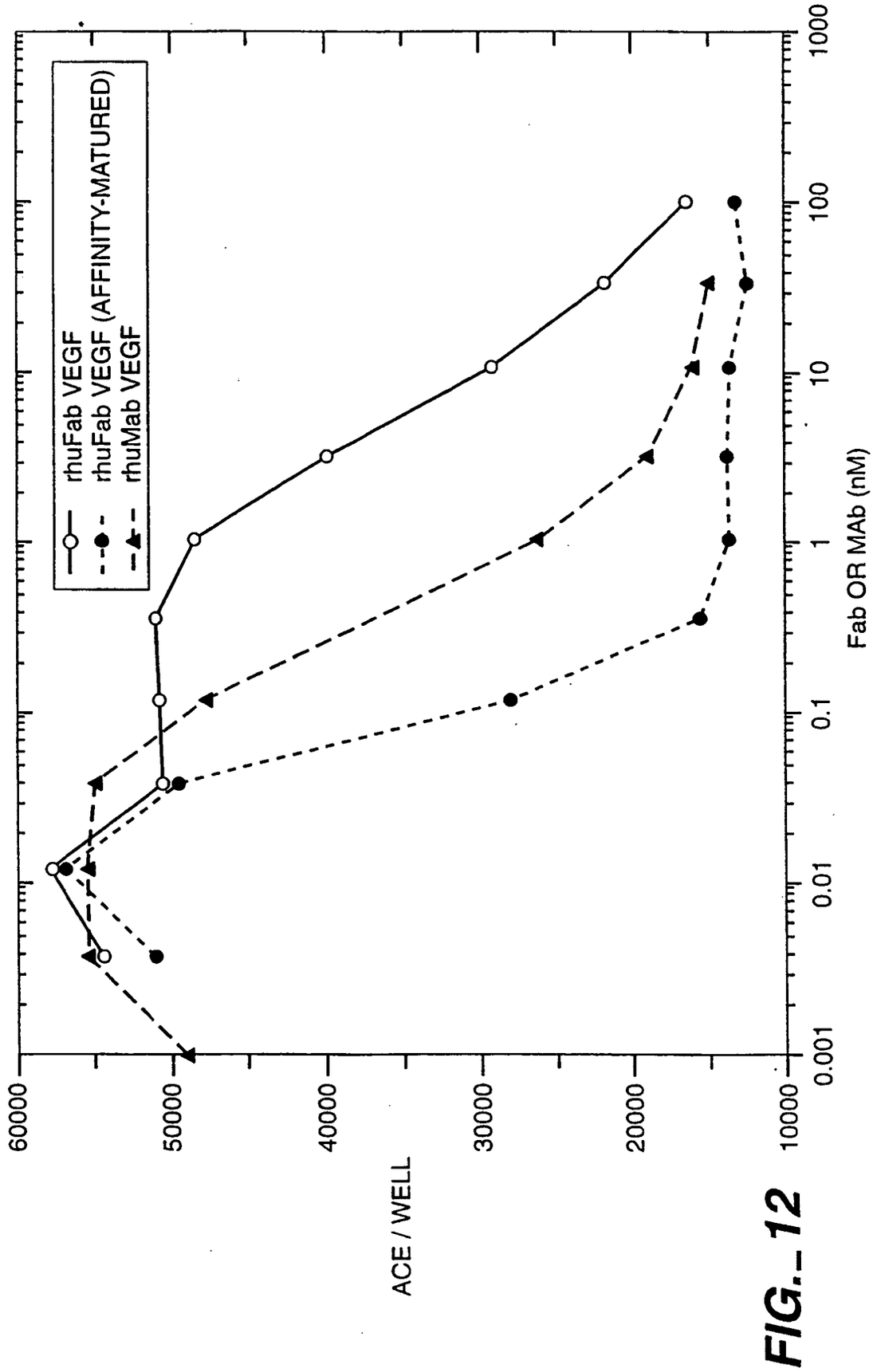
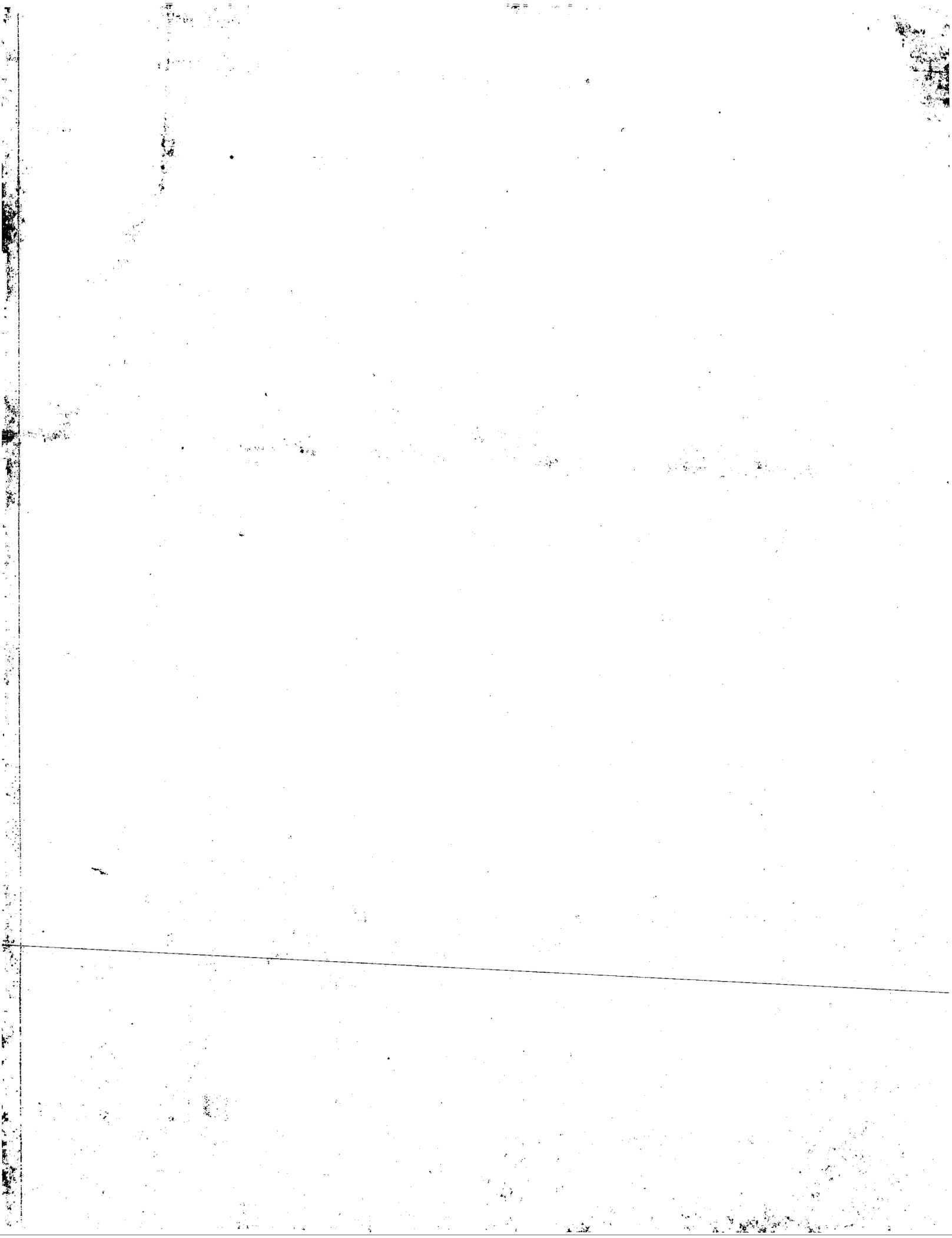


FIG._12



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 16/22, C12N 15/13, 15/63, 15/70, A61K 39/395 | A3

(11) International Publication Number: **WO 98/45331**

(43) International Publication Date: 15 October 1998 (15.10.98)

(21) International Application Number: PCT/US98/06604

(22) International Filing Date: 3 April 1998 (03.04.98)

(30) Priority Data:

08/833,504	7 April 1997 (07.04.97)	US
08/908,469	6 August 1997 (06.08.97)	US

(71) Applicant *(for all designated States except US)*: GENENTECH, INC. [US/US]; One DNA Way, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BACA, Manuel [AU/US]; Apartment #H3, 888 Foster City Boulevard, Foster City, CA 94404 (US). WELLS, James, A. [US/US]; 1341 Columbus Avenue, Burlingame, CA 94010 (US). PRESTA, Leonard, G. [US/US]; Apartment 206, 1900 Gough Street, San Francisco, CA 94109 (US). LOWMAN, Henry, B. [US/US]; 400 San Juan Avenue, El Granada, CA 94018 (US). CHEN, Yvonne, Man-Yee [CA/US]; 1951 O'Farrell Street #321, San Mateo, CA 94403 (US).

(74) Agents: DREGER, Walter, H. et al.; Flehr, Hohbach, Test, Albritton & Herbert LLP, Suite 400, 4 Embarcadero Center, San Francisco, CA 94111-4187 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

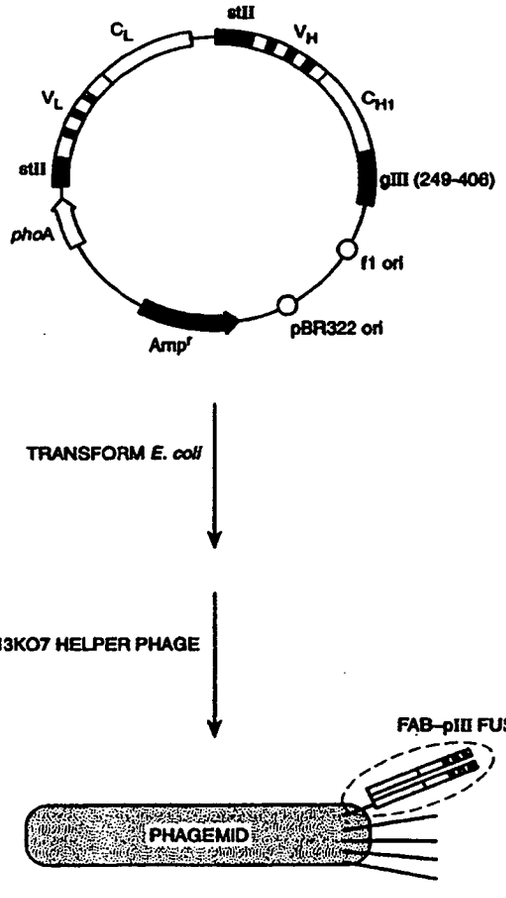
Published
With international search report.

(88) Date of publication of the international search report: 3 December 1998 (03.12.98)

(54) Title: ANTI-VEGF ANTIBODIES

(57) Abstract

Humanized and variant anti-VEGF antibodies and various uses therefor are disclosed. The anti-VEGF antibodies have strong binding affinities for VEGF; inhibit VEGF-induced proliferation of endothelial cells *in vitro*, and inhibit tumor growth *in vivo*.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 98/06604

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/22 C12N15/13 C12N15/63 C12N15/70 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 92 22653 A (GENENTECH INC) 23 December 1992 the whole document and specially: see SEQ.ID.N. 17,18 and 25 see page 5, line 24 - page 7, line 35 see page 9, line 22 - page 10, line 4; figure 5 ---	7,10-13, 25-27 1,4,14, 15,22, 28-31, 34-38
Y	KIM ET AL.,: "Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth in vivo" NATURE, vol. 362, 1993, page 841 XP002013864 London, GB cited in the application see abstract --- -/--	1,4,14, 15,22, 28-31, 34-38

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 1998	02/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PCT/US 98/06604

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 268 744 A (CELLTECH LTD) 19 January 1994 see abstract see page 4, paragraph 3 - page 6, paragraph 1	1,32-41
A	M.M. BENDIG: "Humanization of rodent monoclonal antibodies" METHODS: A COMPANION TO METHODS IN ENZYMOLOGY, vol. 8, 1995, pages 83-93, XP000647344 New York, NY, US see the whole document	1,34-38
P,X	M. BACA ET AL., : "Antibody humanization using monovalent phage display" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 16, 18 April 1997, pages 10678-10684, XP002077471 see the whole document	1-42

1

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/US 98/06604

Box I Observations where certain claims were found uns archable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 5 AND 39-42
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 5 and 39-42 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/US 98/06604

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9222653 A	23-12-1992	AU 675916 B	27-02-1997
		AU 2250992 A	12-01-1993
		CA 2103059 A	15-12-1992
		EP 0590058 A	06-04-1994
		JP 6508267 T	22-09-1994
		WO 9404679 A	03-03-1994
GB 2268744 A	19-01-1994	AT 129017 T	15-10-1995
		AT 124459 T	15-07-1995
		AT 159299 T	15-11-1997
		AU 664801 B	30-11-1995
		AU 6461294 A	22-12-1994
		AU 646009 B	03-02-1994
		AU 6974091 A	24-07-1991
		AU 649645 B	02-06-1994
		AU 7033091 A	24-07-1991
		AU 631481 B	26-11-1992
		AU 7048691 A	24-07-1991
		BG 60462 B	28-04-1995
		CA 2037607 A	07-09-1992
		CA 2046904 A	22-06-1991
		CA 2050479 A,C	22-06-1991
		DE 69020544 D	03-08-1995
		DE 69020544 T	18-01-1996
		DE 69022982 D	16-11-1995
		DE 69022982 T	28-03-1996
		DE 69031591 D	20-11-1997
		DE 69031591 T	12-03-1998
		DK 460167 T	20-11-1995
		DK 460171 T	28-08-1995
		DK 460178 T	22-12-1997
		EP 0460167 A	11-12-1991
		EP 0460171 A	11-12-1991
		EP 0460178 A	11-12-1991
		EP 0620276 A	19-10-1994
		EP 0626390 A	30-11-1994
		ES 2079638 T	16-01-1996
		ES 2074701 T	16-09-1995
		ES 2112270 T	01-04-1998
		WO 9109966 A	11-07-1991

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/US 98/06604

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 2268744 A		WO 9109967 A	11-07-1991
		WO 9109968 A	11-07-1991
		GB 2246781 A,B	12-02-1992
		GB 2246570 A,B	05-02-1992
		GB 2268745 A,B	19-01-1994
		GR 3017734 T	31-01-1996
		GR 3025781 T	31-03-1998
		JP 4505398 T	24-09-1992
		JP 4506458 T	12-11-1992
		JP 5500312 T	28-01-1993

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 For: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 CUSTOMER NO: 09157 **CERTIFICATE OF MAILING** I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on March 26, 2004 Eileen Ly

NOTICE OF APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant hereby appeals to the Board of Appeals and Interferences from the decision dated September 26, 2003, of the Primary Examiner finally rejecting claims 47, 49, 50 and 53-60.

The Commissioner is hereby authorized to charge Deposit Account No. 07-0630 in the amount of $330 to cover the fees for this appeal and to charge the deposit account for any further fees in regard to this patent application. **A duplicate copy of this Notice is enclosed for this purpose.**

Respectfully submitted,
GENENTECH, INC.

03/30/2004 AWONDAF1 00000089 070630 09723752
01 FC:1401 330.00 DA

Date: March 26, 2004

By: _____
Steven X. Cui
Reg. No. 44,637
Telephone No. (650)225- 8674

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 For: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 CUSTOMER NO: 09157

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

March 26, 2004

Eileen Ly

PETITION AND FEE FOR THREE MONTH EXTENSION OF TIME
(37 CFR 1.136(a))

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Applicant petitions the Commissioner of Patents and Trademarks to extend the time for response to the Final Office Action dated September 26, 2003 for three (3) month(s) from December 26, 2003 to March 26, 2004. The extended time for response does not exceed the statutory period.

Please charge Deposit Account No. 07-0630 in the amount of $950 to cover the cost of the extension. Any deficiency or overpayment should be charged or credited to this deposit account. A duplicate of this sheet is enclosed.

Respectfully submitted,

GENENTECH, INC.

03/30/2004 AWONDAF1 00000089 070630 09723752
02 FC:1253 950.00 DA

Date: March 26, 2004

By: _____
Steven X. Cui
Reg. No. 44,637
Telephone No. (650) 225-8674

#152334

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 For: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 **Customer No: 09157**

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on

March 26, 2004

Eileen Ly

TRANSMITTAL LETTER

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Transmitted herewith are the following documents:

1. Notice of Appeal (duplicate);
2. Petition for Three Months Extension of Time (duplicate);
3. Fees ($950 + $330); and
4. Return Postcard

In the event any additional fees are due in connection with the filing of these documents, the Commissioner is authorized to charge such fees to our Deposit Account No. 07-0630.

Respectfully submitted,

GENENTECH, INC.

Date: March 26, 2004 By: _____

Steven X. Cui
Reg. No. 44,637
Telephone No. (650) 225-8674

#152336

Revised (10/11/95)

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

9157 7590 12/13/2004

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HELMS, LARRY RONALD	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 12/13/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
09/723752			

EXAMINER	
LARRY R. HELMS	
ART UNIT	**PAPER**
1642	20041210

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

In view of applicant's failure to file a brief within the time prescribed by 37 CFR 1.192(a), the appeal stands dismissed and the proceedings as to the rejected claims are considered terminated. See 37 CFR 1.197(c).

This application will be passed to issue on allowed claims 51-52 provided the following formal matters are corrected. Prosecution is otherwise closed.

Claims 47, 49, 50, 53-60 are the rejected claims and need to be canceled in order to allow claims 51-52.

Applicant is required to make the necessary corrections within a shortened statutory period set to expire ONE MONTH or THIRTY DAYS, whichever is longer, from the mailing date of this letter. Extensions of time may be granted under 37 CFR 1.136.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Larry R. Helms, Ph.D, whose telephone number is (571) 272-0832. The examiner can normally be reached on Monday through Friday from 6:30 am to 4:00 pm, with alternate Fridays off. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jeffrey Siew, can be reached at (571) 272-0787.

Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The Fax Center telephone number is 703-872-9306.

Larry R. Helms

571-272-0832

LARRY R. HELMS, PH.D
PRIMARY EXAMINER

1-14-05

1642
1fw

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 Title: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 Customer No: 09157 **EXPRESS MAIL LABEL NO.: EV 351 927 211 US** **DATE OF DEPOSIT: JANUARY 13, 2005**

RESPONSE TO OFFICE COMMUNICATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This document is responsive to the Communication mailed December 13, 2004 (Paper No. 20041210) for which a one-month period for response was given. Applicant is required to cancel the currently rejected claims in order for claims 51-52 to be allowed. Otherwise, the prosecution is deemed closed.

Amendments to the Specification and Claims begin on page 2 of this paper.

Amendments to the Specification

Please replace the paragraph beginning at page 1, line 9 with the following amended paragraph:

This application is a divisional of the co-pending U.S. Application Serial No. 08/908,469, filed August 6, 1997, ~~which claims priority under 35 USC 119 to the provisional U.S. Application Serial No. 60/126,446, filed April 7, 1997,~~ which ~~applications are~~ is incorporated herein by reference.

Amendments to the Claims

Please cancel claims 43-47, 49, 50, 53-60.

Remarks

Claims 43-47 and 49-60 were pending. All the rejected claims have been cancelled in favour of the issuance of claims 51-52. Applicants reserve the right to pursue prosecution of the canceled claims in currently pending or future continuation and/or divisional applications.

Furthermore, the priority claim has been amended in light of the allowed claims and in response to the Office Action mailed September 26, 2003.

Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

GENENTECH, INC.

Date: January 13, 2005

By: _____

Steven X. Cui
Reg. No. 44,637
Telephone No. (650) 225-8674

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

9157 7590 02/04/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HELMS, LARRY RONALD	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 02/04/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450
www.uspto.gov

Notice of Non-Compliant Amendment (37 CFR 1.121)

The amendment document filed on 1-13-05 is considered non-compliant because it has failed to meet the requirements of 37 CFR 1.121. In order for the amendment document to be compliant, correction of the following item(s) is required. **Only the corrected section of the non-compliant amendment document must be resubmitted (in its entirety), e.g., the entire "Amendments to the claims" section of applicant's amendment document must be re-submitted.** 37 CFR 1.121(h).

THE FOLLOWING CHECKED (X) ITEM(S) CAUSE THE AMENDMENT DOCUMENT TO BE NON-COMPLIANT:

☒ 1. Amendments to the specification:
- ☐ A. Amended paragraph(s) do not include markings.
- ☐ B. New paragraph(s) should not be underlined.
- ☒ C. Other specification on separate sheet

☐ 2. Abstract:
- ☐ A. Not presented on a separate sheet. 37 CFR 1.72.
- ☐ B. Other _____

☐ 3. Amendments to the drawings: _____

☒ 4. Amendments to the claims:
- ☒ A. A complete listing of all of the claims is not present.
- ☐ B. The listing of claims does not include the text of all pending claims (including withdrawn claims)
- ☒ C. Each claim has not been provided with the proper status identifier, and as such, the individual status of each claim cannot be identified. Note: the status of every claim must be indicated after its claim number by using one of the following 7 status identifiers: (Original), (Currently amended), (Canceled), (Withdrawn), (Previously presented), (New) and (Not entered).
- ☐ D. The claims of this amendment paper have not been presented in ascending numerical order.
- ☐ E. Other: _____

For further explanation of the amendment format required by 37 CFR 1.121, see MPEP Sec. 714 and the USPTO website at http://www.uspto.gov/web/offices/pac/dapp/opla/preognotice/officeflyer.pdf .

If the non-compliant amendment is a **PRELIMINARY AMENDMENT,** applicant is given ONE MONTH from the mail date of this letter to supply the corrected section which complies with 37 CFR 1.121. Failure to comply with 37 CFR 1.121 will result in non-entry of the preliminary amendment and examination on the merits will commence without consideration of the proposed changes in the preliminary amendment(s). This notice is not an action under 35 U.S.C. 132, and **this ONE MONTH time limit is not extendable.**

If the non-compliant amendment is a reply to a **NON-FINAL OFFICE ACTION (including a submission for an RCE),** and since the amendment appears to be a *bona fide* attempt to be a reply (37 CFR 1.135(c)), applicant is given a TIME PERIOD of ONE MONTH from the mailing of this notice within which to re-submit the corrected section which complies with 37 CFR 1.121 in order to avoid abandonment. **EXTENSIONS OF THIS TIME PERIOD ARE AVAILABLE UNDER 37 CFR 1.136(a).**

If the amendment is a reply to a **FINAL REJECTION,** this form may be an attachment to an Advisory Action. **The period for response to a final rejection continues to run from the date set in the final rejection**, and is not affected by the non-compliant status of the amendment.

Linda Gallo — 571-272-0555

Legal Instruments Examiner (LIE) — Telephone No.

Rev. 6/04

1642

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 Title: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 Customer No: 09157 **EXPRESS MAIL LABEL NO.: EV 385 659 925 US** **DATE OF DEPOSIT: MARCH 1, 2005**

RESPONSE TO NOTICE OF NON-COMPLIANT AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This document is responsive to the Communication mailed December 13, 2004 (Paper No. 20041210) and the Notice of Non-Compliant Amendment mailed February 4, 2005 for which a one-month period for response was given. Applicant is required to cancel the currently rejected claims in order for claims 51-52 to be allowed.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 4 of this paper.

Amendments to the Specification

Please replace the paragraph beginning at page 1, line 9 with the following amended paragraph:

This application is a divisional of the co-pending U.S. Application Serial No. 08/908,469, filed August 6, 1997, ~~which claims priority under 35 USC 119 to the provisional U.S. Application Serial No. 60/126,446, filed April 7, 1997,~~ which ~~applications are~~ is incorporated herein by reference.

Amendments to the Claims

Please cancel claims 43-47, 49, 50, 53-60.

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.-50. (Canceled)

51. (Previously presented) A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:116 and a light chain variable domain sequence of SEQ ID NO:115.

52. (Previously presented) A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:7 and a light chain variable domain sequence of SEQ ID NO:8.

53.-60. (Canceled)

REMARKS

Claims 43-47 and 49-60 were pending. All the rejected claims have been cancelled in favour of the issuance of claims 51-52. Applicants reserve the right to pursue prosecution of the canceled claims in currently pending or future continuation and/or divisional applications.

The priority claim in the specification has been amended in light of the allowed claims and in response to the Office Action mailed September 26, 2003.

Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

GENENTECH, INC.

Date: March 1, 2005

By: _____

Steven X. Cui
Reg. No. 44,637
Telephone No. (650) 225-8674

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:21:17 ; Search time 88.0482 Seconds
(without alignments)
483.186 Million cell updates/sec

Title: US-09-723-752B-115
Perfect score: 575
Sequence: 1 DIQLTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 2105692 seqs, 386760381 residues

Total number of hits satisfying chosen parameters: 2105692

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : A_Geneseq_16Dec04:*
1: geneseqp1980s:*
2: geneseqp1990s:*
3: geneseqp2000s:*
4: geneseqp2001s:*
5: geneseqp2002s:*
6: geneseqp2003as:*
7: geneseqp2003bs:*
8: geneseqp2004s:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	575	100.0	110	2	AAW70677	Aaw70677 Anti-VEGF
2	575	100.0	110	2	AAW70687	Aaw70687 Anti-VEGF
3	575	100.0	110	3	AAB13380	Aab13380 Anti-VEGF
4	575	100.0	110	5	ABP61256	Abp61256 Humanised
5	575	100.0	110	5	ABP61246	Abp61246 Humanised
6	575	100.0	214	7	ADC26154	Adc26154 Parent an
7	575	100.0	237	5	ABB81107	Abb81107 Anti-VEGF
8	575	100.0	237	5	ABP51952	Abp51952 Plasmid p
9	575	100.0	237	8	ADO14128	Ado14128 Plasmid p
10	575	100.0	237	8	ADO14131	Ado14131 Plasmid p
11	575	100.0	237	8	ADQ90703	Adq90703 Anti-VEGF
12	575	100.0	237	8	ADQ90701	Adq90701 Anti-VEGF
13	575	100.0	237	8	ADQ90705	Adq90705 Anti-VEGF
14	575	100.0	237	8	ADQ90709	Adq90709 Anti-VEGF
15	575	100.0	237	8	ADQ90723	Adq90723 Anti-VEGF
16	575	100.0	237	8	ADQ90707	Adq90707 Anti-VEGF
17	573	99.7	110	3	AAB05897	Aab05897 Humanised
18	573	99.7	110	3	AAB13376	Aab13376 F(ab)-12
19	573	99.7	237	8	ADQ90721	Adq90721 Anti-VEGF
20	572	99.5	110	2	AAW70675	Aaw70675 Anti-VEGF
21	572	99.5	110	5	ABP61244	Abp61244 Humanised
22	569	99.0	110	2	AAW70673	Aaw70673 Anti-VEGF
23	569	99.0	110	5	ABP61242	Abp61242 Humanised
24	569	99.0	237	2	AAW70703	Aaw70703 Protein e
25	569	99.0	650	5	ABP61241	Abp61241 Phage-dis
26	566	98.4	108	8	ADG31770	Adg31770 V(L) doma
27	564	98.1	108	2	AAW70618	Aaw70618 Anti-VEGF
28	564	98.1	108	5	ABP61187	Abp61187 Humanised
29	564	98.1	108	8	ADG31782	Adg31782 V(L) doma
30	564	98.1	108	8	ADG31768	Adg31768 V(L) doma
31	564	98.1	108	8	ADG31893	Adg31893 V(L) prot
32	561	97.6	108	2	AAW70696	Aaw70696 Anti-VEGF
33	561	97.6	108	5	ABP61265	Abp61265 Humanised
34	558	97.0	214	7	ADC26157	Adc26157 Anti-VEGF
35	557	96.9	214	7	ADC26156	Adc26156 Anti-VEGF
36	556	96.7	107	2	AAW86804	Aaw86804 Variable
37	556	96.7	107	2	AAW70623	Aaw70623 Humanised
38	556	96.7	107	5	ABP61192	Abp61192 Humanised
39	556	96.7	110	2	AAW70685	Aaw70685 Anti-VEGF
40	556	96.7	110	2	AAW70681	Aaw70681 Anti-VEGF
41	556	96.7	110	2	AAW70683	Aaw70683 Anti-VEGF
42	556	96.7	110	2	AAW70679	Aaw70679 Anti-VEGF
43	556	96.7	110	3	AAB05898	Aab05898 Humanised
44	556	96.7	110	3	AAB13386	Aab13386 Anti-VEGF
45	556	96.7	110	3	AAB13387	Aab13387 Anti-VEGF

ALIGNMENTS

RESULT 1
AAW70677
ID AAW70677 standard; peptide; 110 AA.
XX
AC AAW70677;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable light domain of variant Y0101.
XX
KW Light variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 9A; 100pp; English.
XX
CC The present sequence represents a variable light domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can

```
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 575;  DB 2;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 5.4e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 2
AAW70687
ID   AAW70687 standard; peptide; 110 AA.
XX
AC   AAW70687;
XX
DT   27-JAN-1999  (first entry)
XX
DE   Anti-VEGF humanised antibody variable light domain of variant Y0317.
XX
KW   Light variable domain; murine; humanised antibody;
KW   anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW   VEGF-induced angiogenesis; tumour; retinal disorder;
KW   age-related macular degeneration; diabetic retinopathy;
KW   rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS   Synthetic.
OS   Mus sp.
OS   Homo sapiens.
XX
PN   WO9845331-A2.
XX
PD   15-OCT-1998.
XX
PF   03-APR-1998;   98WO-US006604.
XX
PR   07-APR-1997;   97US-00833504.
PR   06-AUG-1997;   97US-00908469.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 1998-568337/48.
XX
PT   New humanised antibody with affinity for vascular endothelial growth
PT   factor - for treatment of tumours, retinal disease and other angiogenic
PT   states, also related nucleic acid, vectors and transformed cells.
XX
PS   Claim 27; Fig 10A; 100pp; English.
XX
CC   The present sequence represents a variable light domain of an affinity-
CC   matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC   variant. The sequence is used in the course of the invention to produce
CC   the humanised anti-VEGF antibody of the invention. The humanised
CC   antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC   for treating or preventing tumours (of any type) and retinal disorders
CC   (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 575;  DB 2;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 5.4e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 3
AAB13380
ID   AAB13380 standard; protein; 110 AA.
XX
AC   AAB13380;
XX
DT   21-NOV-2000  (first entry)
XX
DE   Anti-VEGF antibody YO317 light chain variable domain.
XX
KW   YO317; vascular endothelial cell growth factor; VEGF; antibody;
KW   antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Unidentified.
XX
FH   Key             Location/Qualifiers
FT   Region          24. .33
FT                   /label= CDR-L1
FT   Region          50. .56
FT                   /label= CDR-L2
FT   Region          89. .97
FT                   /label= CDR-L3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14A; 60pp; English.
XX
CC   The present sequence is the light chain variable region of the affinity
CC   matured anti-vascular endothelial cell growth factor (anti-VEGF) antibody
CC   YO317. Humanised F(ab)-12 and affinity matured anti-VEGF antibodies may
CC   be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Monoclonal antibodies are generated in
```

```
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 575;  DB 3;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 5.4e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 4
ABP61256
ID   ABP61256 standard; protein; 110 AA.
XX
AC   ABP61256;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0317 antibody variable light domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0317; light chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          24. .34
FT                   /label= CDR-L1
FT   Domain          50. .56
FT                   /label= CDR-L2
FT   Domain          89. .97
FT                   /label= CDR-L3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Claim 27; Fig 10; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary light chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 575;  DB 5;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 5.4e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 5
ABP61246
ID   ABP61246 standard; protein; 110 AA.
XX
AC   ABP61246;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0101 antibody variable light domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0101; light chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          24. .34
FT                   /label= CDR-L1
FT   Domain          50. .57
FT                   /label= CDR-L2
FT   Domain          89. .97
FT                   /label= CDR-L3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Example 3; Fig 9; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
```

```
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary light chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 575;  DB 5;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 5.4e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 6
ADC26154
ID   ADC26154 standard; protein; 214 AA.
XX
AC   ADC26154;
XX
DT   18-DEC-2003  (first entry)
XX
DE   Parent anti-VEGF Y0101 antibody wild-type light chain protein.
XX
KW   antibody variant; cytostatic; cancer; parent; anti-VEGF;
KW   vascular endothelial growth factor; Y0101; light chain; wild-type.
XX
OS   Unidentified.
XX
PN   WO2003068801-A2.
XX
PD   21-AUG-2003.
XX
PF   11-FEB-2003; 2003WO-US004184.
XX
PR   11-FEB-2002; 2002US-0355895P.
PR   10-SEP-2002; 2002US-0409685P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Lowman HB,  Marvin JS;
XX
DR   WPI; 2003-697521/66.
XX
PT   Making an antibody variant of a parent antibody specific to an antigen by
PT   identifying a target amino acid residue within the variable domain of the
PT   parent antibody and substituting the target residue with a different
PT   amino acid residue.
XX
PS   Example 1; SEQ ID NO 1; 81pp; English.
XX
CC   The invention relates to a novel method for making an antibody variant of
CC   a parent antibody specific to an antigen. This is achieved via
CC   identifying a target amino acid residue within the variable domain of the
CC   parent antibody and substituting the target residue with a different
CC   replacement amino acid residue such that the charge complementarity
CC   between the antibody and antigen is increased. The antibody variant of
CC   the invention demonstrates cytostatic activity whilst the method may be
CC   useful for treating cancer. The current sequence is that of the parent
CC   anti-VEGF (vascular endothelial growth factor) Y0101 antibody wild-type
CC   light chain protein of the invention.
XX
SQ   Sequence 214 AA;

  Query Match             100.0%;  Score 575;  DB 7;  Length 214;
  Best Local Similarity   100.0%;  Pred. No. 9.7e-34;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 7
ABB81107
ID   ABB81107 standard; protein; 237 AA.
XX
AC   ABB81107;
XX
DT   05-NOV-2002  (first entry)
XX
DE   Anti-VEGF light chain fragment.
XX
KW   Immunoglobulin; promoter; cytostatic; antiinflammatory; immunomodulator;
KW   neuroprotective; CD11; tissue factor; vascular endothelial growth factor;
KW   VEGF.
XX
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /note= "STII signal sequence TIR-1"
FT   Protein         24. .237
FT                   /note= "anti-VEGF light chain"
XX
PN   WO200261090-A2.
XX
PD   08-AUG-2002.
XX
PF   13-DEC-2001; 2001WO-US048691.
XX
PR   14-DEC-2000; 2000US-0256164P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons LC,  Klimowski L,  Reilly DE,  Yansura DG;
XX
DR   WPI; 2002-619253/66.
DR   N-PSDB; ABN86646.
XX
PT   New polynucleotide comprising first and second promoter-cistron pairs,
PT   useful for diagnosing, treating or preventing diseases associated with
PT   abnormal expression and/or activity of antigens such as inflammatory
PT   disorders.
XX
PS   Disclosure; Fig 21A-C; 104pp; English.
XX
CC   The invention provides a polynucleotide, which encodes an immunoglobulin
CC   (Ig), comprising a first or second promoter-cistron pair consisting of a
CC   first or second promoter and cistron, respectively. The first cistron of
CC   the first promoter-cistron pair comprises a first translational
CC   initiation region (TIR-L) operably linked to a nucleic acid sequence
CC   encoding an Ig light chain and the second cistron of the second promoter-
CC   cistron pair comprises a second translational initiation region (TIR-H)
CC   operably linked to a nucleic acid sequence encoding an Ig heavy chain.
CC   Upon expression of the polynucleotide in a prokaryotic host cell, light
CC   and heavy chains are folded and assembled to form a biologically active
CC   Ig. The antibody of the invention is useful for diagnosing, treating or
CC   preventing diseases or conditions associated with abnormal expression and
CC   /or activity of one or more antigen molecules e.g. lymphoid malignancies,
CC   inflammatory, angiogenic, immunologic, neuronal, glial, astrocytal,
CC   hypothalamic or other glandular disorders. The present sequence
CC   represents the amino acid sequence of an anti-vascular endothelial growth
CC   factor (VEGF) light chain fragment of the cistron vector pxVG2AP11
XX
SQ   Sequence 237 AA;
```

```
  Query Match           100.0%;  Score 575;  DB 5;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 8
ABP51952
ID   ABP51952 standard; protein; 237 AA.
XX
AC   ABP51952;
XX
DT   09-OCT-2002  (first entry)
XX
DE   Plasmid pY0317 anti-VEGF Fab amino acid sequence SEQ ID NO:2 #1.
XX
KW   Bacterial host; protease; degP; prc; spr; anti-VEGF antibody; antibody;
KW   humanised; Apo2 ligand; anti-CD18; anti-tissue factor; 2C4; anti-CD20;
KW   anti-vascular endothelial growth factor; anti-Her-2; anti-CD40; Fab;
KW   anti-CD11a; Fab'; Fab'2; Fab'2-leucine zipper fusion; anti-VEGF Fab.
XX
OS   Mus sp.
OS   Escherichia coli.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /label= signal
FT   Protein         24. .237
FT                   /label= anti-VEGF_Fab
XX
PN   WO200248376-A2.
XX
PD   20-JUN-2002.
XX
PF   07-DEC-2001; 2001WO-US047581.
XX
PR   14-DEC-2000; 2000US-0256162P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Chen CY;
XX
DR   WPI; 2002-583522/62.
DR   N-PSDB; ABQ73919.
XX
PT   Novel Escherichia coli strain useful for producing polypeptide, deficient
PT   in degP and prc encoding protease, and harboring mutant spr gene, product
PT   of gene suppresses growth phenotypes of strains harboring prc mutants.
XX
PS   Example 1; Fig 1A-C; 63pp; English.
XX
CC   The present invention describes an Escherichia coli strain (I) deficient
CC   in chromosomal degP and prc encoding protease DegP and Prc, respectively,
CC   and harbouring a mutant spr gene, the product of mutant spr gene
CC   suppresses growth phenotypes exhibited by strains harbouring prc mutants.
CC   (I) is useful for producing a polypeptide, by culturing (I) comprising
CC   nucleic acid encoding the polypeptide, which is heterologous to the
CC   strain, such that the nucleic acid is expressed, and recovering the
CC   heterologous polypeptide from the strain. The heterologous polypeptide is
CC   proteolytically sensitive. Culturing of (I) is performed in a fermentor
CC   under conditions of high- or low-cell density fermentation. The
CC   polypeptide is recovered from the periplasm or culture medium of the
CC   strain. The polypeptide is an antibody (humanised or full-length
CC   antibody) or Apo2 ligand. The antibody is an anti-CD18, anti-vascular
CC   endothelial growth factor (VEGF), anti-tissue factor, 2C4, anti-Her-2,
CC   anti-CD20, anti-CD40, or anti-CD11a antibody. The antibody is also an
CC   antibody fragment having a light chain (kappa light chain). The antibody
CC   fragment is a Fab, Fab', Fab'2 or Fab'2-leucine zipper fusion, anti-CD18
CC   Fab'2-leucine zipper fusion, anti-tissue factor Fab'2-leucine zipper
CC   fusion or anti-VEGF Fab, with or without a histidine or lysine tag, anti-
CC   tissue factor Fab'2-leucine zipper fusion with a 6-histidine tag, or anti
CC   -CD18 Fab'2-leucine zipper fusion with a 6-histidine tag, and anti-CD18
CC   Fab'2-leucine zipper fusion with a 6-lysine tag. The present sequence
CC   represents an anti-VEGF Fab amino acid sequence from the present
CC   invention
XX
SQ   Sequence 237 AA;

  Query Match           100.0%;  Score 575;  DB 5;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 9
ADO14128
ID   ADO14128 standard; protein; 237 AA.
XX
AC   ADO14128;
XX
DT   12-AUG-2004  (first entry)
XX
DE   Plasmid pxVG2AP11 expression cassette light chain protein SEQ ID NO:8.
XX
KW   antibody; variant heavy chain hinge region; immunoconjugate; cytostatic;
KW   immunosuppressive; immunotherapy; tumour; cancer; immune disorder;
KW   expression cassette; plasmid pxVG2AP11; anti-VEGF light chain.
XX
OS   Synthetic.
XX
PN   WO2004042017-A2.
XX
PD   21-MAY-2004.
XX
PF   30-OCT-2003; 2003WO-US034610.
XX
PR   31-OCT-2002; 2002US-0422952P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Reilly D,  Yansura DG;
XX
DR   WPI; 2004-390607/36.
DR   N-PSDB; ADO14127.
XX
PT   New antibody comprising a variant heavy chain hinge region incapable of
PT   inter-heavy chain disulfide linkage, useful for treating, preventing,
PT   diagnosing, delaying or preventing a disease, e.g. tumor, cancer or
PT   immune disorder.
XX
PS   Example 1; SEQ ID NO 8; 124pp; English.
XX
CC   The present invention describes an antibody comprising a variant heavy
CC   chain hinge region incapable of inter-heavy chain disulfide linkage. Also
CC   described: (1) an antibody lacking inter-heavy chain disulfide linkage;
CC   (2) an immunoconjugate comprising the antibody conjugated with a
CC   heterologous moiety; (3) a composition comprising the antibody or
CC   immunoconjugate, and carrier; (4) an article of manufacture comprising
CC   the composition in a container; (5) a polynucleotide encoding the
CC   antibody or immunoconjugate, or a variant immunoglobulin heavy chain
```

```
CC   incapable of inter-heavy chain disulfide linkage; (6) a recombinant
CC   vector for expressing the antibody or immunoconjugate; (7) a host cell
CC   comprising the recombinant vector; (8) expressing in a host cell an
CC   antibody of interest in which at least one inter-heavy chain disulfide
CC   linkage is eliminated, and recovering the antibody from the host cell;
CC   (9) an aglycosylated antibody produced by the method; and (10) treating,
CC   preventing, diagnosing, delaying or preventing a disease in a subject.
CC   The antibody has cytostatic and immunosuppressive activities, and can be
CC   used in immunotherapy. The antibody, immunoconjugate and methods are
CC   useful for treating, preventing, diagnosing, delaying or preventing a
CC   disease, e.g. tumour, cancer or immune disorder. The present sequence
CC   represents the anti-VEGF light chain from the expression cassette of
CC   plasmid pxVG2AP11, which is used in the exemplification of the present
CC   invention.
XX
SQ   Sequence 237 AA;

  Query Match          100.0%;  Score 575;  DB 8;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 10
ADO14131
ID   ADO14131 standard; protein; 237 AA.
XX
AC   ADO14131;
XX
DT   12-AUG-2004  (first entry)
XX
DE   Plasmid pxVG11VNERK expression cassette light chain protein SEQ ID NO:11.
XX
KW   antibody; variant heavy chain hinge region; immunoconjugate; cytostatic;
KW   immunosuppressive; immunotherapy; tumour; cancer; immune disorder;
KW   expression cassette; plasmid pxVG11VNERK; anti-VEGF light chain.
XX
OS   Synthetic.
XX
PN   WO2004042017-A2.
XX
PD   21-MAY-2004.
XX
PF   30-OCT-2003; 2003WO-US034610.
XX
PR   31-OCT-2002; 2002US-0422952P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Reilly D,  Yansura DG;
XX
DR   WPI; 2004-390607/36.
DR   N-PSDB; ADO14130.
XX
PT   New antibody comprising a variant heavy chain hinge region incapable of
PT   inter-heavy chain disulfide linkage, useful for treating, preventing,
PT   diagnosing, delaying or preventing a disease, e.g. tumor, cancer or
PT   immune disorder.
XX
PS   Example 1; SEQ ID NO 11; 124pp; English.
XX
CC   The present invention describes an antibody comprising a variant heavy
CC   chain hinge region incapable of inter-heavy chain disulfide linkage. Also
CC   described: (1) an antibody lacking inter-heavy chain disulfide linkage;
CC   (2) an immunoconjugate comprising the antibody conjugated with a
CC   heterologous moiety; (3) a composition comprising the antibody or
CC   immunoconjugate, and carrier; (4) an article of manufacture comprising
CC   the composition in a container; (5) a polynucleotide encoding the
CC   antibody or immunoconjugate, or a variant immunoglobulin heavy chain
CC   incapable of inter-heavy chain disulfide linkage; (6) a recombinant
CC   vector for expressing the antibody or immunoconjugate; (7) a host cell
CC   comprising the recombinant vector; (8) expressing in a host cell an
CC   antibody of interest in which at least one inter-heavy chain disulfide
CC   linkage is eliminated, and recovering the antibody from the host cell;
CC   (9) an aglycosylated antibody produced by the method; and (10) treating,
CC   preventing, diagnosing, delaying or preventing a disease in a subject.
CC   The antibody has cytostatic and immunosuppressive activities, and can be
CC   used in immunotherapy. The antibody, immunoconjugate and methods are
CC   useful for treating, preventing, diagnosing, delaying or preventing a
CC   disease, e.g. tumour, cancer or immune disorder. The present sequence
CC   represents the anti-VEGF light chain from the expression cassette of
CC   plasmid pxVG11VNERK, which is used in the exemplification of the present
CC   invention.
XX
SQ   Sequence 237 AA;

  Query Match          100.0%;  Score 575;  DB 8;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 11
ADQ90703
ID   ADQ90703 standard; protein; 237 AA.
XX
AC   ADQ90703;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody YO317 light chain protein SEQ ID NO:7.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; light chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
XX
PD   05-AUG-2004.
XX
PF   23-JAN-2004; 2004WO-US001844.
XX
PR   23-JAN-2003; 2003US-0442484P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons L;
XX
DR   WPI; 2004-562149/54.
DR   N-PSDB; ADQ90702.
XX
PT   Producing an antibody or antigen binding fragment in high yield in a cell
PT   culture, comprises expressing a variable domain with a modified framework
PT   region in a host cell.
XX
PS   Example 2; SEQ ID NO 7; 161pp; English.
```

```
XX
CC   The present invention describes a method for producing an antibody or
CC   antigen binding fragment in high yield in a cell culture. The method
CC   comprises expressing a variable domain of the antibody or antigen binding
CC   fragment comprising a modified framework region (FR) in a host cell, and
CC   recovering the antibody or antigen binding fragment variable domain
CC   comprising the modified framework from the host cell. The modified FR in
CC   the method described above has a substitution of at least one amino acid
CC   position with a different amino acid, where the different amino acid is
CC   the amino acid found at the corresponding FR position of a human subgroup
CC   variable domain consensus sequence that has a hypervariable region 1
CC   (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC   with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC   The antibody or antigen binding fragment variable domain comprises the
CC   modified FR that has improved yield in cell culture compared to an
CC   unmodified antibody or antigen-binding fragment. The antibody and antigen
CC   binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC   immunomodulatory activities, and can be used in antibody therapy. The
CC   methods and compositions of the present invention are useful for
CC   producing antibodies or antigen binding fragments in cell culture, in
CC   particular for improving the yield of recombinant antibodies or antigen
CC   binding fragments in cell culture. The antibodies of the invention can be
CC   used to diagnose, treat, inhibit or prevent e.g. tumours and
CC   inflammatory, angiogenic and immunological disorders. The present
CC   sequence represents the light chain of an anti-VEGF (vascular endothelial
CC   cell growth factor) antibody, which is used in the exemplification of the
CC   present invention.
XX
SQ   Sequence 237 AA;

  Query Match             100.0%;  Score 575;  DB 8;  Length 237;
  Best Local Similarity   100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 12
ADQ90701
ID   ADQ90701 standard; protein; 237 AA.
XX
AC   ADQ90701;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody VNERK light chain protein SEQ ID NO:5.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; light chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
XX
PD   05-AUG-2004.
XX
PF   23-JAN-2004; 2004WO-US001844.
XX
PR   23-JAN-2003; 2003US-0442484P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons L;
XX
DR   WPI; 2004-562149/54.
DR   N-PSDB; ADQ90700.
XX
PT   Producing an antibody or antigen binding fragment in high yield in a cell
PT   culture, comprises expressing a variable domain with a modified framework
PT   region in a host cell.
XX
PS   Example 2; SEQ ID NO 5; 161pp; English.
XX
CC   The present invention describes a method for producing an antibody or
CC   antigen binding fragment in high yield in a cell culture. The method
CC   comprises expressing a variable domain of the antibody or antigen binding
CC   fragment comprising a modified framework region (FR) in a host cell, and
CC   recovering the antibody or antigen binding fragment variable domain
CC   comprising the modified framework from the host cell. The modified FR in
CC   the method described above has a substitution of at least one amino acid
CC   position with a different amino acid, where the different amino acid is
CC   the amino acid found at the corresponding FR position of a human subgroup
CC   variable domain consensus sequence that has a hypervariable region 1
CC   (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC   with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC   The antibody or antigen binding fragment variable domain comprises the
CC   modified FR that has improved yield in cell culture compared to an
CC   unmodified antibody or antigen-binding fragment. The antibody and antigen
CC   binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC   immunomodulatory activities, and can be used in antibody therapy. The
CC   methods and compositions of the present invention are useful for
CC   producing antibodies or antigen binding fragments in cell culture, in
CC   particular for improving the yield of recombinant antibodies or antigen
CC   binding fragments in cell culture. The antibodies of the invention can be
CC   used to diagnose, treat, inhibit or prevent e.g. tumours and
CC   inflammatory, angiogenic and immunological disorders. The present
CC   sequence represents the light chain of an anti-VEGF (vascular endothelial
CC   cell growth factor) antibody, which is used in the exemplification of the
CC   present invention.
XX
SQ   Sequence 237 AA;

  Query Match             100.0%;  Score 575;  DB 8;  Length 237;
  Best Local Similarity   100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 13
ADQ90705
ID   ADQ90705 standard; protein; 237 AA.
XX
AC   ADQ90705;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody VNERK light chain protein SEQ ID NO:9.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; light chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
```

PN WO2004065417-A2.
XX
PD 05-AUG-2004.
XX
PF 23-JAN-2004; 2004WO-US001844.
XX
PR 23-JAN-2003; 2003US-0442484P.
XX
PA (GETH) GENENTECH INC.
XX
PI Simmons L;
XX
DR WPI; 2004-562149/54.
DR N-PSDB; ADQ90704.
XX
PT Producing an antibody or antigen binding fragment in high yield in a cell
PT culture, comprises expressing a variable domain with a modified framework
PT region in a host cell.
XX
PS Example 2; SEQ ID NO 9; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the light chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 237 AA;

Query Match 100.0%; Score 575; DB 8; Length 237;
Best Local Similarity 100.0%; Pred. No. 1.1e-33;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

RESULT 14
ADQ90709
ID ADQ90709 standard; protein; 237 AA.
XX
AC ADQ90709;
XX
DT 21-OCT-2004 (first entry)
XX
DE Anti-VEGF antibody VNERK light chain protein SEQ ID NO:13.
XX
KW antibody; antigen binding fragment; cell culture; variable domain;
KW modified framework region; hypervariable region; cytostatic;
KW antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW tumour; inflammatory disorder; angiogenic disorder;
KW immunological disorder; anti-VEGF antibody;
KW anti vascular endothelial cell growth factor antibody; light chain.
XX
OS Homo sapiens.
OS Synthetic.
XX
PN WO2004065417-A2.
XX
PD 05-AUG-2004.
XX
PF 23-JAN-2004; 2004WO-US001844.
XX
PR 23-JAN-2003; 2003US-0442484P.
XX
PA (GETH) GENENTECH INC.
XX
PI Simmons L;
XX
DR WPI; 2004-562149/54.
DR N-PSDB; ADQ90708.
XX
PT Producing an antibody or antigen binding fragment in high yield in a cell
PT culture, comprises expressing a variable domain with a modified framework
PT region in a host cell.
XX
PS Example 2; SEQ ID NO 13; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the light chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 237 AA;

Query Match 100.0%; Score 575; DB 8; Length 237;
Best Local Similarity 100.0%; Pred. No. 1.1e-33;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

```
RESULT 15
ADQ90723
ID   ADQ90723 standard; protein; 237 AA.
XX
AC   ADQ90723;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody VNERK light chain protein SEQ ID NO:27.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; light chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
XX
PD   05-AUG-2004.
XX
PF   23-JAN-2004; 2004WO-US001844.
XX
PR   23-JAN-2003; 2003US-0442484P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons L;
XX
DR   WPI; 2004-562149/54.
DR   N-PSDB; ADQ90722.
XX
PT   Producing an antibody or antigen binding fragment in high yield in a cell
PT   culture, comprises expressing a variable domain with a modified framework
PT   region in a host cell.
XX
PS   Example 7; SEQ ID NO 27; 161pp; English.
XX
CC   The present invention describes a method for producing an antibody or
CC   antigen binding fragment in high yield in a cell culture. The method
CC   comprises expressing a variable domain of the antibody or antigen binding
CC   fragment comprising a modified framework region (FR) in a host cell, and
CC   recovering the antibody or antigen binding fragment variable domain
CC   comprising the modified framework from the host cell. The modified FR in
CC   the method described above has a substitution of at least one amino acid
CC   position with a different amino acid, where the different amino acid is
CC   the amino acid found at the corresponding FR position of a human subgroup
CC   variable domain consensus sequence that has a hypervariable region 1
CC   (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC   with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC   The antibody or antigen binding fragment variable domain comprises the
CC   modified FR that has improved yield in cell culture compared to an
CC   unmodified antibody or antigen-binding fragment. The antibody and antigen
CC   binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC   immunomodulatory activities, and can be used in antibody therapy. The
CC   methods and compositions of the present invention are useful for
CC   producing antibodies or antigen binding fragments in cell culture, in
CC   particular for improving the yield of recombinant antibodies or antigen
CC   binding fragments in cell culture. The antibodies of the invention can be
CC   used to diagnose, treat, inhibit or prevent e.g. tumours and
CC   inflammatory, angiogenic and immunological disorders. The present
CC   sequence represents the light chain of an anti-VEGF (vascular endothelial
CC   cell growth factor) antibody, which is used in the exemplification of the
CC   present invention.
XX
SQ   Sequence 237 AA;

  Query Match            100.0%;  Score 575;  DB 8;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 1.1e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

Search completed: March 14, 2005, 20:39:16
Job time : 88.0482 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:30:13 ; Search time 22.6754 Seconds
(without alignments)
362.127 Million cell updates/sec

Title: US-09-723-752B-115
Perfect score: 575
Sequence: 1 DIQLTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 513545 seqs, 74649064 residues

Total number of hits satisfying chosen parameters: 513545

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Issued_Patents_AA:*
1: /cgn2_6/ptodata/1/iaa/5A_COMB.pep:*
2: /cgn2_6/ptodata/1/iaa/5B_COMB.pep:*
3: /cgn2_6/ptodata/1/iaa/6A_COMB.pep:*
4: /cgn2_6/ptodata/1/iaa/6B_COMB.pep:*
5: /cgn2_6/ptodata/1/iaa/PCTUS_COMB.pep:*
6: /cgn2_6/ptodata/1/iaa/backfiles1.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	573	99.7	110	4	US-09-440-781-94	Sequence 94, Appl
2	569	99.0	491	4	US-10-011-125A-2	Sequence 2, Appli
3	556	96.7	110	4	US-09-440-781-95	Sequence 95, Appl
4	522	90.8	214	2	US-07-934-373C-40	Sequence 40, Appl
5	522	90.8	214	2	US-08-788-800-11	Sequence 11, Appl
6	522	90.8	214	3	US-08-437-642B-40	Sequence 40, Appl
7	522	90.8	214	3	US-09-097-309-2	Sequence 2, Appli
8	522	90.8	214	3	US-09-097-171A-2	Sequence 2, Appli
9	522	90.8	214	3	US-09-460-587-2	Sequence 2, Appli
10	522	90.8	214	4	US-09-940-166A-2	Sequence 2, Appli
11	522	90.8	214	5	PCT-US93-07832-40	Sequence 40, Appl
12	522	90.8	233	2	US-07-934-373C-25	Sequence 25, Appl
13	522	90.8	233	3	US-08-437-642B-25	Sequence 25, Appl
14	522	90.8	233	4	US-08-146-206C-25	Sequence 25, Appl
15	522	90.8	233	4	US-09-705-686-25	Sequence 25, Appl
16	522	90.8	233	4	US-09-705-392A-25	Sequence 25, Appl
17	522	90.8	233	4	US-09-705-398-25	Sequence 25, Appl
18	522	90.8	233	5	PCT-US93-07832-25	Sequence 25, Appl
19	522	90.8	237	3	US-09-097-309-6	Sequence 6, Appli
20	522	90.8	237	3	US-09-097-171A-10	Sequence 10, Appl
21	522	90.8	237	3	US-09-422-712B-2	Sequence 2, Appli
22	522	90.8	237	3	US-09-607-756-2	Sequence 2, Appli
23	522	90.8	237	3	US-09-460-587-6	Sequence 6, Appli
24	522	90.8	237	4	US-09-940-166A-6	Sequence 6, Appli
25	519	90.3	214	1	US-08-458-516-12	Sequence 12, Appl
26	518	90.1	109	2	US-07-934-373C-47	Sequence 47, Appl
27	518	90.1	109	3	US-08-437-642B-47	Sequence 47, Appl
28	514	89.4	214	2	US-07-934-373C-39	Sequence 39, Appl
29	514	89.4	214	3	US-08-437-642B-39	Sequence 39, Appl
30	514	89.4	214	5	PCT-US93-07832-39	Sequence 39, Appl
31	511	88.9	107	2	US-07-934-373C-17	Sequence 17, Appl
32	511	88.9	107	3	US-08-437-642B-17	Sequence 17, Appl
33	511	88.9	107	4	US-08-146-206C-17	Sequence 17, Appl
34	511	88.9	107	4	US-09-705-686-17	Sequence 17, Appl
35	511	88.9	107	4	US-09-705-392A-17	Sequence 17, Appl
36	511	88.9	107	4	US-09-705-398-17	Sequence 17, Appl
37	511	88.9	107	5	PCT-US93-07832-17	Sequence 17, Appl
38	511	88.9	108	3	US-08-974-899-3	Sequence 3, Appli
39	511	88.9	108	4	US-09-795-798-3	Sequence 3, Appli
40	511	88.9	127	3	US-08-649-100-33	Sequence 33, Appl
41	507	88.2	109	2	US-07-934-373C-3	Sequence 3, Appli
42	507	88.2	109	3	US-08-437-642B-3	Sequence 3, Appli
43	507	88.2	109	4	US-08-146-206C-3	Sequence 3, Appli
44	507	88.2	109	4	US-09-705-686-3	Sequence 3, Appli
45	507	88.2	109	4	US-09-705-392A-3	Sequence 3, Appli

ALIGNMENTS

RESULT 1
US-09-440-781-94
; Sequence 94, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
; APPLICANT: Yvonne Man-yee Chen et al.
; TITLE OF INVENTION: ANTIBODY VARIANTS
; FILE REFERENCE: P1469R1
; CURRENT APPLICATION NUMBER: US/09/440,781
; CURRENT FILING DATE: 1999-11-16
; NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 94
; LENGTH: 110
; TYPE: PRT
; ORGANISM: artificial sequence
; FEATURE:
; NAME/KEY: artificial
; LOCATION: 1-110
; OTHER INFORMATION: humanized antibody light chain variable domain
US-09-440-781-94

Query Match 99.7%; Score 573; DB 4; Length 110;
Best Local Similarity 99.1%; Pred. No. 8.6e-47;
Matches 109; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
```

RESULT 2
US-10-011-125A-2
; Sequence 2, Application US/10011125A
; Patent No. 6828121
; GENERAL INFORMATION:
; APPLICANT: Chen, Christina Yu-Ching
; TITLE OF INVENTION: BACTERIAL HOST STRAINS
; FILE REFERENCE: P1804R1
; CURRENT APPLICATION NUMBER: US/10/011,125A
; CURRENT FILING DATE: 2001-12-07
; PRIOR APPLICATION NUMBER: US 60/256,162
; PRIOR FILING DATE: 2000-12-14
; NUMBER OF SEQ ID NOS: 12
; SEQ ID NO 2
; LENGTH: 491
; TYPE: PRT

```
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: Sequence is synthesized.
; Patent No. 6828121
US-10-011-125A-2

  Query Match             99.0%;  Score 569;  DB 4;  Length 491;
  Best Local Similarity   98.2%;  Pred. No. 1.1e-45;
  Matches  108;  Conservative    2;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||:|||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKLLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 3
US-09-440-781-95
; Sequence 95, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 95
;   LENGTH: 110
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-110
;   OTHER INFORMATION: humanized antibody light chain variable domain
US-09-440-781-95

  Query Match             96.7%;  Score 556;  DB 4;  Length 110;
  Best Local Similarity   95.5%;  Pred. No. 3.4e-45;
  Matches  105;  Conservative    3;  Mismatches    2;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| |:: :|||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 4
US-07-934-373C-40
; Sequence 40, Application US/07934373C
; Patent No. 5821337
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  48
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/07/934,373C
;      FILING DATE:  21-Aug-1992
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-07-934-373C-40

  Query Match             90.8%;  Score 522;  DB 2;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 5
US-08-788-800-11
; Sequence 11, Application US/08788800
; Patent No. 5914112
;  GENERAL INFORMATION:
;    APPLICANT:  Bednar, Martin M.
;    APPLICANT:  Thomas, G. Roger
;    APPLICANT:  Gross, Cordell E.
;    TITLE OF INVENTION:  ANTI-CD18 ANTIBODIES IN STROKE
;    NUMBER OF SEQUENCES:  15
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  460 Point San Bruno Blvd
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/788,800
;      FILING DATE:  22-Jan-1997
;      CLASSIFICATION:  424
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0987r1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  415/225-1994
;      TELEFAX:  415/952-9881
;      TELEX:  910/371-7168
;  INFORMATION FOR SEQ ID NO:  11:
```

```
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-788-800-11

  Query Match             90.8%;  Score 522;  DB 2;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 6
US-08-437-642B-40
; Sequence 40, Application US/08437642B
; Patent No. 6054297
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  47
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/437,642B
;      FILING DATE:  09-May-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/934373
;      FILING DATE:  21-AUG-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/146206
;      FILING DATE:  17-NOV-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2C1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-437-642B-40

  Query Match             90.8%;  Score 522;  DB 3;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 7
US-09-097-309-2
; Sequence 2, Application US/09097309
; Patent No. 6121428
;  GENERAL INFORMATION:
;    APPLICANT:  Blank, Gregory S.
;    APPLICANT:  Narindray, Daljit S.
;    APPLICANT:  Zapata, Gerardo A.
;    TITLE OF INVENTION:  Protein Recovery
;    NUMBER OF SEQUENCES:  7
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/097,309
;      FILING DATE:  12-Jun-1998
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/050951
;      FILING DATE:  13-JUN-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Schwartz, Timothy R.
;      REGISTRATION NUMBER:  32171
;      REFERENCE/DOCKET NUMBER:  P1105R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-7467
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-097-309-2

  Query Match             90.8%;  Score 522;  DB 3;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 8
US-09-097-171A-2
; Sequence 2, Application US/09097171A
; Patent No. 6171586
;  GENERAL INFORMATION:
;    APPLICANT:  Lam, Xanthe M.
;    APPLICANT:  Oeswein, James Q.
```

```
;    APPLICANT:  Ongpipattanakul, Boonsri
;    APPLICANT:  Shahrokh, Zahra
;    APPLICANT:  Wang, Sharon X.
;    APPLICANT:  Weissburg, Robert P.
;    APPLICANT:  Wong, Rita L.
;    TITLE OF INVENTION:  Antibody Formulation
;    NUMBER OF SEQUENCES:  11
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/097,171A
;      FILING DATE:
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/874897
;      FILING DATE:  13-JUN-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P1089R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-097-171A-2

  Query Match             90.8%;  Score 522;  DB 3;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| |||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 9
US-09-460-587-2
; Sequence 2, Application US/09460587
; Patent No. 6322997
;  GENERAL INFORMATION:
;    APPLICANT:  Blank, Gregory S.
;    APPLICANT:  Narindray, Daljit S.
;    APPLICANT:  Zapata, Gerardo A.
;    TITLE OF INVENTION:  Protein Recovery
;    NUMBER OF SEQUENCES:  7
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/460,587
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  09/097,309
;      FILING DATE:
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Schwartz, Timothy R.
;      REGISTRATION NUMBER:  32171
;      REFERENCE/DOCKET NUMBER:  P1105R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-7467
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-460-587-2

  Query Match             90.8%;  Score 522;  DB 3;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| |||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 10
US-09-940-166A-2
; Sequence 2, Application US/09940166A
; Patent No. 6716598
;  GENERAL INFORMATION:
;       APPLICANT: Blank, Gregory S.
;                  Narindray, Daljit S.
;                  Zapata, Gerardo A.
;       TITLE OF INVENTION: Protein Recovery
;       NUMBER OF SEQUENCES: 7
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/09/940,166A
;            FILING DATE: 27-Aug-2001
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/097,309
;            FILING DATE: 13-JUN-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Schwartz, Timothy R.
;            REGISTRATION NUMBER: 32171
;            REFERENCE/DOCKET NUMBER: P1105R1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-7467
;            TELEFAX: 650/952-9881
;  INFORMATION FOR SEQ ID NO: 2:
```

```
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 214 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 2:
US-09-940-166A-2

  Query Match             90.8%;  Score 522;  DB 4;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||| :|:| |||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 11
PCT-US93-07832-40
; Sequence 40, Application PC/TUS9307832
;  GENERAL INFORMATION:
;    APPLICANT:  Genentech, Inc.
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  40
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  460 Point San Bruno Blvd
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  5.25 inch, 360 Kb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  patin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US93/07832
;      FILING DATE:  19930820
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/934373
;      FILING DATE:  21-AUG-1992
;    ATTORNEY/AGENT INFORMATION:
;      NAME:
;      REGISTRATION NUMBER:
;      REFERENCE/DOCKET NUMBER:  709P2PCT
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:
;      TELEFAX:  415/952-9881
;      TELEX:  910/371-7168
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
PCT-US93-07832-40

  Query Match             90.8%;  Score 522;  DB 5;  Length 214;
  Best Local Similarity   90.0%;  Pred. No. 1.1e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||| :|:| |||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 12
US-07-934-373C-25
; Sequence 25, Application US/07934373C
; Patent No. 5821337
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  48
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/07/934,373C
;      FILING DATE:  21-Aug-1992
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  25:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  233 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-07-934-373C-25

  Query Match             90.8%;  Score 522;  DB 2;  Length 233;
  Best Local Similarity   90.0%;  Pred. No. 1.3e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||| :|:| |||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 13
US-08-437-642B-25
; Sequence 25, Application US/08437642B
; Patent No. 6054297
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
```

```
;    NUMBER OF SEQUENCES:  47
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/437,642B
;      FILING DATE:  09-May-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/934373
;      FILING DATE:  21-AUG-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/146206
;      FILING DATE:  17-NOV-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2C1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  25:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  233 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-437-642B-25

  Query Match             90.8%;  Score 522;  DB 3;  Length 233;
  Best Local Similarity   90.0%;  Pred. No. 1.3e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 14
US-08-146-206C-25
; Sequence 25, Application US/08146206C
; Patent No. 6407213
;  GENERAL INFORMATION:
;    APPLICANT:  Carter, Paul J.
;    APPLICANT:  Presta, Leonard G.
;    TITLE OF INVENTION:  Method for Making Humanized Antibodies
;    NUMBER OF SEQUENCES:  26
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/146,206C
;      FILING DATE:  17-No. 6407213-1993
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  25:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  233 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-146-206C-25

  Query Match             90.8%;  Score 522;  DB 4;  Length 233;
  Best Local Similarity   90.0%;  Pred. No. 1.3e-41;
  Matches   99;  Conservative    8;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |||||:||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 15
US-09-705-686-25
; Sequence 25, Application US/09705686
; Patent No. 6639055
;  GENERAL INFORMATION:
;       APPLICANT: Carter, Paul J.
;                  Presta, Leonard G.
;       TITLE OF INVENTION: Method for Making Humanized Antibodies
;       NUMBER OF SEQUENCES: 26
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/09/705,686
;            FILING DATE: 02-No. 6639055-2000
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 08/146206
;            FILING DATE: 17-NOV-1993
;            APPLICATION NUMBER: 07/715272
;            FILING DATE: 14-JUN-1991
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Lee, Wendy M.
;            REGISTRATION NUMBER: 40,378
;            REFERENCE/DOCKET NUMBER: P0709P1D3
;       TELECOMMUNICATION INFORMATION:
```

; TELEPHONE: 650/225-1994
; TELEFAX: 650/952-9881
; INFORMATION FOR SEQ ID NO: 25:
; SEQUENCE CHARACTERISTICS:
; LENGTH: 233 amino acids
; TYPE: Amino Acid
; TOPOLOGY: Linear
; SEQUENCE DESCRIPTION: SEQ ID NO: 25:
US-09-705-686-25

Query Match 90.8%; Score 522; DB 4; Length 233;
Best Local Similarity 90.0%; Pred. No. 1.3e-41;
Matches 99; Conservative 8; Mismatches 3; Indels 0; Gaps 0;

```
Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| ||||:||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| |||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129
```

Search completed: March 14, 2005, 20:43:52
Job time : 22.6754 secs

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:22:02 ; Search time 41.0088 Seconds
(without alignments)
884.760 Million cell updates/sec

Title: US-09-723-752B-115
Perfect score: 575
Sequence: 1 DIQLTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1396920 seqs, 329844858 residues

Total number of hits satisfying chosen parameters: 1396920

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA:*
1: /cgn2_6/ptodata/2/pubpaa/US07_PUBCOMB.pep:*
2: /cgn2_6/ptodata/2/pubpaa/PCT_NEW_PUB.pep:*
3: /cgn2_6/ptodata/2/pubpaa/US06_NEW_PUB.pep:*
4: /cgn2_6/ptodata/2/pubpaa/US06_PUBCOMB.pep:*
5: /cgn2_6/ptodata/2/pubpaa/US07_NEW_PUB.pep:*
6: /cgn2_6/ptodata/2/pubpaa/PCTUS_PUBCOMB.pep:*
7: /cgn2_6/ptodata/2/pubpaa/US08_NEW_PUB.pep:*
8: /cgn2_6/ptodata/2/pubpaa/US08_PUBCOMB.pep:*
9: /cgn2_6/ptodata/2/pubpaa/US09A_PUBCOMB.pep:*
10: /cgn2_6/ptodata/2/pubpaa/US09B_PUBCOMB.pep:*
11: /cgn2_6/ptodata/2/pubpaa/US09C_PUBCOMB.pep:*
12: /cgn2_6/ptodata/2/pubpaa/US09_NEW_PUB.pep:*
13: /cgn2_6/ptodata/2/pubpaa/US10A_PUBCOMB.pep:*
14: /cgn2_6/ptodata/2/pubpaa/US10B_PUBCOMB.pep:*
15: /cgn2_6/ptodata/2/pubpaa/US10C_PUBCOMB.pep:*
16: /cgn2_6/ptodata/2/pubpaa/US10D_PUBCOMB.pep:*
17: /cgn2_6/ptodata/2/pubpaa/US10_NEW_PUB.pep:*
18: /cgn2_6/ptodata/2/pubpaa/US11_NEW_PUB.pep:*
19: /cgn2_6/ptodata/2/pubpaa/US60_NEW_PUB.pep:*
20: /cgn2_6/ptodata/2/pubpaa/US60_PUBCOMB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	575	100.0	110	9	US-09-056-160B-107	Sequence 107, App
2	575	100.0	110	9	US-09-056-160B-117	Sequence 117, App
3	575	100.0	110	14	US-10-234-671-105	Sequence 105, App
4	575	100.0	110	14	US-10-234-671-115	Sequence 115, App
5	575	100.0	213	16	US-10-379-392-135	Sequence 135, App
6	575	100.0	213	16	US-10-379-392-137	Sequence 137, App
7	575	100.0	213	16	US-10-379-392-139	Sequence 139, App
8	575	100.0	214	15	US-10-364-953-1	Sequence 1, Appli
9	575	100.0	237	14	US-10-020-786-10	Sequence 10, Appl
10	575	100.0	237	17	US-10-697-995-8	Sequence 8, Appli
11	575	100.0	237	17	US-10-697-995-11	Sequence 11, Appl
12	573	99.7	110	14	US-10-234-671-8	Sequence 8, Appli
13	573	99.7	110	15	US-10-624-153-94	Sequence 94, Appl
14	572	99.5	110	9	US-09-056-160B-105	Sequence 105, App
15	572	99.5	110	14	US-10-234-671-103	Sequence 103, App
16	571	99.3	213	16	US-10-379-392-155	Sequence 155, App
17	570	99.1	213	16	US-10-379-392-153	Sequence 153, App
18	569	99.0	110	9	US-09-056-160B-103	Sequence 103, App
19	569	99.0	110	14	US-10-234-671-101	Sequence 101, App
20	569	99.0	237	9	US-09-056-160B-100	Sequence 100, App
21	569	99.0	237	14	US-10-234-671-100	Sequence 100, App
22	569	99.0	491	13	US-10-011-125-2	Sequence 2, Appli
23	567	98.6	213	16	US-10-379-392-157	Sequence 157, App
24	566	98.4	108	13	US-10-153-159-4	Sequence 4, Appli
25	566	98.4	108	14	US-10-153-176-4	Sequence 4, Appli
26	566	98.4	108	15	US-10-443-134A-4	Sequence 4, Appli
27	564	98.1	108	9	US-09-056-160B-8	Sequence 8, Appli
28	564	98.1	108	13	US-10-153-159-2	Sequence 2, Appli
29	564	98.1	108	13	US-10-153-159-16	Sequence 16, Appl
30	564	98.1	108	14	US-10-153-176-2	Sequence 2, Appli
31	564	98.1	108	14	US-10-153-176-16	Sequence 16, Appl
32	564	98.1	108	15	US-10-443-134A-2	Sequence 2, Appli
33	564	98.1	108	15	US-10-443-134A-16	Sequence 16, Appl
34	564	98.1	108	15	US-10-443-134A-127	Sequence 127, App
35	564	98.1	108	17	US-10-877-532-7	Sequence 7, Appli
36	561	97.6	108	9	US-09-056-160B-126	Sequence 126, App
37	561	97.6	108	14	US-10-234-671-124	Sequence 124, App
38	559	97.2	107	16	US-10-723-434-1	Sequence 1, Appli
39	558	97.0	214	15	US-10-364-953-4	Sequence 4, Appli
40	557	96.9	214	15	US-10-364-953-3	Sequence 3, Appli
41	556	96.7	107	9	US-09-056-160B-13	Sequence 13, Appl
42	556	96.7	107	14	US-10-234-671-13	Sequence 13, Appl
43	556	96.7	110	9	US-09-056-160B-109	Sequence 109, App
44	556	96.7	110	9	US-09-056-160B-111	Sequence 111, App
45	556	96.7	110	9	US-09-056-160B-113	Sequence 113, App

ALIGNMENTS

RESULT 1
US-09-056-160B-107
; Sequence 107, Application US/09056160B
; Patent No. US20020032315A1
; GENERAL INFORMATION:
; APPLICANT: Baca, Manuel
; APPLICANT: Wells, James A.
; APPLICANT: Presta, Leonard G:
; APPLICANT: Lowman, Henry B.
; APPLICANT: Chen, Yvonne M.
; TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
; NUMBER OF SEQUENCES: 131
; CORRESPONDENCE ADDRESS:
; ADDRESSEE: Genentech, Inc.
; STREET: 1 DNA Way
; CITY: South San Francisco
; STATE: California
; COUNTRY: USA
; ZIP: 94080
; COMPUTER READABLE FORM:
; MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
; COMPUTER: IBM PC compatible
; OPERATING SYSTEM: PC-DOS/MS-DOS
; SOFTWARE: WinPatin (Genentech)
; CURRENT APPLICATION DATA:
; APPLICATION NUMBER: US/09/056,160B
; FILING DATE: 06-Apr-1998
; CLASSIFICATION: 424
; PRIOR APPLICATION DATA:
; APPLICATION NUMBER: 60/054,856
; FILING DATE: 06-AUG-1997
; ATTORNEY/AGENT INFORMATION:
; NAME: Hasak, Janet E.
; REGISTRATION NUMBER: 28,616
; REFERENCE/DOCKET NUMBER: P1093R2
; TELECOMMUNICATION INFORMATION:

```
;       TELEPHONE:  650/225-1896
;       TELEFAX:  650/952-9881
;   INFORMATION FOR SEQ ID NO:  107:
;     SEQUENCE CHARACTERISTICS:
;       LENGTH:  110 amino acids
;       TYPE:  Amino Acid
;       TOPOLOGY:  Linear
US-09-056-160B-107

  Query Match             100.0%;  Score 575;  DB 9;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 1.8e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 2
US-09-056-160B-117
; Sequence 117, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  117:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  110 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-117

  Query Match             100.0%;  Score 575;  DB 9;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 1.8e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 3
US-10-234-671-105
; Sequence 105, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 105:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 110 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 105:
US-10-234-671-105

  Query Match             100.0%;  Score 575;  DB 14;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 1.8e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 4
US-10-234-671-115
; Sequence 115, Application US/10234671
```

```
; Publication No. US20030190317A1
;  GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 115:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 110 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 115:
US-10-234-671-115

  Query Match             100.0%;  Score 575;  DB 14;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 1.8e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 5
US-10-379-392-135
; Sequence 135, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 135
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Unknown
;   FEATURE:
;   OTHER INFORMATION: Humanized
US-10-379-392-135

  Query Match             100.0%;  Score 575;  DB 16;  Length 213;
  Best Local Similarity   100.0%;  Pred. No. 3.3e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 6
US-10-379-392-137
; Sequence 137, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 137
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
US-10-379-392-137

  Query Match             100.0%;  Score 575;  DB 16;  Length 213;
  Best Local Similarity   100.0%;  Pred. No. 3.3e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 7
US-10-379-392-139
; Sequence 139, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
```

```
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 139
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (116)..(116)
;   OTHER INFORMATION: Xaa at position 116 can be Phe or Tyr
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (133)..(133)
;   OTHER INFORMATION: Xaa at position 133 can be Ile, Met or Val
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (135)..(135)
;   OTHER INFORMATION: Xaa at position 135 can be Leu, Ile or Met
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (176)..(176)
;   OTHER INFORMATION: Xaa at position 176 can be Met, Val, Ala or Ser
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (178)..(178)
;   OTHER INFORMATION: Xaa at position 178 can be Met, Thr or Val
US-10-379-392-139

  Query Match           100.0%;  Score 575;  DB 16;  Length 213;
  Best Local Similarity  100.0%;  Pred. No. 3.3e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 8
US-10-364-953-1
; Sequence 1, Application US/10364953
; Publication No. US20030224397A1
; GENERAL INFORMATION:
;  APPLICANT: LOWMAN, HENRY B.
;  APPLICANT:  MARVIN, JONATHAN S.
;  TITLE OF INVENTION: ANTIBODY VARIANTS WITH FASTER ANTIGEN ASSOCIATION RATES
;  FILE REFERENCE: P1951R1
;  CURRENT APPLICATION NUMBER: US/10/364,953
;  CURRENT FILING DATE:  2003-02-11
;  PRIOR APPLICATION NUMBER: US 60/355,895
;  PRIOR FILING DATE: 2002-02-11
;  PRIOR APPLICATION NUMBER: US 60/409,685
;  PRIOR FILING DATE: 2002-09-10
;  NUMBER OF SEQ ID NOS: 14
; SEQ ID NO 1
;   LENGTH: 214
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   NAME/KEY: Artificial Sequence
;   LOCATION: Full
;   OTHER INFORMATION: Y0101-VL
US-10-364-953-1

  Query Match           100.0%;  Score 575;  DB 15;  Length 214;
  Best Local Similarity  100.0%;  Pred. No. 3.4e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 9
US-10-020-786-10
; Sequence 10, Application US/10020786
; Publication No. US20030073164A1
; GENERAL INFORMATION:
;  APPLICANT: Simmons, Laura C.
;  APPLICANT:  Klimowski, Laura
;  APPLICANT:  Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: PROKARYOTICALLY PRODUCED ANTIBODIES AND USES THEREOF
;  FILE REFERENCE: P1793R1
;  CURRENT APPLICATION NUMBER: US/10/020,786
;  CURRENT FILING DATE:  2002-03-26
;  PRIOR APPLICATION NUMBER: US 60/256,164
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 11
; SEQ ID NO 10
;   LENGTH: 237
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF light chain
US-10-020-786-10

  Query Match           100.0%;  Score 575;  DB 14;  Length 237;
  Best Local Similarity  100.0%;  Pred. No. 3.7e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 10
US-10-697-995-8
; Sequence 8, Application US/10697995
; Publication No. US20050048572A1
; GENERAL INFORMATION:
;  APPLICANT: Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR INCREASING ANTIBODY PRODUCTION
;  FILE REFERENCE: 11669.195USU1
;  CURRENT APPLICATION NUMBER: US/10/697,995
;  CURRENT FILING DATE:  2003-10-30
;  PRIOR APPLICATION NUMBER: US 60/422,952
;  PRIOR FILING DATE: 2002-10-31
;  NUMBER OF SEQ ID NOS: 37
; SEQ ID NO 8
;   LENGTH: 237
```

```
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF light chain
US-10-697-995-8

  Query Match             100.0%;  Score 575;  DB 17;  Length 237;
  Best Local Similarity   100.0%;  Pred. No. 3.7e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 11
US-10-697-995-11
; Sequence 11, Application US/10697995
; Publication No. US20050048572A1
; GENERAL INFORMATION:
;  APPLICANT: Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR INCREASING ANTIBODY PRODUCTION
;  FILE REFERENCE: 11669.195USU1
;  CURRENT APPLICATION NUMBER: US/10/697,995
;  CURRENT FILING DATE:  2003-10-30
;  PRIOR APPLICATION NUMBER: US 60/422,952
;  PRIOR FILING DATE: 2002-10-31
;  NUMBER OF SEQ ID NOS: 37
; SEQ ID NO 11
;   LENGTH: 237
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Anti-VEGF light chain
US-10-697-995-11

  Query Match             100.0%;  Score 575;  DB 17;  Length 237;
  Best Local Similarity   100.0%;  Pred. No. 3.7e-40;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 12
US-10-234-671-8
; Sequence 8, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;        APPLICANT: Baca, Manuel
;                   Wells, James A.
;                   Presta, Leonard G.
;                   Lowman, Henry B.
;                   Chen, Yvonne M.
;        TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;        NUMBER OF SEQUENCES: 131
;        CORRESPONDENCE ADDRESS:
;             ADDRESSEE: Genentech, Inc.
;             STREET: 1 DNA Way
;             CITY: South San Francisco
;             STATE: California
;             COUNTRY: USA
;             ZIP: 94080
;        COMPUTER READABLE FORM:
;             MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;             COMPUTER: IBM PC compatible
;             OPERATING SYSTEM: PC-DOS/MS-DOS
;             SOFTWARE: WinPatin (Genentech)
;        CURRENT APPLICATION DATA:
;             APPLICATION NUMBER: US/10/234,671
;             FILING DATE: 03-Sep-2002
;             CLASSIFICATION: <Unknown>
;        PRIOR APPLICATION DATA:
;             APPLICATION NUMBER: 09/056160
;             FILING DATE: 06-APR-1998
;             APPLICATION NUMBER: 60/126446
;             FILING DATE: 07-APR-1997
;             APPLICATION NUMBER: 60/054856
;             FILING DATE: 06-AUG-1997
;        ATTORNEY/AGENT INFORMATION:
;             NAME: Cui, Steven X.
;             REGISTRATION NUMBER: 44,637
;             REFERENCE/DOCKET NUMBER: P1093R2C1
;        TELECOMMUNICATION INFORMATION:
;             TELEPHONE: 650/225-8674
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 8:
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 110 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 8:
US-10-234-671-8

  Query Match             99.7%;  Score 573;  DB 14;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 2.6e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 13
US-10-624-153-94
; Sequence 94, Application US/10624153
; Publication No. US20040086502A1
; GENERAL INFORMATION:
;  APPLICANT: CHEN, YVONNE M.
;  APPLICANT:  LOWMAN, HENRY B.
;  APPLICANT:  MULLER, YVES
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1C1
;  CURRENT APPLICATION NUMBER: US/10/624,153
;  CURRENT FILING DATE:  2003-07-21
;  PRIOR APPLICATION NUMBER: US 09/440,781
;  PRIOR FILING DATE: 1999-11-16
;  PRIOR APPLICATION NUMBER: US 60/108,945
;  PRIOR FILING DATE: 1998-11-18
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 94
;   LENGTH: 110
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   OTHER INFORMATION: sequence is synthesized
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-110
;   OTHER INFORMATION: humanized antibody light chain variable domain
US-10-624-153-94
```

```
  Query Match             99.7%;  Score 573;  DB 15;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 2.6e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 14
US-09-056-160B-105
; Sequence 105, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  105:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  110 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-105

  Query Match             99.5%;  Score 572;  DB 9;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 3.1e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 15
US-10-234-671-103
; Sequence 103, Application US/10234671
; Publication No. US20030190317A1
;  GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;  INFORMATION FOR SEQ ID NO: 103:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 110 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 103:
US-10-234-671-103

  Query Match             99.5%;  Score 572;  DB 14;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 3.1e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


Search completed: March 14, 2005, 20:42:12
Job time : 41.0088 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:39:29 ; Search time 16.6447 Seconds
(without alignments)
635.867 Million cell updates/sec

Title: US-09-723-752B-115
Perfect score: 575
Sequence: 1 DIQLTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 283416 seqs, 96216763 residues

Total number of hits satisfying chosen parameters: 283416

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : PIR_79:*
1: pir1:*
2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	510	88.7	127	2	S40367	Ig kappa chain V-J
2	487	84.7	108	1	K1HUAU	Ig kappa chain V-I
3	483	84.0	125	2	S40333	Ig kappa chain V-J
4	482	83.8	123	2	S40331	Ig kappa chain - h
5	481	83.7	108	2	B49047	Ig kappa chain V r
6	481	83.7	131	2	S40352	Ig kappa chain V-J
7	479	83.3	108	2	S44122	Ig kappa chain V r
8	479	83.3	109	2	S31998	Ig kappa chain - h
9	477	83.0	107	2	S36264	Ig lambda chain V
10	477	83.0	129	2	S52789	Ig kappa chain V r
11	473.5	82.3	124	2	S40336	Ig kappa chain V-J
12	473	82.3	129	2	S40369	Ig kappa chain - h
13	471	81.9	108	1	K1HUHU	Ig kappa chain V-I
14	470	81.7	108	2	S19674	Ig kappa chain V r
15	470	81.7	132	2	S40334	Ig kappa chain - h
16	469	81.6	108	1	K1HUWE	Ig kappa chain V-I
17	469	81.6	109	2	S31981	Ig kappa chain - h
18	469	81.6	111	2	A38740	Ig kappa chain V r
19	469	81.6	125	2	S40349	Ig kappa chain V-J
20	467	81.2	110	2	S44118	Ig kappa chain V-J
21	466	81.0	108	1	K1HURE	Ig kappa chain V-I
22	466	81.0	139	2	S40365	Ig kappa chain - h
23	465.5	81.0	107	2	S36275	Ig lambda chain V
24	464	80.7	108	1	K1HUAG	Ig kappa chain V-I
25	464	80.7	108	1	K1HUBN	Ig kappa chain V-I
26	463	80.5	108	2	S47182	Ig kappa chain - h
27	463	80.5	130	2	S40368	Ig kappa chain - h
28	462	80.3	125	2	S40316	Ig kappa chain - h
29	461	80.2	108	2	I39154	Ig kappa chain (BR
30	461	80.2	111	2	E38740	Ig kappa chain V r
31	461	80.2	122	2	S40370	Ig kappa chain - h
32	459	79.8	108	1	K1HURY	Ig kappa chain V-I
33	459	79.8	111	2	C38740	Ig kappa chain V r
34	459	79.8	122	2	S40314	Ig kappa chain - h
35	459	79.8	129	2	S40317	Ig kappa chain - h
36	459	79.8	129	2	S52793	Ig kappa chain V r
37	459	79.8	135	2	S24320	Ig kappa chain pre
38	458	79.7	107	2	I69017	anti-HIV1 envelope
39	457.5	79.6	125	2	S40315	Ig kappa chain - h
40	457	79.5	107	2	S36269	Ig lambda chain V
41	457	79.5	117	2	S46371	Ig kappa chain V-J
42	457	79.5	129	1	K1HUWK	Ig kappa chain pre
43	456	79.3	111	2	G38740	Ig kappa chain V r
44	456	79.3	117	2	S46376	Ig kappa chain V-J
45	456	79.3	128	2	S46372	IG light chain var

ALIGNMENTS

RESULT 1
S40367
Ig kappa chain V-J-C region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40367
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40367
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-127 <KLE>
A;Cross-references: EMBL:X72477
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;33-107/Domain: immunoglobulin homology <IMM>

Query Match 88.7%; Score 510; DB 2; Length 127;
Best Local Similarity 89.1%; Pred. No. 5.3e-38;
Matches 98; Conservative 3; Mismatches 9; Indels 0; Gaps 0;

```
Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| ||| |||||||||:|||||||:|||  ||| |||||
Db         18 DIQMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQRKPGKAPKLLIYAASSLQSGVPS 77

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||    ||||||||||||||
Db         78 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPWTFGQGTKVEIKRTV 127
```

RESULT 2
K1HUAU
Ig kappa chain V-I region (Au) - human
C;Species: Homo sapiens (man)
C;Date: 24-Apr-1984 #sequence_revision 02-Jul-1998 #text_change 09-Jul-2004
C;Accession: A91653; A01862; S02573
R;Schiechl, H.; Hilschmann, N.
Hoppe-Seyler's Z. Physiol. Chem. 353, 345-370, 1972
A;Title: Die Primaerstruktur einer monoklonalen Immunglobulin-L-Kette vom kappa-Typ, Sub
A;Reference number: A91653; MUID:72189444; PMID:5028201
A;Accession: A91653
A;Molecule type: protein
A;Residues: 1-108 <SCH>
A;Cross-references: UNIPROT:P01594
A;Note: the C region of this chain has the Inv (3) marker
R;Fehlhammer, H.; Schiffer, M.; Epp, O.; Colman, P.M.; Lattman, E.E.; Schwager, P.; Ste
Biophys. Struct. Mech. 1, 139-146, 1975
A;Title: The structure determination of the variable portion of the Bence-Jones protein
A;Reference number: A90729; MUID:77022433; PMID:1234024
A;Contents: annotation; X-ray crystallography

A;Note: the structure of the V region was determined by molecular replacement methods us
R;Steiner, V.; Chang, J.Y.
FEBS Lett. 222, 6-10, 1987
A;Title: Chemical modification of the carboxyl groups of protein substrates enhances the
A;Reference number: S02572; MUID:88005152; PMID:3115831
A;Contents: annotation
C;Comment: This is a Bence Jones protein.
C;Genetics:
A;Gene: GDB:IGKV1
A;Cross-references: GDB:136264
A;Map position: 2p12-2p12
C;Complex: an immunoglobulin heterotetramer subunit consists of two identical light (kap
hain disulfide bonds; in some cases, such as IgA and IgM, the subunits associate into la
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>
F;23-88/Disulfide bonds: #status predicted

Query Match 84.7%; Score 487; DB 1; Length 108;
Best Local Similarity 85.2%; Pred. No. 4.7e-36;
Matches 92; Conservative 5; Mismatches 11; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 1 DIQMTQSPSSLSASVGDRVTITCQASQDISDYLNWYQQKPGKAPKLLIYDASNLESGVPS 60

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
Db 61 RFSGGGSGAHFTFTISSLQPEDIATYYCQQYDYLPWTFGQGTKVEIKR 108

RESULT 3
S40333
Ig kappa chain V-J region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40333
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40333
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-125 <KLE>
A;Cross-references: EMBL:X72443; NID:g441354; PIDN:CAA51111.1; PID:g441355
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-108/Domain: immunoglobulin homology <IMM>

Query Match 84.0%; Score 483; DB 2; Length 125;
Best Local Similarity 85.0%; Pred. No. 1.2e-35;
Matches 91; Conservative 9; Mismatches 7; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 19 DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPS 78

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
Db 79 RFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPWTFGQGTKVEIK 125

RESULT 4
S40331
Ig kappa chain - human
C;Species: Homo sapiens (man)
C;Date: 06-Mar-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40331
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40331
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-123 <KLE>
A;Cross-references: EMBL:X72441; NID:g441350; PIDN:CAA51109.1; PID:g441351
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;32-106/Domain: immunoglobulin homology <IMM>

Query Match 83.8%; Score 482; DB 2; Length 123;
Best Local Similarity 87.9%; Pred. No. 1.5e-35;
Matches 94; Conservative 4; Mismatches 9; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 17 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 76

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
Db 77 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPRTFGQGTKVEIK 123

RESULT 5
B49047
Ig kappa chain V region (monoclonal striational autoantibody StrAB SA-1A) - human (fragr
C;Species: Homo sapiens (man)
C;Date: 19-Dec-1993 #sequence_revision 18-Nov-1994 #text_change 09-Jul-2004
C;Accession: B49047
R;Victor, K.D.; Pascual, V.; Williams, C.L.; Lennon, V.A.; Capra, J.D.
Eur. J. Immunol. 22, 2231-2236, 1992
A;Title: Human monoclonal striational autoantibodies isolated from thymic B lymphocytes
A;Reference number: A49047; MUID:92387224; PMID:1516616
A;Accession: B49047
A;Status: preliminary
A;Molecule type: nucleic acid
A;Residues: 1-108 <VIC>
A;Cross-references: UNIPROT:Q96SA9; UNIPROT:Q9UL77
A;Experimental source: thymic B lymphocytes
A;Note: sequence extracted from NCBI backbone (NCBIN:113208, NCBIP:113209)
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;16-90/Domain: immunoglobulin homology <IMM>

Query Match 83.7%; Score 481; DB 2; Length 108;
Best Local Similarity 87.0%; Pred. No. 1.6e-35;
Matches 94; Conservative 4; Mismatches 10; Indels 0; Gaps 0;

Qy 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
Db 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKR 108

RESULT 6
S40352
Ig kappa chain V-J-C region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40352
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40352
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-131 <KLE>
A;Cross-references: EMBL:X72462; NID:g441392; PIDN:CAA51130.1; PID:g441393
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;36-110/Domain: immunoglobulin homology <IMM>

```
  Query Match             83.7%;  Score 481;  DB 2;  Length 131;
  Best Local Similarity   84.5%;  Pred. No. 1.9e-35;
  Matches   93;  Conservative    8;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||:|||||| ||| ||||| |||||||| ||:|||  |:| |||||
Db         21 DIQMTQSPSSLSASVGNRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLQSGVPS 80

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||| ||||||:|::|| ||||||||||||||
Db         81 RFSGSGSGTDFSLTISSLQPEDVATYYCQKYNSVPRTFGQGTKVEIKRTV 130


RESULT 7
S44122
Ig kappa chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 24-May-2001
C;Accession: S44122
R;Hawkins, R.E.; Zhu, D.; Ovecka, M.; Winter, G.; Hamblin, T.J.; Stevenson, F.K.
submitted to the EMBL Data Library, March 1994
A;Description: Idiotypic vaccination against human B-cell lymphoma: rescue of variable r
A;Reference number: S44105
A;Accession: S44122
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-108 <HAW>
A;Cross-references: EMBL:Z31390; NID:g472976; PIDN:CAA83265.1; PID:g940533
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             83.3%;  Score 479;  DB 2;  Length 108;
  Best Local Similarity   86.1%;  Pred. No. 2.4e-35;
  Matches   93;  Conservative    4;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||||| ||| ||:|||||||| |||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKLGKAPKLLIYSASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||| |||||  : ||||||||||||||
Db         61 TFSGSGSGTDFTLTISSLQPEDFAIYYCQQSYSTPWTFGQGTKVEIKR 108


RESULT 8
S31998
Ig kappa chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 06-Feb-1995 #sequence_revision 06-Feb-1995 #text_change 21-Jan-2000
C;Accession: S31998
R;Portolano, S.; Chazenbalk, G.D.; Hutchison, S.J.; McLachlan, S.M.; Rapoport, B.
submitted to the EMBL Data Library, June 1992
A;Description: Lack of promiscuity in autoantigen-specific H and L chain combinations as
A;Reference number: S31977
A;Accession: S31998
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-109 <POR>
A;Cross-references: EMBL:Z15081; NID:g38501; PIDN:CAA78790.1; PID:g38502
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             83.3%;  Score 479;  DB 2;  Length 109;
  Best Local Similarity   84.4%;  Pred. No. 2.4e-35;
  Matches   92;  Conservative    5;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :: :|||||||||||||||||| ||| || ||||||:|||||||:|||  ||| |||||
Db          1 ELVMTQSPSSLSASVGDRVTITCRASQSISAYLNWYQKKPGKAPKLLIYSASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRT 109
              |||||||||||||||||||||||||||||    ||||| |||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYDTPWTFGHGTKVEIKRT 109


RESULT 9
S36264
Ig lambda chain V region (clone alpha-CEA4-8A) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 03-Feb-1994 #sequence_revision 03-Feb-1994 #text_change 21-Jan-2000
C;Accession: S36264
R;Griffiths, A.D.; Malmqvist, M.; Marks, J.D.; Bye, J.M.; Embleton, M.J.; McCafferty, J.
EMBO J. 12, 725-734, 1993
A;Title: Human anti-self antibodies with high specificity from phage display libraries.
A;Reference number: S36256; MUID:93178448; PMID:7679990
A;Accession: S36264
A;Status: preliminary; nucleic acid sequence not shown
A;Molecule type: mRNA
A;Residues: 1-107 <GRI>
A;Cross-references: EMBL:Z18845; NID:g33426; PIDN:CAA79297.1; PID:g939919
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             83.0%;  Score 477;  DB 2;  Length 107;
  Best Local Similarity   86.9%;  Pred. No. 3.5e-35;
  Matches   93;  Conservative    4;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :| |||||||||||||||||||| ||| ||:|||||||||||||:|||  ||| |||||
Db          1 EIVLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
              |||||||||||||||||||||||||||||||  | ||| ||||:||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSNYPLTFGGGTKVDIK 107


RESULT 10
S52789
Ig kappa chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 19-May-1995 #sequence_revision 21-Jul-1995 #text_change 21-Jan-2000
C;Accession: S52789
R;Rocca, A.; Khamlichi, A.A.; Touchard, G.; Mougenot, B.; Ronco, P.; Denoroy, L.; Deret,
submitted to the EMBL Data Library, March 1995
A;Description: Light chain V region gene usage restriction and peculiarities in myeloma
A;Reference number: S52789
A;Accession: S52789
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-129 <ROC>
A;Cross-references: EMBL:X85995; NID:g758588; PIDN:CAA59987.1; PID:g758589
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;38-112/Domain: immunoglobulin homology <IMM>

  Query Match             83.0%;  Score 477;  DB 2;  Length 129;
  Best Local Similarity   85.0%;  Pred. No. 4.3e-35;
  Matches   91;  Conservative    6;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| ||||||||||||||||||:||:  ||| :||||
Db         23 DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIHAASSLETGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
              ||||||||||: |||||||| ||||||||  :| ||| |||||||
Db         83 RFSGSGSGTDFSFTISSLQPEDLATYYCQQYDNLPLTFGGGTKVEIK 129


RESULT 11
S40336
Ig kappa chain V-J region - human
C;Species: Homo sapiens (man)
```

```
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40336
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40336
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-124 <KLE>
A;Cross-references: EMBL:X72446; NID:g441360; PIDN:CAA51114.1; PID:g441361
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;31-105/Domain: immunoglobulin homology <IMM>

  Query Match             82.3%;  Score 473.5;  DB 2;  Length 124;
  Best Local Similarity   85.3%;  Pred. No. 8.3e-35;
  Matches   93;  Conservative    6;  Mismatches    9;  Indels    1;  Gaps    1;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||| |||||||||||| ||| ||:|| |||||||||||:|||  |:| |||||
Db         16 DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPKLLIYAASTLQSGVPS 75

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTV-PWTFGQGTKVEIKR 108
              ||||||||:|:|||||||||||||||||| :|  ||||||||||||||
Db         76 RFSGSGSGTEFSLTISSLQPEDFATYYCQQLNTYPPWTFGQGTKVEIKR 124


RESULT 12
S40369
Ig kappa chain - human
C;Species: Homo sapiens (man)
C;Date: 06-Mar-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40369
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40369
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-129 <KLE>
A;Cross-references: EMBL:X72479; NID:g441426; PIDN:CAA51147.1; PID:g441427
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;37-111/Domain: immunoglobulin homology <IMM>

  Query Match             82.3%;  Score 473;  DB 2;  Length 129;
  Best Local Similarity   84.3%;  Pred. No. 9.6e-35;
  Matches   91;  Conservative    8;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| ||  |||:| |:|||||||| |||  ||| |||||
Db         22 DIQMTQSPSSLSASVGDRVTITCRASHVISNHLVWFQQKPGKAPKSLIYAASSLQSGVPS 81

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              :|||||||||||||||||||||||||:: |:|||||:||||
Db         82 KFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIKR 129


RESULT 13
K1HUHU
Ig kappa chain V-I region (Hau) - human
C;Species: Homo sapiens (man)
C;Date: 24-Apr-1984 #sequence_revision 02-Jul-1998 #text_change 09-Jul-2004
C;Accession: A01868; S02574
R;Watanabe, S.; Hilschmann, N.
Hoppe-Seyler's Z. Physiol. Chem. 351, 1291-1295, 1970
A;Title: The primary structure of a monoclonal kappa-type immunoglobulin L-chain of subg
A;Reference number: A01868; MUID:71032830; PMID:4097974
A;Accession: A01868
A;Molecule type: protein
A;Residues: 1-108 <WAT>
A;Cross-references: UNIPROT:P01600
A;Note: the C region of this chain has the Inv (3) marker
R;Steiner, V.; Chang, J.Y.
FEBS Lett. 222, 6-10, 1987
A;Title: Chemical modification of the carboxyl groups of protein substrates enhances th
A;Reference number: S02572; MUID:88005152; PMID:3115831
A;Contents: annotation
C;Comment: This is a Bence Jones protein.
C;Genetics:
A;Gene: GDB:IGKV1
A;Cross-references: GDB:136264
A;Map position: 2p12-2p12
C;Complex: an immunoglobulin heterotetramer subunit consists of two identical light (ka
hain disulfide bonds; in some cases, such as IgA and IgM, the subunits associate into l
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>
F;23-88/Disulfide bonds: #status predicted

  Query Match             81.9%;  Score 471;  DB 1;  Length 108;
  Best Local Similarity   85.2%;  Pred. No. 1.2e-34;
  Matches   92;  Conservative    6;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| ||| ||:||:||||||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLSWYQQKPGKAPQVLIYAASSLPSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||||    | :|||||:|||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQNYITPTSFGQGTRVEIKR 108


RESULT 14
S19674
Ig kappa chain V region (clone alpha-TEL9) - human
C;Species: Homo sapiens (man)
C;Date: 22-Jan-1993 #sequence_revision 22-Jan-1993 #text_change 20-Jun-2000
C;Accession: S19674
R;Marks, J.D.; Hoogenboom, H.R.; Bonnert, T.P.; McCafferty, J.; Griffiths, A.D.; Winter,
J. Mol. Biol. 222, 581-597, 1991
A;Title: By-passing immunization. Human antibodies from V-gene libraries displayed on ph
A;Reference number: S19663; MUID:92085276; PMID:1748994
A;Accession: S19674
A;Molecule type: mRNA
A;Residues: 1-108 <MAR>
A;Cross-references: EMBL:X61642; NID:g37860; PIDN:CAA43823.1; PID:g1335386
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             81.7%;  Score 470;  DB 2;  Length 108;
  Best Local Similarity   84.3%;  Pred. No. 1.5e-34;
  Matches   91;  Conservative    7;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :| |||||||||||||||||| ||| |||||||||||||||:|||  |:| |||||
Db          1 EIVLTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQKPGKAPKLLIYAASTLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||:|||||||||||||| :: | ||| |||:|||||
Db         61 RFSGSGSGTDFTLTINSLQPEDFATYYCQQTNSFPLTFGGGTKLEIKR 108


RESULT 15
S40334
Ig kappa chain - human
C;Species: Homo sapiens (man)
C;Date: 06-Mar-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40334
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
```

A;Accession: S40334
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-132 <KLE>
A;Cross-references: EMBL:X72444
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;37-111/Domain: immunoglobulin homology <IMM>

Query Match 81.7%; Score 470; DB 2; Length 132;
Best Local Similarity 80.9%; Pred. No. 1.8e-34;
Matches 89; Conservative 12; Mismatches 9; Indels 0; Gaps 0;

```
Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||| |||||||||| ||| |::|| |||||||||||:|||  |:| |||||
Db         22 DIQLTQSPSFLSASIGDRVTITCRASQGINSYLAWYQQKPGKAPKLLIYVASTLQSGVPS 81

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||:|||||||||||||||:|||||::: |:||| ||||||:|||
Db         82 RFSGSGSGTEFTLTISSLQPEDFASYYCQQFNSYPFTFGGGTKVEIRRTV 131
```

Search completed: March 14, 2005, 21:08:52
Job time : 17.6447 secs

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:32:33 ; Search time 77.193 Seconds
(without alignments)
729.713 Million cell updates/sec

Title: US-09-723-752B-115
Perfect score: 575
Sequence: 1 DIQLTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1612378 seqs, 512079187 residues

Total number of hits satisfying chosen parameters: 1612378

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : UniProt_03:*
1: uniprot_sprot:*
2: uniprot_trembl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	487	84.7	108	1	KV1B_HUMAN	P01594 homo sapien
2	485	84.3	108	2	Q9UL77	Q9ul77 homo sapien
3	483	84.0	236	2	Q6GMX9	Q6gmx9 homo sapien
4	482	83.8	236	2	Q6GMW1	Q6gmw1 homo sapien
5	481	83.7	236	2	Q7Z3Y4	Q7z3y4 homo sapien
6	474.5	82.5	107	2	Q96SA9	Q96sa9 homo sapien
7	474	82.4	236	2	Q6GMX8	Q6gmx8 homo sapien
8	471	81.9	108	1	KV1H_HUMAN	P01600 homo sapien
9	471	81.9	108	1	KV1Y_HUMAN	P80362 homo sapien
10	471	81.9	236	2	Q6GMX0	Q6gmx0 homo sapien
11	469	81.6	108	1	KV1R_HUMAN	P01610 homo sapien
12	466	81.0	108	1	KV1O_HUMAN	P01607 homo sapien
13	465	80.9	108	2	Q9UL70	Q9ul70 homo sapien
14	465	80.9	236	2	Q6PIH7	Q6pih7 homo sapien
15	464	80.7	108	1	KV1A_HUMAN	P01593 homo sapien
16	464	80.7	108	1	KV1V_HUMAN	P04430 homo sapien
17	462	80.3	234	2	Q7Z473	Q7z473 homo sapien
18	459	79.8	108	1	KV1P_HUMAN	P01608 homo sapien
19	457	79.5	129	1	KV1W_HUMAN	P04431 homo sapien
20	455	79.1	108	1	KV1E_HUMAN	P01597 homo sapien
21	454.5	79.0	107	1	KV1D_HUMAN	P01596 homo sapien
22	454	79.0	108	1	KV1M_HUMAN	P01605 homo sapien
23	453	78.8	108	1	KV1K_HUMAN	P01603 homo sapien
24	452.5	78.7	107	2	Q9UL81	Q9ul81 homo sapien
25	452	78.6	244	2	Q65ZC8	Q65zc8 homo sapien
26	449	78.1	108	1	KV1Q_HUMAN	P01609 homo sapien
27	448	77.9	108	1	KV1S_HUMAN	P01611 homo sapien
28	448	77.9	116	2	Q96PF6	Q96pf6 homo sapien
29	447	77.7	108	1	KV1N_HUMAN	P01606 homo sapien
30	446	77.6	108	1	KV1C_HUMAN	P01595 homo sapien
31	445	77.4	108	1	KV5J_MOUSE	P01643 mus musculu
32	445	77.4	236	2	Q6PIT5	Q6pit5 homo sapien
33	445	77.4	240	2	Q65ZC9	Q65zc9 homo sapien
34	443	77.0	108	1	KV1G_HUMAN	P01599 homo sapien
35	439	76.3	108	1	KV1F_HUMAN	P01598 homo sapien
36	439	76.3	108	1	KV1L_HUMAN	P01604 homo sapien
37	439	76.3	108	2	Q9UL79	Q9ul79 homo sapien
38	438	76.2	236	2	Q6PIH4	Q6pih4 homo sapien
39	428	74.4	108	1	KV5K_MOUSE	P01644 mus musculu
40	428	74.4	108	1	KV5N_MOUSE	P01647 mus musculu
41	426	74.1	108	1	KV5L_MOUSE	P01645 mus musculu
42	426	74.1	108	1	KV5M_MOUSE	P01646 mus musculu
43	425	73.9	108	1	KV5O_MOUSE	P01648 mus musculu
44	424.5	73.8	109	1	KV1T_HUMAN	P01612 homo sapien
45	422	73.4	108	1	KV5U_MOUSE	P04946 mus musculu

ALIGNMENTS

RESULT 1
KV1B_HUMAN
ID KV1B_HUMAN STANDARD; PRT; 108 AA.
AC P01594;
DT 21-JUL-1986 (Rel. 01, Created)
DT 21-JUL-1986 (Rel. 01, Last sequence update)
DT 25-OCT-2004 (Rel. 45, Last annotation update)
DE Ig kappa chain V-I region AU.
OS Homo sapiens (Human).
OC Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX NCBI_TaxID=9606;
RN [1]
RP SEQUENCE.
RX MEDLINE=72189444; PubMed=5028201;
RA Schiechl H., Hilschmann N.;
RT "Rule of antibody structure. The primary structure of a monoclonal
RT immunoglobulin L-chain of the kappa-type, subgroup I (Bence-Jones
RT protein Au).";
RL Hoppe-Seyler's Z. Physiol. Chem. 353:345-370(1972).
RN [2]
RP X-RAY CRYSTALLOGRAPHY (2.2 ANGSTROMS).
RX MEDLINE=77022433; PubMed=1234024;
RA Fehlhammer H., Schiffer M., Epp O., Colman P.M., Lattman E.E.,
RA Schwager P., Steigemann W., Schramm H.J.;
RT "The structure determination of the variable portion of the Bence-
RT Jones protein Au.";
RL Biophys. Struct. Mech. 1:139-146(1975).
CC -!- MISCELLANEOUS: The structure of the V region was determined by
CC molecular replacement methods using the known structure of the V
CC region of the kappa chain REI.
CC -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR PIR; A91653; K1HUAU.
DR PDB; 1JV5; X-ray; A=1-107.
DR GO; GO:0005576; C:extracellular; NAS.
DR GO; GO:0003823; F:antigen binding; NAS.
DR GO; GO:0006955; P:immune response; NAS.
DR InterPro; IPR007110; Ig-like.
DR InterPro; IPR003596; Ig_v.
DR Pfam; PF00047; ig; 1.
DR SMART; SM00406; IGv; 1.
DR PROSITE; PS50835; IG_LIKE; 1.
KW 3D-structure; Bence-Jones protein; Direct protein sequencing;
KW Immunoglobulin V region.
FT DOMAIN 1 23 Framework-1.
FT DOMAIN 24 34 Complementarity-determining-1.
FT DOMAIN 35 49 Framework-2.
FT DOMAIN 50 56 Complementarity-determining-2.
FT DOMAIN 57 88 Framework-3.
FT DOMAIN 89 97 Complementarity-determining-3.
FT DOMAIN 98 107 Framework-4.
FT DISULFID 23 88 By similarity.
FT STRAND 4 5

```
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       44     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       85     90
FT   STRAND       97     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11939 MW;  E8011187EE6F6FB9 CRC64;

  Query Match             84.7%;  Score 487;  DB 1;  Length 108;
  Best Local Similarity   85.2%;  Pred. No. 2.3e-42;
  Matches   92;  Conservative    5;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| ||||||:|||||||||||||:|||  |:| ||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDISDYLNWYQQKPGKAPKLLIYDASNLESGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||| |||  || ||||||||| ||||||||  :|||||||||||||
Db         61 RFSGGGSGAHFTFTISSLQPEDIATYYCQQYDYLPWTFGQGTKVEIKR 108


RESULT 2
Q9UL77
ID   Q9UL77      PRELIMINARY;      PRT;   108 AA.
AC   Q9UL77;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Myosin-reactive immunoglobulin light chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035037; AAD56273.1; -.
DR   PIR; B49047; B49047.
DR   PIR; S34083; S34083.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11738 MW;  C06681716C4D16F3 CRC64;

  Query Match             84.3%;  Score 485;  DB 2;  Length 108;
  Best Local Similarity   86.1%;  Pred. No. 3.8e-42;
  Matches   93;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||| ||| ||:|||||||||||||:|||  ||| ||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              ||||||||||||||||||||||||||||||  :  ||||:||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTSWTFGEGTKVEIKR 108


RESULT 3
Q6GMX9
ID   Q6GMX9      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX9;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073763; AAH73763.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25924 MW;  FDE2093DC560CFF7 CRC64;

  Query Match             84.0%;  Score 483;  DB 2;  Length 236;
  Best Local Similarity   85.5%;  Pred. No. 1.5e-41;
  Matches   94;  Conservative    6;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||| |||||| |||::| :| ||||:| |||| ||| ||||||||||
Db         23 DIQMTQSPSSLSASVGHRVTITCRASQNVSRWLAWYQQRPEKAPKSLIYATSSLHSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
```

```
                     ||||||||||||||||||||||||||||||:| | ||| ||||||||||
Db         83 RPSGSGSGTDFTLTISSLQPEDFATYYCQQYNTYPLTFGGGTKVEIKRTV 132


RESULT 4
Q6GMW1
ID   Q6GMW1      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMW1;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073791; AAH73791.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25751 MW;  5BFE6A087AFAC437 CRC64;

  Query Match             83.8%;  Score 482;  DB 2;  Length 236;
  Best Local Similarity   87.2%;  Pred. No. 1.9e-41;
  Matches   95;  Conservative    2;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          2 IQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSR 61
              ||:|||||||||||||||||| ||| ||| | |||||||||||:|||  ||| ||||||
Db         24 IQMTQSPSSLSASVGDRVTITCRASQGISNDLGWYQQKPGKAPKLLIYAASSLQSGVPSR 83

Qy         62 FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||||||||||||||||| |    ||||||||||||||||
Db         84 FSGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPWTFGQGTKVEIKRTV 132


RESULT 5
Q7Z3Y4
ID   Q7Z3Y4      PRELIMINARY;      PRT;   236 AA.
AC   Q7Z3Y4;
DT   01-OCT-2003 (TrEMBLrel. 25, Created)
DT   01-OCT-2003 (TrEMBLrel. 25, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Skeletal Muscle;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Skeletal Muscle;
RA   Strausberg R.;
RL   Submitted (MAR-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC005332; AAH05332.1; -.
DR   HSSP; P01834; 1HEZ.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25702 MW;  7FBFE4ED23084BC6 CRC64;

  Query Match             83.7%;  Score 481;  DB 2;  Length 236;
  Best Local Similarity   85.5%;  Pred. No. 2.4e-41;
  Matches   94;  Conservative    5;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||| ||||| ||||||||| |:||||||||| |||  ||| ||| |
Db         23 DIQMTQSPSSLSASVGDTVTITCRASQDISNYLAWFQQKPGKAPKSLIYGASSLQSGVQS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              :|||||||||||||||||||||||||||| : | |||||||:||||||
Db         83 KFSGSGSGTDFTLTISSLQPEDFATYYCQQYKSYPVTFGQGTKLEIKRTV 132


RESULT 6
Q96SA9
ID   Q96SA9      PRELIMINARY;      PRT;   107 AA.
AC   Q96SA9;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
```

```
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Anti-streptococcal/anti-myosin immunoglobulin kappa light chain
DE   variable region (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98375893; PubMed=9712075;
RA   Adderson E.E., Shikhman A.R., Ward K.E., Cunningham M.W.;
RT   "Molecular analysis of polyreactive monoclonal antibodies from
RT   rheumatic carditis: human anti-N-acetylglucosamine/anti-myosin
RT   antibody V region genes.";
RL   J. Immunol. 161:2020-2031(1998).
DR   EMBL; U96396; AAB68785.1; -.
DR   PIR; B49047; B49047.
DR   PIR; PH0867; PH0867.
DR   PIR; S16840; S16840.
DR   PIR; S31977; S31977.
DR   PIR; S34083; S34083.
DR   PIR; S34086; S34086.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     107    107
SQ   SEQUENCE   107 AA;  11520 MW;  4BB43E9C5B577F16 CRC64;

  Query Match             82.5%;  Score 474.5;  DB 2;  Length 107;
  Best Local Similarity   88.1%;  Pred. No. 4.5e-41;
  Matches   96;  Conservative    4;  Mismatches    6;  Indels    3;  Gaps    2;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| ||| ||:|||||||||||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQ-YSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||| |||:  ||| ||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTL--TFGGGTKVEIKR 107


RESULT 7
Q6GMX8
ID   Q6GMX8      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX8;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073764; AAH73764.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25707 MW;  4FC8E14B6559EFC9 CRC64;

  Query Match             82.4%;  Score 474;  DB 2;  Length 236;
  Best Local Similarity   83.6%;  Pred. No. 1.3e-40;
  Matches   92;  Conservative    8;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||:|||||||||||| ||| ||::| ||||||||||:|||  ||| |||||
Db         23 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||  : |:||| ||||:|||||
Db         83 RFSGSGSGTDFTLTISSLQPEDFATYYCQQAHSFPFTFGPGTKVDIKRTV 132


RESULT 8
KV1H_HUMAN
ID   KV1H_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01600;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region Hau.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=71032830; PubMed=4097974;
RA   Watanabe S., Hilschmann N.;
RT   "The primary structure of a monoclonal kappa-type immunoglobulin L-
RT   chain of subgroup I (Bence-Jones Protein Hau): subdivision within
RT   subgroups.";
RL   Hoppe-Seyler's Z. Physiol. Chem. 351:1291-1295(1970).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A01868; K1HUHU.
DR   PDB; 1F6L; X-ray; L=1-108.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
```

```
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88       By similarity.
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11671 MW;  08D3A6160D8D0618 CRC64;

  Query Match             81.9%;  Score 471;  DB 1;  Length 108;
  Best Local Similarity   85.2%;  Pred. No. 1e-40;
  Matches   92;  Conservative    6;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| ||| ||:||:|||||||||||:|||| ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLSWYQQKPGKAPQVLIYAASSLPSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||    | :|||||:|||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQNYITPTSFGQGTRVEIKR 108


RESULT 9
KV1Y_HUMAN
ID   KV1Y_HUMAN     STANDARD;      PRT;   108 AA.
AC   P80362;
DT   01-NOV-1995 (Rel. 32, Created)
DT   01-NOV-1995 (Rel. 32, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region WAT.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE, AND X-RAY CRYSTALLOGRAPHY (1.9 ANGSTROMS).
RX   MEDLINE=95086080; PubMed=7993911;
RA   Huang D.-B., Chang C.-H., Ainsworth C., Bruenger A.T., Eulitz M.,
RA   Solomon A., Stevens F.J., Schiffer M.;
RT   "Comparison of crystal structures of two homologous proteins:
RT   structural origin of altered domain interactions in immunoglobulin
RT   light-chain dimers.";
RL   Biochemistry 33:14848-14857(1994).
RN   [2]
RP   SEQUENCE OF 1-35.
RX   MEDLINE=81267384; PubMed=6167731;
RA   Stevens F.J., Westholm F.A., Panagiotopoulos N., Schiffer M.,
RA   Popp R.A., Solomon A.;
RT   "Characterization and preliminary crystallographic data on the VL-
RT   related fragment of the human kI Bence Jones protein Wat.";
RL   J. Mol. Biol. 147:185-193(1981).
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PDB; 1WTL; X-ray; A/B=1-108.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88       By similarity.
FT   CONFLICT     30     31       TN -> SD (in Ref. 2).
FT   STRAND        4      7
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       45     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       84     90
FT   STRAND       98     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11737 MW;  D9D941B3F0FAE697 CRC64;

  Query Match             81.9%;  Score 471;  DB 1;  Length 108;
  Best Local Similarity   82.4%;  Pred. No. 1e-40;
  Matches   89;  Conservative    9;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| |||||:||:||:||:|||||||  | | :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDITNYVNWFQQRPGQAPKVLIYGASILETGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||| ||||||||| |||||||| |:| ||| ||||:|||
Db         61 RFSGSGSGTDFTFTISSLQPEDIATYYCQQYDTLPLTFGGGTKVDIKR 108


RESULT 10
Q6GMX0
ID   Q6GMX0      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX0;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
```

```
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073775; AAH73775.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25807 MW;  864EA08C7E92BF8F CRC64;

  Query Match             81.9%;  Score 471;  DB 2;  Length 236;
  Best Local Similarity   82.7%;  Pred. No. 2.6e-40;
  Matches   91;  Conservative    7;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| |||:|:||||||| |||||| :|||  ||| |||||
Db         23 DIQMTQSPSSLSASVGDRVTITCRASQNINNYLNWYQLKPGKAPNLLIYAASSLQSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||:|:|||||||||   :| ||| || |||||||
Db         83 RFSGSGSGTDFTLTISSLRPDDFATYYCQQSYNIPLTFGGGTNVEIKRTV 132


RESULT 11
KV1R_HUMAN
ID   KV1R_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01610;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig kappa chain V-I region WEA.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=83273707; PubMed=6410398;
RA   Goni F., Frangione B.;
RT   "Amino acid sequence of the Fv region of a human monoclonal IgM
RT   (protein WEA) with antibody activity against 3,4-pyruvylated galactose
RT   in Klebsiella polysaccharides K30 and K33.";
RL   Proc. Natl. Acad. Sci. U.S.A. 80:4837-4841(1983).
CC   -!- MISCELLANEOUS: This chain was obtained from a monoclonal antibody
CC       against 3,4-pyruvylated galactose and isolated from a patient with
CC       Waldenstrom's macroglobulinemia.
DR   PIR; A01876; K1HUWE.
DR   HSSP; P80362; 1WTL.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region;
KW   Monoclonal antibody.
FT   DOMAIN       1     23       Framework-1.
FT   DOMAIN       24    34       Complementarity-determining-1.
FT   DOMAIN       35    49       Framework-2.
FT   DOMAIN       50    56       Complementarity-determining-2.
FT   DOMAIN       57    88       Framework-3.
FT   DOMAIN       89    97       Complementarity-determining-3.
FT   DOMAIN       98    107      Framework-4.
FT   DISULFID     23    88       By similarity.
FT   NON_TER      108   108
SQ   SEQUENCE   108 AA;  11840 MW;  9249B61F0945618C CRC64;

  Query Match             81.6%;  Score 469;  DB 1;  Length 108;
  Best Local Similarity   83.3%;  Pred. No. 1.7e-40;
  Matches   90;  Conservative    6;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| ||| | | | ||||||| ||| |||  :|| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLTWYQQKPGTAPKRLIYGATSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||:|||||:|||||||||||| |||: ||||||||||:||
Db         61 RFSGSGSGTEFTLTINSLQPEDFATYYCLQYSSFPWTFGQGTKVEVKR 108


RESULT 12
KV1O_HUMAN
ID   KV1O_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01607;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region Rei.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=76023758; PubMed=809329;
RA   Palm W., Hilschmann N.;
RT   "The primary structure of a crystalline monoclonal immunoglobulin
RT   kappa-type L-chain, subgroup I (Bence-Jones protein Rei); isolation
RT   and characterization of the tryptic peptides; the complete amino acid
RT   sequence of the protein; a contribution to the elucidation of the
RT   three-dimensional structure of antibodies, in particular their
RT   combining site.";
RL   Hoppe-Seyler's Z. Physiol. Chem. 356:167-191(1975).
RN   [2]
RP   X-RAY CRYSTALLOGRAPHY (2.0 ANGSTROMS).
RX   MEDLINE=76039968; PubMed=1182131;
RA   Epp O., Lattman E.E., Schiffer M., Huber R., Palm W.;
RT   "The molecular structure of a dimer composed of the variable portions
RT   of the Bence-Jones protein REI refined at 2.0-A resolution.";
RL   Biochemistry 14:4943-4952(1975).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (1,2)
CC       marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A91663; K1HURE.
DR   PDB; 1AR2; X-ray; @=1-107.
DR   PDB; 1BWW; X-ray; A/B=1-107.
DR   PDB; 1REI; X-ray; A/B=1-107.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN       1     23       Framework-1.
FT   DOMAIN       24    34       Complementarity-determining-1.
FT   DOMAIN       35    49       Framework-2.
FT   DOMAIN       50    56       Complementarity-determining-2.
```

```
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88
FT   STRAND        4      7
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       45     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       84     90
FT   STRAND       97     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11902 MW;  9E8143E1188BCE2A CRC64;

  Query Match             81.0%;  Score 466;  DB 1;  Length 108;
  Best Local Similarity   80.6%;  Pred. No. 3.4e-40;
  Matches   87;  Conservative   10;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||||| |||||  ||||||| ||||||:|||  |:| :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGKAPKLLIYEASNLQAGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              ||||||||||:| |||||||||| |||||||| ::|:||||||::| |
Db         61 RFSGSGSGTDYTFTISSLQPEDIATYYCQQYQSLPYTFGQGTKLQITR 108


RESULT 13
Q9UL70
ID   Q9UL70      PRELIMINARY;      PRT;   108 AA.
AC   Q9UL70;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Myosin-reactive immunoglobulin light chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035044; AAD56280.1; -.
DR   PIR; PH0863; PH0863.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11633 MW;  B7BEDC3E41FCCA37 CRC64;

  Query Match             80.9%;  Score 465;  DB 2;  Length 108;
  Best Local Similarity   83.3%;  Pred. No. 4.3e-40;
  Matches   90;  Conservative    6;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||||| ||| ||||| |||||||| || |||  |:| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKSLIYAASTLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||| ||||||:|:: | ||| |||:|||
Db         61 RFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPRTFGPGTKLEIKR 108


RESULT 14
Q6PIH7
ID   Q6PIH7      PRELIMINARY;      PRT;   236 AA.
AC   Q6PIH7;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Lung;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Lung;
RA   Strausberg R.;
RL   Submitted (JUL-2002) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC034141; AAH34141.1; -.
DR   HSSP; P01607; 1AR2.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25603 MW;  8BC561106861213F CRC64;

  Query Match             80.9%;  Score 465;  DB 2;  Length 236;
  Best Local Similarity   83.6%;  Pred. No. 1.1e-39;
  Matches   92;  Conservative    6;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
```

```
              ||||||||| |||||||||||||| ||| ||:|| |||||||||| :|||  |:| |||||
Db         23 DIQLTQSPSFLSASVGDRVTITCRASQGISSYLAWYQQKPGKAPNLLIYAASTLQSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||:|||||||||||||||||||| :: | ||| ||||||||||
Db         83 RFSGSGSGTEFTLTISSLQPEDFATYYCQQLNSSPPTFGGGTKVEIKRTV 132


RESULT 15
KV1A_HUMAN
ID   KV1A_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01593;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig kappa chain V-I region AG.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=69234734; PubMed=4893682;
RA   Titani K., Shinoda T., Putnam F.W.;
RT   "The amino acid sequence of a kappa type Bence-Jones protein. 3. The
RT   complete sequence and the location of the disulfide bridges.";
RL   J. Biol. Chem. 244:3550-3560(1969).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A01861; K1HUAG.
DR   HSSP; P01607; 1BWW.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11992 MW;  E3B3B246C18F0C4F CRC64;

  Query Match             80.7%;  Score 464;  DB 1;  Length 108;
  Best Local Similarity   81.5%;  Pred. No. 5.5e-40;
  Matches   88;  Conservative    8;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:|||||||||||||||||| ||||||::|||||| | ||||:|||  |:| :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDINHYLNWYQQGPKKAPKILIYDASNLETGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||| ||||| ||| ||||| |||||||| |:| |||||||:||||
Db         61 RFSGSGFGTDFTFTISGLQPEDIATYYCQQYDTLPRTFGQGTKLEIKR 108


Search completed: March 14, 2005, 20:49:19
Job time : 78.193 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:21:17 ; Search time 94.4518 Seconds
(without alignments)
483.186 Million cell updates/sec

Title: US-09-723-752B-116
Perfect score: 658
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPYYYGTSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 2105692 seqs, 386760381 residues

Total number of hits satisfying chosen parameters: 2105692

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : A_Geneseq_16Dec04:*
1: geneseqp1980s:*
2: geneseqp1990s:*
3: geneseqp2000s:*
4: geneseqp2001s:*
5: geneseqp2002s:*
6: geneseqp2003as:*
7: geneseqp2003bs:*
8: geneseqp2004s:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	658	100.0	118	2	AAW70686	Aaw70686 Anti-VEGF
2	658	100.0	118	2	AAW70688	Aaw70688 Anti-VEGF
3	658	100.0	118	3	AAB13385	Aab13385 Anti-VEGF
4	658	100.0	118	3	AAB13384	Aab13384 Anti-VEGF
5	658	100.0	118	5	ABP61255	Abp61255 Humanised
6	658	100.0	118	5	ABP61257	Abp61257 Humanised
7	658	100.0	123	8	ADG31769	Adg31769 V(H) doma
8	658	100.0	254	5	ABP51953	Abp51953 Plasmid p
9	658	100.0	476	5	ABB81110	Abb81110 Anti-VEGF
10	658	100.0	476	8	ADO14129	Ado14129 Plasmid p
11	658	100.0	476	8	ADQ90730	Adq90730 Anti-VEGF
12	654	99.4	117	7	ADF09953	Adf09953 Antibody
13	654	99.4	117	7	ADF10058	Adf10058 VEGF anti
14	649	98.6	118	2	AAW70682	Aaw70682 Anti-VEGF
15	649	98.6	118	3	AAB05900	Aab05900 F(ab)-12
16	649	98.6	118	3	AAB13382	Aab13382 Anti-VEGF
17	649	98.6	118	5	ABP61251	Abp61251 Humanised
18	645	98.0	231	7	ADC26162	Adc26162 Anti-VEGF
19	640	97.3	118	2	AAW70684	Aaw70684 Anti-VEGF
20	640	97.3	118	3	AAB13383	Aab13383 Anti-VEGF
21	640	97.3	118	5	ABP61253	Abp61253 Humanised
22	640	97.3	231	7	ADC26158	Adc26158 Anti-VEGF
23	638	97.0	123	8	ADG31894	Adg31894 V(H) prot
24	635	96.5	118	2	AAW70678	Aaw70678 Anti-VEGF
25	635	96.5	118	3	AAB05899	Aab05899 Humanised
26	635	96.5	118	3	AAB13381	Aab13381 F(ab)-12
27	635	96.5	118	3	AAB13389	Aab13389 Anti-VEGF
28	635	96.5	118	5	ABP61247	Abp61247 Humanised
29	635	96.5	123	2	AAW70617	Aaw70617 Anti-VEGF
30	635	96.5	123	5	ABP61186	Abp61186 Humanised
31	635	96.5	123	8	ADG31767	Adg31767 V(H) doma
32	635	96.5	123	8	ADG31780	Adg31780 V(H) doma
33	635	96.5	231	7	ADC26155	Adc26155 Parent an
34	635	96.5	476	8	ADQ90736	Adq90736 Anti-VEGF
35	633	96.2	123	8	ADG31892	Adg31892 V(H) prot
36	631	95.9	118	2	AAW70680	Aaw70680 Anti-VEGF
37	631	95.9	118	5	ABP61249	Abp61249 Humanised
38	629	95.6	123	2	AAW70697	Aaw70697 Anti-VEGF
39	629	95.6	123	5	ABP61266	Abp61266 Humanised
40	629	95.6	123	8	ADG31895	Adg31895 V(H) prot
41	627.5	95.4	121	3	AAB05902	Aab05902 F(ab)-12
42	627.5	95.4	121	3	AAB13391	Aab13391 Anti-VEGF
43	625.5	95.1	234	7	ADC26161	Adc26161 Anti-VEGF
44	620.5	94.3	234	7	ADC26163	Adc26163 Anti-VEGF
45	618	93.9	123	2	AAW70626	Aaw70626 Humanised

ALIGNMENTS

RESULT 1
AAW70686
ID AAW70686 standard; peptide; 118 AA.
XX
AC AAW70686;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable heavy domain of variant Y0313-1.
XX
KW Heavy variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 10B; 100pp; English.
XX
CC The present sequence represents a variable heavy domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can

```
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 2;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 2
AAW70688
ID   AAW70688 standard; peptide; 118 AA.
XX
AC   AAW70688;
XX
DT   27-JAN-1999  (first entry)
XX
DE   Anti-VEGF humanised antibody variable heavy domain of variant Y0317.
XX
KW   Heavy variable domain; murine; humanised antibody;
KW   anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW   VEGF-induced angiogenesis; tumour; retinal disorder;
KW   age-related macular degeneration; diabetic retinopathy;
KW   rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS   Synthetic.
OS   Mus sp.
OS   Homo sapiens.
XX
PN   WO9845331-A2.
XX
PD   15-OCT-1998.
XX
PF   03-APR-1998;   98WO-US006604.
XX
PR   07-APR-1997;   97US-00833504.
PR   06-AUG-1997;   97US-00908469.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 1998-568337/48.
XX
PT   New humanised antibody with affinity for vascular endothelial growth
PT   factor - for treatment of tumours, retinal disease and other angiogenic
PT   states, also related nucleic acid, vectors and transformed cells.
XX
PS   Claim 25; Fig 10B; 100pp; English.
XX
CC   The present sequence represents a variable heavy domain of an affinity-
CC   matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC   variant. The sequence is used in the course of the invention to produce
CC   the humanised anti-VEGF antibody of the invention. The humanised
CC   antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC   for treating or preventing tumours (of any type) and retinal disorders
CC   (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 2;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 3
AAB13385
ID   AAB13385 standard; protein; 118 AA.
XX
AC   AAB13385;
XX
DT   21-NOV-2000  (first entry)
XX
DE   Anti-VEGF antibody YO317 heavy chain variable domain.
XX
KW   YO317; vascular endothelial cell growth factor; VEGF; antibody;
KW   antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Unidentified.
XX
FH   Key             Location/Qualifiers
FT   Region          26. .35
FT                   /label= CDR-H1
FT   Region          50. .66
FT                   /label= CDR-H2
FT   Region          70. .79
FT                   /label= CDR-7
FT   Region          99. .112
FT                   /label= CDR-H3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14B; 60pp; English.
XX
CC   The present sequence is the heavy chain variable region of the affinity
CC   matured anti-vascular endothelial cell growth factor (anti-VEGF) antibody
CC   YO317. Humanised F(ab)-12 and affinity matured anti-VEGF antibodies may
CC   be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
```

```
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 3;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 4
AAB13384
ID   AAB13384 standard; protein; 118 AA.
XX
AC   AAB13384;
XX
DT   21-NOV-2000  (first entry)
XX
DE   Anti-VEGF antibody YO313-1 heavy chain variable domain.
XX
KW   YO313-1; vascular endothelial cell growth factor; VEGF; antibody;
KW   antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Unidentified.
XX
FH   Key             Location/Qualifiers
FT   Region          26. .35
FT                   /label= CDR-H1
FT   Region          50. .66
FT                   /label= CDR-H2
FT   Region          70. .79
FT                   /label= CDR-7
FT   Region          99. .112
FT                   /label= CDR-H3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14B; 60pp; English.
XX
CC   The present sequence is the heavy chain variable region of the affinity
CC   matured anti-vascular endothelial cell growth factor (anti-VEGF) antibody
CC   YO313-1. Humanised F(ab)-12 and affinity matured anti-VEGF antibodies may
CC   be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 3;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 5
ABP61255
ID   ABP61255 standard; protein; 118 AA.
XX
AC   ABP61255;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF YO313-1 antibody variable heavy domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; YO313-1; heavy chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          26. .35
FT                   /label= CDR-H1
FT   Domain          50. .66
FT                   /label= CDR-H2
FT   Domain          70. .79
FT                   /label= CDR-7
FT   Domain          99. .112
FT                   /label= CDR-H3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
```

```
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Example 3; Fig 10; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary heavy chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 5;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 6
ABP61257
ID   ABP61257 standard; protein; 118 AA.
XX
AC   ABP61257;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0317 antibody variable heavy domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0317; heavy chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          26. .35
FT                   /label= CDR-H1
FT   Domain          50. .66
FT                   /label= CDR-H2
FT   Domain          70. .79
FT                   /label= CDR-7
FT   Domain          99. .112
FT                   /label= CDR-H3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Claim 25; Fig 10; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary heavy chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 658;  DB 5;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.6e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 7
ADG31769
ID   ADG31769 standard; protein; 123 AA.
XX
AC   ADG31769;
XX
DT   26-FEB-2004  (first entry)
XX
DE   V(H) domain of matured humanised murine anti-VEGF antibody SeqID3.
XX
KW   protein library; in silico; VEGF; vascular endothelial growth factor;
KW   antibody; computational prediction; V(H) domain; mouse; murine.
XX
OS   Synthetic.
OS   Mus sp.
XX
PN   WO2003099999-A2.
XX
PD   04-DEC-2003.
XX
PF   20-MAY-2003; 2003WO-US016037.
XX
PR   20-MAY-2002; 2002US-00153159.
PR   20-MAY-2002; 2002US-00153176.
XX
PA   (ABMA-) ABMAXIS INC.
XX
PI   Luo P,  Hsieh M,  Zhong P,  Wang C,  Cao Y,  Liu S;
XX
DR   WPI; 2004-035117/03.
XX
PT   Constructing antibody libraries for generating protein libraries with
PT   improved biological function comprising selecting from tester protein
PT   sequences two peptide segments having 15% sequence identity with the lead
PT   sequence.
XX
PS   Disclosure; SEQ ID NO 3; 354pp; English.
XX
```

```
CC   This invention relates to a novel method for the generation and screening
CC   of a protein library in silico. Specifically, it refers to a high-
CC   throughput method optimised for the identification of anti-VEGF (vascular
CC   endothelial growth factor) antibodies with improved binding affinities
CC   for their target antigen (VEGF), using computational prediction. The
CC   present invention describes selecting proteins with a desirable function
CC   based on their structural similarity to the target structural or
CC   functional motif of a lead protein of interest. Accordingly, these
CC   protein libraries are functionally biased with increased diversity so as
CC   to increase the chance of identifying novel hits or combinations of
CC   mutants with enhanced binding affinity. Furthermore, the sequence profile
CC   based on the multiple structure alignment of the available lead structure
CC   allows the sampling of a larger sequence space than by traditional,
CC   multiple sequence alignment approaches. This polypeptide sequence is the
CC   V(H) domain of affinity matured humanised murine anti-VEGF antibody, used
CC   in an exemplification of the invention.
XX
SQ   Sequence 123 AA;

  Query Match             100.0%;  Score 658;  DB 8;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.7e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 8
ABP51953
ID   ABP51953 standard; protein; 254 AA.
XX
AC   ABP51953;
XX
DT   09-OCT-2002  (first entry)
XX
DE   Plasmid pY0317 anti-VEGF Fab amino acid sequence SEQ ID NO:2 #2.
XX
KW   Bacterial host; protease; degP; prc; spr; anti-VEGF antibody; antibody;
KW   humanised; Apo2 ligand; anti-CD18; anti-tissue factor; 2C4; anti-CD20;
KW   anti-vascular endothelial growth factor; anti-Her-2; anti-CD40; Fab;
KW   anti-CD11a; Fab'; Fab'2; Fab'2-leucine zipper fusion; anti-VEGF Fab.
XX
OS   Mus sp.
OS   Escherichia coli.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /label= signal
FT   Protein         24. .254
FT                   /label= anti-VEGF_Fab
XX
PN   WO200248376-A2.
XX
PD   20-JUN-2002.
XX
PF   07-DEC-2001; 2001WO-US047581.
XX
PR   14-DEC-2000; 2000US-0256162P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Chen CY;
XX
DR   WPI; 2002-583522/62.
DR   N-PSDB; ABQ73919.
XX
PT   Novel Escherichia coli strain useful for producing polypeptide, deficient
PT   in degP and prc encoding protease, and harboring mutant spr gene, product
PT   of gene suppresses growth phenotypes of strains harboring prc mutants.
XX
PS   Example 1; Fig 1D-E; 63pp; English.
XX
CC   The present invention describes an Escherichia coli strain (I) deficient
CC   in chromosomal degP and prc encoding protease DegP and Prc, respectively,
CC   and harbouring a mutant spr gene, the product of mutant spr gene
CC   suppresses growth phenotypes exhibited by strains harbouring prc mutants.
CC   (I) is useful for producing a polypeptide, by culturing (I) comprising
CC   nucleic acid encoding the polypeptide, which is heterologous to the
CC   strain, such that the nucleic acid is expressed, and recovering the
CC   heterologous polypeptide from the strain. The heterologous polypeptide is
CC   proteolytically sensitive. Culturing of (I) is performed in a fermentor
CC   under conditions of high- or low-cell density fermentation. The
CC   polypeptide is recovered from the periplasm or culture medium of the
CC   strain. The polypeptide is an antibody (humanised or full-length
CC   antibody) or Apo2 ligand. The antibody is an anti-CD18, anti-vascular
CC   endothelial growth factor (VEGF), anti-tissue factor, 2C4, anti-Her-2,
CC   anti-CD20, anti-CD40, or anti-CD11a antibody. The antibody is also an
CC   antibody fragment having a light chain (kappa light chain). The antibody
CC   fragment is a Fab, Fab', Fab'2 or Fab'2-leucine zipper fusion, anti-CD18
CC   Fab'2-leucine zipper fusion, anti-tissue factor Fab'2-leucine zipper
CC   fusion or anti-VEGF Fab, with or without a histidine or lysine tag, anti-
CC   tissue factor Fab'2-leucine zipper fusion with a 6-histidine tag, or anti
CC   -CD18 Fab'2-leucine zipper fusion with a 6-histidine tag, and anti-CD18
CC   Fab'2-leucine zipper fusion with a 6-lysine tag. The present sequence
CC   represents an anti-VEGF Fab amino acid sequence from the present
CC   invention
XX
SQ   Sequence 254 AA;

  Query Match             100.0%;  Score 658;  DB 5;  Length 254;
  Best Local Similarity   100.0%;  Pred. No. 6.1e-56;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141


RESULT 9
ABB81110
ID   ABB81110 standard; protein; 476 AA.
XX
AC   ABB81110;
XX
DT   05-NOV-2002  (first entry)
XX
DE   Anti-VEGF heavy chain fragment.
XX
KW   Immunoglobulin; promoter; cytostatic; antiinflammatory; immunomodulator;
KW   neuroprotective; CD11; tissue factor; vascular endothelial growth factor;
KW   VEGF.
XX
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /note= "STII signal sequence TIR-1"
FT   Protein         24. .476
FT                   /note= "anti-VEGF heavy chain"
XX
PN   WO200261090-A2.
XX
PD   08-AUG-2002.
XX
PF   13-DEC-2001; 2001WO-US048691.
XX
```

```
PR   14-DEC-2000; 2000US-0256164P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons LC,  Klimowski L,  Reilly DE,  Yansura DG;
XX
DR   WPI; 2002-619253/66.
DR   N-PSDB; ABN86646.
XX
PT   New polynucleotide comprising first and second promoter-cistron pairs,
PT   useful for diagnosing, treating or preventing diseases associated with
PT   abnormal expression and/or activity of antigens such as inflammatory
PT   disorders.
XX
PS   Disclosure; Fig 21A-C; 104pp; English.
XX
CC   The invention provides a polynucleotide, which encodes an immunoglobulin
CC   (Ig), comprising a first or second promoter-cistron pair consisting of a
CC   first or second promoter and cistron, respectively. The first cistron of
CC   the first promoter-cistron pair comprises a first translational
CC   initiation region (TIR-L) operably linked to a nucleic acid sequence
CC   encoding an Ig light chain and the second cistron of the second promoter-
CC   cistron pair comprises a second translational initiation region (TIR-H)
CC   operably linked to a nucleic acid sequence encoding an Ig heavy chain.
CC   Upon expression of the polynucleotide in a prokaryotic host cell, light
CC   and heavy chains are folded and assembled to form a biologically active
CC   Ig. The antibody of the invention is useful for diagnosing, treating or
CC   preventing diseases or conditions associated with abnormal expression and
CC   /or activity of one or more antigen molecules e.g. lymphoid malignancies,
CC   inflammatory, angiogenic, immunologic, neuronal, glial, astrocytal,
CC   hypothalamic or other glandular disorders. The present sequence
CC   represents the amino acid sequence of an anti-vascular endothelial growth
CC   factor (VEGF) heavy chain fragment of the cistron vector pxVG2AP11
XX
SQ   Sequence 476 AA;

  Query Match             100.0%;  Score 658;  DB 5;  Length 476;
  Best Local Similarity   100.0%;  Pred. No. 1.2e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141


RESULT 10
ADO14129
ID   ADO14129 standard; protein; 476 AA.
XX
AC   ADO14129;
XX
DT   12-AUG-2004  (first entry)
XX
DE   Plasmid pxVG2AP11 expression cassette heavy chain protein SEQ ID NO:9.
XX
KW   antibody; variant heavy chain hinge region; immunoconjugate; cytostatic;
KW   immunosuppressive; immunotherapy; tumour; cancer; immune disorder;
KW   expression cassette; plasmid pxVG2AP11; anti-VEGF heavy chain.
XX
OS   Synthetic.
XX
PN   WO2004042017-A2.
XX
PD   21-MAY-2004.
XX
PF   30-OCT-2003; 2003WO-US034610.
XX
PR   31-OCT-2002; 2002US-0422952P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Reilly D,  Yansura DG;
XX
DR   WPI; 2004-390607/36.
DR   N-PSDB; ADO14127.
XX
PT   New antibody comprising a variant heavy chain hinge region incapable of
PT   inter-heavy chain disulfide linkage, useful for treating, preventing,
PT   diagnosing, delaying or preventing a disease, e.g. tumor, cancer or
PT   immune disorder.
XX
PS   Example 1; SEQ ID NO 9; 124pp; English.
XX
CC   The present invention describes an antibody comprising a variant heavy
CC   chain hinge region incapable of inter-heavy chain disulfide linkage. Also
CC   described: (1) an antibody lacking inter-heavy chain disulfide linkage;
CC   (2) an immunoconjugate comprising the antibody conjugated with a
CC   heterologous moiety; (3) a composition comprising the antibody or
CC   immunoconjugate, and carrier; (4) an article of manufacture comprising
CC   the composition in a container; (5) a polynucleotide encoding the
CC   antibody or immunoconjugate, or a variant immunoglobulin heavy chain
CC   incapable of inter-heavy chain disulfide linkage; (6) a recombinant
CC   vector for expressing the antibody or immunoconjugate; (7) a host cell
CC   comprising the recombinant vector; (8) expressing in a host cell an
CC   antibody of interest in which at least one inter-heavy chain disulfide
CC   linkage is eliminated, and recovering the antibody from the host cell;
CC   (9) an aglycosylated antibody produced by the method; and (10) treating,
CC   preventing, diagnosing, delaying or preventing a disease in a subject.
CC   The antibody has cytostatic and immunosuppressive activities, and can be
CC   used in immunotherapy. The antibody, immunoconjugate and methods are
CC   useful for treating, preventing, diagnosing, delaying or preventing a
CC   disease, e.g. tumour, cancer or immune disorder. The present sequence
CC   represents the anti-VEGF heavy chain from the expression cassette of
CC   plasmid pxVG2AP11, which is used in the exemplification of the present
CC   invention.
XX
SQ   Sequence 476 AA;

  Query Match             100.0%;  Score 658;  DB 8;  Length 476;
  Best Local Similarity   100.0%;  Pred. No. 1.2e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141


RESULT 11
ADQ90730
ID   ADQ90730 standard; protein; 476 AA.
XX
AC   ADQ90730;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody YO317 heavy chain protein SEQ ID NO:7.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; heavy chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
```

XX
PD 05-AUG-2004.
XX
PF 23-JAN-2004; 2004WO-US001844.
XX
PR 23-JAN-2003; 2003US-0442484P.
XX
PA (GETH) GENENTECH INC.
XX
PI Simmons L;
XX
DR WPI; 2004-562149/54.
DR N-PSDB; ADQ90702.
XX
PT Producing an antibody or antigen binding fragment in high yield in a cell
PT culture, comprises expressing a variable domain with a modified framework
PT region in a host cell.
XX
PS Example 2; SEQ ID NO 7; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the heavy chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 476 AA;

Query Match 100.0%; Score 658; DB 8; Length 476;
Best Local Similarity 100.0%; Pred. No. 1.2e-55;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141
```

RESULT 12
ADF09953
ID ADF09953 standard; protein; 117 AA.
XX
AC ADF09953;
XX
DT 12-FEB-2004 (first entry)
XX
DE Antibody heavy chain variable region 1CZ8(7-4-1).
XX
KW Antibody; stability; solubility; antigen binding affinity;
KW variable region; human.
XX
OS Homo sapiens.
XX
PN WO2003074679-A2.
XX
PD 12-SEP-2003.
XX
PF 03-MAR-2003; 2003WO-US006598.
XX
PR 01-MAR-2002; 2002US-0360843P.
PR 29-MAY-2002; 2002US-0384197P.
XX
PA (XENC-) XENCOR.
XX
PI Lazar GA, Desjarlais JR, Marshall SA, Dahiyat B;
XX
DR WPI; 2003-722066/68.
XX
PT Computer optimization of physicochemical properties of antibodies
PT comprises analyzing the interactions of amino acids at variable
PT positions.
XX
PS Disclosure; Fig 2a; 135pp; English.
XX
CC The present invention relates to a method for optimizing at least one
CC physico-chemical property of an antibody by a computational screening
CC method. The method comprises: receiving a template antibody structure;
CC selecting at least one variable position belonging to the antibody
CC structure; selecting at least one amino acid to be considered at the
CC variable position(s); analyzing the interaction of each selected amino
CC acid at each variable position with at least part of the remainder of the
CC antibody, including the selected amino acids at other variable positions;
CC and identifying a set of at least one antibody sequence with at least one
CC optimized physico-chemical property. The method is useful for optimizing
CC the physico-chemical properties of an antibody, especially the stability,
CC solubility, or antigen binding affinity. The optimized antibody may be
CC useful for treating a patient. The present sequence is an antibody
CC variable region sequence used to illustrate the invention.
XX
SQ Sequence 117 AA;

Query Match 99.4%; Score 654; DB 7; Length 117;
Best Local Similarity 100.0%; Pred. No. 6.3e-56;
Matches 117; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
```

RESULT 13
ADF10058
ID ADF10058 standard; protein; 117 AA.
XX
AC ADF10058;
XX
DT 12-FEB-2004 (first entry)
XX
DE VEGF antibody heavy chain variable region 1CZ8.
XX
KW Antibody; stability; solubility; antigen binding affinity;
KW variable region; human; VEGF.
XX
OS Homo sapiens.
XX
PN WO2003074679-A2.
XX
PD 12-SEP-2003.

XX
PF 03-MAR-2003; 2003WO-US006598.
XX
PR 01-MAR-2002; 2002US-0360843P.
PR 29-MAY-2002; 2002US-0384197P.
XX
PA (XENC-) XENCOR.
XX
PI Lazar GA, Desjarlais JR, Marshall SA, Dahiyat B;
XX
DR WPI; 2003-722066/68.
XX
PT Computer optimization of physicochemical properties of antibodies
PT comprises analyzing the interactions of amino acids at variable
PT positions.
XX
PS Example 6; Fig 16a; 135pp; English.
XX
CC The present invention relates to a method for optimizing at least one
CC physico-chemical property of an antibody by a computational screening
CC method. The method comprises: receiving a template antibody structure;
CC selecting at least one variable position belonging to the antibody
CC structure; selecting at least one amino acid to be considered at the
CC variable position(s); analyzing the interaction of each selected amino
CC acid at each variable position with at least part of the remainder of the
CC antibody, including the selected amino acids at other variable positions;
CC and identifying a set of at least one antibody sequence with at least one
CC optimized physico-chemical property. The method is useful for optimizing
CC the physico-chemical properties of an antibody, especially the stability,
CC solubility, or antigen binding affinity. The optimized antibody may be
CC useful for treating a patient. The present sequence is an antibody
CC variable region sequence used to illustrate the invention.
XX
SQ Sequence 117 AA;

Query Match 99.4%; Score 654; DB 7; Length 117;
Best Local Similarity 100.0%; Pred. No. 6.3e-56;
Matches 117; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117

RESULT 14
AAW70682
ID AAW70682 standard; peptide; 118 AA.
XX
AC AAW70682;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable heavy domain of variant Y0243-1.
XX
KW Heavy variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 10B; 100pp; English.
XX
CC The present sequence represents a variable heavy domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders.
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC also be used to treat other conditions that involve angiogenesis, e.g.
CC rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ Sequence 118 AA;

Query Match 98.6%; Score 649; DB 2; Length 118;
Best Local Similarity 98.3%; Pred. No. 2e-55;
Matches 116; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118

RESULT 15
AAB05900
ID AAB05900 standard; peptide; 118 AA.
XX
AC AAB05900;
XX
DT 17-OCT-2000 (first entry)
XX
DE F(ab)-12 antibody variant Y0238-3 heavy chain variable domain.
XX
KW Humanised; F(ab)-12; heavy chain variable domain; antibody variant;
KW phage display; randomised library; cytostatic; antiarthritic;
KW antipsoriatic; antidiabetic; antiinflammatory; antiarteriosclerotic;
KW vascular endothelial growth factor; VEGF; breast cancer; lung cancer;
KW retinoblastoma; rheumatoid arthritis; psoriasis; atherosclerosis;
KW diabetic retinopathy; complementarity determining region; CDR.
XX
OS Homo sapiens.
OS Synthetic.
XX
PN WO200029584-A1.
XX
PD 25-MAY-2000.
XX
PF 16-NOV-1999; 99WO-US027153.
XX
PR 18-NOV-1998; 98US-0108945P.
XX
PA (GETH) GENENTECH INC.
XX
PI Chen YM, Lowman HB, Muller Y;
XX

DR WPI; 2000-387797/33.
XX
PT Antibody variants with higher binding affinity than native antibodies
PT useful for diagnosis, prevention and treatment of neoplastic and non-
PT neoplastic diseases comprises amino acid insertion in hypervariable
PT region.
XX
PS Disclosure; Fig 1B; 110pp; English.
XX
CC The present sequence is the heavy chain variable domain of the F(ab)-12
CC antibody variant Y0238-3. F(ab)-12 is a humanised anti-vascular
CC endothelial growth factor (VEGF) antibody. F(ab)-12 was the parent
CC antibody used in the production of a large number of antibody variants
CC containing randomised peptide inserts within the complementarity
CC determining regions (CDRs). Phage display libraries were subjected to
CC eight rounds of selection to isolate variants with an antigen binding
CC affinity at least two-fold stronger than the binding affinity of parent
CC antibody for the target VEGF antibody. The anti-VEGF antibody variants
CC may be useful in diagnostic assays for detecting expression of VEGF in
CC cells, tissue or serum. They may also be used in the prevention and
CC treatment of neoplastic diseases such as breast cancer, lung cancer and
CC retinoblastoma, and non-neoplastic diseases including rheumatoid
CC arthritis, psoriasis, atherosclerosis, and diabetic and other
CC proliferative retinopathies
XX
SQ Sequence 118 AA;

Query Match 98.6%; Score 649; DB 3; Length 118;
Best Local Similarity 98.3%; Pred. No. 2e-55;
Matches 116; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||:|||:||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
```

Search completed: March 14, 2005, 20:39:17
Job time : 95.4518 secs

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:30:13 ; Search time 24.3246 Seconds
(without alignments)
362.127 Million cell updates/sec

Title: US-09-723-752B-116
Perfect score: 658
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPYYYGTSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 513545 seqs, 74649064 residues

Total number of hits satisfying chosen parameters: 513545

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Issued_Patents_AA:*
1: /cgn2_6/ptodata/1/iaa/5A_COMB.pep:*
2: /cgn2_6/ptodata/1/iaa/5B_COMB.pep:*
3: /cgn2_6/ptodata/1/iaa/6A_COMB.pep:*
4: /cgn2_6/ptodata/1/iaa/6B_COMB.pep:*
5: /cgn2_6/ptodata/1/iaa/PCTUS_COMB.pep:*
6: /cgn2_6/ptodata/1/iaa/backfiles1.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	649	98.6	118	4	US-09-440-781-97	Sequence 97, Appl
2	635	96.5	118	4	US-09-440-781-96	Sequence 96, Appl
3	627.5	95.4	121	4	US-09-440-781-99	Sequence 99, Appl
4	613.5	93.2	121	4	US-09-440-781-98	Sequence 98, Appl
5	611	92.9	491	4	US-10-011-125A-2	Sequence 2, Appli
6	507.5	77.1	118	1	US-08-425-336-126	Sequence 126, App
7	507.5	77.1	118	1	US-08-488-113B-126	Sequence 126, App
8	507.5	77.1	118	1	US-08-477-484B-126	Sequence 126, App
9	507.5	77.1	118	2	US-08-646-360-126	Sequence 126, App
10	507.5	77.1	118	3	US-08-839-765-126	Sequence 126, App
11	507.5	77.1	118	3	US-09-136-389-126	Sequence 126, App
12	507.5	77.1	118	3	US-09-610-838-126	Sequence 126, App
13	507.5	77.1	118	4	US-09-711-485-126	Sequence 126, App
14	507.5	77.1	240	1	US-08-488-113B-147	Sequence 147, App
15	507.5	77.1	240	1	US-08-488-113B-148	Sequence 148, App
16	507.5	77.1	240	1	US-08-477-484B-147	Sequence 147, App
17	507.5	77.1	240	1	US-08-477-484B-148	Sequence 148, App
18	507.5	77.1	240	2	US-08-646-360-147	Sequence 147, App
19	507.5	77.1	240	2	US-08-646-360-148	Sequence 148, App
20	507.5	77.1	240	3	US-08-839-765-147	Sequence 147, App
21	507.5	77.1	240	3	US-08-839-765-148	Sequence 148, App
22	507.5	77.1	240	3	US-09-136-389-147	Sequence 147, App
23	507.5	77.1	240	3	US-09-136-389-148	Sequence 148, App
24	507.5	77.1	240	3	US-09-610-838-147	Sequence 147, App
25	507.5	77.1	240	3	US-09-610-838-148	Sequence 148, App
26	507.5	77.1	240	4	US-09-711-485-147	Sequence 147, App
27	507.5	77.1	240	4	US-09-711-485-148	Sequence 148, App
28	493.5	75.0	118	1	US-08-107-669D-29	Sequence 29, Appl
29	493.5	75.0	118	1	US-08-472-788A-29	Sequence 29, Appl
30	493.5	75.0	118	2	US-08-477-531B-29	Sequence 29, Appl
31	493.5	75.0	118	2	US-08-082-842A-29	Sequence 29, Appl
32	492.5	74.8	118	1	US-08-107-669D-67	Sequence 67, Appl
33	492.5	74.8	118	1	US-08-472-788A-89	Sequence 89, Appl
34	492.5	74.8	118	2	US-08-477-531B-67	Sequence 67, Appl
35	492.5	74.8	118	2	US-08-082-842A-89	Sequence 89, Appl
36	491.5	74.7	122	2	US-07-934-373C-20	Sequence 20, Appl
37	491.5	74.7	122	3	US-08-437-642B-20	Sequence 20, Appl
38	491.5	74.7	122	4	US-08-146-206C-20	Sequence 20, Appl
39	491.5	74.7	122	4	US-09-705-686-20	Sequence 20, Appl
40	491.5	74.7	122	4	US-09-705-392A-20	Sequence 20, Appl
41	491.5	74.7	122	4	US-09-705-398-20	Sequence 20, Appl
42	491.5	74.7	122	5	PCT-US93-07832-20	Sequence 20, Appl
43	490.5	74.5	122	2	US-07-934-373C-45	Sequence 45, Appl
44	490.5	74.5	122	3	US-08-437-642B-45	Sequence 45, Appl
45	490.5	74.5	122	4	US-08-146-206C-26	Sequence 26, Appl

ALIGNMENTS

RESULT 1
US-09-440-781-97
; Sequence 97, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
; APPLICANT: Yvonne Man-yee Chen et al.
; TITLE OF INVENTION: ANTIBODY VARIANTS
; FILE REFERENCE: P1469R1
; CURRENT APPLICATION NUMBER: US/09/440,781
; CURRENT FILING DATE: 1999-11-16
; NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 97
; LENGTH: 118
; TYPE: PRT
; ORGANISM: artificial sequence
; FEATURE:
; NAME/KEY: artificial
; LOCATION: 1-118
; OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-97

Query Match 98.6%; Score 649; DB 4; Length 118;
Best Local Similarity 98.3%; Pred. No. 7.8e-59;
Matches 116; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||:|||:||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
```

RESULT 2
US-09-440-781-96
; Sequence 96, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
; APPLICANT: Yvonne Man-yee Chen et al.
; TITLE OF INVENTION: ANTIBODY VARIANTS
; FILE REFERENCE: P1469R1
; CURRENT APPLICATION NUMBER: US/09/440,781
; CURRENT FILING DATE: 1999-11-16
; NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 96
; LENGTH: 118
; TYPE: PRT
; ORGANISM: artificial sequence
; FEATURE:

```
;   NAME/KEY: artificial
;   LOCATION: 1-118
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-96

  Query Match             96.5%;  Score 635;  DB 4;  Length 118;
  Best Local Similarity   96.6%;  Pred. No. 2.1e-57;
  Matches  114;  Conservative    3;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||| ||:||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||:|||:||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 3
US-09-440-781-99
; Sequence 99, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 99
;   LENGTH: 121
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-121
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-99

  Query Match             95.4%;  Score 627.5;  DB 4;  Length 121;
  Best Local Similarity   95.0%;  Pred. No. 1.3e-56;
  Matches  115;  Conservative    1;  Mismatches    2;  Indels    3;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYY---GTSHWYFDVWGQGT 117
              ||||||||||||||||||||||||||||||||||||||||:||     ||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYVNERKSHWYFDVWGQGT 120

Qy        118 L 118
              |
Db        121 L 121


RESULT 4
US-09-440-781-98
; Sequence 98, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 98
;   LENGTH: 121
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-121
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-98

  Query Match             93.2%;  Score 613.5;  DB 4;  Length 121;
  Best Local Similarity   93.4%;  Pred. No. 3.4e-55;
  Matches  113;  Conservative    2;  Mismatches    3;  Indels    3;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||| ||:||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYY---GTSHWYFDVWGQGT 117
              ||||||||||||||||||||||||||||||||||||||||:||     ||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYVNERKSHWYFDVWGQGT 120

Qy        118 L 118
              |
Db        121 L 121


RESULT 5
US-10-011-125A-2
; Sequence 2, Application US/10011125A
; Patent No. 6828121
; GENERAL INFORMATION:
;  APPLICANT: Chen, Christina Yu-Ching
;  TITLE OF INVENTION: BACTERIAL HOST STRAINS
;  FILE REFERENCE: P1804R1
;  CURRENT APPLICATION NUMBER: US/10/011,125A
;  CURRENT FILING DATE:  2001-12-07
;  PRIOR APPLICATION NUMBER: US 60/256,162
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 12
; SEQ ID NO 2
;   LENGTH: 491
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: Sequence is synthesized.
; Patent No. 6828121
US-10-011-125A-2

  Query Match             92.9%;  Score 611;  DB 4;  Length 491;
  Best Local Similarity   91.5%;  Pred. No. 3.2e-54;
  Matches  108;  Conservative    5;  Mismatches    5;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||| ||:|||||:|||||||||||||||||||||||
Db        261 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWIRQAPGKGLEWVGWINTYTGEPTY 320

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |||||||| | ||| :| ||||||||||||||||||:|||:||||||||||||||
Db        321 AADFKRRFTISADTSSNTVYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 378


RESULT 6
US-08-425-336-126
; Sequence 126, Application US/08425336
; Patent No. 5621083
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribsome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  140
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Marshall, O'Toole, Gerstein, Murray & Borun
;      STREET:  6300 Sears Tower, 233 South Wacker Drive
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
```

```
;      ZIP:  60606-6402
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/425,336
;      FILING DATE:  18-APR-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/064,691
;      FILING DATE:  12-MAY-1993
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Meyers, Thomas C.
;      REGISTRATION NUMBER:  P-36,989
;      REFERENCE/DOCKET NUMBER:  31394
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/474-6300
;      TELEFAX:  312/474-0448
;      TELEX:  25-3856
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-425-336-126

  Query Match             77.1%;  Score 507.5;  DB 1;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
                |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 7
US-08-488-113B-126
; Sequence 126, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-126

  Query Match             77.1%;  Score 507.5;  DB 1;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
                |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 8
US-08-477-484B-126
; Sequence 126, Application US/08477484B
; Patent No. 5756699
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/477,484B
;      FILING DATE:  07-JUN-1995
```

```
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-477-484B-126

  Query Match             77.1%;  Score 507.5;  DB 1;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  |       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 9
US-08-646-360-126
; Sequence 126, Application US/08646360
; Patent No. 5837491
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/646,360
;      FILING DATE:  13-MAY-1996
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-646-360-126

  Query Match             77.1%;  Score 507.5;  DB 2;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  |       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 10
US-08-839-765-126
; Sequence 126, Application US/08839765
; Patent No. 6146631
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/839,765
;      FILING DATE:  15-APR-1997
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
```

```
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US09/200-70.P3.C3
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-839-765-126

  Query Match             77.1%;  Score 507.5;  DB 3;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:|||||||  ||:|||||||||||||||:|:||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || |||||| ||:|||||:||||||||||:|  :  |     ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 11
US-09-136-389-126
; Sequence 126, Application US/09136389
; Patent No. 6146850
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/136,389
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/646,360
;      FILING DATE:  13-MAY-1996
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-136-389-126

  Query Match             77.1%;  Score 507.5;  DB 3;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:|||||||  ||:|||||||||||||||:|:||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || |||||| ||:|||||:||||||||||:|  :  |     ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 12
US-09-610-838-126
; Sequence 126, Application US/09610838
; Patent No. 6376217
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/610,838
;      FILING DATE:  06-JUL-2000
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
```

```
;      APPLICATION NUMBER:  US/09/136,389
;      FILING DATE:  18-AUG-1998
;      APPLICATION NUMBER:  08/646,360
;      FILING DATE:  13-MAY-1996
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-610-838-126

  Query Match             77.1%;  Score 507.5;  DB 3;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:|||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
                |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 13
US-09-711-485-126
; Sequence 126, Application US/09711485
; Patent No. 6649742
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/711,485
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/839,765
;      FILING DATE:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US09/200-70.P3.C3
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-711-485-126

  Query Match             77.1%;  Score 507.5;  DB 4;  Length 118;
  Best Local Similarity   77.8%;  Pred. No. 2.1e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:|||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
                |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 14
US-08-488-113B-147
; Sequence 147, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
```

```
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  147:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  240 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-147

  Query Match             77.1%;  Score 507.5;  DB 1;  Length 240;
  Best Local Similarity   77.8%;  Pred. No. 4.8e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db        123 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 182

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db        183 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 234


RESULT 15
US-08-488-113B-148
; Sequence 148, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  148:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  240 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-148

  Query Match             77.1%;  Score 507.5;  DB 1;  Length 240;
  Best Local Similarity   77.8%;  Pred. No. 4.8e-44;
  Matches   91;  Conservative   12;  Mismatches    9;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||| ||:||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  |      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


Search completed: March 14, 2005, 20:43:53
Job time : 25.3246 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:22:02 ; Search time 43.9912 Seconds
(without alignments)
884.760 Million cell updates/sec

Title: US-09-723-752B-116
Perfect score: 658
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPYYYGTSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1396920 seqs, 329844858 residues

Total number of hits satisfying chosen parameters: 1396920

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA:*
1: /cgn2_6/ptodata/2/pubpaa/US07_PUBCOMB.pep:*
2: /cgn2_6/ptodata/2/pubpaa/PCT_NEW_PUB.pep:*
3: /cgn2_6/ptodata/2/pubpaa/US06_NEW_PUB.pep:*
4: /cgn2_6/ptodata/2/pubpaa/US06_PUBCOMB.pep:*
5: /cgn2_6/ptodata/2/pubpaa/US07_NEW_PUB.pep:*
6: /cgn2_6/ptodata/2/pubpaa/PCTUS_PUBCOMB.pep:*
7: /cgn2_6/ptodata/2/pubpaa/US08_NEW_PUB.pep:*
8: /cgn2_6/ptodata/2/pubpaa/US08_PUBCOMB.pep:*
9: /cgn2_6/ptodata/2/pubpaa/US09A_PUBCOMB.pep:*
10: /cgn2_6/ptodata/2/pubpaa/US09B_PUBCOMB.pep:*
11: /cgn2_6/ptodata/2/pubpaa/US09C_PUBCOMB.pep:*
12: /cgn2_6/ptodata/2/pubpaa/US09_NEW_PUB.pep:*
13: /cgn2_6/ptodata/2/pubpaa/US10A_PUBCOMB.pep:*
14: /cgn2_6/ptodata/2/pubpaa/US10B_PUBCOMB.pep:*
15: /cgn2_6/ptodata/2/pubpaa/US10C_PUBCOMB.pep:*
16: /cgn2_6/ptodata/2/pubpaa/US10D_PUBCOMB.pep:*
17: /cgn2_6/ptodata/2/pubpaa/US10_NEW_PUB.pep:*
18: /cgn2_6/ptodata/2/pubpaa/US11_NEW_PUB.pep:*
19: /cgn2_6/ptodata/2/pubpaa/US60_NEW_PUB.pep:*
20: /cgn2_6/ptodata/2/pubpaa/US60_PUBCOMB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	658	100.0	118	9	US-09-056-160B-116	Sequence 116, App
2	658	100.0	118	9	US-09-056-160B-118	Sequence 118, App
3	658	100.0	118	14	US-10-234-671-114	Sequence 114, App
4	658	100.0	118	14	US-10-234-671-116	Sequence 116, App
5	658	100.0	123	13	US-10-153-159-3	Sequence 3, Appli
6	658	100.0	123	14	US-10-153-176-3	Sequence 3, Appli
7	658	100.0	123	15	US-10-443-134A-3	Sequence 3, Appli
8	658	100.0	123	16	US-10-723-434-56	Sequence 56, Appl
9	658	100.0	224	16	US-10-379-392-136	Sequence 136, App
10	658	100.0	224	16	US-10-379-392-138	Sequence 138, App
11	658	100.0	224	16	US-10-379-392-140	Sequence 140, App
12	658	100.0	476	14	US-10-020-786-11	Sequence 11, Appl
13	658	100.0	476	17	US-10-697-995-9	Sequence 9, Appli
14	654	99.4	117	16	US-10-379-392-58	Sequence 58, Appl
15	653	99.2	224	16	US-10-379-392-148	Sequence 148, App
16	650	98.8	117	16	US-10-379-392-122	Sequence 122, App
17	649	98.6	118	9	US-09-056-160B-112	Sequence 112, App
18	649	98.6	118	14	US-10-234-671-110	Sequence 110, App
19	649	98.6	118	15	US-10-624-153-97	Sequence 97, Appl
20	648	98.5	224	16	US-10-379-392-156	Sequence 156, App
21	645	98.0	117	16	US-10-379-392-124	Sequence 124, App
22	645	98.0	231	15	US-10-364-953-9	Sequence 9, Appli
23	644	97.9	123	16	US-10-723-434-103	Sequence 103, App
24	641	97.4	117	16	US-10-379-392-130	Sequence 130, App
25	640	97.3	118	9	US-09-056-160B-114	Sequence 114, App
26	640	97.3	118	14	US-10-234-671-112	Sequence 112, App
27	640	97.3	231	15	US-10-364-953-5	Sequence 5, Appli
28	638	97.0	123	15	US-10-443-134A-128	Sequence 128, App
29	638	97.0	123	16	US-10-723-434-105	Sequence 105, App
30	635	96.5	118	9	US-09-056-160B-108	Sequence 108, App
31	635	96.5	118	14	US-10-234-671-7	Sequence 7, Appli
32	635	96.5	118	14	US-10-234-671-106	Sequence 106, App
33	635	96.5	118	15	US-10-624-153-96	Sequence 96, Appl
34	635	96.5	123	9	US-09-056-160B-7	Sequence 7, Appli
35	635	96.5	123	13	US-10-153-159-1	Sequence 1, Appli
36	635	96.5	123	13	US-10-153-159-14	Sequence 14, Appl
37	635	96.5	123	14	US-10-153-176-1	Sequence 1, Appli
38	635	96.5	123	14	US-10-153-176-14	Sequence 14, Appl
39	635	96.5	123	15	US-10-443-134A-1	Sequence 1, Appli
40	635	96.5	123	15	US-10-443-134A-14	Sequence 14, Appl
41	635	96.5	123	16	US-10-723-434-55	Sequence 55, Appl
42	635	96.5	123	17	US-10-877-532-8	Sequence 8, Appli
43	635	96.5	231	15	US-10-364-953-2	Sequence 2, Appli
44	633	96.2	123	15	US-10-443-134A-126	Sequence 126, App
45	633	96.2	123	16	US-10-723-434-104	Sequence 104, App

ALIGNMENTS

RESULT 1
US-09-056-160B-116
; Sequence 116, Application US/09056160B
; Patent No. US20020032315A1
; GENERAL INFORMATION:
; APPLICANT: Baca, Manuel
; APPLICANT: Wells, James A.
; APPLICANT: Presta, Leonard G.
; APPLICANT: Lowman, Henry B.
; APPLICANT: Chen, Yvonne M.
; TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
; NUMBER OF SEQUENCES: 131
; CORRESPONDENCE ADDRESS:
; ADDRESSEE: Genentech, Inc.
; STREET: 1 DNA Way
; CITY: South San Francisco
; STATE: California
; COUNTRY: USA
; ZIP: 94080
; COMPUTER READABLE FORM:
; MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
; COMPUTER: IBM PC compatible
; OPERATING SYSTEM: PC-DOS/MS-DOS
; SOFTWARE: WinPatin (Genentech)
; CURRENT APPLICATION DATA:
; APPLICATION NUMBER: US/09/056,160B
; FILING DATE: 06-Apr-1998
; CLASSIFICATION: 424
; PRIOR APPLICATION DATA:
; APPLICATION NUMBER: 60/054,856
; FILING DATE: 06-AUG-1997
; ATTORNEY/AGENT INFORMATION:
; NAME: Hasak, Janet E.
; REGISTRATION NUMBER: 28,616
; REFERENCE/DOCKET NUMBER: P1093R2
; TELECOMMUNICATION INFORMATION:

```
;       TELEPHONE:  650/225-1896
;       TELEFAX:  650/952-9881
;   INFORMATION FOR SEQ ID NO:  116:
;     SEQUENCE CHARACTERISTICS:
;       LENGTH:  118 amino acids
;       TYPE:  Amino Acid
;       TOPOLOGY:  Linear
US-09-056-160B-116

  Query Match             100.0%;  Score 658;  DB 9;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.3e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 2
US-09-056-160B-118
; Sequence 118, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  118:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-118

  Query Match             100.0%;  Score 658;  DB 9;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.3e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 3
US-10-234-671-114
; Sequence 114, Application US/10234671
; Publication No. US20030190317A1
;  GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;  INFORMATION FOR SEQ ID NO: 114:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 118 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 114:
US-10-234-671-114

  Query Match             100.0%;  Score 658;  DB 14;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.3e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 4
US-10-234-671-116
; Sequence 116, Application US/10234671
```

```
; Publication No. US20030190317A1
;  GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 116:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 118 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 116:
US-10-234-671-116

  Query Match             100.0%;  Score 658;  DB 14;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.3e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 5
US-10-153-159-3
; Sequence 3, Application US/10153159
; Publication No. US20020177170A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  TITLE OF INVENTION: STRUCTURE-BASED SELECTION AND AFFINITY MATURATION OF ANTIBODY LIB
;  TITLE OF INVENTION:  SILICO
;  FILE REFERENCE: 26050-704
;  CURRENT APPLICATION NUMBER: US/10/153,159
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 3
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of matured anti-VEGF antibody
US-10-153-159-3

  Query Match             100.0%;  Score 658;  DB 13;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.4e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 6
US-10-153-176-3
; Sequence 3, Application US/10153176
; Publication No. US20030022240A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  APPLICANT:  Cao, Yicheng
;  APPLICANT:  Li, Shengfeng
;  APPLICANT:  Liu, Shengjiang
;  TITLE OF INVENTION: GENERATION AND AFFINITY MATURATION OF ANTIBODY LIBRARY IN SILICO
;  FILE REFERENCE: 26050-701
;  CURRENT APPLICATION NUMBER: US/10/153,176
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 3
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of matured anti-VEGF antibody
US-10-153-176-3

  Query Match             100.0%;  Score 658;  DB 14;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.4e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 7
US-10-443-134A-3
```

; Sequence 3, Application US/10443134A
; Publication No. US20040010376A1
; GENERAL INFORMATION:
; APPLICANT: Luo, Peizhi
; APPLICANT: Hsieh, Mark
; APPLICANT: Zhong, Pingyu
; APPLICANT: Wang, Caili
; APPLICANT: Cao, Yicheng
; APPLICANT: Liu, Shengjiang
; TITLE OF INVENTION: GENERATION AND SELECTION OF PROTEIN LIBRARY IN SILICO
; FILE REFERENCE: 26050-709
; CURRENT APPLICATION NUMBER: US/10/443,134A
; CURRENT FILING DATE: 2003-05-20
; PRIOR APPLICATION NUMBER: US 10/125,687
; PRIOR FILING DATE: 2002-04-17
; PRIOR APPLICATION NUMBER: US 60/284,407
; PRIOR FILING DATE: 2001-04-17
; PRIOR APPLICATION NUMBER: US 10/153,176
; PRIOR FILING DATE: 2002-05-20
; PRIOR APPLICATION NUMBER: US 10/153,159
; PRIOR FILING DATE: 2002-05-20
; NUMBER OF SEQ ID NOS: 131
; SOFTWARE: PatentIn version 3.1
; SEQ ID NO 3
; LENGTH: 123
; TYPE: PRT
; ORGANISM: Artificial Sequence
; FEATURE:
; OTHER INFORMATION: VH of matured anti-VEGF antibody
US-10-443-134A-3

Query Match 100.0%; Score 658; DB 15; Length 123;
Best Local Similarity 100.0%; Pred. No. 2.4e-47;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118

RESULT 8
US-10-723-434-56
; Sequence 56, Application US/10723434
; Publication No. US20040133357A1
; GENERAL INFORMATION:
; APPLICANT: Zhong, Pingyu
; APPLICANT: Luo, Peizhi
; APPLICANT: Wang, Kevin C.
; APPLICANT: Hsieh, Mark
; APPLICANT: Li, Yan
; TITLE OF INVENTION: HUMANIZED ANTIBODIES AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR
; FILE REFERENCE: 26050-709.501
; CURRENT APPLICATION NUMBER: US/10/723,434
; CURRENT FILING DATE: 2003-11-26
; PRIOR APPLICATION NUMBER: US 60/284,407
; PRIOR FILING DATE: 2001-04-17
; PRIOR APPLICATION NUMBER: US 10/125,687
; PRIOR FILING DATE: 2002-04-17
; PRIOR APPLICATION NUMBER: US 10/153,176
; PRIOR FILING DATE: 2002-05-20
; PRIOR APPLICATION NUMBER: US 10/443,134
; PRIOR FILING DATE: 2003-05-20
; NUMBER OF SEQ ID NOS: 156
; SOFTWARE: PatentIn version 3.1
; SEQ ID NO 56
; LENGTH: 123
; TYPE: PRT
; ORGANISM: Artificial Sequence
; FEATURE:
; OTHER INFORMATION: VH
US-10-723-434-56

Query Match 100.0%; Score 658; DB 16; Length 123;
Best Local Similarity 100.0%; Pred. No. 2.4e-47;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118

RESULT 9
US-10-379-392-136
; Sequence 136, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
; APPLICANT: Lazar, Gregory Alan
; APPLICANT: Desjarlais, John Rudolf
; APPLICANT: Marshall, Shannon Alicia
; APPLICANT: Dahiyat, Bassil I.
; TITLE OF INVENTION: ANTIBODY OPTIMIZATION
; FILE REFERENCE: A-71386-3 463077-236
; CURRENT APPLICATION NUMBER: US/10/379,392
; CURRENT FILING DATE: 2003-03-03
; PRIOR APPLICATION NUMBER: US 60/360,843
; PRIOR FILING DATE: 2002-03-01
; PRIOR APPLICATION NUMBER: US 60/384,197
; PRIOR FILING DATE: 2002-05-29
; NUMBER OF SEQ ID NOS: 184
; SOFTWARE: PatentIn version 3.2
; SEQ ID NO 136
; LENGTH: 224
; TYPE: PRT
; ORGANISM: Unknown
; FEATURE:
; OTHER INFORMATION: Humanized
US-10-379-392-136

Query Match 100.0%; Score 658; DB 16; Length 224;
Best Local Similarity 100.0%; Pred. No. 4.1e-47;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118

RESULT 10
US-10-379-392-138
; Sequence 138, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
; APPLICANT: Lazar, Gregory Alan
; APPLICANT: Desjarlais, John Rudolf
; APPLICANT: Marshall, Shannon Alicia
; APPLICANT: Dahiyat, Bassil I.
; TITLE OF INVENTION: ANTIBODY OPTIMIZATION
; FILE REFERENCE: A-71386-3 463077-236
; CURRENT APPLICATION NUMBER: US/10/379,392
; CURRENT FILING DATE: 2003-03-03
; PRIOR APPLICATION NUMBER: US 60/360,843
; PRIOR FILING DATE: 2002-03-01
; PRIOR APPLICATION NUMBER: US 60/384,197
; PRIOR FILING DATE: 2002-05-29

```
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 138
;   LENGTH: 224
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
US-10-379-392-138

  Query Match            100.0%;  Score 658;  DB 16;  Length 224;
  Best Local Similarity  100.0%;  Pred. No. 4.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 11
US-10-379-392-140
; Sequence 140, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 140
;   LENGTH: 224
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (134)..(134)
;   OTHER INFORMATION: Xaa at position 134 can be Leu or Met
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (189)..(189)
;   OTHER INFORMATION: Xaa at position 189 can be Val, Met, Ala or Ser
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (191)..(191)
;   OTHER INFORMATION: Xaa at position 191 can be Val, Met or Ile
US-10-379-392-140

  Query Match            100.0%;  Score 658;  DB 16;  Length 224;
  Best Local Similarity  100.0%;  Pred. No. 4.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


RESULT 12
US-10-020-786-11
; Sequence 11, Application US/10020786
; Publication No. US20030073164A1
; GENERAL INFORMATION:
;  APPLICANT: Simmons, Laura C.
;  APPLICANT:  Klimowski, Laura
;  APPLICANT:  Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: PROKARYOTICALLY PRODUCED ANTIBODIES AND USES THEREOF
;  FILE REFERENCE: P1793R1
;  CURRENT APPLICATION NUMBER: US/10/020,786
;  CURRENT FILING DATE:  2002-03-26
;  PRIOR APPLICATION NUMBER: US 60/256,164
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 11
; SEQ ID NO 11
;   LENGTH: 476
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF heavy chain
US-10-020-786-11

  Query Match            100.0%;  Score 658;  DB 14;  Length 476;
  Best Local Similarity  100.0%;  Pred. No. 8e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141


RESULT 13
US-10-697-995-9
; Sequence 9, Application US/10697995
; Publication No. US20050048572A1
; GENERAL INFORMATION:
;  APPLICANT: Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR INCREASING ANTIBODY PRODUCTION
;  FILE REFERENCE: 11669.195USU1
;  CURRENT APPLICATION NUMBER: US/10/697,995
;  CURRENT FILING DATE:  2003-10-30
;  PRIOR APPLICATION NUMBER: US 60/422,952
;  PRIOR FILING DATE: 2002-10-31
;  NUMBER OF SEQ ID NOS: 37
; SEQ ID NO 9
;   LENGTH: 476
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF heavy chain
US-10-697-995-9

  Query Match            100.0%;  Score 658;  DB 17;  Length 476;
  Best Local Similarity  100.0%;  Pred. No. 8e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 141
```

```
RESULT 14
US-10-379-392-58
; Sequence 58, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 58
;   LENGTH: 117
;   TYPE: PRT
;   ORGANISM: Unknown
;   FEATURE:
;   OTHER INFORMATION: Humanized
US-10-379-392-58

  Query Match             99.4%;  Score 654;  DB 16;  Length 117;
  Best Local Similarity   100.0%;  Pred. No. 4.8e-47;
  Matches  117;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117


RESULT 15
US-10-379-392-148
; Sequence 148, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 148
;   LENGTH: 224
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (5)..(5)
;   OTHER INFORMATION: Xaa at position 5 can be Val or Arg
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (132)..(132)
;   OTHER INFORMATION: Xaa at position 132 can be Phe or Arg
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (180)..(180)
;   OTHER INFORMATION: Xaa at position 180 can be Leu, Asp, Glu, Asn, Gln, or Arg
US-10-379-392-148

  Query Match             99.2%;  Score 653;  DB 16;  Length 224;
  Best Local Similarity   99.2%;  Pred. No. 1.1e-46;
  Matches  117;  Conservative    0;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLXESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118


Search completed: March 14, 2005, 20:42:13
Job time : 44.9912 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:39:29 ; Search time 17.8553 Seconds
(without alignments)
635.867 Million cell updates/sec

Title: US-09-723-752B-116
Perfect score: 658
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPYYYGTSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 283416 seqs, 96216763 residues

Total number of hits satisfying chosen parameters: 283416

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : PIR_79:*
1: pir1:*
2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	427	64.9	138	2	S31666	Ig heavy chain V r
2	426	64.7	123	2	S31114	Ig heavy chain - h
3	426	64.7	131	2	S26792	Ig heavy chain V r
4	424	64.4	119	2	S31107	Ig heavy chain - h
5	418.5	63.6	120	2	B42848	L6 mAb heavy chain
6	418.5	63.6	128	2	S48797	Ig heavy chain V r
7	418.5	63.6	146	4	S33905	Ig heavy chain pre
8	418	63.5	125	2	S30531	Ig heavy chain V r
9	416	63.2	123	2	S26794	Ig heavy chain V.r
10	416	63.2	142	2	S19245	Ig heavy chain pre
11	415	63.1	127	2	S38489	Ig heavy chain - h
12	415	63.1	140	2	S31588	Ig heavy chain V r
13	414	62.9	140	2	S31686	Ig heavy chain V r
14	413.5	62.8	140	2	S70442	Ig heavy chain pre
15	412	62.6	119	2	A53285	Ig heavy chain V a
16	411.5	62.5	115	2	S19968	Ig heavy chain V r
17	411	62.5	121	2	S19666	Ig heavy chain V r
18	411	62.5	121	2	S31104	Ig heavy chain (su
19	411	62.5	160	2	S05271	Ig heavy chain pre
20	410.5	62.4	147	2	I37780	Ig variable region
21	409.5	62.2	119	2	S37453	Ig mu chain - huma
22	409	62.2	119	2	S31108	Ig heavy chain - h
23	408	62.0	119	2	D36005	Ig heavy chain V r
24	408	62.0	121	2	I55673	Ig heavy chain - h
25	407.5	61.9	114	2	D32967	Ig heavy chain V r
26	407	61.9	135	2	S31598	Ig heavy chain V r
27	407	61.9	143	2	S23624	Ig heavy chain V r
28	406.5	61.8	114	2	C32967	Ig heavy chain V r
29	406.5	61.8	124	2	S20782	Ig heavy chain V r
30	406	61.7	132	2	S31603	Ig heavy chain V r
31	405	61.6	120	1	M3HUBW	Ig heavy chain V-I
32	405	61.6	121	2	S31113	Ig heavy chain - h
33	402.5	61.2	120	2	S48798	Ig heavy chain V r
34	402	61.1	119	2	C36005	Ig heavy chain V r
35	401.5	61.0	124	1	AVMS51	Ig heavy chain V r
36	401.5	61.0	128	2	S26786	Ig heavy chain V r
37	401.5	61.0	141	2	S31669	Ig heavy chain V r
38	401	60.9	117	2	S31109	Ig heavy chain - h
39	401	60.9	117	2	S36259	Ig heavy chain V r
40	400	60.8	123	1	AVMST5	Ig heavy chain VH1
41	399.5	60.7	120	2	S26789	Ig heavy chain V r
42	399.5	60.7	122	2	S31117	Ig heavy chain - h
43	399	60.6	117	2	S32190	Ig heavy chain V r
44	399	60.6	123	2	PL0017	Ig heavy chain V-D
45	399	60.6	136	2	S35759	BHD9D10 protein -

ALIGNMENTS

RESULT 1
S31666
Ig heavy chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 10-Nov-1995 #text_change 23-Jul-1999
C;Accession: S31666
R;Cuisinier, A.M.; Gauthier, L.; Boubli, L.; Fougereau, M.; Tonnelle, C.
submitted to the EMBL Data Library, June 1992
A;Description: Mechanisms that generate human immunoglobulin diversity operate from the
A;Reference number: S31585
A;Accession: S31666
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-138 <CUI>
A;Cross-references: EMBL:Z14202; NID:g30963; PIDN:CAA78571.1; PID:g30964
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

Query Match 64.9%; Score 427; DB 2; Length 138;
Best Local Similarity 70.3%; Pred. No. 1.3e-32;
Matches 83; Conservative 9; Mismatches 22; Indels 4; Gaps 1;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||||: |: | |:||||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||||      | :||||:||:|||
Db         80 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK----ARTGYWYFDLWGRGTL 133
```

RESULT 2
S31114
Ig heavy chain - human
C;Species: Homo sapiens (man)
C;Date: 02-Dec-1993 #sequence_revision 26-May-1995 #text_change 17-Mar-1999
C;Accession: S31114
R;Raaphorst, F.M.; Timmers, E.; Kenter, M.J.H.; van Tol, M.J.D.; Vossen, J.M.; Schuurman
Eur. J. Immunol. 22, 247-251, 1992
A;Title: Restricted utilization of germ-line V(H)3 genes and short diverse third complem
A;Reference number: S31104; MUID:92111633; PMID:1730252
A;Accession: S31114
A;Status: preliminary; nucleic acid sequence not shown; translation not shown
A;Molecule type: mRNA
A;Residues: 1-123 <RAA>
A;Cross-references: EMBL:X62963
A;Note: the nucleotide sequence was submitted to the EMBL Data Library, October 1991
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

```
  Query Match             64.7%;  Score 426;  DB 2;  Length 123;
  Best Local Similarity   70.3%;  Pred. No. 1.5e-32;
  Matches   83;  Conservative    6;  Mismatches   29;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:|||||||||||||||||||||: |: | |:|||||||||||||  |:   |   |
Db          1 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||    |      | || ||||||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKASLYLRFLEWLFDYWGQGTL 118


RESULT 3
S26792
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 20-Jun-2000
C;Accession: S26792
R;Mortari, F.; Newton, J.A.; Wang, J.Y.; Schroeder Jr., H.W.
Eur. J. Immunol. 22, 241-245, 1992
A;Title: The human cord blood antibody repertoire. Frequent usage of the V(H)7 gene fami
A;Reference number: S26786; MUID:92111632; PMID:1730251
A;Accession: S26792
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-131 <MOR>
A;Cross-references: EMBL:X61012; NID:g32804; PIDN:CAA43346.1; PID:g1335131
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             64.7%;  Score 426;  DB 2;  Length 131;
  Best Local Similarity   64.8%;  Pred. No. 1.6e-32;
  Matches   81;  Conservative   15;  Mismatches   21;  Indels    8;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :||||:||  | :|| |:::|| |||| || | |||||||||:||||:||||| || |||
Db          1 QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK----YPYYYGTS----HWYFDV 112
              |  |  || |||||| ||||||::||:||||||||||:    | ||   |    ::| ||
Db         61 AQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDSRGYSYYDFWSGYFYYYYMDV 120

Qy        113 WGQGT 117
              ||:||
Db        121 WGKGT 125


RESULT 4
S31107
Ig heavy chain - human
C;Species: Homo sapiens (man)
C;Date: 02-Dec-1993 #sequence_revision 26-May-1995 #text_change 17-Mar-1999
C;Accession: S31107
R;Raaphorst, F.M.; Timmers, E.; Kenter, M.J.H.; van Tol, M.J.D.; Vossen, J.M.; Schuurman
Eur. J. Immunol. 22, 247-251, 1992
A;Title: Restricted utilization of germ-line V(H)3 genes and short diverse third complem
A;Reference number: S31104; MUID:92111633; PMID:1730252
A;Accession: S31107
A;Status: preliminary; nucleic acid sequence not shown; translation not shown
A;Molecule type: mRNA
A;Residues: 1-119 <RAA>
A;Cross-references: EMBL:X62955
A;Note: the nucleotide sequence was submitted to the EMBL Data Library, October 1991
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             64.4%;  Score 424;  DB 2;  Length 119;
  Best Local Similarity   72.9%;  Pred. No. 2.2e-32;
  Matches   86;  Conservative    6;  Mismatches   22;  Indels    4;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||: |: | |:|||||||||||||  |:   |   |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||    |      | || ||||||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDP---GAS-YYFDYWGQGTL 114


RESULT 5
B42848
L6 mAb heavy chain V region - mouse (fragment)
C;Species: Mus musculus (house mouse)
C;Date: 27-Apr-1993 #sequence_revision 18-Nov-1994 #text_change 21-Jan-2000
C;Accession: B42848; S33903
R;Fell, H.P.; Gayle, M.A.; Yelton, D.; Lipsich, L.; Schieven, G.L.; Marken, J.S.; Aruffo
J. Biol. Chem. 267, 15552-15558, 1992
A;Title: Chimeric L6 anti-tumor antibody. Genomic construction, expression, and characte
A;Reference number: A42848; MUID:92348410; PMID:1639794
A;Accession: B42848
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-120 <FEL>
A;Cross-references: GB:M90690; NID:g195065; PIDN:AAA38146.1; PID:g195066
A;Note: sequence extracted from NCBI backbone (NCBIN:109960, NCBIP:109961)
A;Accession: S33903
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-120 <FE2>
A;Cross-references: EMBL:M90691
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             63.6%;  Score 418.5;  DB 2;  Length 120;
  Best Local Similarity   64.1%;  Pred. No. 7.1e-32;
  Matches   75;  Conservative   20;  Mismatches   19;  Indels    3;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| |||| ||:|||||:|||||||:|:|||||||:|||
Db          1 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              | ||| || |||:||  |||||:|:|: || | |:||::   || |  | | |||||
Db         61 ADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFS--YGNSR-YADYWGQGT 114


RESULT 6
S48797
Ig heavy chain V region (anti-Sm, VH3/Dxp4/JH6) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Sep-1998 #text_change 23-Jul-1999
C;Accession: S48797; S26893
R;Mahmoudi, M.; Edwards, J.; Cairns, E.; Bell, D.
submitted to the EMBL Data Library, October 1994
A;Description: Molecular characterization of natural human anti-Sm autoantibodies.
A;Reference number: S48797
A;Accession: S48797
A;Molecule type: mRNA
A;Residues: 1-128 <MAH>
A;Cross-references: EMBL:Z46379; NID:g587147; PIDN:CAA86512.1; PID:g1340168
R;Tomlinson, I.M.; Walter, G.; Marks, J.D.; Llewelyn, M.B.; Winter, G.
J. Mol. Biol. 227, 776-798, 1992
A;Title: The repertoire of human germline V(H) sequences reveals about fifty groups of
A;Reference number: S26885; MUID:93021117; PMID:1404388
A;Accession: S26893
A;Molecule type: DNA
A;Residues: 1-98 <TOM>
A;Cross-references: EMBL:Z12350; NID:g32922; PIDN:CAA78220.1; PID:g32923
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             63.6%;  Score 418.5;  DB 2;  Length 128;
```

```
  Best Local Similarity   69.1%;  Pred. No. 7.6e-32;
  Matches   85;  Conservative   10;  Mismatches   21;  Indels    7;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||| |||||||||||: |: |||:||||||||||||| |  | |  |
Db          1 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI-WYDGSNKY 59

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYF-----DVWG 114
               ||  | ||| | | ||:| ||||||||||||||||||:  ||| :| :|:     ||||
Db         60 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDNYYYDSSGYYYYYYGMDVWG 119

Qy        115 QGT 117
              |||
Db        120 QGT 122


RESULT 7
S33905
Ig heavy chain precursor V region - synthetic
C;Species: synthetic
C;Date: 13-Jan-1995 #sequence_revision 30-Apr-1998 #text_change 20-Oct-2000
C;Accession: S33905
R;Liu, A.Y.; Robinson, R.R.; Hellstroem, K.E.; Murray Jr., E.D.; Chang, C.P.; Hellstroem
Proc. Natl. Acad. Sci. U.S.A. 84, 3439-3443, 1987
A;Title: Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells.
A;Reference number: S33905; MUID:87204152; PMID:3106970
A;Accession: S33905
A;Molecule type: mRNA
A;Residues: 1-146 <LIU>
A;Cross-references: EMBL:M16072; NID:g195270; PIDN:AAA38229.1; PID:g195271

  Query Match             63.6%;  Score 418.5;  DB 4;  Length 146;
  Best Local Similarity   64.1%;  Pred. No. 8.7e-32;
  Matches   75;  Conservative   20;  Mismatches   19;  Indels    3;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| |||| ||:|||||:|||||||:|:|||||||:|||
Db         20 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              | ||| || |||:||  |||||:|:|: || | |:||::   || |  | | |||||
Db         80 ADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFS--YGNSR-YSDYWGQGT 133


RESULT 8
S30531
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 06-Jan-1995 #sequence_revision 06-Jan-1995 #text_change 09-Jul-2004
C;Accession: S30531
R;Mariette, X.
submitted to the EMBL Data Library, October 1992
A;Reference number: S30520
A;Accession: S30531
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-125 <MAR>
A;Cross-references: UNIPROT:Q9UL91; EMBL:Z18317
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             63.5%;  Score 418;  DB 2;  Length 125;
  Best Local Similarity   66.7%;  Pred. No. 8.2e-32;
  Matches   80;  Conservative   14;  Mismatches   24;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||: |: | ||||||||||||| :|:: :    |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWISYISSSSSTIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHW--YFDVWGQGTL 118
              |    | ||| | | :|:: ||||||||||||||||:    |  : ::  ||| ||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSRNYDSSGYYSHYFDYWGQGTL 120


RESULT 9
S26794
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 17-Mar-1999
C;Accession: S26794
R;Mortari, F.; Newton, J.A.; Wang, J.Y.; Schroeder Jr., H.W.
Eur. J. Immunol. 22, 241-245, 1992
A;Title: The human cord blood antibody repertoire. Frequent usage of the V(H)7 gene fa
A;Reference number: S26786; MUID:92111632; PMID:1730251
A;Accession: S26794
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-123 <MOR>
A;Cross-references: EMBL:X61011
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             63.2%;  Score 416;  DB 2;  Length 123;
  Best Local Similarity   67.5%;  Pred. No. 1.2e-31;
  Matches   79;  Conservative   13;  Mismatches   25;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||||: |: | |||||||||||||| :|:: :    |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSSSSTIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              |   | ||| | | :|:: ||||||||| |||||||||:   ||  :::  |||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARSIKYYDENYYGMDVWGQGT 117


RESULT 10
S19245
Ig heavy chain precursor V region (10P1) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 21-Jul-1995 #text_change 21-Jan-2000
C;Accession: S19245
R;Kirkham, P.M.; Mortari, F.; Newton, J.A.; Schroeder, H.W.
EMBO J. 11, 603-609, 1992
A;Title: Immunoglobulin V(H) clan and family identity predicts variable domain structu
A;Reference number: S19245; MUID:92164649; PMID:1537339
A;Accession: S19245
A;Status: preliminary; translation not shown
A;Molecule type: DNA
A;Residues: 1-142 <KIR>
A;Cross-references: EMBL:X59906; NID:g37791; PIDN:CAA42547.1; PID:g37792
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             63.2%;  Score 416;  DB 2;  Length 142;
  Best Local Similarity   66.1%;  Pred. No. 1.4e-31;
  Matches   78;  Conservative   14;  Mismatches   26;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :||||:|   | :|| |:::|| |||| || | |||||||||:||||:||||| || ||:
Db         20 QVQLVQSASELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTF 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | || ||||||| |||||::|||||||||||||:      |:  :|| ||||||
Db         80 AQGFTGRFVFSLDTSVSTAYLQISSLRAEDTAVYYCARAYTLMMTAVTHFDFWGQGTL 137


RESULT 11
S38489
Ig heavy chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 06-Jan-1995 #sequence_revision 06-Jan-1995 #text_change 23-Jul-1999
C;Accession: S38489
R;Marks, J.D.; Ouwehand, W.H.; Bye, J.M.; Finnern, R.; Gorick, B.D.; Voak, D.; Thorpe,
```

```
submitted to the EMBL Data Library, June 1993
A;Description: Human antibody fragments specific for human blood group antigens from a p
A;Reference number: S38488
A;Accession: S38489
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-127 <MAR>
A;Cross-references: EMBL:Z23028; NID:g414025; PIDN:CAA80563.1; PID:g414026
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             63.1%;  Score 415;  DB 2;  Length 127;
  Best Local Similarity   66.1%;  Pred. No. 1.6e-31;
  Matches   82;  Conservative   12;  Mismatches   24;  Indels    6;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :||||:||||:|||||||||||||||: |: | |:|||||||||||||  |:   |   |
Db          1 QVQLVQSGGGVVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK---YP---YYYGTSHWYFDVWG 114
              |   | ||| | | ||:| ||||||||||||||||||   :|   ||  : :: || ||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKEGPFPASDYYDSSGYYSFDYWG 120

Qy        115 QGTL 118
              ||||
Db        121 QGTL 124


RESULT 12
S31588
Ig heavy chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 10-Nov-1995 #text_change 23-Jul-1999
C;Accession: S31588
R;Cuisinier, A.M.; Gauthier, L.; Boubli, L.; Fougereau, M.; Tonnelle, C.
submitted to the EMBL Data Library, June 1992
A;Description: Mechanisms that generate human immunoglobulin diversity operate from the
A;Reference number: S31585
A;Accession: S31588
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-140 <CUI>
A;Cross-references: EMBL:Z14200; NID:g30957; PIDN:CAA78569.1; PID:g30958
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             63.1%;  Score 415;  DB 2;  Length 140;
  Best Local Similarity   70.3%;  Pred. No. 1.8e-31;
  Matches   83;  Conservative    9;  Mismatches   24;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||: |: | |:|||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||   ::: ||| ||||||
Db         80 ADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCAKDHDY--SNYIYFDYWGQGTL 135


RESULT 13
S31686
Ig heavy chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 10-Nov-1995 #text_change 23-Jul-1999
C;Accession: S31686
R;Cuisinier, A.M.; Gauthier, L.; Boubli, L.; Fougereau, M.; Tonnelle, C.
submitted to the EMBL Data Library, June 1992
A;Description: Mechanisms that generate human immunoglobulin diversity operate from the
A;Reference number: S31585
A;Accession: S31686
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-140 <CUI>
A;Cross-references: EMBL:Z14205; NID:g30969; PIDN:CAA78574.1; PID:g30970
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             62.9%;  Score 414;  DB 2;  Length 140;
  Best Local Similarity   69.5%;  Pred. No. 2.2e-31;
  Matches   82;  Conservative    9;  Mismatches   25;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||: |: | |:|||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              :   | ||| | | ||:| ||||||||||||||||||| |:  |:    || ||||||
Db         80 SDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKCPPAGGSPS--FDYWGQGTL 135


RESULT 14
S70442
Ig heavy chain precursor V region (mu) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 24-Jul-1998 #sequence_revision 24-Jul-1998 #text_change 09-Jul-2004
C;Accession: S70442
R;Cuisinier, A.M.; Fumoux, F.; Fougereau, M.; Tonnelle, C.
Mol. Immunol. 29, 1363-1373, 1992
A;Title: IgM kappa/lambda EBV human B cell clone: an early step of differentiation of f
A;Reference number: S70442; MUID:93024508; PMID:1383695
A;Accession: S70442
A;Status: not compared with conceptual translation
A;Molecule type: mRNA
A;Residues: 1-140 <CUI>
A;Cross-references: UNIPROT:Q8WUK1
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             62.8%;  Score 413.5;  DB 2;  Length 140;
  Best Local Similarity   70.6%;  Pred. No. 2.4e-31;
  Matches   84;  Conservative   11;  Mismatches   19;  Indels    5;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||||||||||||||: |::|||:|||||||||||||| :|  | |   |
Db         20 QVQLVESGGGVVQPGGSLRLSCAASGFTFSNYGMHWVRQAPGKGLEWVAFIR-YDGSNKY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              ||  | ||| | | ||:| ||||||||||||||||||:  :  | :  ||| ||||||
Db         79 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR-DHIVGAT--YFDYWGQGTL 134


RESULT 15
A53285
Ig heavy chain V and J regions, monoclonal antibody SCET.M8.1 - mouse (fragment)
C;Species: Mus musculus (house mouse)
C;Date: 02-May-1994 #sequence_revision 18-Nov-1994 #text_change 20-Jun-2000
C;Accession: A53285
R;Sawada, J.; Mizusawa, S.; Terao, T.; Naito, M.; Kurosawa, Y.
Mol. Immunol. 28, 1063-1072, 1991
A;Title: Molecular characterization of monoclonal anti-steroid antibodies: primary stru
 and their pH-reactivity profiles.
A;Reference number: A53285; MUID:92017897; PMID:1922102
A;Accession: A53285
A;Status: preliminary
A;Molecule type: DNA; protein
A;Residues: 1-119 <SAW>
A;Cross-references: GB:D12736; NID:g220595; PIDN:BAA02228.1; PID:g220596
A;Note: sequence extracted from NCBI backbone (NCBIN:63271, NCBIP:63299)
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             62.6%;  Score 412;  DB 2;  Length 119;
```

```
  Best Local Similarity   64.4%;  Pred. No. 2.8e-31;
  Matches   76;  Conservative   16;  Mismatches   20;  Indels    6;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| |||| ||:||||||:|||||||| ||||||||||
Db          1 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLEWMDWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWY-FDVWGQGT 117
              | ||| || |||:|| ||||||:| |: |||| |:||:     |    |  | |||||
Db         61 ADDFKGRFAFSLETSASTAYLQINDLKNEDTATYFCAR-----GNVRVYAMDYWGQGT 113


Search completed: March 14, 2005, 21:08:52
Job time : 17.8553 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:32:33 ; Search time 82.807 Seconds
(without alignments)
729.713 Million cell updates/sec

Title: US-09-723-752B-116
Perfect score: 658
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPYYYGTSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1612378 seqs, 512079187 residues

Total number of hits satisfying chosen parameters: 1612378

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : UniProt_03:*
1: uniprot_sprot:*
2: uniprot_trembl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	414.5	63.0	475	2	Q6MZQ6	Q6mzq6 homo sapien
2	414	62.9	597	2	Q96BB9	Q96bb9 homo sapien
3	413.5	62.8	241	2	Q921A6	Q921a6 mus musculu
4	406.5	61.8	118	2	Q9UL91	Q9ul91 homo sapien
5	405	61.6	120	1	HV3E_HUMAN	P01766 homo sapien
6	404.5	61.5	122	1	HV21_MOUSE	P01790 mus musculu
7	402.5	61.2	606	2	Q6GMY2	Q6gmy2 homo sapien
8	401.5	61.0	573	2	Q8WU38	Q8wu38 homo sapien
9	400	60.8	123	1	HV18_MOUSE	P01787 mus musculu
10	399	60.6	113	2	Q9UL90	Q9ul90 homo sapien
11	398	60.5	123	1	HV19_MOUSE	P01788 mus musculu
12	398	60.5	472	2	Q6N089	Q6n089 homo sapien
13	396.5	60.3	473	2	Q6MZV7	Q6mzv7 homo sapien
14	395.5	60.1	112	2	Q9HCC1	Q9hcc1 homo sapien
15	395.5	60.1	613	2	Q8WUK1	Q8wuk1 homo sapien
16	394	59.9	478	2	Q6PI81	Q6pi81 homo sapien
17	391	59.4	123	1	HV22_MOUSE	P01791 mus musculu
18	391	59.4	240	2	Q65ZC9	Q65zc9 homo sapien
19	389.5	59.2	544	2	Q6PJ95	Q6pj95 homo sapien
20	389	59.1	123	1	HV23_MOUSE	P01792 mus musculu
21	389	59.1	125	2	Q6PIL0	Q6pil0 homo sapien
22	389	59.1	470	2	Q6PJA4	Q6pja4 homo sapien
23	388.5	59.0	119	1	HV38_MOUSE	P01808 mus musculu
24	387	58.8	121	2	Q9UL71	Q9ul71 homo sapien
25	386	58.7	464	2	Q6MZU6	Q6mzu6 homo sapien
26	384.5	58.4	122	1	HV20_MOUSE	P01789 mus musculu
27	384	58.4	487	2	Q80ZI7	Q80zi7 mus musculu
28	382	58.1	499	2	Q8N5K4	Q8n5k4 homo sapien
29	381	57.9	115	1	HV3D_HUMAN	P01765 homo sapien
30	381	57.9	123	1	HV24_MOUSE	P01793 mus musculu
31	379.5	57.7	119	1	HV37_MOUSE	P01807 mus musculu
32	379.5	57.7	147	2	Q9Y509	Q9y509 homo sapien
33	379.5	57.7	487	2	Q6ZVX0	Q6zvx0 homo sapien
34	378.5	57.5	483	2	Q6MZX9	Q6mzx9 homo sapien
35	378	57.4	493	2	Q6GMX2	Q6gmx2 homo sapien
36	375.5	57.1	122	1	HV3G_HUMAN	P01768 homo sapien
37	375	57.0	123	1	HV25_MOUSE	P01794 mus musculu
38	374.5	56.9	119	1	HV40_MOUSE	P01810 mus musculu
39	374	56.8	484	2	Q99LA6	Q99la6 mus musculu
40	374	56.8	485	2	Q6PDB8	Q6pdb8 mus musculu
41	372.5	56.6	116	2	Q683Y8	Q683y8 mus musculu
42	372.5	56.6	118	2	Q9UL72	Q9ul72 homo sapien
43	372.5	56.6	122	2	Q9UL84	Q9ul84 homo sapien
44	372.5	56.6	136	1	HV16_MOUSE	P01783 mus musculu
45	371	56.4	470	2	Q7TMK1	Q7tmk1 mus musculu

ALIGNMENTS

RESULT 1
Q6MZQ6
ID Q6MZQ6 PRELIMINARY; PRT; 475 AA.
AC Q6MZQ6;
DT 05-JUL-2004 (TrEMBLrel. 27, Created)
DT 05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT 05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE Hypothetical protein DKFZp686G11190.
GN Name=DKFZp686G11190;
OS Homo sapiens (Human).
OC Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX NCBI_TaxID=9606;
RN [1]
RP SEQUENCE FROM N.A.
RC TISSUE=Human esophagus tumor;
RG The German Human cDNA Consortium;
RA Lauber J., Bahr A., Mewes H.W., Weil B., Amid C., Osanger A., Fobo G.,
RA Han M., Wiemann S.;
RL Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR EMBL; BX640947; CAE45972.1; -.
DR HSSP; P01861; 1ADQ.
DR InterPro; IPR003599; Ig.
DR InterPro; IPR007110; Ig-like.
DR InterPro; IPR003597; Ig_c1.
DR InterPro; IPR003006; Ig_MHC.
DR InterPro; IPR003596; Ig_v.
DR Pfam; PF07654; C1-set; 3.
DR SMART; SM00409; IG; 2.
DR SMART; SM00407; IGc1; 3.
DR SMART; SM00406; IGv; 1.
DR PROSITE; PS50835; IG_LIKE; 4.
DR PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW Hypothetical protein.
SQ SEQUENCE 475 AA; 52043 MW; B7EAE255A26F4B8E CRC64;

Query Match 63.0%; Score 414.5; DB 2; Length 475;
Best Local Similarity 68.0%; Pred. No. 7.7e-35;
Matches 83; Conservative 13; Mismatches 21; Indels 5; Gaps 3;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:|||||||||||||||||||: | :| ||||||||||||||  |:: :|  ||
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFRNYAMNWVRQAPGKGLEWVSGISS-SGVNTY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK---YPYYYGTSHWYFDVWGQG 116
               ||  | ||| | | | :| ||||:||||:||||||||:     |    ::|||||||:|
Db         79 YADSVKGRFTISGDISTNTLYLQMHSLRADDTAVYYCARADYRDYQVSPAYWYFDVWGRG 138

Qy        117 TL 118
              ||
Db        139 TL 140
```

RESULT 2

```
Q96BB9
ID   Q96BB9      PRELIMINARY;      PRT;   597 AA.
AC   Q96BB9;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   IGHM protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (OCT-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC015760; AAH15760.1; -.
DR   PIR; S05271; S05271.
DR   PIR; S24260; S24260.
DR   HSSP; P01861; 1ADQ.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
SQ   SEQUENCE   597 AA;  65039 MW;  4FCA3AD8ECE263D9 CRC64;

  Query Match             62.9%;  Score 414;  DB 2;  Length 597;
  Best Local Similarity   70.0%;  Pred. No. 1.1e-34;
  Matches   84;  Conservative    5;  Mismatches   29;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||||: |: | ||||||||||||||| |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGPSFSSYAMNWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYF--DVWGQGTL 118
                 |   | ||| | | |: | ||||||||||||||||||||| |  |   |   |    | ||||||
Db         80 ADSVKGRFTISRDNSRDTLYLQMNSLRAEDTAVYYCAKDPRGYSASGNYTREDYWGQGTL 139


RESULT 3
Q921A6
ID   Q921A6      PRELIMINARY;      PRT;   241 AA.
AC   Q921A6;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Anti-CEA 79 single chain Fv (Fragment).
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98170165; PubMed=9509426;
RA   Chung J.H., Choi S.J., Kim H.J., Kim I.J., Choi I.H., Lee S.D.,
RA   Yi K.S., Suh P.G., Ryu S.H., Chung H.K.;
RT   "Cloning and characterization of cDNAs encoding VH and VL of a
RT   monoclonal anti-CEA antibody (CEA 79) cross-reactive with NCA-95 and
RT   generation of a single-chain Fv molecule (scFv).";
RL   Mol. Cells 7:816-819(1997).
DR   EMBL; U88067; AAB48044.1; -.
DR   PIR; S19965; S19965.
DR   PIR; S19967; S19967.
DR   PIR; S19968; S19968.
DR   PIR; S26325; S26325.
DR   HSSP; P01607; 1BWW.
DR   SMART; SM00406; IGv; 2.
DR   PROSITE; PS50835; IG_LIKE; 2.
FT   NON_TER       1      1
FT   NON_TER     241    241
SQ   SEQUENCE   241 AA;  26086 MW;  0276887248E9C771 CRC64;

  Query Match             62.8%;  Score 413.5;  DB 2;  Length 241;
  Best Local Similarity   64.1%;  Pred. No. 4.7e-35;
  Matches   75;  Conservative   17;  Mismatches   20;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|:| :||  | :|| ::::|| |||| || |||||||:|||||||:|:||||||||||||
Db          1 QVKLQQSGPELKKPGETVKISCKASGYTFTDYGMNWVKQAPGKGLKWMGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGT 117
              | ||| || |||:|| |||||||:|:|: |||| |:||:          ||| |||||
Db         61 ADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARKDLLR-----YFDYWGQGT 112


RESULT 4
Q9UL91
ID   Q9UL91      PRELIMINARY;      PRT;   118 AA.
AC   Q9UL91;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Myosin-reactive immunoglobulin heavy chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035023; AAD56259.1; -.
DR   PIR; PH0875; PH0875.
DR   PIR; S21205; S21205.
DR   PIR; S30531; S30531.
DR   HSSP; P01783; 1IGC.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     118    118
SQ   SEQUENCE   118 AA;  12843 MW;  D0633949F2AC149D CRC64;
```

```
  Query Match             61.8%;  Score 406.5;  DB 2;  Length 118;
  Best Local Similarity   67.8%;  Pred. No. 1.2e-34;
  Matches   80;  Conservative   11;  Mismatches   22;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||: |: | |||||||||||||| :|::      |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSTIITIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   |,||| | | :|:: ||||||||||||||||||:     | |   ||:|||||:
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR-----GDSSEAFDIWGQGTM 113


RESULT 5
HV3E_HUMAN
ID   HV3E_HUMAN     STANDARD;      PRT;   120 AA.
AC   P01766;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V-III region BRO.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=77117674; PubMed=65324; DOI=10.1016/0019-2791(76)90271-8;
RA   Capra J.D., Hopper J.E.;
RT   "Comparative studies on monotypic IgM lambda and IgG kappa from an
RT   individual patient. III. The complete amino acid sequence of the VH
RT   region of the IgM paraprotein.";
RL   Immunochemistry 13:995-999(1976).
CC   -!- MISCELLANEOUS: This chain was obtained from IgM isolated from the
CC       serum of a patient with malignant lymphoma of the Waldenstrom
CC       type.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   PIR; A02049; M3HUBW.
DR   HSSP; P01783; 1IGC.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    111       Ig-like.
FT   NON_TER     120    120
SQ   SEQUENCE   120 AA;  13227 MW;  D3F0428F7C2E6410 CRC64;

  Query Match             61.6%;  Score 405;  DB 1;  Length 120;
  Best Local Similarity   65.4%;  Pred. No. 1.7e-34;
  Matches   83;  Conservative    8;  Mismatches   20;  Indels   16;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||: |::| ||||||  |||||||  | |   |:  |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSYYNMNWVRQVTGKGLEWVSAIGT-AGDQYY 59

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP---------YYYGTSHWYFD 111
              |   | ||| | : ||:| || ||||||||||||||: |         ||||:
Db         60 ADSVKGRFTISRNDSKNTLYLNMNSLRAEDTAVYYCARSPVSLVDGWLYYYYGS------ 113

Qy        112 VWGQGTL 118
              |||||||
Db        114 VWGQGTL 120


RESULT 6
HV21_MOUSE
ID   HV21_MOUSE     STANDARD;      PRT;   122 AA.
AC   P01790;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V region M511.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=81054880; PubMed=6776528;
RA   Robinson E.A., Appella E.;
RT   "Complete amino acid sequence of a mouse immunoglobulin alpha chain
RT   (MOPC 511).";
RL   Proc. Natl. Acad. Sci. U.S.A. 77:4909-4913(1980).
CC   -!- MISCELLANEOUS: This chain was isolated from a myeloma protein that
CC       binds phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     122    122
SQ   SEQUENCE   122 AA;  13652 MW;  9F4837731EA50207 CRC64;

  Query Match             61.5%;  Score 404.5;  DB 1;  Length 122;
  Best Local Similarity   65.3%;  Pred. No. 1.9e-34;
  Matches   79;  Conservative   13;  Mismatches   20;  Indels    9;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:|||||||||||||||||||| ||: |: : | ||||:||| |||:        | ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQSPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   |||:|:|||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR---YYGSSYWYFDVWGAG 115

Qy        117 T 117
              |
Db        116 T 116


RESULT 7
Q6GMY2
ID   Q6GMY2      PRELIMINARY;      PRT;   606 AA.
AC   Q6GMY2;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
```

```
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073758; AAH73758.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 4.
DR   Pfam; PF00047; ig; 4.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
KW   Hypothetical protein.
SQ   SEQUENCE   606 AA;  66184 MW;  B6B38B51114E4C55 CRC64;

  Query Match             61.2%;  Score 402.5;  DB 2;  Length 606;
  Best Local Similarity   59.4%;  Pred. No. 1.8e-33;
  Matches   79;  Conservative   14;  Mismatches   19;  Indels   21;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||||:|||||||||||||: |: | |:|:|||||||||| :|:: :    |
Db         20 QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSSSYTNY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK----------------YPYYYG 104
              |   | ||| | | :|:: ||||||||||||||||||:                 | ||||
Db         80 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGNGIAAAGRVVYAEDYYYYYG 139

Qy        105 TSHWYFDVWGQGT 117
                |||||||
Db        140 -----MDVWGQGT 147


RESULT 8
Q8WU38
ID   Q8WU38      PRELIMINARY;      PRT;   573 AA.
AC   Q8WU38;
DT   01-MAR-2002 (TrEMBLrel. 20, Created)
DT   01-MAR-2002 (TrEMBLrel. 20, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JAN-2002) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC021276; AAH21276.1; -.
DR   PIR; S21205; S21205.
DR   PIR; S30532; S30532.
DR   HSSP; P18529; 1I8K.
DR   Pfam; PF07654; C1-set; 2.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 4.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW   Hypothetical protein.
SQ   SEQUENCE   573 AA;  62967 MW;  FD072344033AC530 CRC64;

  Query Match             61.0%;  Score 401.5;  DB 2;  Length 573;
  Best Local Similarity   65.9%;  Pred. No. 2.2e-33;
  Matches   81;  Conservative    9;  Mismatches   22;  Indels   11;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||| |||||||||: |  | |:|||||||||||||  |:  :|   |
Db         20 EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIGY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKY------PYYYGTSHWYFDVWG 114
              |   | ||| | | :|:: ||||||||||||:|||||:       ||||      ||||
Db         80 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKHGSGSYIGYYYG-----MDVWG 134

Qy        115 QGT 117
              |||
Db        135 QGT 137


RESULT 9
HV18_MOUSE
ID   HV18_MOUSE     STANDARD;      PRT;   123 AA.
AC   P01787;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V regions TEPC 15/S107/HPCM1/HPCM2/HPCM3.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE (TEPC 15).
RX   MEDLINE=76222762; PubMed=819932;
RA   Rudikoff S., Potter M.;
RT   "Size differences among immunoglobulin heavy chains from
RT   phosphorylcholine-binding proteins.";
RL   Proc. Natl. Acad. Sci. U.S.A. 73:2109-2112(1976).
RN   [2]
RP   SEQUENCE FROM N.A. (H107).
RX   MEDLINE=80199926; PubMed=6769593; DOI=10.1016/0092-8674(80)90089-6;
RA   Early P., Huang H., Davis M., Calame K., Hood L.;
RT   "An immunoglobulin heavy chain variable region gene is generated from
RT   three segments of DNA: VH, D and JH.";
RL   Cell 19:981-992(1980).
RN   [3]
RP   SEQUENCE (S107).
```

```
RX   MEDLINE=76110488; PubMed=813561;
RX   DOI=10.1146/annurev.ge.09.120175.001513;
RA   Rudikoff S., Barstad P., Potter M., Hood L.;
RL   Unpublished results, cited by:
RL   Hood L., Campbell J.H., Elgin S.C.R.;
RL   Annu. Rev. Genet. 9:305-353(1975).
RN   [4]
RP   SEQUENCE (HPCM1; HPCM2 AND HPCM3).
RX   MEDLINE=81197602; PubMed=7231520;
RA   Gearhart P.J., Johnson N.D., Douglas R., Hood L.;
RT   "IgG antibodies to phosphorylcholine exhibit more diversity than their
RT   IgM counterparts.";
RL   Nature 291:29-34(1981).
CC   -!- MISCELLANEOUS: All those sequence appears to be identical.
CC   -!- MISCELLANEOUS: These chains were isolated from myeloma and
CC       hybridoma proteins that bind phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   PIR; A93804; AVMST5.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Hybridoma; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     123    123
SQ   SEQUENCE   123 AA;  13777 MW;  9D58086DE12F7000 CRC64;

  Query Match             60.8%;  Score 400;  DB 1;  Length 123;
  Best Local Similarity   65.3%;  Pred. No. 5.8e-34;
  Matches   79;  Conservative   12;  Mismatches   22;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:||||||||||||||||||| ||: |: : | |||| ||| |||:       | ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQPPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   |||:|:||||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR--DYYGSSYWYFDVWGAG 116

Qy        117 T 117
              |
Db        117 T 117


RESULT 10
Q9UL90
ID   Q9UL90      PRELIMINARY;      PRT;   113 AA.
AC   Q9UL90;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Myosin-reactive immunoglobulin heavy chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035024; AAD56260.1; -.
DR   PIR; S78486; S78486.
DR   HSSP; P01772; 2FB4.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     113    113
SQ   SEQUENCE   113 AA;  12437 MW;  ED57FDD19086D07F CRC64;

  Query Match             60.6%;  Score 399;  DB 2;  Length 113;
  Best Local Similarity   69.7%;  Pred. No. 6.7e-34;
  Matches   83;  Conservative    6;  Mismatches   18;  Indels   12;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||:|||||||||||||||: |: |||:|||||||||||||| :|  | |   |
Db          1 EVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIR-YDGSNKY 59

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
                ||  | ||| | | ||:| |||||||||||||||||||    |           ||||||
Db         60 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDLNY----------WGQGTL 108


RESULT 11
HV19_MOUSE
ID   HV19_MOUSE     STANDARD;      PRT;   123 AA.
AC   P01788;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V region H8.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE.
RA   Barstad P.;
RL   Thesis (1975), California Institute of Technology / Pasadena, U.S.A.
CC   -!- MISCELLANEOUS: This chain was isolated from a myeloma protein that
CC       binds phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     123    123
SQ   SEQUENCE   123 AA;  13805 MW;  9D581401912F7000 CRC64;

  Query Match             60.5%;  Score 398;  DB 1;  Length 123;
  Best Local Similarity   65.3%;  Pred. No. 9.4e-34;
  Matches   79;  Conservative   11;  Mismatches   23;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:|||||||||||||||||| ||: |: : | |||| ||| |||:       | ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQPPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   ||| |:||||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR--DYYGBSYWYFDVWGAG 116

Qy        117 T 117
              |
Db        117 T 117


RESULT 12
Q6N089
ID   Q6N089      PRELIMINARY;      PRT;   472 AA.
AC   Q6N089;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein DKFZp686P15220.
```

```
GN   Name=DKFZp686P15220;
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Human rectum tumor;
RG   The German Human cDNA Consortium;
RA   Wambutt R., Heubner D., Mewes H.W., Weil B., Amid C., Osanger A.,
RA   Fobo G., Han M., Wiemann S.;
RL   Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BX640627; CAE45781.1; -.
DR   HSSP; P01861; 1ADQ.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 3.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 3.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 4.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW   Hypothetical protein.
SQ   SEQUENCE   472 AA;  51724 MW;  26CB340D0046D279 CRC64;

  Query Match             60.5%;  Score 398;  DB 2;  Length 472;
  Best Local Similarity   66.4%;  Pred. No. 4.1e-33;
  Matches   81;  Conservative    7;  Mismatches   24;  Indels   10;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||| |||||||||: |  | |:|||||||||||||  |:  :|   |
Db         20 EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIAY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP-----YYYGTSHWYFDVWGQ 115
              |   | ||| | |  |:: |||||||||||||:|||||       ||||      |||||
Db         80 ADSVKGRFTISRDNGKNSLYLQMNSLRAEDTALYYCAKEIGAHNFYYYG-----MDVWGQ 134

Qy        116 GT 117
              ||
Db        135 GT 136


RESULT 13
Q6MZV7
ID   Q6MZV7      PRELIMINARY;      PRT;   473 AA.
AC   Q6MZV7;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein DKFZp686C11235.
GN   Name=DKFZp686C11235;
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Human small intestine;
RG   The German Human cDNA Consortium;
RA   Bloecker H., Boecher M., Mewes H.W., Weil B., Amid C., Osanger A.,
RA   Fobo G., Han M., Wiemann S.;
RL   Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BX640853; CAE45920.1; -.
DR   HSSP; P01861; 1ADQ.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 3.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 3.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 4.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW   Hypothetical protein.
SQ   SEQUENCE   473 AA;  52121 MW;  9476EAE4C0BFC447 CRC64;

  Query Match             60.3%;  Score 396.5;  DB 2;  Length 473;
  Best Local Similarity   65.6%;  Pred. No. 5.9e-33;
  Matches   80;  Conservative   14;  Mismatches   21;  Indels    7;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||||||||||||||||||||||: |: : |||||||||||||: :| | :|    |
Db         20 EIQLVESGGGLVQPGGSLRLSCAASGFTFSSFEMNWVRQAPGKGLEWLSYI-TRSGNTVY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWY---FDVWGQG 116
              ||  : ||| | | :::: |||||||||||||||||:   :  || ||   || ||||
Db         79 YADSLQGRFTISRDNARNSLYLQMNSLRAEDTAVYYCARQNEH--TSPWYPSFFDYWGQG 136

Qy        117 TL 118
              |
Db        137 IL 138


RESULT 14
Q9HCC1
ID   Q9HCC1      PRELIMINARY;      PRT;   112 AA.
AC   Q9HCC1;
DT   01-MAR-2001 (TrEMBLrel. 16, Created)
DT   01-MAR-2001 (TrEMBLrel. 16, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Single chain Fv (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RA   Kikuchi M., Takeda C., Tsujimoto Y., Asada S., Nagata K.;
RL   Submitted (OCT-2000) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; AB049915; BAB16829.1; -.
DR   HSSP; P01783; 1IGC.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1       1
FT   NON_TER     112     112
SQ   SEQUENCE   112 AA;  12243 MW;  24F1A45EC3B84788 CRC64;

  Query Match             60.1%;  Score 395.5;  DB 2;  Length 112;
  Best Local Similarity   66.1%;  Pred. No. 1.5e-33;
  Matches   78;  Conservative    9;  Mismatches   24;  Indels    7;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||:|:||||||:||||||: |  |||:|||||||||||||  ||   |   |
Db          1 EVQLVESGGGVVRPGGSLRISCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHWYFDVWGQGTL 118
              |   | ||| | | :|:: |||||||||||||||||:  |          | ||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARRRY-------ALDYWGQGTL 111


RESULT 15
Q8WUK1
ID   Q8WUK1      PRELIMINARY;      PRT;   613 AA.
AC   Q8WUK1;
DT   01-MAR-2002 (TrEMBLrel. 20, Created)
DT   01-MAR-2002 (TrEMBLrel. 20, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   IGHM protein.
OS   Homo sapiens (Human).
```

```
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (DEC-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC020240; AAH20240.1; -.
DR   PIR; F36005; F36005.
DR   PIR; G36005; G36005.
DR   PIR; PH1642; PH1642.
DR   PIR; PH1643; PH1643.
DR   PIR; PH1645; PH1645.
DR   PIR; PH1646; PH1646.
DR   PIR; PL0098; PL0098.
DR   PIR; PL0120; PL0120.
DR   PIR; S15590; S15590.
DR   PIR; S31116; S31116.
DR   PIR; S31119; S31119.
DR   PIR; S70442; S70442.
DR   HSSP; P01861; 1ADQ.
DR   Pfam; PF07654; C1-set; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
SQ   SEQUENCE   613 AA;  67295 MW;  60C7F5950671E315 CRC64;

  Query Match           60.1%;  Score 395.5;  DB 2;  Length 613;
  Best Local Similarity   66.1%;  Pred. No. 9.9e-33;
  Matches   82;  Conservative    9;  Mismatches   18;  Indels   15;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||| ||||||||||: |: |||:|||||||||||||  | :| |   |
Db         20 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI-SYDGSNKY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPYYYGTSHW-----YFDVWG 114
               ||  | ||| | | ||:| ||||||||||||||||||     |       ||:||
Db         79 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK--------DWSEGVETFDIWG 130

Qy        115 QGTL 118
              |||:
Db        131 QGTM 134


Search completed: March 14, 2005, 20:49:19
Job time : 82.807 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:39:29 ; Search time 17.8553 Seconds
(without alignments)
635.867 Million cell updates/sec

Title: US-09-723-752B-7
Perfect score: 655
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPHYYGSSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 283416 seqs, 96216763 residues

Total number of hits satisfying chosen parameters: 283416

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : PIR_79:*
1: pir1:*
2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	433	66.1	119	2	S31107	Ig heavy chain - h
2	432	66.0	123	2	S31114	Ig heavy chain - h
3	432	66.0	125	2	S30531	Ig heavy chain V r
4	431.5	65.9	140	2	S70442	Ig heavy chain pre
5	431	65.8	138	2	S31666	Ig heavy chain V r
6	430.5	65.7	120	2	B42848	L6 mAb heavy chain
7	430.5	65.7	146	4	S33905	Ig heavy chain pre
8	430	65.6	131	2	S26792	Ig heavy chain V r
9	428	65.3	140	2	S31588	Ig heavy chain V r
10	426	65.0	123	2	S26794	Ig heavy chain V r
11	426	65.0	127	2	S38489	Ig heavy chain - h
12	424.5	64.8	128	2	S48797	Ig heavy chain V r
13	424	64.7	119	2	A53285	Ig heavy chain V a
14	423.5	64.7	115	2	S19968	Ig heavy chain V r
15	421	64.3	121	2	I55673	Ig heavy chain - h
16	421	64.3	140	2	S31686	Ig heavy chain V r
17	420	64.1	142	2	S19245	Ig heavy chain pre
18	419	64.0	121	2	S31104	Ig heavy chain (su
19	417.5	63.7	147	2	I37780	Ig variable region
20	417	63.7	121	2	S19666	Ig heavy chain V r
21	416.5	63.6	141	2	S31669	Ig heavy chain V r
22	415.5	63.4	114	2	D32967	Ig heavy chain V r
23	415	63.4	117	2	S36259	Ig heavy chain V r
24	415	63.4	119	2	S31108	Ig heavy chain - h
25	414.5	63.3	114	2	C32967	Ig heavy chain V r
26	414.5	63.3	122	2	E36005	Ig heavy chain V r
27	414.5	63.3	124	2	S20782	Ig heavy chain V r
28	414	63.2	119	2	D36005	Ig heavy chain V r
29	414	63.2	121	2	S31113	Ig heavy chain - h
30	414	63.2	160	2	S05271	Ig heavy chain pre
31	413.5	63.1	124	1	AVMS51	Ig heavy chain V r
32	413	63.1	123	1	AVMST5	Ig heavy chain VH1
33	413	63.1	135	2	S31598	Ig heavy chain V r
34	413	63.1	143	2	S23624	Ig heavy chain V r
35	412.5	63.0	120	2	S48798	Ig heavy chain V r
36	412	62.9	119	2	C36005	Ig heavy chain V r
37	412	62.9	123	2	PL0017	Ig heavy chain V-D
38	410.5	62.7	119	2	S37453	Ig mu chain - huma
39	410	62.6	132	2	S31603	Ig heavy chain V r
40	409.5	62.5	120	2	S26789	Ig heavy chain V r
41	409.5	62.5	122	2	S31117	Ig heavy chain - h
42	408.5	62.4	114	2	S36280	Ig heavy chain V r
43	408.5	62.4	124	2	E30539	Ig heavy chain V r
44	408	62.3	117	2	S31109	Ig heavy chain - h
45	408	62.3	124	2	PH1404	Ig heavy chain V r

ALIGNMENTS

RESULT 1
S31107
Ig heavy chain - human
C;Species: Homo sapiens (man)
C;Date: 02-Dec-1993 #sequence_revision 26-May-1995 #text_change 17-Mar-1999
C;Accession: S31107
R;Raaphorst, F.M.; Timmers, E.; Kenter, M.J.H.; van Tol, M.J.D.; Vossen, J.M.; Schuurma
Eur. J. Immunol. 22, 247-251, 1992
A;Title: Restricted utilization of germ-line V(H)3 genes and short diverse third comple
A;Reference number: S31104; MUID:92111633; PMID:1730252
A;Accession: S31107
A;Status: preliminary; nucleic acid sequence not shown; translation not shown
A;Molecule type: mRNA
A;Residues: 1-119 <RAA>
A;Cross-references: EMBL:X62955
A;Note: the nucleotide sequence was submitted to the EMBL Data Library, October 1991
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

Query Match 66.1%; Score 433; DB 2; Length 119;
Best Local Similarity 73.7%; Pred. No. 1.8e-32;
Matches 87; Conservative 8; Mismatches 19; Indels 4; Gaps 2;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||||:||::| |:|||||||||||||  |:   |   |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | ||:| |||||||||||||||||| |   |:| :||| ||||||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDP---GAS-YYFDYWGQGTL 114
```

RESULT 2
S31114
Ig heavy chain - human
C;Species: Homo sapiens (man)
C;Date: 02-Dec-1993 #sequence_revision 26-May-1995 #text_change 17-Mar-1999
C;Accession: S31114
R;Raaphorst, F.M.; Timmers, E.; Kenter, M.J.H.; van Tol, M.J.D.; Vossen, J.M.; Schuurma
Eur. J. Immunol. 22, 247-251, 1992
A;Title: Restricted utilization of germ-line V(H)3 genes and short diverse third comple
A;Reference number: S31104; MUID:92111633; PMID:1730252
A;Accession: S31114
A;Status: preliminary; nucleic acid sequence not shown; translation not shown
A;Molecule type: mRNA
A;Residues: 1-123 <RAA>
A;Cross-references: EMBL:X62963
A;Note: the nucleotide sequence was submitted to the EMBL Data Library, October 1991
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

```
  Query Match             66.0%;  Score 432;  DB 2;  Length 123;
  Best Local Similarity   71.2%;  Pred. No. 2.3e-32;
  Matches   84;  Conservative    7;  Mismatches   27;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||||:||::| |:|||||||||||||  |:   |   |
Db          1 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||    |      | || ||||||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKASLYLRFLEWLFDYWGQGTL 118


RESULT 3
S30531
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 06-Jan-1995 #sequence_revision 06-Jan-1995 #text_change 09-Jul-2004
C;Accession: S30531
R;Mariette, X.
submitted to the EMBL Data Library, October 1992
A;Reference number: S30520
A;Accession: S30531
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-125 <MAR>
A;Cross-references: UNIPROT:Q9UL91; EMBL:Z18317
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             66.0%;  Score 432;  DB 2;  Length 125;
  Best Local Similarity   68.3%;  Pred. No. 2.4e-32;
  Matches   82;  Conservative   15;  Mismatches   21;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||:||::| |||||||||||||: :|:: :    |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWISYISSSSSTIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHW--YFDVWGQGTL 118
              |   | ||| | | :|:: ||||||||||||||||||:  :|  | ::  ||| ||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSRNYDSSGYYSHYFDYWGQGTL 120


RESULT 4
S70442
Ig heavy chain precursor V region (mu) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 24-Jul-1998 #sequence_revision 24-Jul-1998 #text_change 09-Jul-2004
C;Accession: S70442
R;Cuisinier, A.M.; Pumoux, F.; Fougereau, M.; Tonnelle, C.
Mol. Immunol. 29, 1363-1373, 1992
A;Title: IgM kappa/lambda EBV human B cell clone: an early step of differentiation of fe
A;Reference number: S70442; MUID:93024508; PMID:1383695
A;Accession: S70442
A;Status: not compared with conceptual translation
A;Molecule type: mRNA
A;Residues: 1-140 <CUI>
A;Cross-references: UNIPROT:Q8WUK1
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             65.9%;  Score 431.5;  DB 2;  Length 140;
  Best Local Similarity   73.1%;  Pred. No. 3e-32;
  Matches   87;  Conservative   10;  Mismatches   17;  Indels    5;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||||||||||||||:||:||||:||||||||||||| :|  | |   |
Db         20 QVQLVESGGGVVQPGGSLRLSCAASGFTFSNYGMHWVRQAPGKGLEWVAFIR-YDGSNKY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
                 ||  | ||| | | ||:| ||||||||||||||||||:  |  |::  ||| ||||||
Db         79 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR-DHIVGAT--YFDYWGQGTL 134


RESULT 5
S31666
Ig heavy chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 10-Nov-1995 #text_change 23-Jul-1999
C;Accession: S31666
R;Cuisinier, A.M.; Gauthier, L.; Boubli, L.; Fougereau, M.; Tonnelle, C.
submitted to the EMBL Data Library, June 1992
A;Description: Mechanisms that generate human immunoglobulin diversity operate from the
A;Reference number: S31585
A;Accession: S31666
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-138 <CUI>
A;Cross-references: EMBL:Z14202; NID:g30963; PIDN:CAA78571.1; PID:g30964
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             65.8%;  Score 431;  DB 2;  Length 138;
  Best Local Similarity   70.3%;  Pred. No. 3.2e-32;
  Matches   83;  Conservative   11;  Mismatches   20;  Indels    4;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||||:||::| |:|||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||      : :||||:||:|||
Db         80 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK----ARTGYWYFDLWGRGTL 133


RESULT 6
B42848
L6 mAb heavy chain V region - mouse (fragment)
C;Species: Mus musculus (house mouse)
C;Date: 27-Apr-1993 #sequence_revision 18-Nov-1994 #text_change 21-Jan-2000
C;Accession: B42848; S33903
R;Fell, H.P.; Gayle, M.A.; Yelton, D.; Lipsich, L.; Schieven, G.L.; Marken, J.S.; Aruff
J. Biol. Chem. 267, 15552-15558, 1992
A;Title: Chimeric L6 anti-tumor antibody. Genomic construction, expression, and charact
A;Reference number: A42848; MUID:92348410; PMID:1639794
A;Accession: B42848
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-120 <FEL>
A;Cross-references: GB:M90690; NID:g195065; PIDN:AAA38146.1; PID:g195066
A;Note: sequence extracted from NCBI backbone (NCBIN:109960, NCBIP:109961)
A;Accession: S33903
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-120 <FE2>
A;Cross-references: EMBL:M90691
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             65.7%;  Score 430.5;  DB 2;  Length 120;
  Best Local Similarity   65.8%;  Pred. No. 3.1e-32;
  Matches   77;  Conservative   20;  Mismatches   17;  Indels    3;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| ||||||||||||||:|||||||:|:|||||||:|||
Db          1 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              | ||| || |||:||  |||||:|:|: || | |:||::   ||:|  | | |||||
Db         61 ADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFS--YGNSR-YADYWGQGT 114


RESULT 7
```

```
S33905
Ig heavy chain precursor V region - synthetic
C;Species: synthetic
C;Date: 13-Jan-1995 #sequence_revision 30-Apr-1998 #text_change 20-Oct-2000
C;Accession: S33905
R;Liu, A.Y.; Robinson, R.R.; Hellstroem, K.E.; Murray Jr., E.D.; Chang, C.P.; Hellstroem
Proc. Natl. Acad. Sci. U.S.A. 84, 3439-3443, 1987
A;Title: Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells.
A;Reference number: S33905; MUID:87204152; PMID:3106970
A;Accession: S33905
A;Molecule type: mRNA
A;Residues: 1-146 <LIU>
A;Cross-references: EMBL:M16072; NID:g195270; PIDN:AAA38229.1; PID:g195271

  Query Match             65.7%;  Score 430.5;  DB 4;  Length 146;
  Best Local Similarity   65.8%;  Pred. No. 3.8e-32;
  Matches   77;  Conservative   20;  Mismatches   17;  Indels    3;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| |||||||||||||||:|||||||:|:|||||||:|||
Db         20 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGQPTY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              | ||| || |||:||  |||||:|:|: || | |:||::   ||:|  | | |||||
Db         80 ADDFKGRFAFSLETSAYTAYLQINNLKNEDMATYFCARFS--YGNSR-YSDYWGQGT 133


RESULT 8
S26792
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 20-Jun-2000
C;Accession: S26792
R;Mortari, F.; Newton, J.A.; Wang, J.Y.; Schroeder Jr., H.W.
Eur. J. Immunol. 22, 241-245, 1992
A;Title: The human cord blood antibody repertoire. Frequent usage of the V(H)7 gene fami
A;Reference number: S26786; MUID:92111632; PMID:1730251
A;Accession: S26792
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-131 <MOR>
A;Cross-references: EMBL:X61012; NID:g32804; PIDN:CAA43346.1; PID:g1335131
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             65.6%;  Score 430;  DB 2;  Length 131;
  Best Local Similarity   64.8%;  Pred. No. 3.8e-32;
  Matches   81;  Conservative   19;  Mismatches   17;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :||||:||  | :|| |:::|| |||||||:| |||||||||:||||:||||| || |||
Db          1 QVQLVQSGSELKKPGASVKVSCKASGYTFTSYAMNWVRQAPGQGLEWMGWINTNTGNPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK----YPHY-YGSSHWYF---DV 112
              |  |  || |||||| ||||||::||:||||||||||:    | :| : | ::|:   ||
Db         61 AQGFTGRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARDSRGYSYYDFWSGYFYYYYMDV 120

Qy        113 WGQGT 117
              ||:||
Db        121 WGKGT 125


RESULT 9
S31588
Ig heavy chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 22-Nov-1993 #sequence_revision 10-Nov-1995 #text_change 23-Jul-1999
C;Accession: S31588
R;Cuisinier, A.M.; Gauthier, L.; Boubli, L.; Fougereau, M.; Tonnelle, C.
submitted to the EMBL Data Library, June 1992
A;Description: Mechanisms that generate human immunoglobulin diversity operate from the
A;Reference number: S31585
A;Accession: S31588
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-140 <CUI>
A;Cross-references: EMBL:Z14200; NID:g30957; PIDN:CAA78569.1; PID:g30958
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-117/Domain: immunoglobulin homology <IMM>

  Query Match             65.3%;  Score 428;  DB 2;  Length 140;
  Best Local Similarity   72.9%;  Pred. No. 6.1e-32;
  Matches   86;  Conservative    9;  Mismatches   21;  Indels    2;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||||||||:||::| |:|||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | ||:| ||||||||||||||||||  | | |:: ||| ||||||
Db         80 ADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCAK-DHDY-SNYIYFDYWGQGTL 135


RESULT 10
S26794
Ig heavy chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 17-Mar-1999
C;Accession: S26794
R;Mortari, F.; Newton, J.A.; Wang, J.Y.; Schroeder Jr., H.W.
Eur. J. Immunol. 22, 241-245, 1992
A;Title: The human cord blood antibody repertoire. Frequent usage of the V(H)7 gene fam
A;Reference number: S26786; MUID:92111632; PMID:1730251
A;Accession: S26794
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-123 <MOR>
A;Cross-references: EMBL:X61011
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             65.0%;  Score 426;  DB 2;  Length 123;
  Best Local Similarity   68.4%;  Pred. No. 8.2e-32;
  Matches   80;  Conservative   14;  Mismatches   23;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||:||::| |||||||||||||| :|:: :    |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSSSSTIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              |   | ||| | | :|:: |||||||| ||||||||:   ||  :::  |||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRDEDTAVYYCARSIKYYDENYYGMDVWGQGT 117


RESULT 11
S38489
Ig heavy chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 06-Jan-1995 #sequence_revision 06-Jan-1995 #text_change 23-Jul-1999
C;Accession: S38489
R;Marks, J.D.; Ouwehand, W.H.; Bye, J.M.; Finnern, R.; Gorick, B.D.; Voak, D.; Thorpe,
submitted to the EMBL Data Library, June 1993
A;Description: Human antibody fragments specific for human blood group antigens from a
A;Reference number: S38488
A;Accession: S38489
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-127 <MAR>
A;Cross-references: EMBL:Z23028; NID:g414025; PIDN:CAA80563.1; PID:g414026
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>
```

```
  Query Match             65.0%;  Score 426;  DB 2;  Length 127;
  Best Local Similarity   69.4%;  Pred. No. 8.4e-32;
  Matches   86;  Conservative   11;  Mismatches   21;  Indels    6;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :||||:||||:|||||||||||||||||:||::| |:||||||||||||  |:   |   |
Db          1 QVQLVQSGGGVVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK---YP--HYYGSSHWY-FDVWG 114
              |   | ||| | | ||:| ||||||||||||||||||   :|   || || :| || ||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKEGPFPASDYYDSSGYYSFDYWG 120

Qy        115 QGTL 118
              ||||
Db        121 QGTL 124


RESULT 12
S48797
Ig heavy chain V region (anti-Sm, VH3/Dxp4/JH6) - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Sep-1998 #text_change 23-Jul-1999
C;Accession: S48797; S26893
R;Mahmoudi, M.; Edwards, J.; Cairns, E.; Bell, D.
submitted to the EMBL Data Library, October 1994
A;Description: Molecular characterization of natural human anti-Sm autoantibodies.
A;Reference number: S48797
A;Accession: S48797
A;Molecule type: mRNA
A;Residues: 1-128 <MAH>
A;Cross-references: EMBL:Z46379; NID:g587147; PIDN:CAA86512.1; PID:g1340168
R;Tomlinson, I.M.; Walter, G.; Marks, J.D.; Llewelyn, M.B.; Winter, G.
J. Mol. Biol. 227, 776-798, 1992
A;Title: The repertoire of human germline V(H) sequences reveals about fifty groups of V
A;Reference number: S26885; MUID:93021117; PMID:1404388
A;Accession: S26893
A;Molecule type: DNA
A;Residues: 1-98 <TOM>
A;Cross-references: EMBL:Z12350; NID:g32922; PIDN:CAA78220.1; PID:g32923
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             64.8%;  Score 424.5;  DB 2;  Length 128;
  Best Local Similarity   69.9%;  Pred. No. 1.2e-31;
  Matches   86;  Conservative   11;  Mismatches   19;  Indels    7;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||| ||||||||||:||::|||:|||||||||||||  | | |   |
Db          1 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI-WYDGSNKY 59

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYF-----DVWG 114
                ||  | ||| | | ||:| ||||||||||||||||||:  :|| || :|:      ||||
Db         60 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDNYYYDSSGYYYYYYGMDVWG 119

Qy        115 QGT 117
              |||
Db        120 QGT 122


RESULT 13
A53285
Ig heavy chain V and J regions, monoclonal antibody SCET.M8.1 - mouse (fragment)
C;Species: Mus musculus (house mouse)
C;Date: 02-May-1994 #sequence_revision 18-Nov-1994 #text_change 20-Jun-2000
C;Accession: A53285
R;Sawada, J.; Mizusawa, S.; Terao, T.; Naito, M.; Kurosawa, Y.
Mol..Immunol. 28, 1063-1072, 1991
A;Title: Molecular characterization of monoclonal anti-steroid antibodies: primary struc
 and their pH-reactivity profiles.
A;Reference number: A53285; MUID:92017897; PMID:1922102
A;Accession: A53285
A;Status: preliminary
A;Molecule type: DNA; protein
A;Residues: 1-119 <SAW>
A;Cross-references: GB:D12736; NID:g220595; PIDN:BAA02228.1; PID:g220596
A;Note: sequence extracted from NCBI backbone (NCBIN:63271, NCBIP:63299)
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;15-98/Domain: immunoglobulin homology <IMM>

  Query Match             64.7%;  Score 424;  DB 2;  Length 119;
  Best Local Similarity   66.1%;  Pred. No. 1.2e-31;
  Matches   78;  Conservative   16;  Mismatches   18;  Indels    6;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ::|||:||  | :|| ::::|| ||||||||||||||:|||||||||: ||||||||||
Db          1 QIQLVQSGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLEWMDWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWY-FDVWGQGT 117
              | ||| || |||:|| ||||||:| |: |||| |:||:     |:   |  | |||||
Db         61 ADDFKGRFAFSLETSASTAYLQINDLKNEDTATYFCAR-----GNVRVYAMDYWGQGT 113


RESULT 14
S19968
Ig heavy chain V region (M-T408) - mouse (fragment)
C;Species: Mus musculus (house mouse)
C;Date: 06-Feb-1995 #sequence_revision 06-Feb-1995 #text_change 09-Jul-2004
C;Accession: S19968
R;Weissenhorn, W.; Riethmueller, G.; Weiss, E.M.; Rieber, E.P.
submitted to the EMBL Data Library, March 1992
A;Description: Structural characterization of CD4 mAb.
A;Reference number: S19963
A;Accession: S19968
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-115 <WEI>
A;Cross-references: UNIPROT:Q921A6; EMBL:X65089
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;10-93/Domain: immunoglobulin homology <IMM>

  Query Match             64.7%;  Score 423.5;  DB 2;  Length 115;
  Best Local Similarity   67.9%;  Pred. No. 1.3e-31;
  Matches   76;  Conservative   15;  Mismatches   18;  Indels    3;  Gaps    1;

Qy          6 ESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTYAADFK 65
              :||  |  || ::::|| |||||||||||||::||||| |||:||||||||:|||| |||
Db          1 QSGPELKTPGETVKISCKASGYTFTNYGMNWMKQAPGKDLEWMGWINTYTGQPTYADDFK 60

Qy         66 RRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              || |||:|| |||||:|:|: |||| |:||: | ||  |   || |||||
Db         61 GRFAFSLETSASTAYLQINNLKNEDTAAYFCARGPPYYYRFS---FDYWGQGT 109


RESULT 15
I55673
Ig heavy chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 02-Jul-1996 #sequence_revision 02-Jul-1996 #text_change 21-Jan-2000
C;Accession: I55673
R;Knight, G.B.; Agnello, V.; Bonagura, V.; Barnes, J.L.; Panka, D.J.; Zhang, Q.X.
J. Exp. Med. 178, 1903-1911, 1993
A;Title: Human rheumatoid factor cross-idiotypes. IV. Studies on WA XId-positive IgM wi
tinct from the 17.109 and G6 XIds.
A;Reference number: I55673; MUID:94065558; PMID:8245772
A;Accession: I55673
A;Status: preliminary; translated from GB/EMBL/DDBJ
A;Molecule type: mRNA
A;Residues: 1-121 <RES>
A;Cross-references: GB:M87268; NID:g186197; PIDN:AAC37536.1; PID:g186198
C;Genetics:
A;Gene: GDB:IGHM
A;Cross-references: GDB:120086; OMIM:147020
A;Map position: 14q32.33-14q32.33
```

C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;15-98/Domain: immunoglobulin homology <IMM>

Query Match 64.3%; Score 421; DB 2; Length 121;
Best Local Similarity 70.3%; Pred. No. 2.3e-31;
Matches 83; Conservative 8; Mismatches 25; Indels 2; Gaps 1;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:||||||||||||||| |||:||: |||:|||||||||||| |:   |   |
Db          1 EVQLLESGGGLVQPGGSLRLSCTASGFTFSTYGMSWVRQAPGKGLEWVSAISGSGGSTYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | ||:| |||||||||||||||||| | : ||    :| ||||||
Db         61 ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAAAPRHAGSPP--YDYWGQGTL 116
```

Search completed: March 14, 2005, 21:08:50
Job time : 18.8553 secs

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:32:33 ; Search time 82.807 Seconds
(without alignments)
729.713 Million cell updates/sec

Title: US-09-723-752B-7
Perfect score: 655
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPHYYGSSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1612378 seqs, 512079187 residues

Total number of hits satisfying chosen parameters: 1612378

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : UniProt_03:*
1: uniprot_sprot:*
2: uniprot_trembl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	425.5	65.0	475	2	Q6MZQ6	Q6mzq6 homo sapien
2	420	64.1	597	2	Q96BB9	Q96bb9 homo sapien
3	419.5	64.0	241	2	Q921A6	Q921a6 mus musculu
4	415.5	63.4	118	2	Q9UL91	Q9ul91 homo sapien
5	415.5	63.4	122	1	HV21_MOUSE	P01790 mus musculu
6	413	63.1	123	1	HV18_MOUSE	P01787 mus musculu
7	410	62.6	113	2	Q9UL90	Q9ul90 homo sapien
8	409	62.4	123	1	HV19_MOUSE	P01788 mus musculu
9	405.5	61.9	606	2	Q6GMY2	Q6gmy2 homo sapien
10	405	61.8	120	1	HV3E_HUMAN	P01766 homo sapien
11	404.5	61.8	573	2	Q8WU38	Q8wu38 homo sapien
12	403.5	61.6	613	2	Q8WUK1	Q8wuk1 homo sapien
13	402.5	61.5	473	2	Q6MZV7	Q6mzv7 homo sapien
14	402	61.4	123	1	HV23_MOUSE	P01792 mus musculu
15	401.5	61.3	112	2	Q9HCC1	Q9hcc1 homo sapien
16	401	61.2	123	1	HV22_MOUSE	P01791 mus musculu
17	401	61.2	472	2	Q6N089	Q6n089 homo sapien
18	400	61.1	240	2	Q65ZC9	Q65zc9 homo sapien
19	399	60.9	470	2	Q6PJA4	Q6pja4 homo sapien
20	398.5	60.8	122	1	HV20_MOUSE	P01789 mus musculu
21	397.5	60.7	544	2	Q6PJ95	Q6pj95 homo sapien
22	397	60.6	478	2	Q6PI81	Q6pi81 homo sapien
23	397	60.6	487	2	Q80ZI7	Q80zi7 mus musculu
24	395	60.3	464	2	Q6MZU6	Q6mzu6 homo sapien
25	393.5	60.1	147	2	Q9Y509	Q9y509 homo sapien
26	393	60.0	121	2	Q9UL71	Q9ul71 homo sapien
27	393	60.0	499	2	Q8N5K4	Q8n5k4 homo sapien
28	388	59.2	115	1	HV3D_HUMAN	P01765 homo sapien
29	388	59.2	123	1	HV24_MOUSE	P01793 mus musculu
30	387.5	59.2	122	1	HV3G_HUMAN	P01768 homo sapien
31	387.5	59.2	483	2	Q6MZX9	Q6mzx9 homo sapien
32	387	59.1	125	2	Q6PIL0	Q6pil0 homo sapien
33	386	58.9	485	2	Q6PDB8	Q6pdb8 mus musculu
34	383.5	58.5	479	2	Q6MZV6	Q6mzv6 homo sapien
35	383.5	58.5	487	2	Q6ZVX0	Q6zvx0 homo sapien
36	382	58.3	493	2	Q6GMX2	Q6gmx2 homo sapien
37	381.5	58.2	122	2	Q9UL84	Q9ul84 homo sapien
38	381	58.2	470	2	Q7TMK1	Q7tmk1 mus musculu
39	380	58.0	121	1	HV3J_HUMAN	P01771 homo sapien
40	379.5	57.9	119	1	HV38_MOUSE	P01808 mus musculu
41	379	57.9	123	1	HV25_MOUSE	P01794 mus musculu
42	378.5	57.8	118	2	Q9UL72	Q9ul72 homo sapien
43	378.5	57.8	119	1	HV37_MOUSE	P01807 mus musculu
44	377	57.6	116	2	Q9UL93	Q9ul93 homo sapien
45	376.5	57.5	136	1	HV16_MOUSE	P01783 mus musculu

ALIGNMENTS

RESULT 1
Q6MZQ6
ID Q6MZQ6 PRELIMINARY; PRT; 475 AA.
AC Q6MZQ6;
DT 05-JUL-2004 (TrEMBLrel. 27, Created)
DT 05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT 05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE Hypothetical protein DKFZp686G11190.
GN Name=DKFZp686G11190;
OS Homo sapiens (Human).
OC Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX NCBI_TaxID=9606;
RN [1]
RP SEQUENCE FROM N.A.
RC TISSUE=Human esophagus tumor;
RG The German Human cDNA Consortium;
RA Lauber J., Bahr A., Mewes H.W., Weil B., Amid C., Osanger A., Fobo G.,
RA Han M., Wiemann S.;
RL Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR EMBL; BX640947; CAE45972.1; -.
DR HSSP; P01861; 1ADQ.
DR InterPro; IPR003599; Ig.
DR InterPro; IPR007110; Ig-like.
DR InterPro; IPR003597; Ig_c1.
DR InterPro; IPR003006; Ig_MHC.
DR InterPro; IPR003596; Ig_v.
DR Pfam; PF07654; C1-set; 3.
DR SMART; SM00409; IG; 2.
DR SMART; SM00407; IGc1; 3.
DR SMART; SM00406; IGv; 1.
DR PROSITE; PS50835; IG_LIKE; 4.
DR PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW Hypothetical protein.
SQ SEQUENCE 475 AA; 52043 MW; B7EAE255A26F4B8E CRC64;

Query Match 65.0%; Score 425.5; DB 2; Length 475;
Best Local Similarity 71.3%; Pred. No. 2.7e-36;
Matches 87; Conservative 12; Mismatches 18; Indels 5; Gaps 4;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:|||||||||||||||||||:|| || ||||||||||||||  |:: :|  ||
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFTFRNYAMNWVRQAPGKGLEWVSGISS-SGVNTY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK--YPHYYGS-SHWYFDVWGQG 116
                ||  | ||| | | | :| |||:||||:||||||||:  |  |  | ::||||||:|
Db         79 YADSVKGRFTISGDISTNTLYLQMHSLRADDTAVYYCARADYRDYQVSPAYWYFDVWGRG 138

Qy        117 TL 118
              ||
Db        139 TL 140
```

RESULT 2

```
Q96BB9
ID   Q96BB9      PRELIMINARY;      PRT;   597 AA.
AC   Q96BB9;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   IGHM protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (OCT-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC015760; AAH15760.1; -.
DR   PIR; S05271; S05271.
DR   PIR; S24260; S24260.
DR   HSSP; P01861; 1ADQ.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
SQ   SEQUENCE   597 AA;  65039 MW;  4FCA3AD8ECE263D9 CRC64;

  Query Match             64.1%;  Score 420;  DB 2;  Length 597;
  Best Local Similarity   70.0%;  Pred. No. 1.3e-35;
  Matches   84;  Conservative    8;  Mismatches   26;  Indels    2;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||:|||||||||||||||||||||::|::| ||||||||||||||  |:   |   |
Db         20 EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYAMNWVRQAPGKGLEWVSAISGSGGSTYY 79

Qy         61 AADPKRRPTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYF--DVWGQGTL 118
                 |   | ||| | | |: | ||||||||||||||||||| |  | :|  |    | ||||||
Db         80 ADSVKGRFTISRDNSRDTLYLQMNSLRAEDTAVYYCAKDPRGYSASGNYTREDYWGQGTL 139


RESULT 3
Q921A6
ID   Q921A6      PRELIMINARY;      PRT;   241 AA.
AC   Q921A6;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Anti-CEA 79 single chain Fv (Fragment).
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98170165; PubMed=9509426;
RA   Chung J.H., Choi S.J., Kim H.J., Kim I.J., Choi I.H., Lee S.D.,
RA   Yi K.S., Suh P.G., Ryu S.H., Chung H.K.;
RT   "Cloning and characterization of cDNAs encoding VH and VL of a
RT   monoclonal anti-CEA antibody (CEA 79) cross-reactive with NCA-95 and
RT   generation of a single-chain Fv molecule (scFv).";
RL   Mol. Cells 7:816-819(1997).
DR   EMBL; U88067; AAB48044.1; -.
DR   PIR; S19965; S19965.
DR   PIR; S19967; S19967.
DR   PIR; S19968; S19968.
DR   PIR; S26325; S26325.
DR   HSSP; P01607; 1BWW.
DR   SMART; SM00406; IGv; 2.
DR   PROSITE; PS50835; IG_LIKE; 2.
FT   NON_TER       1      1
FT   NON_TER     241    241
SQ   SEQUENCE   241 AA;  26086 MW;  0276887248E9C771 CRC64;

  Query Match             64.0%;  Score 419.5;  DB 2;  Length 241;
  Best Local Similarity   65.0%;  Pred. No. 5.5e-36;
  Matches   76;  Conservative   18;  Mismatches   18;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|:| :||  | :|| ::::|| |||||||:|||||||:|||||||:|:||||||||||||
Db          1 QVKLQQSGPELKKPGETVKISCKASGYTFTDYGMNWVKQAPGKGLKWMGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              | ||| || |||:|| ||||||:|:|: |||| |:||:          ||| |||||
Db         61 ADDFKGRFAFSLETSASTAYLQINNLKNEDTATYFCARKDLLR-----YFDYWGQGT 112


RESULT 4
Q9UL91
ID   Q9UL91      PRELIMINARY;      PRT;   118 AA.
AC   Q9UL91;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Myosin-reactive immunoglobulin heavy chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035023; AAD56259.1; -.
DR   PIR; PH0875; PH0875.
DR   PIR; S21205; S21205.
DR   PIR; S30531; S30531.
DR   HSSP; P01783; 1IGC.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     118    118
SQ   SEQUENCE   118 AA;  12843 MW;  D0633949F2AC149D CRC64;
```

```
  Query Match            63.4%;  Score 415.5;  DB 2;  Length 118;
  Best Local Similarity  68.6%;  Pred. No. 6.6e-36;
  Matches   81;  Conservative   12;  Mismatches   20;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||:||::| |||||||||||||| :|::      |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSTIITIYY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | :|:: ||||||||||||||||||:     | |   ||:|||||:
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR-----GDSSEAFDIWGQGTM 113


RESULT 5
HV21_MOUSE
ID   HV21_MOUSE     STANDARD;      PRT;   122 AA.
AC   P01790;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V region M511.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=81054880; PubMed=6776528;
RA   Robinson E.A., Appella E.;
RT   "Complete amino acid sequence of a mouse immunoglobulin alpha chain
RT   (MOPC 511).";
RL   Proc. Natl. Acad. Sci. U.S.A. 77:4909-4913(1980).
CC   -!- MISCELLANEOUS: This chain was isolated from a myeloma protein that
CC       binds phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     122    122
SQ   SEQUENCE   122 AA;  13652 MW;  9F4837731EA50207 CRC64;

  Query Match            63.4%;  Score 415.5;  DB 1;  Length 122;
  Best Local Similarity  66.9%;  Pred. No. 6.8e-36;
  Matches   81;  Conservative   13;  Mismatches   18;  Indels    9;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:|||||||||||||||||||| ||:||::: | ||||:||| |||:       | ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQSPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   |||||:||||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR---YYGSSYWYFDVWGAG 115

Qy        117 T 117
              |
Db        116 T 116


RESULT 6
HV18_MOUSE
ID   HV18_MOUSE     STANDARD;      PRT;   123 AA.
AC   P01787;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V regions TEPC 15/S107/HPCM1/HPCM2/HPCM3.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE (TEPC 15).
RX   MEDLINE=76222762; PubMed=819932;
RA   Rudikoff S., Potter M.;
RT   "Size differences among immunoglobulin heavy chains from
RT   phosphorylcholine-binding proteins.";
RL   Proc. Natl. Acad. Sci. U.S.A. 73:2109-2112(1976).
RN   [2]
RP   SEQUENCE FROM N.A. (H107).
RX   MEDLINE=80199926; PubMed=6769593; DOI=10.1016/0092-8674(80)90089-6;
RA   Early P., Huang H., Davis M., Calame K., Hood L.;
RT   "An immunoglobulin heavy chain variable region gene is generated from
RT   three segments of DNA: VH, D and JH.";
RL   Cell 19:981-992(1980).
RN   [3]
RP   SEQUENCE (S107).
RX   MEDLINE=76110488; PubMed=813561;
RX   DOI=10.1146/annurev.ge.09.120175.001513;
RA   Rudikoff S., Barstad P., Potter M., Hood L.;
RL   Unpublished results, cited by:
RL   Hood L., Campbell J.H., Elgin S.C.R.;
RL   Annu. Rev. Genet. 9:305-353(1975).
RN   [4]
RP   SEQUENCE (HPCM1; HPCM2 AND HPCM3).
RX   MEDLINE=81197602; PubMed=7231520;
RA   Gearhart P.J., Johnson N.D., Douglas R., Hood L.;
RT   "IgG antibodies to phosphorylcholine exhibit more diversity than their
RT   IgM counterparts.";
RL   Nature 291:29-34(1981).
CC   -!- MISCELLANEOUS: All those sequence appears to be identical.
CC   -!- MISCELLANEOUS: These chains were isolated from myeloma and
CC       hybridoma proteins that bind phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   PIR; A93804; AVMST5.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Hybridoma; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     123    123
SQ   SEQUENCE   123 AA;  13777 MW;  9D58086DE12F7000 CRC64;

  Query Match            63.1%;  Score 413;  DB 1;  Length 123;
  Best Local Similarity  66.9%;  Pred. No. 1.3e-35;
  Matches   81;  Conservative   12;  Mismatches   20;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:|||||||||||||||||||| ||:||::: | ||||:||| |||:       | ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQPPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   |||||:||||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR--DYYGSSYWYFDVWGAG 116

Qy        117 T 117
              |
Db        117 T 117


RESULT 7
Q9UL90
ID   Q9UL90      PRELIMINARY;      PRT;   113 AA.
AC   Q9UL90;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Myosin-reactive immunoglobulin heavy chain variable region
```

```
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035024; AAD56260.1; -.
DR   PIR; S78486; S78486.
DR   HSSP; P01772; 2FB4.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     113    113
SQ   SEQUENCE   113 AA;  12437 MW;  ED57FDD19086D07F CRC64;

  Query Match             62.6%;  Score 410;  DB 2;  Length 113;
  Best Local Similarity   70.6%;  Pred. No. 2.4e-35;
  Matches   84;  Conservative    8;  Mismatches   15;  Indels   12;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||:|||||||||||||||:||::|||:|||||||||||||| :|  | |   |
Db          1 EVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAFIR-YDGSNKY 59

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
               ||  | ||| | | ||:| ||||||||||||||||||  :|          ||||||
Db         60 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDLNY----------WGQGTL 108


RESULT 8
HV19_MOUSE
ID   HV19_MOUSE     STANDARD;      PRT;   123 AA.
AC   P01788;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V region H8.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE.
RA   Barstad P.;
RL   Thesis (1975), California Institute of Technology / Pasadena, U.S.A.
CC   -!- MISCELLANEOUS: This chain was isolated from a myeloma protein that
CC       binds phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     123    123
SQ   SEQUENCE   123 AA;  13805 MW;  9D581401912F7000 CRC64;

  Query Match             62.4%;  Score 409;  DB 1;  Length 123;
  Best Local Similarity   66.1%;  Pred. No. 3.3e-35;
  Matches   80;  Conservative   12;  Mismatches   21;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWV----GWINTYTG 56
              ||:|||||||||||||||||||| ||:||::: | |||| ||| |||:       | ||

Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQPPGKRLEWIAASRNKANDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   ||| |:|||||| |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR--DYYGBSYWYFDVWGAG 116

Qy        117 T 117
              |
Db        117 T 117


RESULT 9
Q6GMY2
ID   Q6GMY2      PRELIMINARY;      PRT;   606 AA.
AC   Q6GMY2;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073758; AAH73758.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 4.
DR   Pfam; PF00047; ig; 4.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
KW   Hypothetical protein.
SQ   SEQUENCE   606 AA;  66184 MW;  B6B38B51114E4C55 CRC64;

  Query Match             61.9%;  Score 405.5;  DB 2;  Length 606;
  Best Local Similarity   59.4%;  Pred. No. 4.6e-34;
  Matches   79;  Conservative   16;  Mismatches   17;  Indels   21;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
```

```
           :|||||||||||:|||||||||||||||:||::| |:|:|||||||||||| :|:: :    |
Db         20 QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGKGLEWVSYISSSSSYTNY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAK----------------YPHYYG 104
              |   | ||| | | :|:: ||||||||||||||||:                | :|||
Db         80 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARGGNGIAAAGRVVYAEDYYYYYG 139

Qy        105 SSHWYFDVWGQGT 117
                 |||||||
Db        140 -----MDVWGQGT 147


RESULT 10
HV3E_HUMAN
ID   HV3E_HUMAN     STANDARD;      PRT;   120 AA.
AC   P01766;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V-III region BRO.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=77117674; PubMed=65324; DOI=10.1016/0019-2791(76)90271-8;
RA   Capra J.D., Hopper J.E.;
RT   "Comparative studies on monotypic IgM lambda and IgG kappa from an
RT   individual patient. III. The complete amino acid sequence of the VH
RT   region of the IgM paraprotein.";
RL   Immunochemistry 13:995-999(1976).
CC   -!- MISCELLANEOUS: This chain was obtained from IgM isolated from the
CC       serum of a patient with malignant lymphoma of the Waldenstrom
CC       type.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   PIR; A02049; M3HUBW.
DR   HSSP; P01783; 1IGC.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region.
FT   DOMAIN        1    111       Ig-like.
FT   NON_TER     120    120
SQ   SEQUENCE   120 AA;  13227 MW;  D3F0428F7C2E6410 CRC64;

  Query Match             61.8%;  Score 405;  DB 1;  Length 120;
  Best Local Similarity   66.1%;  Pred. No. 8.6e-35;
  Matches   84;  Conservative    7;  Mismatches   20;  Indels   16;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||||:||: | ||||||  |||||||  | |  |:  |
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSYYNMNWVRQVTGKGLEWVSAIGT-AGDQYY 59

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP---------HYYGSSHWYFD 111
              |   | ||| | : ||:| || ||||||||||||||: |         :||||
Db         60 ADSVKGRFTISRNDSKNTLYLNMNSLRAEDTAVYYCARSPVSLVDGWLYYYYGS------ 113

Qy        112 VWGQGTL 118
              |||||||
Db        114 VWGQGTL 120


RESULT 11
Q8WU38
ID   Q8WU38      PRELIMINARY;      PRT;   573 AA.
AC   Q8WU38;
DT   01-MAR-2002 (TrEMBLrel. 20, Created)
DT   01-MAR-2002 (TrEMBLrel. 20, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JAN-2002) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC021276; AAH21276.1; -.
DR   PIR; S21205; S21205.
DR   PIR; S30532; S30532.
DR   HSSP; P18529; 1I8K.
DR   Pfam; PF07654; C1-set; 2.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 4.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW   Hypothetical protein.
SQ   SEQUENCE   573 AA;  62967 MW;  FD072344033AC530 CRC64;

  Query Match             61.8%;  Score 404.5;  DB 2;  Length 573;
  Best Local Similarity   65.9%;  Pred. No. 5.5e-34;
  Matches   81;  Conservative   11;  Mismatches   20;  Indels   11;  Gaps    2;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||| ||||||||||:|| :| |:|||||||||||||  |:  :|   |
Db         20 EVQLVESGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSGISWNSGSIGY 79

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKY------PHYYGSSHWYFDVWG 114
              |   | ||| | | :|:: ||||||||||||:|||||:      :|||      ||||
Db         80 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYCAKHGSGSYIGYYYG-----MDVWG 134

Qy        115 QGT 117
              |||
Db        135 QGT 137


RESULT 12
Q8WUK1
ID   Q8WUK1      PRELIMINARY;      PRT;   613 AA.
AC   Q8WUK1;
DT   01-MAR-2002 (TrEMBLrel. 20, Created)
DT   01-MAR-2002 (TrEMBLrel. 20, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   IGHM protein.
```

```
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (DEC-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC020240; AAH20240.1; -.
DR   PIR; F36005; F36005.
DR   PIR; G36005; G36005.
DR   PIR; PH1642; PH1642.
DR   PIR; PH1643; PH1643.
DR   PIR; PH1645; PH1645.
DR   PIR; PH1646; PH1646.
DR   PIR; PL0098; PL0098.
DR   PIR; PL0120; PL0120.
DR   PIR; S15590; S15590.
DR   PIR; S31116; S31116.
DR   PIR; S31119; S31119.
DR   PIR; S70442; S70442.
DR   HSSP; P01861; 1ADQ.
DR   Pfam; PF07654; C1-set; 4.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 5.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_3.
SQ   SEQUENCE   613 AA;  67295 MW;  60C7F5950671E315 CRC64;

  Query Match             61.6%;  Score 403.5;  DB 2;  Length 613;
  Best Local Similarity   66.9%;  Pred. No. 7.6e-34;
  Matches   83;  Conservative   10;  Mismatches   16;  Indels   15;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              :|||||||||:|||| ||||||||||:||::|||:|||||||||||||  | :| |   |
Db         20 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI-SYDGSNKY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHW-----YFDVWG 114
               ||  | ||| | | ||:| ||||||||||||||||||||      |      ||:||
Db         79 YADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK--------DWSEGVETFDIWG 130

Qy        115 QGTL 118
              |||:
Db        131 QGTM 134


RESULT 13
Q6MZV7
ID   Q6MZV7      PRELIMINARY;      PRT;   473 AA.
AC   Q6MZV7;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein DKFZp686C11235.
GN   Name=DKFZp686C11235;
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Human small intestine;
RG   The German Human cDNA Consortium;
RA   Bloecker H., Boecher M., Mewes H.W., Weil B., Amid C., Osanger A.,
RA   Fobo G., Han M., Wiemann S.;
RL   Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BX640853; CAE45920.1; -.
DR   HSSP; P01861; 1ADQ.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 3.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 3.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 4.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_2.
KW   Hypothetical protein.
SQ   SEQUENCE   473 AA;  52121 MW;  9476EAE4C0BFC447 CRC64;

  Query Match             61.5%;  Score 402.5;  DB 2;  Length 473;
  Best Local Similarity   65.6%;  Pred. No. 7.3e-34;
  Matches   80;  Conservative   16;  Mismatches   19;  Indels    7;  Gaps    4;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||||||||||||||||||||||:||::: |||||||||||||: :| | :|   |
Db         20 EIQLVESGGGLVQPGGSLRLSCAASGFTFSSFEMNWVRQAPGKGLEWLSYI-TRSGNTVY 78

Qy         61 AAD-FKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWY---FDVWGQG 116
               ||  : ||| | | :::: ||||||||||||||||:   :  :| ||   || ||||
Db         79 YADSLQGRFTISRDNARNSLYLQMNSLRAEDTAVYYCARQNEH--TSPWYPSFFDYWGQG 136

Qy        117 TL 118
              |
Db        137 IL 138


RESULT 14
HV23_MOUSE
ID   HV23_MOUSE     STANDARD;      PRT;   123 AA.
AC   P01792;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig heavy chain V region HPCG8.
OS   Mus musculus (Mouse).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
OX   NCBI_TaxID=10090;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=81197602; PubMed=7231520;
RA   Gearhart P.J., Johnson N.D., Douglas R., Hood L.;
RT   "IgG antibodies to phosphorylcholine exhibit more diversity than their
RT   IgM counterparts.";
RL   Nature 291:29-34(1981).
CC   -!- MISCELLANEOUS: This chain was isolated from a myeloma protein that
CC       binds phosphorylcholine.
CC   -!- SIMILARITY: Contains 1 immunoglobulin-like domain.
DR   PIR; E93256; AVMSH8.
```

```
DR   HSSP; P01789; 1MCP.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Hybridoma; Immunoglobulin V region.
FT   DOMAIN        1    114       Ig-like.
FT   NON_TER     123    123
SQ   SEQUENCE   123 AA;  13879 MW;  4559D3106CAF7D8D CRC64;

  Query Match             61.4%;  Score 402;  DB 1;  Length 123;
  Best Local Similarity   65.3%;  Pred. No. 1.8e-34;
  Matches   79;  Conservative   12;  Mismatches   22;  Indels    8;  Gaps    3;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINT----YTG 56
              ||:|||||||||||||||||||| ||:||::: | |||| ||| |||:          ||
Db          1 EVKLVESGGGLVQPGGSLRLSCATSGFTFSDFYMEWVRQPPGKRLEWIAASRNKAFDYTT 60

Qy         57 EPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQG 116
              |  |:|  | ||  | |||:|  |||||:|||||||:||||:   |||| :|||||||  |
Db         61 E--YSASVKGRFIVSRDTSQSILYLQMNALRAEDTAIYYCAR--DYYGSRYWYFDVWGAG 116

Qy        117 T 117
              |
Db        117 T 117


RESULT 15
Q9HCC1
ID   Q9HCC1      PRELIMINARY;      PRT;   112 AA.
AC   Q9HCC1;
DT   01-MAR-2001 (TrEMBLrel. 16, Created)
DT   01-MAR-2001 (TrEMBLrel. 16, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Single chain Fv (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RA   Kikuchi M., Takeda C., Tsujimoto Y., Asada S., Nagata K.;
RL   Submitted (OCT-2000) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; AB049915; BAB16829.1; -.
DR   HSSP; P01783; 1IGC.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     112    112
SQ   SEQUENCE   112 AA;  12243 MW;  24F1A45EC3B84788 CRC64;

  Query Match             61.3%;  Score 401.5;  DB 2;  Length 112;
  Best Local Similarity   66.1%;  Pred. No. 1.9e-34;
  Matches   78;  Conservative   11;  Mismatches   22;  Indels    7;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||:|:||||||:||||||:|| :|||:|||||||||||||  ||   |   |
Db          1 EVQLVESGGGVVRPGGSLRISCAASGFTFDDYGMSWVRQAPGKGLEWVSGINWNGGSTGY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |   | ||| | | :|:: |||||||||||||||||:         :  | ||||||
Db         61 ADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR-------RRYALDYWGQGTL 111


Search completed: March 14, 2005, 20:49:18
Job time : 84.807 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:21:17 ; Search time 88.0482 Seconds
(without alignments)
483.186 Million cell updates/sec

Title: US-09-723-752B-8
Perfect score: 576
Sequence: 1 DIQMTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 2105692 seqs, 386760381 residues

Total number of hits satisfying chosen parameters: 2105692

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : A_Geneseq_16Dec04:*
1: geneseqp1980s:*
2: geneseqp1990s:*
3: geneseqp2000s:*
4: geneseqp2001s:*
5: geneseqp2002s:*
6: geneseqp2003as:*
7: geneseqp2003bs:*
8: geneseqp2004s:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	576	100.0	110	3	AAB05897	Aab05897 Humanised
2	576	100.0	110	3	AAB13376	Aab13376 F(ab)-12
3	576	100.0	237	8	ADQ90721	Adq90721 Anti-VEGF
4	573	99.5	110	2	AAW70677	Aaw70677 Anti-VEGF
5	573	99.5	110	2	AAW70687	Aaw70687 Anti-VEGF
6	573	99.5	110	3	AAB13380	Aab13380 Anti-VEGF
7	573	99.5	110	5	ABP61256	Abp61256 Humanised
8	573	99.5	110	5	ABP61246	Abp61246 Humanised
9	573	99.5	214	7	ADC26154	Adc26154 Parent an
10	573	99.5	237	5	ABB81107	Abb81107 Anti-VEGF
11	573	99.5	237	5	ABP51952	Abp51952 Plasmid p
12	573	99.5	237	8	ADO14128	Ado14128 Plasmid p
13	573	99.5	237	8	ADO14131	Ado14131 Plasmid p
14	573	99.5	237	8	ADQ90703	Adq90703 Anti-VEGF
15	573	99.5	237	8	ADQ90701	Adq90701 Anti-VEGF
16	573	99.5	237	8	ADQ90705	Adq90705 Anti-VEGF
17	573	99.5	237	8	ADQ90709	Adq90709 Anti-VEGF
18	573	99.5	237	8	ADQ90723	Adq90723 Anti-VEGF
19	573	99.5	237	8	ADQ90707	Adq90707 Anti-VEGF
20	570	99.0	110	2	AAW70675	Aaw70675 Anti-VEGF
21	570	99.0	110	5	ABP61244	Abp61244 Humanised
22	567	98.4	108	2	AAW70618	Aaw70618 Anti-VEGF
23	567	98.4	108	5	ABP61187	Abp61187 Humanised
24	567	98.4	108	8	ADG31782	Adg31782 V(L) doma
25	567	98.4	108	8	ADG31768	Adg31768 V(L) doma
26	567	98.4	108	8	ADG31893	Adg31893 V(L) prot
27	567	98.4	110	2	AAW70673	Aaw70673 Anti-VEGF
28	567	98.4	110	5	ABP61242	Abp61242 Humanised
29	567	98.4	237	2	AAW70703	Aaw70703 Protein e
30	567	98.4	650	5	ABP61241	Abp61241 Phage-dis
31	564	97.9	108	8	ADG31770	Adg31770 V(L) doma
32	561	97.4	108	2	AAW70696	Aaw70696 Anti-VEGF
33	561	97.4	108	5	ABP61265	Abp61265 Humanised
34	559	97.0	107	2	AAW86804	Aaw86804 Variable
35	559	97.0	107	2	AAW70623	Aaw70623 Humanised
36	559	97.0	107	5	ABP61192	Abp61192 Humanised
37	556	96.5	107	2	AAW86805	Aaw86805 Variable
38	556	96.5	107	2	AAW70625	Aaw70625 Humanised
39	556	96.5	107	5	ABP61194	Abp61194 Humanised
40	556	96.5	214	7	ADC26157	Adc26157 Anti-VEGF
41	555	96.4	214	7	ADC26156	Adc26156 Anti-VEGF
42	554	96.2	110	2	AAW70685	Aaw70685 Anti-VEGF
43	554	96.2	110	2	AAW70681	Aaw70681 Anti-VEGF
44	554	96.2	110	2	AAW70683	Aaw70683 Anti-VEGF
45	554	96.2	110	2	AAW70679	Aaw70679 Anti-VEGF

ALIGNMENTS

RESULT 1
AAB05897
ID AAB05897 standard; peptide; 110 AA.
XX
AC AAB05897;
XX
DT 17-OCT-2000 (first entry)
XX
DE Humanised anti-VEGF antibody F(ab)-12 light chain variable domain.
XX
KW Humanised; F(ab)-12; light chain variable domain; antibody variant;
KW phage display; randomised library; cytostatic; antiarthritic;
KW antipsoriatic; antidiabetic; antiinflammatory; antiarteriosclerotic;
KW vascular endothelial growth factor; VEGF; breast cancer; lung cancer;
KW retinoblastoma; rheumatoid arthritis; psoriasis; atherosclerosis;
KW diabetic retinopathy; complementarity determining region; CDR.
XX
OS Homo sapiens.
OS Synthetic.
XX
PN WO200029584-A1.
XX
PD 25-MAY-2000.
XX
PF 16-NOV-1999; 99WO-US027153.
XX
PR 18-NOV-1998; 98US-0108945P.
XX
PA (GETH) GENENTECH INC.
XX
PI Chen YM, Lowman HB, Muller Y;
XX
DR WPI; 2000-387797/33.
XX
PT Antibody variants with higher binding affinity than native antibodies
PT useful for diagnosis, prevention and treatment of neoplastic and non-
PT neoplastic diseases comprises amino acid insertion in hypervariable
PT region.
XX
PS Disclosure; Fig 1A; 110pp; English.
XX
CC The present sequence is the light chain variable domain of F(ab)-12, a
CC humanised anti-vascular endothelial growth factor (VEGF) antibody. F(ab)-
CC 12 was the parent antibody used in the production of a large number of
CC antibody variants containing randomised peptide inserts within the
CC complementarity determining regions (CDRs). Phage display libraries were
CC subjected to eight rounds of selection to isolate variants with an
CC antigen binding affinity at least two-fold stronger than the binding

```
CC   affinity of parent antibody for the target VEGF antibody. The anti-VEGF
CC   antibody variants may be useful in diagnostic assays for detecting
CC   expression of VEGF in cells, tissue or serum. They may also be used in
CC   the prevention and treatment of neoplastic diseases such as breast
CC   cancer, lung cancer and retinoblastoma, and non-neoplastic diseases
CC   including rheumatoid arthritis, psoriasis, atherosclerosis, and diabetic
CC   and other proliferative retinopathies
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 576;  DB 3;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 7.5e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 2
AAB13376
ID   AAB13376 standard; protein; 110 AA.
XX
AC   AAB13376;
XX
DT   12-SEP-2003  (revised)
DT   21-NOV-2000  (first entry)
XX
DE   F(ab)-12 anti-VEGF antibody light chain variable domain.
XX
KW   Humanised; F(ab)-12; vascular endothelial cell growth factor; VEGF;
KW   antibody; antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Chimeric.
XX
FH   Key             Location/Qualifiers
FT   Region          24. .34
FT                   /label= CDR-L1
FT   Region          50. .56
FT                   /label= CDR-L2
FT   Region          89. .97
FT                   /label= CDR-L3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14A; 60pp; English.
XX
CC   The present sequence is the light chain variable domain of humanised anti
CC   -vascular endothelial cell growth factor (anti-VEGF) antibody F(ab)-12.
CC   It may be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Affinity matured anti-VEGF antibodies are
CC   also used as therapeutic agents. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay. (Updated on 12-SEP-
CC   2003 to standardise OS field)
XX
SQ   Sequence 110 AA;

  Query Match             100.0%;  Score 576;  DB 3;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 7.5e-33;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 3
ADQ90721
ID   ADQ90721 standard; protein; 237 AA.
XX
AC   ADQ90721;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody light chain protein SEQ ID NO:25.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; light chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
XX
PD   05-AUG-2004.
XX
PF   23-JAN-2004; 2004WO-US001844.
XX
PR   23-JAN-2003; 2003US-0442484P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons L;
XX
DR   WPI; 2004-562149/54.
DR   N-PSDB; ADQ90720.
XX
PT   Producing an antibody or antigen binding fragment in high yield in a cell
PT   culture, comprises expressing a variable domain with a modified framework
PT   region in a host cell.
XX
```

PS Example 6; SEQ ID NO 25; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the light chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 237 AA;

Query Match 100.0%; Score 576; DB 8; Length 237;
Best Local Similarity 100.0%; Pred. No. 1.5e-32;
Matches 110; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 24 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

RESULT 4
AAW70677
ID AAW70677 standard; peptide; 110 AA.
XX
AC AAW70677;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable light domain of variant Y0101.
XX
KW Light variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 9A; 100pp; English.
XX
CC The present sequence represents a variable light domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC also be used to treat other conditions that involve angiogenesis, e.g.
CC rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ Sequence 110 AA;

Query Match 99.5%; Score 573; DB 2; Length 110;
Best Local Similarity 99.1%; Pred. No. 1.2e-32;
Matches 109; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110

RESULT 5
AAW70687
ID AAW70687 standard; peptide; 110 AA.
XX
AC AAW70687;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable light domain of variant Y0317.
XX
KW Light variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth

```
PT   factor - for treatment of tumours, retinal disease and other angiogenic
PT   states, also related nucleic acid, vectors and transformed cells.
XX
PS   Claim 27; Fig 10A; 100pp; English.
XX
CC   The present sequence represents a variable light domain of an affinity-
CC   matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC   variant. The sequence is used in the course of the invention to produce
CC   the humanised anti-VEGF antibody of the invention. The humanised
CC   antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC   for treating or preventing tumours (of any type) and retinal disorders
CC   (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 110 AA;

  Query Match             99.5%;  Score 573;  DB 2;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.2e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 6
AAB13380
ID   AAB13380 standard; protein; 110 AA.
XX
AC   AAB13380;
XX
DT   21-NOV-2000  (first entry)
XX
DE   Anti-VEGF antibody YO317 light chain variable domain.
XX
KW   YO317; vascular endothelial cell growth factor; VEGF; antibody;
KW   antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Unidentified.
XX
FH   Key             Location/Qualifiers
FT   Region          24. .33
FT                   /label= CDR-L1
FT   Region          50. .56
FT                   /label= CDR-L2
FT   Region          89. .97
FT                   /label= CDR-L3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14A; 60pp; English.
XX
CC   The present sequence is the light chain variable region of the affinity
CC   matured anti-vascular endothelial cell growth factor (anti-VEGF) antibody
CC   YO317. Humanised F(ab)-12 and affinity matured anti-VEGF antibodies may
CC   be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay
XX
SQ   Sequence 110 AA;

  Query Match             99.5%;  Score 573;  DB 3;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.2e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 7
ABP61256
ID   ABP61256 standard; protein; 110 AA.
XX
AC   ABP61256;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0317 antibody variable light domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0317; light chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          24. .34
FT                   /label= CDR-L1
FT   Domain          50. .56
FT                   /label= CDR-L2
FT   Domain          89. .97
FT                   /label= CDR-L3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
```

```
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Claim 27; Fig 10; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary light chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 110 AA;

  Query Match             99.5%;  Score 573;  DB 5;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.2e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 8
ABP61246
ID   ABP61246 standard; protein; 110 AA.
XX
AC   ABP61246;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0101 antibody variable light domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0101; light chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          24. .34
FT                   /label= CDR-L1
FT   Domain          50. .57
FT                   /label= CDR-L2
FT   Domain          89. .97
FT                   /label= CDR-L3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
PR   06-AUG-1997;   97US-0054856P.
XX
PA   (BACA/) BACA M.
PA   (WELL/) WELLS J A.
PA   (PRES/) PRESTA L G.
PA   (LOWM/) LOWMAN H B.
PA   (CHEN/) CHEN Y M.
XX
PI   Baca M,  Wells JA,  Presta LG,  Lowman HB,  Chen YM;
XX
DR   WPI; 2002-517920/55.
XX
PT   New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT   or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Example 3; Fig 9; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary light chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 110 AA;

  Query Match             99.5%;  Score 573;  DB 5;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.2e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 9
ADC26154
ID   ADC26154 standard; protein; 214 AA.
XX
AC   ADC26154;
XX
DT   18-DEC-2003  (first entry)
XX
DE   Parent anti-VEGF Y0101 antibody wild-type light chain protein.
XX
KW   antibody variant; cytostatic; cancer; parent; anti-VEGF;
KW   vascular endothelial growth factor; Y0101; light chain; wild-type.
XX
OS   Unidentified.
XX
PN   WO2003068801-A2.
XX
PD   21-AUG-2003.
XX
PF   11-FEB-2003; 2003WO-US004184.
XX
PR   11-FEB-2002; 2002US-0355895P.
PR   10-SEP-2002; 2002US-0409685P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Lowman HB,  Marvin JS;
XX
DR   WPI; 2003-697521/66.
XX
PT   Making an antibody variant of a parent antibody specific to an antigen by
PT   identifying a target amino acid residue within the variable domain of the
PT   parent antibody and substituting the target residue with a different
PT   amino acid residue.
```

```
XX
PS   Example 1; SEQ ID NO 1; 81pp; English.
XX
CC   The invention relates to a novel method for making an antibody variant of
CC   a parent antibody specific to an antigen. This is achieved via
CC   identifying a target amino acid residue within the variable domain of the
CC   parent antibody and substituting the target residue with a different
CC   replacement amino acid residue such that the charge complementarity
CC   between the antibody and antigen is increased. The antibody variant of
CC   the invention demonstrates cytostatic activity whilst the method may be
CC   useful for treating cancer. The current sequence is that of the parent
CC   anti-VEGF (vascular endothelial growth factor) Y0101 antibody wild-type
CC   light chain protein of the invention.
XX
SQ   Sequence 214 AA;

  Query Match             99.5%;  Score 573;  DB 7;  Length 214;
  Best Local Similarity   99.1%;  Pred. No. 2.2e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 10
ABB81107
ID   ABB81107 standard; protein; 237 AA.
XX
AC   ABB81107;
XX
DT   05-NOV-2002  (first entry)
XX
DE   Anti-VEGF light chain fragment.
XX
KW   Immunoglobulin; promoter; cytostatic; antiinflammatory; immunomodulator;
KW   neuroprotective; CD11; tissue factor; vascular endothelial growth factor;
KW   VEGF.
XX
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /note= "STII signal sequence TIR-1"
FT   Protein         24. .237
FT                   /note= "anti-VEGF light chain"
XX
PN   WO200261090-A2.
XX
PD   08-AUG-2002.
XX
PF   13-DEC-2001; 2001WO-US048691.
XX
PR   14-DEC-2000; 2000US-0256164P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons LC,  Klimowski L,  Reilly DE,  Yansura DG;
XX
DR   WPI; 2002-619253/66.
DR   N-PSDB; ABN86646.
XX
PT   New polynucleotide comprising first and second promoter-cistron pairs,
PT   useful for diagnosing, treating or preventing diseases associated with
PT   abnormal expression and/or activity of antigens such as inflammatory
PT   disorders.
XX
PS   Disclosure; Fig 21A-C; 104pp; English.
XX
CC   The invention provides a polynucleotide, which encodes an immunoglobulin
CC   (Ig), comprising a first or second promoter-cistron pair consisting of a
CC   first or second promoter and cistron, respectively. The first cistron of
CC   the first promoter-cistron pair comprises a first translational
CC   initiation region (TIR-L) operably linked to a nucleic acid sequence
CC   encoding an Ig light chain and the second cistron of the second promoter-
CC   cistron pair comprises a second translational initiation region (TIR-H)
CC   operably linked to a nucleic acid sequence encoding an Ig heavy chain.
CC   Upon expression of the polynucleotide in a prokaryotic host cell, light
CC   and heavy chains are folded and assembled to form a biologically active
CC   Ig. The antibody of the invention is useful for diagnosing, treating or
CC   preventing diseases or conditions associated with abnormal expression and
CC   /or activity of one or more antigen molecules e.g. lymphoid malignancies,
CC   inflammatory, angiogenic, immunologic, neuronal, glial, astrocytal,
CC   hypothalamic or other glandular disorders. The present sequence
CC   represents the amino acid sequence of an anti-vascular endothelial growth
CC   factor (VEGF) light chain fragment of the cistron vector pxVG2AP11
XX
SQ   Sequence 237 AA;

  Query Match             99.5%;  Score 573;  DB 5;  Length 237;
  Best Local Similarity   99.1%;  Pred. No. 2.4e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 11
ABP51952
ID   ABP51952 standard; protein; 237 AA.
XX
AC   ABP51952;
XX
DT   09-OCT-2002  (first entry)
XX
DE   Plasmid pY0317 anti-VEGF Fab amino acid sequence SEQ ID NO:2 #1.
XX
KW   Bacterial host; protease; degP; prc; spr; anti-VEGF antibody; antibody;
KW   humanised; Apo2 ligand; anti-CD18; anti-tissue factor; 2C4; anti-CD20;
KW   anti-vascular endothelial growth factor; anti-Her-2; anti-CD40; Fab;
KW   anti-CD11a; Fab'; Fab'2; Fab'2-leucine zipper fusion; anti-VEGF Fab.
XX
OS   Mus sp.
OS   Escherichia coli.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Peptide         1. .23
FT                   /label= signal
FT   Protein         24. .237
FT                   /label= anti-VEGF_Fab
XX
PN   WO200248376-A2.
XX
PD   20-JUN-2002.
XX
PF   07-DEC-2001; 2001WO-US047581.
XX
PR   14-DEC-2000; 2000US-0256162P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Chen CY;
XX
DR   WPI; 2002-583522/62.
DR   N-PSDB; ABQ73919.
XX
```

PT Novel Escherichia coli strain useful for producing polypeptide, deficient
PT in degP and prc encoding protease, and harboring mutant spr gene, product
PT of gene suppresses growth phenotypes of strains harboring prc mutants.
XX
PS Example 1; Fig 1A-C; 63pp; English.
XX
CC The present invention describes an Escherichia coli strain (I) deficient
CC in chromosomal degP and prc encoding protease DegP and Prc, respectively,
CC and harbouring a mutant spr gene, the product of mutant spr gene
CC suppresses growth phenotypes exhibited by strains harbouring prc mutants.
CC (I) is useful for producing a polypeptide, by culturing (I) comprising
CC nucleic acid encoding the polypeptide, which is heterologous to the
CC strain, such that the nucleic acid is expressed, and recovering the
CC heterologous polypeptide from the strain. The heterologous polypeptide is
CC proteolytically sensitive. Culturing of (I) is performed in a fermentor
CC under conditions of high- or low-cell density fermentation. The
CC polypeptide is recovered from the periplasm or culture medium of the
CC strain. The polypeptide is an antibody (humanised or full-length
CC antibody) or Apo2 ligand. The antibody is an anti-CD18, anti-vascular
CC endothelial growth factor (VEGF), anti-tissue factor, 2C4, anti-Her-2,
CC anti-CD20, anti-CD40, or anti-CD11a antibody. The antibody is also an
CC antibody fragment having a light chain (kappa light chain). The antibody
CC fragment is a Fab, Fab', Fab'2 or Fab'2-leucine zipper fusion, anti-CD18
CC Fab'2-leucine zipper fusion, anti-tissue factor Fab'2-leucine zipper
CC fusion or anti-VEGF Fab, with or without a histidine or lysine tag, anti-
CC tissue factor Fab'2-leucine zipper fusion with a 6-histidine tag, or anti
CC -CD18 Fab'2-leucine zipper fusion with a 6-histidine tag, and anti-CD18
CC Fab'2-leucine zipper fusion with a 6-lysine tag. The present sequence
CC represents an anti-VEGF Fab amino acid sequence from the present
CC invention
XX
SQ Sequence 237 AA;

```
  Query Match             99.5%;  Score 573;  DB 5;  Length 237;
  Best Local Similarity   99.1%;  Pred. No. 2.4e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133
```

RESULT 12
ADO14128
ID ADO14128 standard; protein; 237 AA.
XX
AC ADO14128;
XX
DT 12-AUG-2004 (first entry)
XX
DE Plasmid pxVG2AP11 expression cassette light chain protein SEQ ID NO:8.
XX
KW antibody; variant heavy chain hinge region; immunoconjugate; cytostatic;
KW immunosuppressive; immunotherapy; tumour; cancer; immune disorder;
KW expression cassette; plasmid pxVG2AP11; anti-VEGF light chain.
XX
OS Synthetic.
XX
PN WO2004042017-A2.
XX
PD 21-MAY-2004.
XX
PF 30-OCT-2003; 2003WO-US034610.
XX
PR 31-OCT-2002; 2002US-0422952P.
XX
PA (GETH) GENENTECH INC.
XX
PI Reilly D, Yansura DG;
XX
DR WPI; 2004-390607/36.
DR N-PSDB; ADO14127.
XX
PT New antibody comprising a variant heavy chain hinge region incapable of
PT inter-heavy chain disulfide linkage, useful for treating, preventing,
PT diagnosing, delaying or preventing a disease, e.g. tumor, cancer or
PT immune disorder.
XX
PS Example 1; SEQ ID NO 8; 124pp; English.
XX
CC The present invention describes an antibody comprising a variant heavy
CC chain hinge region incapable of inter-heavy chain disulfide linkage. Also
CC described: (1) an antibody lacking inter-heavy chain disulfide linkage;
CC (2) an immunoconjugate comprising the antibody conjugated with a
CC heterologous moiety; (3) a composition comprising the antibody or
CC immunoconjugate, and carrier; (4) an article of manufacture comprising
CC the composition in a container; (5) a polynucleotide encoding the
CC antibody or immunoconjugate, or a variant immunoglobulin heavy chain
CC incapable of inter-heavy chain disulfide linkage; (6) a recombinant
CC vector for expressing the antibody or immunoconjugate; (7) a host cell
CC comprising the recombinant vector; (8) expressing in a host cell an
CC antibody of interest in which at least one inter-heavy chain disulfide
CC linkage is eliminated, and recovering the antibody from the host cell;
CC (9) an aglycosylated antibody produced by the method; and (10) treating,
CC preventing, diagnosing, delaying or preventing a disease in a subject.
CC The antibody has cytostatic and immunosuppressive activities, and can be
CC used in immunotherapy. The antibody, immunoconjugate and methods are
CC useful for treating, preventing, diagnosing, delaying or preventing a
CC disease, e.g. tumour, cancer or immune disorder. The present sequence
CC represents the anti-VEGF light chain from the expression cassette of
CC plasmid pxVG2AP11, which is used in the exemplification of the present
CC invention.
XX
SQ Sequence 237 AA;

```
  Query Match             99.5%;  Score 573;  DB 8;  Length 237;
  Best Local Similarity   99.1%;  Pred. No. 2.4e-32;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133
```

RESULT 13
ADO14131
ID ADO14131 standard; protein; 237 AA.
XX
AC ADO14131;
XX
DT 12-AUG-2004 (first entry)
XX
DE Plasmid pxVG11VNERK expression cassette light chain protein SEQ ID NO:11.
XX
KW antibody; variant heavy chain hinge region; immunoconjugate; cytostatic;
KW immunosuppressive; immunotherapy; tumour; cancer; immune disorder;
KW expression cassette; plasmid pxVG11VNERK; anti-VEGF light chain.
XX
OS Synthetic.
XX
PN WO2004042017-A2.
XX
PD 21-MAY-2004.
XX
PF 30-OCT-2003; 2003WO-US034610.
XX
PR 31-OCT-2002; 2002US-0422952P.
XX

PA (GETH) GENENTECH INC.
XX
PI Reilly D, Yansura DG;
XX
DR WPI; 2004-390607/36.
DR N-PSDB; ADO14130.
XX
PT New antibody comprising a variant heavy chain hinge region incapable of
PT inter-heavy chain disulfide linkage, useful for treating, preventing,
PT diagnosing, delaying or preventing a disease, e.g. tumor, cancer or
PT immune disorder.
XX
PS Example 1; SEQ ID NO 11; 124pp; English.
XX
CC The present invention describes an antibody comprising a variant heavy
CC chain hinge region incapable of inter-heavy chain disulfide linkage. Also
CC described: (1) an antibody lacking inter-heavy chain disulfide linkage;
CC (2) an immunoconjugate comprising the antibody conjugated with a
CC heterologous moiety; (3) a composition comprising the antibody or
CC immunoconjugate, and carrier; (4) an article of manufacture comprising
CC the composition in a container; (5) a polynucleotide encoding the
CC antibody or immunoconjugate, or a variant immunoglobulin heavy chain
CC incapable of inter-heavy chain disulfide linkage; (6) a recombinant
CC vector for expressing the antibody or immunoconjugate; (7) a host cell
CC comprising the recombinant vector; (8) expressing in a host cell an
CC antibody of interest in which at least one inter-heavy chain disulfide
CC linkage is eliminated, and recovering the antibody from the host cell;
CC (9) an aglycosylated antibody produced by the method; and (10) treating,
CC preventing, diagnosing, delaying or preventing a disease in a subject.
CC The antibody has cytostatic and immunosuppressive activities, and can be
CC used in immunotherapy. The antibody, immunoconjugate and methods are
CC useful for treating, preventing, diagnosing, delaying or preventing a
CC disease, e.g. tumour, cancer or immune disorder. The present sequence
CC represents the anti-VEGF light chain from the expression cassette of
CC plasmid pxVG11VNERK, which is used in the exemplification of the present
CC invention.
XX
SQ Sequence 237 AA;

Query Match 99.5%; Score 573; DB 8; Length 237;
Best Local Similarity 99.1%; Pred. No. 2.4e-32;
Matches 109; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

RESULT 14
ADQ90703
ID ADQ90703 standard; protein; 237 AA.
XX
AC ADQ90703;
XX
DT 21-OCT-2004 (first entry)
XX
DE Anti-VEGF antibody YO317 light chain protein SEQ ID NO:7.
XX
KW antibody; antigen binding fragment; cell culture; variable domain;
KW modified framework region; hypervariable region; cytostatic;
KW antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW tumour; inflammatory disorder; angiogenic disorder;
KW immunological disorder; anti-VEGF antibody;
KW anti vascular endothelial cell growth factor antibody; light chain.
XX
OS Homo sapiens.
OS Synthetic.
XX
PN WO2004065417-A2.
XX
PD 05-AUG-2004.
XX
PF 23-JAN-2004; 2004WO-US001844.
XX
PR 23-JAN-2003; 2003US-0442484P.
XX
PA (GETH) GENENTECH INC.
XX
PI Simmons L;
XX
DR WPI; 2004-562149/54.
DR N-PSDB; ADQ90702.
XX
PT Producing an antibody or antigen binding fragment in high yield in a cell
PT culture, comprises expressing a variable domain with a modified framework
PT region in a host cell.
XX
PS Example 2; SEQ ID NO 7; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the light chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 237 AA;

Query Match 99.5%; Score 573; DB 8; Length 237;
Best Local Similarity 99.1%; Pred. No. 2.4e-32;
Matches 109; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
Db 84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133

RESULT 15
ADQ90701
ID ADQ90701 standard; protein; 237 AA.
XX
AC ADQ90701;
XX
DT 21-OCT-2004 (first entry)
XX
DE Anti-VEGF antibody VNERK light chain protein SEQ ID NO:5.
XX
KW antibody; antigen binding fragment; cell culture; variable domain;

KW modified framework region; hypervariable region; cytostatic;
KW antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW tumour; inflammatory disorder; angiogenic disorder;
KW immunological disorder; anti-VEGF antibody;
KW anti vascular endothelial cell growth factor antibody; light chain.
XX
OS Homo sapiens.
OS Synthetic.
XX
PN WO2004065417-A2.
XX
PD 05-AUG-2004.
XX
PF 23-JAN-2004; 2004WO-US001844.
XX
PR 23-JAN-2003; 2003US-0442484P.
XX
PA (GETH) GENENTECH INC.
XX
PI Simmons L;
XX
DR WPI; 2004-562149/54.
DR N-PSDB; ADQ90700.
XX
PT Producing an antibody or antigen binding fragment in high yield in a cell
PT culture, comprises expressing a variable domain with a modified framework
PT region in a host cell.
XX
PS Example 2; SEQ ID NO 5; 161pp; English.
XX
CC The present invention describes a method for producing an antibody or
CC antigen binding fragment in high yield in a cell culture. The method
CC comprises expressing a variable domain of the antibody or antigen binding
CC fragment comprising a modified framework region (FR) in a host cell, and
CC recovering the antibody or antigen binding fragment variable domain
CC comprising the modified framework from the host cell. The modified FR in
CC the method described above has a substitution of at least one amino acid
CC position with a different amino acid, where the different amino acid is
CC the amino acid found at the corresponding FR position of a human subgroup
CC variable domain consensus sequence that has a hypervariable region 1
CC (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC The antibody or antigen binding fragment variable domain comprises the
CC modified FR that has improved yield in cell culture compared to an
CC unmodified antibody or antigen-binding fragment. The antibody and antigen
CC binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC immunomodulatory activities, and can be used in antibody therapy. The
CC methods and compositions of the present invention are useful for
CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the light chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 237 AA;

Query Match 99.5%; Score 573; DB 8; Length 237;
Best Local Similarity 99.1%; Pred. No. 2.4e-32;
Matches 109; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133
```

Search completed: March 14, 2005, 20:39:16

Job time : 89.0482 secs

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:30:13 ; Search time 22.6754 Seconds
(without alignments)
362.127 Million cell updates/sec

Title: US-09-723-752B-8
Perfect score: 576
Sequence: 1 DIQMTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 513545 seqs, 74649064 residues

Total number of hits satisfying chosen parameters: 513545

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Issued_Patents_AA:*
1: /cgn2_6/ptodata/1/iaa/5A_COMB.pep:*
2: /cgn2_6/ptodata/1/iaa/5B_COMB.pep:*
3: /cgn2_6/ptodata/1/iaa/6A_COMB.pep:*
4: /cgn2_6/ptodata/1/iaa/6B_COMB.pep:*
5: /cgn2_6/ptodata/1/iaa/PCTUS_COMB.pep:*
6: /cgn2_6/ptodata/1/iaa/backfiles1.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	576	100.0	110	4	US-09-440-781-94	Sequence 94, Appl
2	567	98.4	491	4	US-10-011-125A-2	Sequence 2, Appli
3	554	96.2	110	4	US-09-440-781-95	Sequence 95, Appl
4	525	91.1	214	2	US-07-934-373C-40	Sequence 40, Appl
5	525	91.1	214	2	US-08-788-800-11	Sequence 11, Appl
6	525	91.1	214	3	US-08-437-642B-40	Sequence 40, Appl
7	525	91.1	214	3	US-09-097-309-2	Sequence 2, Appli
8	525	91.1	214	3	US-09-097-171A-2	Sequence 2, Appli
9	525	91.1	214	3	US-09-460-587-2	Sequence 2, Appli
10	525	91.1	214	4	US-09-940-166A-2	Sequence 2, Appli
11	525	91.1	214	5	PCT-US93-07832-40	Sequence 40, Appl
12	525	91.1	233	2	US-07-934-373C-25	Sequence 25, Appl
13	525	91.1	233	3	US-08-437-642B-25	Sequence 25, Appl
14	525	91.1	233	4	US-08-146-206C-25	Sequence 25, Appl
15	525	91.1	233	4	US-09-705-686-25	Sequence 25, Appl
16	525	91.1	233	4	US-09-705-392A-25	Sequence 25, Appl
17	525	91.1	233	4	US-09-705-398-25	Sequence 25, Appl
18	525	91.1	233	5	PCT-US93-07832-25	Sequence 25, Appl
19	525	91.1	237	3	US-09-097-309-6	Sequence 6, Appli
20	525	91.1	237	3	US-09-097-171A-10	Sequence 10, Appl
21	525	91.1	237	3	US-09-422-712B-2	Sequence 2, Appli
22	525	91.1	237	3	US-09-607-756-2	Sequence 2, Appli
23	525	91.1	237	3	US-09-460-587-6	Sequence 6, Appli
24	525	91.1	237	4	US-09-940-166A-6	Sequence 6, Appli
25	522	90.6	214	1	US-08-458-516-12	Sequence 12, Appl
26	521	90.5	109	2	US-07-934-373C-47	Sequence 47, Appl
27	521	90.5	109	3	US-08-437-642B-47	Sequence 47, Appl
28	517	89.8	214	2	US-07-934-373C-39	Sequence 39, Appl
29	517	89.8	214	3	US-08-437-642B-39	Sequence 39, Appl
30	517	89.8	214	5	PCT-US93-07832-39	Sequence 39, Appl
31	514	89.2	107	2	US-07-934-373C-17	Sequence 17, Appl
32	514	89.2	107	3	US-08-437-642B-17	Sequence 17, Appl
33	514	89.2	107	4	US-08-146-206C-17	Sequence 17, Appl
34	514	89.2	107	4	US-09-705-686-17	Sequence 17, Appl
35	514	89.2	107	4	US-09-705-392A-17	Sequence 17, Appl
36	514	89.2	107	4	US-09-705-398-17	Sequence 17, Appl
37	514	89.2	107	5	PCT-US93-07832-17	Sequence 17, Appl
38	514	89.2	108	3	US-08-974-899-3	Sequence 3, Appli
39	514	89.2	108	4	US-09-795-798-3	Sequence 3, Appli
40	514	89.2	127	3	US-08-649-100-33	Sequence 33, Appl
41	510	88.5	109	2	US-07-934-373C-3	Sequence 3, Appli
42	510	88.5	109	3	US-08-437-642B-3	Sequence 3, Appli
43	510	88.5	109	4	US-08-146-206C-3	Sequence 3, Appli
44	510	88.5	109	4	US-09-705-686-3	Sequence 3, Appli
45	510	88.5	109	4	US-09-705-392A-3	Sequence 3, Appli

ALIGNMENTS

```
RESULT 1
US-09-440-781-94
; Sequence 94, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 94
;   LENGTH: 110
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-110
;   OTHER INFORMATION: humanized antibody light chain variable domain
US-09-440-781-94

  Query Match             100.0%;  Score 576;  DB 4;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 2.8e-47;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 2
US-10-011-125A-2
; Sequence 2, Application US/10011125A
; Patent No. 6828121
; GENERAL INFORMATION:
;  APPLICANT: Chen, Christina Yu-Ching
;  TITLE OF INVENTION: BACTERIAL HOST STRAINS
;  FILE REFERENCE: P1804R1
;  CURRENT APPLICATION NUMBER: US/10/011,125A
;  CURRENT FILING DATE:  2001-12-07
;  PRIOR APPLICATION NUMBER: US 60/256,162
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 12
; SEQ ID NO 2
;   LENGTH: 491
;   TYPE: PRT
```

```
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: Sequence is synthesized.
; Patent No. 6828121
US-10-011-125A-2

  Query Match             98.4%;  Score 567;  DB 4;  Length 491;
  Best Local Similarity   97.3%;  Pred. No. 1.1e-45;
  Matches  107;  Conservative    3;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||:|||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKLLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 3
US-09-440-781-95
; Sequence 95, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 95
;   LENGTH: 110
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-110
;   OTHER INFORMATION: humanized antibody light chain variable domain
US-09-440-781-95

  Query Match             96.2%;  Score 554;  DB 4;  Length 110;
  Best Local Similarity   94.5%;  Pred. No. 3.4e-45;
  Matches  104;  Conservative    4;  Mismatches    2;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||| |:: :||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 4
US-07-934-373C-40
; Sequence 40, Application US/07934373C
; Patent No. 5821337
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  48
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/07/934,373C
;      FILING DATE:  21-Aug-1992
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-07-934-373C-40

  Query Match             91.1%;  Score 525;  DB 2;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 5
US-08-788-800-11
; Sequence 11, Application US/08788800
; Patent No. 5914112
;  GENERAL INFORMATION:
;    APPLICANT:  Bednar, Martin M.
;    APPLICANT:  Thomas, G. Roger
;    APPLICANT:  Gross, Cordell E.
;    TITLE OF INVENTION:  ANTI-CD18 ANTIBODIES IN STROKE
;    NUMBER OF SEQUENCES:  15
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  460 Point San Bruno Blvd
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/788,800
;      FILING DATE:  22-Jan-1997
;      CLASSIFICATION:  424
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0987r1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  415/225-1994
;      TELEFAX:  415/952-9881
;      TELEX:  910/371-7168
;  INFORMATION FOR SEQ ID NO:  11:
```

```
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-788-800-11

  Query Match           91.1%;  Score 525;  DB 2;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 6
US-08-437-642B-40
; Sequence 40, Application US/08437642B
; Patent No. 6054297
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  47
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/437,642B
;      FILING DATE:  09-May-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/934373
;      FILING DATE:  21-AUG-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/146206
;      FILING DATE:  17-NOV-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2C1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-437-642B-40

  Query Match           91.1%;  Score 525;  DB 3;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 7
US-09-097-309-2
; Sequence 2, Application US/09097309
; Patent No. 6121428
;  GENERAL INFORMATION:
;    APPLICANT:  Blank, Gregory S.
;    APPLICANT:  Narindray, Daljit S.
;    APPLICANT:  Zapata, Gerardo A.
;    TITLE OF INVENTION:  Protein Recovery
;    NUMBER OF SEQUENCES:  7
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/097,309
;      FILING DATE:  12-Jun-1998
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/050951
;      FILING DATE:  13-JUN-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Schwartz, Timothy R.
;      REGISTRATION NUMBER:  32171
;      REFERENCE/DOCKET NUMBER:  P1105R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-7467
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-097-309-2

  Query Match           91.1%;  Score 525;  DB 3;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 8
US-09-097-171A-2
; Sequence 2, Application US/09097171A
; Patent No. 6171586
;  GENERAL INFORMATION:
;    APPLICANT:  Lam, Xanthe M.
;    APPLICANT:  Oeswein, James Q.
```

```
;    APPLICANT:  Ongpipattanakul, Boonsri
;    APPLICANT:  Shahrokh, Zahra
;    APPLICANT:  Wang, Sharon X.
;    APPLICANT:  Weissburg, Robert P.
;    APPLICANT:  Wong, Rita L.
;    TITLE OF INVENTION:  Antibody Formulation
;    NUMBER OF SEQUENCES:  11
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/097,171A
;      FILING DATE:
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/874897
;      FILING DATE:  13-JUN-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P1089R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-097-171A-2

  Query Match             91.1%;  Score 525;  DB 3;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 9
US-09-460-587-2
; Sequence 2, Application US/09460587
; Patent No. 6322997
;  GENERAL INFORMATION:
;    APPLICANT:  Blank, Gregory S.
;    APPLICANT:  Narindray, Daljit S.
;    APPLICANT:  Zapata, Gerardo A.
;    TITLE OF INVENTION:  Protein Recovery
;    NUMBER OF SEQUENCES:  7
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/460,587
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  09/097,309
;      FILING DATE:
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Schwartz, Timothy R.
;      REGISTRATION NUMBER:  32171
;      REFERENCE/DOCKET NUMBER:  P1105R1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-7467
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  2:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-460-587-2

  Query Match             91.1%;  Score 525;  DB 3;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 10
US-09-940-166A-2
; Sequence 2, Application US/09940166A
; Patent No. 6716598
;  GENERAL INFORMATION:
;       APPLICANT: Blank, Gregory S.
;                  Narindray, Daljit S.
;                  Zapata, Gerardo A.
;       TITLE OF INVENTION: Protein Recovery
;       NUMBER OF SEQUENCES: 7
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/09/940,166A
;            FILING DATE: 27-Aug-2001
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/097,309
;            FILING DATE: 13-JUN-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Schwartz, Timothy R.
;            REGISTRATION NUMBER: 32171
;            REFERENCE/DOCKET NUMBER: P1105R1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-7467
;            TELEFAX: 650/952-9881
;  INFORMATION FOR SEQ ID NO: 2:
```

```
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 214 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 2:
US-09-940-166A-2

  Query Match             91.1%;  Score 525;  DB 4;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 11
PCT-US93-07832-40
; Sequence 40, Application PC/TUS9307832
;  GENERAL INFORMATION:
;    APPLICANT:  Genentech, Inc.
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  40
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  460 Point San Bruno Blvd
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  5.25 inch, 360 Kb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  patin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US93/07832
;      FILING DATE:  19930820
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/934373
;      FILING DATE:  21-AUG-1992
;    ATTORNEY/AGENT INFORMATION:
;      NAME:
;      REGISTRATION NUMBER:
;      REFERENCE/DOCKET NUMBER:  709P2PCT
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:
;      TELEFAX:  415/952-9881
;      TELEX:  910/371-7168
;  INFORMATION FOR SEQ ID NO:  40:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  214 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
PCT-US93-07832-40

  Query Match             91.1%;  Score 525;  DB 5;  Length 214;
  Best Local Similarity   90.9%;  Pred. No. 3.9e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 110


RESULT 12
US-07-934-373C-25
; Sequence 25, Application US/07934373C
; Patent No. 5821337
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
;    NUMBER OF SEQUENCES:  48
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/07/934,373C
;      FILING DATE:  21-Aug-1992
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US92/05126
;      FILING DATE:  15-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  25:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  233 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-07-934-373C-25

  Query Match             91.1%;  Score 525;  DB 2;  Length 233;
  Best Local Similarity   90.9%;  Pred. No. 4.3e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:||||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 13
US-08-437-642B-25
; Sequence 25, Application US/08437642B
; Patent No. 6054297
;  GENERAL INFORMATION:
;    APPLICANT:  Paul J. Carter
;    APPLICANT:  Leonard G. Presta
;    TITLE OF INVENTION:  Immunoglobulin Variants
```

```
;     NUMBER OF SEQUENCES:  47
;     CORRESPONDENCE ADDRESS:
;       ADDRESSEE:  Genentech, Inc.
;       STREET:  1 DNA Way
;       CITY:  South San Francisco
;       STATE:  California
;       COUNTRY:  USA
;       ZIP:  94080
;     COMPUTER READABLE FORM:
;       MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;       COMPUTER:  IBM PC compatible
;       OPERATING SYSTEM:  PC-DOS/MS-DOS
;       SOFTWARE:  WinPatin (Genentech)
;     CURRENT APPLICATION DATA:
;       APPLICATION NUMBER:  US/08/437,642B
;       FILING DATE:  09-May-1995
;       CLASSIFICATION:  530
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  07/934373
;       FILING DATE:  21-AUG-1992
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  08/146206
;       FILING DATE:  17-NOV-1993
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  PCT/US92/05126
;       FILING DATE:  15-JUN-1992
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  07/715272
;       FILING DATE:  14-JUN-1991
;     ATTORNEY/AGENT INFORMATION:
;       NAME:  Lee, Wendy M.
;       REGISTRATION NUMBER:  40,378
;       REFERENCE/DOCKET NUMBER:  P0709P2C1
;     TELECOMMUNICATION INFORMATION:
;       TELEPHONE:  650/225-1994
;       TELEFAX:  650/952-9881
;   INFORMATION FOR SEQ ID NO:  25:
;     SEQUENCE CHARACTERISTICS:
;       LENGTH:  233 amino acids
;       TYPE:  Amino Acid
;       TOPOLOGY:  Linear
US-08-437-642B-25

  Query Match             91.1%;  Score 525;  DB 3;  Length 233;
  Best Local Similarity   90.9%;  Pred. No. 4.3e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 14
US-08-146-206C-25
; Sequence 25, Application US/08146206C
; Patent No. 6407213
;  GENERAL INFORMATION:
;    APPLICANT:  Carter, Paul J.
;    APPLICANT:  Presta, Leonard G.
;    TITLE OF INVENTION:  Method for Making Humanized Antibodies
;    NUMBER OF SEQUENCES:  26
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/146,206C
;      FILING DATE:  17-No. 6407213-1993
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  07/715272
;      FILING DATE:  14-JUN-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Lee, Wendy M.
;      REGISTRATION NUMBER:  40,378
;      REFERENCE/DOCKET NUMBER:  P0709P1
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1994
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  25:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  233 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-08-146-206C-25

  Query Match             91.1%;  Score 525;  DB 4;  Length 233;
  Best Local Similarity   90.9%;  Pred. No. 4.3e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| |||||:|||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


RESULT 15
US-09-705-686-25
; Sequence 25, Application US/09705686
; Patent No. 6639055
;  GENERAL INFORMATION:
;       APPLICANT: Carter, Paul J.
;                  Presta, Leonard G.
;       TITLE OF INVENTION: Method for Making Humanized Antibodies
;       NUMBER OF SEQUENCES: 26
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/09/705,686
;            FILING DATE: 02-No. 6639055-2000
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 08/146206
;            FILING DATE: 17-NOV-1993
;            APPLICATION NUMBER: 07/715272
;            FILING DATE: 14-JUN-1991
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Lee, Wendy M.
;            REGISTRATION NUMBER: 40,378
;            REFERENCE/DOCKET NUMBER: P0709P1D3
;       TELECOMMUNICATION INFORMATION:
```

```
;             TELEPHONE: 650/225-1994
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 25:
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 233 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 25:
US-09-705-686-25

  Query Match            91.1%;  Score 525;  DB 4;  Length 233;
  Best Local Similarity  90.9%;  Pred. No. 4.3e-42;
  Matches  100;  Conservative    7;  Mismatches    3;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| ||||:|||||||||||||||:|||:||:|||||||
Db         20 DIQMTQSPSSLSASVGDRVTITCRASQDINNYLNWYQQKPGKAPKLLIYYTSTLHSGVPS 79

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:|||||||||||||||||| :|:| ||||||||||||||
Db         80 RFSGSGSGTDYTLTISSLQPEDFATYYCQQGNTLPPTFGQGTKVEIKRTV 129


Search completed: March 14, 2005, 20:43:52
Job time : 23.6754 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:22:02 ; Search time 41.0088 Seconds
(without alignments)
884.760 Million cell updates/sec

Title: US-09-723-752B-8
Perfect score: 576
Sequence: 1 DIQMTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1396920 seqs, 329844858 residues

Total number of hits satisfying chosen parameters: 1396920

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA:*
1: /cgn2_6/ptodata/2/pubpaa/US07_PUBCOMB.pep:*
2: /cgn2_6/ptodata/2/pubpaa/PCT_NEW_PUB.pep:*
3: /cgn2_6/ptodata/2/pubpaa/US06_NEW_PUB.pep:*
4: /cgn2_6/ptodata/2/pubpaa/US06_PUBCOMB.pep:*
5: /cgn2_6/ptodata/2/pubpaa/US07_NEW_PUB.pep:*
6: /cgn2_6/ptodata/2/pubpaa/PCTUS_PUBCOMB.pep:*
7: /cgn2_6/ptodata/2/pubpaa/US08_NEW_PUB.pep:*
8: /cgn2_6/ptodata/2/pubpaa/US08_PUBCOMB.pep:*
9: /cgn2_6/ptodata/2/pubpaa/US09A_PUBCOMB.pep:*
10: /cgn2_6/ptodata/2/pubpaa/US09B_PUBCOMB.pep:*
11: /cgn2_6/ptodata/2/pubpaa/US09C_PUBCOMB.pep:*
12: /cgn2_6/ptodata/2/pubpaa/US09_NEW_PUB.pep:*
13: /cgn2_6/ptodata/2/pubpaa/US10A_PUBCOMB.pep:*
14: /cgn2_6/ptodata/2/pubpaa/US10B_PUBCOMB.pep:*
15: /cgn2_6/ptodata/2/pubpaa/US10C_PUBCOMB.pep:*
16: /cgn2_6/ptodata/2/pubpaa/US10D_PUBCOMB.pep:*
17: /cgn2_6/ptodata/2/pubpaa/US10_NEW_PUB.pep:*
18: /cgn2_6/ptodata/2/pubpaa/US11_NEW_PUB.pep:*
19: /cgn2_6/ptodata/2/pubpaa/US60_NEW_PUB.pep:*
20: /cgn2_6/ptodata/2/pubpaa/US60_PUBCOMB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	576	100.0	110	14	US-10-234-671-8	Sequence 8, Appli
2	576	100.0	110	15	US-10-624-153-94	Sequence 94, Appl
3	573	99.5	110	9	US-09-056-160B-107	Sequence 107, App
4	573	99.5	110	9	US-09-056-160B-117	Sequence 117, App
5	573	99.5	110	14	US-10-234-671-105	Sequence 105, App
6	573	99.5	110	14	US-10-234-671-115	Sequence 115, App
7	573	99.5	213	16	US-10-379-392-135	Sequence 135, App
8	573	99.5	213	16	US-10-379-392-137	Sequence 137, App
9	573	99.5	213	16	US-10-379-392-139	Sequence 139, App
10	573	99.5	214	15	US-10-364-953-1	Sequence 1, Appli
11	573	99.5	237	14	US-10-020-786-10	Sequence 10, Appl
12	573	99.5	237	17	US-10-697-995-8	Sequence 8, Appli
13	573	99.5	237	17	US-10-697-995-11	Sequence 11, Appl
14	570	99.0	110	9	US-09-056-160B-105	Sequence 105, App
15	570	99.0	110	14	US-10-234-671-103	Sequence 103, App
16	569	98.8	213	16	US-10-379-392-155	Sequence 155, App
17	568	98.6	213	16	US-10-379-392-153	Sequence 153, App
18	567	98.4	108	9	US-09-056-160B-8	Sequence 8, Appli
19	567	98.4	108	13	US-10-153-159-2	Sequence 2, Appli
20	567	98.4	108	13	US-10-153-159-16	Sequence 16, Appl
21	567	98.4	108	14	US-10-153-176-2	Sequence 2, Appli
22	567	98.4	108	14	US-10-153-176-16	Sequence 16, Appl
23	567	98.4	108	15	US-10-443-134A-2	Sequence 2, Appli
24	567	98.4	108	15	US-10-443-134A-16	Sequence 16, Appl
25	567	98.4	108	15	US-10-443-134A-127	Sequence 127, App
26	567	98.4	108	17	US-10-877-532-7	Sequence 7, Appli
27	567	98.4	110	9	US-09-056-160B-103	Sequence 103, App
28	567	98.4	110	14	US-10-234-671-101	Sequence 101, App
29	567	98.4	237	9	US-09-056-160B-100	Sequence 100, App
30	567	98.4	237	14	US-10-234-671-100	Sequence 100, App
31	567	98.4	491	13	US-10-011-125-2	Sequence 2, Appli
32	565	98.1	213	16	US-10-379-392-157	Sequence 157, App
33	564	97.9	108	13	US-10-153-159-4	Sequence 4, Appli
34	564	97.9	108	14	US-10-153-176-4	Sequence 4, Appli
35	564	97.9	108	15	US-10-443-134A-4	Sequence 4, Appli
36	562	97.6	107	16	US-10-723-434-1	Sequence 1, Appli
37	561	97.4	108	9	US-09-056-160B-126	Sequence 126, App
38	561	97.4	108	14	US-10-234-671-124	Sequence 124, App
39	559	97.0	107	9	US-09-056-160B-13	Sequence 13, Appl
40	559	97.0	107	14	US-10-234-671-13	Sequence 13, Appl
41	556	96.5	107	9	US-09-056-160B-15	Sequence 15, Appl
42	556	96.5	107	14	US-10-234-671-15	Sequence 15, Appl
43	556	96.5	214	15	US-10-364-953-4	Sequence 4, Appli
44	555	96.4	214	15	US-10-364-953-3	Sequence 3, Appli
45	554	96.2	110	9	US-09-056-160B-109	Sequence 109, App

ALIGNMENTS

RESULT 1
US-10-234-671-8
; Sequence 8, Application US/10234671
; Publication No. US20030190317A1
; GENERAL INFORMATION:
; APPLICANT: Baca, Manuel
; Wells, James A.
; Presta, Leonard G.
; Lowman, Henry B.
; Chen, Yvonne M.
; TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
; NUMBER OF SEQUENCES: 131
; CORRESPONDENCE ADDRESS:
; ADDRESSEE: Genentech, Inc.
; STREET: 1 DNA Way
; CITY: South San Francisco
; STATE: California
; COUNTRY: USA
; ZIP: 94080
; COMPUTER READABLE FORM:
; MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
; COMPUTER: IBM PC compatible
; OPERATING SYSTEM: PC-DOS/MS-DOS
; SOFTWARE: WinPatin (Genentech)
; CURRENT APPLICATION DATA:
; APPLICATION NUMBER: US/10/234,671
; FILING DATE: 03-Sep-2002
; CLASSIFICATION: <Unknown>
; PRIOR APPLICATION DATA:
; APPLICATION NUMBER: 09/056160
; FILING DATE: 06-APR-1998
; APPLICATION NUMBER: 60/126446
; FILING DATE: 07-APR-1997
; APPLICATION NUMBER: 60/054856
; FILING DATE: 06-AUG-1997
; ATTORNEY/AGENT INFORMATION:

```
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 8:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 110 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 8:
US-10-234-671-8

  Query Match             100.0%;  Score 576;  DB 14;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 8.4e-41;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 2
US-10-624-153-94
; Sequence 94, Application US/10624153
; Publication No. US20040086502A1
; GENERAL INFORMATION:
;  APPLICANT: CHEN, YVONNE M.
;  APPLICANT:  LOWMAN, HENRY B.
;  APPLICANT:  MULLER, YVES
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1C1
;  CURRENT APPLICATION NUMBER: US/10/624,153
;  CURRENT FILING DATE:  2003-07-21
;  PRIOR APPLICATION NUMBER: US 09/440,781
;  PRIOR FILING DATE: 1999-11-16
;  PRIOR APPLICATION NUMBER: US 60/108,945
;  PRIOR FILING DATE: 1998-11-18
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 94
;   LENGTH: 110
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   OTHER INFORMATION: sequence is synthesized
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-110
;   OTHER INFORMATION: humanized antibody light chain variable domain
US-10-624-153-94

  Query Match             100.0%;  Score 576;  DB 15;  Length 110;
  Best Local Similarity   100.0%;  Pred. No. 8.4e-41;
  Matches  110;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 3
US-09-056-160B-107
; Sequence 107, Application US/09056160B
; Patent No. US20020032315A1
; GENERAL INFORMATION:
;   APPLICANT:  Baca, Manuel
;   APPLICANT:  Wells, James A.
;   APPLICANT:  Presta, Leonard G.
;   APPLICANT:  Lowman, Henry B.
;   APPLICANT:  Chen, Yvonne M.
;   TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;   NUMBER OF SEQUENCES:  131
;   CORRESPONDENCE ADDRESS:
;     ADDRESSEE:  Genentech, Inc.
;     STREET:  1 DNA Way
;     CITY:  South San Francisco
;     STATE:  California
;     COUNTRY:  USA
;     ZIP:  94080
;   COMPUTER READABLE FORM:
;     MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;     COMPUTER:  IBM PC compatible
;     OPERATING SYSTEM:  PC-DOS/MS-DOS
;     SOFTWARE:  WinPatin (Genentech)
;   CURRENT APPLICATION DATA:
;     APPLICATION NUMBER:  US/09/056,160B
;     FILING DATE:  06-Apr-1998
;     CLASSIFICATION:  424
;   PRIOR APPLICATION DATA:
;     APPLICATION NUMBER:  60/054,856
;     FILING DATE:  06-AUG-1997
;   ATTORNEY/AGENT INFORMATION:
;     NAME:  Hasak, Janet E.
;     REGISTRATION NUMBER:  28,616
;     REFERENCE/DOCKET NUMBER:  P1093R2
;   TELECOMMUNICATION INFORMATION:
;     TELEPHONE:  650/225-1896
;     TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  107:
;   SEQUENCE CHARACTERISTICS:
;     LENGTH:  110 amino acids
;     TYPE:  Amino Acid
;     TOPOLOGY:  Linear
US-09-056-160B-107

  Query Match             99.5%;  Score 573;  DB 9;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.5e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 4
US-09-056-160B-117
; Sequence 117, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
```

```
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  117:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  110 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-117

  Query Match             99.5%;  Score 573;  DB 9;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.5e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 5
US-10-234-671-105
; Sequence 105, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;        APPLICANT: Baca, Manuel
;                   Wells, James A.
;                   Presta, Leonard G.
;                   Lowman, Henry B.
;                   Chen, Yvonne M.
;        TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;        NUMBER OF SEQUENCES: 131
;        CORRESPONDENCE ADDRESS:
;             ADDRESSEE: Genentech, Inc.
;             STREET: 1 DNA Way
;             CITY: South San Francisco
;             STATE: California
;             COUNTRY: USA
;             ZIP: 94080
;        COMPUTER READABLE FORM:
;             MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;             COMPUTER: IBM PC compatible
;             OPERATING SYSTEM: PC-DOS/MS-DOS
;             SOFTWARE: WinPatin (Genentech)
;        CURRENT APPLICATION DATA:
;             APPLICATION NUMBER: US/10/234,671
;             FILING DATE: 03-Sep-2002
;             CLASSIFICATION: <Unknown>
;        PRIOR APPLICATION DATA:
;             APPLICATION NUMBER: 09/056160
;             FILING DATE: 06-APR-1998
;             APPLICATION NUMBER: 60/126446
;             FILING DATE: 07-APR-1997
;             APPLICATION NUMBER: 60/054856
;             FILING DATE: 06-AUG-1997
;        ATTORNEY/AGENT INFORMATION:
;             NAME: Cui, Steven X.
;             REGISTRATION NUMBER: 44,637
;             REFERENCE/DOCKET NUMBER: P1093R2C1
;        TELECOMMUNICATION INFORMATION:
;             TELEPHONE: 650/225-8674
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 105:
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 110 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 105:
US-10-234-671-105

  Query Match             99.5%;  Score 573;  DB 14;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.5e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 6
US-10-234-671-115
; Sequence 115, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;        APPLICANT: Baca, Manuel
;                   Wells, James A.
;                   Presta, Leonard G.
;                   Lowman, Henry B.
;                   Chen, Yvonne M.
;        TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;        NUMBER OF SEQUENCES: 131
;        CORRESPONDENCE ADDRESS:
;             ADDRESSEE: Genentech, Inc.
;             STREET: 1 DNA Way
;             CITY: South San Francisco
;             STATE: California
;             COUNTRY: USA
;             ZIP: 94080
;        COMPUTER READABLE FORM:
;             MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;             COMPUTER: IBM PC compatible
;             OPERATING SYSTEM: PC-DOS/MS-DOS
;             SOFTWARE: WinPatin (Genentech)
;        CURRENT APPLICATION DATA:
;             APPLICATION NUMBER: US/10/234,671
;             FILING DATE: 03-Sep-2002
;             CLASSIFICATION: <Unknown>
;        PRIOR APPLICATION DATA:
;             APPLICATION NUMBER: 09/056160
;             FILING DATE: 06-APR-1998
;             APPLICATION NUMBER: 60/126446
;             FILING DATE: 07-APR-1997
;             APPLICATION NUMBER: 60/054856
;             FILING DATE: 06-AUG-1997
;        ATTORNEY/AGENT INFORMATION:
;             NAME: Cui, Steven X.
;             REGISTRATION NUMBER: 44,637
;             REFERENCE/DOCKET NUMBER: P1093R2C1
;        TELECOMMUNICATION INFORMATION:
;             TELEPHONE: 650/225-8674
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 115:
;        SEQUENCE CHARACTERISTICS:
```

```
;             LENGTH: 110 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 115:
US-10-234-671-115

  Query Match             99.5%;  Score 573;  DB 14;  Length 110;
  Best Local Similarity   99.1%;  Pred. No. 1.5e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;. Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 7
US-10-379-392-135
; Sequence 135, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 135
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Unknown
;   FEATURE:
;   OTHER INFORMATION: Humanized
US-10-379-392-135

  Query Match             99.5%;  Score 573;  DB 16;  Length 213;
  Best Local Similarity   99.1%;  Pred. No. 2.8e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 8
US-10-379-392-137
; Sequence 137, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 137
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
US-10-379-392-137

  Query Match             99.5%;  Score 573;  DB 16;  Length 213;
  Best Local Similarity   99.1%;  Pred. No. 2.8e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 9
US-10-379-392-139
; Sequence 139, Application US/10379392
; Publication No. US20040110226A1
; GENERAL INFORMATION:
;  APPLICANT: Lazar, Gregory Alan
;  APPLICANT:  Desjarlais, John Rudolf
;  APPLICANT:  Marshall, Shannon Alicia
;  APPLICANT:  Dahiyat, Bassil I.
;  TITLE OF INVENTION: ANTIBODY OPTIMIZATION
;  FILE REFERENCE: A-71386-3 463077-236
;  CURRENT APPLICATION NUMBER: US/10/379,392
;  CURRENT FILING DATE:  2003-03-03
;  PRIOR APPLICATION NUMBER: US 60/360,843
;  PRIOR FILING DATE: 2002-03-01
;  PRIOR APPLICATION NUMBER: US 60/384,197
;  PRIOR FILING DATE: 2002-05-29
;  NUMBER OF SEQ ID NOS: 184
;  SOFTWARE: PatentIn version 3.2
; SEQ ID NO 139
;   LENGTH: 213
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Synthetic
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (116)..(116)
;   OTHER INFORMATION: Xaa at position 116 can be Phe or Tyr
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (133)..(133)
;   OTHER INFORMATION: Xaa at position 133 can be Ile, Met or Val
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (135)..(135)
;   OTHER INFORMATION: Xaa at position 135 can be Leu, Ile or Met
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (176)..(176)
;   OTHER INFORMATION: Xaa at position 176 can be Met, Val, Ala or Ser
;   FEATURE:
;   NAME/KEY: MISC_FEATURE
;   LOCATION: (178)..(178)
;   OTHER INFORMATION: Xaa at position 178 can be Met, Thr or Val
US-10-379-392-139
```

```
  Query Match           99.5%;  Score 573;  DB 16;  Length 213;
  Best Local Similarity  99.1%;  Pred. No. 2.8e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 10
US-10-364-953-1
; Sequence 1, Application US/10364953
; Publication No. US20030224397A1
; GENERAL INFORMATION:
;  APPLICANT: LOWMAN, HENRY B.
;  APPLICANT:  MARVIN, JONATHAN S.
;  TITLE OF INVENTION: ANTIBODY VARIANTS WITH FASTER ANTIGEN ASSOCIATION RATES
;  FILE REFERENCE: P1951R1
;  CURRENT APPLICATION NUMBER: US/10/364,953
;  CURRENT FILING DATE:  2003-02-11
;  PRIOR APPLICATION NUMBER: US 60/355,895
;  PRIOR FILING DATE: 2002-02-11
;  PRIOR APPLICATION NUMBER: US 60/409,685
;  PRIOR FILING DATE: 2002-09-10
;  NUMBER OF SEQ ID NOS: 14
; SEQ ID NO 1
;   LENGTH: 214
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   NAME/KEY: Artificial Sequence
;   LOCATION: Full
;   OTHER INFORMATION: Y0101-VL
US-10-364-953-1

  Query Match           99.5%;  Score 573;  DB 15;  Length 214;
  Best Local Similarity  99.1%;  Pred. No. 2.8e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 11
US-10-020-786-10
; Sequence 10, Application US/10020786
; Publication No. US20030073164A1
; GENERAL INFORMATION:
;  APPLICANT: Simmons, Laura C.
;  APPLICANT:  Klimowski, Laura
;  APPLICANT:  Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: PROKARYOTICALLY PRODUCED ANTIBODIES AND USES THEREOF
;  FILE REFERENCE: P1793R1
;  CURRENT APPLICATION NUMBER: US/10/020,786
;  CURRENT FILING DATE:  2002-03-26
;  PRIOR APPLICATION NUMBER: US 60/256,164
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 11
; SEQ ID NO 10
;   LENGTH: 237
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF light chain
US-10-020-786-10

  Query Match           99.5%;  Score 573;  DB 14;  Length 237;
  Best Local Similarity  99.1%;  Pred. No. 3.1e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 12
US-10-697-995-8
; Sequence 8, Application US/10697995
; Publication No. US20050048572A1
; GENERAL INFORMATION:
;  APPLICANT: Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR INCREASING ANTIBODY PRODUCTION
;  FILE REFERENCE: 11669.195USU1
;  CURRENT APPLICATION NUMBER: US/10/697,995
;  CURRENT FILING DATE:  2003-10-30
;  PRIOR APPLICATION NUMBER: US 60/422,952
;  PRIOR FILING DATE: 2002-10-31
;  NUMBER OF SEQ ID NOS: 37
; SEQ ID NO 8
;   LENGTH: 237
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: anti-VEGF light chain
US-10-697-995-8

  Query Match           99.5%;  Score 573;  DB 17;  Length 237;
  Best Local Similarity  99.1%;  Pred. No. 3.1e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 13
US-10-697-995-11
; Sequence 11, Application US/10697995
; Publication No. US20050048572A1
; GENERAL INFORMATION:
;  APPLICANT: Reilly, Dorothea
;  APPLICANT:  Yansura, Daniel G.
;  TITLE OF INVENTION: METHODS AND COMPOSITIONS FOR INCREASING ANTIBODY PRODUCTION
;  FILE REFERENCE: 11669.195USU1
;  CURRENT APPLICATION NUMBER: US/10/697,995
;  CURRENT FILING DATE:  2003-10-30
;  PRIOR APPLICATION NUMBER: US 60/422,952
;  PRIOR FILING DATE: 2002-10-31
;  NUMBER OF SEQ ID NOS: 37
; SEQ ID NO 11
;   LENGTH: 237
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: Anti-VEGF light chain
US-10-697-995-11
```

```
  Query Match             99.5%;  Score 573;  DB 17;  Length 237;
  Best Local Similarity   99.1%;  Pred. No. 3.1e-40;
  Matches  109;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         24 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 83

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||||||||||||||||||||||
Db         84 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 133


RESULT 14
US-09-056-160B-105
; Sequence 105, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  105:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  110 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-105

  Query Match             99.0%;  Score 570;  DB 9;  Length 110;
  Best Local Similarity   98.2%;  Pred. No. 2.7e-40;
  Matches  108;  Conservative    2;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


RESULT 15
US-10-234-671-103
; Sequence 103, Application US/10234671
; Publication No. US20030190317A1
;  GENERAL INFORMATION:
;       APPLICANT: Baca, Manuel
;                  Wells, James A.
;                  Presta, Leonard G.
;                  Lowman, Henry B.
;                  Chen, Yvonne M.
;       TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;       NUMBER OF SEQUENCES: 131
;       CORRESPONDENCE ADDRESS:
;            ADDRESSEE: Genentech, Inc.
;            STREET: 1 DNA Way
;            CITY: South San Francisco
;            STATE: California
;            COUNTRY: USA
;            ZIP: 94080
;       COMPUTER READABLE FORM:
;            MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;            COMPUTER: IBM PC compatible
;            OPERATING SYSTEM: PC-DOS/MS-DOS
;            SOFTWARE: WinPatin (Genentech)
;       CURRENT APPLICATION DATA:
;            APPLICATION NUMBER: US/10/234,671
;            FILING DATE: 03-Sep-2002
;            CLASSIFICATION: <Unknown>
;       PRIOR APPLICATION DATA:
;            APPLICATION NUMBER: 09/056160
;            FILING DATE: 06-APR-1998
;            APPLICATION NUMBER: 60/126446
;            FILING DATE: 07-APR-1997
;            APPLICATION NUMBER: 60/054856
;            FILING DATE: 06-AUG-1997
;       ATTORNEY/AGENT INFORMATION:
;            NAME: Cui, Steven X.
;            REGISTRATION NUMBER: 44,637
;            REFERENCE/DOCKET NUMBER: P1093R2C1
;       TELECOMMUNICATION INFORMATION:
;            TELEPHONE: 650/225-8674
;            TELEFAX: 650/952-9881
;  INFORMATION FOR SEQ ID NO: 103:
;       SEQUENCE CHARACTERISTICS:
;            LENGTH: 110 amino acids
;            TYPE: Amino Acid
;            TOPOLOGY: Linear
;       SEQUENCE DESCRIPTION: SEQ ID NO: 103:
US-10-234-671-103

  Query Match             99.0%;  Score 570;  DB 14;  Length 110;
  Best Local Similarity   98.2%;  Pred. No. 2.7e-40;
  Matches  108;  Conservative    2;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||:||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||:||||||||||||||||||||||||||||||||||||||||
Db         61 RFSGSGSGTDYTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110


Search completed: March 14, 2005, 20:42:12
Job time : 42.0088 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:39:29 ; Search time 16.6447 Seconds
(without alignments)
635.867 Million cell updates/sec

Title: US-09-723-752B-8
Perfect score: 576
Sequence: 1 DIQMTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 283416 seqs, 96216763 residues

Total number of hits satisfying chosen parameters: 283416

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : PIR_79:*
1: pir1:*
2: pir2:*
3: pir3:*
4: pir4:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	513	89.1	127	2	S40367	Ig kappa chain V-J
2	490	85.1	108	1	K1HUAU	Ig kappa chain V-I
3	486	84.4	125	2	S40333	Ig kappa chain V-J
4	485	84.2	123	2	S40331	Ig kappa chain - h
5	484	84.0	108	2	B49047	Ig kappa chain V r
6	484	84.0	131	2	S40352	Ig kappa chain V-J
7	482	83.7	108	2	S44122	Ig kappa chain V r
8	482	83.7	109	2	S31998	Ig kappa chain - h
9	480	83.3	129	2	S52789	Ig kappa chain V r
10	476	82.6	129	2	S40369	Ig kappa chain - h
11	475	82.5	107	2	S36264	Ig lambda chain V
12	474	82.3	108	1	K1HUHU	Ig kappa chain V-I
13	472	81.9	108	1	K1HUWE	Ig kappa chain V-I
14	472	81.9	109	2	S31981	Ig kappa chain - h
15	472	81.9	111	2	A38740	Ig kappa chain V r
16	471.5	81.9	124	2	S40336	Ig kappa chain V-J
17	470	81.6	110	2	S44118	Ig kappa chain V-J
18	469	81.4	108	1	K1HURE	Ig kappa chain V-I
19	469	81.4	139	2	S40365	Ig kappa chain - h
20	468.5	81.3	107	2	S36275	Ig lambda chain V
21	468	81.2	108	2	S19674	Ig kappa chain V r
22	468	81.2	132	2	S40334	Ig kappa chain - h
23	467	81.1	108	1	K1HUAG	Ig kappa chain V-I
24	467	81.1	125	2	S40349	Ig kappa chain V-J
25	466	80.9	130	2	S40368	Ig kappa chain - h
26	464	80.6	108	2	I39154	Ig kappa chain (BR
27	464	80.6	111	2	E38740	Ig kappa chain V r
28	462	80.2	108	1	K1HUBN	Ig kappa chain V-I
29	462	80.2	108	1	K1HURY	Ig kappa chain V-I
30	462	80.2	111	2	C38740	Ig kappa chain V r
31	462	80.2	122	2	S40314	Ig kappa chain - h
32	462	80.2	129	2	S40317	Ig kappa chain - h
33	462	80.2	129	2	S52793	Ig kappa chain V r
34	462	80.2	135	2	S24320	Ig kappa chain pre
35	461	80.0	108	2	S47182	Ig kappa chain - h
36	460.5	79.9	125	2	S40315	Ig kappa chain - h
37	460	79.9	107	2	S36269	Ig lambda chain V
38	460	79.9	117	2	S46371	Ig kappa chain V-J
39	460	79.9	125	2	S40316	Ig kappa chain - h
40	460	79.9	129	1	K1HUWK	Ig kappa chain pre
41	459	79.7	111	2	G38740	Ig kappa chain V r
42	459	79.7	117	2	S46376	Ig kappa chain V-J
43	459	79.7	122	2	S40370	Ig kappa chain - h
44	458	79.5	107	2	I69017	anti-HIV1 envelope
45	458	79.5	108	1	K1HUDE	Ig kappa chain V-I

ALIGNMENTS

RESULT 1
S40367
Ig kappa chain V-J-C region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40367
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40367
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-127 <KLE>
A;Cross-references: EMBL:X72477
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;33-107/Domain: immunoglobulin homology <IMM>

Query Match 89.1%; Score 513; DB 2; Length 127;
Best Local Similarity 90.0%; Pred. No. 1.9e-38;
Matches 99; Conservative 2; Mismatches 9; Indels 0; Gaps 0;

```
Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||||||||| ||| |||||||||:|||||||:|||  ||| |||||
Db         18 DIQMTQSPSSLSASVGDRVTITCRASQSISNYLNWYQRKPGKAPKLLIYAASSLQSGVPS 77

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||||||||||||||    ||||||||||||||||
Db         78 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYNTPWTFGQGTKVEIKRTV 127
```

RESULT 2
K1HUAU
Ig kappa chain V-I region (Au) - human
C;Species: Homo sapiens (man)
C;Date: 24-Apr-1984 #sequence_revision 02-Jul-1998 #text_change 09-Jul-2004
C;Accession: A91653; A01862; S02573
R;Schiechl, H.; Hilschmann, N.
Hoppe-Seyler's Z. Physiol. Chem. 353, 345-370, 1972
A;Title: Die Primaerstruktur einer monoklonalen Immunglobulin-L-Kette vom kappa-Typ, Sul
A;Reference number: A91653; MUID:72189444; PMID:5028201
A;Accession: A91653
A;Molecule type: protein
A;Residues: 1-108 <SCH>
A;Cross-references: UNIPROT:P01594
A;Note: the C region of this chain has the Inv (3) marker
R;Fehlhammer, H.; Schiffer, M.; Epp, O.; Colman, P.M.; Lattman, E.E.; Schwager, P.; Ste
Biophys. Struct. Mech. 1, 139-146, 1975
A;Title: The structure determination of the variable portion of the Bence-Jones protein
A;Reference number: A90729; MUID:77022433; PMID:1234024
A;Contents: annotation; X-ray crystallography

A;Note: the structure of the V region was determined by molecular replacement methods us
R;Steiner, V.; Chang, J.Y.
FEBS Lett. 222, 6-10, 1987
A;Title: Chemical modification of the carboxyl groups of protein substrates enhances the
A;Reference number: S02572; MUID:88005152; PMID:3115831
A;Contents: annotation
C;Comment: This is a Bence Jones protein.
C;Genetics:
A;Gene: GDB:IGKV1
A;Cross-references: GDB:136264
A;Map position: 2p12-2p12
C;Complex: an immunoglobulin heterotetramer subunit consists of two identical light (kap
hain disulfide bonds; in some cases, such as IgA and IgM, the subunits associate into la
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>
F;23-88/Disulfide bonds: #status predicted

Query Match 85.1%; Score 490; DB 1; Length 108;
Best Local Similarity 86.1%; Pred. No. 1.7e-36;
Matches 93; Conservative 4; Mismatches 11; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 1 DIQMTQSPSSLSASVGDRVTITCQASQDISDYLNWYQQKPGKAPKLLIYDASNLESGVPS 60

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
Db 61 RFSGGGSGAHFTFTISSLQPEDIATYYCQQYDYLPWTFGQGTKVEIKR 108

RESULT 3
S40333
Ig kappa chain V-J region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40333
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40333
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-125 <KLE>
A;Cross-references: EMBL:X72443; NID:g441354; PIDN:CAA51111.1; PID:g441355
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;34-108/Domain: immunoglobulin homology <IMM>

Query Match 84.4%; Score 486; DB 2; Length 125;
Best Local Similarity 86.0%; Pred. No. 4.5e-36;
Matches 92; Conservative 8; Mismatches 7; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 19 DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAPKLLIYKASSLESGVPS 78

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
Db 79 RFSGSGSGTEFTLTISSLQPDDFATYYCQQYNSYPWTFGQGTKVEIK 125

RESULT 4
S40331
Ig kappa chain - human
C;Species: Homo sapiens (man)
C;Date: 06-Mar-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40331
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40331
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-123 <KLE>
A;Cross-references: EMBL:X72441; NID:g441350; PIDN:CAA51109.1; PID:g441351
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;32-106/Domain: immunoglobulin homology <IMM>

Query Match 84.2%; Score 485; DB 2; Length 123;
Best Local Similarity 88.8%; Pred. No. 5.4e-36;
Matches 95; Conservative 3; Mismatches 9; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 17 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 76

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
Db 77 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPRTFGQGTKVEIK 123

RESULT 5
B49047
Ig kappa chain V region (monoclonal striational autoantibody StrAB SA-1A) - human (fragm
C;Species: Homo sapiens (man)
C;Date: 19-Dec-1993 #sequence_revision 18-Nov-1994 #text_change 09-Jul-2004
C;Accession: B49047
R;Victor, K.D.; Pascual, V.; Williams, C.L.; Lennon, V.A.; Capra, J.D.
Eur. J. Immunol. 22, 2231-2236, 1992
A;Title: Human monoclonal striational autoantibodies isolated from thymic B lymphocytes
A;Reference number: A49047; MUID:92387224; PMID:1516616
A;Accession: B49047
A;Status: preliminary
A;Molecule type: nucleic acid
A;Residues: 1-108 <VIC>
A;Cross-references: UNIPROT:Q96SA9; UNIPROT:Q9UL77
A;Experimental source: thymic B lymphocytes
A;Note: sequence extracted from NCBI backbone (NCBIN:113208, NCBIP:113209)
C;Superfamily: immunoglobulin V region; immunoglobulin homology
F;16-90/Domain: immunoglobulin homology <IMM>

Query Match 84.0%; Score 484; DB 2; Length 108;
Best Local Similarity 88.0%; Pred. No. 5.8e-36;
Matches 95; Conservative 3; Mismatches 10; Indels 0; Gaps 0;

Qy 1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
Db 1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
Db 61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKR 108

RESULT 6
S40352
Ig kappa chain V-J-C region - human
C;Species: Homo sapiens (man)
C;Date: 19-May-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40352
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40352
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-131 <KLE>
A;Cross-references: EMBL:X72462; NID:g441392; PIDN:CAA51130.1; PID:g441393
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;36-110/Domain: immunoglobulin homology <IMM>

```
  Query Match           84.0%;  Score 484;  DB 2;  Length 131;
  Best Local Similarity   85.5%;  Pred. No. 7.1e-36;
  Matches   94;  Conservative    7;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||||||||:|||||| ||| ||||| |||||||| ||:|||  |:| |||||
Db         21 DIQMTQSPSSLSASVGNRVTITCRASQGISNYLAWYQQKPGKVPKLLIYAASTLQSGVPS 80

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||:|||||||||| ||||||:|::|| ||||||||||||||
Db         81 RFSGSGSGTDFSLTISSLQPEDVATYYCQKYNSVPRTFGQGTKVEIKRTV 130


RESULT 7
S44122
Ig kappa chain V region - human
C;Species: Homo sapiens (man)
C;Date: 13-Jan-1995 #sequence_revision 13-Jan-1995 #text_change 24-May-2001
C;Accession: S44122
R;Hawkins, R.E.; Zhu, D.; Ovecka, M.; Winter, G.; Hamblin, T.J.; Stevenson, F.K.
submitted to the EMBL Data Library, March 1994
A;Description: Idiotypic vaccination against human B-cell lymphoma: rescue of variable r
A;Reference number: S44105
A;Accession: S44122
A;Status: preliminary
A;Molecule type: DNA
A;Residues: 1-108 <HAW>
A;Cross-references: EMBL:Z31390; NID:g472976; PIDN:CAA83265.1; PID:g940533
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match           83.7%;  Score 482;  DB 2;  Length 108;
  Best Local Similarity   87.0%;  Pred. No. 8.7e-36;
  Matches   94;  Conservative    3;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||| ||:||||||| |||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKLGKAPKLLIYSASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||| |||||  : |||||||||||||||
Db         61 TFSGSGSGTDFTLTISSLQPEDFAIYYCQQSYSTPWTFGQGTKVEIKR 108


RESULT 8
S31998
Ig kappa chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 06-Feb-1995 #sequence_revision 06-Feb-1995 #text_change 21-Jan-2000
C;Accession: S31998
R;Portolano, S.; Chazenbalk, G.D.; Hutchison, S.J.; McLachlan, S.M.; Rapoport, B.
submitted to the EMBL Data Library, June 1992
A;Description: Lack of promiscuity in autoantigen-specific H and L chain combinations as
A;Reference number: S31977
A;Accession: S31998
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-109 <POR>
A;Cross-references: EMBL:Z15081; NID:g38501; PIDN:CAA78790.1; PID:g38502
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match           83.7%;  Score 482;  DB 2;  Length 109;
  Best Local Similarity   85.3%;  Pred. No. 8.8e-36;
  Matches   93;  Conservative    4;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :: |||||||||||||||||||| ||| || ||||||:|||||||:|||  ||| |||||
Db          1 ELVMTQSPSSLSASVGDRVTITCRASQSISAYLNWYQKKPGKAPKLLIYSASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRT 109
              |||||||||||||||||||||||||||||    ||||| |||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYDTPWTFGHGTKVEIKRT 109


RESULT 9
S52789
Ig kappa chain V region - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 19-May-1995 #sequence_revision 21-Jul-1995 #text_change 21-Jan-2000
C;Accession: S52789
R;Rocca, A.; Khamlichi, A.A.; Touchard, G.; Mougenot, B.; Ronco, P.; Denoroy, L.; Deret,
submitted to the EMBL Data Library, March 1995
A;Description: Light chain V region gene usage restriction and peculiarities in myeloma-
A;Reference number: S52789
A;Accession: S52789
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-129 <ROC>
A;Cross-references: EMBL:X85995; NID:g758588; PIDN:CAA59987.1; PID:g758589
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;38-112/Domain: immunoglobulin homology <IMM>

  Query Match           83.3%;  Score 480;  DB 2;  Length 129;
  Best Local Similarity   86.0%;  Pred. No. 1.6e-35;
  Matches   92;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| ||||||||||||||||||:||:  ||| :||||
Db         23 DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIHAASSLETGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
              ||||||||||: |||||||||  ||||||||  :| ||| |||||||
Db         83 RFSGSGSGTDFSFTISSLQPEDLATYYCQQYDNLPLTFGGGTKVEIK 129


RESULT 10
S40369
Ig kappa chain - human
C;Species: Homo sapiens (man)
C;Date: 06-Mar-1994 #sequence_revision 26-May-1995 #text_change 21-Jan-2000
C;Accession: S40369
R;Klein, R.; Jaenichen, R.; Zachau, H.G.
Eur. J. Immunol. 23, 3248-3271, 1993
A;Title: Expressed human immunoglobulin chi genes and their hypermutation.
A;Reference number: S40312; MUID:94080891; PMID:8258341
A;Accession: S40369
A;Status: preliminary; translation not shown
A;Molecule type: mRNA
A;Residues: 1-129 <KLE>
A;Cross-references: EMBL:X72479; NID:g441426; PIDN:CAA51147.1; PID:g441427
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;37-111/Domain: immunoglobulin homology <IMM>

  Query Match           82.6%;  Score 476;  DB 2;  Length 129;
  Best Local Similarity   85.2%;  Pred. No. 3.5e-35;
  Matches   92;  Conservative    7;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||  |||:| |:||||||||| |||  ||| |||||
Db         22 DIQMTQSPSSLSASVGDRVTITCRASHVISNHLVWFQQKPGKAPKSLIYAASSLQSGVPS 81

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              :|||||||||||||||||||||||||:: |:|||||||:||||
Db         82 KFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIKR 129


RESULT 11
S36264
Ig lambda chain V region (clone alpha-CEA4-8A) - human (fragment)
C;Species: Homo sapiens (man)
```

```
C;Date: 03-Feb-1994 #sequence_revision 03-Feb-1994 #text_change 21-Jan-2000
C;Accession: S36264
R;Griffiths, A.D.; Malmqvist, M.; Marks, J.D.; Bye, J.M.; Embleton, M.J.; McCafferty, J.
EMBO J. 12, 725-734, 1993
A;Title: Human anti-self antibodies with high specificity from phage display libraries.
A;Reference number: S36256; MUID:93178448; PMID:7679990
A;Accession: S36264
A;Status: preliminary; nucleic acid sequence not shown
A;Molecule type: mRNA
A;Residues: 1-107 <GRI>
A;Cross-references: EMBL:Z18845; NID:g33426; PIDN:CAA79297.1; PID:g939919
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             82.5%;  Score 475;  DB 2;  Length 107;
  Best Local Similarity   86.0%;  Pred. No. 3.6e-35;
  Matches   92;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :| :|||||||||||||||||||| ||| ||:|||||||||||||:|||  ||| |||||
Db          1 EIVLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIK 107
              |||||||||||||||||||||||||||||  | ||| ||||:||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSNYPLTFGGGTKVDIK 107


RESULT 12
K1HUHU
Ig kappa chain V-I region (Hau) - human
C;Species: Homo sapiens (man)
C;Date: 24-Apr-1984 #sequence_revision 02-Jul-1998 #text_change 09-Jul-2004
C;Accession: A01868; S02574
R;Watanabe, S.; Hilschmann, N.
Hoppe-Seyler's Z. Physiol. Chem. 351, 1291-1295, 1970
A;Title: The primary structure of a monoclonal kappa-type immunoglobulin L-chain of subg
A;Reference number: A01868; MUID:71032830; PMID:4097974
A;Accession: A01868
A;Molecule type: protein
A;Residues: 1-108 <WAT>
A;Cross-references: UNIPROT:P01600
A;Note: the C region of this chain has the Inv (3) marker
R;Steiner, V.; Chang, J.Y.
FEBS Lett. 222, 6-10, 1987
A;Title: Chemical modification of the carboxyl groups of protein substrates enhances the
A;Reference number: S02572; MUID:88005152; PMID:3115831
A;Contents: annotation
C;Comment: This is a Bence Jones protein.
C;Genetics:
A;Gene: GDB:IGKV1
A;Cross-references: GDB:136264
A;Map position: 2p12-2p12
C;Complex: an immunoglobulin heterotetramer subunit consists of two identical light (kap
hain disulfide bonds; in some cases, such as IgA and IgM, the subunits associate into la
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>
F;23-88/Disulfide bonds: #status predicted

  Query Match             82.3%;  Score 474;  DB 1;  Length 108;
  Best Local Similarity   86.1%;  Pred. No. 4.4e-35;
  Matches   93;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||| ||:||:|||||||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLSWYQQKPGKAPQVLIYAASSLPSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||    | :|||||:|||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQNYITPTSFGQGTRVEIKR 108


RESULT 13
K1HUWE
Ig kappa chain V-I region (WEA) - human
C;Species: Homo sapiens (man)
C;Date: 03-Aug-1984 #sequence_revision 03-Aug-1984 #text_change 09-Jul-2004
C;Accession: A01876
R;Goni, F.; Frangione, B.
Proc. Natl. Acad. Sci. U.S.A. 80, 4837-4841, 1983
A;Title: Amino acid sequence of the Fv region of a human monoclonal IgM (protein WEA) wi
A;Reference number: A93964; MUID:83273707; PMID:6410398
A;Accession: A01876
A;Molecule type: protein
A;Residues: 1-108 <GON>
A;Cross-references: UNIPROT:P01610
C;Comment: This chain was obtained from a monoclonal antibody against 3,4-pyruvylated ga
C;Genetics:
A;Gene: GDB:IGKV1
A;Cross-references: GDB:136264
A;Map position: 2p12-2p12
C;Complex: An immunoglobulin heterotetramer subunit consists of two identical light (kap
hain disulfide bonds. In some cases, such as IgA and IgM, the subunits associate into la
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer
F;16-90/Domain: immunoglobulin homology <IMM>
F;23-88/Disulfide bonds: #status predicted

  Query Match             81.9%;  Score 472;  DB 1;  Length 108;
  Best Local Similarity   84.3%;  Pred. No. 6.6e-35;
  Matches   91;  Conservative    5;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||| | | | ||||||| ||| |||  :|| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLTWYQQKPGTAPKRLIYGATSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||:|||||:|||||||||||| ||||: |||||||||||:||
Db         61 RFSGSGSGTEFTLTINSLQPEDFATYYCLQYSSFPWTFGQGTKVEVKR 108


RESULT 14
S31981
Ig kappa chain - human (fragment)
C;Species: Homo sapiens (man)
C;Date: 06-Jan-1995 #sequence_revision 06-Jan-1995 #text_change 21-Jan-2000
C;Accession: S31981
R;Portolano, S.; Chazenbalk, G.D.; Hutchison, S.J.; McLachlan, S.M.; Rapoport, B.
submitted to the EMBL Data Library, June 1992
A;Description: Lack of promiscuity in autoantigen-specific H and L chain combinations as
A;Reference number: S31977
A;Accession: S31981
A;Status: preliminary
A;Molecule type: mRNA
A;Residues: 1-109 <POR>
A;Cross-references: EMBL:Z15077; NID:g38493; PIDN:CAA78786.1; PID:g38494
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;16-90/Domain: immunoglobulin homology <IMM>

  Query Match             81.9%;  Score 472;  DB 2;  Length 109;
  Best Local Similarity   82.6%;  Pred. No. 6.7e-35;
  Matches   90;  Conservative    9;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              :: |||||||||||||||||||| |||||| |||||||||||||:||:  |:| ||||
Db          1 ELVMTQSPSSLSASVGDRVTITCRASQDISRYLNWYQQKPGKAPKLLIHGASTLESGVPG 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRT 109
              |||||||||||||||||||||:|||||||  : |:|||||||:|||||
Db         61 RFSGSGSGTDFTLTISSLQPEDYATYYCQQSYSTPFTFGQGTKLEIKRT 109


RESULT 15
A38740
```

```
Ig kappa chain V region (Py20) - mouse
C;Species: Mus musculus (house mouse)
C;Date: 18-Oct-1991 #sequence_revision 18-Oct-1991 #text_change 09-Jul-2004
C;Accession: A38740
R;Ruff-Jamison, S.; Campos-Gonzalez, R.; Glenney Jr., J.R.
J. Biol. Chem. 266, 6607-6613, 1991
A;Title: Heavy and light chain variable region sequences and antibody properties of anti
A;Reference number: A38740; MUID:91177923; PMID:1706720
A;Accession: A38740
A;Status: preliminary; nucleic acid sequence not shown; not compared with conceptual tra
A;Molecule type: mRNA
A;Residues: 1-111 <RUF>
A;Cross-references: UNIPROT:Q91WS9
C;Superfamily: immunoglobulin V region; immunoglobulin homology
C;Keywords: heterotetramer; immunoglobulin
F;19-93/Domain: immunoglobulin homology <IMM>

  Query Match            81.9%;  Score 472;  DB 2;  Length 111;
  Best Local Similarity  81.5%;  Pred. No. 6.8e-35;
  Matches   88;  Conservative   11;  Mismatches    9;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |:||||: ||||||:||||||:||||| ||||||||||     |:|||:||||||||||
Db          4 DVQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTVKLLIYYTSSLHSGVPS 63

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              ||||||||||::||||:|:||| ||||||||| |||||| |||:||||
Db         64 RFSGSGSGTDYSLTISNLEPEDVATYYCQQYSKVPWTFGGGTKLEIKR 111


Search completed: March 14, 2005, 21:08:51
Job time : 17.6447 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:32:33 ; Search time 77.193 Seconds
(without alignments)
729.713 Million cell updates/sec

Title: US-09-723-752B-8
Perfect score: 576
Sequence: 1 DIQMTQSPSSLSASVGDRVT.....YSTVPWTFGQGTKVEIKRTV 110

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1612378 seqs, 512079187 residues

Total number of hits satisfying chosen parameters: 1612378

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : UniProt_03:*
1: uniprot_sprot:*
2: uniprot_trembl:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	490	85.1	108	1	KV1B_HUMAN	P01594 homo sapien
2	488	84.7	108	2	Q9UL77	Q9ul77 homo sapien
3	486	84.4	236	2	Q6GMX9	Q6gmx9 homo sapien
4	485	84.2	236	2	Q6GMW1	Q6gmw1 homo sapien
5	484	84.0	236	2	Q7Z3Y4	Q7z3y4 homo sapien
6	477.5	82.9	107	2	Q96SA9	Q96sa9 homo sapien
7	477	82.8	236	2	Q6GMX8	Q6gmx8 homo sapien
8	474	82.3	108	1	KV1H_HUMAN	P01600 homo sapien
9	474	82.3	108	1	KV1Y_HUMAN	P80362 homo sapien
10	474	82.3	236	2	Q6GMX0	Q6gmx0 homo sapien
11	472	81.9	108	1	KV1R_HUMAN	P01610 homo sapien
12	469	81.4	108	1	KV1O_HUMAN	P01607 homo sapien
13	468	81.2	108	2	Q9UL70	Q9ul70 homo sapien
14	467	81.1	108	1	KV1A_HUMAN	P01593 homo sapien
15	465	80.7	234	2	Q7Z473	Q7z473 homo sapien
16	463	80.4	236	2	Q6PIH7	Q6pih7 homo sapien
17	462	80.2	108	1	KV1P_HUMAN	P01608 homo sapien
18	462	80.2	108	1	KV1V_HUMAN	P04430 homo sapien
19	460	79.9	129	1	KV1W_HUMAN	P04431 homo sapien
20	458	79.5	108	1	KV1E_HUMAN	P01597 homo sapien
21	457.5	79.4	107	1	KV1D_HUMAN	P01596 homo sapien
22	457	79.3	108	1	KV1M_HUMAN	P01605 homo sapien
23	456	79.2	108	1	KV1K_HUMAN	P01603 homo sapien
24	455.5	79.1	107	2	Q9UL81	Q9ul81 homo sapien
25	455	79.0	244	2	Q65ZC8	Q65zc8 homo sapien
26	452	78.5	108	1	KV1Q_HUMAN	P01609 homo sapien
27	451	78.3	108	1	KV1S_HUMAN	P01611 homo sapien
28	451	78.3	116	2	Q96PF6	Q96pf6 homo sapien
29	450	78.1	108	1	KV1N_HUMAN	P01606 homo sapien
30	449	78.0	108	1	KV1C_HUMAN	P01595 homo sapien
31	448	77.8	108	1	KV5J_MOUSE	P01643 mus musculu
32	448	77.8	240	2	Q65ZC9	Q65zc9 homo sapien
33	446	77.4	108	1	KV1G_HUMAN	P01599 homo sapien
34	443	76.9	236	2	Q6PIT5	Q6pit5 homo sapien
35	442	76.7	108	1	KV1F_HUMAN	P01598 homo sapien
36	442	76.7	108	1	KV1L_HUMAN	P01604 homo sapien
37	442	76.7	108	2	Q9UL79	Q9ul79 homo sapien
38	441	76.6	236	2	Q6PIH4	Q6pih4 homo sapien
39	431	74.8	108	1	KV5K_MOUSE	P01644 mus musculu
40	431	74.8	108	1	KV5N_MOUSE	P01647 mus musculu
41	429	74.5	108	1	KV5L_MOUSE	P01645 mus musculu
42	429	74.5	108	1	KV5M_MOUSE	P01646 mus musculu
43	428	74.3	108	1	KV5O_MOUSE	P01648 mus musculu
44	427.5	74.2	109	1	KV1T_HUMAN	P01612 homo sapien
45	425	73.8	108	1	KV5U_MOUSE	P04946 mus musculu

ALIGNMENTS

RESULT 1
KV1B_HUMAN
ID KV1B_HUMAN STANDARD; PRT; 108 AA.
AC P01594;
DT 21-JUL-1986 (Rel. 01, Created)
DT 21-JUL-1986 (Rel. 01, Last sequence update)
DT 25-OCT-2004 (Rel. 45, Last annotation update)
DE Ig kappa chain V-I region AU.
OS Homo sapiens (Human).
OC Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX NCBI_TaxID=9606;
RN [1]
RP SEQUENCE.
RX MEDLINE=72189444; PubMed=5028201;
RA Schiechl H., Hilschmann N.;
RT "Rule of antibody structure. The primary structure of a monoclonal
RT immunoglobulin L-chain of the kappa-type, subgroup I (Bence-Jones
RT protein Au).";
RL Hoppe-Seyler's Z. Physiol. Chem. 353:345-370(1972).
RN [2]
RP X-RAY CRYSTALLOGRAPHY (2.2 ANGSTROMS).
RX MEDLINE=77022433; PubMed=1234024;
RA Fehlhammer H., Schiffer M., Epp O., Colman P.M., Lattman E.E.,
RA Schwager P., Steigemann W., Schramm H.J.;
RT "The structure determination of the variable portion of the Bence-
RT Jones protein Au.";
RL Biophys. Struct. Mech. 1:139-146(1975).
CC -!- MISCELLANEOUS: The structure of the V region was determined by
CC molecular replacement methods using the known structure of the V
CC region of the kappa chain REI.
CC -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR PIR; A91653; K1HUAU.
DR PDB; 1JV5; X-ray; A=1-107.
DR GO; GO:0005576; C:extracellular; NAS.
DR GO; GO:0003823; F:antigen binding; NAS.
DR GO; GO:0006955; P:immune response; NAS.
DR InterPro; IPR007110; Ig-like.
DR InterPro; IPR003596; Ig_v.
DR Pfam; PF00047; ig; 1.
DR SMART; SM00406; IGv; 1.
DR PROSITE; PS50835; IG_LIKE; 1.
KW 3D-structure; Bence-Jones protein; Direct protein sequencing;
KW Immunoglobulin V region.
FT DOMAIN 1 23 Framework-1.
FT DOMAIN 24 34 Complementarity-determining-1.
FT DOMAIN 35 49 Framework-2.
FT DOMAIN 50 56 Complementarity-determining-2.
FT DOMAIN 57 88 Framework-3.
FT DOMAIN 89 97 Complementarity-determining-3.
FT DOMAIN 98 107 Framework-4.
FT DISULFID 23 88 By similarity.
FT STRAND 4 5

```
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       44     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       85     90
FT   STRAND       97     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11939 MW;  E8011187EE6F6FB9 CRC64;

  Query Match             85.1%;  Score 490;  DB 1;  Length 108;
  Best Local Similarity   86.1%;  Pred. No. 1.2e-42;
  Matches   93;  Conservative    4;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| |||||:|||||||||||||||:|||  |:| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDISDYLNWYQQKPGKAPKLLIYDASNLESGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              ||||  |||  || |||||||| ||||||||  :|||||||||||||
Db         61 RFSGGGSGAHFTFTISSLQPEDIATYYCQQYDYLPWTFGQGTKVEIKR 108


RESULT 2
Q9UL77
ID   Q9UL77      PRELIMINARY;      PRT;   108 AA.
AC   Q9UL77;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Myosin-reactive immunoglobulin light chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035037; AAD56273.1; -.
DR   PIR; B49047; B49047.
DR   PIR; S34083; S34083.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11738 MW;  C06681716C4D16F3 CRC64;

  Query Match             84.7%;  Score 488;  DB 2;  Length 108;
  Best Local Similarity   87.0%;  Pred. No. 2e-42;
  Matches   94;  Conservative    4;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| ||| ||:|||||||||||||| :|||  ||| |||||

Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPNLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              ||||||||||||||||||||||||||||| :  ||||:||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTSWTFGEGTKVEIKR 108


RESULT 3
Q6GMX9
ID   Q6GMX9      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX9;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073763; AAH73763.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25924 MW;  FDE2093DC560CFF7 CRC64;

  Query Match             84.4%;  Score 486;  DB 2;  Length 236;
  Best Local Similarity   86.4%;  Pred. No. 7.8e-42;
  Matches   95;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||| |||||| |||::| :| ||||:| |||| ||| ||||||||||
Db         23 DIQMTQSPSSLSASVGHRVTITCRASQNVSRWLAWYQQRPEKAPKSLIYATSSLHSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
```

```
                     ||||||||||||||||||||||||||||||:| | ||| ||||||||||
Db         83 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYNTYPLTFGGGTKVEIKRTV 132


RESULT 4
Q6GMW1
ID   Q6GMW1      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMW1;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073791; AAH73791.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25751 MW;  5BFE6A087AFAC437 CRC64;

  Query Match            84.2%;  Score 485;  DB 2;  Length 236;
  Best Local Similarity  88.1%;  Pred. No. 9.9e-42;
  Matches   96;  Conservative    1;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          2 IQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSR 61
              |||||||||||||||||||||||| ||| ||| | ||||||||||||:|||  ||| ||||||
Db         24 IQMTQSPSSLSASVGDRVTITCRASQGISNDLGWYQQKPGKAPKLLIYAASSLQSGVPSR 83

Qy         62 FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||||||||||||||||| |    ||||||||||||||||
Db         84 FSGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPWTFGQGTKVEIKRTV 132


RESULT 5
Q7Z3Y4
ID   Q7Z3Y4      PRELIMINARY;      PRT;   236 AA.
AC   Q7Z3Y4;
DT   01-OCT-2003 (TrEMBLrel. 25, Created)
DT   01-OCT-2003 (TrEMBLrel. 25, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Skeletal Muscle;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Skeletal Muscle;
RA   Strausberg R.;
RL   Submitted (MAR-2001) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC005332; AAH05332.1; -.
DR   HSSP; P01834; 1HEZ.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25702 MW;  7FBFE4ED23084BC6 CRC64;

  Query Match            84.0%;  Score 484;  DB 2;  Length 236;
  Best Local Similarity  86.4%;  Pred. No. 1.3e-41;
  Matches   95;  Conservative    4;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||| ||||| ||||||||| |:||||||||| |||  ||| ||| |
Db         23 DIQMTQSPSSLSASVGDTVTITCRASQDISNYLAWFQQKPGKAPKSLIYGASSLQSGVQS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              :|||||||||||||||||||||||||||| : | |||||||:||||||
Db         83 KFSGSGSGTDFTLTISSLQPEDFATYYCQQYKSYPVTFGQGTKLEIKRTV 132


RESULT 6
Q96SA9
ID   Q96SA9      PRELIMINARY;      PRT;   107 AA.
AC   Q96SA9;
DT   01-DEC-2001 (TrEMBLrel. 19, Created)
```

```
DT   01-DEC-2001 (TrEMBLrel. 19, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Anti-streptococcal/anti-myosin immunoglobulin kappa light chain
DE   variable region (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98375893; PubMed=9712075;
RA   Adderson E.E., Shikhman A.R., Ward K.E., Cunningham M.W.;
RT   "Molecular analysis of polyreactive monoclonal antibodies from
RT   rheumatic carditis: human anti-N-acetylglucosamine/anti-myosin
RT   antibody V region genes.";
RL   J. Immunol. 161:2020-2031(1998).
DR   EMBL; U96396; AAB68785.1; -.
DR   PIR; B49047; B49047.
DR   PIR; PH0867; PH0867.
DR   PIR; S16840; S16840.
DR   PIR; S31977; S31977.
DR   PIR; S34083; S34083.
DR   PIR; S34086; S34086.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     107    107
SQ   SEQUENCE   107 AA;  11520 MW;  4BB43E9C5B577F16 CRC64;

  Query Match             82.9%;  Score 477.5;  DB 2;  Length 107;
  Best Local Similarity   89.0%;  Pred. No. 2.3e-41;
  Matches   97;  Conservative    3;  Mismatches    6;  Indels    3;  Gaps    2;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||| ||:|||||||||||||:|||  ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQ-YSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||| |||:  ||| ||||||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTL--TFGGGTKVEIKR 107


RESULT 7
Q6GMX8
ID   Q6GMX8      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX8;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Primary B-Cells;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073764; AAH73764.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25707 MW;  4FC8E14B6559EFC9 CRC64;

  Query Match             82.8%;  Score 477;  DB 2;  Length 236;
  Best Local Similarity   84.5%;  Pred. No. 6.6e-41;
  Matches   93;  Conservative    7;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              ||||||||||:|||||||||||| ||| ||::| ||||||||||:|||  ||| |||||
Db         23 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||||||||||||||||||||||||||||  : |:||| ||||:|||||
Db         83 RFSGSGSGTDFTLTISSLQPEDFATYYCQQAHSFPFTFGPGTKVDIKRTV 132


RESULT 8
KV1H_HUMAN
ID   KV1H_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01600;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region Hau.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=71032830; PubMed=4097974;
RA   Watanabe S., Hilschmann N.;
RT   "The primary structure of a monoclonal kappa-type immunoglobulin L-
RT   chain of subgroup I (Bence-Jones Protein Hau): subdivision within
RT   subgroups.";
RL   Hoppe-Seyler's Z. Physiol. Chem. 351:1291-1295(1970).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A01868; K1HUHU.
DR   PDB; 1F6L; X-ray; L=1-108.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
```

```
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88       By similarity.
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11671 MW;  08D3A6160D8D0618 CRC64;

  Query Match             82.3%;  Score 474;  DB 1;  Length 108;
  Best Local Similarity   86.1%;  Pred. No. 5.4e-41;
  Matches   93;  Conservative    5;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| ||| ||:||:||||||||||:|||| ||| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQSISSYLSWYQQKPGKAPQVLIYAASSLPSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||||||||||||    | :|||||:|||||
Db         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQNYITPTSFGQGTRVEIKR 108


RESULT 9
KV1Y_HUMAN
ID   KV1Y_HUMAN     STANDARD;      PRT;   108 AA.
AC   P80362;
DT   01-NOV-1995 (Rel. 32, Created)
DT   01-NOV-1995 (Rel. 32, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region WAT.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE, AND X-RAY CRYSTALLOGRAPHY (1.9 ANGSTROMS).
RX   MEDLINE=95086080; PubMed=7993911;
RA   Huang D.-B., Chang C.-H., Ainsworth C., Bruenger A.T., Eulitz M.,
RA   Solomon A., Stevens F.J., Schiffer M.;
RT   "Comparison of crystal structures of two homologous proteins:
RT   structural origin of altered domain interactions in immunoglobulin
RT   light-chain dimers.";
RL   Biochemistry 33:14848-14857(1994).
RN   [2]
RP   SEQUENCE OF 1-35.
RX   MEDLINE=81267384; PubMed=6167731;
RA   Stevens F.J., Westholm F.A., Panagiotopoulos N., Schiffer M.,
RA   Popp R.A., Solomon A.;
RT   "Characterization and preliminary crystallographic data on the VL-
RT   related fragment of the human kI Bence Jones protein Wat.";
RL   J. Mol. Biol. 147:185-193(1981).
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PDB; 1WTL; X-ray; A/B=1-108.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88       By similarity.
FT   CONFLICT     30     31       TN -> SD (in Ref. 2).
FT   STRAND        4      7
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       45     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       84     90
FT   STRAND       98     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11737 MW;  D9D941B3F0FAE697 CRC64;

  Query Match             82.3%;  Score 474;  DB 1;  Length 108;
  Best Local Similarity   83.3%;  Pred. No. 5.4e-41;
  Matches   90;  Conservative    8;  Mismatches   10;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||| |||||:||:||:||:|||||||  | | :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQDITNYVNWFQQRPGQAPKVLIYGASILETGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||| ||||||||| |||||||| |:| ||| ||||:|||
Db         61 RFSGSGSGTDFTFTISSLQPEDIATYYCQQYDTLPLTFGGGTKVDIKR 108


RESULT 10
Q6GMX0
ID   Q6GMX0      PRELIMINARY;      PRT;   236 AA.
AC   Q6GMX0;
DT   05-JUL-2004 (TrEMBLrel. 27, Created)
DT   05-JUL-2004 (TrEMBLrel. 27, Last sequence update)
DT   05-JUL-2004 (TrEMBLrel. 27, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
```

```
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Spleen;
RA   Strausberg R.;
RL   Submitted (JUN-2004) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC073775; AAH73775.1; -.
DR   InterPro; IPR003599; Ig.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_C1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_V.
DR   Pfam; PF07654; C1-set; 1.
DR   Pfam; PF00047; ig; 2.
DR   SMART; SM00409; IG; 2.
DR   SMART; SM00407; IGc1; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   236 AA;  25807 MW;  864EA08C7E92BF8F CRC64;

  Query Match             82.3%;  Score 474;  DB 2;  Length 236;
  Best Local Similarity   83.6%;  Pred. No. 1.3e-40;
  Matches   92;  Conservative    6;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| |||:|:||||||| |||||| :|||  ||| |||||
Db         23 DIQMTQSPSSLSASVGDRVTITCRASQNINNYLNWYQLKPGKAPNLLIYAASSLQSGVPS 82

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              ||||||||||||||||||:|:|||||||||   :| ||| || |||||||
Db         83 RFSGSGSGTDFTLTISSLRPDDFATYYCQQSYNIPLTFGGGTNVEIKRTV 132


RESULT 11
KV1R_HUMAN
ID   KV1R_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01610;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig kappa chain V-I region WEA.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=83273707; PubMed=6410398;
RA   Goni F., Frangione B.;
RT   "Amino acid sequence of the Fv region of a human monoclonal IgM
RT   (protein WEA) with antibody activity against 3,4-pyruvylated galactose
RT   in Klebsiella polysaccharides K30 and K33.";
RL   Proc. Natl. Acad. Sci. U.S.A. 80:4837-4841(1983).
CC   -!- MISCELLANEOUS: This chain was obtained from a monoclonal antibody
CC       against 3,4-pyruvylated galactose and isolated from a patient with
CC       Waldenstrom's macroglobulinemia.
DR   PIR; A01876; K1HUWE.
DR   HSSP; P80362; 1WTL.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_V.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Direct protein sequencing; Immunoglobulin V region;
KW   Monoclonal antibody.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88       By similarity.
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11840 MW;  9249B61F0945618C CRC64;

  Query Match             81.9%;  Score 472;  DB 1;  Length 108;
  Best Local Similarity   84.3%;  Pred. No. 8.7e-41;
  Matches   91;  Conservative    5;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| ||| | | | ||||||| ||| |||  :|| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLTWYQQKPGTAPKRLIYGATSLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||:|||||:|||||||||||| |||: ||||||||||:||
Db         61 RFSGSGSGTEFTLTINSLQPEDFATYYCLQYSSFPWTFGQGTKVEVKR 108


RESULT 12
KV1O_HUMAN
ID   KV1O_HUMAN     STANDARD;      PRT;   108 AA.
AC   P01607;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   25-OCT-2004 (Rel. 45, Last annotation update)
DE   Ig kappa chain V-I region Rei.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=76023758; PubMed=809329;
RA   Palm W., Hilschmann N.;
RT   "The primary structure of a crystalline monoclonal immunoglobulin
RT   kappa-type L-chain, subgroup I (Bence-Jones protein Rei); isolation
RT   and characterization of the tryptic peptides; the complete amino acid
RT   sequence of the protein; a contribution to the elucidation of the
RT   three-dimensional structure of antibodies, in particular their
RT   combining site.";
RL   Hoppe-Seyler's Z. Physiol. Chem. 356:167-191(1975).
RN   [2]
RP   X-RAY CRYSTALLOGRAPHY (2.0 ANGSTROMS).
RX   MEDLINE=76039968; PubMed=1182131;
RA   Epp O., Lattman E.E., Schiffer M., Huber R., Palm W.;
RT   "The molecular structure of a dimer composed of the variable portions
RT   of the Bence-Jones protein REI refined at 2.0-A resolution.";
RL   Biochemistry 14:4943-4952(1975).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (1,2)
CC       marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A91663; K1HURE.
DR   PDB; 1AR2; X-ray; @=1-107.
DR   PDB; 1BWW; X-ray; A/B=1-107.
DR   PDB; 1REI; X-ray; A/B=1-107.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_V.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   3D-structure; Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
```

```
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88
FT   STRAND        4      7
FT   STRAND       10     13
FT   TURN         15     16
FT   STRAND       19     25
FT   TURN         30     31
FT   STRAND       33     38
FT   TURN         40     41
FT   STRAND       45     49
FT   TURN         50     52
FT   STRAND       53     54
FT   TURN         56     57
FT   TURN         60     61
FT   STRAND       62     67
FT   TURN         68     69
FT   STRAND       70     75
FT   HELIX        80     82
FT   STRAND       84     90
FT   STRAND       97     98
FT   STRAND      102    106
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11902 MW;  9E8143E1188BCE2A CRC64;

  Query Match             81.4%;  Score 469;  DB 1;  Length 108;
  Best Local Similarity   81.5%;  Pred. No. 1.8e-40;
  Matches   88;  Conservative    9;  Mismatches   11;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| |||||  ||||||| ||||||:|||  |:| :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDIIKYLNWYQQTPGKAPKLLIYEASNLQAGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||:| ||||||||| |||||||| ::|:|||||||::| |
Db         61 RFSGSGSGTDYTFTISSLQPEDIATYYCQQYQSLPYTFGQGTKLQITR 108


RESULT 13
Q9UL70
ID   Q9UL70     . PRELIMINARY;      PRT;   108 AA.
AC   Q9UL70;
DT   01-MAY-2000 (TrEMBLrel. 13, Created)
DT   01-MAY-2000 (TrEMBLrel. 13, Last sequence update)
DT   01-OCT-2003 (TrEMBLrel. 25, Last annotation update)
DE   Myosin-reactive immunoglobulin light chain variable region
DE   (Fragment).
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RX   MEDLINE=98277139; PubMed=9614934; DOI=10.1006/clin.1998.4531;
RA   Wu X., Liu B., Van der Merwe P.L., Kalis N.N., Berney S.M.,
RA   Young D.C.;
RT   "Myosin-reactive autoantibodies in rheumatic carditis and normal
RT   fetus.";
RL   Clin. Immunol. Immunopathol. 87:184-192(1998).
DR   EMBL; AF035044; AAD56280.1; -.
DR   PIR; PH0863; PH0863.
DR   HSSP; P01607; 1BWW.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
FT   NON_TER       1      1
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11633 MW;  B7BEDC3E41FCCA37 CRC64;

  Query Match             81.2%;  Score 468;  DB 2;  Length 108;
  Best Local Similarity   84.3%;  Pred. No. 2.2e-40;
  Matches   91;  Conservative    5;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| ||| ||||| |||||||| || |||  |:| |||||
Db          1 DIQMTQSPSSLSASVGDRVTITCRASQGISNYLAWYQQKPGKVPKSLIYAASTLQSGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||||||||||||||||| ||||||:|:: | ||| |||:|||| 
Db         61 RFSGSGSGTDFTLTISSLQPEDVATYYCQKYNSAPRTFGPGTKLEIKR 108


RESULT 14
KV1A_HUMAN
ID   KV1A_HUMAN     STANDARD;      PRT;  . 108 AA.
AC   P01593;
DT   21-JUL-1986 (Rel. 01, Created)
DT   21-JUL-1986 (Rel. 01, Last sequence update)
DT   05-JUL-2004 (Rel. 44, Last annotation update)
DE   Ig kappa chain V-I region AG.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE.
RX   MEDLINE=69234734; PubMed=4893682;
RA   Titani K., Shinoda T., Putnam F.W.;
RT   "The amino acid sequence of a kappa type Bence-Jones protein. 3. The
RT   complete sequence and the location of the disulfide bridges.";
RL   J. Biol. Chem. 244:3550-3560(1969).
CC   -!- MISCELLANEOUS: The C region of this chain has the INV (3) marker.
CC   -!- MISCELLANEOUS: This is a Bence-Jones protein.
DR   PIR; A01861; K1HUAG.
DR   HSSP; P01607; 1BWW.
DR   GO; GO:0005576; C:extracellular; NAS.
DR   GO; GO:0003823; F:antigen binding; NAS.
DR   GO; GO:0006955; P:immune response; NAS.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF00047; ig; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 1.
KW   Bence-Jones protein; Direct protein sequencing;
KW   Immunoglobulin V region.
FT   DOMAIN        1     23       Framework-1.
FT   DOMAIN       24     34       Complementarity-determining-1.
FT   DOMAIN       35     49       Framework-2.
FT   DOMAIN       50     56       Complementarity-determining-2.
FT   DOMAIN       57     88       Framework-3.
FT   DOMAIN       89     97       Complementarity-determining-3.
FT   DOMAIN       98    107       Framework-4.
FT   DISULFID     23     88
FT   NON_TER     108    108
SQ   SEQUENCE   108 AA;  11992 MW;  E3B3B246C18F0C4F CRC64;

  Query Match             81.1%;  Score 467;  DB 1;  Length 108;
  Best Local Similarity   82.4%;  Pred. No. 2.8e-40;
  Matches   89;  Conservative    7;  Mismatches   12;  Indels    0;  Gaps    0;

Qy          1 DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPS 60
              |||||||||||||||||||||||| |||||::||||||| | ||||:|||  |:| :||||
Db          1 DIQMTQSPSSLSASVGDRVTITCQASQDINHYLNWYQQGPKKAPKILIYDASNLETGVPS 60

Qy         61 RFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKR 108
              |||||| ||||| ||| ||||| |||||||| |:| |||||||:|||| 
Db         61 RFSGSGFGTDFTFTISGLQPEDIATYYCQQYDTLPRTFGQGTKLEIKR 108


RESULT 15
Q7Z473
ID   Q7Z473      PRELIMINARY;      PRT;   234 AA.
AC   Q7Z473;
DT   01-OCT-2003 (TrEMBLrel. 25, Created)
```

```
DT   01-OCT-2003 (TrEMBLrel. 25, Last sequence update)
DT   01-MAR-2004 (TrEMBLrel. 26, Last annotation update)
DE   Hypothetical protein.
OS   Homo sapiens (Human).
OC   Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
OC   Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
OX   NCBI_TaxID=9606;
RN   [1]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Lung;
RX   MEDLINE=22388257; PubMed=12477932; DOI=10.1073/pnas.242603899;
RA   Strausberg R.L., Feingold E.A., Grouse L.H., Derge J.G.,
RA   Klausner R.D., Collins F.S., Wagner L., Shenmen C.M., Schuler G.D.,
RA   Altschul S.F., Zeeberg B., Buetow K.H., Schaefer C.F., Bhat N.K.,
RA   Hopkins R.F., Jordan H., Moore T., Max S.I., Wang J., Hsieh F.,
RA   Diatchenko L., Marusina K., Farmer A.A., Rubin G.M., Hong L.,
RA   Stapleton M., Soares M.B., Bonaldo M.F., Casavant T.L., Scheetz T.E.,
RA   Brownstein M.J., Usdin T.B., Toshiyuki S., Carninci P., Prange C.,
RA   Raha S.S., Loquellano N.A., Peters G.J., Abramson R.D., Mullahy S.J.,
RA   Bosak S.A., McEwan P.J., McKernan K.J., Malek J.A., Gunaratne P.H.,
RA   Richards S., Worley K.C., Hale S., Garcia A.M., Gay L.J., Hulyk S.W.,
RA   Villalon D.K., Muzny D.M., Sodergren E.J., Lu X., Gibbs R.A.,
RA   Fahey J., Helton E., Ketteman M., Madan A., Rodrigues S., Sanchez A.,
RA   Whiting M., Madan A., Young A.C., Shevchenko Y., Bouffard G.G.,
RA   Blakesley R.W., Touchman J.W., Green E.D., Dickson M.C.,
RA   Rodriguez A.C., Grimwood J., Schmutz J., Myers R.M., Butterfield Y.S.,
RA   Krzywinski M.I., Skalska U., Smailus D.E., Schnerch A., Schein J.E.,
RA   Jones S.J., Marra M.A.;
RT   "Generation and initial analysis of more than 15,000 full-length human
RT   and mouse cDNA sequences.";
RL   Proc. Natl. Acad. Sci. U.S.A. 99:16899-16903(2002).
RN   [2]
RP   SEQUENCE FROM N.A.
RC   TISSUE=Lung;
RA   Strausberg R.;
RL   Submitted (AUG-2003) to the EMBL/GenBank/DDBJ databases.
DR   EMBL; BC056256; AAH56256.1; -.
DR   HSSP; P01834; 1HEZ.
DR   InterPro; IPR007110; Ig-like.
DR   InterPro; IPR003597; Ig_c1.
DR   InterPro; IPR003006; Ig_MHC.
DR   InterPro; IPR003596; Ig_v.
DR   Pfam; PF07654; C1-set; 1.
DR   SMART; SM00406; IGv; 1.
DR   PROSITE; PS50835; IG_LIKE; 2.
DR   PROSITE; PS00290; IG_MHC; UNKNOWN_1.
KW   Hypothetical protein.
SQ   SEQUENCE   234 AA;  25674 MW;  1A2C259BAB51BC0F CRC64;

  Query Match             80.7%;  Score 465;  DB 2;  Length 234;
  Best Local Similarity   81.7%;  Pred. No. 1.1e-39;
  Matches   89;  Conservative    6;  Mismatches   14;  Indels    0;  Gaps    0;

Qy          2 IQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYFTSSLHSGVPSR 61
              |:||||||| ||| |||||||| ||| | :|| ||||||||::|||  |:| ||||||
Db         22 IRMTQSPSSFSASTGDRVTITCRASQSIGSYLAWYQQKPGKAPQLLIYAASTLQSGVPSR 81

Qy         62 FSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQGTKVEIKRTV 110
              |||| ||||||||:|| || |||||||||||| | ||||||||||||||||
Db         82 FSGSASGTDFTLSISCLQSEDFATYYCQQYYTYPWTFGQGTKVEIKRTV 130


Search completed: March 14, 2005, 20:49:18
Job time : 77.193 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:22:02 ; Search time 43.9912 Seconds
(without alignments)
884.760 Million cell updates/sec

Title: US-09-723-752B-7
Perfect score: 655
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPHYYGSSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 1396920 seqs, 329844858 residues

Total number of hits satisfying chosen parameters: 1396920

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Published_Applications_AA:*
1: /cgn2_6/ptodata/2/pubpaa/US07_PUBCOMB.pep:*
2: /cgn2_6/ptodata/2/pubpaa/PCT_NEW_PUB.pep:*
3: /cgn2_6/ptodata/2/pubpaa/US06_NEW_PUB.pep:*
4: /cgn2_6/ptodata/2/pubpaa/US06_PUBCOMB.pep:*
5: /cgn2_6/ptodata/2/pubpaa/US07_NEW_PUB.pep:*
6: /cgn2_6/ptodata/2/pubpaa/PCTUS_PUBCOMB.pep:*
7: /cgn2_6/ptodata/2/pubpaa/US08_NEW_PUB.pep:*
8: /cgn2_6/ptodata/2/pubpaa/US08_PUBCOMB.pep:*
9: /cgn2_6/ptodata/2/pubpaa/US09A_PUBCOMB.pep:*
10: /cgn2_6/ptodata/2/pubpaa/US09B_PUBCOMB.pep:*
11: /cgn2_6/ptodata/2/pubpaa/US09C_PUBCOMB.pep:*
12: /cgn2_6/ptodata/2/pubpaa/US09_NEW_PUB.pep:*
13: /cgn2_6/ptodata/2/pubpaa/US10A_PUBCOMB.pep:*
14: /cgn2_6/ptodata/2/pubpaa/US10B_PUBCOMB.pep:*
15: /cgn2_6/ptodata/2/pubpaa/US10C_PUBCOMB.pep:*
16: /cgn2_6/ptodata/2/pubpaa/US10D_PUBCOMB.pep:*
17: /cgn2_6/ptodata/2/pubpaa/US10_NEW_PUB.pep:*
18: /cgn2_6/ptodata/2/pubpaa/US11_NEW_PUB.pep:*
19: /cgn2_6/ptodata/2/pubpaa/US60_NEW_PUB.pep:*
20: /cgn2_6/ptodata/2/pubpaa/US60_PUBCOMB.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	655	100.0	118	9	US-09-056-160B-108	Sequence 108, App
2	655	100.0	118	14	US-10-234-671-7	Sequence 7, Appli
3	655	100.0	118	14	US-10-234-671-106	Sequence 106, App
4	655	100.0	118	15	US-10-624-153-96	Sequence 96, Appl
5	655	100.0	123	9	US-09-056-160B-7	Sequence 7, Appli
6	655	100.0	123	13	US-10-153-159-1	Sequence 1, Appli
7	655	100.0	123	13	US-10-153-159-14	Sequence 14, Appl
8	655	100.0	123	14	US-10-153-176-1	Sequence 1, Appli
9	655	100.0	123	14	US-10-153-176-14	Sequence 14, Appl
10	655	100.0	123	15	US-10-443-134A-1	Sequence 1, Appli
11	655	100.0	123	15	US-10-443-134A-14	Sequence 14, Appl
12	655	100.0	123	16	US-10-723-434-55	Sequence 55, Appl
13	655	100.0	123	17	US-10-877-532-8	Sequence 8, Appli
14	655	100.0	231	15	US-10-364-953-2	Sequence 2, Appli
15	651	99.4	118	9	US-09-056-160B-110	Sequence 110, App
16	651	99.4	118	14	US-10-234-671-108	Sequence 108, App
17	650	99.2	123	15	US-10-443-134A-126	Sequence 126, App
18	650	99.2	123	16	US-10-723-434-104	Sequence 104, App
19	649	99.1	123	15	US-10-443-134A-129	Sequence 129, App
20	649	99.1	123	16	US-10-723-434-70	Sequence 70, Appl
21	646	98.6	123	16	US-10-723-434-103	Sequence 103, App
22	645	98.5	123	16	US-10-723-434-59	Sequence 59, Appl
23	644	98.3	118	9	US-09-056-160B-112	Sequence 112, App
24	644	98.3	118	14	US-10-234-671-110	Sequence 110, App
25	644	98.3	118	15	US-10-624-153-97	Sequence 97, Appl
26	644	98.3	123	15	US-10-443-134A-128	Sequence 128, App
27	644	98.3	123	16	US-10-723-434-105	Sequence 105, App
28	644	98.3	231	15	US-10-364-953-5	Sequence 5, Appli
29	643	98.2	123	16	US-10-723-434-69	Sequence 69, Appl
30	642	98.0	118	9	US-09-056-160B-114	Sequence 114, App
31	642	98.0	118	14	US-10-234-671-112	Sequence 112, App
32	642	98.0	123	16	US-10-723-434-65	Sequence 65, Appl
33	638	97.4	123	9	US-09-056-160B-16	Sequence 16, Appl
34	638	97.4	123	14	US-10-234-671-16	Sequence 16, Appl
35	638	97.4	123	15	US-10-443-134A-130	Sequence 130, App
36	638	97.4	123	16	US-10-723-434-67	Sequence 67, Appl
37	638	97.4	123	16	US-10-723-434-101	Sequence 101, App
38	638	97.4	123	16	US-10-723-434-102	Sequence 102, App
39	638	97.4	231	15	US-10-364-953-9	Sequence 9, Appli
40	637	97.3	118	9	US-09-056-160B-106	Sequence 106, App
41	637	97.3	118	14	US-10-234-671-104	Sequence 104, App
42	636	97.1	123	15	US-10-443-134A-131	Sequence 131, App
43	636	97.1	123	16	US-10-723-434-75	Sequence 75, Appl
44	636	97.1	123	16	US-10-723-434-77	Sequence 77, Appl
45	636	97.1	123	16	US-10-723-434-84	Sequence 84, Appl

ALIGNMENTS

RESULT 1
US-09-056-160B-108
; Sequence 108, Application US/09056160B
; Patent No. US20020032315A1
; GENERAL INFORMATION:
; APPLICANT: Baca, Manuel
; APPLICANT: Wells, James A.
; APPLICANT: Presta, Leonard G.
; APPLICANT: Lowman, Henry B.
; APPLICANT: Chen, Yvonne M.
; TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
; NUMBER OF SEQUENCES: 131
; CORRESPONDENCE ADDRESS:
; ADDRESSEE: Genentech, Inc.
; STREET: 1 DNA Way
; CITY: South San Francisco
; STATE: California
; COUNTRY: USA
; ZIP: 94080
; COMPUTER READABLE FORM:
; MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
; COMPUTER: IBM PC compatible
; OPERATING SYSTEM: PC-DOS/MS-DOS
; SOFTWARE: WinPatin (Genentech)
; CURRENT APPLICATION DATA:
; APPLICATION NUMBER: US/09/056,160B
; FILING DATE: 06-Apr-1998
; CLASSIFICATION: 424
; PRIOR APPLICATION DATA:
; APPLICATION NUMBER: 60/054,856
; FILING DATE: 06-AUG-1997
; ATTORNEY/AGENT INFORMATION:
; NAME: Hasak, Janet E.
; REGISTRATION NUMBER: 28,616
; REFERENCE/DOCKET NUMBER: P1093R2
; TELECOMMUNICATION INFORMATION:

```
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  108:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-108

  Query Match           100.0%;  Score 655;  DB 9;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 2
US-10-234-671-7
; Sequence 7, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;        APPLICANT: Baca, Manuel
;                   Wells, James A.
;                   Presta, Leonard G.
;                   Lowman, Henry B.
;                   Chen, Yvonne M.
;        TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;        NUMBER OF SEQUENCES: 131
;        CORRESPONDENCE ADDRESS:
;             ADDRESSEE: Genentech, Inc.
;             STREET: 1 DNA Way
;             CITY: South San Francisco
;             STATE: California
;             COUNTRY: USA
;             ZIP: 94080
;        COMPUTER READABLE FORM:
;             MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;             COMPUTER: IBM PC compatible
;             OPERATING SYSTEM: PC-DOS/MS-DOS
;             SOFTWARE: WinPatin (Genentech)
;        CURRENT APPLICATION DATA:
;             APPLICATION NUMBER: US/10/234,671
;             FILING DATE: 03-Sep-2002
;             CLASSIFICATION: <Unknown>
;        PRIOR APPLICATION DATA:
;             APPLICATION NUMBER: 09/056160
;             FILING DATE: 06-APR-1998
;             APPLICATION NUMBER: 60/126446
;             FILING DATE: 07-APR-1997
;             APPLICATION NUMBER: 60/054856
;             FILING DATE: 06-AUG-1997
;        ATTORNEY/AGENT INFORMATION:
;             NAME: Cui, Steven X.
;             REGISTRATION NUMBER: 44,637
;             REFERENCE/DOCKET NUMBER: P1093R2C1
;        TELECOMMUNICATION INFORMATION:
;             TELEPHONE: 650/225-8674
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 7:
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 118 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 7:
US-10-234-671-7

  Query Match           100.0%;  Score 655;  DB 14;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 3
US-10-234-671-106
; Sequence 106, Application US/10234671
; Publication No. US20030190317A1
;   GENERAL INFORMATION:
;        APPLICANT: Baca, Manuel
;                   Wells, James A.
;                   Presta, Leonard G.
;                   Lowman, Henry B.
;                   Chen, Yvonne M.
;        TITLE OF INVENTION: ANTI-VEGF ANTIBODIES
;        NUMBER OF SEQUENCES: 131
;        CORRESPONDENCE ADDRESS:
;             ADDRESSEE: Genentech, Inc.
;             STREET: 1 DNA Way
;             CITY: South San Francisco
;             STATE: California
;             COUNTRY: USA
;             ZIP: 94080
;        COMPUTER READABLE FORM:
;             MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
;             COMPUTER: IBM PC compatible
;             OPERATING SYSTEM: PC-DOS/MS-DOS
;             SOFTWARE: WinPatin (Genentech)
;        CURRENT APPLICATION DATA:
;             APPLICATION NUMBER: US/10/234,671
;             FILING DATE: 03-Sep-2002
;             CLASSIFICATION: <Unknown>
;        PRIOR APPLICATION DATA:
;             APPLICATION NUMBER: 09/056160
;             FILING DATE: 06-APR-1998
;             APPLICATION NUMBER: 60/126446
;             FILING DATE: 07-APR-1997
;             APPLICATION NUMBER: 60/054856
;             FILING DATE: 06-AUG-1997
;        ATTORNEY/AGENT INFORMATION:
;             NAME: Cui, Steven X.
;             REGISTRATION NUMBER: 44,637
;             REFERENCE/DOCKET NUMBER: P1093R2C1
;        TELECOMMUNICATION INFORMATION:
;             TELEPHONE: 650/225-8674
;             TELEFAX: 650/952-9881
;   INFORMATION FOR SEQ ID NO: 106:
;        SEQUENCE CHARACTERISTICS:
;             LENGTH: 118 amino acids
;             TYPE: Amino Acid
;             TOPOLOGY: Linear
;        SEQUENCE DESCRIPTION: SEQ ID NO: 106:
US-10-234-671-106

  Query Match           100.0%;  Score 655;  DB 14;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
```

```
RESULT 4
US-10-624-153-96
; Sequence 96, Application US/10624153
; Publication No. US20040086502A1
; GENERAL INFORMATION:
;  APPLICANT: CHEN, YVONNE M.
;  APPLICANT:  LOWMAN, HENRY B.
;  APPLICANT:  MULLER, YVES
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1C1
;  CURRENT APPLICATION NUMBER: US/10/624,153
;  CURRENT FILING DATE:  2003-07-21
;  PRIOR APPLICATION NUMBER: US 09/440,781
;  PRIOR FILING DATE: 1999-11-16
;  PRIOR APPLICATION NUMBER: US 60/108,945
;  PRIOR FILING DATE: 1998-11-18
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 96
;   LENGTH: 118
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   OTHER INFORMATION: sequence is synthesized
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-118
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-10-624-153-96

  Query Match             100.0%;  Score 655;  DB 15;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 5
US-09-056-160B-7
; Sequence 7, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  7:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  123 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-7

  Query Match             100.0%;  Score 655;  DB 9;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 6
US-10-153-159-1
; Sequence 1, Application US/10153159
; Publication No. US20020177170A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  TITLE OF INVENTION: STRUCTURE-BASED SELECTION AND AFFINITY MATURATION OF ANTIBODY LIE
;  TITLE OF INVENTION:  SILICO
;  FILE REFERENCE: 26050-704
;  CURRENT APPLICATION NUMBER: US/10/153,159
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 1
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of parental anti-VEGF antibody
US-10-153-159-1

  Query Match             100.0%;  Score 655;  DB 13;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 7
US-10-153-159-14
; Sequence 14, Application US/10153159
```

```
; Publication No. US20020177170A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  TITLE OF INVENTION: STRUCTURE-BASED SELECTION AND AFFINITY MATURATION OF ANTIBODY LIB
;  TITLE OF INVENTION:  SILICO
;  FILE REFERENCE: 26050-704
;  CURRENT APPLICATION NUMBER: US/10/153,159
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 14
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of AM2-ccFv
US-10-153-159-14

  Query Match             100.0%;  Score 655;  DB 13;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 8
US-10-153-176-1
; Sequence 1, Application US/10153176
; Publication No. US20030022240A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  APPLICANT:  Cao, Yicheng
;  APPLICANT:  Li, Shengfeng
;  APPLICANT:  Liu, Shengjiang
;  TITLE OF INVENTION: GENERATION AND AFFINITY MATURATION OF ANTIBODY LIBRARY IN SILICO
;  FILE REFERENCE: 26050-701
;  CURRENT APPLICATION NUMBER: US/10/153,176
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 1
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of parental anti-VEGF antibody
US-10-153-176-1

  Query Match             100.0%;  Score 655;  DB 14;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 9
US-10-153-176-14
; Sequence 14, Application US/10153176
; Publication No. US20030022240A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peter
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  APPLICANT:  Cao, Yicheng
;  APPLICANT:  Li, Shengfeng
;  APPLICANT:  Liu, Shengjiang
;  TITLE OF INVENTION: GENERATION AND AFFINITY MATURATION OF ANTIBODY LIBRARY IN SILICO
;  FILE REFERENCE: 26050-701
;  CURRENT APPLICATION NUMBER: US/10/153,176
;  CURRENT FILING DATE:  2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  NUMBER OF SEQ ID NOS: 125
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 14
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of AM2-ccFv
US-10-153-176-14

  Query Match             100.0%;  Score 655;  DB 14;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 10
US-10-443-134A-1
; Sequence 1, Application US/10443134A
; Publication No. US20040010376A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peizhi
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  APPLICANT:  Cao, Yicheng
;  APPLICANT:  Liu, Shengjiang
;  TITLE OF INVENTION: GENERATION AND SELECTION OF PROTEIN LIBRARY IN SILICO
;  FILE REFERENCE: 26050-709
;  CURRENT APPLICATION NUMBER: US/10/443,134A
;  CURRENT FILING DATE:  2003-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  PRIOR APPLICATION NUMBER: US 10/153,176
;  PRIOR FILING DATE: 2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/153,159
```

```
;  PRIOR FILING DATE: 2002-05-20
;  NUMBER OF SEQ ID NOS: 131
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 1
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of parental anti-VEGF antibody
US-10-443-134A-1

  Query Match            100.0%;  Score 655;  DB 15;  Length 123;
  Best Local Similarity  100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 11
US-10-443-134A-14
; Sequence 14, Application US/10443134A
; Publication No. US20040010376A1
; GENERAL INFORMATION:
;  APPLICANT: Luo, Peizhi
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Zhong, Pingyu
;  APPLICANT:  Wang, Caili
;  APPLICANT:  Cao, Yicheng
;  APPLICANT:  Liu, Shengjiang
;  TITLE OF INVENTION: GENERATION AND SELECTION OF PROTEIN LIBRARY IN SILICO
;  FILE REFERENCE: 26050-709
;  CURRENT APPLICATION NUMBER: US/10/443,134A
;  CURRENT FILING DATE:  2003-05-20
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  PRIOR APPLICATION NUMBER: US 10/153,176
;  PRIOR FILING DATE: 2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/153,159
;  PRIOR FILING DATE: 2002-05-20
;  NUMBER OF SEQ ID NOS: 131
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 14
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH of AM2-ccFv
US-10-443-134A-14

  Query Match            100.0%;  Score 655;  DB 15;  Length 123;
  Best Local Similarity  100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 12
US-10-723-434-55
; Sequence 55, Application US/10723434
; Publication No. US20040133357A1
; GENERAL INFORMATION:
;  APPLICANT: Zhong, Pingyu
;  APPLICANT:  Luo, Peizhi
;  APPLICANT:  Wang, Kevin C.
;  APPLICANT:  Hsieh, Mark
;  APPLICANT:  Li, Yan
;  TITLE OF INVENTION: HUMANIZED ANTIBODIES AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR
;  FILE REFERENCE: 26050-709.501
;  CURRENT APPLICATION NUMBER: US/10/723,434
;  CURRENT FILING DATE:  2003-11-26
;  PRIOR APPLICATION NUMBER: US 60/284,407
;  PRIOR FILING DATE: 2001-04-17
;  PRIOR APPLICATION NUMBER: US 10/125,687
;  PRIOR FILING DATE: 2002-04-17
;  PRIOR APPLICATION NUMBER: US 10/153,176
;  PRIOR FILING DATE: 2002-05-20
;  PRIOR APPLICATION NUMBER: US 10/443,134
;  PRIOR FILING DATE: 2003-05-20
;  NUMBER OF SEQ ID NOS: 156
;  SOFTWARE: PatentIn version 3.1
; SEQ ID NO 55
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: VH
US-10-723-434-55

  Query Match            100.0%;  Score 655;  DB 16;  Length 123;
  Best Local Similarity  100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 13
US-10-877-532-8
; Sequence 8, Application US/10877532
; Publication No. US20050038231A1
; GENERAL INFORMATION:
;  APPLICANT: FAHRNER, ROBERT L.
;  APPLICANT:  LAVERDIERE, AMY
;  APPLICANT:  MCDONALD, PAUL J.
;  APPLICANT:  O'LEARY, RHONA M.
;  TITLE OF INVENTION: REDUCING PROTEIN A LEACHING DURING PROTEIN A AFFINITY CHROMATOGRA
;  FILE REFERENCE: P2015R1
;  CURRENT APPLICATION NUMBER: US/10/877,532
;  CURRENT FILING DATE:  2004-06-24
;  PRIOR APPLICATION NUMBER: US 60/490,500
;  PRIOR FILING DATE: 2003-07-28
;  NUMBER OF SEQ ID NOS: 8
; SEQ ID NO 8
;   LENGTH: 123
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   OTHER INFORMATION: sequence is synthesized
US-10-877-532-8

  Query Match            100.0%;  Score 655;  DB 17;  Length 123;
  Best Local Similarity  100.0%;  Pred. No. 2.1e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
```

```
Qy          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 14
US-10-364-953-2
; Sequence 2, Application US/10364953
; Publication No. US20030224397A1
; GENERAL INFORMATION:
;  APPLICANT: LOWMAN, HENRY B.
;  APPLICANT:  MARVIN, JONATHAN S.
;  TITLE OF INVENTION: ANTIBODY VARIANTS WITH FASTER ANTIGEN ASSOCIATION RATES
;  FILE REFERENCE: P1951R1
;  CURRENT APPLICATION NUMBER: US/10/364,953
;  CURRENT FILING DATE:  2003-02-11
;  PRIOR APPLICATION NUMBER: US 60/355,895
;  PRIOR FILING DATE: 2002-02-11
;  PRIOR APPLICATION NUMBER: US 60/409,685
;  PRIOR FILING DATE: 2002-09-10
;  NUMBER OF SEQ ID NOS: 14
; SEQ ID NO 2
;   LENGTH: 231
;   TYPE: PRT
;   ORGANISM: Artificial sequence
;   FEATURE:
;   NAME/KEY: Artificial Sequence
;   LOCATION: Full
;   OTHER INFORMATION: Y0101-VH
US-10-364-953-2

  Query Match           100.0%;  Score 655;  DB 15;  Length 231;
  Best Local Similarity  100.0%;  Pred. No. 3.8e-47;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy           1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db           1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 15
US-09-056-160B-110
; Sequence 110, Application US/09056160B
; Patent No. US20020032315A1
;  GENERAL INFORMATION:
;    APPLICANT:  Baca, Manuel
;    APPLICANT:  Wells, James A.
;    APPLICANT:  Presta, Leonard G.
;    APPLICANT:  Lowman, Henry B.
;    APPLICANT:  Chen, Yvonne M.
;    TITLE OF INVENTION:  ANTI-VEGF ANTIBODIES
;    NUMBER OF SEQUENCES:  131
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Genentech, Inc.
;      STREET:  1 DNA Way
;      CITY:  South San Francisco
;      STATE:  California
;      COUNTRY:  USA
;      ZIP:  94080
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  3.5 inch, 1.44 Mb floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  WinPatin (Genentech)
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/056,160B
;      FILING DATE:  06-Apr-1998
;      CLASSIFICATION:  424
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  60/054,856
;      FILING DATE:  06-AUG-1997
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  Hasak, Janet E.
;      REGISTRATION NUMBER:  28,616
;      REFERENCE/DOCKET NUMBER:  P1093R2
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  650/225-1896
;      TELEFAX:  650/952-9881
;  INFORMATION FOR SEQ ID NO:  110:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  Amino Acid
;      TOPOLOGY:  Linear
US-09-056-160B-110

  Query Match           99.4%;  Score 651;  DB 9;  Length 118;
  Best Local Similarity  99.2%;  Pred. No. 4.4e-47;
  Matches  117;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy           1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
               ||||||||||||||||||||||||||||||||:|||||||||||||||||||||||||||
Db           1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


Search completed: March 14, 2005, 20:42:11
Job time : 44.9912 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:30:13 ; Search time 24.3246 Seconds
(without alignments)
362.127 Million cell updates/sec

Title: US-09-723-752B-7
Perfect score: 655
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPHYYGSSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 513545 seqs, 74649064 residues

Total number of hits satisfying chosen parameters: 513545

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : Issued_Patents_AA:*
1: /cgn2_6/ptodata/1/iaa/5A_COMB.pep:*
2: /cgn2_6/ptodata/1/iaa/5B_COMB.pep:*
3: /cgn2_6/ptodata/1/iaa/6A_COMB.pep:*
4: /cgn2_6/ptodata/1/iaa/6B_COMB.pep:*
5: /cgn2_6/ptodata/1/iaa/PCTUS_COMB.pep:*
6: /cgn2_6/ptodata/1/iaa/backfiles1.pep:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

6632926

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	655	100.0	118	4	US-09-440-781-96	Sequence 96, Appl
2	644	98.3	118	4	US-09-440-781-97	Sequence 97, Appl
3	631.5	96.4	121	4	US-09-440-781-98	Sequence 98, Appl
4	631	96.3	491	4	US-10-011-125A-2	Sequence 2, Appli
5	620.5	94.7	121	4	US-09-440-781-99	Sequence 99, Appl
6	513.5	78.4	118	1	US-08-425-336-126	Sequence 126, App
7	513.5	78.4	118	1	US-08-488-113B-126	Sequence 126, App
8	513.5	78.4	118	1	US-08-477-484B-126	Sequence 126, App
9	513.5	78.4	118	2	US-08-646-360-126	Sequence 126, App
10	513.5	78.4	118	3	US-08-839-765-126	Sequence 126, App
11	513.5	78.4	118	3	US-09-136-389-126	Sequence 126, App
12	513.5	78.4	118	3	US-09-610-838-126	Sequence 126, App
13	513.5	78.4	118	4	US-09-711-485-126	Sequence 126, App
14	513.5	78.4	240	1	US-08-488-113B-147	Sequence 147, App
15	513.5	78.4	240	1	US-08-488-113B-148	Sequence 148, App
16	513.5	78.4	240	1	US-08-477-484B-147	Sequence 147, App
17	513.5	78.4	240	1	US-08-477-484B-148	Sequence 148, App
18	513.5	78.4	240	2	US-08-646-360-147	Sequence 147, App
19	513.5	78.4	240	2	US-08-646-360-148	Sequence 148, App
20	513.5	78.4	240	3	US-08-839-765-147	Sequence 147, App
21	513.5	78.4	240	3	US-08-839-765-148	Sequence 148, App
22	513.5	78.4	240	3	US-09-136-389-147	Sequence 147, App
23	513.5	78.4	240	3	US-09-136-389-148	Sequence 148, App
24	513.5	78.4	240	3	US-09-610-838-147	Sequence 147, App
25	513.5	78.4	240	3	US-09-610-838-148	Sequence 148, App
26	513.5	78.4	240	4	US-09-711-485-147	Sequence 147, App
27	513.5	78.4	240	4	US-09-711-485-148	Sequence 148, App
28	499.5	76.3	118	1	US-08-107-669D-29	Sequence 29, Appl
29	499.5	76.3	118	1	US-08-472-788A-29	Sequence 29, Appl
30	499.5	76.3	118	2	US-08-477-531B-29	Sequence 29, Appl
31	499.5	76.3	118	2	US-08-082-842A-29	Sequence 29, Appl
32	498.5	76.1	118	1	US-08-107-669D-67	Sequence 67, Appl
33	498.5	76.1	118	1	US-08-472-788A-89	Sequence 89, Appl
34	498.5	76.1	118	2	US-08-477-531B-67	Sequence 67, Appl
35	498.5	76.1	118	2	US-08-082-842A-89	Sequence 89, Appl
36	496.5	75.8	122	2	US-07-934-373C-20	Sequence 20, Appl
37	496.5	75.8	122	3	US-08-437-642B-20	Sequence 20, Appl
38	496.5	75.8	122	4	US-08-146-206C-20	Sequence 20, Appl
39	496.5	75.8	122	4	US-09-705-686-20	Sequence 20, Appl
40	496.5	75.8	122	4	US-09-705-392A-20	Sequence 20, Appl
41	496.5	75.8	122	4	US-09-705-398-20	Sequence 20, Appl
42	496.5	75.8	122	5	PCT-US93-07832-20	Sequence 20, Appl
43	495.5	75.6	122	2	US-07-934-373C-45	Sequence 45, Appl
44	495.5	75.6	122	3	US-08-437-642B-45	Sequence 45, Appl
45	495.5	75.6	122	4	US-08-146-206C-26	Sequence 26, Appl

ALIGNMENTS

RESULT 1
US-09-440-781-96
; Sequence 96, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
; APPLICANT: Yvonne Man-yee Chen et al.
; TITLE OF INVENTION: ANTIBODY VARIANTS
; FILE REFERENCE: P1469R1
; CURRENT APPLICATION NUMBER: US/09/440,781
; CURRENT FILING DATE: 1999-11-16
; NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 96
; LENGTH: 118
; TYPE: PRT
; ORGANISM: artificial sequence
; FEATURE:
; NAME/KEY: artificial
; LOCATION: 1-118
; OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-96

Query Match 100.0%; Score 655; DB 4; Length 118;
Best Local Similarity 100.0%; Pred. No. 3.2e-59;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
```

RESULT 2
US-09-440-781-97
; Sequence 97, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
; APPLICANT: Yvonne Man-yee Chen et al.
; TITLE OF INVENTION: ANTIBODY VARIANTS
; FILE REFERENCE: P1469R1
; CURRENT APPLICATION NUMBER: US/09/440,781
; CURRENT FILING DATE: 1999-11-16
; NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 97
; LENGTH: 118
; TYPE: PRT
; ORGANISM: artificial sequence
; FEATURE:

```
;   NAME/KEY: artificial
;   LOCATION: 1-118
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-97

  Query Match             98.3%;  Score 644;  DB 4;  Length 118;
  Best Local Similarity   98.3%;  Pred. No. 4.2e-58;
  Matches  116;  Conservative    1;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||| ||:||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 3
US-09-440-781-98
; Sequence 98, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 98
;   LENGTH: 121
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-121
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-98

  Query Match             96.4%;  Score 631.5;  DB 4;  Length 121;
  Best Local Similarity   95.9%;  Pred. No. 8e-57;
  Matches  116;  Conservative    0;  Mismatches    2;  Indels    3;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYY---GSSHWYFDVWGQGT 117
              |||||||||||||||||||||||||||||||||||||||||||||   ||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYVNERKSHWYFDVWGQGT 120

Qy        118 L 118
              |
Db        121 L 121


RESULT 4
US-10-011-125A-2
; Sequence 2, Application US/10011125A
; Patent No. 6828121
; GENERAL INFORMATION:
;  APPLICANT: Chen, Christina Yu-Ching
;  TITLE OF INVENTION: BACTERIAL HOST STRAINS
;  FILE REFERENCE: P1804R1
;  CURRENT APPLICATION NUMBER: US/10/011,125A
;  CURRENT FILING DATE:  2001-12-07
;  PRIOR APPLICATION NUMBER: US 60/256,162
;  PRIOR FILING DATE: 2000-12-14
;  NUMBER OF SEQ ID NOS: 12
; SEQ ID NO 2
;   LENGTH: 491
;   TYPE: PRT
;   ORGANISM: Artificial Sequence
;   FEATURE:
;   OTHER INFORMATION: Sequence is synthesized.
; Patent No. 6828121
US-10-011-125A-2

  Query Match             96.3%;  Score 631;  DB 4;  Length 491;
  Best Local Similarity   94.9%;  Pred. No. 4.7e-56;
  Matches  112;  Conservative    2;  Mismatches    4;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||:|||||||||||||||||||||||||
Db        261 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWIRQAPGKGLEWVGWINTYTGEPTY 320

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |||||||| | ||| :| ||||||||||||||||||||||||||||||||||||||||||
Db        321 AADFKRRFTISADTSSNTVYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 378


RESULT 5
US-09-440-781-99
; Sequence 99, Application US/09440781
; Patent No. 6632926
; GENERAL INFORMATION:
;  APPLICANT: Yvonne Man-yee Chen et al.
;  TITLE OF INVENTION: ANTIBODY VARIANTS
;  FILE REFERENCE: P1469R1
;  CURRENT APPLICATION NUMBER: US/09/440,781
;  CURRENT FILING DATE: 1999-11-16
;  NUMBER OF SEQ ID NOS: 99
; SEQ ID NO 99
;   LENGTH: 121
;   TYPE: PRT
;   ORGANISM: artificial sequence
;   FEATURE:
;   NAME/KEY: artificial
;   LOCATION: 1-121
;   OTHER INFORMATION: humanized antibody heavy chain variable domain
US-09-440-781-99

  Query Match             94.7%;  Score 620.5;  DB 4;  Length 121;
  Best Local Similarity   94.2%;  Pred. No. 1e-55;
  Matches  114;  Conservative    1;  Mismatches    3;  Indels    3;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||||||||||||||||||||||| ||:||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYY---GSSHWYFDVWGQGT 117
              |||||||||||||||||||||||||||||||||||||||||||   ||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYVNERKSHWYFDVWGQGT 120

Qy        118 L 118
              |
Db        121 L 121


RESULT 6
US-08-425-336-126
; Sequence 126, Application US/08425336
; Patent No. 5621083
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribsome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  140
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  Marshall, O'Toole, Gerstein, Murray & Borun
;      STREET:  6300 Sears Tower, 233 South Wacker Drive
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
```

```
;       ZIP:  60606-6402
;     COMPUTER READABLE FORM:
;       MEDIUM TYPE:  Floppy disk
;       COMPUTER:  IBM PC compatible
;       OPERATING SYSTEM:  PC-DOS/MS-DOS
;       SOFTWARE:  PatentIn Release #1.0, Version #1.25
;     CURRENT APPLICATION DATA:
;       APPLICATION NUMBER:  US/08/425,336
;       FILING DATE:  18-APR-1995
;       CLASSIFICATION:  530
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  08/064,691
;       FILING DATE:  12-MAY-1993
;       APPLICATION NUMBER:  US 07/901,707
;       FILING DATE:  19-JUN-1992
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  US 07/787,567
;       FILING DATE:  04-NOV-1991
;     ATTORNEY/AGENT INFORMATION:
;       NAME:  Meyers, Thomas C.
;       REGISTRATION NUMBER:  P-36,989
;       REFERENCE/DOCKET NUMBER:  31394
;     TELECOMMUNICATION INFORMATION:
;       TELEPHONE:  312/474-6300
;       TELEFAX:  312/474-0448
;       TELEX:  25-3856
;   INFORMATION FOR SEQ ID NO:  126:
;     SEQUENCE CHARACTERISTICS:
;       LENGTH:  118 amino acids
;       TYPE:  amino acid
;       STRANDEDNESS:  single
;       TOPOLOGY:  linear
;     MOLECULE TYPE:  protein
US-08-425-336-126

  Query Match             78.4%;  Score 513.5;  DB 1;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 ||  ||||||| ||:|||||:|||||||||||:| :  :      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 7
US-08-488-113B-126
; Sequence 126, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-126

  Query Match             78.4%;  Score 513.5;  DB 1;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 ||  ||||||| ||:|||||:|||||||||||:| :  :      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 8
US-08-477-484B-126
; Sequence 126, Application US/08477484B
; Patent No. 5756699
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/477,484B
;      FILING DATE:  07-JUN-1995
```

```
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-477-484B-126

  Query Match             78.4%;  Score 513.5;  DB 1;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              |  || |||||||  ||:|||||:||||||||||:| :  :       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 9
US-08-646-360-126
; Sequence 126, Application US/08646360
; Patent No. 5837491
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/646,360
;      FILING DATE:  13-MAY-1996
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-646-360-126

  Query Match             78.4%;  Score 513.5;  DB 2;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              |  || |||||||  ||:|||||:||||||||||:| :  :       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 10
US-08-839-765-126
; Sequence 126, Application US/08839765
; Patent No. 6146631
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/839,765
;      FILING DATE:  15-APR-1997
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
```

```
;       APPLICATION NUMBER:  US 08/425,336
;       FILING DATE:  18-APR-1995
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  US 08/064,691
;       FILING DATE:  12-MAY-1993
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  US 07/988,430
;       FILING DATE:  09-DEC-1992
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  US 07/901,707
;       FILING DATE:  19-JUN-1992
;     PRIOR APPLICATION DATA:
;       APPLICATION NUMBER:  US 07/787,567
;       FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;       NAME:  McNicholas, Janet M.
;       REGISTRATION NUMBER:  32,918
;       REFERENCE/DOCKET NUMBER:  11022US09/200-70.P3.C3
;    TELECOMMUNICATION INFORMATION:
;       TELEPHONE:  312/707-8889
;       TELEFAX:  312/707-9155
;       TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;       LENGTH:  118 amino acids
;       TYPE:  amino acid
;       STRANDEDNESS:  single
;       TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-839-765-126

  Query Match             78.4%;  Score 513.5;  DB 3;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:|||||||:||||:|:||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 || ||||||| ||:||||||:||||||||||:| :  :       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 11
US-09-136-389-126
; Sequence 126, Application US/09136389
; Patent No. 6146850
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/136,389
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/646,360
;      FILING DATE:  13-MAY-1996
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-136-389-126

  Query Match             78.4%;  Score 513.5;  DB 3;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:|||||||:||||:|:||||||||||||||||||||||||||:||||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 || ||||||| ||:||||||:||||||||||:| :  :       ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 12
US-09-610-838-126
; Sequence 126, Application US/09610838
; Patent No. 6376217
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  173
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/610,838
;      FILING DATE:  06-JUL-2000
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
```

```
;      APPLICATION NUMBER:  US/09/136,389
;      FILING DATE:  18-AUG-1998
;      APPLICATION NUMBER:  08/646,360
;      FILING DATE:  13-MAY-1996
;      APPLICATION NUMBER:  PCT/US94/05348
;      FILING DATE:  12-MAY-1994
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  200-70.P4
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-610-838-126

  Query Match             78.4%;  Score 513.5;  DB 3;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:||||||||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 || ||||||| ||:|||||:||||||||||:| :  :      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 13
US-09-711-485-126
; Sequence 126, Application US/09711485
; Patent No. 6649742
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/09/711,485
;      FILING DATE:
;      CLASSIFICATION:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  08/839,765
;      FILING DATE:
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US09/200-70.P3.C3
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  126:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  118 amino acids
;      TYPE:  amino acid
;      STRANDEDNESS:  single
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-09-711-485-126

  Query Match             78.4%;  Score 513.5;  DB 4;  Length 118;
  Best Local Similarity   78.6%;  Pred. No. 7.5e-45;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:||||||||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
                 || ||||||| ||:|||||:||||||||||:| :  :      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


RESULT 14
US-08-488-113B-147
; Sequence 147, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
```

```
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  147:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  240 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-147

  Query Match             78.4%;  Score 513.5;  DB 1;  Length 240;
  Best Local Similarity   78.6%;  Pred. No. 1.7e-44;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:|||||:||||||
Db        123 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 182

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  :      ||||||||||
Db        183 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 234


RESULT 15
US-08-488-113B-148
; Sequence 148, Application US/08488113B
; Patent No. 5744580
;  GENERAL INFORMATION:
;    APPLICANT:  Better, Marc D.
;    APPLICANT:  Carroll, Stephen F.
;    APPLICANT:  Studnika, Gary M.
;    TITLE OF INVENTION:  Immunotoxins Comprising Ribosome-Inactivating
;    TITLE OF INVENTION:  Proteins
;    NUMBER OF SEQUENCES:  169
;    CORRESPONDENCE ADDRESS:
;      ADDRESSEE:  McAndrews, Held & Malloy, Ltd.
;      STREET:  500 West Madison Street, 34th floor
;      CITY:  Chicago
;      STATE:  Illinois
;      COUNTRY:  USA
;      ZIP:  60661
;    COMPUTER READABLE FORM:
;      MEDIUM TYPE:  Floppy disk
;      COMPUTER:  IBM PC compatible
;      OPERATING SYSTEM:  PC-DOS/MS-DOS
;      SOFTWARE:  PatentIn Release #1.0, Version #1.25
;    CURRENT APPLICATION DATA:
;      APPLICATION NUMBER:  US/08/488,113B
;      FILING DATE:  07-JUN-1995
;      CLASSIFICATION:  530
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/425,336
;      FILING DATE:  18-APR-1995
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 08/064,691
;      FILING DATE:  12-MAY-1993
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/988,430
;      FILING DATE:  09-DEC-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/901,707
;      FILING DATE:  19-JUN-1992
;    PRIOR APPLICATION DATA:
;      APPLICATION NUMBER:  US 07/787,567
;      FILING DATE:  04-NOV-1991
;    ATTORNEY/AGENT INFORMATION:
;      NAME:  McNicholas, Janet M.
;      REGISTRATION NUMBER:  32,918
;      REFERENCE/DOCKET NUMBER:  11022US07/200-70.P3.C2A
;    TELECOMMUNICATION INFORMATION:
;      TELEPHONE:  312/707-8889
;      TELEFAX:  312/707-9155
;      TELEX:  650 388-1248
;  INFORMATION FOR SEQ ID NO:  148:
;    SEQUENCE CHARACTERISTICS:
;      LENGTH:  240 amino acids
;      TYPE:  amino acid
;      TOPOLOGY:  linear
;    MOLECULE TYPE:  protein
US-08-488-113B-148

  Query Match             78.4%;  Score 513.5;  DB 1;  Length 240;
  Best Local Similarity   78.6%;  Pred. No. 1.7e-44;
  Matches   92;  Conservative   12;  Mismatches    8;  Indels    5;  Gaps    1;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |:|||:||||||:||||:|:||||||||||||||||||||||||||:|||||:||||||
Db          1 EIQLVQSGGGLVKPGGSVRISCAASGYTFTNYGMNWVRQAPGKGLEWMGWINTHTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGT 117
              |  || ||||||| ||:|||||:||||||||||:| :  :      ||||||||||
Db         61 ADSFKGRFTFSLDDSKNTAYLQINSLRAEDTAVYFCTRRGY-----DWYFDVWGQGT 112


Search completed: March 14, 2005, 20:43:51
Job time : 25.3246 secs
```

GenCore version 5.1.6

OM protein - protein search, using sw model

Run on: March 14, 2005, 20:21:17 ; Search time 94.4518 Seconds
(without alignments)
483.186 Million cell updates/sec

Title: US-09-723-752B-7
Perfect score: 655
Sequence: 1 EVQLVESGGGLVQPGGSLRL.....YPHYYGSSHWYFDVWGQGTL 118

Scoring table: BLOSUM62
Gapop 10.0 , Gapext 0.5

Searched: 2105692 seqs, 386760381 residues

Total number of hits satisfying chosen parameters: 2105692

Minimum DB seq length: 0
Maximum DB seq length: 2000000000

Post-processing: Minimum Match 0%
Maximum Match 100%
Listing first 45 summaries

Database : A_Geneseq_16Dec04:*
1: geneseqp1980s:*
2: geneseqp1990s:*
3: geneseqp2000s:*
4: geneseqp2001s:*
5: geneseqp2002s:*
6: geneseqp2003as:*
7: geneseqp2003bs:*
8: geneseqp2004s:*

Pred. No. is the number of results predicted by chance to have a score greater than or equal to the score of the result being printed, and is derived by analysis of the total score distribution.

SUMMARIES

Result No.	Score	% Query Match	Length	DB	ID	Description
1	655	100.0	118	2	AAW70678	Aaw70678 Anti-VEGF
2	655	100.0	118	3	AAB05899	Aab05899 Humanised
3	655	100.0	118	3	AAB13381	Aab13381 F(ab)-12
4	655	100.0	118	3	AAB13389	Aab13389 Anti-VEGF
5	655	100.0	118	5	ABP61247	Abp61247 Humanised
6	655	100.0	123	2	AAW70617	Aaw70617 Anti-VEGF
7	655	100.0	123	5	ABP61186	Abp61186 Humanised
8	655	100.0	123	8	ADG31767	Adg31767 V(H) doma
9	655	100.0	123	8	ADG31780	Adg31780 V(H) doma
10	655	100.0	231	7	ADC26155	Adc26155 Parent an
11	655	100.0	476	8	ADQ90736	Adq90736 Anti-VEGF
12	651	99.4	118	2	AAW70680	Aaw70680 Anti-VEGF
13	651	99.4	118	5	ABP61249	Abp61249 Humanised
14	650	99.2	123	8	ADG31892	Adg31892 V(H) prot
15	649	99.1	123	8	ADG31895	Adg31895 V(H) prot
16	644	98.3	118	2	AAW70682	Aaw70682 Anti-VEGF
17	644	98.3	118	3	AAB05900	Aab05900 F(ab)-12
18	644	98.3	118	3	AAB13382	Aab13382 Anti-VEGF
19	644	98.3	118	5	ABP61251	Abp61251 Humanised
20	644	98.3	123	8	ADG31894	Adg31894 V(H) prot
21	644	98.3	231	7	ADC26158	Adc26158 Anti-VEGF
22	642	98.0	118	2	AAW70684	Aaw70684 Anti-VEGF
23	642	98.0	118	3	AAB13383	Aab13383 Anti-VEGF
24	642	98.0	118	5	ABP61253	Abp61253 Humanised
25	638	97.4	123	2	AAW70626	Aaw70626 Humanised
26	638	97.4	123	5	ABP61195	Abp61195 Humanised
27	638	97.4	123	8	ADG31896	Adg31896 V(H) prot
28	638	97.4	231	7	ADC26162	Adc26162 Anti-VEGF
29	637	97.3	118	2	AAW70676	Aaw70676 Anti-VEGF
30	637	97.3	118	5	ABP61245	Abp61245 Humanised
31	636	97.1	123	8	ADG31897	Adg31897 V(H) prot
32	635	96.9	118	2	AAW70686	Aaw70686 Anti-VEGF
33	635	96.9	118	2	AAW70688	Aaw70688 Anti-VEGF
34	635	96.9	118	3	AAB13385	Aab13385 Anti-VEGF
35	635	96.9	118	3	AAB13384	Aab13384 Anti-VEGF
36	635	96.9	118	5	ABP61255	Abp61255 Humanised
37	635	96.9	118	5	ABP61257	Abp61257 Humanised
38	635	96.9	123	8	ADG31769	Adg31769 V(H) doma
39	635	96.9	254	5	ABP51953	Abp51953 Plasmid p
40	635	96.9	476	5	ABB81110	Abb81110 Anti-VEGF
41	635	96.9	476	8	ADO14129	Ado14129 Plasmid p
42	635	96.9	476	8	ADQ90730	Adq90730 Anti-VEGF
43	634	96.8	123	2	AAW86808	Aaw86808 Variable
44	631.5	96.4	121	3	AAB05901	Aab05901 F(ab)-12
45	631.5	96.4	121	3	AAB13390	Aab13390 Anti-VEGF

ALIGNMENTS

RESULT 1
AAW70678
ID AAW70678 standard; peptide; 118 AA.
XX
AC AAW70678;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable heavy domain of variant Y0101.
XX
KW Heavy variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 9B; 100pp; English.
XX
CC The present sequence represents a variable heavy domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can

```
CC   also be used to treat other conditions that involve angiogenesis, e.g.
CC   rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 655;  DB 2;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 4.5e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 2
AAB05899
ID   AAB05899 standard; peptide; 118 AA.
XX
AC   AAB05899;
XX
DT   17-OCT-2000  (first entry)
XX
DE   Humanised anti-VEGF antibody F(ab)-12 heavy chain variable domain.
XX
KW   Humanised; F(ab)-12; heavy chain variable domain; antibody variant;
KW   phage display; randomised library; cytostatic; antiarthritic;
KW   antipsoriatic; antidiabetic; antiinflammatory; antiarteriosclerotic;
KW   vascular endothelial growth factor; VEGF; breast cancer; lung cancer;
KW   retinoblastoma; rheumatoid arthritis; psoriasis; atherosclerosis;
KW   diabetic retinopathy; complementarity determining region; CDR.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO200029584-A1.
XX
PD   25-MAY-2000.
XX
PF   16-NOV-1999;   99WO-US027153.
XX
PR   18-NOV-1998;   98US-0108945P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Chen YM,  Lowman HB,  Muller Y;
XX
DR   WPI; 2000-387797/33.
XX
PT   Antibody variants with higher binding affinity than native antibodies
PT   useful for diagnosis, prevention and treatment of neoplastic and non-
PT   neoplastic diseases comprises amino acid insertion in hypervariable
PT   region.
XX
PS   Disclosure; Fig 1B; 110pp; English.
XX
CC   The present sequence is the heavy chain variable domain of F(ab)-12, a
CC   humanised anti-vascular endothelial growth factor (VEGF) antibody. F(ab)-
CC   12 was the parent antibody used in the production of a large number of
CC   antibody variants containing randomised peptide inserts within the
CC   complementarity determining regions (CDRs). Phage display libraries were
CC   subjected to eight rounds of selection to isolate variants with an
CC   antigen binding affinity at least two-fold stronger than the binding
CC   affinity of parent antibody for the target VEGF antibody. The anti-VEGF
CC   antibody variants may be useful in diagnostic assays for detecting
CC   expression of VEGF in cells, tissue or serum. They may also be used in
CC   the prevention and treatment of neoplastic diseases such as breast
CC   cancer, lung cancer and retinoblastoma, and non-neoplastic diseases
CC   including rheumatoid arthritis, psoriasis, atherosclerosis, and diabetic
CC   and other proliferative retinopathies
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 655;  DB 3;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 4.5e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 3
AAB13381
ID   AAB13381 standard; protein; 118 AA.
XX
AC   AAB13381;
XX
DT   12-SEP-2003  (revised)
DT   21-NOV-2000  (first entry)
XX
DE   F(ab)-12 anti-VEGF antibody heavy chain variable domain.
XX
KW   Humanised; F(ab)-12; vascular endothelial cell growth factor; VEGF;
KW   antibody; antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Chimeric.
XX
FH   Key             Location/Qualifiers
FT   Region          26. .35
FT                   /label= CDR-H1
FT   Region          50. .66
FT                   /label= CDR-H2
FT   Region          70. .79
FT                   /label= CDR-7
FT   Region          99. .112
FT                   /label= CDR-H3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 14B; 60pp; English.
XX
CC   The present sequence is the heavy chain variable domain of humanised anti
CC   -vascular endothelial cell growth factor (anti-VEGF) antibody F(ab)-12.
CC   It may be used to treat conditions characterised by undesirable excessive
```

```
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Affinity matured anti-VEGF antibodies are
CC   also used as therapeutic agents. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay. (Updated on 12-SEP-
CC   2003 to standardise OS field)
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 655;  DB 3;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 4.5e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 4
AAB13389
ID   AAB13389 standard; protein; 118 AA.
XX
AC   AAB13389;
XX
DT   21-NOV-2000  (first entry)
XX
DE   Anti-VEGF antibody YO192 heavy chain variable domain.
XX
KW   YO192; vascular endothelial cell growth factor; VEGF; antibody;
KW   antiinflammatory; cerebroprotective; cytostatic; antirheumatic;
KW   antiarthritic; antipsoriatic; antiarteriosclerotic; antidiabetic;
KW   antithyroid; excessive neovascularisation; tumour; rheumatoid arthritis;
KW   psoriasis; atherosclerosis; diabetes; retrolental fibroplasia;
KW   neovascular glaucoma; haemangioma; thyroid hyperplasia; Grave's disease;
KW   tissue transplantation; inflammation; oedema; trauma;
KW   complementarity determining region; CDR.
XX
OS   Unidentified.
XX
FH   Key             Location/Qualifiers
FT   Region          26. .35
FT                   /label= CDR-H1
FT   Region          50. .66
FT                   /label= CDR-H2
FT   Region          70. .79
FT                   /label= CDR-7
FT   Region          99. .112
FT                   /label= CDR-H3
XX
PN   WO200037502-A2.
XX
PD   29-JUN-2000.
XX
PF   09-DEC-1999;   99WO-US029475.
XX
PR   22-DEC-1998;   98US-00218481.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Van Bruggen N,  Ferrara N;
XX
DR   WPI; 2000-442646/38.
XX
PT   Treating edema, tumors, rheumatoid arthritis, psoriasis, atherosclerosis,
PT   diabetes and chronic inflammation in a mammal, comprises administering a
PT   human vascular endothelial cell growth factor antagonist.
XX
PS   Disclosure; Fig 15B; 60pp; English.
XX
CC   The present sequence is the heavy chain variable region of the affinity
CC   matured anti-vascular endothelial cell growth factor (anti-VEGF) antibody
CC   YO192. Humanised F(ab)-12 and affinity matured anti-VEGF antibodies may
CC   be used to treat conditions characterised by undesirable excessive
CC   neovascularisation. Such conditions include tumours (especially solid
CC   ones), rheumatoid arthritis, psoriasis, atherosclerosis, diabetes and
CC   other retinopathies, retrolental fibroplasia, age-related macular
CC   degeneration, neovascular glaucoma, haemangiomas, thyroid hyperplasias
CC   (including Grave's disease), corneal and other tissue transplantation,
CC   and chronic inflammation. Oedemas associated with tumours, strokes and
CC   head trauma, and ascites associated with malignancies, meig's syndrome,
CC   lung inflammation, nephrotic syndrome, pericardial effusion and pleural
CC   effusion, may also be treated. Monoclonal antibodies are generated in
CC   hybridoma cells and those with affinity for VEGF are identified by
CC   immunoprecipitation or by an in vitro binding assay
XX
SQ   Sequence 118 AA;

  Query Match             100.0%;  Score 655;  DB 3;  Length 118;
  Best Local Similarity   100.0%;  Pred. No. 4.5e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 5
ABP61247
ID   ABP61247 standard; protein; 118 AA.
XX
AC   ABP61247;
XX
DT   20-SEP-2002  (first entry)
XX
DE   Humanised anti-VEGF Y0101 antibody variable heavy domain.
XX
KW   Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW   vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW   retinal disorder; intraocular neovascular disorder; Y0101; heavy chain;
KW   variable domain.
XX
OS   Homo sapiens.
OS   Mus sp.
OS   Synthetic.
XX
FH   Key             Location/Qualifiers
FT   Domain          26. .35
FT                   /label= CDR-H1
FT   Domain          50. .66
FT                   /label= CDR-H2
FT   Domain          70. .79
FT                   /label= CDR-7
FT   Domain          99. .112
FT                   /label= CDR-H3
XX
PN   US2002032315-A1.
XX
PD   14-MAR-2002.
XX
PF   06-APR-1998;   98US-00056160.
XX
```

PR 06-AUG-1997; 97US-0054856P.
XX
PA (BACA/) BACA M.
PA (WELL/) WELLS J A.
PA (PRES/) PRESTA L G.
PA (LOWM/) LOWMAN H B.
PA (CHEN/) CHEN Y M.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 2002-517920/55.
XX
PT New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT mammal, particularly for treating tumor or retinal disorders.
XX
PS Example 3; Fig 9; 47pp; English.
XX
CC The present invention relates to humanised anti-VEGF (vascular
CC endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC particularly those having a tumour or a retinal disorder e.g. intraocular
CC neovascular disorders. The present sequence is an exemplary heavy chain
CC variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ Sequence 118 AA;

Query Match 100.0%; Score 655; DB 5; Length 118;
Best Local Similarity 100.0%; Pred. No. 4.5e-55;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118

RESULT 6
AAW70617
ID AAW70617 standard; peptide; 123 AA.
XX
AC AAW70617;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody F(ab)-12 variable heavy domain.
XX
KW Heavy variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Claim 7; Fig 1A; 100pp; English.
XX
CC The present sequence represents a variable heavy domain of the humanised
CC anti-vascular endothelial growth factor (anti-VEGF) antibody F(ab)-12.
CC The sequence is used to construct the humanised anti-VEGF antibody of the
CC invention. The humanised antibodies are used to inhibit VEGF-induced
CC angiogenesis, particularly for treating or preventing tumours (of any
CC type) and retinal disorders (e.g. age-related macular degeneration or
CC diabetic retinopathy). They can also be used to treat other conditions
CC that involve angiogenesis, e.g. rheumatoid arthritis, psoriasis,
CC atherosclerosis, Grave's disease, etc
XX
SQ Sequence 123 AA;

Query Match 100.0%; Score 655; DB 2; Length 123;
Best Local Similarity 100.0%; Pred. No. 4.8e-55;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118

RESULT 7
ABP61186
ID ABP61186 standard; protein; 123 AA.
XX
AC ABP61186;
XX
DT 20-SEP-2002 (first entry)
XX
DE Humanised anti-VEGF F(ab) (F(ab)-12) antibody variable heavy domain.
XX
KW Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW retinal disorder; intraocular neovascular disorder; F(ab) (F(ab)-12);
KW heavy chain; variable domain.
XX
OS Homo sapiens.
OS Mus sp.
OS Synthetic.
XX
PN US2002032315-A1.
XX
PD 14-MAR-2002.
XX
PF 06-APR-1998; 98US-00056160.
XX
PR 06-AUG-1997; 97US-0054856P.
XX
PA (BACA/) BACA M.
PA (WELL/) WELLS J A.
PA (PRES/) PRESTA L G.
PA (LOWM/) LOWMAN H B.
PA (CHEN/) CHEN Y M.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 2002-517920/55.
XX
PT New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT or their variants, useful for inhibiting VEGF-induced angiogenesis in a

```
PT   mammal, particularly for treating tumor or retinal disorders.
XX
PS   Claim 7; Fig 1; 47pp; English.
XX
CC   The present invention relates to humanised anti-VEGF (vascular
CC   endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC   antibody, which binds human VEGF. The anti-VEGF antibodies are useful for
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary heavy chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 123 AA;

  Query Match             100.0%;  Score 655;  DB 5;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 4.8e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 8
ADG31767
ID   ADG31767 standard; protein; 123 AA.
XX
AC   ADG31767;
XX
DT   26-FEB-2004  (first entry)
XX
DE   V(H) domain of parental humanised murine anti-VEGF antibody SeqID1.
XX
KW   protein library; in silico; VEGF; vascular endothelial growth factor;
KW   antibody; computational prediction; V(H) domain; mouse; murine.
XX
OS   Synthetic.
OS   Mus sp.
XX
PN   WO2003099999-A2.
XX
PD   04-DEC-2003.
XX
PF   20-MAY-2003; 2003WO-US016037.
XX
PR   20-MAY-2002; 2002US-00153159.
PR   20-MAY-2002; 2002US-00153176.
XX
PA   (ABMA-) ABMAXIS INC.
XX
PI   Luo P,  Hsieh M,  Zhong P,  Wang C,  Cao Y,  Liu S;
XX
DR   WPI; 2004-035117/03.
XX
PT   Constructing antibody libraries for generating protein libraries with
PT   improved biological function comprising selecting from tester protein
PT   sequences two peptide segments having 15% sequence identity with the lead
PT   sequence.
XX
PS   Disclosure; SEQ ID NO 1; 354pp; English.
XX
CC   This invention relates to a novel method for the generation and screening
CC   of a protein library in silico. Specifically, it refers to a high-
CC   throughput method optimised for the identification of anti-VEGF (vascular
CC   endothelial growth factor) antibodies with improved binding affinities
CC   for their target antigen (VEGF), using computational prediction. The
CC   present invention describes selecting proteins with a desirable function
CC   based on their structural similarity to the target structural or
CC   functional motif of a lead protein of interest. Accordingly, these
CC   protein libraries are functionally biased with increased diversity so as
CC   to increase the chance of identifying novel hits or combinations of
CC   mutants with enhanced binding affinity. Furthermore, the sequence profile
CC   based on the multiple structure alignment of the available lead structure
CC   allows the sampling of a larger sequence space than by traditional,
CC   multiple sequence alignment approaches. This polypeptide sequence is the
CC   V(H) domain of parental humanised murine anti-VEGF antibody, used in an
CC   exemplification of the invention.
XX
SQ   Sequence 123 AA;

  Query Match             100.0%;  Score 655;  DB 8;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 4.8e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 9
ADG31780
ID   ADG31780 standard; protein; 123 AA.
XX
AC   ADG31780;
XX
DT   26-FEB-2004  (first entry)
XX
DE   V(H) domain of the anti-VEGF two chain antibody AM2 protein SeqID 14.
XX
KW   protein library; in silico; VEGF; vascular endothelial growth factor;
KW   antibody; computational prediction; V(H) domain; flexon; AM2;
KW   two chain antibody; murine; mouse.
XX
OS   Synthetic.
OS   Unidentified.
OS   Mus sp.
XX
PN   WO2003099999-A2.
XX
PD   04-DEC-2003.
XX
PF   20-MAY-2003; 2003WO-US016037.
XX
PR   20-MAY-2002; 2002US-00153159.
PR   20-MAY-2002; 2002US-00153176.
XX
PA   (ABMA-) ABMAXIS INC.
XX
PI   Luo P,  Hsieh M,  Zhong P,  Wang C,  Cao Y,  Liu S;
XX
DR   WPI; 2004-035117/03.
DR   N-PSDB; ADG31779.
XX
PT   Constructing antibody libraries for generating protein libraries with
PT   improved biological function comprising selecting from tester protein
PT   sequences two peptide segments having 15% sequence identity with the lead
PT   sequence.
XX
PS   Disclosure; SEQ ID NO 14; 354pp; English.
XX
CC   This invention relates to a novel method for the generation and screening
CC   of a protein library in silico. Specifically, it refers to a high-
CC   throughput method optimised for the identification of anti-VEGF (vascular
CC   endothelial growth factor) antibodies with improved binding affinities
CC   for their target antigen (VEGF), using computational prediction. The
CC   present invention describes selecting proteins with a desirable function
CC   based on their structural similarity to the target structural or
CC   functional motif of a lead protein of interest. Accordingly, these
```

```
CC   protein libraries are functionally biased with increased diversity so as
CC   to increase the chance of identifying novel hits or combinations of
CC   mutants with enhanced binding affinity. Furthermore, the sequence profile
CC   based on the multiple structure alignment of the available lead structure
CC   allows the sampling of a larger sequence space than by traditional,
CC   multiple sequence alignment approaches. This polypeptide sequence is the
CC   V(H) domain of the anti-VEGF two chain antibody AMU protein, used in an
CC   exemplification of the invention.
XX
SQ   Sequence 123 AA;

  Query Match             100.0%;  Score 655;  DB 8;  Length 123;
  Best Local Similarity   100.0%;  Pred. No. 4.8e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 10
ADC26155
ID   ADC26155 standard; protein; 231 AA.
XX
AC   ADC26155;
XX
DT   18-DEC-2003  (first entry)
XX
DE   Parent anti-VEGF Y0101 antibody wild-type heavy chain protein.
XX
KW   antibody variant; cytostatic; cancer; parent; anti-VEGF;
KW   vascular endothelial growth factor; Y0101; heavy chain; wild-type.
XX
OS   Unidentified.
XX
PN   WO2003068801-A2.
XX
PD   21-AUG-2003.
XX
PF   11-FEB-2003; 2003WO-US004184.
XX
PR   11-FEB-2002; 2002US-0355895P.
PR   10-SEP-2002; 2002US-0409685P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Lowman HB,  Marvin JS;
XX
DR   WPI; 2003-697521/66.
XX
PT   Making an antibody variant of a parent antibody specific to an antigen by
PT   identifying a target amino acid residue within the variable domain of the
PT   parent antibody and substituting the target residue with a different
PT   amino acid residue.
XX
PS   Example 1; SEQ ID NO 2; 81pp; English.
XX
CC   The invention relates to a novel method for making an antibody variant of
CC   a parent antibody specific to an antigen. This is achieved via
CC   identifying a target amino acid residue within the variable domain of the
CC   parent antibody and substituting the target residue with a different
CC   replacement amino acid residue such that the charge complementarity
CC   between the antibody and antigen is increased. The antibody variant of
CC   the invention demonstrates cytostatic activity whilst the method may be
CC   useful for treating cancer. The current sequence is that of the parent
CC   anti-VEGF (vascular endothelial growth factor) Y0101 antibody Fab
CC   fragment heavy chain protein of the invention.
XX
SQ   Sequence 231 AA;

  Query Match             100.0%;  Score 655;  DB 7;  Length 231;
  Best Local Similarity   100.0%;  Pred. No. 9.6e-55;
  Matches  118;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 11
ADQ90736
ID   ADQ90736 standard; protein; 476 AA.
XX
AC   ADQ90736;
XX
DT   21-OCT-2004  (first entry)
XX
DE   Anti-VEGF antibody heavy chain protein SEQ ID NO:19.
XX
KW   antibody; antigen binding fragment; cell culture; variable domain;
KW   modified framework region; hypervariable region; cytostatic;
KW   antiinflammatory; antiangiogenic; immunomodulatory; antibody therapy;
KW   tumour; inflammatory disorder; angiogenic disorder;
KW   immunological disorder; anti-VEGF antibody;
KW   anti vascular endothelial cell growth factor antibody; heavy chain.
XX
OS   Homo sapiens.
OS   Synthetic.
XX
PN   WO2004065417-A2.
XX
PD   05-AUG-2004.
XX
PF   23-JAN-2004; 2004WO-US001844.
XX
PR   23-JAN-2003; 2003US-0442484P.
XX
PA   (GETH ) GENENTECH INC.
XX
PI   Simmons L;
XX
DR   WPI; 2004-562149/54.
DR   N-PSDB; ADQ90720.
XX
PT   Producing an antibody or antigen binding fragment in high yield in a cell
PT   culture, comprises expressing a variable domain with a modified framework
PT   region in a host cell.
XX
PS   Example 6; SEQ ID NO 25; 161pp; English.
XX
CC   The present invention describes a method for producing an antibody or
CC   antigen binding fragment in high yield in a cell culture. The method
CC   comprises expressing a variable domain of the antibody or antigen binding
CC   fragment comprising a modified framework region (FR) in a host cell, and
CC   recovering the antibody or antigen binding fragment variable domain
CC   comprising the modified framework from the host cell. The modified FR in
CC   the method described above has a substitution of at least one amino acid
CC   position with a different amino acid, where the different amino acid is
CC   the amino acid found at the corresponding FR position of a human subgroup
CC   variable domain consensus sequence that has a hypervariable region 1
CC   (HVR1) and/or HVR2 amino acid sequence with the most sequence identity
CC   with a corresponding HVR1 and/or HVR2 sequence of the variable domain.
CC   The antibody or antigen binding fragment variable domain comprises the
CC   modified FR that has improved yield in cell culture compared to an
CC   unmodified antibody or antigen-binding fragment. The antibody and antigen
CC   binding fragment have cytostatic, antiinflammatory, antiangiogenic and
CC   immunomodulatory activities, and can be used in antibody therapy. The
CC   methods and compositions of the present invention are useful for
```

CC producing antibodies or antigen binding fragments in cell culture, in
CC particular for improving the yield of recombinant antibodies or antigen
CC binding fragments in cell culture. The antibodies of the invention can be
CC used to diagnose, treat, inhibit or prevent e.g. tumours and
CC inflammatory, angiogenic and immunological disorders. The present
CC sequence represents the heavy chain of an anti-VEGF (vascular endothelial
CC cell growth factor) antibody, which is used in the exemplification of the
CC present invention.
XX
SQ Sequence 476 AA;

Query Match 100.0%; Score 655; DB 8; Length 476;
Best Local Similarity 100.0%; Pred. No. 2.2e-54;
Matches 118; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 24 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 83

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
Db 84 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 141

RESULT 12
AAW70680
ID AAW70680 standard; peptide; 118 AA.
XX
AC AAW70680;
XX
DT 27-JAN-1999 (first entry)
XX
DE Anti-VEGF humanised antibody variable heavy domain of variant Y0192.
XX
KW Heavy variable domain; murine; humanised antibody;
KW anti-vascular endothelial growth factor antibody; anti-VEGF antibody;
KW VEGF-induced angiogenesis; tumour; retinal disorder;
KW age-related macular degeneration; diabetic retinopathy;
KW rheumatoid arthritis; psoriasis; atherosclerosis; Grave's disease.
XX
OS Synthetic.
OS Mus sp.
OS Homo sapiens.
XX
PN WO9845331-A2.
XX
PD 15-OCT-1998.
XX
PF 03-APR-1998; 98WO-US006604.
XX
PR 07-APR-1997; 97US-00833504.
PR 06-AUG-1997; 97US-00908469.
XX
PA (GETH) GENENTECH INC.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 1998-568337/48.
XX
PT New humanised antibody with affinity for vascular endothelial growth
PT factor - for treatment of tumours, retinal disease and other angiogenic
PT states, also related nucleic acid, vectors and transformed cells.
XX
PS Example 3; Fig 9B; 100pp; English.
XX
CC The present sequence represents a variable heavy domain of an affinity-
CC matured anti-vascular endothelial growth factor (anti-VEGF) antibody
CC variant. The sequence is used in the course of the invention to produce
CC the humanised anti-VEGF antibody of the invention. The humanised
CC antibodies are used to inhibit VEGF-induced angiogenesis, particularly
CC for treating or preventing tumours (of any type) and retinal disorders
CC (e.g. age-related macular degeneration or diabetic retinopathy). They can
CC also be used to treat other conditions that involve angiogenesis, e.g.
CC rheumatoid arthritis, psoriasis, atherosclerosis, Grave's disease, etc
XX
SQ Sequence 118 AA;

Query Match 99.4%; Score 651; DB 2; Length 118;
Best Local Similarity 99.2%; Pred. No. 1.1e-54;
Matches 117; Conservative 1; Mismatches 0; Indels 0; Gaps 0;

Qy 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
Db 1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
Db 61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118

RESULT 13
ABP61249
ID ABP61249 standard; protein; 118 AA.
XX
AC ABP61249;
XX
DT 20-SEP-2002 (first entry)
XX
DE Humanised anti-VEGF Y0192 antibody variable heavy domain.
XX
KW Cytostatic; ophthalmological; humanised; antibody; anti-VEGF; VEGF;
KW vascular endothelial growth factor; angiogenesis inhibitor; tumour;
KW retinal disorder; intraocular neovascular disorder; Y0192; heavy chain;
KW variable domain.
XX
OS Homo sapiens.
OS Mus sp.
OS Synthetic.
XX
FH Key Location/Qualifiers
FT Domain 26. .35
FT /label= CDR-H1
FT Domain 50. .66
FT /label= CDR-H2
FT Domain 70. .79
FT /label= CDR-7
FT Domain 99. .112
FT /label= CDR-H3
XX
PN US2002032315-A1.
XX
PD 14-MAR-2002.
XX
PF 06-APR-1998; 98US-00056160.
XX
PR 06-AUG-1997; 97US-0054856P.
XX
PA (BACA/) BACA M.
PA (WELL/) WELLS J A.
PA (PRES/) PRESTA L G.
PA (LOWM/) LOWMAN H B.
PA (CHEN/) CHEN Y M.
XX
PI Baca M, Wells JA, Presta LG, Lowman HB, Chen YM;
XX
DR WPI; 2002-517920/55.
XX
PT New humanized anti-VEGF (vascular endothelial growth factor) antibodies
PT or their variants, useful for inhibiting VEGF-induced angiogenesis in a
PT mammal, particularly for treating tumor or retinal disorders.
XX
PS Example 3; Fig 9; 47pp; English.
XX
CC The present invention relates to humanised anti-VEGF (vascular
CC endothelial growth factor) antibodies or a variant of a parent anti-VEGF
CC antibody, which binds human VEGF. The anti-VEGF antibodies are useful for

```
CC   inhibiting VEGF-induced angiogenesis in a mammal (particularly a human),
CC   particularly those having a tumour or a retinal disorder e.g. intraocular
CC   neovascular disorders. The present sequence is an exemplary heavy chain
CC   variable domain of the humanised anti-VEGF antibody of the invention
XX
SQ   Sequence 118 AA;

  Query Match             99.4%;  Score 651;  DB 5;  Length 118;
  Best Local Similarity   99.2%;  Pred. No. 1.1e-54;
  Matches  117;  Conservative    1;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||:||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118


RESULT 14
ADG31892
ID   ADG31892 standard; peptide; 123 AA.
XX
AC   ADG31892;
XX
DT   26-FEB-2004  (first entry)
XX
DE   V(H) protein sequence of anti-VEGF antibody X64 SeqID 126.
XX
KW   protein library; in silico; VEGF; vascular endothelial growth factor;
KW   antibody; computational prediction; V(H) domain.
XX
OS   Unidentified.
XX
PN   WO2003099999-A2.
XX
PD   04-DEC-2003.
XX
PF   20-MAY-2003; 2003WO-US016037.
XX
PR   20-MAY-2002; 2002US-00153159.
PR   20-MAY-2002; 2002US-00153176.
XX
PA   (ABMA-) ABMAXIS INC.
XX
PI   Luo P,  Hsieh M,  Zhong P,  Wang C,  Cao Y,  Liu S;
XX
DR   WPI; 2004-035117/03.
XX
PT   Constructing antibody libraries for generating protein libraries with
PT   improved biological function comprising selecting from tester protein
PT   sequences two peptide segments having 15% sequence identity with the lead
PT   sequence.
XX
PS   Disclosure; SEQ ID NO 126; 354pp; English.
XX
CC   This invention relates to a novel method for the generation and screening
CC   of a protein library in silico. Specifically, it refers to a high-
CC   throughput method optimised for the identification of anti-VEGF (vascular
CC   endothelial growth factor) antibodies with improved binding affinities
CC   for their target antigen (VEGF), using computational prediction. The
CC   present invention describes selecting proteins with a desirable function
CC   based on their structural similarity to the target structural or
CC   functional motif of a lead protein of interest. Accordingly, these
CC   protein libraries are functionally biased with increased diversity so as
CC   to increase the chance of identifying novel hits or combinations of
CC   mutants with enhanced binding affinity. Furthermore, the sequence profile
CC   based on the multiple structure alignment of the available lead structure
CC   allows the sampling of a larger sequence space than by traditional,
CC   multiple sequence alignment approaches. This polypeptide sequence is the
CC   V(H) protein sequence of anti-VEGF antibody X64, used in an
CC   exemplification of the invention.
XX
SQ   Sequence 123 AA;

  Query Match             99.2%;  Score 650;  DB 8;  Length 123;
  Best Local Similarity   99.2%;  Pred. No. 1.4e-54;
  Matches  117;  Conservative    0;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              |||||||||||||||||||||||||||||||||||||||| ||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGRSHWYFDVWGQGTL 118


RESULT 15
ADG31895
ID   ADG31895 standard; peptide; 123 AA.
XX
AC   ADG31895;
XX
DT   26-FEB-2004  (first entry)
XX
DE   V(H) protein sequence of anti-VEGF antibody D36 SeqID 129.
XX
KW   protein library; in silico; VEGF; vascular endothelial growth factor;
KW   antibody; computational prediction; V(H) domain.
XX
OS   Unidentified.
XX
PN   WO2003099999-A2.
XX
PD   04-DEC-2003.
XX
PF   20-MAY-2003; 2003WO-US016037.
XX
PR   20-MAY-2002; 2002US-00153159.
PR   20-MAY-2002; 2002US-00153176.
XX
PA   (ABMA-) ABMAXIS INC.
XX
PI   Luo P,  Hsieh M,  Zhong P,  Wang C,  Cao Y,  Liu S;
XX
DR   WPI; 2004-035117/03.
XX
PT   Constructing antibody libraries for generating protein libraries with
PT   improved biological function comprising selecting from tester protein
PT   sequences two peptide segments having 15% sequence identity with the lead
PT   sequence.
XX
PS   Disclosure; SEQ ID NO 129; 354pp; English.
XX
CC   This invention relates to a novel method for the generation and screening
CC   of a protein library in silico. Specifically, it refers to a high-
CC   throughput method optimised for the identification of anti-VEGF (vascular
CC   endothelial growth factor) antibodies with improved binding affinities
CC   for their target antigen (VEGF), using computational prediction. The
CC   present invention describes selecting proteins with a desirable function
CC   based on their structural similarity to the target structural or
CC   functional motif of a lead protein of interest. Accordingly, these
CC   protein libraries are functionally biased with increased diversity so as
CC   to increase the chance of identifying novel hits or combinations of
CC   mutants with enhanced binding affinity. Furthermore, the sequence profile
CC   based on the multiple structure alignment of the available lead structure
CC   allows the sampling of a larger sequence space than by traditional,
CC   multiple sequence alignment approaches. This polypeptide sequence is the
CC   V(H) protein sequence of anti-VEGF antibody D36, used in an
CC   exemplification of the invention.
XX
SQ   Sequence 123 AA;

  Query Match             99.1%;  Score 649;  DB 8;  Length 123;
```

Best Local Similarity 98.3%; Pred. No. 1.8e-54;
Matches 116; Conservative 2; Mismatches 0; Indels 0; Gaps 0;

```
Qy          1 EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60
              |||||:||||:||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 EVQLVQSGGGVVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGWINTYTGEPTY 60

Qy         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 AADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYPHYYGSSHWYFDVWGQGTL 118
```

Search completed: March 14, 2005, 20:39:15
Job time : 95.4518 secs

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

BIBDATASHEET

Bib Data Sheet

CONFIRMATION NO. 6340

SERIAL NUMBER	FILING OR 371(c) DATE / RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/723,752	11/27/2000	424	1642	P1093P1D1

APPLICANTS

Manuel Baca, Foster City, CA;
James A. Wells, Burlingame, CA;
Leonard G. Presta, San Francisco, CA;
Henry B. Lowman, El Granada, CA;
Yvonne Man-Yee Chen, San Mateo, CA;

**** CONTINUING DATA ***************************

This application is a DIV of 08/908,469 08/06/1997 PAT 6,884,879

**** FOREIGN APPLICATIONS *********************

IF REQUIRED, FOREIGN FILING LICENSE GRANTED
** 04/18/2001

Foreign Priority claimed	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☐ no	CA	16	17	1

35 USC 119 (a-d) conditions met: ☐ yes ☐ no ☐ Met after Allowance

Verified and Acknowledged: _____ Examiner's Signature Initials

ADDRESS

9157

TITLE

Anti-VEGF antibodies

FILING FEE RECEIVED	FEES: Authority has been given in Paper
840	No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:

☐ All Fees
☐ 1.16 Fees (Filing)
☐ 1.17 Fees (Processing Ext. of time)
☐ 1.18 Fees (Issue)
☐ Other _____
☐ Credit

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

9157 7590 05/06/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER
HELMS, LARRY RONALD

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 05/06/2005

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

TITLE OF INVENTION: ANTI-VEGF ANTIBODIES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1400	$0	$1400	08/08/2005

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

A. If the status is the same, pay the TOTAL FEE(S) DUE shown above.

B. If the status above is to be removed, check box 5b on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 12/04) Approved for use through 04/30/2007.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail **Mail Stop ISSUE FEE**
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (703) 746-4000

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

9157 7590 05/06/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

TITLE OF INVENTION: ANTI-VEGF ANTIBODIES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1400	$0	$1400	08/08/2005

EXAMINER	ART UNIT	CLASS-SUBCLASS
HELMS, LARRY RONALD	1642	424-133100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.
1 _____
2 _____
3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are enclosed:
- ☐ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of Copies _____

4b. Payment of Fee(s):
- ☐ A check in the amount of the fee(s) is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☐ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
- ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.
NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date _____

Typed or printed name _____ Registration No. _____

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

9157 7590 05/06/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HELMS, LARRY RONALD	

ART UNIT	PAPER NUMBER
1642	

DATE MAILED: 05/06/2005

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)
(application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 118 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 118 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571) 272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 12/04) Approved for use through 04/30/2007.

Notice of Allowability	Application No.	Applicant(s)	
	09/723,752	BACA ET AL.	
	Examiner	Art Unit	
	Larry R. Helms	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment filed 3/1/05*.

2. ☒ The allowed claim(s) is/are *51 and 52*.

3. ☐ The drawings filed on _____ are accepted by the Examiner.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
   1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date 11/3/03
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☐ Examiner's Amendment/Comment
8. ☐ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

LARRY R. HELMS, PH.D
PRIMARY EXAMINER

FORM PTO-1449

U.S. Dept. of Commerce
Patent and Trademark Office

Atty Docket No.	Serial No.
P1093P1D1	09/723752
Applicant: Baca et al.	
Filing Date: 27 Nov 2000	Group: 1642

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
LCD	79	2002/032315	14.03.02	Baca et al.	—	—	
LAO	80	6,037,454	14.03.00	Jardieu et al.	—	—	

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
LAO	81	WO 98/45331	15.10.98	PCT	—	—		
LAO	82	WO 98/45332	15.10.98	PCT	—	—		

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

LAO	83	Presta et al., "Humanization of an Anti-Vascular Endothelial Growth Factor Monoclonal Antibody for the Therapy of Solid Tumors and Other Disorders" Cancer Research 57(20):4593-4599 (Oct 15, 1997)

Examiner: [signature]

Date Considered: 3/15/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

Issue Classification	Application/Control No.	Applicant(s)/Patent under Reexamination	
	09/723,752	BACA ET AL.	
	Examiner	Art Unit	
	Larry R. Helms	1642	

ISSUE CLASSIFICATION

ORIGINAL					CLASS	CROSS REFERENCE(S)						
CLASS				SUBCLASS		SUBCLASS (ONE SUBCLASS PER BLOCK)						
424				133.1	424	156.1						
INTERNATIONAL CLASSIFICATION					530	387.3	388.85					
A	6	1	K	39/395								
				/								
				/								
				/								
				/								

(Assistant Examiner) (Date)

(Legal Instruments Examiner) (Date)

LARRY R. HELMS, PH.D
PRIMARY EXAMINER

Larry R. Helms 5/5/05

(Primary Examiner) (Date)

Total Claims Allowed: 2

O.G. Print Claim(s)	O.G. Print Fig.
1	none

☐ **Claims renumbered in the same order as presented by applicant** ☐ CPA ☐ T.D. ☐ R.1.47

Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
	1		31		61		91		121		151		181
	2		32		62		92		122		152		182
	3		33		63		93		123		153		183
	4		34		64		94		124		154		184
	5		35		65		95		125		155		185
	6		36		66		96		126		156		186
	7		37		67		97		127		157		187
	8		38		68		98		128		158		188
	9		39		69		99		129		159		189
	10		40		70		100		130		160		190
	11		41		71		101		131		161		191
	12		42		72		102		132		162		192
	13		43		73		103		133		163		193
	14		44		74		104		134		164		194
	15		45		75		105		135		165		195
	16		46		76		106		136		166		196
	17		47		77		107		137		167		197
	18		48		78		108		138		168		198
	19		49		79		109		139		169		199
	20		50		80		110		140		170		200
	21	1	51		81		111		141		171		201
	22	2	52		82		112		142		172		202
	23		53		83		113		143		173		203
	24		54		84		114		144		174		204
	25		55		85		115		145		175		205
	26		56		86		116		146		176		206
	27		57		87		117		147		177		207
	28		58		88		118		148		178		208
	29		59		89		119		149		179		209
	30		60		90		120		150		180		210

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

BIBDATASHEET

Bib Data Sheet

CONFIRMATION NO. 6340

SERIAL NUMBER	FILING OR 371(c) DATE / RULE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/723,752	11/27/2000	424	1642	P1093P1D1

APPLICANTS

Manuel Baca, Foster City, CA;
James A. Wells, Burlingame, CA;
Leonard G. Presta, San Francisco, CA;
Henry B. Lowman, El Granada, CA;
Yvonne Man-Yee Chen, San Mateo, CA;

**** CONTINUING DATA ***************************

This application is a DIV of 08/908,469 08/06/1997 PAT 6,884,879

**** FOREIGN APPLICATIONS *********************

IF REQUIRED, FOREIGN FILING LICENSE GRANTED
** 04/18/2001

Foreign Priority claimed	35 USC 119 (a-d) conditions met	Verified and Acknowledged	STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS
☐ yes ☒ no	☐ yes ☒ no ☐ Met after Allowance	Examiner's Signature / Initials	CA	16	17	1

ADDRESS
9157

TITLE
Anti-VEGF antibodies

FILING FEE RECEIVED	FEES: Authority has been given in Paper
840	No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:

☐ All Fees
☐ 1.16 Fees (Filing)
☐ 1.17 Fees (Processing Ext. of time)
☐ 1.18 Fees (Issue)
☐ Other _____
☐ Credit

MAR 0 3 2005

1642

Patent Docket P1093P1D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of Manuel Baca et al. Serial No.: 09/723,752 Filed: November 27, 2000 Title: ANTI-VEGF ANTIBODIES	Group Art Unit: 1642 Examiner: Helms, Larry Ronald Confirmation No: 6340 Customer No: 09157 **EXPRESS MAIL LABEL NO.: EV 385 659 925 US** **DATE OF DEPOSIT: MARCH 1, 2005**

OK TO ENTER LRH 3/15/05

RESPONSE TO NOTICE OF NON-COMPLIANT AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This document is responsive to the Communication mailed December 13, 2004 (Paper No. 20041210) and the Notice of Non-Compliant Amendment mailed February 4, 2005 for which a one-month period for response was given. Applicant is required to cancel the currently rejected claims in order for claims 51-52 to be allowed.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 4 of this paper.

Amendments to the Claims

Please cancel claims 43-47, 49, 50, 53-60.

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1.-50. (Canceled)

1 51. (Previously presented) A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:116 and a light chain variable domain sequence of SEQ ID NO:115.

2 52. (Previously presented) A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:7 and a light chain variable domain sequence of SEQ ID NO:8.

53.-60. (Canceled)

Index of Claims

Application/Control No.	Applicant(s)/Patent under Reexamination
09/723,752	BACA ET AL.
Examiner	**Art Unit**
Larry R. Helms	1642

√	Rejected	–	(Through numeral) Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

Claim		Date								
Final	Original									
	1									
	2									
	3									
	4									
	5									
	6									
	7									
	8									
	9									
	10									
	11									
	12									
	13									
	14									
	15									
	16									
	17									
	18									
	19									
	20									
	21									
	22									
	23									
	24									
	25									
	26									
	27									
	28									
	29									
	30									
	31									
	32									
	33									
	34									
	35									
	36									
	37									
	38									
	39									
	40									
	41									
	42									
	43									
	44									
	45									
	46									
	47									
	48									
	49									
	50									

Claim		Date								
Final	Original	3/25/05								
1	51	=								
2	52	=								
	53									
	54									
	55									
	56									
	57									
	58									
	59									
	60									
	61									
	62									
	63									
	64									
	65									
	66									
	67									
	68									
	69									
	70									
	71									
	72									
	73									
	74									
	75									
	76									
	77									
	78									
	79									
	80									
	81									
	82									
	83									
	84									
	85									
	86									
	87									
	88									
	89									
	90									
	91									
	92									
	93									
	94									
	95									
	96									
	97									
	98									
	99									
	100									

Claim		Date								
Final	Original									
	101									
	102									
	103									
	104									
	105									
	106									
	107									
	108									
	109									
	110									
	111									
	112									
	113									
	114									
	115									
	116									
	117									
	118									
	119									
	120									
	121									
	122									
	123									
	124									
	125									
	126									
	127									
	128									
	129									
	130									
	131									
	132									
	133									
	134									
	135									
	136									
	137									
	138									
	139									
	140									
	141									
	142									
	143									
	144									
	145									
	146									
	147									
	148									
	149									
	150									

Search Notes

Application/Control No.	Applicant(s)/Patent under Reexamination	
09/723,752	BACA ET AL.	
Examiner	**Art Unit**	
Larry R. Helms	1642	

SEARCHED			
Class	Subclass	Date	Examiner
UPDATED		3/15/05	LRH

SEARCH NOTES (INCLUDING SEARCH STRATEGY)		
	DATE	EXMR
UPDATED 562 10 115, 116 7, 8	3/15/05	LRH

INTERFERENCE SEARCHED			
Class	Subclass	Date	Examiner
562 10#	7, 8, 115, 116	3/15/05	LRH
424	130.1, 131.1, 135.1, 144.1, 155.1, 156.1		
530	387.1, 387.3, 388.1, 388.24, 388.8, 388.85		

AUG 0 1 2005

PART B - FEE(S) TRANSMITTAL

EXPRESS MAIL LABEL NO: EV 385 659 783 US

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
or Fax (703) 746-4000

DATE OF DEPOSIT: JULY 29, 2005

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

9157 7590 05/06/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

08/02/2005 CNGUYEN1 00000018 070630 09723752

01 FC:1501 1400.00 DA

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (703) 746-4000, on the date indicated below.

Eileen Ly (Depositor's name)
(Signature)
July 29, 2005 (Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

TITLE OF INVENTION: ANTI-VEGF ANTIBODIES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1400	$0	$1400	08/08/2005

EXAMINER	ART UNIT	CLASS-SUBCLASS
HELMS, LARRY RONALD	1642	424-133100

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
(1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
(2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Steven X. Cui
Genentech, Inc.
2
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: Genentech, Inc.

(B) RESIDENCE: (CITY and STATE OR COUNTRY): South San Francisco, CA

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are enclosed:
- ☒ Issue Fee
- ☐ Publication Fee (No small entity discount permitted)
- ☐ Advance Order - # of copies _____

4b. Payment of Fee(s):
- ☐ A check in the amount of the fee(s) is enclosed.
- ☐ Payment by credit card. Form PTO-2038 is attached.
- ☒ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number 07-0630 (enclose an extra copy of this form).

5. **Change in Entity Status** (from status indicated above)
- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27.
- ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

The Director of the USPTO is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.
NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

Authorized Signature _____ Date July 29, 2005

Typed or printed name Steven X. Cui Registration No. 44,637

This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

● PRINTER RUSH ●

(PTO ASSISTANCE)

Application : 09/723752 **Examiner :** Helms **GAU :** 1642

From: NW93 **Location:** (IDC) FMF FDC **Date:** 10-17-05

Tracking #: epm 09732752 Week Date: 5-16-05

DOC CODE	DOC DATE	MISCELLANEOUS
☐ 1449		☐ Continuing Data
☑ IDS	3-6-2001	☐ Foreign Priority
☐ CLM		☐ Document Legibility
☐ IIFW		☐ Fees
☐ SRFW		☐ Other
☐ DRW		
☐ OATH		
☐ 312		
☐ SPEC		

[RUSH] **MESSAGE:** PTO-1449: Please either initial/line through citations.
[TIFS 353, 354, 355, 356]

Thank You
NW93

[XRUSH] **RESPONSE:**

INITIALS:

NOTE: This form will be included as part of the official USPTO record, with the Response document coded as XRUSH.

REV 10/04

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/723,752	11/27/2000	Manuel Baca	P1093P1D1	6340

9157 7590 11/08/2005

GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

EXAMINER	
HELMS, LARRY RONALD	
ART UNIT	**PAPER NUMBER**
1642	

DATE MAILED: 11/08/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

PTO-90C (Rev. 10/03)

UNITED STATES DEPARTMENT OF COMMERCE
U.S. Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450

APPLICATION NO./ CONTROL NO.	FILING DATE	FIRST NAMED INVENTOR / PATENT IN REEXAMINATION	ATTORNEY DOCKET NO.
09/723752			

EXAMINER
LARRY HELMS

ART UNIT	PAPER
1643	20051101

DATE MAILED:

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner for Patents

The IDS filed 3/6/01 has been considered and is attatched.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Larry R. Helms, Ph.D, whose telephone number is (571) 272-0832.

ATTATCHED
4
PAGES OF
105

LARRY R. HELMS, PH.D.
SUPERVISORY PATENT EXAMINER

PTO-90C (Rev.04-03)

FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1093P1D1	09/723752
Applicant: Baca et al.	
Filing Date: 27 Nov 2000	Group

U.S. PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Name	Class	Subclass	Filing Date
LD	* 1	4,816,567	28.03.89	Cabilly et al.			
LD	* 2	5,530,101	25.06.96	Queen et al.			19.12.90
LD	* 3	5,580,723	01.12.96	Wells et al.			

FOREIGN PATENT DOCUMENTS

Examiner Initials		Document Number	Date	Country	Class	Subclass	Translation Yes	Translation No
LD	* 4	0,451,216	01.01.96	EPO				
	* 5	WO 91/09967	11.07.91	PCT				
	* 6	WO 92/22653	23.12.92	PCT				
	* 7	WO 94/04679	03.03.94	PCT				
	* 8	WO 94/10202	11.05.94	PCT				
	* 9	WO 96/30046	03.10.96	PCT				
	*10	2,268,744	19.12.94	UNITED KINGDOM				
LD	*11	2188638	07.10.87	UNITED KINGDOM				

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

LD	*12	Adamis et al., "Inhibition of Vascular Endothelial Growth Factor Prevents Retinal Ischemia-Associated Iris Neovascularization in a Nonhuman Primate" Arch. Ophthalmology 114(1):66-71 (1996)
	*13	Aiello et al., "Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders" New England J. of Medicine 331(22):1480-1487 (1994)
	*14	Alberts et al., "Molecular Biology of the Cell", 3rd edition, Garland Publishing pps. 1154 (1994)
	*15	Allen et al., "Specificity of the T cell Receptor: Two Different Determinants are Generated by the Same Peptide and the IA^k Molecule" J. Immunol. 135(1):368-373 (Jul 1985)
	*16	Baca et al., "Antibody Humanization Using Monovalent Phage Display" Journal of Biological Chemistry 272(16):10678-10684 (1997)
	*17	Bass et al., "Hormone Phage: An Enrichment Method for Variant Proteins with Altered Binding Properties" Proteins: Structure, Function, and Genetics 8(4):309-314 (1990)
	*18	Bendig, M. M., "Humanization of Rodent Monoclonal Antibodies" Methods: A Companion to Methods in Enzymology 8:83-93 (1994)
	*19	Berkman et al., "Expression of the vascular permeability factor/vascular endothelial growth factor gene in central nervous system neoplasms" J. Clin. Invest. 91(1):153-159 (1993)
	*20	Borgstrom et al., "Complete inhibition of angiogenesis and growth of microtumors by anti-vascular endothelial growth factor neutralizing antibody: novel concepts of angiostatic therapy from intravital videomicroscopy" Cancer Research 56(17):4032-4039 (1996)
	*21	Brown et al., "Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract" Cancer Research 53(19):4727-4735 (1993)
LD	*22	Brown et al., "Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer" Human Pathology 26(1):86-91 (1995)

Examiner: [signature] Date Considered 11/1/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1093P1D1	09/723752
Applicant: Baca et al.	
Filing Date: 27 Nov 2000	Group

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

	*23	Carter et al., "Humanization of an anti-p185^{HER2} antibody for human cancer therapy" Proc. Natl. Acad. Sci. 89:4285-4289 (1992)
	*24	Chang et al., "High-level secretion of human growth hormone by Escherichia coli" Gene 55:189-196 (1987)
	*25	Chisholm, "High Efficiency Gene Transfer into Mammaliam Cells" DNA Cloning 4. Mammaliam Systems pps. 1-41 (1995)
	*26	Chothia et al., "Domain Association in Immunoglobulin Molecules. The Packing of Variable Domains" Journal of Molecular Biology 186:651-663 (1985)
	*27	Clapp et al., "The 16-kilodalton N-terminal fragment of human prolactin is a potent inhibitor of angiogenesis" Endocrinology 133(3):1292-1299 (1993)
	*28	Cunningham et al., "Production of an Atrial Natriuretic Peptide Variant that is Specific for Type A Receptor" EMBO Journal 13(11):2508-2515 (1994)
	*29	de Vries et al., "The fms-like tyrosine kinase, a receptor for vascular endothelial growth factor" Science 255:989-991 (1992)
	*30	Dvorak et al., "Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis" American Journal of Pathology 146(5):1029-1039 (1995).
	*31	Eaton et al., "Construction and characterization of an active factor VIII variant lacking the central one-third of the molecule" Biochemistry 25:8343-8347 (1986)
	*32	Eigenbrot et al., "X-Ray Structures of Fragments From Binding and Nonbinding Versions of a Humanized Anti-CD18 Antibody: Structural Indications of the Key Role of V_H Residues 59 to 65" Proteins: Structure, Function, and Genetics 18:49-62 (1994)
	*33	Eigenbrot et al., "X-ray structures of the antigen-binding domains from three variants of humanized anti-p185^{HER2} antibody 4D5 and comparison with molecular modeling" J. Mol. Biol. 229:969-995 (1993)
	*34	Ferrara and Davis-Smyth, "The biology of vascular endothelial growth factor" Endocrine Reviews 18(1):4-25 (1997)
	*35	Folkman and Shing, "Angiogenesis" Journal of Biological Chemistry 267:10931-10934 (1992)
	*36	Foote et al., "Antibody Framework Residues Affecting the Conformation of the Hypervariable Loops" J. Mol. Biol. 224:487-499 (1992)
	*37	Garner, A., "Vascular Diseases" Pathobiology of Ocular Disease. A Dynamic Approach, Garner, A., Klintworth GK Eds., 2nd edition, NY:Marcel Dekker pps. 1625-1710 (1994)
	*38	Garrard et al., "Fab assembly and enrichment in a monovalent phage display system" Bio/technology 9:1373-1377 (1991)
	*39	Good et al., "A tumor suppressor-dependent inhibitor of angiogenesis is immunologically and functionally indistinguishable from a fragment of thrombospondin" Proc. Natl. Acad. Sci. USA 87(17):6624-6628 (1990)
	*40	Gorman et al., "Transient Production of Proteins Using an Adenovirus Transformed Cell Line" DNA Prot. Eng. Tech. 2(1):3-10 (1990)
	*41	Graham et al., "Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5" J. Gen. Virol. 36:59-74 (1977)
	*42	Hawkins et al., "Selection of Phage Antibodies by Binding Affinity Mimicking Affinity Maturation" J. Mol. Biol. 226:889-896 (1992)

Examiner: [signature] Date Considered: 11/1/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

FORM PTO-1449 U.S. Dept. of Commerce Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1093P1D1	09/723752
Applicant: Baca et al.	
Filing Date: 27 Nov 2000	Group

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

	*43	Horak et al., "Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival in breast cancer" Lancet 340(8828):1120-1124 (1992)
	*44	Kabat et al. Sequences of Proteins of Immunological Interest, U.S. Dept. of Health and Human Services, NIH, 5th edition Vol. 1:103-108, 324-331 (1991)
	*45	Karlsson et al., "Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system" J. Immun. Methods 145:229-240 (1991)
	*46	Karlsson et al., "Kinetic and Concentration Analysis Using BIA Technology" Methods: A Comparison to Methods in Enzymology 6:99-110 (1994)
	*47	Kettleborough et al., "Humanization of a Mouse Monoclonal Antibody by CDR-grafting: the Importance of Framework Residues on Loop Conformation" Protein Engineering 4(7):773-783 (1991)
	*48	Kim et al., "Inhibition of Vascular Endothelial Growth Factor-Induced Angiogenesis Suppresses Tumour Growth in vivo" Nature 362:841-844 (1993)
	*49	Kim et al., "The Vascular Endothelial Growth Factor Proteins: Identification of Biologically Relevant Regions by Neutralizing Monoclonal Antibodies" Growth Factors 7(1):53-64 (1992)
	*50	Klagsbrun and D'Amore, "Regulators of angiogenesis" Ann. Rev. Physiol. 53:217-239 (1991)
	*51	Kunkel et al., "Efficient site-directed mutagenesis using uracil-containing DNA" Methods in Enzymology 204:125-139 (1991)
	*52	Kunkel, T., "Rapid and Efficient Site-Specific Mutagenesis Without Phenotypic Selection" Proc. Natl. Acad. Sci. 82:488-492 (1985)
	*53	Leung et al., "Vascular Endothelial Growth Factor is a Secreted Angiogenic Mitogen" Science 246:1306-1309 (1989)
	*54	Lopez et al., "Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes" Invest. Ophthalmol. Vis. Sci. 37(5):855-868 (1996)
	*55	Lowman et al., "Selecting High-Affinity Binding Proteins by Monovalent Phage Display" Biochemistry 30(45):10832-10838 (1991)
	*56	Lucas et al., "High-level production of recombinant proteins in CHO cells using a dicistronic DHFR intron expression vector" Nucleic Acids Research 24(9):1774-1779 (1996)
	*57	Macchiarini et al., "Relation of neovascularisation to metastasis of non-small-cell lung cancer" Lancet 340(8812):145-146 (1992)
	*58	Mattern et al., "Association of vascular endothelial growth factor expression with intratumoral microvessel density and tumour cell proliferation in human epidermoid lung carcinoma" Brit. J. Cancer 73(7):931-934 (1996)
	*59	Melnyk et al., "Vascular endothelial growth factor promotes tumor dissemination by a mechanism distinct from its effect on primary tumor growth" Cancer Research 56(4):921-924 (1996)
	*60	Novotny et al., "Structural invariants of antigen binding: comparison of immunoglobulin V_L-V_H and V_L-V_L domain dimers" Proc. Natl. Acad. Sci. USA 82(14):4592-4596 (Jul 1985)
	*61	O'Reilly et al., "Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma" Cell 79(2):315-328 (1994)
	*62	O'Reilly et al., "Endostatin: an endogenous inhibitor of angiogenesis and tumor growth" Cell 88(2):277-285 (1997)

Examiner: [signature] Date Considered: 11/1/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

FORM PTO-1449

U.S. Department of Commerce
Patent and Trademark Office

LIST OF DISCLOSURES CITED BY APPLICANT

(Use several sheets if necessary)

Atty Docket No.	Serial No.
P1093P1D1	09/723752
Applicant: Baca et al.	
Filing Date: 27 Nov 2000	Group

OTHER DISCLOSURES (Including Author, Title, Date, Pertinent Pages, etc.)

LP	*63	Padlan, E., "A Possible Procedure for Reducing the Immunogenicity of Antibody Variable Domains While Preserving Their Ligand-Binding Properties" Molecular Immunology 28(4/4):489-498 (1991)
	*64	Park et al., "Placenta growth factor. Potentiation of vascular endothelial growth factor bioactivity, in vitro and in vivo, and high affinity binding to Flt-1 but not to Flk-1/KDR" Journal of Biological Chemistry 269(41):25646-25654 (1994)
	*65	Presta et al., "Humanization of an Antibody Directed Against IgE" J. Immunol. 151(5):2623-2632 (September 1, 1993)
	*66	Queen et al., "A humanized antibody that binds to the interleukin 2 receptor" Proc. Natl. Acad. Sci. USA 86(24):10029-10033 (December 1989)
	*67	Roguska et al., "Humanization of murine monoclonal antibodies through variable domain resurfacing" Proc. Natl. Acad. Sci. USA 91:969-973 (Feb 1994)
	*68	Rosok et al., "A Combinatorial Library Strategy for the Rapid Humanization of Anticarcinoma BR96 Fab" Journal of Biological Chemistry 271(37):22611-22618 (Sep 13, 1996)
	*69	Sanger et al., "DNA Sequencing with Chain-terminating Inhibitors" Proc. Natl. Acad. Sci. USA 74(12):5463-5467 (Dec 1977)
	*70	Shalaby et al., "Development of Humanized Bispecific Antibodies Reactive with Cytotoxic Lymphocytes and Tumor Cells Overexpressing the HER2 Protooncogene" Journal of Experimental Medicine 175:217-225 (Jan 1, 1992)
	*71	Studnicka et al., "Human-engineered monoclonal antibodies retain full specific binding activity by preserving non-CDR complementarity-modulating residues" Protein Eng. 7(6):805-814 (1994)
	*72	Tempest et al., "Reshaping a Human Monoclonal Antibody to Inhibit Human Respiratory Syncytial Virus Infection In Vivo" Bio/Technology 9:266-271 (March 1991)
	*73	Vieira et al., "Production of Single-stranded Plasmid DNA" Methods in Enzymology 153:3-11 (1987)
	*74	Warren et al., "Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis" J. Clin. Invest. 95(4):1789-1797 (1995)
	*75	Weidner et al., "Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma" New England J. of Medicine 324(1):1-8 (1991)
	*76	Werther et al., "Humanization of an Anti-Lymphocyte Function-Associated Antigen (LFA)-1 Monoclonal Antibody and Reengineering of the Humanized Antibody for Binding to Rhesus LFA-1" J. of Immunology 157:4986-4995 (1996)
	*77	Winter et al., "Making antibodies by phage display technology" Annual Review of Immunology 12:433-455 (1994)
LP	*78	Yang et al., "CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range" Journal of Molecular Biology 254(3):392-403 (Dec 1, 1995)

Examiner	Date Considered
	11/1/05

*Examiner: Initial if reference considered, whether or not citation is in conformance with MPEP 609; draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

USCOMM-DC 80-398.

SIDLEY AUSTIN LLP
SIDLEY

RECEIVED
CENTRAL FAX CENTER
AUG 2 5 2006

SIDLEY AUSTIN LLP
1501 K STREET, N.W.
WASHINGTON, D.C. 20005
TEL. +1 202 736 8000
FAX +1 202 736 8711

FAX TRANSMISSION

*Total **3 pages**, including cover sheet*

To: Commissioner of Patents
U.S. Patent and Trademark Office

Fax no.: **(571) 273-8300**

From: Jeffrey P. Kushan
Tel. (202) 736-8000

Date: 25 August 2006

Re:

Patent:	7,060,269		
Issued:	June 13, 2006		
Application No:	09/723,752	Atty. Docket No.:	22338-80060
Patentee:	Manuel Baca et al.	Unit:	OPLA; Attn: K. Ferriter

For: ANTI-VEGF ANTIBODIES - Application for § 156 Patent Term Extension

CERTIFICATE OF TRANSMISSION UNDER 37 C.F.R. § 1.8

I CERTIFY THAT THE FOLLOWING DOCUMENTS ARE BEING TRANSMITTED TO THE USPTO AT FAX NUMBER (571) 273-8300 THE DATE SHOWN:

- Power of Attorney by Assignee
- Transmittal

SIGNATURE — Brian McMonagle, PRINTED NAME — 8/25/06, DATE

RECEIVED
CENTRAL FAX CENTER
AUG 25 2006

Serial no. 09/723,752 Attorney Docket No. 22338-80060

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* – § 156
Patent No.: 7,060,269
Issued: June 13, 2006
Application No: 09/723,752

Docket No: 22338-80060
Assignee: Genentech, Inc.
Unit: OPLA; Attn: K. Ferriter

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extenstion

Mail Stop: **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY BY ASSIGNEE

The assignee of the entire right, title, and interest in U.S. Patent No. 7,060,269 (granted on application serial no. 09/723,752), Genentech, Inc., hereby appoints the practitioners associated with

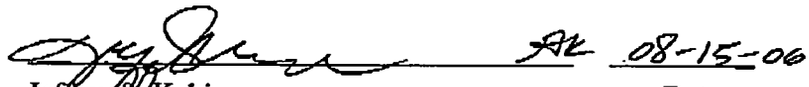
CUSTOMER NUMBER 33694

as its attorneys and agents to prosecute the captioned patent/application, and to transact all business in the U.S. Patent and Trademark Office connected therewith.

Pursuant to 37 C.F.R. § 3.73(b), the undersigned states that Genentech, Inc. is the assignee of the entire right, title, and interest in the captioned patent/application by virtue of an assignment by the inventors to Genentech Inc. recorded at Reel 008872/ Frame 0429.

The undersigned, whose title is supplied below, is authorized to act on behalf of the assignee.

Respectfully submitted,
GENENTECH, INC.



08-15-06

Jeffrey S. Kubinec
Associate General Counsel – Patent Law

Date

Serial no. 09/723,752 Attorney Docket No. 22338-80060

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* – § 156	Docket No: 22338-80060
Patent No.: 7,060,269	Assignee: Genentech, Inc.
Issued: June 13, 2006	Unit: OPLA; Attn: K. Ferriter
Application No: 09/723,752	

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extension

Mail Stop: **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL

Transmitted for filing in the captioned application is a Power of Attorney by the assignee. We believe that no fee is due in connection with this submission.

Should any fee under 37 C.F.R. §§ 1.16 or 1.17 be required to render this or any other paper filed during the pendency of this application timely or proper, the Director is requested to charge the appropriate amount to our Deposit Account No. 18-1260.

Respectfully submitted,

Jeffrey P. Kushan, Reg. No. 43,401
Attorney for Patentees

Date: 8-24-06

SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005
tel. (202) 736-8914
fax (202) 736-8711

08-28-06

DAC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* -- § 156

Patent No.: 7,060,269

Issued: June 13, 2006

Application No: 09/723,752

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extension

Docket No: 22338-80060

Assignee: Genentech, Inc.

Unit: OPLA

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919899 US

I hereby certify this correspondence is being deposited with the U.S. Postal Service with sufficient postage as "Express Mail – Post Office to Addressee" addressed to: Mail Stop Patent Ext., Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Signature — YVONNE T. REYES (Printed Name) — Aug. 25, 2006 (Date)

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 7,060,269 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 7,060,269, granted to Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen (Baca *et al.*) by virtue of an assignment of such patent to Genentech, Inc., recorded December 29, 1997, at Reel 8872, Frame 0429.[1]

[1] The assignment recorded at the noted location in the Office's records identifies U.S.S.N. 08/908,469 ("the '469 application") and states that the conveyance includes the entire "right, title and interest ... in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor...." U.S.S.N. 09/723,752, from which the '269 patent issued, is a continuation application (divisional) of the '469 application.

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is LUCENTIS™. The name of the active ingredient of LUCENTIS ™ is ranibizumab. Ranibizumab is a recombinant humanized monoclonal IgG_1 antibody antigen-binding fragment (Fab) based on a humanized framework with complementarity-determining regions (CDRs) derived from a murine monoclonal antibody that binds to human Vascular Endothelial Growth Factor (VEGF).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

LUCENTIS™ was approved for commercial marketing or use under § 351 of the Public Heath Service Act on **June 30, 2006**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a humanized monoclonal IgG_1 antibody antigen-binding fragment produced by an *E. coli* expression system. It contains human framework regions (FRs) and the complementarity-determining regions (CDRs) derived from a murine antibody that binds to VEGF.

(b) Applicant certifies that ranibizumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 30, 2006 to the present Applicant.

(c) Ranibizumab has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. *See* LUCENTIS™ product label, provided as Attachment A.

(d) LUCENTIS™ was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* LUCENTIS™ approval letter, provided as Attachment B.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 28, 2006.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a)	Names of the inventors:	Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen.
(b)	Patent Number:	7,060,269
(c)	Date of Issue:	June 13, 2006
(d)	Date of Expiration:	July 4, 2019[2]

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 7,060,269 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 7,060,269 is not subject to a terminal disclaimer.

(b) A Certificate of Correction has not been issued for U.S. Patent No. 7,060,269.

(c) The first maintenance fee for U.S. Patent No. 7,060,269 will be due on December 13, 2009.

(d) U.S. Patent No. 7,060,269 has not been the subject of a reexamination proceeding.

---

[2] The term of the '269 patent has been extended, under 35 USC § 154(b) by 697 days. The 697 days have been included in calculating the July 4, 2019 expiration date.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claim 1 of U.S. Patent No. 7,060,269 ("the '269 patent") claims the active pharmaceutical ingredient in the approved product or a method that may be used to make or use that ingredient.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how the above-listed claim of the '269 patent claims a method of using the approved product.

(1) Description of the approved product and its method of use

The approved product is described in Section 11 of the approved label for LUCENTIS™ as follows, a copy of which is provided as Attachment A.

> LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.
>
> LUCENTIS™ is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS™ is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS™ aqueous solution with 10 mM histidine HCL, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

Ranibizumab is further characterized in a scientific reference by Chen *et al.* published in 1999 in the Journal of Molecular Biology (JMB) entitled "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen."[3] For example, the heavy

[3] 293:865-881 (1999) (Attachment E)

and light chain sequences of ranibizumab, designated as Y0317 in the article, are displayed in Figure 1. In addition, the article provides data regarding the binding affinity of the Y0317 antibody fragment to VEGF. *See, e.g.,* Table 6 on p. 870.

(2) Claim 1

Claim 1 of the '269 patent reads as follows:

1. A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about $1x10^{-8}$ M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:116 and a light chain variable domain sequence of SEQ ID NO:115.

Comparison of Ranibizumab to the limitations of claim 1

Claim 1 pertains to a method of inhibiting VEGF-induced angiogenesis in a subject by administering an effective amount of a humanized anti-VEGF antibody that binds to human VEGF at a defined Kd value and that contains designated light and heavy chain variable domains. Applicant asserts that the use of ranibizumab for the treatment of age-related macular degeneration falls within the scope of claim 1 for at least the following reasons.

According to the label, ranibizumab is a humanized anti-VEGF antibody fragment that has been found effective in the treatment of patients with neovascular (wet) age-related macular degeneration. Ranibizumab binds to and inhibits the biological activity of human vascular endothelial growth factor A (VEGF-A), which has been shown to cause neovascularization and leakage in models of ocular angiogenesis. The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation (i.e., angiogenesis). *See* Label ¶¶11 and 12.1. Accordingly, administration of an effective amount of ranibizumab inhibits VEGF-induced angiogenesis in a subject to which it is administered. Applicant notes that the term "antibody" as defined in the '269 patent includes, in addition to full-length antibodies, antibody fragments such as Fab, Fab', $F(ab)_2$ and Fv as long as the fragments exhibit the desired biological activity, i.e., binding to human VEGF (*See, e.g.,* Col 8, lines 43-54). Ranibizumab, being a Fab fragment that binds human VEGF, falls within the scope of the term "antibody" as it is used in claim 1.

Claim 1 also pertains to administering an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about

$1x10^{-8}$, wherein the antibody contains the variable light and heavy chains of SEQ ID NOS: 115 and 116. The article by Chen *et al* presents data demonstrating that ranibizumab (designated as Y0317) does, in fact, bind human VEGF with a K_d value of no more than about $1x10^{-8}$ M. For example, Table 6 on page 870 of the reference shows that ranibizumab has a K_d value of about $1.4 x10^{-10}$ and thus falls within the scope of claim 1. Finally, Figures 10A and 10B of the '269 patent provide the sequence of the light chain variable and heavy chain variable domains of, *inter alia*, ranibizumab (noted therein as Fab Y0317). The light chain variable and heavy chain variable domains depicted in Figures 10A and 10B are identical to SEQ ID NO:115 and SEQ ID NO:116, respectively, of the '269 patent. Accordingly, ranibizumab contains the heavy chain variable domain (SEQ ID NO:116) and the light chain variable domain (SEQ ID NO:115) recited in claim 1.

For at least the reasons discussed above, claim 1 of the '269 patent covers, *inter alia*, a method of using the approved drug product, ranibizumab.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 7,060,269 was issued on June 13, 2006.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for LUCENTIS ™ (referred to as "Humanized Monoclonal Antibody Fragment (rhuFab V2)(E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal") was October 7, 1999. The IND was assigned number BB-IND # 8633. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment E. The application date for the IND was October 6, 1999.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 29, 2005. The BLA was assigned number BL# 125156/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment F.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125156/0 authorizing the marketing of LUCENTIS™ on June 30, 2006. LUCENTIS™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc., before the FDA in relation to the regulatory review of LUCENTIS™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On October 6, 1999, Genentech submitted to FDA (*See* Attachment E) an investigational new drug application for a recombinant humanized monoclonal antibody fragment (rhuFab V2, now known as ranibizumab) against Vascular Endothelial Growth Factor (VEGF). The antibody was developed as a potential new therapeutic in treating patients with the exudative (wet or neovascular) form for age-related macular degeneration (AMD).

- On October 7, 1999 FDA made BB-IND #8633 effective via a communication mailed to Genentech on October 13, 1999 (*See* Attachment E). According to the FDA, initiation of trials could begin 30 days after October 7, 1999.

- The first human clinical trial (Phase I) was initiated on February 8, 2000 followed by Phase II human trials and Phase III human trials, some of which remain ongoing at the time of this application.

- On February 5, 2002, representatives of Genentech and the FDA (CBER and CDER) participated in a Type C meeting to discuss the proposed clinical development plan for ranibizumab in AMD.

- On October 31, 2002 representatives of Genentech and FDA (CBER and CDER) participated in an Type B End-of-Phase II meeting.

- Beginning in approximately March 2003, and continuing at the time of this application, Phase III studies have been conducted. The three Phase III trials forming the basis of the Biologics License Application (BLA), FVF2598g, FVF2587g, and FVF3192g are studies of two year duration with primary endpoints of one year. FVF2587g and FVF3192g, along with extension study FVF3426g and safety study FVF3689g, remain ongoing at the time of this application.

- On September 21, 2005 representatives of Genentech and CDER participated in a Type B Pre-BLA submission meeting to discuss information requirements for the BLA.

- Genentech submitted a BLA for ranibizumab for the treatment of patients with wet AMD on December 29, 2005. (*See* Attachment F)

- FDA acknowledged receipt of the BLA for ranibizumab via a communication mailed to Genentech dated January 27, 2006. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BL #125156/0 to the BLA (*See* Attachment F).

- By way of a communication mailed to Genentech on March 14, 2006 FDA made Genentech aware that the BLA for ranibizumab was filed on February 28, 2006 and that FDA had assigned a user fee goal date of June 30, 2006 (*See* Attachment G).

- On June 30, 2006 FDA approved BLA 125156/0, issuing marketing authorization for LUCENTIS™ (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 7,060,269 is eligible for an extension under § 156 because:

(i) one or more claims of the '269 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '269 patent has not been previously extended on the basis of § 156;

(iii) the '269 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, LUCENTIS™;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '269 patent is requested by Applicant to be extended is **17 days**.

(c) The requested period of extension of term for the '269 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product LUCENTIS™ and the issuance of the patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective	October 7, 1999
Biologics License Application (BLA) under PHSA § 351 was filed	December 29, 2005
Patent was granted	June 13, 2006
BLA was approved	June 30, 2006

(ii) The '269 patent was granted after the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(c), the calculated regulatory review period therefore does not include a component of time between when the IND became effective and when the BLA was submitted.

(iii) The patent was granted during the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component equal to the total number of days in that period that are after the issuance of the patent (17 days).

(iv) The period determined according to § 156(b), (c), and (g)(1) for the approved product (*i.e.*, the number of days following the date of patent issuance until the approval of the BLA) is 17 days.

(v) The '269 patent will expire on July 4, 2019.

(vi) The date of approval of the approved product is June 30, 2006.

(vii) The date that is fourteen years from the date of approval of the approved product is June 30, 2020.

(viii) The period measured from the date the patent expires (*i.e.*, July 4, 2019) until the end of the fourteen-year period specified in §156 (c)(3) (*i.e.*, June 30, 2020) is approximately 361 days.

(ix) The number of days in the regulatory review period determined pursuant to § 156(g)(1)(B)(ii) does not exceed the number of days that the patent may be extended pursuant to §156(c)(3). As such, the period by which the

patent may be extended is not limited by the fourteen-year rule of §156(c)(3).

(x) The '269 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Our check in payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 accompanies this application. Please deduct any additional required fees from, or credit any overpayments to our deposit account no. 18-1260.

15. Name and Address for Correspondence [§ 1.740(a)(14)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005
Phone: 202-736-8914
Fax: 202-736-8111
email: jkushan@sidley.com

The correspondence address for U.S. Patent No. 7,060,269 is unchanged for all other purposes. A Power of Attorney granted to the undersigned by the patent assignee, a copy of which is included with this application as Attachment H, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b).

Sincerely,

Jeffrey P. Kushan
Attorney for Applicant
Registration No. 43,401

Sidley Austin LLP
1501 K Street, N.W.
Washington, D.C. 20005

Dated: August 24, 2006

INDEX OF ATTACHMENTS

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use LUCENTIS safely and effectively. See full prescribing information for LUCENTIS.

LUCENTIS™ (ranibizumab injection)

Initial U.S. Approval: 2006

----------INDICATIONS AND USAGE----------

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (1).

----------DOSAGE AND ADMINISTRATION----------

- FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY (2.1)
- LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month (2.2).
- Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly (2.2).

----------DOSAGE FORMS AND STRENGTHS----------

- 10 mg/mL single-use vial (3)

----------CONTRAINDICATIONS----------

- Ocular or periocular infections (4.1)
- Hypersensitivity (4.2)

----------WARNINGS AND PRECAUTIONS----------

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be monitored during the week following the injection (5.1).
- Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection (5.2).

----------ADVERSE REACTIONS----------

The most common adverse reactions (reported ≥ 6% higher in LUCENTIS-treated subjects than control subjects) are conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation (6.2).

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See Section 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY.

2.2 Dosing

LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month.

Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly [*see Clinical Studies (14.2)*].

2.3 Preparation for Administration

Using aseptic technique, all (0.2 mL) of the LUCENTIS vial contents are withdrawn through a 5-micron 19-gauge filter needle attached to a 1-cc tuberculin syringe. The filter needle should be discarded after withdrawal of the vial contents and should not be used for intravitreal injection. The filter needle should be replaced with a sterile 30-gauge × 1/2-inch needle for the intravitreal injection. The contents should be expelled until the plunger tip is aligned with the line that marks 0.05 mL on the syringe.

2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a broad-spectrum microbicide should be given prior to the injection.

Following the intravitreal injection, patients should be monitored for elevation in intraocular pressure and for endophthalmitis. Monitoring may consist of a check for perfusion of the optic nerve head immediately after the injection, tonometry within 30 minutes following the injection, and biomicroscopy between two and seven days following the injection. Patients should be instructed to report any symptoms suggestive of endophthalmitis without delay.

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before LUCENTIS is administered to the other eye.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

2.5 Stability and Storage

LUCENTIS should be refrigerated at 2°-8°C (36°-46°F). DO NOT FREEZE. Do not use beyond the date stamped on the label. LUCENTIS vials should be protected from light. Store in the original carton until time of use.

3 DOSAGE FORMS AND STRENGTHS

Single-use glass vial designed to deliver 0.05 mL of 10 mg/mL.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

LUCENTIS is contraindicated in patients with ocular or periocular infections.

4.2 Hypersensitivity

LUCENTIS is contraindicated in patients with known hypersensitivity to ranibizumab or any of the excipients in LUCENTIS.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with LUCENTIS, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique should always be used when administering LUCENTIS. In addition, patients should be monitored during the week following the injection to permit early treatment should an infection occur [*see Dosage and Administration (2.3, 2.4) and Patient Counseling Information (17)*].

5.2 Increases in Intraocular Pressure

Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection with LUCENTIS. Therefore, intraocular pressure as well as the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

Although there was a low rate (<4%) of arterial thromboembolic events observed in the LUCENTIS clinical trials, there is a theoretical risk of arterial thromboembolic events following intravitreal use of inhibitors of VEGF [*see Adverse Reactions (6.3)*].

6 ADVERSE REACTIONS

6.1 Injection Procedure

Serious adverse events related to the injection procedure have occurred in < 0.1% of intravitreal injections, including endophthalmitis [*see Warnings and Precautions (5.1)*], rhegmatogenous retinal detachments, and iatrogenic traumatic cataracts.

6.2 Clinical Trials Experience – Ocular Events

Other serious ocular adverse events observed among LUCENTIS-treated patients occurring in <2% of patients

included intraocular inflammation and increased intraocular pressure [*see Warnings and Precautions (5.1, 5.2)*].

The available safety data include exposure to LUCENTIS in 874 patients with neovascular age-related macular degeneration in three double-masked, controlled studies with dosage regimens of 0.3 mg (375 patients) or 0.5 mg (379 patients) administered monthly by intravitreal injection (Studies 1 and 2) [*see Clinical Studies (14.1)*] and dosage regimens of 0.3 mg (59 patients) or 0.5 mg (61 patients) administered once a month for 3 consecutive doses followed by a dose administered once every 3 months (Study 3) [*see Clinical Studies (14.2)*].

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in one clinical trial of a drug cannot be directly compared with rates in the clinical trials of the same or another drug and may not reflect the rates observed in practice.

Table 1 shows the most frequently reported ocular adverse events that were reported with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 1

Adverse Event	LUCENTIS	Control
Conjunctival hemorrhage	77%-43%	66%-29%
Eye pain	37%-17%	33%-11%
Vitreous floaters	32%-3%	10%-3%
Retinal hemorrhage	26%-15%	56%-37%
Intraocular pressure increased	24%-8%	7%-3%
Vitreous detachment	22%-7%	18%-13%
Intraocular inflammation	18%-5%	11%-3%
Eye irritation	19%-4%	20%-6%
Cataract	16%-5%	16%-6%
Foreign body sensation in eyes	19%-6%	14%-6%
Lacrimation increased	17%-3%	16%-0%
Eye pruritis	13%-0%	12%-3%
Visual disturbance	14%-0%	9%-2%
Blepharitis	13%-3%	9%-4%
Subretinal fibrosis	13%-0%	19%-10%
Ocular hyperemia	10%-5%	10%-1%
Maculopathy	10%-3%	11%-3%
Visual acuity blurred/decreased	17%-4%	24%-10%
Detachment of the retinal pigment epithelium	11%-1%	15%-3%
Dry eye	10%-3%	8%-5%
Ocular discomfort	8%-0%	5%-0%
Conjunctival hyperemia	9%-0%	7%-0%
Posterior capsule opacification	8%-0%	5%-0%
Retinal exudates	9%-1%	11%-3%

6.3 Clinical Trials Experience – Non-Ocular Events

Table 2 shows the most frequently reported non-ocular adverse events with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 2

Adverse Event	LUCENTIS	Control
Hypertension/elevated blood pressure	23%-5%	23%-8%
Nasopharyngitis	16%-5%	13%-5%
Arthralgia	11%-3%	9%-0%
Headache	15%-2%	10%-3%
Bronchitis	10%-3%	8%-2%
Cough	10%-3%	7%-2%
Anemia	8%-3%	8%-0%
Nausea	9%-2%	6%-4%
Sinusitis	8%-2%	6%-4%
Upper respiratory tract infection	15%-2%	10%-4%
Back pain	10%-1%	9%-0%
Urinary tract infection	9%-4%	8%-5%
Influenza	10%-2%	5%-1%
Arthritis	8%-0%	8%-2%
Dizziness	8%-2%	10%-2%
Constipation	7%-3%	8%-2%

The rate of arterial thromboembolic events in the three studies in the first year was 2.1% of patients (18 out of 874) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 1.1% of patients (5 out of 441) in the control arms of the studies. In the second year of Study 1, the rate of arterial thromboembolic events was 3.0% of patients (14 out of 466) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 3.2% of patients (7 out of 216) in the control arm [*see Warnings and Precautions (5.3)*].

6.4 Immunogenicity

The pre-treatment incidence of immunoreactivity to LUCENTIS was 0%-3% across treatment groups. After monthly dosing with LUCENTIS for 12 to 24 months, low titers of antibodies to LUCENTIS were detected in approximately 1%-6% of patients. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to LUCENTIS in an electrochemiluminescence assay and are highly dependent on the sensitivity and specificity of the assay. The clinical significance of immunoreactivity to LUCENTIS is unclear at this time, although some patients with the highest levels of immunoreactivity were noted to have iritis or vitritis.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with LUCENTIS.

LUCENTIS intravitreal injection has been used adjunctively with verteporfin photodynamic therapy (PDT). Twelve of 105 (11%) patients developed serious intraocular inflammation; in 10 of the 12 patients, this occurred when LUCENTIS was administered 7 days (± 2 days) after verteporfin PDT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with ranibizumab. It is also not known whether ranibizumab can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. LUCENTIS should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers
It is not known whether ranibizumab is excreted in human milk. Because many drugs are excreted in human milk, and because the potential for absorption and harm to infant growth and development exists, caution should be exercised when LUCENTIS is administered to a nursing woman.

8.4 Pediatric Use
The safety and effectiveness of LUCENTIS in pediatric patients has not been established.

8.5 Geriatric Use
In the controlled clinical studies, approximately 94% (822/879) of the patients randomized to treatment with LUCENTIS were ≥ 65 years of age and approximately 68% (601/879) were ≥ 75 years of age. No notable difference in treatment effect was seen with increasing age in any of the studies. Age did not have a significant effect on systemic exposure in a population pharmacokinetic analysis after correcting for creatinine clearance.

8.6 Patients with Renal Impairment
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with renal impairment. Sixty-eight percent of patients (136 of 200) in the population pharmacokinetic analysis had renal impairment (46.5% mild, 20% moderate, and 1.5% severe). Reduction in ranibizumab clearance is minimal in patients with renal impairment and is considered clinically insignificant. Dose adjustment is not expected to be needed for patients with renal impairment.

8.7 Patients with Hepatic Dysfunction
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with hepatic impairment. Dose adjustment is not expected to be needed for patients with hepatic dysfunction.

10 OVERDOSAGE
Planned initial single doses of ranibizumab injection 1.0 mg were associated with clinically significant intraocular inflammation in 2 of 2 patients injected. With an escalating regimen of doses beginning with initial doses of ranibizumab injection 0.3 mg, doses as high as 2.0 mg were tolerated in 15 of 20 patients.

11 DESCRIPTION
LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.

LUCENTIS is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS aqueous solution with 10 mM histidine HCl, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Ranibizumab binds to the receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecule, $VEGF_{110}$. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and is thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD). The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation.

12.2 Pharmacodynamics
Neovascular AMD is associated with foveal retinal thickening as assessed by optical coherence tomography (OCT) and leakage from CNV as assessed by fluorescein angiography.

In Study 3, foveal retinal thickness was assessed by OCT in 118/184 patients. OCT measurements were collected at baseline, Months 1, 2, 3, 5, 8, and 12. In patients treated with LUCENTIS, foveal retinal thickness decreased, on average, more than the sham group from baseline through Month 12. Retinal thickness decreased by Month 1 and decreased further at Month 3, on average. Foveal retinal thickness data did not provide information useful in influencing treatment decisions [*see Clinical Studies (14.2)*].

In patients treated with LUCENTIS, the area of vascular leakage, on average, decreased by Month 3 as assessed by fluorescein angiography. The area of vascular leakage for an individual patient was not correlated with visual acuity.

12.3 Pharmacokinetics
In animal studies, following intravitreal injection, ranibizumab was cleared from the vitreous with a half-life of approximately 3 days. After reaching a maximum at approximately 1 day,

the serum concentration of ranibizumab declined in parallel with the vitreous concentration. In these animal studies, systemic exposure of ranibizumab is more than 2000-fold lower than in the vitreous.

In patients with neovascular AMD, following monthly intravitreal administration, maximum ranibizumab serum concentrations were low (0.3 ng/mL to 2.36 ng/mL). These levels were below the concentration of ranibizumab (11 ng/mL to 27 ng/mL) thought to be necessary to inhibit the biological activity of VEGF-A by 50%, as measured in an in vitro cellular proliferation assay. The maximum observed serum concentration was dose proportional over the dose range of 0.05 to 1.0 mg/eye. Based on a population pharmacokinetic analysis, maximum serum concentrations of 1.5 ng/mL are predicted to be reached at approximately 1 day after monthly intravitreal administration of LUCENTIS 0.5 mg/eye. Based on the disappearance of ranibizumab from serum, the estimated average vitreous elimination half-life was approximately 9 days. Steady-state minimum concentration is predicted to be 0.22 ng/mL with a monthly dosing regimen. In humans, serum ranibizumab concentrations are predicted to be approximately 90,000-fold lower than vitreal concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or mutagenicity data are available for ranibizumab injection in animals or humans.

No studies on the effects of ranibizumab on fertility have been conducted.

14 CLINICAL STUDIES

The safety and efficacy of LUCENTIS were assessed in three randomized, double-masked, sham- or active-controlled studies in patients with neovascular AMD. A total of 1323 patients (LUCENTIS 879, Control 444) were enrolled in the three studies.

14.1 Study 1 and Study 2

In Study 1, patients with minimally classic or occult (without classic) CNV lesions received monthly LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or monthly sham injections. Data are available through Month 24. Patients treated with LUCENTIS in Study 1 received a mean of 22 total treatments out of a possible 24 from Day 0 to Month 24.

In Study 2, patients with predominantly classic CNV lesions received one of the following: 1) monthly LUCENTIS 0.3 mg intravitreal injections and sham PDT; 2) monthly LUCENTIS 0.5 mg intravitreal injections and sham PDT; or 3) sham intravitreal injections and active verteporfin PDT. Sham PDT (or active verteporfin PDT) was given with the initial LUCENTIS (or sham) intravitreal injection and every 3 months thereafter if fluorescein angiography showed persistence or recurrence of leakage. Data are available through Month 12. Patients treated with LUCENTIS in Study 2 received a mean of 12 total treatments out of a possible 13 from Day 0 through Month 12.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at 12 months compared with baseline. Almost all LUCENTIS-treated patients (approximately 95%) maintained their visual acuity. 34%-40% of LUCENTIS-treated patients experienced a clinically significant improvement in vision, defined as gaining 15 or more letters at 12 months. The size of the lesion did not significantly affect the results. Detailed results are shown in the tables below.

Table 3
Outcomes at Month 12 and Month 24 in Study 1

Outcome Measure	Month	Sham n = 238	LUCENTIS 0.5 mg n = 240	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	Month 12	62%	95%	32% (26%, 39%)
	Month 24	53%	90%	37% (29%, 44%)
Gain of ≥ 15 letters in visual acuity (%)[b]	Month 12	5%	34%	29% (22%,-35%)
	Month 24	4%	33%	29% (23%, 35%)
Mean change in visual acuity (letters) (SD)[b]	Month 12	-10.5 (16.6)	+7.2 (14.4)	17.5 (14.8, 20.2)
	Month 24	-14.9 (18.7)	+6.6 (16.5)	21.1 (18.1, 24.2)

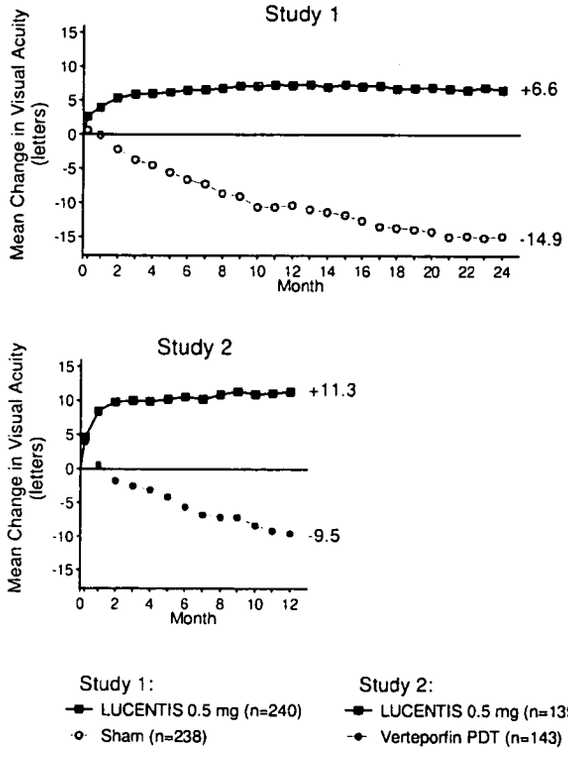
[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Table 4
Outcomes at Month 12 in Study 2

Outcome Measure	Verteporfin PDT n = 143	LUCENTIS 0.5 mg n = 140	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	64%	96%	33% (25%, 41%)
Gain of ≥ 15 letters in visual acuity (%)[b]	6%	40%	35% (26%, 44%)
Mean change in visual acuity (letters) (SD)[b]	-9.5 (16.4)	+11.3 (14.6)	21.1 (17.5, 24.6)

[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Figure 1
Mean Change in Visual Acuity from Baseline to Month 24 in Study 1 and to Month 12 in Study 2



Patients in the group treated with LUCENTIS had minimal observable CNV lesion growth, on average. At Month 12, the mean change in the total area of the CNV lesion was 0.1-0.3 DA for LUCENTIS versus 2.3-2.6 DA for the control arms.

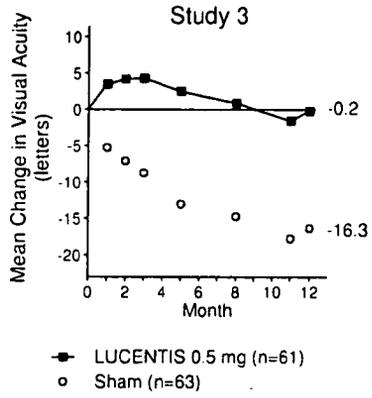
The use of LUCENTIS beyond 24 months has not been studied.

14.2 Study 3

Study 3 was a randomized, double-masked, sham-controlled, two-year study designed to assess the safety and efficacy of LUCENTIS in patients with neovascular AMD (with or without a classic CNV component). Data are available through Month 12. Patients received LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or sham injections once a month for 3 consecutive doses, followed by a dose administered once every 3 months. A total of 184 patients were enrolled in this study (LUCENTIS 0.3 mg, 60; LUCENTIS 0.5 mg, 61; sham, 63); 171 (93%) completed 12 months of this study. Patients treated with LUCENTIS in Study 3 received a mean of 6 total treatments out of possible 6 from Day 0 through Month 12.

In Study 3, the primary efficacy endpoint was mean change in visual acuity at 12 months compared with baseline (see Figure 2). After an initial increase in visual acuity (following monthly dosing), on average, patients dosed once every three months with LUCENTIS lost visual acuity, returning to baseline at Month 12. In Study 3, almost all LUCENTIS-treated patients (90%) maintained their visual acuity at Month 12.

Figure 2
Mean Change in Visual Acuity from Baseline to Month 12 in Study 3



16 HOW SUPPLIED/STORAGE AND HANDLING

Each LUCENTIS carton, NDC 50242-080-01, contains one 2-cc glass vial of ranibizumab, one 5-micron, 19-gauge × 1-1/2-inch filter needle for withdrawal of the vial contents, one 30-gauge × 1/2-inch injection needle for the intravitreal injection, and one package insert [*see Dosage and*

Administration (2.4)]. VIALS ARE FOR SINGLE EYE USE ONLY.

17 PATIENT COUNSELING INFORMATION

In the days following LUCENTIS administration, patients are at risk of developing endophthalmitis. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

LUCENTIS™ [ranibizumab injection]

Manufactured by:
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

8277700
LL1404
4833801
FDA Approval Date:
June 2006

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

We have approved your biologics' license application for Lucentis (ranibizumab injection) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ranibizumab injection under your existing Department of Health and Human Services U.S. License No. 1048. Lucentis (ranibizumab injection) is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture ranibizumab drug substance at Genentech, Inc., South San Francisco, California; fill the final formulated product at (b) (4) (b) (4); and label and package filled vials at Genentech, Inc., South San Francisco, California. You may label your product with the proprietary name Lucentis and market it in 10 mg/mL single use glass vials.

We acknowledge receipt of your submissions dated December 29, 2005, and January 31, February 10, 17, 21, and 24, March 17, 23, and 31, April 10, and 28, May 5, 10, 25 (2), 26 (2), and 31, and June 1, 5 (2), 6, 9, 13, 16, 23, 26, 27, 28 (3), and 29, 2006.

The final printed labeling (FPL) must be identical in content to the enclosed labeling text for the package insert, submitted June 28, 2006; the immediate vial container submitted March 31, 2006; and the carton labels submitted June 5, 2006. The statement "No U.S. standard of potency" should be added with the next printing of carton labels. Marketing this product with FPL that is not identical in content to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for formulated drug product shall be 18 months from the date of manufacture when stored at 2°-8°C (36°-46°F). The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for ranibizumab drug substance shall be (b) (4) when stored at -20 °C.

You currently are not required to submit samples of future lots of Lucentis to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Lucentis, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

The following are Postmarketing Studies that are subject to reporting requirements of 21 CFR 601.70:

1. Submit the final Clinical Study Report from Study FVF3689g by June 30, 2008.

2. Provide safety and efficacy data from a 2-year adequate and well-controlled clinical trial of a mutually acceptable design exploring multiple dosing frequencies of Lucentis.

   Date of submission of protocol: November 14, 2008.

   Date of start of study: September 21, 2009.

   Date of final clinical study report: April 1, 2013.

3. To detect and characterize immune responses to ranibizumab:

   a. Develop and validate a confirmatory assay capable of detecting both IgG and IgM isotype responses.

   b. Develop and validate an assay to detect neutralizing anti-ranibizumab antibodies.

   The assay methodology and validation reports: September 28, 2007.

4. To characterize further the immune response to ranibizumab, serum samples collected in studies FVF2587g, FVF2598g, FVF3192g will be assayed using the validated methods described above in Postmarketing Commitment #3. The data obtained will be analyzed to discover and evaluate any association between immunoreactivity and dosing frequency as well as any potential impact of immunoreactivity on efficacy or safety outcomes.

   The need for an additional clinical study will be determined based on the results from the analysis described above.

   Date of submission of protocol and statistical analysis plan: February 28, 2007.

   Date of submission of final study report: September 30, 2008.

The following are Postmarketing Studies that are not subject to reporting requirements of 21 CFR 601.70:

5. To revise release specifications, shelf-life specifications and in-process limits for ranibizumab drug substance and drug product after [redacted] commercial manufacturing runs to reflect increased manufacturing experience.

   These revisions to the Quality control system, the corresponding data from the [redacted] commercial manufacturing runs and the analysis plan used to create the revisions will be submitted as a supplement on or before June 30, 2008.

6. To perform additional Lucentis stability studies at 40°C using Ion Exchange Chromatography (IEC) to demonstrate that the corrective actions taken at [redacted] to address the atypical accelerated stability profile observed in the Lucentis 2005 qualification campaign have been sufficient.

   Specifically, a one time stability study consisting of [redacted] centis Drug Product launch lots are placed at 40°C and tested by IEC at [redacted] months. These [redacted] Lucentis Drug Product lots are derived from the following:

   - [redacted] of these Lucentis Drug Product lots are manufactured from distinct lots of [redacted].
   - At least [redacted] these [redacted] lots are aliquoted and used to manufacture [redacted] ucentis drug product lots.

   Data will be submitted as a supplement on or before March 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this application. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),

- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post040401.htm) for further information.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all FPL at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Mark J. Goldberger, M.D., M.P.H.
Director
Office of Antimicrobial Products
Center for Drug Evaluation and Research

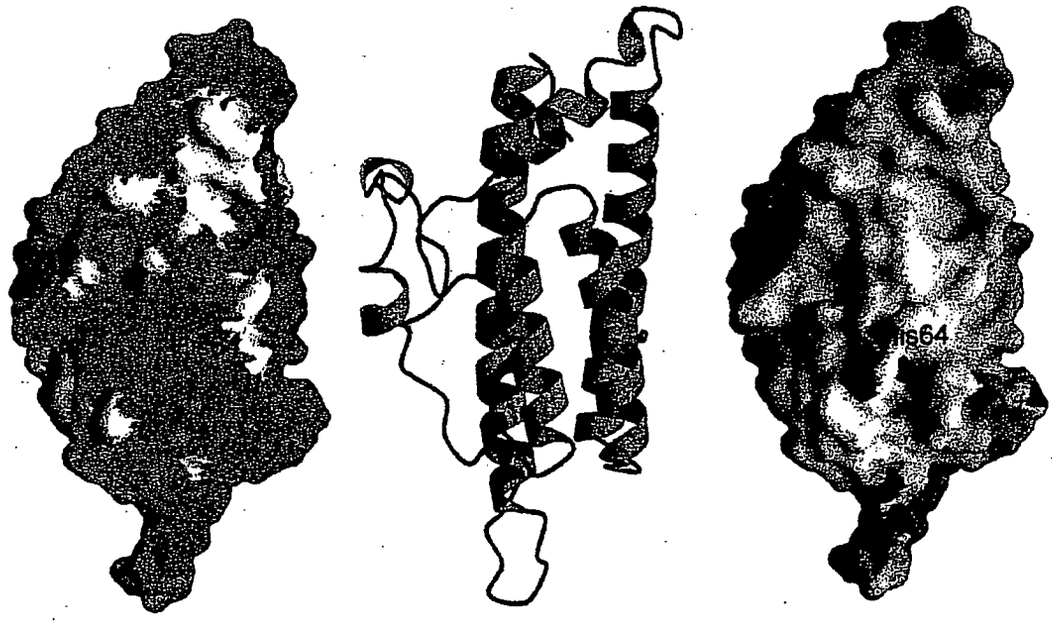
Enclosure

Volume 293
Number 4
5 November 1999

CELEBRATING 40 YEARS 1959–1999

JMB

JOURNAL OF MOLECULAR BIOLOGY



IDEAL

ACADEMIC PRESS

Article No. jmbi.1999.3192 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (1999) 293, 865–881

JMB

Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen

Yvonne Chen[1], Christian Wiesmann[1], Germaine Fuh[1], Bing Li[1], Hans W. Christinger[1], Patrick McKay[2], Abraham M. de Vos[1] and Henry B. Lowman[1]*

[1]*Department of Protein Engineering, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

[2]*Department of Process Sciences, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

**Corresponding author*

The Fab portion of a humanized antibody (Fab-12; IgG form known as rhuMAb VEGF) to vascular endothelial growth factor (VEGF) has been affinity-matured through complementarity-determining region (CDR) mutation, followed by affinity selection using monovalent phage display. After stringent binding selections at 37 °C, with dissociation (off-rate) selection periods of several days, high affinity variants were isolated from CDR-H1, H2, and H3 libraries. Mutations were combined to obtain cumulatively tighter-binding variants. The final variant identified here, Y0317, contained six mutations from the parental antibody. *In vitro* cell-based assays show that four mutations yielded an improvement of about 100-fold in potency for inhibition of VEGF-dependent cell proliferation by this variant, consistent with the equilibrium binding constant determined from kinetics experiments at 37 °C. Using X-ray crystallography, we determined a high-resolution structure of the complex between VEGF and the affinity-matured Fab fragment. The overall features of the binding interface seen previously with wild-type are preserved, and many contact residues are maintained in precise alignment in the superimposed structures. However, locally, we see evidence for improved contacts between antibody and antigen, and two mutations result in increased van der Waals contact and improved hydrogen bonding. Site-directed mutants confirm that the most favorable improvements as judged by examination of the complex structure, in fact, have the greatest impact on free energy of binding. In general, the final antibody has improved affinity for several VEGF variants as compared with the parental antibody; however, some contact residues on VEGF differ in their contribution to the energetics of Fab binding. The results show that small changes even in a large protein-protein binding interface can have significant effects on the energetics of interaction.

Keywords: angiogenesis; humanized antibody-antigen complex; affinity maturation; phage display; X-ray crystallography

Introduction

Angiogenic factors (Folkman & Klagsbrun, 1987), which stimulate endothelial cells leading to new vascularization, have roles in such disease states as cancer, rheumatoid arthritis, and macular degeneration (reviewed by Ferrara, 1995; Folkman, 1995; Iruela-Arispe & Dvorak, 1997). Vascular endothelial growth factor (VEGF), a heparin-binding protein initially identified from pituitary cells (Ferrara & Henzel, 1989), is clearly a key angio-

Abbreviations used: CDR, complementarity-determining region; FR, framework region; HuVEC, human umbilical vein endothelial cell; $K_d^{25°}$, equilibrium dissociation constant determined at 25 °C; mAb, IgG form of monoclonal antibody; PBS, phosphate-buffered saline; SPR, surface plasmon resonance; VEGF, vascular endothelial growth factor; VEGF(109), receptor-binding fragment of VEGF with residues 8-109; VEGF(165), VEGF form with residues 1-165.

E-mail address of the corresponding author: hbl@gene.com

0022-2836/99/440865–17 $30.00/0

genic factor in development as well as in certain disease states, including the growth of solid tumors (reviewed by Ferrara, 1999). A murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo* (Kim *et al.*, 1993). The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12 (Presta *et al.*, 1997). Both chimeric and humanized antibodies retained high affinity binding to VEGF, with an apparent equilibrium dissociation constant, $K_d^{25°}$, of 0.9 to 3 nM (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a). The corresponding full-length IgG form of this antibody, rhumAb VEGF, is being developed as a possible therapeutic agent for the treatment of human solid tumors (Mordenti *et al.*, 1999).

We became interested in obtaining higher affinity variants of Fab-12 in order to test whether affinity improvements of this antibody might improve its potency and efficacy. Phage display of randomized libraries of antibodies and other proteins has been extensively used to engineer proteins with improved affinity and specificity (Lowman *et al.*, 1991; reviewed by Kay & Hoess, 1996; Rader & Barbas, 1997; Griffiths & Duncan, 1998). In particular, a phage-based *in vitro* affinity maturation process has been successful in improving the binding affinity of antibodies previously identified from traditional monoclonal or naive-library sources (e.g. Hawkins *et al.*, 1992; Marks *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Thompson *et al.*, 1996).

In previous work, the humanized anti-VEGF antibody Fab-12 was adapted for improved monovalent phage display through selection of a CDR-L1 variant, designated Y0192 (Muller *et al.*, 1998a). To select target residues for randomization and affinity optimization, we also previously screened all CDR residues, as defined by a combination of the hypervariable (Kabat *et al.*, 1987) and structurally defined (Chothia & Lesk, 1987) CDR residues. Fab variants of Y0192 generated by alanine scanning were analyzed for side-chain contributions to antigen binding (Muller *et al.*, 1998a). In addition, a crystal structure of Fab-12 in complex with the receptor-binding domain of VEGF, VEGF(109), was determined (Muller *et al.*, 1998a). The results of these studies showed that the antigen binding site is almost entirely composed of residues from the heavy chain CDRs, CDR-H1, H2, and H3. Therefore, these CDRs appeared most likely to provide the opportunity for improved binding interactions with antigen.

Here, we describe the selection of an affinity-improved anti-VEGF antibody by phage display and off-rate selection. We show that the affinity-matured antibody binds VEGF with at least 20-fold improved affinity and inhibits VEGF-induced cell proliferation with enhanced potency in a cell-based assay. We also report the crystal structure of an affinity-optimized antibody in complex with VEGF, to our knowledge, representing the first reported structure of an *in vitro* affinity-matured antibody:antigen complex. The structure, together with mutational analysis, shows that subtle changes in the antibody-antigen interface account for improved affinity.

Results

Library design

We used the results of an alanine-scanning analysis, combined with a crystal structure of the wild-type Fab fragment in complex with VEGF (Muller *et al.*, 1998a), to design targeted libraries within the antibody CDRs for random mutagenesis and affinity selection. This strategy enabled us to construct theoretically complete libraries with a small number of residues randomized in each CDR. Although sites remote from the antigen-combining region or buried within the protein could modulate antigen binding affinity indirectly and have in fact been exploited for affinity improvement (Hawkins *et al.*, 1993), clearly residues shown to be important by alanine scanning are useful starting points for binding-affinity optimization (Lowman *et al.*, 1991; Lowman & Wells, 1993). Furthermore, we reasoned that by making mutations at residues of the antibody CDRs which were known to affect antigen binding and were located at or near points of contact in the bound complex, we could minimize the possibility of other indirect effects which might alter stability, immunogenicity, or other properties of the antibody.

Both Ala-scanning and crystallography (Muller *et al.*, 1998a) identified CDR-H3 as the predominant contact segment for VEGF, consistent with the general observation that CDR-H3 is often key to antigen binding (Chothia & Lesk, 1987). Within CDR-H3, residues Y95, P96, H97, Y98, Y99, S100b, H100c, W100d, Y100e, and F100f (numbering is as described by Kabat *et al.* (1987)), all showed effects on binding over a range of twofold to >150-fold when mutated to Ala, and Ala substitution at S100a caused a slight improvement in binding. However, H100c, Y100e, and F100f were found to have little or no direct contact with VEGF and presumed to have indirect effects on binding. On the other hand, Y95 and W100d have significant contacts with VEGF, and Ala substitutions resulted in no detectable binding to VEGF. Therefore, these residues were excluded from optimization. Inspection of the complex structure suggested that substitutions at P96 and Y98 could be disruptive to the antibody structure, while G100, where Ala mutation had little effect, might tolerate further substitutions. We therefore constructed a library (YC81) which fully randomized positions H97, Y99, G100, S100a, and S100b, within CDR-H3.

Significant effects of Ala substitution were also found in CDR-H2. Here, W50, I51, N52, T52a, Y53, T54, T58 alanine mutants all showed >twofold loss in binding affinity, with the greatest residue surface area buried at positions W50, I51, Y53, and

T58 (Muller *et al.*, 1998a). Indeed, W50 along with other aromatic side-chains was observed to form a deep pocket into which a loop of VEGF inserts in the complex, and was excluded from further optimization. Residue I51, on the other hand, showed no direct contact with VEGF and was also excluded. Residue T58 had multiple interactions within the interface, including contacts with VEGF and with the critical W50 of the CDR pocket. Although E56 showed no contact with VEGF and little effect (<twofold) upon alanine substitution, its side-chain lies at the periphery of the interface, near several hydrophobic residues of VEGF. We reasoned that these might be exploited for additional binding interactions. Two CDR-H2 libraries were constructed: YC266, randomizing positions T52a, Y53, T54, and E56; and YC103, randomizing positions N52, T52a, Y53, and T54.

In CDR-H1 G26, Y27, F29, N31, Y32, G33, M34, and N35 were implicated by alanine mutagenesis as important for binding VEGF; however, only N31, Y32, and G33 had significant direct contacts with VEGF. Since Ala substitution of G33 showed reduced binding, larger side-chains seemed less desirable; for this reason, this position was not randomized. Residues 27 (buried in the antibody structure) and T28 and T30 (which are mutually contacting) were included at the end of the H1 loop as possible indirect determinants of binding. Residues 27, 28, and 30-32 were randomized in a library designated YC265.

Framework residues, especially heavy chain residues 71 and 93, normally outside the region of contact with antigen, have also been found to affect antibody binding affinity (Tramontano *et al.*, 1990; Foote & Winter, 1992; Hawkins *et al.*, 1993; Xiang *et al.*, 1995), and sometimes participate in antigen contacts (reviewed by Nezlin, 1998). Therefore, an additional region of the anti-VEGF Fab, within FR-H3 and including position 71, was also targeted for randomization. Since the residue 71-76 region has contacts with CDR-H1 (at F29) and CDR-H2 (at I51 and T52a), these represented potential sites for affinity improvement through secondary effects on the interface residues. Residues L71, T73, and S76 were randomized in this FR-H3 library.

Phage selections

Fab libraries were constructed using a fusion to the g3p minor coat protein in a monovalent phage display (phagemid) vector (Bass *et al.*, 1990; Lowman *et al.*, 1991). For each library, stop codons were introduced by mutagenesis into the Y0192 phage template (Muller *et al.*, 1998a) at each residue position to be randomized. Each stop-codon construct was then used for construction of a fully randomized (using NNS codons) library as described in Materials and Methods. Phage were precipitated from overnight *Escherichia coli* shake-flask cultures and applied to VEGF-coated immunosorbant plates for binding selections. Cycles of selection followed by amplification were carried out essentially as described (Lowman, 1998).

We used an off-rate selection process (see Materials and Methods) similar to previously described procedures (Hawkins *et al.*, 1992; Yang *et al.*, 1995), modified by gradually increasing the selective pressure for binding to antigen during successive cycles of enrichment. The enrichment factor (ratio of displaying phage to non-displaying phage eluted *versus* applied) was used to monitor the stringency of selection at each step (Table 1). As a control, and to obtain a relative measure of affinity improvement, Y0192-phage were subjected to the same procedure at each cycle.

Fab-phage clones were sequenced from several phage-binding selection rounds that showed enrichment for Fab-phage over non-displaying phage. From round 6 of the CDR-H1 library selections, a dominant clone, Y0243-1 was found, having wild-type residues at Y27, T30, and Y32, and substitutions T28D and N31H (Table 2). Additional clones had related sequences, with N31H found in all selectants; Asp or Glu substituting for T28; and Thr, Ser, Gln, or Gly found at position T30.

Table 1. Enrichment factors from phage-displayed Fab libraries

Round	Wash time (hours)	CDR-H1 YC265	CDR-H2 YC266	CDR-H2 YC103	CDR-H3 YC81	FR-H3 YC101	Control Y0192
1	0	8.2	1.7	1.3	3.3	4	1.5
2	1	1.6	25	0.7	10	110	90
3	2	340	880	100	570	2300	22000
4	18	6800	880	5200	3700	600	2700
5	37[a]	210	900	920	1300	480	32
6	47[a]	130	80	100	3500	30	20
7	63[a]	1	1	>3	>25	1	>8

Libraries are designated by CDR region and oligonucleotide label (see the text for details). Library Fab-phage (ampicillin-resistant) were mixed with non-displaying control phage (chloramphenicol-resistant) in each starting pool, and subjected to VEGF binding selection, washing, and elution as described in the text.

The enrichment factor for each library is reported here as the ratio of Amp/Cam colony-forming units in the eluted pool, divided by the ratio of Amp/Cam colony-forming units in the starting pool. Starting phage concentrations were about 10^{12}/ml, except 10^{13}/ml in round 1. The wild-type Fab-phage, Y0192 was included at each round for comparison of enrichment under the particular conditions used.

[a] In some cases, the wash-step included incubation at 37 °C.

Table 2. Anti-VEGF Fab variants selected from a CDR-H1 library (HL-265)

Variant	*n*	Y 27	T 28	T 30	N 31	Y 32	I 34[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
Y0243-1	5	Y	D	T	H	Y	M	3.1
Y0243-2	1	Y	E	Q	H	Y	M	
Y0243-3	1	Y	E	T	H	Y	M	
Y0243-4	1	Y	D	G	H	Y	M	
Y0243-5	1	Y	D	S	H	Y	M	
Y0243-6	1	Y	E	S	H	Y	M	
Consensus:		Y	D	T	H	Y	M	3.1

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n* indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987).

[a] Position 34 was not randomized, but was changed to Met (as in Fab-12) in this library. The consensus reported here, equivalent to clone Y0243-1, represents the most abundant amino acid residue at each position (including clones with multiple representation ($n > 1$)). K_d(Y0192)/K_d (variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

Clones from two independently constructed CDR-H2 libraries were remarkable in that all sequenced library clones conserved wild-type residues at virtually all positions mutated, except at position Y53, where all clones contained a Trp substitution (Table 3).

Because of the strong enrichment observed from the CDR-H3 library, a number of clones were sequenced from rounds 5 and 7 (Table 4). Of 39 sequenced clones, 37 retained the wild-type residue S100b, and all contained the mutation H97Y. The remaining positions showed greater diversity, even after seven cycles of selection. The dominant clone at round 7, Y0238-3, contained the mutation S100aT (in addition to H97Y), with wild-type residues Y99 and G100. Other substitutions observed included Lys or Arg for Y99 (in 18 of 39 clones), G100N (11 of 39 clones), and a variety of substitutions including Arg, Glu, Gln, and Asn at S100a. In this library, the consensus sequence is represented by the dominant clone, Y0238-1 (Table 4).

Clones from round 6 of the FR-H3 library (Table 5) showed conservation of wild-type residue S76, with wild-type residues or various substitutions at the remaining positions: Val or Ile substituting for L71, and Val or Lys substitutions at T73.

Binding affinity of selected variants

For measurements of binding affinity, we made use of an amber stop codon placed between the genes for the Fab heavy chain and the g3p C-terminal domain, and expressed soluble Fab variants from *E. coli* shake-flask or fermentation cultures. Fab variants purified from protein-G affinity chromatography were characterized for binding affinity using an SPR-based assay on a BIAcore™-2000 instrument. The binding-kinetics assay has been described (Muller *et al.*, 1998a).

Association kinetics (k_{on}) for the wild-type antibody binding to immobilized VEGF are slow (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a), and none of the variants tested had significantly improved on-rates. On the other hand, dissociation kinetics varied over a range of 10^{-4} s^{-1} to $\leqslant 4 \times 10^{-6}$ s^{-1} at 25 °C (Table 6). Based on measurements of instrumental drift, we could not accurately measure k_{off} (and consequently K_d)

Table 3. Anti-VEGF Fab variants selected from CDR-H2 libraries (HL-266, YC103)

Variant	*n*	N 52[a]	T 52a	Y 53	T 54	G 55[a,b]	E 56[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
HL266-A[b]	6	N	T	W	T	G	E	1.3
HL266-E	1	N	T	W	T	G	T	
HL266-I	1	N	T	W	T	G	Q	
YC103-A[b]	7	N	T	W	T	G	E	1.3
YC103-C	1	N	T	W	D	G	E	
Consensus		N	T	W	T	G	E	1.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n* indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clones HL266A and YC103A, represents the most abundant amino acid at each position (including clones with multiple representation; i.e. $n > 1$). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] Constant positions were position 52 in the HL-266 library and position 56 in the YC103 library.

[b] Equivalent clones are assumed to have equal affinity.

Table 4. Anti-VEGF Fab variants selected from a CDR-H3 library (YC81)

Variant	*n*	H 97	Y 99	G 100	S 100a	S 100b	K_d(Y0192)/K_d(variant)
Round 5 (VEGF)							
Y0228-21	1	Y	R	N	A	S	
Y0228-22	1	Y	T	T	R	S	
Y0228-23	1	Y	E	G	S	S	
Y0228-24	1	Y	R	Q	R	G	
Y0228-26	1	Y	T	G	R	S	
Y0228-27	1	Y	T	N	T	S	
Y0228-28	1	Y	R	K	G	S	
Y0228-29	1	Y	T	G	S	S	
Y0228-30	1	Y	R	S	G	S	
Round 5 (HCl)							
Y0229-20	1	Y	T	N	R	S	
Y0229-21	1	Y	R	N	S	S	
Y0229-22	1	Y	K	E	S	S	
Y0229-23	1	Y	R	D	A	S	
Y0229-24	1	Y	R	Q	K	G	
Y0229-25	1	Y	K	G	G	S	
Y0229-26	1	Y	Y	G	A	S	
Y0229-27	1	Y	R	G	E	S	
Y0229-28	1	Y	R	S	T	S	
Y0238-10[a]	1	Y	R	N	T	S	3.8
Round 7 (HCl)							
Y0238-3	6	Y	Y	G	T	S	⩾9.4
Y0238-1	2	Y	R	G	T	S	7.3
Y0238-2	2	Y	I	N	K	S	
Y0238-10[a]	2	Y	R	N	T	S	3.8
Y0238-4	1	Y	Y	N	Q	S	
Y0238-5	1	Y	I	A	K	S	2.1
Y0238-6	1	Y	R	D	N	S	⩾5.4
Y0238-7	1	Y	W	G	T	S	
Y0238-8	1	Y	R	Q	N	S	
Y0238-9	1	Y	R	Q	S	S	
Y0238-11	1	Y	K	N	T	S	
Y0238-12	1	Y	I	E	R	S	
Consensus		Y	R	G	T	S	7.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). The clones are grouped according to the round of selection (5 or 7) and the type of elution (VEGF competition or HCl elution) used for recovery of bound phage. *n*, indicates the number of clones found with identical DNA sequence within each group. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clone Y0238-1, represents the most abundant amino acid at each position (including clones with multiple representation (n > 1)). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One clone was identified at both rounds 5 and 7. Equivalent clones are assumed to have equal affinity.

under these conditions, but instead used the kinetics data to place an upper limit on K_d.

The phage-derived Fab variants tested showed a range of small (within experimental error of about twofold) to significant (>fivefold) improvements in binding affinity over the wild-type (parental phage) antibody Y0192 (Table 6). From the CDR-H1 library, the dominant clone (Y0243-1) showed threefold improved affinity. Variant Y0242-1, the dominant clone in each of three CDR-H2 libraries, showed an affinity equivalent to wild-type within experimental error, and two clones derived from the FR-H3 library (Y0244-1 and Y0244-4) were equivalent or slightly weaker in affinity. Small

Table 5. Anti-VEGF Fab variants selected from a FR-H3 library

Variant	*n*	L 71	T 73	S 76	K_d(Y0192)/K_d(variant)
Round 6 (HCl)					
Y0244-1	1	V	V	S	0.3
Y0244-2	1	L	K	S	
Y0244-3[a]	1	L	V	S	
Y0244-4	1	I	K	S	0.9

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n*, indicates the number of clones found with identical DNA sequence. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One variant contained a spontaneous mutations, S74W.

Table 6. Binding kinetics of anti-VEGF Fab variants at 25 °C

Variant	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Y0192)/K_d(variant)
Y0192[a]	4.1	1.2	2.9	1
A. *Library-derived*				
Y0238-1	2.6	0.09	0.4	7.3
Y0238-3	1.3	≤0.04[b]	≤0.3[b]	≥9.4[b]
Y0238-5	0.57	0.08	1.4	2.1
Y0238-7	1.1	≤0.06[b]	≤0.5[b]	≥5.4[b]
Y0238-10	1.2	0.09	0.8	3.8
Y0242-1	3.8	0.86	2.3	1.3
Y0243-1	4.8	0.45	0.9	3.1
Y0244-1	3.0	2.7	9.0	0.3
Y0244-4	5.2	1.7	3.3	0.9
B. *Engineered*				
Y0268-1	4.0	0.15	0.38	7.6
Y0313-1	3.5	≤0.05[b]	≤0.15[b]	≥20[b]
Y0192(T28D)	6.8	1.4	2.0	1.4
Y0192(N31H)	4.8	0.37	0.8	3.6
Y0192(H97Y)	2.5	0.045	0.2	14
Y0192(S100aT)	6.8	1.0	1.5	1.9
Y0317	3.6	≤0.05[b]	≤0.14[b]	≥20[b]

Kinetic constants were determined from measurements using a BIAcore™-2000 instrument with a biosensor chip containing immobilized VEGF(109). Measurements were performed at 25 °C. Fab concentrations were calculated from quantitative amino acid analysis. The equilibrium dissociation constant, K_d, is calculated form the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity versus the wild-type humanized antibody Y0192. Errors in K_d were approximately ±25%. Variant Y0242-1 corresponds to the point mutations Y53W in CDR-H2 of Fab Y0192; for descriptions of the other variants, see Tables 2, 3, 4, 5, and 8.

[a] Data for Y0192 is from Muller *et al.* (1998a,).

[b] In some cases, the dissociation rate constant observed was at or near the limit of detection; therefore, the reported k_{off} and K_d are upper limits, and the relative affinities are an upper limit.

improvements were seen in CDR-H3 variants Y0238-5 and Y0238-10. However, larger improvements (exceeding the limits of measurement (>fivefold to >ninefold)) were observed for the CDR-H3 variants Y0238-1, Y0238-3, and Y0238-7.

All tested variants (in fact all sequenced clones) from the CDR-H3 library contained the mutation H97Y. In the higher affinity group, Gly was conserved at position 100, while the lower affinity variant contained Ala (known to cause 1.8-fold reduction in Y0192 binding; Muller *et al.*, 1998a) or Asn (Table 4). The S100a position, while quite varied among sequenced clones, was changed to Thr in the higher affinity CDR-H3 variants, and Thr or Lys in the lower affinity ones. Substitutions at Y99, though mostly confined to basic or aromatic residues, apparently had little effect since Y0238-1 (representing the consensus CDR-H3 sequence with Y99R) was not significantly different in affinity from Y0238-3, which retained Y99.

Affinity improvements from combinations of CDR mutations

To improve affinity further, several combinations of the phage-selected CDR-H1, H2, and H3 mutations were made by site-directed mutagenesis (Table 7). Among these, the highest affinity was obtained with pY0313-1 (i.e. pY0192 with mutations CDR-H1 (T28D/N31H/I34M) and CDR-H3 (H97Y/S100aT); note I34M is a reversion to Fab-12 wild-type). From BIAcore™ kinetics measurements carried out at 25 °C, this Fab variant had ≥20-fold higher affinity than Y0192 (Table 6).

Addition of the Y53W mutation, which alone produced little or no improvement over Y0192, to Y0313-1 (producing variant Y0268-1) actually reduced binding affinity by >twofold (Table 6).

The final Fab version was constructed by removing the phage-expression enhancing mutations in CDR-L1 from pY0313-1 by site-directed mutagen-

Table 7. Anti-VEGF CDR combination variants

	CDR-L1					CDR-H1			CDR-H2	CDR-H3	
Y0192: Variant	R 24	N 26	E 27	Q 28	L 29	T 28	N 31	I 34	Y 53	H 97	S 100a
Y0313-1	-	-	-	-	-	D	H	M	-	Y	T
Y0268-1	-	-	-	-	-	D	H	M	W	Y	T
Y0317	S	S	Q	D	I	D	H	M	-	Y	T
Fab-12	S	S	Q	D	I	-	-	-	-	-	-

Substitutions are shown relative to Y0192. Fab-12 also contains T221 in the heavy chain. Dashes (-) indicate no substitution. Numbering is according to Kabat *et al.* (1987) for both the light chain (CDR-L1) and heavy chain (CDR-H1, H2, H3).

esis. The M4L substitution was identified during phage-humanization experiments (Baca *et al.*, 1997), and the Leu residue was retained so as to preclude possible oxidation of the Met side-chain. The first libraries were constructed from a Fab-12 phagemid derivative, pY0101, which contained a buried framework mutation, V_L(M4L), as well as a mutation (T221L) at the junction to g3p. Thus the final version, Y0317 (Table 7 and Figure 1) differs from Fab-12 by the following six mutations: V_L(M4L), V_H(T28D/N31H/H97Y/S100aT/T221L).

Each of the CDR mutations in H1 and H3 was tested for its effect on VEGF binding affinity by introducing the corresponding point mutation into the parental Y0192 Fab and measuring binding kinetics. The results (Table 6) show a 14-fold and 3.6-fold improvement with substitution of H97Y or N31H, respectively, into the parental Fab. However, T28D or S100aT had identical affinity to Y0192, within experimental error.

We compared Fab-12 and Y0317 Fab affinities in a solution binding assay, using VEGF competition with [^{125}I]VEGF for binding to Fab. The results showed Fab-12 having $K_d^{25°} = 433$ pM and Y0317 Fab having $K_d^{25°} = 20$ pM, a 22-fold improvement in binding affinity (Figure 2).

Because dissociation kinetics in surface plasmon resonance (SPR) experiments exceeded instrumental capabilities at 25 °C, and in order to assess binding affinity under more physiological conditions, we compared binding affinities of the original humanized antibody Fab-12 with the final variant Y0317 in kinetics experiments at 37 °C. k_{on} and k_{off} were faster for both antibodies than at 25 °C, and k_{off} was clearly measurable above background. Using either immobilized VEGF(109) or immobilized VEGF(165), Y0317 was 120-fold to 140-fold improved in affinity over Fab-12, with a $K_d^{37°}$ of 80-190 pM (Table 8).

VEGF Ala-scan of the Y0317 binding epitope

In order to understand how mutations in the Fab affected binding affinity to VEGF, we also tested VEGF variants for binding to the affinity-improved antibody. For these experiments, we made use of the full-length IgG forms of Fab-12 (known as rhuMab VEGF) and Y0317 (termed Y0317-IgG) produced in CHO cells (V. Chisholm, unpublished results). These VEGF variants were previously used for mapping the parental antibody's binding site on VEGF (Muller *et al.*, 1998a).

In this assay, carried out at 37 °C, VEGF competed with biotin-VEGF with an IC_{50} of 9 nM in binding rhuMab VEGF, compared with an IC_{50} of 1 nM for Y0317-IgG (Table 9). SPR measurements have shown similar affinity improvement of Y0317-IgG over rhuMAb VEGF (H. Lowman, unpublished results).

Alanine mutations of VEGF that affected rhuMab VEGF binding also affected Y0317-IgG. For example, M81A, G88A, and G92A all caused large (100 to >500-fold) losses in binding affinity. And smaller reductions (3 to 30-fold) in binding affinity for both antibodies were seen for I80A, K84A, I91A, E93A, and M94A.

However, significant differences in the magnitude of the effect were observed at certain sites, including Y45A, fourfold reduced in affinity for rhuMAb VEGF *versus* 26-fold for Y0317-IgG; Q89A, 19-fold *versus* sixfold; and M94A, 11-fold versus 25-fold. Most surprisingly, two mutations that led to loss of detectable binding affinity for rhumAb VEGF (>500-fold) had only modest effects (four- to ninefold) on binding to Y0317-IgG. These differences might suggest a shift in the binding epitope of the antibody, and this possibility was addressed with receptor-inhibition assays and structural analysis, both described below.

Inhibition of VEGF activity

Cell-proliferation assays have been described (Fairbrother *et al.*, 1998) for the measurement of VEGF mitogenic activity on human umbilical vein endothelial cells. Here, we compared the potency of Fab-12 and the affinity-improved variants Y0238-3 and Y0313-1.

The results (Figure 3) show both variants Y0238-3 and Y0313-1 inhibit VEGF activity more potently than Y0192 Fab. Comparing the Fab forms, variant Y0313-1 appeared at least 30-fold to 100-fold more potent than the wild-type Fab. In additional experiments, Y0317 activity was similar to that of Y0313-1 (data not shown). It should be noted that the amount of VEGF (0.2 nM) used in this assay is potentially limiting for determination of an accurate IC_{50} for the mutant. For example, if the bind-

Table 8. Binding kinetics of anti-VEGF Fab variants at 37 °C

Variant	Immobilized	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Fab-12)/K_d(variant)
Fab-12	VEGF(109)	5.1	6.6	13 ± 2.2	1
Y0317	VEGF(109)	5.4	0.059	0.11 ± 0.02	120
Fab-12	VEGF(165)	5.5	11	20 ± 3.8	1
Y0317	VEGF(165)	5.3	0.074	0.14 ± 0.05	140

Kinetic constants were determined by injecting Fab solutions onto a BIAcore™-2000 instrument with a biosensor chip containing approximately 190 RU of immobilized VEGF(109) or VEGF(165), as indicated. The equilibrium dissociation constant, K_d, is calculated from the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Fab-12)/K_d(variant) indicates the fold increase in binding affinity *versus* the original humanized antibody (Fab-12; Presta *et al.*, 1997) under the specified conditions.

```
Light chain:

            1       10        20        30        40        50
Fab-12      DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF
Y0192       DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYF
Y0317.      DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF

            1       10        20        30        40        50
                    60        70        80        90       100
Fab-12      TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0192       TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0317       TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ

                    60        70        80        90       100
                   110       120       130       140       150
Fab-12      GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0192       GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0317       GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

                   110       120       130       140       150
                   160       170       180       190       200
Fab-12      DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0192       DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0317       DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

                   160       170       180       190       200
                   210
Fab-12      LSSPVTKSFNRGEC
Y0192       LSSPVTKSFNRGEC
Y0317       LSSPVTKSFNRGEC

                   210
```

Figure 1 (*legend shown opposite*)

ing affinity (K_d) of the mutant is in fact <0.2 nM, then the IC_{50} in this experiment will appear higher than under conditions of lower VEGF concentration. The result therefore supports the conclusion that the affinity-improved variant is at least 30-fold improved in affinity for VEGF, and that it effectively blocks VEGF activity *in vitro*.

Structure of the complex

In order to compare the structure and binding site of the affinity-improved antibody with that of the parental antibody, we determined the complex structure by X-ray crystallography. Crystals of the complex between the receptor binding domain of VEGF (residues 8 to 109) and the affinity-matured Fab Y0317 were grown as described (see Materials and Methods) and diffracted to a maximum resolution of 2.4 Å. The structure was refined starting from the coordinates of the complex between VEGF and the parent of Fab Y0317, Fab-12 (Muller *et al.*, 1998a), and refined to an *R*-value of 19.9% (R_{free} = 27.4%) for the reflections between 20 Å and 2.4 Å resolution.

```
Heavy chain:

                1       10        20        30        40        50
Fab-12          EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGW
Y0192           EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGW
Y0317           EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGW

                1       10        20        30        40        50
                       60        70        80        90       100
Fab-12          INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0192           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0317           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP

                  a     60        70        80  abc   90    96
                     110       120       130       140       150
Fab-12          HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0192           HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0317           YYYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC

                100abcdef       110       120       130       140
                     160       170       180       190       200
Fab-12          LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0192           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0317           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
                     150       160       170       180       190
                     210       220       230
Fab-12          TQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
Y0192           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
Y0317           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

                     200       210       220
```

Figure 1. Sequence alignment of the original humanized antibody (Fab-12; Presta *et al.*, 1997), the phage-displayed antibody (Y0192; Muller *et al.*, 1998a) and the affinity-improved antibody (Y0317). Sequential numbering of each chain is shown above the sequences; numbering according to Kabat *et al.* (1987) is shown below. CDR regions are underlined. Positions at which Y0317 differs from Fab-12 are indicated with double underlining.

The final model consists of two Fab fragments bound to the symmetrical poles of the VEGF dimer. Only residues 14–107 of each VEGF monomer are well defined in the electron density, and therefore the six N-terminal and the two C-terminal residues of each monomer were omitted from the model. Each Fab light chain comprises residues 1 to 213, with the C-terminal residue disordered, whereas for each heavy chain residues 138 to 143 as well as the six C-terminal residues are absent from the model. As in the parental Fab complex, two out of 1050 residues, namely T51 in the V_L chain of each Fab fragment, are located in the "disallowed regions" (Laskowski *et al.*, 1993) of the Ramachandran plot; 85% of all residues have their main-chain torsion angles in the "most favored"

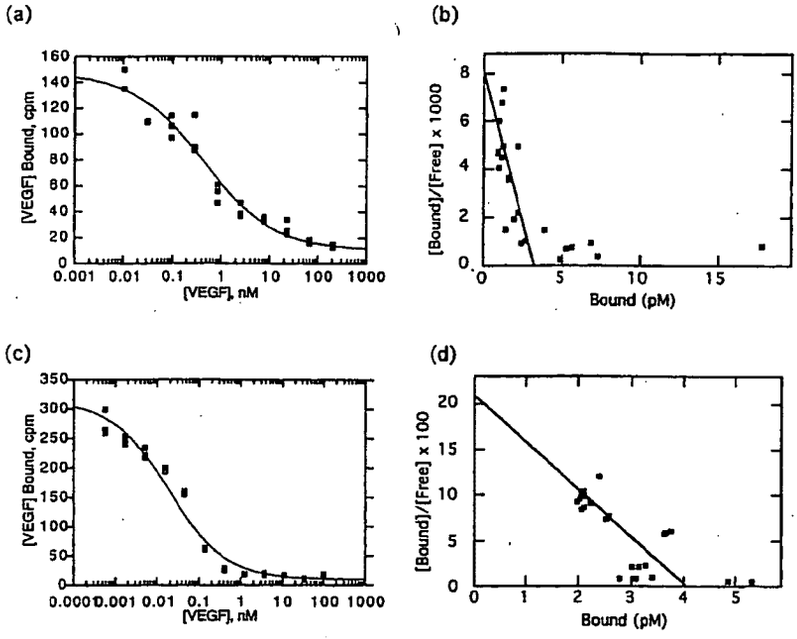


Figure 2. Radiolabeled VEGF binding assay. [^{125}I]VEGF was equilibrated (23 °C) with serial dilutions of unlabeled VEGF and (a) Fab-12 or (c) Y0317. Fabs were captured with an anti-Fab antibody-coated immunosorbant plate. Scatchard analysis (Munson & Rodbard, 1980) with a 1:1 binding model was used to calculate K_d of (b) 433 (±116) pM for Fab-12 and (d) 19.8(±4.3) pM for Y0317.

regions. The average *B*-factor of the model is 51.8 Å^2 and the mobility of the individual domains follows the pattern that was previously observed in the crystal structure of VEGF in complex with the Fab-12, with the constant domain dimer (C_L:C_H1) of one of the Fabs poorly ordered (Muller *et al.*, 1998a).

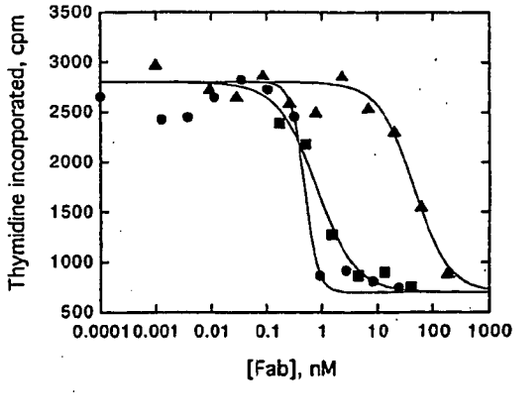


Figure 3. Human umbilical vein endothelial cell (HuVEC) assay of VEGF inhibition. Cells were cultured in the presence of 0.2 nM VEGF and serial dilutions of Fab Y0192 (triangles), Y0238-3 (squares), or Y0313-1 (circles). Cell proliferation was measured by incorporation of [^{3}H]thymidine. Curves show four-parameter fits to the data. Each point represents the mean of three treated wells.

Comparison of the final model with that of the parental Fab-VEGF complex (Muller *et al.*, 1998a) shows that the bound structures are very similar overall (Figure 4(a)) with Y0317 binding to the same site on VEGF as Fab-12 (Figure 4(b)). Side-chains show excellent overlap, and the main-chain structures show very little difference. The most prominent difference in contact residues is at H97Y (Figure 4(c); discussed below), where the tyrosine side-chain packs more favorably with VEGF and a buried water molecule from the parental Fab-VEGF complex is absent in the Y0317-Fab-VEGF complex.

Discussion

Antibody binding selections and affinity improvement

Here we made use of results from alanine-scanning and the previous structure of a humanized antibody-antigen complex to design Fab-phage libraries that randomized the three heavy-chain CDRs as well as one framework region (FR-H3) for improving the binding affinity of an anti-VEGF antibody. Affinity-improved Fab variants were obtained, with the largest effects seen in variants from the CDR-H3 library, although significant improvement was also obtained from mutation of CDR-H1. We therefore combined two mutations from H1 with two from H3, generating a further improved variant, Y0317. By making point mutations, we showed that the 20-fold (Figure 2)

Table 9. Alanine scan of VEGF by ELISA at 37 °C

VEGF(109) variant	IC_{50} (variant)/IC_{50} (VEGF)	
	Fab12-IgG	Y0317-IgG
VEGF(109)	1	1
F17A	1	1
Y21A	1	1
Y45A	4	26
K48A	2	1
Q79A	1	3
I80A	4	5
M81A	>500	930
R82A	>500	4
I83A	>500	9
K84A	3	10
H86A	1	1
Q87A	1	1
G88A	105	87
Q89A	19	6
H90A	1	1
I91A	2	6
G92A	>500	>900
E93A	4	7
M94A	11	25

ELISA assays were carried out using the full-length IgG form of Fab-12 or the IgG form of Y0317 and VEGF(109). Incubation of antibody with VEGF was at 37 °C for five hours. The IC_{50} for inhibition by each Ala mutant was evaluated using a four-parameter equation, and the relative affinities calculated as IC_{50}(mutant VEGF)/IC_{50}(wild-type VEGF). Under these conditions, Fab12-IgG and Y0317-IgG showed IC_{50} values of 9 nM and 1 nM, respectively.

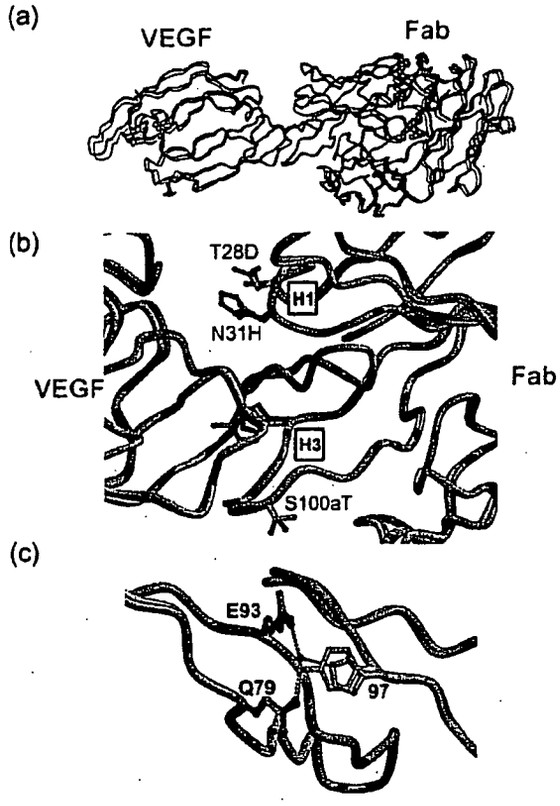


Figure 4. Structure of the affinity-improved Y0317 Fab in complex with VEGF. A superposition of the structure (Muller *et al.*, 1998a) of wild-type humanized antibody Fab-12 (gray) in complex with VEGF (gray) is shown with that of Fab Y0317 (green) in complex with VEGF (yellow). (a) Overall view of the complex, including one Fab molecule bound to one dimer of VEGF (a second Fab molecule is bound at left in the crystal) shows that the binding site for both antibody variants centers on the "80's loop" of VEGF. (b) A view of the four CDR changes between Fab-12 and Y0317 Fab shows that the new D28 and T100a side-chains do not directly contact antigen. However, H31 and Y97 form new contacts. (c) Interactions of H97 and an associated, buried water molecule in the Fab-12 complex, compared with those of Y97 in the Y0317 complex.

to >100-fold (Table 8) affinity improvement in Y0317 can be attributed to two CDR mutations: H97Y and N31H. In fact, H97Y alone improves binding affinty 14-fold.

Despite the relatively slow k_{on} and slow k_{off} of the parental antibody, binding selections described here yielded slower dissociation rates and improved equilibrium dissociation constants. Results of SPR measurements demonstrated that affinity is enhanced mainly through a slower dissociation rate (as opposed to faster association). These results are consistent with the idea of off-rate selection (Hawkins *et al.*, 1992) and with the progressively increased stringency in washing procedures used here (see Materials and Methods and Table 1). Previous binding-optimization efforts have also often yielded larger improvements in k_{off} than in k_{on} (see Lowman & Wells, 1993; Yang *et al.*, 1995; Schier *et al.*, 1996). This may suggest fundamental limitations to the improvements in k_{on} for a given binding site. Even if no conformational changes need occur between free and bound states, the on-rate is limited by the size of the binding interface and the translational and rotational diffusion rates of the binding components (reviewed by Delisi, 1983).

The association rate constants (k_{on}) for both the wild-type Y0192 and the final Y0317 antibodies are relatively slow (about 4×10^4 M^{-1} s^{-1} for both) compared to other antibodies of equal or weaker antigen binding affinity. In fact, the fastest k_{on} identified for any mutant was 6.8×10^4 M^{-1} s^{-1} (Table 6). Typically, k_{on} for antibodies binding to protein antigens, including affinity-matured antibodies, has fallen in the range of 3×10^4 to 1×10^6 M^{-1} s^{-1} (Karlsson *et al.*, 1991; Malmborg *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Wu *et al.*, 1998). In this particular protein-protein interaction, a likely explanation for the slow k_{on} is the high degree of flexibility associated with the binding site both on the Fab and on VEGF. In fact, crystallographic evidence suggests that the "80's loop" region is quite mobile (Muller *et al.*, 1997; Muller *et al.*, 1998b). We are pursuing other strategies to assess whether improvements to k_{on} can be obtained.

The contributions of point mutations in proteins to the free energy of binding or activation are often additive (Wells, 1990). This principle has been used to produce a variety of affinity-improved protein variants based on point or grouped mutations identified by phage display (Lowman & Wells, 1993; Yang *et al.*, 1995) or point-mutant screening (Wu *et al.*, 1998). Considering the initial library selectants Y0238-3 (>ninefold improved in affinity) and Y0243-1 (3.1-fold improved), we would have predicted an improvement of >27-fold for Y0313-1 or Y0317 (Table 7). In fact, a 22-fold improvement is observed (Figure 2) at 25 °C. Addition of the CDR-H1 mutation would be predicted to improve affinity slightly (1.3-fold), but in fact this mutation reduced affinity >twofold (Y0268-1 *versus* Y0313-1; Table 6). Certainly additivity does not always apply, particularly if interacting residues are involved (Wells, 1990). In this case, non-additivity probably results from steric interference between the new Trp in CDR-H2 and the new Tyr in CDR-H3.

To test the energetics of binding by the final Y0317 antibody to VEGF, we made use of a panel of alanine mutants that had been previously constructed for mapping the binding site of the original antibody (Muller *et al.*, 1998a). For these experiments, we made use of the full-length IgG forms of both antibodies. In view of the slow dissociation kinetics for both antibodies, ELISA assays were carried out at 37 °C with incubation for at least five hours to insure that equilibrium was reached. Under these conditions two dramatic differences appear in the Ala-scan of VEGF with respect to Y0317 *versus* Fab-12: both R82A and I83A have small effects on binding in Y0317, but result in large decreases in binding for Fab-12. The reasons for these differences are not clear, but R82 and I83 do have significant surface area (55 Å^2 and 32 Å^2, respectively) buried on binding to VEGF, and make contacts that include residues S100a of CDR-H3 and N52 of CDR-H2 in the wild-type antibody (Muller *et al.*, 1998a).

Structural analysis of the affinity-matured Fab

The structures of a number of antibodies derived from *in vivo* immunization and hybridoma techniques have been determined, in complex with their antigens (reviewed by Nezlin, 1998), and recently, crystallization and preliminary X-ray studies of a chain-shuffled anti-lysozyme scFv antibody in complex with antigen were reported (Küttner *et al.*, 1998). However, to our knowledge, the Y0317 Fab:VEGF structure is the first report of an *in vitro* affinity-matured Fab in complex with antigen. The structural basis of binding affinity improvement is therefore of interest

The Fab fragment of the affinity-matured anti-VEGF antibody Y0317 preserves the structure of the original humanized antibody, Fab-12. Superposition with Fab-12 results in an rmsd of only 0.38 Å for a total of 431 C^{α}-positions, demonstrating the absence of major structural changes between the two molecules. With a total of 1800 Å^2 of solvent-accessible surface buried in each VEGF-Fab interface, the contact area is about 50 Å^2 larger than in the Fab-12 complex. This small increase in buried surface area is mostly due to the exchange of H97 to a tyrosine residue. In the VEGF:Fab-12 complex, H97 buries a solvent-accessible area of 56 Å^2, while the larger tyrosine side-chain of the matured antibody accounts for 86 Å^2 of buried surface. The tyrosine side-chain also affects the hydrogen-bonding pattern and the number of ordered water molecules in the vicinity. In the parental antibody complex, a water molecule near H97 forms two hydrogen bonds to the side-chains of Q79 and E93 of VEGF (Figure 4(c)). In the complex with the affinity-matured Fab, this water molecule is replaced by the hydroxyl group of the newly introduced tyrosine side-chain at position 97. The H97Y mutation therefore not only increases the amount of buried surface area, but also introduces two additional hydrogen bonds between the ligand and Fab-0317 (Figure 4(c)). This is in good agreement with the observation that this single substitution improves VEGF binding affinity by 14-fold (Table 6). We therefore conclude that this single substiution is responsible for the majority of the improvement in binding affinity of Y0317 compared to the parent antibody.

In contrast, despite the availability of the crystal structures of both complexes, it remains uncertain what the structural basis is of the 3.6-fold enhanced binding caused by the N31H mutation. The side-chains of the asparagine and the histidine residues in this position adopt identical conformations in both crystal structures, and the amount of buried surface is not significantly increased in the VEGF:Fab-Y0317 complex. The only difference we can detect is a slight possible improvement in the hydrophobic interactions between the histidine side-chain and the phenyl group of VEGF residue F17, which has rotated slightly compared to the parent complex. It is unclear whether this could contribute to the increased affinity.

Neither of the remaining differences between Fab-12 and Fab-Y0317 has a significant effect on the binding affinity towards VEGF, and the structures show that these residues contribute only marginally to the interface. Some interactions are present between VEGF and the main-chain atoms of the serine and threonine residues in position 100a of the two Fabs, but the side-chains of these residues are not in contact with VEGF. Finally, no contact exist between VEGF and T28 (or D28) of the Fab fragments (the closest point on VEGF to this residue is more than 6 Å distant).

In summary, the analysis and comparison of the two crystal structures are in very good agreement with the results of the binding assays on the various single mutants of the Fab fragments. Although it is not possible to quantify the effects introduced by the amino acid exchanges solely based on the crystal structures, the detailed crystallographic

analysis supports and enables us to interpret the binding data.

Biological implications for antibody inhibition of VEGF

An inhibitory antibody of improved affinity may have improved potency or efficacy in treating diseases associated with VEGF expression. Preceding versions of the anti-VEGF antibody described here, including the murine A4.6.1 (Kim *et al.*, 1993), the humanized version Fab-12 (Presta *et al.*, 1997), as well as Y0192 (Muller *et al.*, 1998a), clearly demonstrated sufficient affinity to effect inhibition of VEGF activity. Here, we show that an affinity-improved variant, Fab Y0317, can inhibit endothelial cell proliferation *in vitro* with least 30-fold greater potency than the parental humanized Fab (Figure 3).

We have limited our optimization strategy to a subset of heavy-chain CDR residues implicated by alanine-scanning and crystallography (Muller *et al.*, 1998a). Furthermore, not all combinations of phage-derived mutations have been tested. One may therefore reasonably ask whether Y0317, with $K_d^{25°} = 20$ pM and $K_d^{37°} = 130$ pM, is the globally optimum variant for binding to this particular epitope (or others) on VEGF. Other affinity optimization efforts have resulted in protein-protein binding affinities in the low picomolar range, from $K_d = 6$ pM to 15 pM (see, e.g. Lowman & Wells, 1993; Schier *et al.*, 1996; Yang *et al.*, 1995). Indeed, we cannot exclude the possibility that higher affinity variants of the A4.6.1 antibody could be produced. However, it seems unlikely that further affinity improvement would greatly enhance biological potency or efficacy because for effective inhibition, the antibody must certainly occupy a significant fraction (perhaps >99 %) of the available (VEGF) binding sites. Serum VEGF concentrations of about 20 pM in normals, and of >300 pM in patients with metastatic carcinoma, have been observed (Kraft *et al.*, 1999). Local or effective concentrations are likely higher. If we conservatively assume the effective concentration of VEGF *in vivo* to be about 400 pM, then 400 pM of even an infinite-affinity Fab would be required to block all sites.

Other factors may limit the improvement in potency of a full-length IgG resulting from an improvement in intrinsic binding affinity of the Fab for antigen. The full-length IgG form of the antibody may benefit from an avidity effect *in vivo*, especially since VEGF is known to associate with proteoglycans on the cell surface (Gitay-Goren *et al.*, 1992). Even in cell-based assays, the IgG form of Fab-12 is a more effective inhibitor than the Fab form (data not shown). Finally, the half-life for dissociation of the affinity-improved antibody is already significant, even on the time-scale of the half-life of clearance for IgG's (days to weeks). The effect of an improved association rate constant for antibody in this system is unknown.

The fact that point (Ala) mutations in the antibody binding site on VEGF sometimes have lesser effects on the binding of Y0317 than on the binding of Fab-12 may suggest that the optimized binding site is more tolerant than the parental one of variations in the antigen. Indeed, Y0317 showed greatly enhanced affinity for murine VEGF over that of Fab-12 (data not shown), though still >100-fold weaker than its affinity for human VEGF. This could provide an advantage against naturally arising VEGF variants.

Materials and Methods

Construction of phage libraries and mutagenesis

A variant of the Fab-12 antibody (a humanized form of murine antibody A4.6.1) was previously identified from phage-displayed Fab libraries for improved expression on phage particles (Muller *et al.*, 1998a). We made use of the plasmid pY0192, a phagemid construct with ampicillin (or carbenicillin) resistance, as the parental ("wild-type") construct for libraries described here. To prevent contamination by wild-type sequence (Lowman *et al.*, 1991; Lowman, 1998), templates with the TAA stop codon at each residue targeted for randomization were prepared from CJ236 *E. coli* cells (Kunkel *et al.*, 1991). Libraries are designated according to the mutagenic oligonucleotides used for their construction: YC265, TCC TGT GCA GCT TCT GGC NNS NNS TTC NNS NNS NNS GGT ATG AAC TGG GTC CG, randomizing residues 27-28, 30-32 in CDR-H1; YC266, GAA TGG GTT GGA TGG ATT AAC NNS NNS NNS GGT NNS CCG ACC TAT GCT GCG G, randomizing residues 52a-54, 56 in CDR-H2; YC103, GAA TGG GTT GGA TGG ATT NNS NNS NNS NNS GGT GAA CCG ACC TAT G, randomizing residues 52-54 in CDR-H2; YC81, C TGT GCA AAG TAC CCG NNS TAT NNS NNS NNS NNS CAC TGG TAT TTC GAC, randomizing residues 97, 99-100b in CDR-H3; and YC101, CGT TTC ACT TTT TCT NNS GAC NNS TCC AAA NNS ACA GCA TAC CTG CAG , randomizing residues 71, 73, and 76 in the "FR-H3" region. An additional library in CDR-H2 was designed to insert three new residues: YC90, GA TGG ATT AAC ACC TAT NNS NNS NNS ACC GGT GAA CCG ACC.

The products of random mutagenesis reactions were electroporated into XL1-Blue *E. coli* cells (Stratagene) and amplified by growing 15-16 hours with M13KO7 helper phage. The complexity of each library, ranging from 2×10^7 to 1.5×10^8 , was estimated based on plating of the initial transformation onto LB plates containing carbenicillin.

Site-directed mutagenesis for point mutations was carried out as above, using appropriate codons to produce the respective mutations, and sequences were confirmed by single-strand DNA sequencing using Sequenase™ (USB).

Phage binding selections

For each round of selection, approximately 10^9-10^{10} phage were screened for binding to plates (Nunc Maxisorp 96-well) coated with 2 μg/ml VEGF(109) in 50 mM carbonate buffer (pH 9.6) and blocked with 5% (w/v) instant milk in 50 mM carbonate buffer, (pH 9.6). Also included were phage prepared from a non-displaying

control phagemid (pCAT), which confers chloramphenicol resistance, as a means of measuring background and enrichment (Lowman & Wells, 1993). Bound phage were eluted with 0.1 M HCl and immediately neutralized with one-third volume of 1 M Tris (pH 8.0). The eluted phage were propagated by infecting XL1 cells for the next selection cycle as described (Lowman, 1998).

In the first cycle, the VEGF plate was incubated with Fab-phage, then was briefly washed to remove bound phage. In the second cycle, binding and washing were followed by a one hour dissociative incubation at room temperature with binding buffer, after which the plate was again washed prior to acid elution. This process was repeated in rounds 3, 4 and 5, except that 1 μM VEGF was included in the dissociative incubation, and the incubation time was increased to 2, 18, and 37 hours, respectively. During these selections, Y0192 phage showed enrichments ranging from 1.5-fold (at the lowest stringency) to 22,000-fold (using a two hour dissociation incubation). However, further increases in stringency (rounds 4-5) resulted in decreasing enrichments for the control phage, with higher enrichments observed for certain libraries, especially the two CDR-H2 libraries and the CDR-H3 library (Table 1).

In cycle 6, a 17 hour dissociative incubation at room temperature was followed by an additional 30 hour incubation at 37 °C (also including VEGF in the buffer). Under these conditions, Y0192-phage showed only slight binding enrichment (20-fold), whereas the CDR-H3 library phage were enriched by 3500-fold. Cycle 7 was carried out with a 63 hour dissociative incubation, after which only small enrichment factors were observed. However, some libraries were continued through eight cycles (with 120 hours of dissociative incubation in the presence of VEGF), after which Fab-phage were still recoverable by acid elution (data not shown).

Purification of Fab

For small-scale preparations, Y0317 Fab and mutants were prepared from *E. coli* shake-flasks as described (Muller *et al.*, 1998a).

For large-scale preparation, whole cell broth was obtained from a ten liter *E. coli* fermentation. The cells were lysed with a Manton-Gaulin homogenizer (two passes at 6000 psi; lysate temperature maintained at 15-25 °C with a heat exchanger). A 5 % (v/v) solution of polyethylene imine (PEI), pH 6.0, was added to the lysate to give a final concentration of 0.25 % (v/v). The lysate was mixed for 30 minutes at room temperature. The suspension was centrifuged, and the supernatant (containing the Fab) was processed further. The pH of the supernatant was adjusted to 6.0 with 6 M HCl, followed by dilution to a conductivity of 5 mS/cm with purified water. The conditioned supernatant was loaded onto a BakerBond ABx ion-exchange column. Following a wash with the column equilibration buffer, the Fab was eluted with an increasing sodium chloride gradient in the equilibration buffer. Fractions containing the Fab were identified by SDS-PAGE. The BakerBond ABx column fractions were pooled, pH adjusted to 5.5 with 1 M Mes and diluted to a conductivity of 5 mS/cm with purified water. The conditioned BakerBond ABx pool was loaded onto a SP Sepharose HP cation exchange column (Pharmacia). Once again, the Fab was eluted with a sodium chloride-containing gradient. Fractions containing the Fab were identified by SDS-PAGE. The level of purity of Fab (as determined by SDS-PAGE) after this two column purification was >95 %.

BIAcore™ binding analysis

The VEGF-binding affinities of Fab fragments were calculated from association and dissociation rate constants measured using a BIAcore™ -2000 surface plasmon resonance system (BIAcore, Inc., Piscataway, NJ). A biosensor chip was activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's (BIAcore, Inc., Piscataway, NJ) instructions. VEGF(109) or VEGF(165) was buffer-exchanged into 20 mM sodium acetate, pH 4.8 and diluted to approximately 50 μg/ml. Aliquots of VEGF were injected at a flow rate of 2 μl/minute to achieve approximately 700-1400 response units (RU) of coupled protein. A solution of 1 M ethanolamine was injected as a blocking agent.

For kinetics measurements, twofold serial dilutions of Fab were injected in PBS/Tween buffer (0.05 % Tween-20 in phosphate-buffered saline) at 25 °C or 37 °C at a flow rate of 10 μl/minute. Equilibrium dissociation constants, K_d values from SPR measurements were calculated as k_{off}/k_{on} (Tables 6 and 8).

Radiolabeled VEGF binding assay

Solution binding affinity of Fabs for VEGF was measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled VEGF(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate.

To establish conditions for the assay, microtiter plates (Dynex) were coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2 % (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [^{125}I]VEGF(109) was mixed with serial dilutions of Fab-12 or Fab Y0317, respectively. Fab-12 was incubated overnight; however, the Fab Y0317 incubation was continued for 65 hours to insure that equilibrium was reached. Thereafter, the mixtures were transferred to the capture plate for incubation at room temperature for one hour. The solution was then removed and the plate washed eight times with 0.1 % Tween-20 in PBS. When the plates had dried, 150 μl/well of scintillant (MicroScint-20; Packard) was added, and the plates were counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab were chosen to give ≤20 % of maximal binding.

For competitive binding assays, Dynex plates were coated and blocked as above, and serial threefold dilutions of unlabeled VEGF(109) were made in PBS/Tween buffer in a Nunc plate. [^{125}I]VEGF(109) was added, followed by addition of a fixed concentration of Fab-12 or Fab Y0317. The final concentrations of Fab-12, and Fab Y0317 were 100 pM and 10 pM, respectively. After incubation (as above), bound VEGF was captured and quantified as described above. The binding data was analyzed using a computer program to perform Scatchard analysis (Munson & Rodbard, 1980) for determination of the dissociation binding constants, K_d, for Fab-12 and Fab Y0317.

ELISA assay of VEGF Ala mutants

The binding affinities of VEGF Ala mutants for full-length Fab-12-IgG (known as rhuMAb VEGF) and Y0317-IgG, a full-length IgG form of the improved antibody expressed in CHO cells (V. Chisholm, unpublished results) were measured as previously described (Muller *et al.*, 1997; Muller *et al.*, 1998a) for the murine antibody A4.6.1, except that the temperature was increased to 37 °C, and the incubation time increased to five hours, to insure that equilibrium was reached with the high-affinity antibody.

Cell-based assay of VEGF inhibition

Several versions of the anti-VEGF antibody were tested for their ability to antagonize VEGF(165) induction of the growth of HuVECs (human umbilical vein endothelial cells). The 96-well plates were seeded with 1000 HuVECs per well and fasted in assay medium (F12:DMEM 50:50 supplemented with 1.5 % (v/v) dia-filtered fetal bovine serum) for 24 hours.

The concentration of VEGF used for inducing the cells was determined by first titrating to identify the amount of VEGF that can stimulate 80 % of maximal DNA synthesis. Fresh assay medium containing fixed amounts of VEGF (0.2 nM final concentration), and increasing concentrations of anti-VEGF Fab or mab were then added. After 40 hours of incubation, DNA synthesis was measured by incorporation of tritiated thymidine. Cells were pulsed with 0.5 μCi per well of [^{3}H]thymidine for 24 hours and harvested for counting, using a TopCount gamma counter.

Crystallization and refinement

The complex between the Fab fragment of affinity-matured, humanized antibody Y0317 Fab and the receptor binding fragment of VEGF (VEGF(109)) was purified and crystallized as described for the analogous complex with the parental humanized Fab-12 fragment (Muller *et al.*, 1998a). The resulting crystals had symmetry consistent with space group $P2_1$ with cell parameters $a = 89.1$ Å, $b = 66.4$ Å, $c = 138.7$ Å, and $\beta = 94.7°$, and were isomorphous with the crystals obtained with the parent complex. A data set was collected from a single frozen crystal at beam line 5.0.2 at the Advanced Light Source, Berkeley, and processed using programs MOSFLM and SCALA (CCP4, 1994). The final data set (R_{merge} = 7.3 %) is described in Table 10. Starting with the model of Brookhaven Protein Data Bank entry 1bj1 (Muller *et al.*, 1998a), the structure was refined using the programs X-PLOR (Brünger *et al.*, 1987) and REFMAC (CCP4, 1994). The free *R*-value was monitored using the identical set of reflections sequestered before refinement of parent complex. The differences in the primary structure between Fab-12 and Fab-Y0317 were modeled using the program O (Jones *et al.*, 1991). After correction for anisotropy and application of a bulk solvent correction, the *R*-value reached its final value of 19.9 % for all reflections greater than 0.2σ (see Table 10; R_{free} = 27.4 %).

Table 10. Crystallographic data and refinement statistics

A. *Data collection*	Overall	Last shell
Resolution range (Å)	30-2.4	2.53-2.40
No. of observations	208,257	22,278
Unique reflections	61,742	8900
Completeness (%)	97.4	96.7
Mean $I/\sigma(I)$	13.6	2.7
R_{sym}	0.073	0.38
B. *Refinement*		
Resolution range (Å)	20-2.4	
No. of reflections	61,689	
No. of atoms	8577	
rmsd bond lengths (Å)	0.013	
rmsd angles (deg.)	1.9	
rmsd improper angles (deg.)	0.92	
rmsd *B*-factors for all bonded atoms, Å^2	3.5	
Number of main-chain torsion angles in disallowed regions of Ramachandran plot[a]	2	

[a] See Laskowski *et al.* (1993).

Protein Data Bank accession number

The coordinates for the VEGF:Y0317 Fab complex have been deposited in the Protein Data Bank, accession number 1cz8.

Acknowledgments

We thank Lyn Deguzman and Tom Zioncheck for providing ^{125}[I]VEGF; Alan Padua and Bill Henzel for quantitative amino acid analysis; James Bourell for mass spectrometry; Vanessa Chisholm and Lynne Krummen for construction of cell lines; and Manuel Baca, Napoleone Ferrara, Yves Muller, Leonard Presta, and James Wells for many helpful discussions.

References

Baca, M., Presta, L. G., O'Connor, S. J. & Wells, J. A. (1997). Antibody humanization using monovalent phage display. *J. Biol. Chem.* **272**, 10678-10684.

Barbas, C. F., III, Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R. M., Nara, P. L. & Burton, D. R. (1994). *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc. Natl Acad. Sci. USA*, **91**, 3809-3813.

Bass, S., Greene, R. & Wells, J. A. (1990). Hormone phage: an enrichment method for variant proteins with altered binding properties. *Proteins: Struct. Funct. Genet.* **8**, 309-314.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). Crystallographic *R* factor refinement by molecular dynamics. *Science*, **235**, 458-460.

CCP4 (1994). Programs for protein crystallography. *Acta Crystallog. sect. D*, **50**, 760-763.

Chothia, C. & Lesk, A. M. (1987). Canonical structures for the hypervariable reigions of immunoglobulins. *J. Mol. Biol.* **196**, 901-917.

Delisi, C. (1983). Role of diffusion regulation in receptor-ligand interactions. *Methods Enzymol.* **93**, 95-109.

Fairbrother, W. J., Christinger, H. W., Cochran, A. G., Fuh, G., Keenan, C. J., Quan, C., Shriver, S. K., Tom, J. Y., Wells, J. A. & Cunningham, B. C. (1998). Novel peptides selected to bind vascular endothelial growth factor target the receptor-binding site. *Biochemistry*, **37**, 17754-17764.

Ferrara, N. (1995). The role of vascular endothelial growth factor in pathological angiogenesis. *Breast Cancer Res. Treat.* **36**, 127-137.

Ferrara, N. (1999). Vascular endothelial growth factor: molecular and biological aspects. *Curr. Top. Microbiol. Immunol.* **237**, 1-30.

Ferrara, N. & Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.* **161**, 851-858.

Folkman, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med.* **1**, 27-31.

Folkman, J. & Klagsbrun, M. (1987). Angiogenic factors. *Science*, **235**, 442-443.

Foote, J. & Winter, G. (1992). Antibody framework residues affecting the conformation of the hypervariable loops. *J. Mol. Biol.* **224**, 487-499.

Gitay-Goren, H., Soker, S., Vlodavsky, I. & Neufeld, G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell-surface-associated heparin-like molecules. *J. Biol. Chem.* **267**, 6093-6098.

Griffiths, A. D. & Duncan, A. R. (1998). Strategies for selection of antibodies by phage display. *Curr. Opin. Biotechnol.* **9**, 102-108.

Hawkins, R. E., Russell, S. J. & Winter, G. (1992). Selection of phage antibodies by binding affinity mimicking affinity maturation. *J. Mol. Biol.* **226**, 889-896.

Hawkins, R. E., Russell, S. J., Baier, M. & Winter, G. (1993). The contribution of contact and non-contact residues of antibody in the affinity of binding to antigen. *J. Mol. Biol.* **234**, 958-964.

Iruela-Arispe, M. L. & Dvorak, H. F. (1997). Angiogenesis: a dynamic balance of stimulators and inhibitors. *Thromb. Haem.* **78**, 672-677.

Jones, T. A., Zhou, J.-Y., Cowan, S. W. & Kjelgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallog. sect. A*, **47**, 110-119.

Kabat, E. A., Wu, T. T., Redi-Miller, M., Perry, H. M. & Gottesman, K. S. (1987). *Sequences of Proteins of Immunological Interest*, 4th edit., National Institutes of Health, Bethesda, MD.

Karlsson, R., Michaelsson, A. & Mattsson, L. (1991). Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods*, **145**, 229-240.

Kay, B. K. & Hoess, R. H. (1996). Principles and applications of phage display. In *Phage Display of Peptides and Proteins* (Kay, B. K., Winter, J. & McCafferty, J., eds), pp. 21-34, Academic Press, San Diego.

Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S. & Ferrara, N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature*, **362**, 841-844.

Kraft, A., Weindel, K., Ochs, A., Marth, C., Zmija, J., Schumacher, P., Unger, C., Marmé, D. & Gastl, G. (1999). Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. *Cancer*, **85**, 178-187.

Kunkel, T. A., Bebenek, K. & McClary, J. (1991). Efficient site-directed mutagenesis using uracil-containing DNA. *Methods Enzymol.* **204**, 125-139.

Küttner, G., Keitel, T., Gießmann, E., Wessner, H., Scholz, C. & Höhne, W. (1998). A phage library-derived single-chain Fv fragment in complex with turkey egg-white lysozyme: characterization, crystallization and preliminary X-ray analysis. *Mol. Immunol.* **35**, 189-194.

Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). Procheck: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallog.* **26**, 283-291.

Lowman, H. B. (1998). Phage display of peptide libraries on protein scaffolds. In *Methods in Molecular Biology, Combinatorial Peptide Library Protocols* (Cabilly, S., ed.), vol. 87, pp. 249-264, Humana Press, Totowa, NJ.

Lowman, H. B. & Wells, J. A. (1993). Affinity maturation of human growth hormone by monovalent phage display. *J. Mol. Biol.* **234**, 564-578.

Lowman, H. B., Bass, S. H., Simpson, N. & Wells, J. A. (1991). Selecting high-affinity binding proteins by monovalent phage display. *Biochemistry*, **30**, 10832-10838.

Malmborg, A.-C., Michaelsson, A., Ohlin, M., Jansson, B. & Borrebaeck, C. A. K. (1992). Real time analysis of antibody-antigen reaction kinetics. *Scan. J. Immunol.* **35**, 643-650.

Marks, J. D., Griffiths, A. D., Malmqvist, M., Clackson, T. P., Bye, J. M. & Winter, G. (1992). By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology*, **10**, 779-783.

Mordenti, J., Thomsen, K., Licko, V., Chen, H., Meng, Y. G. & Ferrara, N. (1999). Efficacy and concentration-response of murine anti-VEGF monoclonal antibody in tumor-bearing mice and extrapolation to humans. *Toxicol. Pathol.* **27**, 14-21.

Muller, Y. A., Li, B., Christinger, H. W., Wells, J. A., Cunningham, B. C. & de Vos, A. M. (1997). Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. *Proc. Natl Acad. Sci. USA*, **94**, 7192-7197.

Muller, Y. A., Chen, Y., Christinger, H. W., Li, B., Cunningham, B. C., Lowman, H. B. & de Vos, A. M. (1998a). VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 Å resolution and mutational analysis of the interface. *Structure*, **6**, 1153-1167.

Muller, Y. A., Christinger, H. W., Keyt, B. A. & de Vos, A. M. (1998b). The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding. *Structure*, **5**, 1325-1338.

Munson, P. & Rodbard, D. (1980). Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**, 220-239.

Nezlin, R. (1998). *The Immunoglobulins: Structure and Function*, pp. 151-204, Academic Press, San Diego.

Presta, L. G., Chen, H., O'Connor, S. J., Chisholm, V., Meng, Y. G., Krummen, L., Winkler, M. & Ferrara, N. (1997). Humanization of a vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.* **47**, 4593-4599.

Rader, C. & Barbas, C. F., III (1997). Phage display of combinatorial antibody libraries. *Curr. Opin. Biotechnol.* **8**, 503-508.

Schier, R., McCall, A., Adams, G. P., Marshall, K. W., Merritt, H., Yim, M., Crawford, R. S., Weiner, L. M., Marks, C. & Marks, J. D. (1996). Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity

determining regions in the center of the antibody binding site. *J. Mol. Biol.* **263**, 551-567.

Thompson, J., Pope, T., Tung, J.-S., Chan, C., Hollis, G., Mark, G. & Johnson, K. S. (1996). Affinity maturation of a high-affinity human monoclonal antibody against the third hypervariable loop of human immunodeficiency virus: use of phage display to improve affinity and broaden strain reactivity. *J. Mol. Biol.* **16**, 77-88.

Tramontano, A., Chothia, C. & Lesk, A. M. (1990). Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. *J. Mol. Biol.* **215**, 175-182.

Wells, J. A. (1990). Additivity of mutational effects in proteins. *Biochemistry*, **29**, 8509-8517.

Wu, H., Beuerlein, G., Nie, Y., Smith, H., Lee, B. A., Hensler, M., Huse, W. D. & Watkins, J. D. (1998). Stepwise *in vitro* affinity maturation of vitaxin, an $\alpha_v\beta_3$-specific humanized mAb. *Proc. Natl Acad. Sci. USA*, **95**, 6037-6042.

Xiang, J., Sha, Y., Jia, Z., Prasad, L. & Delbaere, L. T. (1995). Framework residues 71 and 93 of the chimeric B72.3 antibody are major determinants of the conformation of heavy-chain hypervariable loops. *J. Mol. Biol.* **253**, 385-390.

Yang, W.-P., Green, K., Pinz-Sweeney, S., Briones, A. T., Burton, D. R. & Barbas, C. F., III (1995). CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. *J. Mol. Biol.* **254**, 392-403.

Edited by I. A. Wilson

(Received 19 July 1999; received in revised form 7 September 1999; accepted 13 September 1999)

DEPARTMENT OF HEALTH & HUMAN SERVICES

rhuFab VEGF

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

Our Reference: BB-IND 8633

OCT 13 1999

21579

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Vice President, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 8633

SPONSOR: Genentech, Incorporated

PRODUCT NAME: Humanized Monoclonal Antibody Fragment (rhuFab V2) (E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal

DATE OF SUBMISSION: October 6, 1999

DATE OF RECEIPT: October 7, 1999

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

10-18-99 P02:54 IN
10-18-99 P

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information, and all serious, unexpected adverse experiences must be reported, in writing, to this Division and to all study centers within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Sponsors of INDs for products used to treat life-threatening or severely debilitating diseases are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Kay Schneider

Kay Schneider, M.S.
Consumer Safety Officer
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

JAN 27 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality, and Compliance
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL #125156/0

Name of Biological Product: Lucentis™ (ranibizumab)

Indication: Treatment for patients with neovascular age-related macular degeneration

Date of Application: December 29, 2005

Date of Receipt: December 30, 2005

User Fee Goal Date: June 30, 2006

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We note that you have not fulfilled the requirement. We are waiving the requirement for pediatric studies for this application.

If you have not already done so, promptly submit the *content of labeling* (21 CFR 601.14(b)) in electronic format as described at the following website:
http://www.fda.gov/oc/datacouncil/spl.html.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

We request that you submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions. Effective August 29, 2005, the new address for all submissions to this application is:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, please contact the Regulatory Project Manager, Lori Gorski, at (301) 796-0722.

Sincerely,

Maureen P. Dillon-Parker
Chief, Project Management Staff
Division of Anti-Infective and
Ophthalmology Products
Office of Antimicrobials
Center for Drug Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

MAR 14 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

This letter is in regard to your biologics license application (BLA) submitted under section 351 of the Public Health Service Act.

We have completed an initial review of your application dated December 29, 2005, for Lucentis (ranibizumab injection) to determine its acceptability for filing. Under 21 CFR 601.2(a), your application was filed on February 28, 2006. The user fee goal date is June 30, 2006. This acknowledgment of filing does not mean that we have issued a license nor does it represent any evaluation of the adequacy of the data submitted.

At this time, we have not identified any potential review issues. Our filing review is only a preliminary review, and deficiencies may be identified during substantive review of your application. Following a review of the application, we shall advise you in writing of any action we have taken and request additional information if needed.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

Please use the following address for any amendments to your application:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Maureen Dillon Parker
Chief, Project Management Staff
Division of Anti-Infective and Ophthalmology Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* – § 156	Docket No: 22338-80060
Patent No.: 7,060,269	Assignee: Genentech, Inc.
Issued: June 13, 2006	Unit: OPLA; Attn: K. Ferriter
Application No: 09/723,752	

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extenstion

Mail Stop: **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY BY ASSIGNEE

The assignee of the entire right, title, and interest in U.S. Patent No. 7,060,269 (granted on application serial no. 09/723,752), Genentech, Inc., hereby appoints the practitioners associated with

CUSTOMER NUMBER 33694

as its attorneys and agents to prosecute the captioned patent/application, and to transact all business in the U.S. Patent and Trademark Office connected therewith.

Pursuant to 37 C.F.R. § 3.73(b), the undersigned states that Genentech, Inc. is the assignee of the entire right, title, and interest in the captioned patent/application by virtue of an assignment by the inventors to Genentech Inc. recorded at Reel 008872/ Frame 0429.

The undersigned, whose title is supplied below, is authorized to act on behalf of the assignee.

Respectfully submitted,

GENENTECH, INC.

/JSK/ 08-15-06

Jeffrey S. Kubinec Date
Associate General Counsel – Patent Law

08-28-06

IFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* -- § 156	Docket No: 22338-80060
Patent No.: 7,060,269	Assignee: Genentech, Inc.
Issued: June 13, 2006	Unit: OPLA
Application No: 09/723,752	

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extension

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919942 US

I hereby certify this correspondence is being deposited with the U.S. Postal Service with sufficient postage as "Express Mail – Post Office to Addressee" addressed to: Mail Stop Patent Ext., Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Signature	David Devereaux	Aug. 25, 2006
	Printed Name	Date

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Pursuant to 37 C.F.R. § 1.741(c) Applicant, Genentech, Inc., today filed three (3) copies of the Application for § 156 Patent Term Extension of U.S. Patent No. 7,060,269. Pursuant to MPEP § 2753 Applicant is submitting herewith two (2) additional copies of this Application.

Sincerely,

Jeffrey P. Kushan
Attorney for Applicant
Registration No. 43,401

Sidley Austin LLP
1501 K Street, N.W.
Washington, D.C. 20005

Dated: August 25, 2006

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* -- § 156	Docket No: 22338-80060
Patent No.: 7,060,269	Assignee: Genentech, Inc.
Issued: June 13, 2006	Unit: OPLA
Application No: 09/723,752	

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extension

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919899 US

I hereby certify this correspondence is being deposited with the U.S. Postal Service with sufficient postage as "Express Mail – Post Office to Addressee" addressed to: Mail Stop Patent Ext., Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Signature: _____ Yvonne T. Reyes (Printed Name) Aug. 25, 2006 (Date)

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 7,060,269 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 7,060,269, granted to Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen (Baca *et al.*) by virtue of an assignment of such patent to Genentech, Inc., recorded December 29, 1997, at Reel 8872, Frame 0429.[1]

[1] The assignment recorded at the noted location in the Office's records identifies U.S.S.N. 08/908,469 ("the '469 application") and states that the conveyance includes the entire "right, title and interest ... in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor...." U.S.S.N. 09/723,752, from which the '269 patent issued, is a continuation application (divisional) of the '469 application.

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is LUCENTIS™. The name of the active ingredient of LUCENTIS ™ is ranibizumab. Ranibizumab is a recombinant humanized monoclonal IgG_1 antibody antigen-binding fragment (Fab) based on a humanized framework with complementarity-determining regions (CDRs) derived from a murine monoclonal antibody that binds to human Vascular Endothelial Growth Factor (VEGF).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

LUCENTIS™ was approved for commercial marketing or use under § 351 of the Public Heath Service Act on **June 30, 2006**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a humanized monoclonal IgG_1 antibody antigen-binding fragment produced by an *E. coli* expression system. It contains human framework regions (FRs) and the complementarity-determining regions (CDRs) derived from a murine antibody that binds to VEGF.

(b) Applicant certifies that ranibizumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 30, 2006 to the present Applicant.

(c) Ranibizumab has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. *See* LUCENTIS™ product label, provided as Attachment A.

(d) LUCENTIS™ was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* LUCENTIS™ approval letter, provided as Attachment B.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 28, 2006.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a)	Names of the inventors:	Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen.
(b)	Patent Number:	7,060,269
(c)	Date of Issue:	June 13, 2006
(d)	Date of Expiration:	July 4, 2019[2]

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 7,060,269 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 7,060,269 is not subject to a terminal disclaimer.

(b) A Certificate of Correction has not been issued for U.S. Patent No. 7,060,269.

(c) The first maintenance fee for U.S. Patent No. 7,060,269 will be due on December 13, 2009.

(d) U.S. Patent No. 7,060,269 has not been the subject of a reexamination proceeding.

---

[2] The term of the '269 patent has been extended, under 35 USC § 154(b) by 697 days. The 697 days have been included in calculating the July 4, 2019 expiration date.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claim 1 of U.S. Patent No. 7,060,269 ("the '269 patent") claims the active pharmaceutical ingredient in the approved product or a method that may be used to make or use that ingredient.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how the above-listed claim of the '269 patent claims a method of using the approved product.

(1) Description of the approved product and its method of use

The approved product is described in Section 11 of the approved label for LUCENTIS™ as follows, a copy of which is provided as Attachment A.

> LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.
>
> LUCENTIS™ is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS™ is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS™ aqueous solution with 10 mM histidine HCL, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

Ranibizumab is further characterized in a scientific reference by Chen *et al.* published in 1999 in the Journal of Molecular Biology (JMB) entitled "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen."[3] For example, the heavy

[3] 293:865-881 (1999) (Attachment E)

and light chain sequences of ranibizumab, designated as Y0317 in the article, are displayed in Figure 1. In addition, the article provides data regarding the binding affinity of the Y0317 antibody fragment to VEGF. *See, e.g.*, Table 6 on p. 870.

(2) Claim 1

Claim 1 of the '269 patent reads as follows:

1. A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about $1x10^{-8}$ M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:116 and a light chain variable domain sequence of SEQ ID NO:115.

Comparison of Ranibizumab to the limitations of claim 1

Claim 1 pertains to a method of inhibiting VEGF-induced angiogenesis in a subject by administering an effective amount of a humanized anti-VEGF antibody that binds to human VEGF at a defined Kd value and that contains designated light and heavy chain variable domains. Applicant asserts that the use of ranibizumab for the treatment of age-related macular degeneration falls within the scope of claim 1 for at least the following reasons.

According to the label, ranibizumab is a humanized anti-VEGF antibody fragment that has been found effective in the treatment of patients with neovascular (wet) age-related macular degeneration. Ranibizumab binds to and inhibits the biological activity of human vascular endothelial growth factor A (VEGF-A), which has been shown to cause neovascularization and leakage in models of ocular angiogenesis. The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation (i.e., angiogenesis). *See* Label ¶¶11 and 12.1. Accordingly, administration of an effective amount of ranibizumab inhibits VEGF-induced angiogenesis in a subject to which it is administered. Applicant notes that the term "antibody" as defined in the '269 patent includes, in addition to full-length antibodies, antibody fragments such as Fab, Fab', $F(ab)_2$ and Fv as long as the fragments exhibit the desired biological activity, i.e., binding to human VEGF (*See, e.g.*, Col 8, lines 43-54). Ranibizumab, being a Fab fragment that binds human VEGF, falls within the scope of the term "antibody" as it is used in claim 1.

Claim 1 also pertains to administering an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about

$1x10^{-8}$, wherein the antibody contains the variable light and heavy chains of SEQ ID NOS: 115 and 116. The article by Chen *et al* presents data demonstrating that ranibizumab (designated as Y0317) does, in fact, bind human VEGF with a K_d value of no more than about $1x10^{-8}$ M. For example, Table 6 on page 870 of the reference shows that ranibizumab has a K_d value of about $1.4 x10^{-10}$ and thus falls within the scope of claim 1. Finally, Figures 10A and 10B of the '269 patent provide the sequence of the light chain variable and heavy chain variable domains of, *inter alia*, ranibizumab (noted therein as Fab Y0317). The light chain variable and heavy chain variable domains depicted in Figures 10A and 10B are identical to SEQ ID NO:115 and SEQ ID NO:116, respectively, of the '269 patent. Accordingly, ranibizumab contains the heavy chain variable domain (SEQ ID NO:116) and the light chain variable domain (SEQ ID NO:115) recited in claim 1.

For at least the reasons discussed above, claim 1 of the '269 patent covers, *inter alia*, a method of using the approved drug product, ranibizumab.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 7,060,269 was issued on June 13, 2006.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for LUCENTIS ™ (referred to as "Humanized Monoclonal Antibody Fragment (rhuFab V2)(E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal") was October 7, 1999. The IND was assigned number BB-IND # 8633. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment E. The application date for the IND was October 6, 1999.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 29, 2005. The BLA was assigned number BL# 125156/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment F.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125156/0 authorizing the marketing of LUCENTIS™ on June 30, 2006. LUCENTIS™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc., before the FDA in relation to the regulatory review of LUCENTIS™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On October 6, 1999, Genentech submitted to FDA (*See* Attachment E) an investigational new drug application for a recombinant humanized monoclonal antibody fragment (rhuFab V2, now known as ranibizumab) against Vascular Endothelial Growth Factor (VEGF). The antibody was developed as a potential new therapeutic in treating patients with the exudative (wet or neovascular) form for age-related macular degeneration (AMD).

- On October 7, 1999 FDA made BB-IND #8633 effective via a communication mailed to Genentech on October 13, 1999 (*See* Attachment E). According to the FDA, initiation of trials could begin 30 days after October 7, 1999.

- The first human clinical trial (Phase I) was initiated on February 8, 2000 followed by Phase II human trials and Phase III human trials, some of which remain ongoing at the time of this application.

- On February 5, 2002, representatives of Genentech and the FDA (CBER and CDER) participated in a Type C meeting to discuss the proposed clinical development plan for ranibizumab in AMD.

- On October 31, 2002 representatives of Genentech and FDA (CBER and CDER) participated in an Type B End-of-Phase II meeting.

- Beginning in approximately March 2003, and continuing at the time of this application, Phase III studies have been conducted. The three Phase III trials forming the basis of the Biologics License Application (BLA), FVF2598g, FVF2587g, and FVF3192g are studies of two year duration with primary endpoints of one year. FVF2587g and FVF3192g, along with extension study FVF3426g and safety study FVF3689g, remain ongoing at the time of this application.

- On September 21, 2005 representatives of Genentech and CDER participated in a Type B Pre-BLA submission meeting to discuss information requirements for the BLA.

- Genentech submitted a BLA for ranibizumab for the treatment of patients with wet AMD on December 29, 2005. (*See* Attachment F)

- FDA acknowledged receipt of the BLA for ranibizumab via a communication mailed to Genentech dated January 27, 2006. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BL #125156/0 to the BLA (*See* Attachment F).

- By way of a communication mailed to Genentech on March 14, 2006 FDA made Genentech aware that the BLA for ranibizumab was filed on February 28, 2006 and that FDA had assigned a user fee goal date of June 30, 2006 (*See* Attachment G).

- On June 30, 2006 FDA approved BLA 125156/0, issuing marketing authorization for LUCENTIS™ (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 7,060,269 is eligible for an extension under § 156 because:

(i) one or more claims of the '269 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '269 patent has not been previously extended on the basis of § 156;

(iii) the '269 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, LUCENTIS™;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '269 patent is requested by Applicant to be extended is **17 days**.

(c) The requested period of extension of term for the '269 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product LUCENTIS™ and the issuance of the patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective	October 7, 1999
Biologics License Application (BLA) under PHSA § 351 was filed	December 29, 2005
Patent was granted	June 13, 2006
BLA was approved	June 30, 2006

(ii) The '269 patent was granted after the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(c), the calculated regulatory review period therefore does not include a component of time between when the IND became effective and when the BLA was submitted.

(iii) The patent was granted during the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component equal to the total number of days in that period that are after the issuance of the patent (17 days).

(iv) The period determined according to § 156(b), (c), and (g)(1) for the approved product (*i.e.*, the number of days following the date of patent issuance until the approval of the BLA) is 17 days.

(v) The '269 patent will expire on July 4, 2019.

(vi) The date of approval of the approved product is June 30, 2006.

(vii) The date that is fourteen years from the date of approval of the approved product is June 30, 2020.

(viii) The period measured from the date the patent expires (*i.e.*, July 4, 2019) until the end of the fourteen-year period specified in §156 (c)(3) (*i.e.*, June 30, 2020) is approximately 361 days.

(ix) The number of days in the regulatory review period determined pursuant to § 156(g)(1)(B)(ii) does not exceed the number of days that the patent may be extended pursuant to §156(c)(3). As such, the period by which the

patent may be extended is not limited by the fourteen-year rule of §156(c)(3).

(x) The '269 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Our check in payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 accompanies this application. Please deduct any additional required fees from, or credit any overpayments to our deposit account no. 18-1260.

15. Name and Address for Correspondence [§ 1.740(a)(14)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005
Phone: 202-736-8914
Fax: 202-736-8111
email: jkushan@sidley.com

The correspondence address for U.S. Patent No. 7,060,269 is unchanged for all other purposes. A Power of Attorney granted to the undersigned by the patent assignee, a copy of which is included with this application as Attachment H, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b).

Sincerely,

Jeffrey P. Kushan
Attorney for Applicant
Registration No. 43,401

Sidley Austin LLP
1501 K Street, N.W.
Washington, D.C. 20005

Dated: August 24, 2006

INDEX OF ATTACHMENTS

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use LUCENTIS safely and effectively. See full prescribing information for LUCENTIS.

LUCENTIS™ (ranibizumab injection)

Initial U.S. Approval: 2006

----------INDICATIONS AND USAGE----------

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (1).

----------DOSAGE AND ADMINISTRATION----------

- FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY (2.1)
- LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month (2.2).
- Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly (2.2).

----------DOSAGE FORMS AND STRENGTHS----------

- 10 mg/mL single-use vial (3)

----------CONTRAINDICATIONS----------

- Ocular or periocular infections (4.1)
- Hypersensitivity (4.2)

----------WARNINGS AND PRECAUTIONS----------

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be monitored during the week following the injection (5.1).
- Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection (5.2).

----------ADVERSE REACTIONS----------

The most common adverse reactions (reported ≥ 6% higher in LUCENTIS-treated subjects than control subjects) are conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation (6.2).

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See Section 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

U.S. BLA (BL125156) Ranibizumab injection **Genentech, Inc.**

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY.

2.2 Dosing

LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month.

Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly [*see Clinical Studies (14.2)*].

2.3 Preparation for Administration

Using aseptic technique, all (0.2 mL) of the LUCENTIS vial contents are withdrawn through a 5-micron 19-gauge filter needle attached to a 1-cc tuberculin syringe. The filter needle should be discarded after withdrawal of the vial contents and should not be used for intravitreal injection. The filter needle should be replaced with a sterile 30-gauge × 1/2-inch needle for the intravitreal injection. The contents should be expelled until the plunger tip is aligned with the line that marks 0.05 mL on the syringe.

2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a broad-spectrum microbicide should be given prior to the injection.

Following the intravitreal injection, patients should be monitored for elevation in intraocular pressure and for endophthalmitis. Monitoring may consist of a check for perfusion of the optic nerve head immediately after the injection, tonometry within 30 minutes following the injection, and biomicroscopy between two and seven days following the injection. Patients should be instructed to report any symptoms suggestive of endophthalmitis without delay.

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before LUCENTIS is administered to the other eye.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

2.5 Stability and Storage

LUCENTIS should be refrigerated at 2°-8°C (36°-46°F). DO NOT FREEZE. Do not use beyond the date stamped on the label. LUCENTIS vials should be protected from light. Store in the original carton until time of use.

3 DOSAGE FORMS AND STRENGTHS

Single-use glass vial designed to deliver 0.05 mL of 10 mg/mL.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

LUCENTIS is contraindicated in patients with ocular or periocular infections.

4.2 Hypersensitivity

LUCENTIS is contraindicated in patients with known hypersensitivity to ranibizumab or any of the excipients in LUCENTIS.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with LUCENTIS, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique should always be used when administering LUCENTIS. In addition, patients should be monitored during the week following the injection to permit early treatment should an infection occur [*see Dosage and Administration (2.3, 2.4) and Patient Counseling Information (17)*].

5.2 Increases in Intraocular Pressure

Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection with LUCENTIS. Therefore, intraocular pressure as well as the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

Although there was a low rate (<4%) of arterial thromboembolic events observed in the LUCENTIS clinical trials, there is a theoretical risk of arterial thromboembolic events following intravitreal use of inhibitors of VEGF [*see Adverse Reactions (6.3)*].

6 ADVERSE REACTIONS

6.1 Injection Procedure

Serious adverse events related to the injection procedure have occurred in < 0.1% of intravitreal injections, including endophthalmitis [*see Warnings and Precautions (5.1)*], rhegmatogenous retinal detachments, and iatrogenic traumatic cataracts.

6.2 Clinical Trials Experience – Ocular Events

Other serious ocular adverse events observed among LUCENTIS-treated patients occurring in <2% of patients

included intraocular inflammation and increased intraocular pressure [*see Warnings and Precautions (5.1, 5.2)*].

The available safety data include exposure to LUCENTIS in 874 patients with neovascular age-related macular degeneration in three double-masked, controlled studies with dosage regimens of 0.3 mg (375 patients) or 0.5 mg (379 patients) administered monthly by intravitreal injection (Studies 1 and 2) [*see Clinical Studies (14.1)*] and dosage regimens of 0.3 mg (59 patients) or 0.5 mg (61 patients) administered once a month for 3 consecutive doses followed by a dose administered once every 3 months (Study 3) [*see Clinical Studies (14.2)*].

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in one clinical trial of a drug cannot be directly compared with rates in the clinical trials of the same or another drug and may not reflect the rates observed in practice.

Table 1 shows the most frequently reported ocular adverse events that were reported with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 1

Adverse Event	LUCENTIS	Control
Conjunctival hemorrhage	77%-43%	66%-29%
Eye pain	37%-17%	33%-11%
Vitreous floaters	32%-3%	10%-3%
Retinal hemorrhage	26%-15%	56%-37%
Intraocular pressure increased	24%-8%	7%-3%
Vitreous detachment	22%-7%	18%-13%
Intraocular inflammation	18%-5%	11%-3%
Eye irritation	19%-4%	20%-6%
Cataract	16%-5%	16%-6%
Foreign body sensation in eyes	19%-6%	14%-6%
Lacrimation increased	17%-3%	16%-0%
Eye pruritis	13%-0%	12%-3%
Visual disturbance	14%-0%	9%-2%
Blepharitis	13%-3%	9%-4%
Subretinal fibrosis	13%-0%	19%-10%
Ocular hyperemia	10%-5%	10%-1%
Maculopathy	10%-3%	11%-3%
Visual acuity blurred/decreased	17%-4%	24%-10%
Detachment of the retinal pigment epithelium	11%-1%	15%-3%
Dry eye	10%-3%	8%-5%
Ocular discomfort	8%-0%	5%-0%
Conjunctival hyperemia	9%-0%	7%-0%
Posterior capsule opacification	8%-0%	5%-0%
Retinal exudates	9%-1%	11%-3%

6.3 Clinical Trials Experience – Non-Ocular Events

Table 2 shows the most frequently reported non-ocular adverse events with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 2

Adverse Event	LUCENTIS	Control
Hypertension/elevated blood pressure	23%-5%	23%-8%
Nasopharyngitis	16%-5%	13%-5%
Arthralgia	11%-3%	9%-0%
Headache	15%-2%	10%-3%
Bronchitis	10%-3%	8%-2%
Cough	10%-3%	7%-2%
Anemia	8%-3%	8%-0%
Nausea	9%-2%	6%-4%
Sinusitis	8%-2%	6%-4%
Upper respiratory tract infection	15%-2%	10%-4%
Back pain	10%-1%	9%-0%
Urinary tract infection	9%-4%	8%-5%
Influenza	10%-2%	5%-1%
Arthritis	8%-0%	8%-2%
Dizziness	8%-2%	10%-2%
Constipation	7%-3%	8%-2%

The rate of arterial thromboembolic events in the three studies in the first year was 2.1% of patients (18 out of 874) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 1.1% of patients (5 out of 441) in the control arms of the studies. In the second year of Study 1, the rate of arterial thromboembolic events was 3.0% of patients (14 out of 466) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 3.2% of patients (7 out of 216) in the control arm [*see Warnings and Precautions (5.3)*].

6.4 Immunogenicity

The pre-treatment incidence of immunoreactivity to LUCENTIS was 0%-3% across treatment groups. After monthly dosing with LUCENTIS for 12 to 24 months, low titers of antibodies to LUCENTIS were detected in approximately 1%-6% of patients. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to LUCENTIS in an electrochemiluminescence assay and are highly dependent on the sensitivity and specificity of the assay. The clinical significance of immunoreactivity to LUCENTIS is unclear at this time, although some patients with the highest levels of immunoreactivity were noted to have iritis or vitritis.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with LUCENTIS.

LUCENTIS intravitreal injection has been used adjunctively with verteporfin photodynamic therapy (PDT). Twelve of 105 (11%) patients developed serious intraocular inflammation; in 10 of the 12 patients, this occurred when LUCENTIS was administered 7 days (± 2 days) after verteporfin PDT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with ranibizumab. It is also not known whether ranibizumab can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. LUCENTIS should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers
It is not known whether ranibizumab is excreted in human milk. Because many drugs are excreted in human milk, and because the potential for absorption and harm to infant growth and development exists, caution should be exercised when LUCENTIS is administered to a nursing woman.

8.4 Pediatric Use
The safety and effectiveness of LUCENTIS in pediatric patients has not been established.

8.5 Geriatric Use
In the controlled clinical studies, approximately 94% (822/879) of the patients randomized to treatment with LUCENTIS were ≥ 65 years of age and approximately 68% (601/879) were ≥ 75 years of age. No notable difference in treatment effect was seen with increasing age in any of the studies. Age did not have a significant effect on systemic exposure in a population pharmacokinetic analysis after correcting for creatinine clearance.

8.6 Patients with Renal Impairment
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with renal impairment. Sixty-eight percent of patients (136 of 200) in the population pharmacokinetic analysis had renal impairment (46.5% mild, 20% moderate, and 1.5% severe). Reduction in ranibizumab clearance is minimal in patients with renal impairment and is considered clinically insignificant. Dose adjustment is not expected to be needed for patients with renal impairment.

8.7 Patients with Hepatic Dysfunction
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with hepatic impairment. Dose adjustment is not expected to be needed for patients with hepatic dysfunction.

10 OVERDOSAGE
Planned initial single doses of ranibizumab injection 1.0 mg were associated with clinically significant intraocular inflammation in 2 of 2 patients injected. With an escalating regimen of doses beginning with initial doses of ranibizumab injection 0.3 mg, doses as high as 2.0 mg were tolerated in 15 of 20 patients.

11 DESCRIPTION
LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.

LUCENTIS is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS aqueous solution with 10 mM histidine HCl, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Ranibizumab binds to the receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecule, $VEGF_{110}$. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and is thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD). The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation.

12.2 Pharmacodynamics
Neovascular AMD is associated with foveal retinal thickening as assessed by optical coherence tomography (OCT) and leakage from CNV as assessed by fluorescein angiography.

In Study 3, foveal retinal thickness was assessed by OCT in 118/184 patients. OCT measurements were collected at baseline, Months 1, 2, 3, 5, 8, and 12. In patients treated with LUCENTIS, foveal retinal thickness decreased, on average, more than the sham group from baseline through Month 12. Retinal thickness decreased by Month 1 and decreased further at Month 3, on average. Foveal retinal thickness data did not provide information useful in influencing treatment decisions [*see Clinical Studies (14.2)*].

In patients treated with LUCENTIS, the area of vascular leakage, on average, decreased by Month 3 as assessed by fluorescein angiography. The area of vascular leakage for an individual patient was not correlated with visual acuity.

12.3 Pharmacokinetics
In animal studies, following intravitreal injection, ranibizumab was cleared from the vitreous with a half-life of approximately 3 days. After reaching a maximum at approximately 1 day,

the serum concentration of ranibizumab declined in parallel with the vitreous concentration. In these animal studies, systemic exposure of ranibizumab is more than 2000-fold lower than in the vitreous.

In patients with neovascular AMD, following monthly intravitreal administration, maximum ranibizumab serum concentrations were low (0.3 ng/mL to 2.36 ng/mL). These levels were below the concentration of ranibizumab (11 ng/mL to 27 ng/mL) thought to be necessary to inhibit the biological activity of VEGF-A by 50%, as measured in an in vitro cellular proliferation assay. The maximum observed serum concentration was dose proportional over the dose range of 0.05 to 1.0 mg/eye. Based on a population pharmacokinetic analysis, maximum serum concentrations of 1.5 ng/mL are predicted to be reached at approximately 1 day after monthly intravitreal administration of LUCENTIS 0.5 mg/eye. Based on the disappearance of ranibizumab from serum, the estimated average vitreous elimination half-life was approximately 9 days. Steady-state minimum concentration is predicted to be 0.22 ng/mL with a monthly dosing regimen. In humans, serum ranibizumab concentrations are predicted to be approximately 90,000-fold lower than vitreal concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or mutagenicity data are available for ranibizumab injection in animals or humans.

No studies on the effects of ranibizumab on fertility have been conducted.

14 CLINICAL STUDIES

The safety and efficacy of LUCENTIS were assessed in three randomized, double-masked, sham- or active-controlled studies in patients with neovascular AMD. A total of 1323 patients (LUCENTIS 879, Control 444) were enrolled in the three studies.

14.1 Study 1 and Study 2

In Study 1, patients with minimally classic or occult (without classic) CNV lesions received monthly LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or monthly sham injections. Data are available through Month 24. Patients treated with LUCENTIS in Study 1 received a mean of 22 total treatments out of a possible 24 from Day 0 to Month 24.

In Study 2, patients with predominantly classic CNV lesions received one of the following: 1) monthly LUCENTIS 0.3 mg intravitreal injections and sham PDT; 2) monthly LUCENTIS 0.5 mg intravitreal injections and sham PDT; or 3) sham intravitreal injections and active verteporfin PDT. Sham PDT (or active verteporfin PDT) was given with the initial LUCENTIS (or sham) intravitreal injection and every 3 months thereafter if fluorescein angiography showed persistence or recurrence of leakage. Data are available through Month 12. Patients treated with LUCENTIS in Study 2 received a mean of 12 total treatments out of a possible 13 from Day 0 through Month 12.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at 12 months compared with baseline. Almost all LUCENTIS-treated patients (approximately 95%) maintained their visual acuity. 34%-40% of LUCENTIS-treated patients experienced a clinically significant improvement in vision, defined as gaining 15 or more letters at 12 months. The size of the lesion did not significantly affect the results. Detailed results are shown in the tables below.

Table 3
Outcomes at Month 12 and Month 24 in Study 1

Outcome Measure	Month	Sham n = 238	LUCENTIS 0.5 mg n = 240	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	Month 12	62%	95%	32% (26%, 39%)
	Month 24	53%	90%	37% (29%, 44%)
Gain of ≥ 15 letters in visual acuity (%)[b]	Month 12	5%	34%	29% (22%, 35%)
	Month 24	4%	33%	29% (23%, 35%)
Mean change in visual acuity (letters) (SD)[b]	Month 12	-10.5 (16.6)	+7.2 (14.4)	17.5 (14.8, 20.2)
	Month 24	-14.9 (18.7)	+6.6 (16.5)	21.1 (18.1, 24.2)

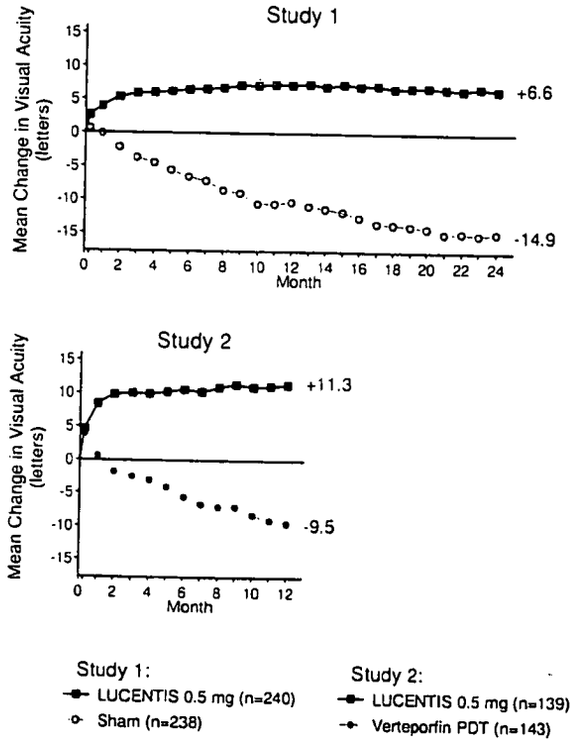
[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Table 4
Outcomes at Month 12 in Study 2

Outcome Measure	Verteporfin PDT n = 143	LUCENTIS 0.5 mg n = 140	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	64%	96%	33% (25%, 41%)
Gain of ≥ 15 letters in visual acuity (%)[b]	6%	40%	35% (26%, 44%)
Mean change in visual acuity (letters) (SD)[b]	-9.5 (16.4)	+11.3 (14.6)	21.1 (17.5, 24.6)

[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Figure 1
Mean Change in Visual Acuity from Baseline to Month 24 in Study 1 and to Month 12 in Study 2



Patients in the group treated with LUCENTIS had minimal observable CNV lesion growth, on average. At Month 12, the mean change in the total area of the CNV lesion was 0.1-0.3 DA for LUCENTIS versus 2.3-2.6 DA for the control arms.

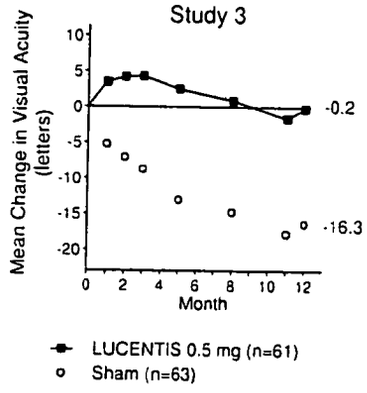
The use of LUCENTIS beyond 24 months has not been studied.

14.2 Study 3

Study 3 was a randomized, double-masked, sham-controlled, two-year study designed to assess the safety and efficacy of LUCENTIS in patients with neovascular AMD (with or without a classic CNV component). Data are available through Month 12. Patients received LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or sham injections once a month for 3 consecutive doses, followed by a dose administered once every 3 months. A total of 184 patients were enrolled in this study (LUCENTIS 0.3 mg, 60; LUCENTIS 0.5 mg, 61; sham, 63); 171 (93%) completed 12 months of this study. Patients treated with LUCENTIS in Study 3 received a mean of 6 total treatments out of possible 6 from Day 0 through Month 12.

In Study 3, the primary efficacy endpoint was mean change in visual acuity at 12 months compared with baseline (see Figure 2). After an initial increase in visual acuity (following monthly dosing), on average, patients dosed once every three months with LUCENTIS lost visual acuity, returning to baseline at Month 12. In Study 3, almost all LUCENTIS-treated patients (90%) maintained their visual acuity at Month 12.

Figure 2
Mean Change in Visual Acuity from Baseline to Month 12 in Study 3



16 HOW SUPPLIED/STORAGE AND HANDLING

Each LUCENTIS carton, NDC 50242-080-01, contains one 2-cc glass vial of ranibizumab, one 5-micron, 19-gauge × 1-1/2-inch filter needle for withdrawal of the vial contents, one 30-gauge × 1/2-inch injection needle for the intravitreal injection, and one package insert [*see Dosage and*

Administration (2.4)]. VIALS ARE FOR SINGLE EYE USE ONLY.

17 PATIENT COUNSELING INFORMATION

In the days following LUCENTIS administration, patients are at risk of developing endophthalmitis. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

LUCENTIS™ [ranibizumab injection]

Manufactured by:
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

8277700
LL1404
4833801
FDA Approval Date:
June 2006

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

We have approved your biologics' license application for Lucentis (ranibizumab injection) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ranibizumab injection under your existing Department of Health and Human Services U.S. License No. 1048. Lucentis (ranibizumab injection) is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture ranibizumab drug substance at Genentech, Inc., South San Francisco, California; fill the final formulated product at (b) (4) and label and package filled vials at Genentech, Inc., South San Francisco, California. You may label your product with the proprietary name Lucentis and market it in 10 mg/mL single use glass vials.

We acknowledge receipt of your submissions dated December 29, 2005, and January 31, February 10, 17, 21, and 24, March 17, 23, and 31, April 10, and 28, May 5, 10, 25 (2), 26 (2), and 31, and June 1, 5 (2), 6, 9, 13, 16, 23, 26, 27, 28 (3), and 29, 2006.

The final printed labeling (FPL) must be identical in content to the enclosed labeling text for the package insert, submitted June 28, 2006; the immediate vial container submitted March 31, 2006; and the carton labels submitted June 5, 2006. The statement "No U.S. standard of potency" should be added with the next printing of carton labels. Marketing this product with FPL that is not identical in content to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for formulated drug product shall be 18 months from the date of manufacture when stored at 2°-8°C (36°-46°F). The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for ranibizumab drug substance shall be (b) (4) when stored at -20 °C.

You currently are not required to submit samples of future lots of Lucentis to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Lucentis, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

The following are Postmarketing Studies that are subject to reporting requirements of 21 CFR 601.70:

1. Submit the final Clinical Study Report from Study FVF3689g by June 30, 2008.

2. Provide safety and efficacy data from a 2-year adequate and well-controlled clinical trial of a mutually acceptable design exploring multiple dosing frequencies of Lucentis.

   Date of submission of protocol: November 14, 2008.

   Date of start of study: September 21, 2009.

   Date of final clinical study report: April 1, 2013.

3. To detect and characterize immune responses to ranibizumab:

   a. Develop and validate a confirmatory assay capable of detecting both IgG and IgM isotype responses.

   b. Develop and validate an assay to detect neutralizing anti-ranibizumab antibodies.

   The assay methodology and validation reports: September 28, 2007.

4. To characterize further the immune response to ranibizumab, serum samples collected in studies FVF2587g, FVF2598g, FVF3192g will be assayed using the validated methods described above in Postmarketing Commitment #3. The data obtained will be analyzed to discover and evaluate any association between immunoreactivity and dosing frequency as well as any potential impact of immunoreactivity on efficacy or safety outcomes.

   The need for an additional clinical study will be determined based on the results from the analysis described above.

   Date of submission of protocol and statistical analysis plan: February 28, 2007.

   Date of submission of final study report: September 30, 2008.

The following are Postmarketing Studies that are not subject to reporting requirements of 21 CFR 601.70:

5. To revise release specifications, shelf-life specifications and in-process limits for ranibizumab drug substance and drug product after (b) (4) nmercial manufacturing runs to reflect increased manufacturing experience.

   These revisions to the Quality control system, the corresponding data from the (b) (4) commercial manufacturing runs and the analysis plan used to create the revisions will be submitted as a supplement on or before June 30, 2008.

6. To perform additional Lucentis stability studies at 40°C using Ion Exchange Chromatography (IEC) to demonstrate that the corrective actions taken at (b) (4) --- --- to address the atypical accelerated stability profile observed in the Lucentis 2005 qualification campaign have been sufficient.

   Specifically, a one time stability study consisting of (b) (4) centis Drug Product launch lots are placed at 40°C and tested by IEC at (b) (4) months. These (b) (4) Lucentis Drug Product lots are derived from the following:

   - (b) (4) of these Lucentis Drug Product lots are manufactured from distinct lots of (b) (4).
   - At least (b) (4) these (b) (4) lots are aliquoted and used to manufacture (b) (4) ucentis drug product lots.

   Data will be submitted as a supplement on or before March 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this application. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),

- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post040401.htm) for further information.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all FPL at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Mark J. Goldberger, M.D., M.P.H.
Director
Office of Antimicrobial Products
Center for Drug Evaluation and Research

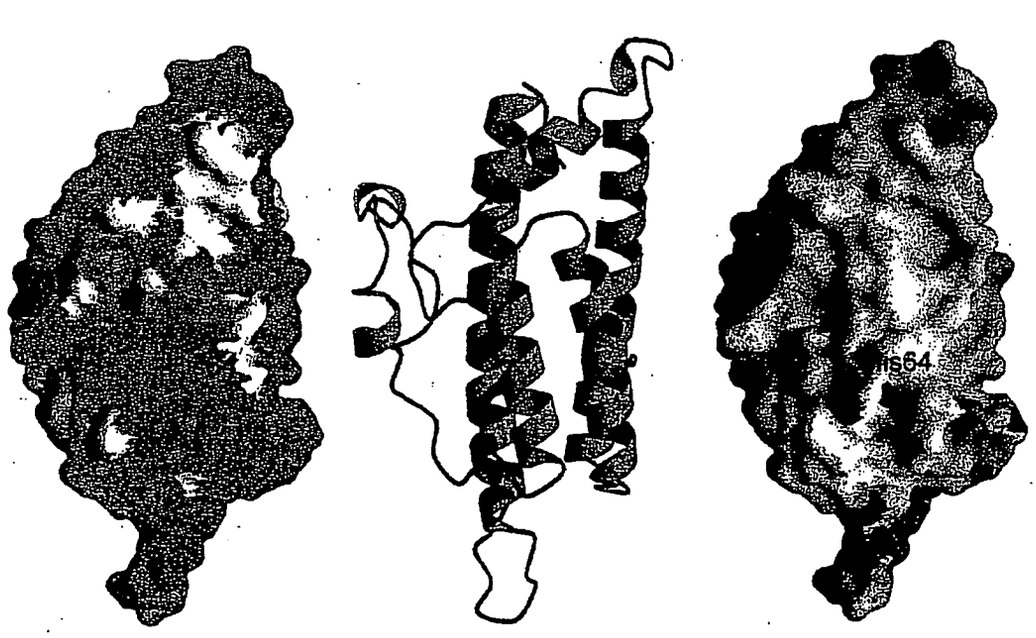
Enclosure

Volume 293
Number 4
5 November 1999

CELEBRATING 40 YEARS 1959–1999

JMB

JOURNAL OF MOLECULAR BIOLOGY



IDEAL

ACADEMIC PRESS

Article No. jmbi.1999.3192 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (1999) 293, 865–881

JMB

Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen

Yvonne Chen[1], Christian Wiesmann[1], Germaine Fuh[1], Bing Li[1], Hans W. Christinger[1], Patrick McKay[2], Abraham M. de Vos[1] and Henry B. Lowman[1]*

[1]*Department of Protein Engineering, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

[2]*Department of Process Sciences, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

**Corresponding author*

The Fab portion of a humanized antibody (Fab-12; IgG form known as rhuMAb VEGF) to vascular endothelial growth factor (VEGF) has been affinity-matured through complementarity-determining region (CDR) mutation, followed by affinity selection using monovalent phage display. After stringent binding selections at 37 °C, with dissociation (off-rate) selection periods of several days, high affinity variants were isolated from CDR-H1, H2, and H3 libraries. Mutations were combined to obtain cumulatively tighter-binding variants. The final variant identified here, Y0317, contained six mutations from the parental antibody. *In vitro* cell-based assays show that four mutations yielded an improvement of about 100-fold in potency for inhibition of VEGF-dependent cell proliferation by this variant, consistent with the equilibrium binding constant determined from kinetics experiments at 37 °C. Using X-ray crystallography, we determined a high-resolution structure of the complex between VEGF and the affinity-matured Fab fragment. The overall features of the binding interface seen previously with wild-type are preserved, and many contact residues are maintained in precise alignment in the superimposed structures. However, locally, we see evidence for improved contacts between antibody and antigen, and two mutations result in increased van der Waals contact and improved hydrogen bonding. Site-directed mutants confirm that the most favorable improvements as judged by examination of the complex structure, in fact, have the greatest impact on free energy of binding. In general, the final antibody has improved affinity for several VEGF variants as compared with the parental antibody; however, some contact residues on VEGF differ in their contribution to the energetics of Fab binding. The results show that small changes even in a large protein-protein binding interface can have significant effects on the energetics of interaction.

Keywords: angiogenesis; humanized antibody-antigen complex; affinity maturation; phage display; X-ray crystallography

Introduction

Angiogenic factors (Folkman & Klagsbrun, 1987), which stimulate endothelial cells leading to new vascularization, have roles in such disease states as cancer, rheumatoid arthritis, and macular degeneration (reviewed by Ferrara, 1995; Folkman, 1995; Iruela-Arispe & Dvorak, 1997). Vascular endothelial growth factor (VEGF), a heparin-binding protein initially identified from pituitary cells (Ferrara & Henzel, 1989), is clearly a key angio-

Abbreviations used: CDR, complementarity-determining region; FR, framework region; HuVEC, human umbilical vein endothelial cell; $K_d^{25°}$, equilibrium dissociation constant determined at 25 °C; mAb, IgG form of monoclonal antibody; PBS, phosphate-buffered saline; SPR, surface plasmon resonance; VEGF, vascular endothelial growth factor; VEGF(109), receptor-binding fragment of VEGF with residues 8-109; VEGF(165), VEGF form with residues 1-165.

E-mail address of the corresponding author: hbl@gene.com

0022-2836/99/440865–17 $30.00/0

genic factor in development as well as in certain disease states, including the growth of solid tumors (reviewed by Ferrara, 1999). A murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo* (Kim *et al.*, 1993). The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12 (Presta *et al.*, 1997). Both chimeric and humanized antibodies retained high affinity binding to VEGF, with an apparent equilibrium dissociation constant, $K_d^{25°}$, of 0.9 to 3 nM (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a). The corresponding full-length IgG form of this antibody, rhumAb VEGF, is being developed as a possible therapeutic agent for the treatment of human solid tumors (Mordenti *et al.*, 1999).

We became interested in obtaining higher affinity variants of Fab-12 in order to test whether affinity improvements of this antibody might improve its potency and efficacy. Phage display of randomized libraries of antibodies and other proteins has been extensively used to engineer proteins with improved affinity and specificity (Lowman *et al.*, 1991; reviewed by Kay & Hoess, 1996; Rader & Barbas, 1997; Griffiths & Duncan, 1998). In particular, a phage-based *in vitro* affinity maturation process has been successful in improving the binding affinity of antibodies previously identified from traditional monoclonal or naive-library sources (e.g. Hawkins *et al.*, 1992; Marks *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Thompson *et al.*, 1996).

In previous work, the humanized anti-VEGF antibody Fab-12 was adapted for improved monovalent phage display through selection of a CDR-L1 variant, designated Y0192 (Muller *et al.*, 1998a). To select target residues for randomization and affinity optimization, we also previously screened all CDR residues, as defined by a combination of the hypervariable (Kabat *et al.*, 1987) and structurally defined (Chothia & Lesk, 1987) CDR residues. Fab variants of Y0192 generated by alanine scanning were analyzed for side-chain contributions to antigen binding (Muller *et al.*, 1998a). In addition, a crystal structure of Fab-12 in complex with the receptor-binding domain of VEGF, VEGF(109), was determined (Muller *et al.*, 1998a). The results of these studies showed that the antigen binding site is almost entirely composed of residues from the heavy chain CDRs, CDR-H1, H2, and H3. Therefore, these CDRs appeared most likely to provide the opportunity for improved binding interactions with antigen.

Here, we describe the selection of an affinity-improved anti-VEGF antibody by phage display and off-rate selection. We show that the affinity-matured antibody binds VEGF with at least 20-fold improved affinity and inhibits VEGF-induced cell proliferation with enhanced potency in a cell-based assay. We also report the crystal structure of an affinity-optimized antibody in complex with VEGF, to our knowledge, representing the first reported structure of an *in vitro* affinity-matured antibody:antigen complex. The structure, together with mutational analysis, shows that subtle changes in the antibody-antigen interface account for improved affinity.

Results

Library design

We used the results of an alanine-scanning analysis, combined with a crystal structure of the wild-type Fab fragment in complex with VEGF (Muller *et al.*, 1998a), to design targeted libraries within the antibody CDRs for random mutagenesis and affinity selection. This strategy enabled us to construct theoretically complete libraries with a small number of residues randomized in each CDR. Although sites remote from the antigen-combining region or buried within the protein could modulate antigen binding affinity indirectly and have in fact been exploited for affinity improvement (Hawkins *et al.*, 1993), clearly residues shown to be important by alanine scanning are useful starting points for binding-affinity optimization (Lowman *et al.*, 1991; Lowman & Wells, 1993). Furthermore, we reasoned that by making mutations at residues of the antibody CDRs which were known to affect antigen binding and were located at or near points of contact in the bound complex, we could minimize the possibility of other indirect effects which might alter stability, immunogenicity, or other properties of the antibody.

Both Ala-scanning and crystallography (Muller *et al.*, 1998a) identified CDR-H3 as the predominant contact segment for VEGF, consistent with the general observation that CDR-H3 is often key to antigen binding (Chothia & Lesk, 1987). Within CDR-H3, residues Y95, P96, H97, Y98, Y99, S100b, H100c, W100d, Y100e, and F100f (numbering is as described by Kabat *et al.* (1987)), all showed effects on binding over a range of twofold to >150-fold when mutated to Ala, and Ala substitution at S100a caused a slight improvement in binding. However, H100c, Y100e, and F100f were found to have little or no direct contact with VEGF and presumed to have indirect effects on binding. On the other hand, Y95 and W100d have significant contacts with VEGF, and Ala substitutions resulted in no detectable binding to VEGF. Therefore, these residues were excluded from optimization. Inspection of the complex structure suggested that substitutions at P96 and Y98 could be disruptive to the antibody structure, while G100, where Ala mutation had little effect, might tolerate further substitutions. We therefore constructed a library (YC81) which fully randomized positions H97, Y99, G100, S100a, and S100b, within CDR-H3.

Significant effects of Ala substitution were also found in CDR-H2. Here, W50, I51, N52, T52a, Y53, T54, T58 alanine mutants all showed >twofold loss in binding affinity, with the greatest residue surface area buried at positions W50, I51, Y53, and

T58 (Muller *et al.*, 1998a). Indeed, W50 along with other aromatic side-chains was observed to form a deep pocket into which a loop of VEGF inserts in the complex, and was excluded from further optimization. Residue I51, on the other hand, showed no direct contact with VEGF and was also excluded. Residue T58 had multiple interactions within the interface, including contacts with VEGF and with the critical W50 of the CDR pocket. Although E56 showed no contact with VEGF and little effect (<twofold) upon alanine substitution, its side-chain lies at the periphery of the interface, near several hydrophobic residues of VEGF. We reasoned that these might be exploited for additional binding interactions. Two CDR-H2 libraries were constructed: YC266, randomizing positions T52a, Y53, T54, and E56; and YC103, randomizing positions N52, T52a, Y53, and T54.

In CDR-H1 G26, Y27, F29, N31, Y32, G33, M34, and N35 were implicated by alanine mutagenesis as important for binding VEGF; however, only N31, Y32, and G33 had significant direct contacts with VEGF. Since Ala substitution of G33 showed reduced binding, larger side-chains seemed less desirable; for this reason, this position was not randomized. Residues 27 (buried in the antibody structure) and T28 and T30 (which are mutually contacting) were included at the end of the H1 loop as possible indirect determinants of binding. Residues 27, 28, and 30-32 were randomized in a library designated YC265.

Framework residues, especially heavy chain residues 71 and 93, normally outside the region of contact with antigen, have also been found to affect antibody binding affinity (Tramontano *et al.*, 1990; Foote & Winter, 1992; Hawkins *et al.*, 1993; Xiang *et al.*, 1995), and sometimes participate in antigen contacts (reviewed by Nezlin, 1998). Therefore, an additional region of the anti-VEGF Fab, within FR-H3 and including position 71, was also targeted for randomization. Since the residue 71-76 region has contacts with CDR-H1 (at F29) and CDR-H2 (at I51 and T52a), these represented potential sites for affinity improvement through secondary effects on the interface residues. Residues L71, T73, and S76 were randomized in this FR-H3 library.

Phage selections

Fab libraries were constructed using a fusion to the g3p minor coat protein in a monovalent phage display (phagemid) vector (Bass *et al.*, 1990; Lowman *et al.*, 1991). For each library, stop codons were introduced by mutagenesis into the Y0192 phage template (Muller *et al.*, 1998a) at each residue position to be randomized. Each stop-codon construct was then used for construction of a fully randomized (using NNS codons) library as described in Materials and Methods. Phage were precipitated from overnight *Escherichia coli* shake-flask cultures and applied to VEGF-coated immunosorbant plates for binding selections. Cycles of selection followed by amplification were carried out essentially as described (Lowman, 1998).

We used an off-rate selection process (see Materials and Methods) similar to previously described procedures (Hawkins *et al.*, 1992; Yang *et al.*, 1995), modified by gradually increasing the selective pressure for binding to antigen during successive cycles of enrichment. The enrichment factor (ratio of displaying phage to non-displaying phage eluted *versus* applied) was used to monitor the stringency of selection at each step (Table 1). As a control, and to obtain a relative measure of affinity improvement, Y0192-phage were subjected to the same procedure at each cycle.

Fab-phage clones were sequenced from several phage-binding selection rounds that showed enrichment for Fab-phage over non-displaying phage. From round 6 of the CDR-H1 library selections, a dominant clone, Y0243-1 was found, having wild-type residues at Y27, T30, and Y32, and substitutions T28D and N31H (Table 2). Additional clones had related sequences, with N31H found in all selectants; Asp or Glu substituting for T28; and Thr, Ser, Gln, or Gly found at position T30.

Table 1. Enrichment factors from phage-displayed Fab libraries

Round	Wash time (hours)	CDR-H1 YC265	CDR-H2 YC266	CDR-H2 YC103	CDR-H3 YC81	FR-H3 YC101	Control Y0192
1	0	8.2	1.7	1.3	3.3	4	1.5
2	1	1.6	25	0.7	10	110	90
3	2	340	880	100	570	2300	22000
4	18	6800	880	5200	3700	600	2700
5	37[a]	210	900	920	1300	480	32
6	47[a]	130	80	100	3500	30	20
7	63[a]	1	1	>3	>25	1	>8

Libraries are designated by CDR region and oligonucleotide label (see the text for details). Library Fab-phage (ampicillin-resistant) were mixed with non-displaying control phage (chloramphenicol-resistant) in each starting pool, and subjected to VEGF binding selection, washing, and elution as described in the text.

The enrichment factor for each library is reported here as the ratio of Amp/Cam colony-forming units in the eluted pool, divided by the ratio of Amp/Cam colony-forming units in the starting pool. Starting phage concentrations were about 10^{12}/ml, except 10^{13}/ml in round 1. The wild-type Fab-phage, Y0192 was included at each round for comparison of enrichment under the particular conditions used.

[a] In some cases, the wash-step included incubation at 37 °C.

Table 2. Anti-VEGF Fab variants selected from a CDR-H1 library (HL-265)

Variant	n	Y 27	T 28	T 30	N 31	Y 32	I 34[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
Y0243-1	5	Y	D	T	H	Y	M	3.1
Y0243-2	1	Y	E	Q	H	Y	M	
Y0243-3	1	Y	E	T	H	Y	M	
Y0243-4	1	Y	D	G	H	Y	M	
Y0243-5	1	Y	D	S	H	Y	M	
Y0243-6	1	Y	E	S	H	Y	M	
Consensus:		Y	D	T	H	Y	M	3.1

All variants are in the background of Y0192 (Muller *et al.*, 1998a). n indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987).

[a] Position 34 was not randomized, but was changed to Met (as in Fab-12) in this library. The consensus reported here, equivalent to clone Y0243-1, represents the most abundant amino acid residue at each position (including clones with multiple representation ($n > 1$)). K_d(Y0192)/K_d (variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

Clones from two independently constructed CDR-H2 libraries were remarkable in that all sequenced library clones conserved wild-type residues at virtually all positions mutated, except at position Y53, where all clones contained a Trp substitution (Table 3).

Because of the strong enrichment observed from the CDR-H3 library, a number of clones were sequenced from rounds 5 and 7 (Table 4). Of 39 sequenced clones, 37 retained the wild-type residue S100b, and all contained the mutation H97Y. The remaining positions showed greater diversity, even after seven cycles of selection. The dominant clone at round 7, Y0238-3, contained the mutation S100aT (in addition to H97Y), with wild-type residues Y99 and G100. Other substitutions observed included Lys or Arg for Y99 (in 18 of 39 clones), G100N (11 of 39 clones), and a variety of substitutions including Arg, Glu, Gln, and Asn at S100a. In this library, the consensus sequence is represented by the dominant clone, Y0238-1 (Table 4).

Clones from round 6 of the FR-H3 library (Table 5) showed conservation of wild-type residue S76, with wild-type residues or various substitutions at the remaining positions: Val or Ile substituting for L71, and Val or Lys substitutions at T73.

Binding affinity of selected variants

For measurements of binding affinity, we made use of an amber stop codon placed between the genes for the Fab heavy chain and the g3p C-terminal domain, and expressed soluble Fab variants from *E. coli* shake-flask or fermentation cultures. Fab variants purified from protein-G affinity chromatography were characterized for binding affinity using an SPR-based assay on a BIAcore™-2000 instrument. The binding-kinetics assay has been described (Muller *et al.*, 1998a).

Association kinetics (k_{on}) for the wild-type antibody binding to immobilized VEGF are slow (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a), and none of the variants tested had significantly improved on-rates. On the other hand, dissociation kinetics varied over a range of 10^{-4} s^{-1} to $\leqslant 4 \times 10^{-6}$ s^{-1} at 25 °C (Table 6). Based on measurements of instrumental drift, we could not accurately measure k_{off} (and consequently K_d)

Table 3. Anti-VEGF Fab variants selected from CDR-H2 libraries (HL-266, YC103)

Variant	n	N 52[a]	T 52a	Y 53	T 54	G 55[a,b]	E 56[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
HL266-A[b]	6	N	T	W	T	G	E	1.3
HL266-E	1	N	T	W	T	G	T	
HL266-I	1	N	T	W	T	G	Q	
YC103-A[b]	7	N	T	W	T	G	E	1.3
YC103-C	1	N	T	W	D	G	E	
Consensus		N	T	W	T	G	E	1.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). n indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clones HL266A and YC103A, represents the most abundant amino acid at each position (including clones with multiple representation; i.e. $n > 1$). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] Constant positions were position 52 in the HL-266 library and position 56 in the YC103 library.

[b] Equivalent clones are assumed to have equal affinity.

Table 4. Anti-VEGF Fab variants selected from a CDR-H3 library (YC81)

Variant	*n*	H 97	Y 99	G 100	S 100a	S 100b	K_d(Y0192)/K_d(variant)
Round 5 (VEGF)							
Y0228-21	1	Y	R	N	A	S	
Y0228-22	1	Y	T	T	R	S	
Y0228-23	1	Y	E	G	S	S	
Y0228-24	1	Y	R	Q	R	G	
Y0228-26	1	Y	T	G	R	S	
Y0228-27	1	Y	T	N	T	S	
Y0228-28	1	Y	R	K	G	S	
Y0228-29	1	Y	T	G	S	S	
Y0228-30	1	Y	R	S	G	S	
Round 5 (HCl)							
Y0229-20	1	Y	T	N	R	S	
Y0229-21	1	Y	R	N	S	S	
Y0229-22	1	Y	K	E	S	S	
Y0229-23	1	Y	R	D	A	S	
Y0229-24	1	Y	R	Q	K	G	
Y0229-25	1	Y	K	G	G	S	
Y0229-26	1	Y	Y	G	A	S	
Y0229-27	1	Y	R	G	E	S	
Y0229-28	1	Y	R	S	T	S	
Y0238-10[a]	1	Y	R	N	T	S	3.8
Round 7 (HCl)							
Y0238-3	6	Y	Y	G	T	S	⩾9.4
Y0238-1	2	Y	R	G	T	S	7.3
Y0238-2	2	Y	I	N	K	S	
Y0238-10[a]	2	Y	R	N	T	S	3.8
Y0238-4	1	Y	Y	N	Q	S	
Y0238-5	1	Y	I	A	K	S	2.1
Y0238-6	1	Y	R	D	N	S	⩾5.4
Y0238-7	1	Y	W	G	T	S	
Y0238-8	1	Y	R	Q	N	S	
Y0238-9	1	Y	R	Q	S	S	
Y0238-11	1	Y	K	N	T	S	
Y0238-12	1	Y	I	E	R	S	
Consensus		Y	R	G	T	S	7.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). The clones are grouped according to the round of selection (5 or 7) and the type of elution (VEGF competition or HCl elution) used for recovery of bound phage. *n*, indicates the number of clones found with identical DNA sequence within each group. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clone Y0238-1, represents the most abundant amino acid at each position (including clones with multiple representation (n > 1)). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One clone was identified at both rounds 5 and 7. Equivalent clones are assumed to have equal affinity.

under these conditions, but instead used the kinetics data to place an upper limit on K_d.

The phage-derived Fab variants tested showed a range of small (within experimental error of about twofold) to significant (>fivefold) improvements in binding affinity over the wild-type (parental phage) antibody Y0192 (Table 6). From the CDR-H1 library, the dominant clone (Y0243-1) showed threefold improved affinity. Variant Y0242-1, the dominant clone in each of three CDR-H2 libraries, showed an affinity equivalent to wild-type within experimental error, and two clones derived from the FR-H3 library (Y0244-1 and Y0244-4) were equivalent or slightly weaker in affinity. Small

Table 5. Anti-VEGF Fab variants selected from a FR-H3 library

Variant	*n*	L 71	T 73	S 76	K_d(Y0192)/K_d(variant)
Round 6 (HCl)					
Y0244-1	1	V	V	S	0.3
Y0244-2	1	L	K	S	
Y0244-3[a]	1	L	V	S	
Y0244-4	1	I	K	S	0.9

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n*, indicates the number of clones found with identical DNA sequence. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One variant contained a spontaneous mutations, S74W.

Table 6. Binding kinetics of anti-VEGF Fab variants at 25 °C

Variant	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Y0192)/K_d(variant)
Y0192[a]	4.1	1.2	2.9	1
A. *Library-derived*				
Y0238-1	2.6	0.09	0.4	7.3
Y0238-3	1.3	≤0.04[b]	≤0.3[b]	≥9.4[b]
Y0238-5	0.57	0.08	1.4	2.1
Y0238-7	1.1	≤0.06[b]	≤0.5[b]	≥5.4[b]
Y0238-10	1.2	0.09	0.8	3.8
Y0242-1	3.8	0.86	2.3	1.3
Y0243-1	4.8	0.45	0.9	3.1
Y0244-1	3.0	2.7	9.0	0.3
Y0244-4	5.2	1.7	3.3	0.9
B. *Engineered*				
Y0268-1	4.0	0.15	0.38	7.6
Y0313-1	3.5	≤0.05[b]	≤0.15[b]	≥20[b]
Y0192(T28D)	6.8	1.4	2.0	1.4
Y0192(N31H)	4.8	0.37	0.8	3.6
Y0192(H97Y)	2.5	0.045	0.2	14
Y0192(S100aT)	6.8	1.0	1.5	1.9
Y0317	3.6	≤0.05[b]	≤0.14[b]	≥20[b]

Kinetic constants were determined from measurements using a BIAcore™-2000 instrument with a biosensor chip containing immobilized VEGF(109). Measurements were performed at 25 °C. Fab concentrations were calculated from quantitative amino acid analysis. The equilibrium dissociation constant, K_d, is calculated form the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity versus the wild-type humanized antibody Y0192. Errors in K_d were approximately ±25 %. Variant Y0242-1 corresponds to the point mutations Y53W in CDR-H2 of Fab Y0192; for descriptions of the other variants, see Tables 2, 3, 4, 5, and 8.

[a] Data for Y0192 is from Muller *et al.* (1998a,).

[b] In some cases, the dissociation rate constant observed was at or near the limit of detection; therefore, the reported k_{off} and K_d are upper limits, and the relative affinities are an upper limit.

improvements were seen in CDR-H3 variants Y0238-5 and Y0238-10. However, larger improvements (exceeding the limits of measurement (>fivefold to >ninefold)) were observed for the CDR-H3 variants Y0238-1, Y0238-3, and Y0238-7.

All tested variants (in fact all sequenced clones) from the CDR-H3 library contained the mutation H97Y. In the higher affinity group, Gly was conserved at position 100, while the lower affinity variant contained Ala (known to cause 1.8-fold reduction in Y0192 binding; Muller *et al.*, 1998a) or Asn (Table 4). The S100a position, while quite varied among sequenced clones, was changed to Thr in the higher affinity CDR-H3 variants, and Thr or Lys in the lower affinity ones. Substitutions at Y99, though mostly confined to basic or aromatic residues, apparently had little effect since Y0238-1 (representing the consensus CDR-H3 sequence with Y99R) was not significantly different in affinity from Y0238-3, which retained Y99.

Affinity improvements from combinations of CDR mutations

To improve affinity further, several combinations of the phage-selected CDR-H1, H2, and H3 mutations were made by site-directed mutagenesis (Table 7). Among these, the highest affinity was obtained with pY0313-1 (i.e. pY0192 with mutations CDR-H1 (T28D/N31H/I34M) and CDR-H3 (H97Y/S100aT); note I34M is a reversion to Fab-12 wild-type). From BIAcore™ kinetics measurements carried out at 25 °C, this Fab variant had ≥20-fold higher affinity than Y0192 (Table 6).

Addition of the Y53W mutation, which alone produced little or no improvement over Y0192, to Y0313-1 (producing variant Y0268-1) actually reduced binding affinity by >twofold (Table 6).

The final Fab version was constructed by removing the phage-expression enhancing mutations in CDR-L1 from pY0313-1 by site-directed mutagen-

Table 7. Anti-VEGF CDR combination variants

	CDR-L1					CDR-H1			CDR-H2	CDR-H3	
Y0192: Variant	R 24	N 26	E 27	Q 28	L 29	T 28	N 31	I 34	Y 53	H 97	S 100a
Y0313-1	-	-	-	-	-	D	H	M	-	Y	T
Y0268-1	-	-	-	-	-	D	H	M	W	Y	T
Y0317	S	S	Q	D	I	D	H	M	-	Y	T
Fab-12	S	S	Q	D	I	-	-	-	-	-	-

Substitutions are shown relative to Y0192. Fab-12 also contains T221 in the heavy chain. Dashes (-) indicate no substitution. Numbering is according to Kabat *et al.* (1987) for both the light chain (CDR-L1) and heavy chain (CDR-H1, H2, H3).

esis. The M4L substitution was identified during phage-humanization experiments (Baca *et al.*, 1997), and the Leu residue was retained so as to preclude possible oxidation of the Met side-chain. The first libraries were constructed from a Fab-12 phagemid derivative, pY0101, which contained a buried framework mutation, V_L(M4L), as well as a mutation (T221L) at the junction to g3p. Thus the final version, Y0317 (Table 7 and Figure 1) differs from Fab-12 by the following six mutations: V_L(M4L), V_H(T28D/N31H/H97Y/S100aT/T221L).

Each of the CDR mutations in H1 and H3 was tested for its effect on VEGF binding affinity by introducing the corresponding point mutation into the parental Y0192 Fab and measuring binding kinetics. The results (Table 6) show a 14-fold and 3.6-fold improvement with substitution of H97Y or N31H, respectively, into the parental Fab. However, T28D or S100aT had identical affinity to Y0192, within experimental error.

We compared Fab-12 and Y0317 Fab affinities in a solution binding assay, using VEGF competition with [^{125}I]VEGF for binding to Fab. The results showed Fab-12 having $K_d^{25°}$ = 433 pM and Y0317 Fab having $K_d^{25°}$ = 20 pM, a 22-fold improvement in binding affinity (Figure 2).

Because dissociation kinetics in surface plasmon resonance (SPR) experiments exceeded instrumental capabilities at 25 °C, and in order to assess binding affinity under more physiological conditions, we compared binding affinities of the original humanized antibody Fab-12 with the final variant Y0317 in kinetics experiments at 37 °C. k_{on} and k_{off} were faster for both antibodies than at 25 °C, and k_{off} was clearly measurable above background. Using either immobilized VEGF(109) or immobilized VEGF(165), Y0317 was 120-fold to 140-fold improved in affinity over Fab-12, with a $K_d^{37°}$ of 80-190 pM (Table 8).

VEGF Ala-scan of the Y0317 binding epitope

In order to understand how mutations in the Fab affected binding affinity to VEGF, we also tested VEGF variants for binding to the affinity-improved antibody. For these experiments, we made use of the full-length IgG forms of Fab-12 (known as rhuMab VEGF) and Y0317 (termed Y0317-IgG) produced in CHO cells (V. Chisholm, unpublished results). These VEGF variants were previously used for mapping the parental antibody's binding site on VEGF (Muller *et al.*, 1998a).

In this assay, carried out at 37 °C, VEGF competed with biotin-VEGF with an IC_{50} of 9 nM in binding rhuMab VEGF, compared with an IC_{50} of 1 nM for Y0317-IgG (Table 9). SPR measurements have shown similar affinity improvement of Y0317-IgG over rhuMAb VEGF (H. Lowman, unpublished results).

Alanine mutations of VEGF that affected rhuMab VEGF binding also affected Y0317-IgG. For example, M81A, G88A, and G92A all caused large (100 to >500-fold) losses in binding affinity. And smaller reductions (3 to 30-fold) in binding affinity for both antibodies were seen for I80A, K84A, I91A, E93A, and M94A.

However, significant differences in the magnitude of the effect were observed at certain sites, including Y45A, fourfold reduced in affinity for rhuMAb VEGF *versus* 26-fold for Y0317-IgG; Q89A, 19-fold *versus* sixfold; and M94A, 11-fold versus 25-fold. Most surprisingly, two mutations that led to loss of detectable binding affinity for rhumAb VEGF (>500-fold) had only modest effects (four- to ninefold) on binding to Y0317-IgG. These differences might suggest a shift in the binding epitope of the antibody, and this possibility was addressed with receptor-inhibition assays and structural analysis, both described below.

Inhibition of VEGF activity

Cell-proliferation assays have been described (Fairbrother *et al.*, 1998) for the measurement of VEGF mitogenic activity on human umbilical vein endothelial cells. Here, we compared the potency of Fab-12 and the affinity-improved variants Y0238-3 and Y0313-1.

The results (Figure 3) show both variants Y0238-3 and Y0313-1 inhibit VEGF activity more potently than Y0192 Fab. Comparing the Fab forms, variant Y0313-1 appeared at least 30-fold to 100-fold more potent than the wild-type Fab. In additional experiments, Y0317 activity was similar to that of Y0313-1 (data not shown). It should be noted that the amount of VEGF (0.2 nM) used in this assay is potentially limiting for determination of an accurate IC_{50} for the mutant. For example, if the bind-

Table 8. Binding kinetics of anti-VEGF Fab variants at 37 °C

Variant	Immobilized	$k_{on}/10^6$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Fab-12)/K_d(variant)
Fab-12	VEGF(109)	5.1	6.6	13 ± 2.2	1
Y0317	VEGF(109)	5.4	0.059	0.11 ± 0.02	120
Fab-12	VEGF(165)	5.5	11	20 ± 3.8	1
Y0317	VEGF(165)	5.3	0.074	0.14 ± 0.05	140

Kinetic constants were determined by injecting Fab solutions onto a BIAcore™-2000 instrument with a biosensor chip containing approximately 190 RU of immobilized VEGF(109) or VEGF(165), as indicated. The equilibrium dissociation constant, K_d, is calculated from the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Fab-12)/K_d(variant) indicates the fold increase in binding affinity *versus* the original humanized antibody (Fab-12; Presta *et al.*, 1997) under the specified conditions.

```
Light chain:

                1        10        20        30        40        50
Fab-12          DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF
Y0192           DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYF
Y0317.          DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF

                1        10        20        30        40        50
                        60        70        80        90       100
Fab-12          TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0192           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0317           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ

                        60        70        80        90       100
                       110       120       130       140       150
Fab-12          GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0192           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0317           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

                       110       120       130       140       150
                       160       170       180       190       200
Fab-12          DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0192           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0317           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

                       160       170       180       190       200
                       210
Fab-12          LSSPVTKSFNRGEC
Y0192           LSSPVTKSFNRGEC
Y0317           LSSPVTKSFNRGEC

                       210
```

Figure 1 (*legend shown opposite*)

ing affinity (K_d) of the mutant is in fact <0.2 nM, then the IC_{50} in this experiment will appear higher than under conditions of lower VEGF concentration. The result therefore supports the conclusion that the affinity-improved variant is at least 30-fold improved in affinity for VEGF, and that it effectively blocks VEGF activity *in vitro*.

Structure of the complex

In order to compare the structure and binding site of the affinity-improved antibody with that of the parental antibody, we determined the complex structure by X-ray crystallography. Crystals of the complex between the receptor binding domain of VEGF (residues 8 to 109) and the affinity-matured Fab Y0317 were grown as described (see Materials and Methods) and diffracted to a maximum resolution of 2.4 Å. The structure was refined starting from the coordinates of the complex between VEGF and the parent of Fab Y0317, Fab-12 (Muller *et al.*, 1998a), and refined to an *R*-value of 19.9% (R_{free} = 27.4%) for the reflections between 20 Å and 2.4 Å resolution.

Heavy chain:

```
            1       10        20        30        40        50
Fab-12      EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGW
Y0192       EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGW
Y0317       EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGW
            1       10        20        30        40        50
                    60        70        80        90       100
Fab-12      INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0192       INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0317       INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
              a     60        70        80  abc   90    96
                   110       120       130       140       150
Fab-12      HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0192       HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0317       YYYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
             100abcdef      110       120       130       140
                   160       170       180       190       200
Fab-12      LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0192       LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0317       LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
                   150       160       170       180       190
                   210       220       230
Fab-12      TQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
Y0192       TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
Y0317       TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
                   200       210       220
```

Figure 1. Sequence alignment of the original humanized antibody (Fab-12; Presta *et al.*, 1997), the phage-displayed antibody (Y0192; Muller *et al.*, 1998a) and the affinity-improved antibody (Y0317). Sequential numbering of each chain is shown above the sequences; numbering according to Kabat *et al.* (1987) is shown below. CDR regions are underlined. Positions at which Y0317 differs from Fab-12 are indicated with double underlining.

The final model consists of two Fab fragments bound to the symmetrical poles of the VEGF dimer. Only residues 14–107 of each VEGF monomer are well defined in the electron density, and therefore the six N-terminal and the two C-terminal residues of each monomer were omitted from the model. Each Fab light chain comprises residues 1 to 213, with the C-terminal residue disordered, whereas for each heavy chain residues 138 to 143 as well as the six C-terminal residues are absent from the model. As in the parental Fab complex, two out of 1050 residues, namely T51 in the V_L chain of each Fab fragment, are located in the "disallowed regions" (Laskowski *et al.*, 1993) of the Ramachandran plot; 85% of all residues have their main-chain torsion angles in the "most favored"

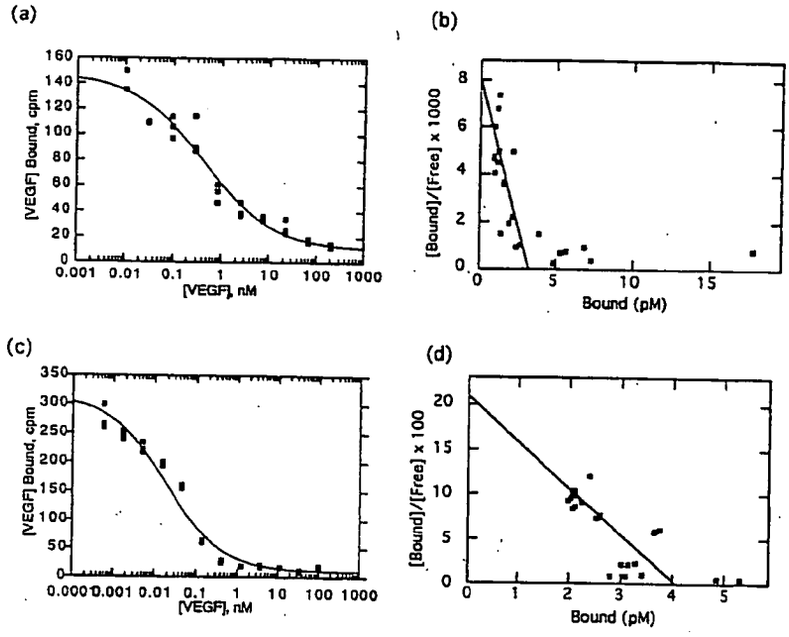


Figure 2. Radiolabeled VEGF binding assay. [^{125}I]VEGF was equilibrated (23 °C) with serial dilutions of unlabeled VEGF and (a) Fab-12 or (c) Y0317. Fabs were captured with an anti-Fab antibody-coated immunosorbant plate. Scatchard analysis (Munson & Rodbard, 1980) with a 1:1 binding model was used to calculate K_d of (b) 433 (±116) pM for Fab-12 and (d) 19.8(±4.3) pM for Y0317.

regions. The average *B*-factor of the model is 51.8 Å^2 and the mobility of the individual domains follows the pattern that was previously observed in the crystal structure of VEGF in complex with the Fab-12, with the constant domain dimer (C_L:C_H1) of one of the Fabs poorly ordered (Muller *et al.*, 1998a).

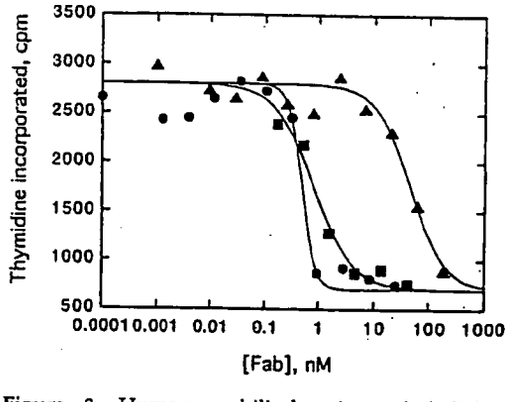


Figure 3. Human umbilical vein endothelial cell (HuVEC) assay of VEGF inhibition. Cells were cultured in the presence of 0.2 nM VEGF and serial dilutions of Fab Y0192 (triangles), Y0238-3 (squares), or Y0313-1 (circles). Cell proliferation was measured by incorporation of [^{3}H]thymidine. Curves show four-parameter fits to the data. Each point represents the mean of three treated wells.

Comparison of the final model with that of the parental Fab-VEGF complex (Muller *et al.*, 1998a) shows that the bound structures are very similar overall (Figure 4(a)) with Y0317 binding to the same site on VEGF as Fab-12 (Figure 4(b)). Side-chains show excellent overlap, and the main-chain structures show very little difference. The most prominent difference in contact residues is at H97Y (Figure 4(c); discussed below), where the tyrosine side-chain packs more favorably with VEGF and a buried water molecule from the parental Fab-VEGF complex is absent in the Y0317-Fab-VEGF complex.

Discussion

Antibody binding selections and affinity improvement

Here we made use of results from alanine-scanning and the previous structure of a humanized antibody-antigen complex to design Fab-phage libraries that randomized the three heavy-chain CDRs as well as one framework region (FR-H3) for improving the binding affinity of an anti-VEGF antibody. Affinity-improved Fab variants were obtained, with the largest effects seen in variants from the CDR-H3 library, although significant improvement was also obtained from mutation of CDR-H1. We therefore combined two mutations from H1 with two from H3, generating a further improved variant, Y0317. By making point mutations, we showed that the 20-fold (Figure 2)

Table 9. Alanine scan of VEGF by ELISA at 37 °C

VEGF(109) variant	IC_{50} (variant)/IC_{50} (VEGF)	
	Fab12-IgG	Y0317-IgG
VEGF(109)	1	1
F17A	1	1
Y21A	1	1
Y45A	4	26
K48A	2	1
Q79A	1	3
I80A	4	5
M81A	>500	930
R82A	>500	4
I83A	>500	9
K84A	3	10
H86A	1	1
Q87A	1	1
G88A	105	87
Q89A	19	6
H90A	1	1
I91A	2	6
G92A	>500	>900
E93A	4	7
M94A	11	25

ELISA assays were carried out using the full-length IgG form of Fab-12 or the IgG form of Y0317 and VEGF(109). Incubation of antibody with VEGF was at 37 °C for five hours. The IC_{50} for inhibition by each Ala mutant was evaluated using a four-parameter equation, and the relative affinities calculated as IC_{50}(mutant VEGF)/IC_{50}(wild-type VEGF). Under these conditions, Fab12-IgG and Y0317-IgG showed IC_{50} values of 9 nM and 1 nM, respectively.

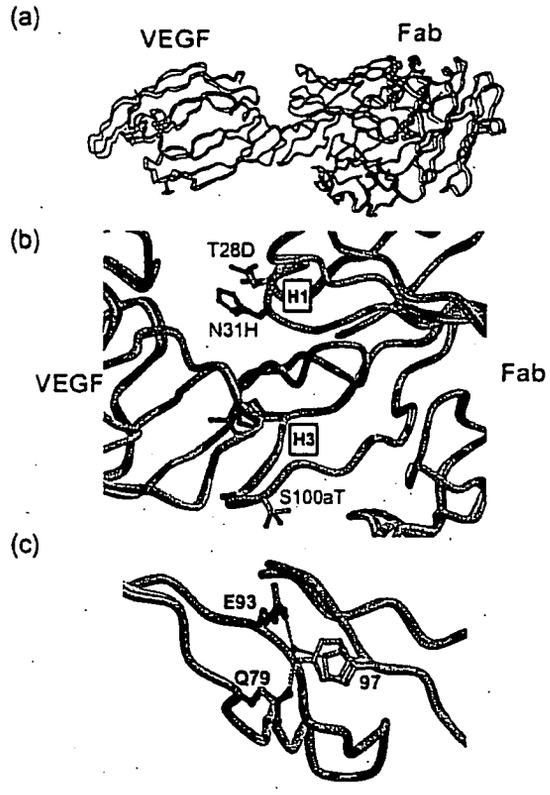


Figure 4. Structure of the affinity-improved Y0317 Fab in complex with VEGF. A superposition of the structure (Muller *et al.*, 1998a) of wild-type humanized antibody Fab-12 (gray) in complex with VEGF (gray) is shown with that of Fab Y0317 (green) in complex with VEGF (yellow). (a) Overall view of the complex, including one Fab molecule bound to one dimer of VEGF (a second Fab molecule is bound at left in the crystal) shows that the binding site for both antibody variants centers on the "80's loop" of VEGF. (b) A view of the four CDR changes between Fab-12 and Y0317 Fab shows that the new D28 and T100a side-chains do not directly contact antigen. However, H31 and Y97 form new contacts. (c) Interactions of H97 and an associated, buried water molecule in the Fab-12 complex, compared with those of Y97 in the Y0317 complex.

to >100-fold (Table 8) affinity improvement in Y0317 can be attributed to two CDR mutations: H97Y and N31H. In fact, H97Y alone improves binding affinty 14-fold.

Despite the relatively slow k_{on} and slow k_{off} of the parental antibody, binding selections described here yielded slower dissociation rates and improved equilibrium dissociation constants. Results of SPR measurements demonstrated that affinity is enhanced mainly through a slower dissociation rate (as opposed to faster association). These results are consistent with the idea of off-rate selection (Hawkins *et al.*, 1992) and with the progressively increased stringency in washing procedures used here (see Materials and Methods and Table 1). Previous binding-optimization efforts have also often yielded larger improvements in k_{off} than in k_{on} (see Lowman & Wells, 1993; Yang *et al.*, 1995; Schier *et al.*, 1996). This may suggest fundamental limitations to the improvements in k_{on} for a given binding site. Even if no conformational changes need occur between free and bound states, the on-rate is limited by the size of the binding interface and the translational and rotational diffusion rates of the binding components (reviewed by Delisi, 1983).

The association rate constants (k_{on}) for both the wild-type Y0192 and the final Y0317 antibodies are relatively slow (about 4×10^4 M^{-1} s^{-1} for both) compared to other antibodies of equal or weaker antigen binding affinity. In fact, the fastest k_{on} identified for any mutant was 6.8×10^4 M^{-1} s^{-1} (Table 6). Typically, k_{on} for antibodies binding to protein antigens, including affinity-matured antibodies, has fallen in the range of 3×10^4 to 1×10^6 M^{-1} s^{-1} (Karlsson *et al.*, 1991; Malmborg *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Wu *et al.*, 1998). In this particular protein-protein interaction, a likely explanation for the slow k_{on} is the high degree of flexibility associated with the binding site both on the Fab and on VEGF. In fact, crystallographic evidence suggests that the "80's loop" region is quite mobile (Muller *et al.*, 1997; Muller *et al.*, 1998b). We are pursuing other strategies to assess whether improvements to k_{on} can be obtained.

The contributions of point mutations in proteins to the free energy of binding or activation are often additive (Wells, 1990). This principle has been used to produce a variety of affinity-improved protein variants based on point or grouped mutations identified by phage display (Lowman & Wells, 1993; Yang *et al.*, 1995) or point-mutant screening (Wu *et al.*, 1998). Considering the initial library selectants Y0238-3 (>ninefold improved in affinity) and Y0243-1 (3.1-fold improved), we would have predicted an improvement of >27-fold for Y0313-1 or Y0317 (Table 7). In fact, a 22-fold improvement is observed (Figure 2) at 25 °C. Addition of the CDR-H1 mutation would be predicted to improve affinity slightly (1.3-fold), but in fact this mutation reduced affinity >twofold (Y0268-1 *versus* Y0313-1; Table 6). Certainly additivity does not always apply, particularly if interacting residues are involved (Wells, 1990). In this case, non-additivity probably results from steric interference between the new Trp in CDR-H2 and the new Tyr in CDR-H3.

To test the energetics of binding by the final Y0317 antibody to VEGF, we made use of a panel of alanine mutants that had been previously constructed for mapping the binding site of the original antibody (Muller *et al.*, 1998a). For these experiments, we made use of the full-length IgG forms of both antibodies. In view of the slow dissociation kinetics for both antibodies, ELISA assays were carried out at 37 °C with incubation for at least five hours to insure that equilibrium was reached. Under these conditions two dramatic differences appear in the Ala-scan of VEGF with respect to Y0317 *versus* Fab-12: both R82A and I83A have small effects on binding in Y0317, but result in large decreases in binding for Fab-12. The reasons for these differences are not clear, but R82 and I83 do have significant surface area (55 Å^2 and 32 Å^2, respectively) buried on binding to VEGF, and make contacts that include residues S100a of CDR-H3 and N52 of CDR-H2 in the wild-type antibody (Muller *et al.*, 1998a).

Structural analysis of the affinity-matured Fab

The structures of a number of antibodies derived from *in vivo* immunization and hybridoma techniques have been determined, in complex with their antigens (reviewed by Nezlin, 1998), and recently, crystallization and preliminary X-ray studies of a chain-shuffled anti-lysozyme scFv antibody in complex with antigen were reported (Küttner *et al.*, 1998). However, to our knowledge, the Y0317 Fab:VEGF structure is the first report of an *in vitro* affinity-matured Fab in complex with antigen. The structural basis of binding affinity improvement is therefore of interest

The Fab fragment of the affinity-matured anti-VEGF antibody Y0317 preserves the structure of the original humanized antibody, Fab-12. Superposition with Fab-12 results in an rmsd of only 0.38 Å for a total of 431 C^{α}-positions, demonstrating the absence of major structural changes between the two molecules. With a total of 1800 Å^2 of solvent-accessible surface buried in each VEGF-Fab interface, the contact area is about 50 Å^2 larger than in the Fab-12 complex. This small increase in buried surface area is mostly due to the exchange of H97 to a tyrosine residue. In the VEGF:Fab-12 complex, H97 buries a solvent-accessible area of 56 Å^2, while the larger tyrosine side-chain of the matured antibody accounts for 86 Å^2 of buried surface. The tyrosine side-chain also affects the hydrogen-bonding pattern and the number of ordered water molecules in the vicinity. In the parental antibody complex, a water molecule near H97 forms two hydrogen bonds to the side-chains of Q79 and E93 of VEGF (Figure 4(c)). In the complex with the affinity-matured Fab, this water molecule is replaced by the hydroxyl group of the newly introduced tyrosine side-chain at position 97. The H97Y mutation therefore not only increases the amount of buried surface area, but also introduces two additional hydrogen bonds between the ligand and Fab-0317 (Figure 4(c)). This is in good agreement with the observation that this single substitution improves VEGF binding affinity by 14-fold (Table 6). We therefore conclude that this single substiution is responsible for the majority of the improvement in binding affinity of Y0317 compared to the parent antibody.

In contrast, despite the availability of the crystal structures of both complexes, it remains uncertain what the structural basis is of the 3.6-fold enhanced binding caused by the N31H mutation. The side-chains of the asparagine and the histidine residues in this position adopt identical conformations in both crystal structures, and the amount of buried surface is not significantly increased in the VEGF:Fab-Y0317 complex. The only difference we can detect is a slight possible improvement in the hydrophobic interactions between the histidine side-chain and the phenyl group of VEGF residue F17, which has rotated slightly compared to the parent complex. It is unclear whether this could contribute to the increased affinity.

Neither of the remaining differences between Fab-12 and Fab-Y0317 has a significant effect on the binding affinity towards VEGF, and the structures show that these residues contribute only marginally to the interface. Some interactions are present between VEGF and the main-chain atoms of the serine and threonine residues in position 100a of the two Fabs, but the side-chains of these residues are not in contact with VEGF. Finally, no contact exist between VEGF and T28 (or D28) of the Fab fragments (the closest point on VEGF to this residue is more than 6 Å distant).

In summary, the analysis and comparison of the two crystal structures are in very good agreement with the results of the binding assays on the various single mutants of the Fab fragments. Although it is not possible to quantify the effects introduced by the amino acid exchanges solely based on the crystal structures, the detailed crystallographic

analysis supports and enables us to interpret the binding data.

Biological Implications for antibody inhibition of VEGF

An inhibitory antibody of improved affinity may have improved potency or efficacy in treating diseases associated with VEGF expression. Preceding versions of the anti-VEGF antibody described here, including the murine A4.6.1 (Kim *et al.*, 1993), the humanized version Fab-12 (Presta *et al.*, 1997), as well as Y0192 (Muller *et al.*, 1998a), clearly demonstrated sufficient affinity to effect inhibition of VEGF activity. Here, we show that an affinity-improved variant, Fab Y0317, can inhibit endothelial cell proliferation *in vitro* with least 30-fold greater potency than the parental humanized Fab (Figure 3).

We have limited our optimization strategy to a subset of heavy-chain CDR residues implicated by alanine-scanning and crystallography (Muller *et al.*, 1998a). Furthermore, not all combinations of phage-derived mutations have been tested. One may therefore reasonably ask whether Y0317, with $K_d^{25^\circ} = 20$ pM and $K_d^{37^\circ} = 130$ pM, is the globally optimum variant for binding to this particular epitope (or others) on VEGF. Other affinity optimization efforts have resulted in protein-protein binding affinities in the low picomolar range, from $K_d = 6$ pM to 15 pM (see, e.g. Lowman & Wells, 1993; Schier *et al.*, 1996; Yang *et al.*, 1995). Indeed, we cannot exclude the possibility that higher affinity variants of the A4.6.1 antibody could be produced. However, it seems unlikely that further affinity improvement would greatly enhance biological potency or efficacy because for effective inhibition, the antibody must certainly occupy a significant fraction (perhaps >99%) of the available (VEGF) binding sites. Serum VEGF concentrations of about 20 pM in normals, and of >300 pM in patients with metastatic carcinoma, have been observed (Kraft *et al.*, 1999). Local or effective concentrations are likely higher. If we conservatively assume the effective concentration of VEGF *in vivo* to be about 400 pM, then 400 pM of even an infinite-affinity Fab would be required to block all sites.

Other factors may limit the improvement in potency of a full-length IgG resulting from an improvement in intrinsic binding affinity of the Fab for antigen. The full-length IgG form of the antibody may benefit from an avidity effect *in vivo*, especially since VEGF is known to associate with proteoglycans on the cell surface (Gitay-Goren *et al.*, 1992). Even in cell-based assays, the IgG form of Fab-12 is a more effective inhibitor than the Fab form (data not shown). Finally, the half-life for dissociation of the affinity-improved antibody is already significant, even on the time-scale of the half-life of clearance for IgG's (days to weeks). The effect of an improved association rate constant for antibody in this system is unknown.

The fact that point (Ala) mutations in the antibody binding site on VEGF sometimes have lesser effects on the binding of Y0317 than on the binding of Fab-12 may suggest that the optimized binding site is more tolerant than the parental one of variations in the antigen. Indeed, Y0317 showed greatly enhanced affinity for murine VEGF over that of Fab-12 (data not shown), though still >100-fold weaker than its affinity for human VEGF. This could provide an advantage against naturally arising VEGF variants.

Materials and Methods

Construction of phage libraries and mutagenesis

A variant of the Fab-12 antibody (a humanized form of murine antibody A4.6.1) was previously identified from phage-displayed Fab libraries for improved expression on phage particles (Muller *et al.*, 1998a). We made use of the plasmid pY0192, a phagemid construct with ampicillin (or carbenicillin) resistance, as the parental ("wild-type") construct for libraries described here. To prevent contamination by wild-type sequence (Lowman *et al.*, 1991; Lowman, 1998), templates with the TAA stop codon at each residue targeted for randomization were prepared from CJ236 *E. coli* cells (Kunkel *et al.*, 1991). Libraries are designated according to the mutagenic oligonucleotides used for their construction: YC265, TCC TGT GCA GCT TCT GGC NNS NNS TTC NNS NNS NNS GGT ATG AAC TGG GTC CG, randomizing residues 27-28, 30-32 in CDR-H1; YC266, GAA TGG GTT GGA TGG ATT AAC NNS NNS NNS GGT NNS CCG ACC TAT GCT GCG G, randomizing residues 52a-54, 56 in CDR-H2; YC103, GAA TGG GTT GGA TGG ATT NNS NNS NNS NNS GGT GAA CCG ACC TAT G, randomizing residues 52-54 in CDR-H2; YC81, C TGT GCA AAG TAC CCG NNS TAT NNS NNS NNS NNS CAC TGG TAT TTC GAC, randomizing residues 97, 99-100b in CDR-H3; and YC101, CGT TTC ACT TTT TCT NNS GAC NNS TCC AAA NNS ACA GCA TAC CTG CAG , randomizing residues 71, 73, and 76 in the "FR-H3" region. An additional library in CDR-H2 was designed to insert three new residues: YC90, GA TGG ATT AAC ACC TAT NNS NNS NNS ACC GGT GAA CCG ACC.

The products of random mutagenesis reactions were electroporated into XL1-Blue *E. coli* cells (Stratagene) and amplified by growing 15-16 hours with M13KO7 helper phage. The complexity of each library, ranging from 2×10^7 to 1.5×10^8 , was estimated based on plating of the initial transformation onto LB plates containing carbenicillin.

Site-directed mutagenesis for point mutations was carried out as above, using appropriate codons to produce the respective mutations, and sequences were confirmed by single-strand DNA sequencing using Sequenase™ (USB).

Phage binding selections

For each round of selection, approximately 10^9-10^{10} phage were screened for binding to plates (Nunc Maxisorp 96-well) coated with 2 μg/ml VEGF(109) in 50 mM carbonate buffer (pH 9.6) and blocked with 5% (w/v) instant milk in 50 mM carbonate buffer, (pH 9.6). Also included were phage prepared from a non-displaying

control phagemid (pCAT), which confers chloramphenicol resistance, as a means of measuring background and enrichment (Lowman & Wells, 1993). Bound phage were eluted with 0.1 M HCl and immediately neutralized with one-third volume of 1 M Tris (pH 8.0). The eluted phage were propagated by infecting XL1 cells for the next selection cycle as described (Lowman, 1998).

In the first cycle, the VEGF plate was incubated with Fab-phage, then was briefly washed to remove bound phage. In the second cycle, binding and washing were followed by a one hour dissociative incubation at room temperature with binding buffer, after which the plate was again washed prior to acid elution. This process was repeated in rounds 3, 4 and 5, except that 1 μM VEGF was included in the dissociative incubation, and the incubation time was increased to 2, 18, and 37 hours, respectively. During these selections, Y0192 phage showed enrichments ranging from 1.5-fold (at the lowest stringency) to 22,000-fold (using a two hour dissociation incubation). However, further increases in stringency (rounds 4-5) resulted in decreasing enrichments for the control phage, with higher enrichments observed for certain libraries, especially the two CDR-H2 libraries and the CDR-H3 library (Table 1).

In cycle 6, a 17 hour dissociative incubation at room temperature was followed by an additional 30 hour incubation at 37 °C (also including VEGF in the buffer). Under these conditions, Y0192-phage showed only slight binding enrichment (20-fold), whereas the CDR-H3 library phage were enriched by 3500-fold. Cycle 7 was carried out with a 63 hour dissociative incubation, after which only small enrichment factors were observed. However, some libraries were continued through eight cycles (with 120 hours of dissociative incubation in the presence of VEGF), after which Fab-phage were still recoverable by acid elution (data not shown).

Purification of Fab

For small-scale preparations, Y0317 Fab and mutants were prepared from *E. coli* shake-flasks as described (Muller *et al.*, 1998a).

For large-scale preparation, whole cell broth was obtained from a ten liter *E. coli* fermentation. The cells were lysed with a Manton-Gaulin homogenizer (two passes at 6000 psi; lysate temperature maintained at 15-25 °C with a heat exchanger). A 5 % (v/v) solution of polyethylene imine (PEI), pH 6.0, was added to the lysate to give a final concentration of 0.25 % (v/v). The lysate was mixed for 30 minutes at room temperature. The suspension was centrifuged, and the supernatant (containing the Fab) was processed further. The pH of the supernatant was adjusted to 6.0 with 6 M HCl, followed by dilution to a conductivity of 5 mS/cm with purified water. The conditioned supernatant was loaded onto a BakerBond ABx ion-exchange column. Following a wash with the column equilibration buffer, the Fab was eluted with an increasing sodium chloride gradient in the equilibration buffer. Fractions containing the Fab were identified by SDS-PAGE. The BakerBond ABx column fractions were pooled, pH adjusted to 5.5 with 1 M Mes and diluted to a conductivity of 5 mS/cm with purified water. The conditioned BakerBond ABx pool was loaded onto a SP Sepharose HP cation exchange column (Pharmacia). Once again, the Fab was eluted with a sodium chloride-containing gradient. Fractions containing the Fab were identified by SDS-PAGE. The level of purity of Fab (as determined by SDS-PAGE) after this two column purification was >95 %.

BIAcore™ binding analysis

The VEGF-binding affinities of Fab fragments were calculated from association and dissociation rate constants measured using a BIAcore™ -2000 surface plasmon resonance system (BIAcore, Inc., Piscataway, NJ). A biosensor chip was activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's (BIAcore, Inc., Piscataway, NJ) instructions. VEGF(109) or VEGF(165) was buffer-exchanged into 20 mM sodium acetate, pH 4.8 and diluted to approximately 50 μg/ml. Aliquots of VEGF were injected at a flow rate of 2 μl/minute to achieve approximately 700-1400 response units (RU) of coupled protein. A solution of 1 M ethanolamine was injected as a blocking agent.

For kinetics measurements, twofold serial dilutions of Fab were injected in PBS/Tween buffer (0.05 % Tween-20 in phosphate-buffered saline) at 25 °C or 37 °C at a flow rate of 10 μl/minute. Equilibrium dissociation constants, K_d values from SPR measurements were calculated as k_{off}/k_{on} (Tables 6 and 8).

Radiolabeled VEGF binding assay

Solution binding affinity of Fabs for VEGF was measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled VEGF(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate.

To establish conditions for the assay, microtiter plates (Dynex) were coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2 % (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [^{125}I]VEGF(109) was mixed with serial dilutions of Fab-12 or Fab Y0317, respectively. Fab-12 was incubated overnight; however, the Fab Y0317 incubation was continued for 65 hours to insure that equilibrium was reached. Thereafter, the mixtures were transferred to the capture plate for incubation at room temperature for one hour. The solution was then removed and the plate washed eight times with 0.1 % Tween-20 in PBS. When the plates had dried, 150 μl/well of scintillant (MicroScint-20; Packard) was added, and the plates were counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab were chosen to give ≤20 % of maximal binding.

For competitive binding assays, Dynex plates were coated and blocked as above, and serial threefold dilutions of unlabeled VEGF(109) were made in PBS/Tween buffer in a Nunc plate. [^{125}I]VEGF(109) was added, followed by addition of a fixed concentration of Fab-12 or Fab Y0317. The final concentrations of Fab-12, and Fab Y0317 were 100 pM and 10 pM, respectively. After incubation (as above), bound VEGF was captured and quantified as described above. The binding data was analyzed using a computer program to perform Scatchard analysis (Munson & Rodbard, 1980) for determination of the dissociation binding constants, K_d, for Fab-12 and Fab Y0317.

ELISA assay of VEGF Ala mutants

The binding affinities of VEGF Ala mutants for full-length Fab-12-IgG (known as rhuMAb VEGF) and Y0317-IgG, a full-length IgG form of the improved antibody expressed in CHO cells (V. Chisholm, unpublished results) were measured as previously described (Muller *et al.*, 1997; Muller *et al.*, 1998a) for the murine antibody A4.6.1, except that the temperature was increased to 37 °C, and the incubation time increased to five hours, to insure that equilibrium was reached with the high-affinity antibody.

Cell-based assay of VEGF Inhibition

Several versions of the anti-VEGF antibody were tested for their ability to antagonize VEGF(165) induction of the growth of HuVECs (human umbilical vein endothelial cells). The 96-well plates were seeded with 1000 HuVECs per well and fasted in assay medium (F12:DMEM 50:50 supplemented with 1.5% (v/v) dia-filtered fetal bovine serum) for 24 hours.

The concentration of VEGF used for inducing the cells was determined by first titrating to identify the amount of VEGF that can stimulate 80% of maximal DNA synthesis. Fresh assay medium containing fixed amounts of VEGF (0.2 nM final concentration), and increasing concentrations of anti-VEGF Fab or mab were then added. After 40 hours of incubation, DNA synthesis was measured by incorporation of tritiated thymidine. Cells were pulsed with 0.5 μCi per well of [^{3}H]thymidine for 24 hours and harvested for counting, using a TopCount gamma counter.

Crystallization and refinement

The complex between the Fab fragment of affinity-matured, humanized antibody Y0317 Fab and the receptor binding fragment of VEGF (VEGF(109)) was purified and crystallized as described for the analogous complex with the parental humanized Fab-12 fragment (Muller *et al.*, 1998a). The resulting crystals had symmetry consistent with space group $P2_1$ with cell parameters $a = 89.1$ Å, $b = 66.4$ Å, $c = 138.7$ Å, and $\beta = 94.7°$, and were isomorphous with the crystals obtained with the parent complex. A data set was collected from a single frozen crystal at beam line 5.0.2 at the Advanced Light Source, Berkeley, and processed using programs MOSFLM and SCALA (CCP4, 1994). The final data set (R_{merge} = 7.3%) is described in Table 10. Starting with the model of Brookhaven Protein Data Bank entry 1bj1 (Muller *et al.*, 1998a), the structure was refined using the programs X-PLOR (Brünger *et al.*, 1987) and REFMAC (CCP4, 1994). The free *R*-value was monitored using the identical set of reflections sequestered before refinement of parent complex. The differences in the primary structure between Fab-12 and Fab-Y0317 were modeled using the program O (Jones *et al.*, 1991). After correction for anisotropy and application of a bulk solvent correction, the *R*-value reached its final value of 19.9% for all reflections greater than 0.2σ (see Table 10; R_{free} = 27.4%).

Table 10. Crystallographic data and refinement statistics

A. *Data collection*	Overall	Last shell
Resolution range (Å)	30-2.4	2.53-2.40
No. of observations	208,257	22,278
Unique reflections	61,742	8900
Completeness (%)	97.4	96.7
Mean $I/\sigma(I)$	13.6	2.7
R_{sym}	0.073	0.38
B. *Refinement*		
Resolution range (Å)	20-2.4	
No. of reflections	61,689	
No. of atoms	8577	
rmsd bond lengths (Å)	0.013	
rmsd angles (deg.)	1.9	
rmsd improper angles (deg.)	0.92	
rmsd *B*-factors for all bonded atoms, Å^2	3.5	
Number of main-chain torsion angles in disallowed regions of Ramachandran plot[a]	2	

[a] See Laskowski *et al.* (1993).

Protein Data Bank accession number

The coordinates for the VEGF:Y0317 Fab complex have been deposited in the Protein Data Bank, accession number 1cz8.

Acknowledgments

We thank Lyn Deguzman and Tom Zioncheck for providing ^{125}[I]VEGF; Alan Padua and Bill Henzel for quantitative amino acid analysis; James Bourell for mass spectrometry; Vanessa Chisholm and Lynne Krummen for construction of cell lines; and Manuel Baca, Napoleone Ferrara, Yves Muller, Leonard Presta, and James Wells for many helpful discussions.

References

Baca, M., Presta, L. G., O'Connor, S. J. & Wells, J. A. (1997). Antibody humanization using monovalent phage display. *J. Biol. Chem.* 272, 10678-10684.

Barbas, C. F., III, Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R. M., Nara, P. L. & Burton, D. R. (1994). *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc. Natl Acad. Sci. USA*, 91, 3809-3813.

Bass, S., Greene, R. & Wells, J. A. (1990). Hormone phage: an enrichment method for variant proteins with altered binding properties. *Proteins: Struct. Funct. Genet.* 8, 309-314.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). Crystallographic *R* factor refinement by molecular dynamics. *Science*, 235, 458-460.

CCP4 (1994). Programs for protein crystallography. *Acta Crystallog. sect. D*, 50, 760-763.

Chothia, C. & Lesk, A. M. (1987). Canonical structures for the hypervariable reigions of immunoglobulins. *J. Mol. Biol.* 196, 901-917.

Delisi, C. (1983). Role of diffusion regulation in receptor-ligand interactions. *Methods Enzymol.* 93, 95-109.

Fairbrother, W. J., Christinger, H. W., Cochran, A. G., Fuh, G., Keenan, C. J., Quan, C., Shriver, S. K., Tom, J. Y., Wells, J. A. & Cunningham, B. C. (1998). Novel peptides selected to bind vascular endothelial growth factor target the receptor-binding site. *Biochemistry*, 37, 17754-17764.

Ferrara, N. (1995). The role of vascular endothelial growth factor in pathological angiogenesis. *Breast Cancer Res. Treat.* 36, 127-137.

Ferrara, N. (1999). Vascular endothelial growth factor: molecular and biological aspects. *Curr. Top. Microbiol. Immunol.* 237, 1-30.

Ferrara, N. & Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 161, 851-858.

Folkman, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med.* 1, 27-31.

Folkman, J. & Klagsbrun, M. (1987). Angiogenic factors. *Science*, 235, 442-443.

Foote, J. & Winter, G. (1992). Antibody framework residues affecting the conformation of the hypervariable loops. *J. Mol. Biol.* 224, 487-499.

Gitay-Goren, H., Soker, S., Vlodavsky, I. & Neufeld, G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell-surface-associated heparin-like molecules. *J. Biol. Chem.* 267, 6093-6098.

Griffiths, A. D. & Duncan, A. R. (1998). Strategies for selection of antibodies by phage display. *Curr. Opin. Biotechnol.* 9, 102-108.

Hawkins, R. E., Russell, S. J. & Winter, G. (1992). Selection of phage antibodies by binding affinity mimicking affinity maturation. *J. Mol. Biol.* 226, 889-896.

Hawkins, R. E., Russell, S. J., Baier, M. & Winter, G. (1993). The contribution of contact and non-contact residues of antibody in the affinity of binding to antigen. *J. Mol. Biol.* 234, 958-964.

Iruela-Arispe, M. L. & Dvorak, H. F. (1997). Angiogenesis: a dynamic balance of stimulators and inhibitors. *Thromb. Haem.* 78, 672-677.

Jones, T. A., Zhou, J.-Y., Cowan, S. W. & Kjelgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallog. sect. A*, 47, 110-119.

Kabat, E. A., Wu, T. T., Redi-Miller, M., Perry, H. M. & Gottesman, K. S. (1987). *Sequences of Proteins of Immunological Interest*, 4th edit., National Institutes of Health, Bethesda, MD.

Karlsson, R., Michaelsson, A. & Mattsson, L. (1991). Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods*, 145, 229-240.

Kay, B. K. & Hoess, R. H. (1996). Principles and applications of phage display. In *Phage Display of Peptides and Proteins* (Kay, B. K., Winter, J. & McCafferty, J., eds), pp. 21-34, Academic Press, San Diego.

Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S. & Ferrara, N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature*, 362, 841-844.

Kraft, A., Weindel, K., Ochs, A., Marth, C., Zmija, J., Schumacher, P., Unger, C., Marmé, D. & Gastl, G. (1999). Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. *Cancer*, 85, 178-187.

Kunkel, T. A., Bebenek, K. & McClary, J. (1991). Efficient site-directed mutagenesis using uracil-containing DNA. *Methods Enzymol.* 204, 125-139.

Küttner, G., Keitel, T., Gießmann, E., Wessner, H., Scholz, C. & Höhne, W. (1998). A phage library-derived single-chain Fv fragment in complex with turkey egg-white lysozyme: characterization, crystallization and preliminary X-ray analysis. *Mol. Immunol.* 35, 189-194.

Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). Procheck: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallog.* 26, 283-291.

Lowman, H. B. (1998). Phage display of peptide libraries on protein scaffolds. In *Methods in Molecular Biology, Combinatorial Peptide Library Protocols* (Cabilly, S., ed.), vol. 87, pp. 249-264, Humana Press, Totowa, NJ.

Lowman, H. B. & Wells, J. A. (1993). Affinity maturation of human growth hormone by monovalent phage display. *J. Mol. Biol.* 234, 564-578.

Lowman, H. B., Bass, S. H., Simpson, N. & Wells, J. A. (1991). Selecting high-affinity binding proteins by monovalent phage display. *Biochemistry*, 30, 10832-10838.

Malmborg, A.-C., Michaelsson, A., Ohlin, M., Jansson, B. & Borrebaeck, C. A. K. (1992). Real time analysis of antibody-antigen reaction kinetics. *Scan. J. Immunol.* 35, 643-650.

Marks, J. D., Griffiths, A. D., Malmqvist, M., Clackson, T. P., Bye, J. M. & Winter, G. (1992). By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology*, 10, 779-783.

Mordenti, J., Thomsen, K., Licko, V., Chen, H., Meng, Y. G. & Ferrara, N. (1999). Efficacy and concentration-response of murine anti-VEGF monoclonal antibody in tumor-bearing mice and extrapolation to humans. *Toxicol. Pathol.* 27, 14-21.

Muller, Y. A., Li, B., Christinger, H. W., Wells, J. A., Cunningham, B. C. & de Vos, A. M. (1997). Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. *Proc. Natl Acad. Sci. USA*, 94, 7192-7197.

Muller, Y. A., Chen, Y., Christinger, H. W., Li, B., Cunningham, B. C., Lowman, H. B. & de Vos, A. M. (1998a). VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 Å resolution and mutational analysis of the interface. *Structure*, 6, 1153-1167.

Muller, Y. A., Christinger, H. W., Keyt, B. A. & de Vos, A. M. (1998b). The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding. *Structure*, 5, 1325-1338.

Munson, P. & Rodbard, D. (1980). Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107, 220-239.

Nezlin, R. (1998). *The Immunoglobulins: Structure and Function*, pp. 151-204, Academic Press, San Diego.

Presta, L. G., Chen, H., O'Connor, S. J., Chisholm, V., Meng, Y. G., Krummen, L., Winkler, M. & Ferrara, N. (1997). Humanization of a vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.* 47, 4593-4599.

Rader, C. & Barbas, C. F., III (1997). Phage display of combinatorial antibody libraries. *Curr. Opin. Biotechnol.* 8, 503-508.

Schier, R., McCall, A., Adams, G. P., Marshall, K. W., Merritt, H., Yim, M., Crawford, R. S., Weiner, L. M., Marks, C. & Marks, J. D. (1996). Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity

determining regions in the center of the antibody binding site. *J. Mol. Biol.* **263**, 551-567.

Thompson, J., Pope, T., Tung, J.-S., Chan, C., Hollis, G., Mark, G. & Johnson, K. S. (1996). Affinity maturation of a high-affinity human monoclonal antibody against the third hypervariable loop of human immunodeficiency virus: use of phage display to improve affinity and broaden strain reactivity. *J. Mol. Biol.* **16**, 77-88.

Tramontano, A., Chothia, C. & Lesk, A. M. (1990). Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. *J. Mol. Biol.* **215**, 175-182.

Wells, J. A. (1990). Additivity of mutational effects in proteins. *Biochemistry*, **29**, 8509-8517.

Wu, H., Beuerlein, G., Nie, Y., Smith, H., Lee, B. A., Hensler, M., Huse, W. D. & Watkins, J. D. (1998). Stepwise *in vitro* affinity maturation of vitaxin, an $\alpha_V\beta_3$-specific humanized mAb. *Proc. Natl Acad. Sci. USA*, **95**, 6037-6042.

Xiang, J., Sha, Y., Jia, Z., Prasad, L. & Delbaere, L. T. (1995). Framework residues 71 and 93 of the chimeric B72.3 antibody are major determinants of the conformation of heavy-chain hypervariable loops. *J. Mol. Biol.* **253**, 385-390.

Yang, W.-P., Green, K., Pinz-Sweeney, S., Briones, A. T., Burton, D. R. & Barbas, C. F., III (1995). CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. *J. Mol. Biol.* **254**, 392-403.

Edited by I. A. Wilson

(Received 19 July 1999; received in revised form 7 September 1999; accepted 13 September 1999)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

rhuFab VEGF

Our Reference: BB-IND 8633

OCT 13 1999

21579

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Vice President, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 8633

SPONSOR: Genentech, Incorporated

PRODUCT NAME: Humanized Monoclonal Antibody Fragment (rhuFab V2) (E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal

DATE OF SUBMISSION: October 6, 1999

DATE OF RECEIPT: October 7, 1999

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

10-18-99 P02:54 IN
10-18-99 P

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information, and all serious, unexpected adverse experiences must be reported, in writing, to this Division and to all study centers within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Sponsors of INDs for products used to treat life-threatening or severely debilitating diseases are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Kay Schneider

Kay Schneider, M.S.
Consumer Safety Officer
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

JAN 27 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality, and Compliance
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL #125156/0

Name of Biological Product: Lucentis™ (ranibizumab)

Indication: Treatment for patients with neovascular age-related macular degeneration

Date of Application: December 29, 2005

Date of Receipt: December 30, 2005

User Fee Goal Date: June 30, 2006

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We note that you have not fulfilled the requirement. We are waiving the requirement for pediatric studies for this application.

If you have not already done so, promptly submit the *content of labeling* (21 CFR 601.14(b)) in electronic format as described at the following website:
http://www.fda.gov/oc/datacouncil/spl.html.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

We request that you submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions. Effective August 29, 2005, the new address for all submissions to this application is:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, please contact the Regulatory Project Manager, Lori Gorski, at (301) 796-0722.

Sincerely,

Maureen P. Dillon-Parker
Chief, Project Management Staff
Division of Anti-Infective and
Ophthalmology Products
Office of Antimicrobials
Center for Drug Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

MAR 14 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

This letter is in regard to your biologics license application (BLA) submitted under section 351 of the Public Health Service Act.

We have completed an initial review of your application dated December 29, 2005, for Lucentis (ranibizumab injection) to determine its acceptability for filing. Under 21 CFR 601.2(a), your application was filed on February 28, 2006. The user fee goal date is June 30, 2006. This acknowledgment of filing does not mean that we have issued a license nor does it represent any evaluation of the adequacy of the data submitted.

At this time, we have not identified any potential review issues. Our filing review is only a preliminary review, and deficiencies may be identified during substantive review of your application. Following a review of the application, we shall advise you in writing of any action we have taken and request additional information if needed.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

Please use the following address for any amendments to your application:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Maureen Dillon Parker
Chief, Project Management Staff
Division of Anti-Infective and Ophthalmology Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* -- § 156

Patent No.: 7,060,269

Issued: June 13, 2006

Application No: 09/723,752

Docket No: 22338-80060

Assignee: Genentech, Inc.

Unit: OPLA

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extension

CERTIFICATE OF MAILING - 37 C.F.R. § 1.10
EXPRESS MAIL LABEL NO. ER 736919899 US

I hereby certify this correspondence is being deposited with the U.S. Postal Service with sufficient postage as "Express Mail – Post Office to Addressee" addressed to: Mail Stop Patent Ext., Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 1450, Alexandria, VA 22313-1450, on the date shown below.

Signature — YVONNE T. REYES Printed Name — Aug. 25, 2006 Date

Mail Stop Patent Ext.
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Dear Sir:

Applicant, Genentech, Inc., hereby submits this application for extension of the term of United States Letters Patent 7,060,269 under 35 U.S.C. § 156 by providing the following information in accordance with the requirements specified in 37 C.F.R. § 1.740.

Applicant represents that it is the assignee of the entire interest in and to United States Letters Patent No. 7,060,269, granted to Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen (Baca *et al.*) by virtue of an assignment of such patent to Genentech, Inc., recorded December 29, 1997, at Reel 8872, Frame 0429.[1]

[1] The assignment recorded at the noted location in the Office's records identifies U.S.S.N. 08/908,469 ("the '469 application") and states that the conveyance includes the entire "right, title and interest ... in and to said invention, and in and to any and all Letters Patents to be granted and issued therefor...." U.S.S.N. 09/723,752, from which the '269 patent issued, is a continuation application (divisional) of the '469 application.

1. Identification of the Approved Product [§ 1.740(a)(1)]

The name of the approved product is LUCENTIS™. The name of the active ingredient of LUCENTIS ™ is ranibizumab. Ranibizumab is a recombinant humanized monoclonal IgG_1 antibody antigen-binding fragment (Fab) based on a humanized framework with complementarity-determining regions (CDRs) derived from a murine monoclonal antibody that binds to human Vascular Endothelial Growth Factor (VEGF).

2. Federal Statute Governing Regulatory Approval of the Approved Product [§ 1.740(a)(2)]

The approved product was subject to regulatory review under, *inter alia*, the Public Health Service Act (42 U.S.C. § 201 *et seq.*) and the Federal Food, Drug and Cosmetic Act (21 U.S.C. § 355 *et seq.*).

3. Date of Approval for Commercial Marketing [§ 1.740(a)(3)]

LUCENTIS™ was approved for commercial marketing or use under § 351 of the Public Heath Service Act on **June 30, 2006**.

4. Identification of Active Ingredient and Certifications Related to Commercial Marketing of Approved Product [§ 1.740(a)(4)]

(a) The active ingredient of LUCENTIS™ is ranibizumab. Ranibizumab is a humanized monoclonal IgG_1 antibody antigen-binding fragment produced by an *E. coli* expression system. It contains human framework regions (FRs) and the complementarity-determining regions (CDRs) derived from a murine antibody that binds to VEGF.

(b) Applicant certifies that ranibizumab had not been approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act prior to the approval granted on June 30, 2006 to the present Applicant.

(c) Ranibizumab has been approved for the treatment of patients with neovascular (wet) age-related macular degeneration. *See* LUCENTIS™ product label, provided as Attachment A.

(d) LUCENTIS™ was approved for commercial marketing pursuant to § 351 of the Public Health Service Act (42 U.S.C. § 262) under Genentech's existing Department of Health and Human Services (DHHS) U.S. License No. 1048. *See* LUCENTIS™ approval letter, provided as Attachment B.

5. Statement Regarding Timeliness of Submission of Patent Term Extension Request [§ 1.740(a)(5)]

Applicant certifies that this application for patent term extension is being timely submitted within the sixty (60) day period permitted for submission specified in 35 U.S.C. § 156(d)(1) and 37 C.F.R. § 1.720(f). The last date on which this application may be submitted is August 28, 2006.

6. Complete Identification of the Patent for Which Extension Is Being Sought [§ 1.740(a)(6)]

The complete identification of the patent for which an extension is being sought is as follows:

(a)	Names of the inventors:	Manuel Baca; James A. Wells; Leonard G. Presta; Henry B. Lowman; and Yvonne Man-yee Chen.
(b)	Patent Number:	7,060,269
(c)	Date of Issue:	June 13, 2006
(d)	Date of Expiration:	July 4, 2019[2]

7. Copy of the Patent for Which an Extension is Being Sought [§ 1.740(a)(7)]

A copy of U.S. Patent No. 7,060,269 is provided as Attachment C to the present application.

8. Copies of Disclaimers, Certificates of Correction, Receipt of Maintenance Fee Payment, or Reexamination Certificate [§ 1.740(a)(8)]

(a) U.S. Patent No. 7,060,269 is not subject to a terminal disclaimer.

(b) A Certificate of Correction has not been issued for U.S. Patent No. 7,060,269.

(c) The first maintenance fee for U.S. Patent No. 7,060,269 will be due on December 13, 2009.

(d) U.S. Patent No. 7,060,269 has not been the subject of a reexamination proceeding.

---

[2] The term of the '269 patent has been extended, under 35 USC § 154(b) by 697 days. The 697 days have been included in calculating the July 4, 2019 expiration date.

9. Statement Regarding Patent Claims Relative to Approved Product [§ 1.740(a)(9)]

The statements below are made solely to comply with the requirements of 37 C.F.R. § 1.740(a)(9). Applicant notes that, as the M.P.E.P. acknowledges, § 1.740(a)(9) does not require an applicant to show whether or how the listed claims would be infringed, and that this question cannot be answered without specific knowledge concerning acts performed by third parties. As such, these comments are not an assertion or an admission of Applicant as to the scope of the listed claims, or whether or how any of the listed claims would be infringed, literally or under the doctrine of equivalents, by the manufacture, use, sale, offer for sale or the importation of any product.

(a) At least claim 1 of U.S. Patent No. 7,060,269 ("the '269 patent") claims the active pharmaceutical ingredient in the approved product or a method that may be used to make or use that ingredient.

(b) Pursuant to M.P.E.P. § 2753 and 37 C.F.R. § 1.740(a)(9), the following explanation is provided which shows how the above-listed claim of the '269 patent claims a method of using the approved product.

(1) Description of the approved product and its method of use

The approved product is described in Section 11 of the approved label for LUCENTIS™ as follows, a copy of which is provided as Attachment A.

> LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.
>
> LUCENTIS™ is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS™ is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS™ aqueous solution with 10 mM histidine HCL, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

Ranibizumab is further characterized in a scientific reference by Chen *et al.* published in 1999 in the Journal of Molecular Biology (JMB) entitled "Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen."[3] For example, the heavy

[3] 293:865-881 (1999) (Attachment E)

and light chain sequences of ranibizumab, designated as Y0317 in the article, are displayed in Figure 1. In addition, the article provides data regarding the binding affinity of the Y0317 antibody fragment to VEGF. *See, e.g.,* Table 6 on p. 870.

(2) Claim 1

Claim 1 of the '269 patent reads as follows:

1. A method for inhibiting VEGF-induced angiogenesis in a subject, comprising administering to said subject an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about $1x10^{-8}$ M, said humanized anti-VEGF antibody comprising a heavy chain variable domain sequence of SEQ ID NO:116 and a light chain variable domain sequence of SEQ ID NO:115.

Comparison of Ranibizumab to the limitations of claim 1

Claim 1 pertains to a method of inhibiting VEGF-induced angiogenesis in a subject by administering an effective amount of a humanized anti-VEGF antibody that binds to human VEGF at a defined Kd value and that contains designated light and heavy chain variable domains. Applicant asserts that the use of ranibizumab for the treatment of age-related macular degeneration falls within the scope of claim 1 for at least the following reasons.

According to the label, ranibizumab is a humanized anti-VEGF antibody fragment that has been found effective in the treatment of patients with neovascular (wet) age-related macular degeneration. Ranibizumab binds to and inhibits the biological activity of human vascular endothelial growth factor A (VEGF-A), which has been shown to cause neovascularization and leakage in models of ocular angiogenesis. The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation (i.e., angiogenesis). *See* Label ¶¶11 and 12.1. Accordingly, administration of an effective amount of ranibizumab inhibits VEGF-induced angiogenesis in a subject to which it is administered. Applicant notes that the term "antibody" as defined in the '269 patent includes, in addition to full-length antibodies, antibody fragments such as Fab, Fab', $F(ab)_2$ and Fv as long as the fragments exhibit the desired biological activity, i.e., binding to human VEGF (*See, e.g.,* Col 8, lines 43-54). Ranibizumab, being a Fab fragment that binds human VEGF, falls within the scope of the term "antibody" as it is used in claim 1.

Claim 1 also pertains to administering an effective amount of a humanized anti-VEGF antibody which binds human VEGF with a K_d value of no more than about

$1x10^{-8}$, wherein the antibody contains the variable light and heavy chains of SEQ ID NOS: 115 and 116. The article by Chen *et al* presents data demonstrating that ranibizumab (designated as Y0317) does, in fact, bind human VEGF with a K_d value of no more than about $1x10^{-8}$ M. For example, Table 6 on page 870 of the reference shows that ranibizumab has a K_d value of about 1.4×10^{-10} and thus falls within the scope of claim 1. Finally, Figures 10A and 10B of the '269 patent provide the sequence of the light chain variable and heavy chain variable domains of, *inter alia*, ranibizumab (noted therein as Fab Y0317). The light chain variable and heavy chain variable domains depicted in Figures 10A and 10B are identical to SEQ ID NO:115 and SEQ ID NO:116, respectively, of the '269 patent. Accordingly, ranibizumab contains the heavy chain variable domain (SEQ ID NO:116) and the light chain variable domain (SEQ ID NO:115) recited in claim 1.

For at least the reasons discussed above, claim 1 of the '269 patent covers, *inter alia*, a method of using the approved drug product, ranibizumab.

10. Relevant Dates Under 35 U.S.C. § 156 for Determination of Applicable Regulatory Review Period [§ 1.740(a)(10)]

(a) Patent Issue Date

U.S. Patent No. 7,060,269 was issued on June 13, 2006.

(b) IND Effective Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(A)]

The date that an exemption under § 505(i) of the Federal Food, Drug and Cosmetic Act became effective (*i.e.*, the date that an investigational new drug application ("IND") became effective) for LUCENTIS ™ (referred to as "Humanized Monoclonal Antibody Fragment (rhuFab V2)(E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal") was October 7, 1999. The IND was assigned number BB-IND # 8633. A copy of the letter from the FDA reflecting the effective date of the IND is provided in Attachment E. The application date for the IND was October 6, 1999.

(c) BLA Submission Date [35 U.S.C. § 156(g)(1)(B)(i); 37 C.F.R. § 1.740(a)(10)(i)(B)]

The BLA was submitted by Genentech to the FDA on December 29, 2005. The BLA was assigned number BL# 125156/0. A copy of the letter from the FDA acknowledging receipt of the BLA and reflecting the BLA submission date is provided in Attachment F.

(d) BLA Issue Date [35 U.S.C. § 156(g)(1)(B)(ii); 37 C.F.R. § 1.740(a)(10)(i)(C)]

The FDA approved biologic license application 125156/0 authorizing the marketing of LUCENTIS™ on June 30, 2006. LUCENTIS™ was approved under Department of Health and Human Services (DHHS) U.S. License No. 1048. A copy of the approval letter from the FDA is provided as Attachment B.

11. Summary of Significant Events During Regulatory Review Period [§ 1.740(a)(11)]

Pursuant to 37 C.F.R. § 1.740(a)(11), the following provides a brief description of the activities of Genentech, Inc., before the FDA in relation to the regulatory review of LUCENTIS™. The brief description lists the significant events that occurred during the regulatory review period for the approved product. In several instances, communications to or from the FDA are referenced. Pursuant to 37 C.F.R. § 1.740(a)(11), 21 C.F.R. § 60.20(a), and M.P.E.P. § 2753, copies of such communications are not provided in this application, but can be obtained from records maintained by the FDA.

- On October 6, 1999, Genentech submitted to FDA (*See* Attachment E) an investigational new drug application for a recombinant humanized monoclonal antibody fragment (rhuFab V2, now known as ranibizumab) against Vascular Endothelial Growth Factor (VEGF). The antibody was developed as a potential new therapeutic in treating patients with the exudative (wet or neovascular) form for age-related macular degeneration (AMD).

- On October 7, 1999 FDA made BB-IND #8633 effective via a communication mailed to Genentech on October 13, 1999 (*See* Attachment E). According to the FDA, initiation of trials could begin 30 days after October 7, 1999.

- The first human clinical trial (Phase I) was initiated on February 8, 2000 followed by Phase II human trials and Phase III human trials, some of which remain ongoing at the time of this application.

- On February 5, 2002, representatives of Genentech and the FDA (CBER and CDER) participated in a Type C meeting to discuss the proposed clinical development plan for ranibizumab in AMD.

- On October 31, 2002 representatives of Genentech and FDA (CBER and CDER) participated in an Type B End-of-Phase II meeting.

- Beginning in approximately March 2003, and continuing at the time of this application, Phase III studies have been conducted. The three Phase III trials forming the basis of the Biologics License Application (BLA), FVF2598g, FVF2587g, and FVF3192g are studies of two year duration with primary endpoints of one year. FVF2587g and FVF3192g, along with extension study FVF3426g and safety study FVF3689g, remain ongoing at the time of this application.

- On September 21, 2005 representatives of Genentech and CDER participated in a Type B Pre-BLA submission meeting to discuss information requirements for the BLA.

- Genentech submitted a BLA for ranibizumab for the treatment of patients with wet AMD on December 29, 2005. (*See* Attachment F)

- FDA acknowledged receipt of the BLA for ranibizumab via a communication mailed to Genentech dated January 27, 2006. The letter indicated that FDA had assigned the Submission Tracking Number (STN) of BL #125156/0 to the BLA (*See* Attachment F).

- By way of a communication mailed to Genentech on March 14, 2006 FDA made Genentech aware that the BLA for ranibizumab was filed on February 28, 2006 and that FDA had assigned a user fee goal date of June 30, 2006 (*See* Attachment G).

- On June 30, 2006 FDA approved BLA 125156/0, issuing marketing authorization for LUCENTIS™ (*See* Attachment B).

12. Statement Concerning Eligibility for and Duration of Extension Sought Under 35 U.S.C. § 156 [37 C.F.R. § 1.740(a)(12)]

(a) In the opinion of the Applicant, U.S. Patent No. 7,060,269 is eligible for an extension under § 156 because:

(i) one or more claims of the '269 patent claim the approved product or a method of making or using the approved product;

(ii) the term of the '269 patent has not been previously extended on the basis of § 156;

(iii) the '269 patent has not expired;

(iv) no other patent has been extended pursuant to § 156 on the basis of the regulatory review process associated with the approved product, LUCENTIS™;

(v) there is an eligible period of regulatory review by which the patent may be extended pursuant to § 156;

(vi) the applicant for marketing approval exercised due diligence within the meaning of § 156(d)(3) during the period of regulatory review;

(vii) the present application has been submitted within the 60-day period following the approval date of the approved product, pursuant to § 156(c); and

(viii) this application otherwise complies with all requirements of 35 U.S.C. § 156 and applicable rules and procedures.

(b) The period by which the term of the '269 patent is requested by Applicant to be extended is **17 days**.

(c) The requested period of extension of term for the '269 patent corresponds to the regulatory review period that is eligible for extension pursuant to § 156, based on the facts and circumstances of the regulatory review associated with the approved product LUCENTIS™ and the issuance of the patent. The period was determined as follows.

(i) The relevant dates for calculating the regulatory review period, based on the events discussed in the section above, are the following.

Exemption under FDCA § 505(i) became effective	October 7, 1999
Biologics License Application (BLA) under PHSA § 351 was filed	December 29, 2005
Patent was granted	June 13, 2006
BLA was approved	June 30, 2006

(ii) The '269 patent was granted after the period specified in § 156(g)(1)(B)(i) (*i.e.*, the period from the date of the grant of the exemption under § 505(i) of the FDCA until the date of submission of the BLA). Pursuant to § 156(c), the calculated regulatory review period therefore does not include a component of time between when the IND became effective and when the BLA was submitted.

(iii) The patent was granted during the period specified in § 156(g)(1)(B)(ii) (*i.e.*, the period from the date of submission of the BLA until the date of approval). The regulatory review period under § 156(b) therefore includes a component equal to the total number of days in that period that are after the issuance of the patent (17 days).

(iv) The period determined according to § 156(b), (c), and (g)(1) for the approved product (*i.e.*, the number of days following the date of patent issuance until the approval of the BLA) is 17 days.

(v) The '269 patent will expire on July 4, 2019.

(vi) The date of approval of the approved product is June 30, 2006.

(vii) The date that is fourteen years from the date of approval of the approved product is June 30, 2020.

(viii) The period measured from the date the patent expires (*i.e.*, July 4, 2019) until the end of the fourteen-year period specified in §156 (c)(3) (*i.e.*, June 30, 2020) is approximately 361 days.

(ix) The number of days in the regulatory review period determined pursuant to § 156(g)(1)(B)(ii) does not exceed the number of days that the patent may be extended pursuant to §156(c)(3). As such, the period by which the

patent may be extended is not limited by the fourteen-year rule of §156(c)(3).

(x) The '269 patent issued after the effective date of Public Law No. 98-417. As such, the two- or three-year limit of 35 U.S.C. § 156(g)(6)(C) does not apply.

13. Statement Pursuant to 37 C.F.R. § 1.740(a)(13)

Pursuant to 37 C.F.R. § 1.740(a)(13), Applicant acknowledges its duty to disclose to the Director of the PTO and to the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought, particularly as that duty is defined in 37 C.F.R. § 1.765.

14. Applicable Fee [§ 1.740(a)(14)]

Our check in payment of the fee prescribed in 37 C.F.R. § 1.20(j) for a patent term extension application under 35 U.S.C. § 156 accompanies this application. Please deduct any additional required fees from, or credit any overpayments to our deposit account no. 18-1260.

15. Name and Address for Correspondence [§ 1.740(a)(14)]

Please direct all inquiries, questions, and communications regarding this application for term extension to:

Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, D.C. 20005
Phone: 202-736-8914
Fax: 202-736-8111
email: jkushan@sidley.com

The correspondence address for U.S. Patent No. 7,060,269 is unchanged for all other purposes. A Power of Attorney granted to the undersigned by the patent assignee, a copy of which is included with this application as Attachment H, accompanies this communication.

Two additional copies of this application are enclosed, in compliance with 37 C.F.R. § 1.740(b).

Sincerely,

Jeffrey P. Kushan
Attorney for Applicant
Registration No. 43,401

Sidley Austin LLP
1501 K Street, N.W.
Washington, D.C. 20005

Dated: August 24, 2006

INDEX OF ATTACHMENTS

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use LUCENTIS safely and effectively. See full prescribing information for LUCENTIS.

LUCENTIS™ (ranibizumab injection)

Initial U.S. Approval: 2006

----------INDICATIONS AND USAGE----------

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration (1).

----------DOSAGE AND ADMINISTRATION----------

- FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY (2.1)
- LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month (2.2).
- Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly (2.2).

----------DOSAGE FORMS AND STRENGTHS----------

- 10 mg/mL single-use vial (3)

----------CONTRAINDICATIONS----------

- Ocular or periocular infections (4.1)
- Hypersensitivity (4.2)

----------WARNINGS AND PRECAUTIONS----------

- Endophthalmitis and retinal detachments may occur following intravitreal injections. Patients should be monitored during the week following the injection (5.1).
- Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection (5.2).

----------ADVERSE REACTIONS----------

The most common adverse reactions (reported ≥ 6% higher in LUCENTIS-treated subjects than control subjects) are conjunctival hemorrhage, eye pain, vitreous floaters, increased intraocular pressure, and intraocular inflammation (6.2).

To report SUSPECTED ADVERSE REACTIONS, contact Genentech at 1-888-835-2555 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

See Section 17 for PATIENT COUNSELING INFORMATION.

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

2 DOSAGE AND ADMINISTRATION

2.1 General Dosing Information

FOR OPHTHALMIC INTRAVITREAL INJECTION ONLY.

2.2 Dosing

LUCENTIS 0.5 mg (0.05 mL) is recommended to be administered by intravitreal injection once a month.

Although less effective, treatment may be reduced to one injection every three months after the first four injections if monthly injections are not feasible. Compared to continued monthly dosing, dosing every 3 months will lead to an approximate 5-letter (1-line) loss of visual acuity benefit, on average, over the following 9 months. Patients should be evaluated regularly [*see Clinical Studies (14.2)*].

2.3 Preparation for Administration

Using aseptic technique, all (0.2 mL) of the LUCENTIS vial contents are withdrawn through a 5-micron 19-gauge filter needle attached to a 1-cc tuberculin syringe. The filter needle should be discarded after withdrawal of the vial contents and should not be used for intravitreal injection. The filter needle should be replaced with a sterile 30-gauge × 1/2-inch needle for the intravitreal injection. The contents should be expelled until the plunger tip is aligned with the line that marks 0.05 mL on the syringe.

2.4 Administration

The intravitreal injection procedure should be carried out under controlled aseptic conditions, which include the use of sterile gloves, a sterile drape, and a sterile eyelid speculum (or equivalent). Adequate anesthesia and a broad-spectrum microbicide should be given prior to the injection.

Following the intravitreal injection, patients should be monitored for elevation in intraocular pressure and for endophthalmitis. Monitoring may consist of a check for perfusion of the optic nerve head immediately after the injection, tonometry within 30 minutes following the injection, and biomicroscopy between two and seven days following the injection. Patients should be instructed to report any symptoms suggestive of endophthalmitis without delay.

Each vial should only be used for the treatment of a single eye. If the contralateral eye requires treatment, a new vial should be used and the sterile field, syringe, gloves, drapes, eyelid speculum, filter, and injection needles should be changed before LUCENTIS is administered to the other eye.

No special dosage modification is required for any of the populations that have been studied (e.g., gender, elderly).

2.5 Stability and Storage

LUCENTIS should be refrigerated at 2°-8°C (36°-46°F). DO NOT FREEZE. Do not use beyond the date stamped on the label. LUCENTIS vials should be protected from light. Store in the original carton until time of use.

3 DOSAGE FORMS AND STRENGTHS

Single-use glass vial designed to deliver 0.05 mL of 10 mg/mL.

4 CONTRAINDICATIONS

4.1 Ocular or Periocular Infections

LUCENTIS is contraindicated in patients with ocular or periocular infections.

4.2 Hypersensitivity

LUCENTIS is contraindicated in patients with known hypersensitivity to ranibizumab or any of the excipients in LUCENTIS.

5 WARNINGS AND PRECAUTIONS

5.1 Endophthalmitis and Retinal Detachments

Intravitreal injections, including those with LUCENTIS, have been associated with endophthalmitis and retinal detachments. Proper aseptic injection technique should always be used when administering LUCENTIS. In addition, patients should be monitored during the week following the injection to permit early treatment should an infection occur [*see Dosage and Administration (2.3, 2.4) and Patient Counseling Information (17)*].

5.2 Increases in Intraocular Pressure

Increases in intraocular pressure have been noted within 60 minutes of intravitreal injection with LUCENTIS. Therefore, intraocular pressure as well as the perfusion of the optic nerve head should be monitored and managed appropriately [*see Dosage and Administration (2.4)*].

5.3 Thromboembolic Events

Although there was a low rate (<4%) of arterial thromboembolic events observed in the LUCENTIS clinical trials, there is a theoretical risk of arterial thromboembolic events following intravitreal use of inhibitors of VEGF [*see Adverse Reactions (6.3)*].

6 ADVERSE REACTIONS

6.1 Injection Procedure

Serious adverse events related to the injection procedure have occurred in < 0.1% of intravitreal injections, including endophthalmitis [*see Warnings and Precautions (5.1)*], rhegmatogenous retinal detachments, and iatrogenic traumatic cataracts.

6.2 Clinical Trials Experience – Ocular Events

Other serious ocular adverse events observed among LUCENTIS-treated patients occurring in <2% of patients

included intraocular inflammation and increased intraocular pressure [*see Warnings and Precautions (5.1, 5.2)*].

The available safety data include exposure to LUCENTIS in 874 patients with neovascular age-related macular degeneration in three double-masked, controlled studies with dosage regimens of 0.3 mg (375 patients) or 0.5 mg (379 patients) administered monthly by intravitreal injection (Studies 1 and 2) [*see Clinical Studies (14.1)*] and dosage regimens of 0.3 mg (59 patients) or 0.5 mg (61 patients) administered once a month for 3 consecutive doses followed by a dose administered once every 3 months (Study 3) [*see Clinical Studies (14.2)*].

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in one clinical trial of a drug cannot be directly compared with rates in the clinical trials of the same or another drug and may not reflect the rates observed in practice.

Table 1 shows the most frequently reported ocular adverse events that were reported with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 1

Adverse Event	LUCENTIS	Control
Conjunctival hemorrhage	77%-43%	66%-29%
Eye pain	37%-17%	33%-11%
Vitreous floaters	32%-3%	10%-3%
Retinal hemorrhage	26%-15%	56%-37%
Intraocular pressure increased	24%-8%	7%-3%
Vitreous detachment	22%-7%	18%-13%
Intraocular inflammation	18%-5%	11%-3%
Eye irritation	19%-4%	20%-6%
Cataract	16%-5%	16%-6%
Foreign body sensation in eyes	19%-6%	14%-6%
Lacrimation increased	17%-3%	16%-0%
Eye pruritis	13%-0%	12%-3%
Visual disturbance	14%-0%	9%-2%
Blepharitis	13%-3%	9%-4%
Subretinal fibrosis	13%-0%	19%-10%
Ocular hyperemia	10%-5%	10%-1%
Maculopathy	10%-3%	11%-3%
Visual acuity blurred/decreased	17%-4%	24%-10%
Detachment of the retinal pigment epithelium	11%-1%	15%-3%
Dry eye	10%-3%	8%-5%
Ocular discomfort	8%-0%	5%-0%
Conjunctival hyperemia	9%-0%	7%-0%
Posterior capsule opacification	8%-0%	5%-0%
Retinal exudates	9%-1%	11%-3%

6.3 Clinical Trials Experience – Non-Ocular Events

Table 2 shows the most frequently reported non-ocular adverse events with LUCENTIS treatment. The ranges represent the maximum and minimum rates across all three studies for control, and across all three studies and both dose groups for LUCENTIS.

Table 2

Adverse Event	LUCENTIS	Control
Hypertension/elevated blood pressure	23%-5%	23%-8%
Nasopharyngitis	16%-5%	13%-5%
Arthralgia	11%-3%	9%-0%
Headache	15%-2%	10%-3%
Bronchitis	10%-3%	8%-2%
Cough	10%-3%	7%-2%
Anemia	8%-3%	8%-0%
Nausea	9%-2%	6%-4%
Sinusitis	8%-2%	6%-4%
Upper respiratory tract infection	15%-2%	10%-4%
Back pain	10%-1%	9%-0%
Urinary tract infection	9%-4%	8%-5%
Influenza	10%-2%	5%-1%
Arthritis	8%-0%	8%-2%
Dizziness	8%-2%	10%-2%
Constipation	7%-3%	8%-2%

The rate of arterial thromboembolic events in the three studies in the first year was 2.1% of patients (18 out of 874) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 1.1% of patients (5 out of 441) in the control arms of the studies. In the second year of Study 1, the rate of arterial thromboembolic events was 3.0% of patients (14 out of 466) in the combined group of patients treated with 0.3 mg or 0.5 mg LUCENTIS compared with 3.2% of patients (7 out of 216) in the control arm [*see Warnings and Precautions (5.3)*].

6.4 Immunogenicity

The pre-treatment incidence of immunoreactivity to LUCENTIS was 0%-3% across treatment groups. After monthly dosing with LUCENTIS for 12 to 24 months, low titers of antibodies to LUCENTIS were detected in approximately 1%-6% of patients. The immunogenicity data reflect the percentage of patients whose test results were considered positive for antibodies to LUCENTIS in an electrochemiluminescence assay and are highly dependent on the sensitivity and specificity of the assay. The clinical significance of immunoreactivity to LUCENTIS is unclear at this time, although some patients with the highest levels of immunoreactivity were noted to have iritis or vitritis.

7 DRUG INTERACTIONS

Drug interaction studies have not been conducted with LUCENTIS.

LUCENTIS intravitreal injection has been used adjunctively with verteporfin photodynamic therapy (PDT). Twelve of 105 (11%) patients developed serious intraocular inflammation; in 10 of the 12 patients, this occurred when LUCENTIS was administered 7 days (± 2 days) after verteporfin PDT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Pregnancy Category C. Animal reproduction studies have not been conducted with ranibizumab. It is also not known whether ranibizumab can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. LUCENTIS should be given to a pregnant woman only if clearly needed.

8.3 Nursing Mothers
It is not known whether ranibizumab is excreted in human milk. Because many drugs are excreted in human milk, and because the potential for absorption and harm to infant growth and development exists, caution should be exercised when LUCENTIS is administered to a nursing woman.

8.4 Pediatric Use
The safety and effectiveness of LUCENTIS in pediatric patients has not been established.

8.5 Geriatric Use
In the controlled clinical studies, approximately 94% (822/879) of the patients randomized to treatment with LUCENTIS were ≥ 65 years of age and approximately 68% (601/879) were ≥ 75 years of age. No notable difference in treatment effect was seen with increasing age in any of the studies. Age did not have a significant effect on systemic exposure in a population pharmacokinetic analysis after correcting for creatinine clearance.

8.6 Patients with Renal Impairment
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with renal impairment. Sixty-eight percent of patients (136 of 200) in the population pharmacokinetic analysis had renal impairment (46.5% mild, 20% moderate, and 1.5% severe). Reduction in ranibizumab clearance is minimal in patients with renal impairment and is considered clinically insignificant. Dose adjustment is not expected to be needed for patients with renal impairment.

8.7 Patients with Hepatic Dysfunction
No formal studies have been conducted to examine the pharmacokinetics of ranibizumab in patients with hepatic impairment. Dose adjustment is not expected to be needed for patients with hepatic dysfunction.

10 OVERDOSAGE
Planned initial single doses of ranibizumab injection 1.0 mg were associated with clinically significant intraocular inflammation in 2 of 2 patients injected. With an escalating regimen of doses beginning with initial doses of ranibizumab injection 0.3 mg, doses as high as 2.0 mg were tolerated in 15 of 20 patients.

11 DESCRIPTION
LUCENTIS™ (ranibizumab injection) is a recombinant humanized IgG1 kappa isotype monoclonal antibody fragment designed for intraocular use. Ranibizumab binds to and inhibits the biologic activity of human vascular endothelial growth factor A (VEGF-A). Ranibizumab has a molecular weight of approximately 48 kilodaltons and is produced by an *E. coli* expression system in a nutrient medium containing the antibiotic tetracycline. Tetracycline is not detectable in the final product.

LUCENTIS is a sterile, colorless to pale yellow solution in a single-use glass vial. LUCENTIS is supplied as a preservative-free, sterile solution in a single-use glass vial designed to deliver 0.05 mL of 10 mg/mL LUCENTIS aqueous solution with 10 mM histidine HCl, 10% α, α-trehalose dihydrate, 0.01% polysorbate 20, pH 5.5.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action
Ranibizumab binds to the receptor binding site of active forms of VEGF-A, including the biologically active, cleaved form of this molecule, $VEGF_{110}$. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and is thought to contribute to the progression of the neovascular form of age-related macular degeneration (AMD). The binding of ranibizumab to VEGF-A prevents the interaction of VEGF-A with its receptors (VEGFR1 and VEGFR2) on the surface of endothelial cells, reducing endothelial cell proliferation, vascular leakage, and new blood vessel formation.

12.2 Pharmacodynamics
Neovascular AMD is associated with foveal retinal thickening as assessed by optical coherence tomography (OCT) and leakage from CNV as assessed by fluorescein angiography.

In Study 3, foveal retinal thickness was assessed by OCT in 118/184 patients. OCT measurements were collected at baseline, Months 1, 2, 3, 5, 8, and 12. In patients treated with LUCENTIS, foveal retinal thickness decreased, on average, more than the sham group from baseline through Month 12. Retinal thickness decreased by Month 1 and decreased further at Month 3, on average. Foveal retinal thickness data did not provide information useful in influencing treatment decisions [*see Clinical Studies (14.2)*].

In patients treated with LUCENTIS, the area of vascular leakage, on average, decreased by Month 3 as assessed by fluorescein angiography. The area of vascular leakage for an individual patient was not correlated with visual acuity.

12.3 Pharmacokinetics
In animal studies, following intravitreal injection, ranibizumab was cleared from the vitreous with a half-life of approximately 3 days. After reaching a maximum at approximately 1 day,

the serum concentration of ranibizumab declined in parallel with the vitreous concentration. In these animal studies, systemic exposure of ranibizumab is more than 2000-fold lower than in the vitreous.

In patients with neovascular AMD, following monthly intravitreal administration, maximum ranibizumab serum concentrations were low (0.3 ng/mL to 2.36 ng/mL). These levels were below the concentration of ranibizumab (11 ng/mL to 27 ng/mL) thought to be necessary to inhibit the biological activity of VEGF-A by 50%, as measured in an in vitro cellular proliferation assay. The maximum observed serum concentration was dose proportional over the dose range of 0.05 to 1.0 mg/eye. Based on a population pharmacokinetic analysis, maximum serum concentrations of 1.5 ng/mL are predicted to be reached at approximately 1 day after monthly intravitreal administration of LUCENTIS 0.5 mg/eye. Based on the disappearance of ranibizumab from serum, the estimated average vitreous elimination half-life was approximately 9 days. Steady-state minimum concentration is predicted to be 0.22 ng/mL with a monthly dosing regimen. In humans, serum ranibizumab concentrations are predicted to be approximately 90,000-fold lower than vitreal concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No carcinogenicity or mutagenicity data are available for ranibizumab injection in animals or humans.

No studies on the effects of ranibizumab on fertility have been conducted.

14 CLINICAL STUDIES

The safety and efficacy of LUCENTIS were assessed in three randomized, double-masked, sham- or active-controlled studies in patients with neovascular AMD. A total of 1323 patients (LUCENTIS 879, Control 444) were enrolled in the three studies.

14.1 Study 1 and Study 2

In Study 1, patients with minimally classic or occult (without classic) CNV lesions received monthly LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or monthly sham injections. Data are available through Month 24. Patients treated with LUCENTIS in Study 1 received a mean of 22 total treatments out of a possible 24 from Day 0 to Month 24.

In Study 2, patients with predominantly classic CNV lesions received one of the following: 1) monthly LUCENTIS 0.3 mg intravitreal injections and sham PDT; 2) monthly LUCENTIS 0.5 mg intravitreal injections and sham PDT; or 3) sham intravitreal injections and active verteporfin PDT. Sham PDT (or active verteporfin PDT) was given with the initial LUCENTIS (or sham) intravitreal injection and every 3 months thereafter if fluorescein angiography showed persistence or recurrence of leakage. Data are available through Month 12. Patients treated with LUCENTIS in Study 2 received a mean of 12 total treatments out of a possible 13 from Day 0 through Month 12.

In both studies, the primary efficacy endpoint was the proportion of patients who maintained vision, defined as losing fewer than 15 letters of visual acuity at 12 months compared with baseline. Almost all LUCENTIS-treated patients (approximately 95%) maintained their visual acuity. 34%-40% of LUCENTIS-treated patients experienced a clinically significant improvement in vision, defined as gaining 15 or more letters at 12 months. The size of the lesion did not significantly affect the results. Detailed results are shown in the tables below.

Table 3
Outcomes at Month 12 and Month 24 in Study 1

Outcome Measure	Month	Sham n = 238	LUCENTIS 0.5 mg n = 240	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	Month 12	62%	95%	32% (26%, 39%)
	Month 24	53%	90%	37% (29%, 44%)
Gain of ≥ 15 letters in visual acuity (%)[b]	Month 12	5%	34%	29% (22%, 35%)
	Month 24	4%	33%	29% (23%, 35%)
Mean change in visual acuity (letters) (SD)[b]	Month 12	-10.5 (16.6)	+7.2 (14.4)	17.5 (14.8, 20.2)
	Month 24	-14.9 (18.7)	+6.6 (16.5)	21.1 (18.1, 24.2)

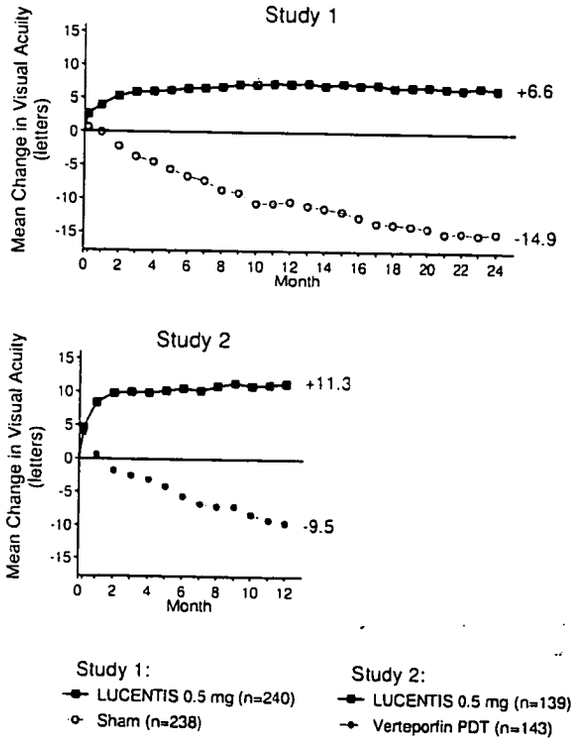
[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Table 4
Outcomes at Month 12 in Study 2

Outcome Measure	Verteporfin PDT n = 143	LUCENTIS 0.5 mg n = 140	Estimated Difference (95% CI)[a]
Loss of < 15 letters in visual acuity (%)[b]	64%	96%	33% (25%, 41%)
Gain of ≥ 15 letters in visual acuity (%)[b]	6%	40%	35% (26%, 44%)
Mean change in visual acuity (letters) (SD)[b]	-9.5 (16.4)	+11.3 (14.6)	21.1 (17.5, 24.6)

[a] Adjusted estimate based on the stratified model.
[b] $p<0.01$.

Figure 1
Mean Change in Visual Acuity from Baseline to Month 24 in Study 1 and to Month 12 in Study 2



Patients in the group treated with LUCENTIS had minimal observable CNV lesion growth, on average. At Month 12, the mean change in the total area of the CNV lesion was 0.1-0.3 DA for LUCENTIS versus 2.3-2.6 DA for the control arms.

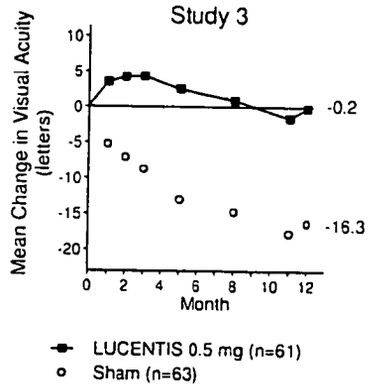
The use of LUCENTIS beyond 24 months has not been studied.

14.2 Study 3

Study 3 was a randomized, double-masked, sham-controlled, two-year study designed to assess the safety and efficacy of LUCENTIS in patients with neovascular AMD (with or without a classic CNV component). Data are available through Month 12. Patients received LUCENTIS 0.3 mg or 0.5 mg intravitreal injections or sham injections once a month for 3 consecutive doses, followed by a dose administered once every 3 months. A total of 184 patients were enrolled in this study (LUCENTIS 0.3 mg, 60; LUCENTIS 0.5 mg, 61; sham, 63); 171 (93%) completed 12 months of this study. Patients treated with LUCENTIS in Study 3 received a mean of 6 total treatments out of possible 6 from Day 0 through Month 12.

In Study 3, the primary efficacy endpoint was mean change in visual acuity at 12 months compared with baseline (see Figure 2). After an initial increase in visual acuity (following monthly dosing), on average, patients dosed once every three months with LUCENTIS lost visual acuity, returning to baseline at Month 12. In Study 3, almost all LUCENTIS-treated patients (90%) maintained their visual acuity at Month 12.

Figure 2
Mean Change in Visual Acuity from Baseline to Month 12 in Study 3



16 HOW SUPPLIED/STORAGE AND HANDLING

Each LUCENTIS carton, NDC 50242-080-01, contains one 2-cc glass vial of ranibizumab, one 5-micron, 19-gauge × 1-1/2-inch filter needle for withdrawal of the vial contents, one 30-gauge × 1/2-inch injection needle for the intravitreal injection, and one package insert [*see Dosage and*

Administration (2.4)]. VIALS ARE FOR SINGLE EYE USE ONLY.

17 PATIENT COUNSELING INFORMATION

In the days following LUCENTIS administration, patients are at risk of developing endophthalmitis. If the eye becomes red, sensitive to light, painful, or develops a change in vision, the patient should seek immediate care from an ophthalmologist [*see Warnings and Precautions (5.1)*].

LUCENTIS™ [ranibizumab injection]

Manufactured by:
Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990

8277700
LL1404
4833801
FDA Approval Date:
June 2006

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

We have approved your biologics' license application for Lucentis (ranibizumab injection) effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, ranibizumab injection under your existing Department of Health and Human Services U.S. License No. 1048. Lucentis (ranibizumab injection) is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration.

Under this license, you are approved to manufacture ranibizumab drug substance at Genentech, Inc., South San Francisco, California; fill the final formulated product at (b) (4) ; and label and package filled vials at Genentech, Inc., South San Francisco, California. You may label your product with the proprietary name Lucentis and market it in 10 mg/mL single use glass vials.

We acknowledge receipt of your submissions dated December 29, 2005, and January 31, February 10, 17, 21, and 24, March 17, 23, and 31, April 10, and 28, May 5, 10, 25 (2), 26 (2), and 31, and June 1, 5 (2), 6, 9, 13, 16, 23, 26, 27, 28 (3), and 29, 2006.

The final printed labeling (FPL) must be identical in content to the enclosed labeling text for the package insert, submitted June 28, 2006; the immediate vial container submitted March 31, 2006; and the carton labels submitted June 5, 2006. The statement "No U.S. standard of potency" should be added with the next printing of carton labels. Marketing this product with FPL that is not identical in content to the approved labeling text may render the product misbranded and an unapproved new drug.

The dating period for formulated drug product shall be 18 months from the date of manufacture when stored at 2°-8°C (36°-46°F). The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. The dating period for ranibizumab drug substance shall be (b)(4) when stored at -20 °C.

You currently are not required to submit samples of future lots of Lucentis to the Center for Drug Evaluation and Research (CDER) for release by the Director, CDER, under 21 CFR 610.2. We will continue to monitor compliance with 21 CFR 610.1 requiring completion of tests for conformity with standards applicable to each product prior to release of each lot.

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Lucentis, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We are waiving the pediatric study requirement for this application.

The following are Postmarketing Studies that are subject to reporting requirements of 21 CFR 601.70:

1. Submit the final Clinical Study Report from Study FVF3689g by June 30, 2008.

2. Provide safety and efficacy data from a 2-year adequate and well-controlled clinical trial of a mutually acceptable design exploring multiple dosing frequencies of Lucentis.

   Date of submission of protocol: November 14, 2008.

   Date of start of study: September 21, 2009.

   Date of final clinical study report: April 1, 2013.

3. To detect and characterize immune responses to ranibizumab:

   a. Develop and validate a confirmatory assay capable of detecting both IgG and IgM isotype responses.

   b. Develop and validate an assay to detect neutralizing anti-ranibizumab antibodies.

   The assay methodology and validation reports: September 28, 2007.

4. To characterize further the immune response to ranibizumab, serum samples collected in studies FVF2587g, FVF2598g, FVF3192g will be assayed using the validated methods described above in Postmarketing Commitment #3. The data obtained will be analyzed to discover and evaluate any association between immunoreactivity and dosing frequency as well as any potential impact of immunoreactivity on efficacy or safety outcomes.

   The need for an additional clinical study will be determined based on the results from the analysis described above.

   Date of submission of protocol and statistical analysis plan: February 28, 2007.

   Date of submission of final study report: September 30, 2008.

The following are Postmarketing Studies that are not subject to reporting requirements of 21 CFR 601.70:

5. To revise release specifications, shelf-life specifications and in-process limits for ranibizumab drug substance and drug product after [redacted] commercial manufacturing runs to reflect increased manufacturing experience.

   These revisions to the Quality control system, the corresponding data from the [redacted] commercial manufacturing runs and the analysis plan used to create the revisions will be submitted as a supplement on or before June 30, 2008.

6. To perform additional Lucentis stability studies at 40°C using Ion Exchange Chromatography (IEC) to demonstrate that the corrective actions taken at [redacted] to address the atypical accelerated stability profile observed in the Lucentis 2005 qualification campaign have been sufficient.

   Specifically, a one time stability study consisting of [redacted] Lucentis Drug Product launch lots are placed at 40°C and tested by IEC at [redacted] months. These [redacted] Lucentis Drug Product lots are derived from the following:

   - [redacted] of these Lucentis Drug Product lots are manufactured from distinct lots of [redacted].
   - At least [redacted] these [redacted] lots are aliquoted and used to manufacture [redacted] Lucentis drug product lots.

   Data will be submitted as a supplement on or before March 31, 2007.

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to this application. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted),

- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment), and
- a revised schedule if the study schedule has changed and an explanation of the basis for the revision.

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (http://www.fda.gov/cder/pmc/default.htm). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see http://www.fda.gov/cber/gdlns/post040401.htm) for further information.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80). You should submit postmarketing adverse experience reports to the Central Document Room, Center for Drug Evaluation and Research, Food and Drug Administration, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

The MedWatch-to-Manufacturer Program provides manufacturers with copies of serious adverse event reports that are received directly by the FDA. New molecular entities and important new biologics qualify for inclusion for three years after approval. Your firm is eligible to receive copies of reports for this product. To participate in the program, please see the enrollment instructions and program description details at www.fda.gov/medwatch/report/mmp.htm.

You must submit distribution reports under the distribution reporting requirements for licensed biological products (21 CFR 600.81).

You must submit reports of biological product deviations under 21 CFR 600.14. You should promptly identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Division of Compliance Risk Management and Surveillance (HFD-330), Center for Drug Evaluation and Research, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857. Biological product deviations sent by courier or overnight mail should be addressed to Food and Drug Administration, CDER, Office of Compliance, Division of Compliance Risk Management and Surveillance, HFD-330, Montrose Metro 2, 11919 Rockville Pike, Rockville, MD 20852.

Please submit all FPL at the time of use and include implementation information on FDA Form 356h. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit draft copies of the proposed introductory advertising and promotional labeling with a cover letter requesting advisory comments to the Food and Drug Administration, Center for Drug Evaluation and Research, Division of Drug Marketing, Advertising and Communication, 5901-B Ammendale Road, Beltsville, MD 20705-1266. Final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have substantial evidence to support that claim.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Mark J. Goldberger, M.D., M.P.H.
Director
Office of Antimicrobial Products
Center for Drug Evaluation and Research

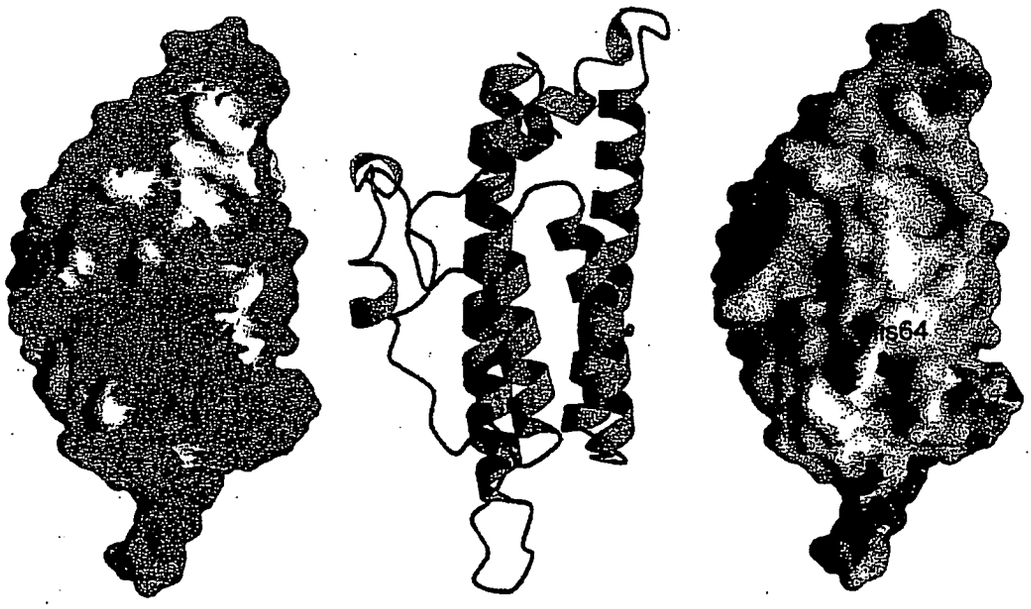
Enclosure

Volume 293
Number 4
5 November 1999

CELEBRATING 40 1959–1999 YEARS

JMB

JOURNAL OF MOLECULAR BIOLOGY



IDEAL

ACADEMIC PRESS

Article No. jmbi.1999.3192 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (1999) 293, 865–881

JMB

Selection and Analysis of an Optimized Anti-VEGF Antibody: Crystal Structure of an Affinity-matured Fab in Complex with Antigen

Yvonne Chen[1], Christian Wiesmann[1], Germaine Fuh[1], Bing Li[1], Hans W. Christinger[1], Patrick McKay[2], Abraham M. de Vos[1] and Henry B. Lowman[1]*

[1]*Department of Protein Engineering, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

[2]*Department of Process Sciences, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080, USA*

**Corresponding author*

The Fab portion of a humanized antibody (Fab-12; IgG form known as rhuMAb VEGF) to vascular endothelial growth factor (VEGF) has been affinity-matured through complementarity-determining region (CDR) mutation, followed by affinity selection using monovalent phage display. After stringent binding selections at 37 °C, with dissociation (off-rate) selection periods of several days, high affinity variants were isolated from CDR-H1, H2, and H3 libraries. Mutations were combined to obtain cumulatively tighter-binding variants. The final variant identified here, Y0317, contained six mutations from the parental antibody. *In vitro* cell-based assays show that four mutations yielded an improvement of about 100-fold in potency for inhibition of VEGF-dependent cell proliferation by this variant, consistent with the equilibrium binding constant determined from kinetics experiments at 37 °C. Using X-ray crystallography, we determined a high-resolution structure of the complex between VEGF and the affinity-matured Fab fragment. The overall features of the binding interface seen previously with wild-type are preserved, and many contact residues are maintained in precise alignment in the superimposed structures. However, locally, we see evidence for improved contacts between antibody and antigen, and two mutations result in increased van der Waals contact and improved hydrogen bonding. Site-directed mutants confirm that the most favorable improvements as judged by examination of the complex structure, in fact, have the greatest impact on free energy of binding. In general, the final antibody has improved affinity for several VEGF variants as compared with the parental antibody; however, some contact residues on VEGF differ in their contribution to the energetics of Fab binding. The results show that small changes even in a large protein-protein binding interface can have significant effects on the energetics of interaction.

Keywords: angiogenesis; humanized antibody-antigen complex; affinity maturation; phage display; X-ray crystallography

Introduction

Angiogenic factors (Folkman & Klagsbrun, 1987), which stimulate endothelial cells leading to new vascularization, have roles in such disease states as cancer, rheumatoid arthritis, and macular degeneration (reviewed by Ferrara, 1995; Folkman, 1995; Iruela-Arispe & Dvorak, 1997). Vascular endothelial growth factor (VEGF), a heparin-binding protein initially identified from pituitary cells (Ferrara & Henzel, 1989), is clearly a key angio-

Abbreviations used: CDR, complementarity-determining region; FR, framework region; HuVEC, human umbilical vein endothelial cell; $K_d^{25°}$, equilibrium dissociation constant determined at 25 °C; mAb, IgG form of monoclonal antibody; PBS, phosphate-buffered saline; SPR, surface plasmon resonance; VEGF, vascular endothelial growth factor; VEGF(109), receptor-binding fragment of VEGF with residues 8-109; VEGF(165), VEGF form with residues 1-165.

E-mail address of the corresponding author: hbl@gene.com

0022-2836/99/440865–17 $30.00/0

genic factor in development as well as in certain disease states, including the growth of solid tumors (reviewed by Ferrara, 1999). A murine monoclonal antibody, A.4.6.1, was found to block VEGF-dependent cell proliferation *in vitro* and to antagonize tumor growth *in vivo* (Kim *et al.*, 1993). The murine mAb was previously humanized in Fab form to yield a variant known as Fab-12 (Presta *et al.*, 1997). Both chimeric and humanized antibodies retained high affinity binding to VEGF, with an apparent equilibrium dissociation constant, $K_d^{25°}$, of 0.9 to 3 nM (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a). The corresponding full-length IgG form of this antibody, rhumAb VEGF, is being developed as a possible therapeutic agent for the treatment of human solid tumors (Mordenti *et al.*, 1999).

We became interested in obtaining higher affinity variants of Fab-12 in order to test whether affinity improvements of this antibody might improve its potency and efficacy. Phage display of randomized libraries of antibodies and other proteins has been extensively used to engineer proteins with improved affinity and specificity (Lowman *et al.*, 1991; reviewed by Kay & Hoess, 1996; Rader & Barbas, 1997; Griffiths & Duncan, 1998). In particular, a phage-based *in vitro* affinity maturation process has been successful in improving the binding affinity of antibodies previously identified from traditional monoclonal or naive-library sources (e.g. Hawkins *et al.*, 1992; Marks *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Thompson *et al.*, 1996).

In previous work, the humanized anti-VEGF antibody Fab-12 was adapted for improved monovalent phage display through selection of a CDR-L1 variant, designated Y0192 (Muller *et al.*, 1998a). To select target residues for randomization and affinity optimization, we also previously screened all CDR residues, as defined by a combination of the hypervariable (Kabat *et al.*, 1987) and structurally defined (Chothia & Lesk, 1987) CDR residues. Fab variants of Y0192 generated by alanine scanning were analyzed for side-chain contributions to antigen binding (Muller *et al.*, 1998a). In addition, a crystal structure of Fab-12 in complex with the receptor-binding domain of VEGF, VEGF(109), was determined (Muller *et al.*, 1998a). The results of these studies showed that the antigen binding site is almost entirely composed of residues from the heavy chain CDRs, CDR-H1, H2, and H3. Therefore, these CDRs appeared most likely to provide the opportunity for improved binding interactions with antigen.

Here, we describe the selection of an affinity-improved anti-VEGF antibody by phage display and off-rate selection. We show that the affinity-matured antibody binds VEGF with at least 20-fold improved affinity and inhibits VEGF-induced cell proliferation with enhanced potency in a cell-based assay. We also report the crystal structure of an affinity-optimized antibody in complex with VEGF, to our knowledge, representing the first reported structure of an *in vitro* affinity-matured antibody:antigen complex. The structure, together with mutational analysis, shows that subtle changes in the antibody-antigen interface account for improved affinity.

Results

Library design

We used the results of an alanine-scanning analysis, combined with a crystal structure of the wild-type Fab fragment in complex with VEGF (Muller *et al.*, 1998a), to design targeted libraries within the antibody CDRs for random mutagenesis and affinity selection. This strategy enabled us to construct theoretically complete libraries with a small number of residues randomized in each CDR. Although sites remote from the antigen-combining region or buried within the protein could modulate antigen binding affinity indirectly and have in fact been exploited for affinity improvement (Hawkins *et al.*, 1993), clearly residues shown to be important by alanine scanning are useful starting points for binding-affinity optimization (Lowman *et al.*, 1991; Lowman & Wells, 1993). Furthermore, we reasoned that by making mutations at residues of the antibody CDRs which were known to affect antigen binding and were located at or near points of contact in the bound complex, we could minimize the possibility of other indirect effects which might alter stability, immunogenicity, or other properties of the antibody.

Both Ala-scanning and crystallography (Muller *et al.*, 1998a) identified CDR-H3 as the predominant contact segment for VEGF, consistent with the general observation that CDR-H3 is often key to antigen binding (Chothia & Lesk, 1987). Within CDR-H3, residues Y95, P96, H97, Y98, Y99, S100b, H100c, W100d, Y100e, and F100f (numbering is as described by Kabat *et al.* (1987)), all showed effects on binding over a range of twofold to >150-fold when mutated to Ala, and Ala substitution at S100a caused a slight improvement in binding. However, H100c, Y100e, and F100f were found to have little or no direct contact with VEGF and presumed to have indirect effects on binding. On the other hand, Y95 and W100d have significant contacts with VEGF, and Ala substitutions resulted in no detectable binding to VEGF. Therefore, these residues were excluded from optimization. Inspection of the complex structure suggested that substitutions at P96 and Y98 could be disruptive to the antibody structure, while G100, where Ala mutation had little effect, might tolerate further substitutions. We therefore constructed a library (YC81) which fully randomized positions H97, Y99, G100, S100a, and S100b, within CDR-H3.

Significant effects of Ala substitution were also found in CDR-H2. Here, W50, I51, N52, T52a, Y53, T54, T58 alanine mutants all showed >twofold loss in binding affinity, with the greatest residue surface area buried at positions W50, I51, Y53, and

T58 (Muller *et al.*, 1998a). Indeed, W50 along with other aromatic side-chains was observed to form a deep pocket into which a loop of VEGF inserts in the complex, and was excluded from further optimization. Residue I51, on the other hand, showed no direct contact with VEGF and was also excluded. Residue T58 had multiple interactions within the interface, including contacts with VEGF and with the critical W50 of the CDR pocket. Although E56 showed no contact with VEGF and little effect (<twofold) upon alanine substitution, its side-chain lies at the periphery of the interface, near several hydrophobic residues of VEGF. We reasoned that these might be exploited for additional binding interactions. Two CDR-H2 libraries were constructed: YC266, randomizing positions T52a, Y53, T54, and E56; and YC103, randomizing positions N52, T52a, Y53, and T54.

In CDR-H1 G26, Y27, F29, N31, Y32, G33, M34, and N35 were implicated by alanine mutagenesis as important for binding VEGF; however, only N31, Y32, and G33 had significant direct contacts with VEGF. Since Ala substitution of G33 showed reduced binding, larger side-chains seemed less desirable; for this reason, this position was not randomized. Residues 27 (buried in the antibody structure) and T28 and T30 (which are mutually contacting) were included at the end of the H1 loop as possible indirect determinants of binding. Residues 27, 28, and 30-32 were randomized in a library designated YC265.

Framework residues, especially heavy chain residues 71 and 93, normally outside the region of contact with antigen, have also been found to affect antibody binding affinity (Tramontano *et al.*, 1990; Foote & Winter, 1992; Hawkins *et al.*, 1993; Xiang *et al.*, 1995), and sometimes participate in antigen contacts (reviewed by Nezlin, 1998). Therefore, an additional region of the anti-VEGF Fab, within FR-H3 and including position 71, was also targeted for randomization. Since the residue 71-76 region has contacts with CDR-H1 (at F29) and CDR-H2 (at I51 and T52a), these represented potential sites for affinity improvement through secondary effects on the interface residues. Residues L71, T73, and S76 were randomized in this FR-H3 library.

Phage selections

Fab libraries were constructed using a fusion to the g3p minor coat protein in a monovalent phage display (phagemid) vector (Bass *et al.*, 1990; Lowman *et al.*, 1991). For each library, stop codons were introduced by mutagenesis into the Y0192 phage template (Muller *et al.*, 1998a) at each residue position to be randomized. Each stop-codon construct was then used for construction of a fully randomized (using NNS codons) library as described in Materials and Methods. Phage were precipitated from overnight *Escherichia coli* shake-flask cultures and applied to VEGF-coated immunosorbant plates for binding selections. Cycles of selection followed by amplification were carried out essentially as described (Lowman, 1998).

We used an off-rate selection process (see Materials and Methods) similar to previously described procedures (Hawkins *et al.*, 1992; Yang *et al.*, 1995), modified by gradually increasing the selective pressure for binding to antigen during successive cycles of enrichment. The enrichment factor (ratio of displaying phage to non-displaying phage eluted *versus* applied) was used to monitor the stringency of selection at each step (Table 1). As a control, and to obtain a relative measure of affinity improvement, Y0192-phage were subjected to the same procedure at each cycle.

Fab-phage clones were sequenced from several phage-binding selection rounds that showed enrichment for Fab-phage over non-displaying phage. From round 6 of the CDR-H1 library selections, a dominant clone, Y0243-1 was found, having wild-type residues at Y27, T30, and Y32, and substitutions T28D and N31H (Table 2). Additional clones had related sequences, with N31H found in all selectants; Asp or Glu substituting for T28; and Thr, Ser, Gln, or Gly found at position T30.

Table 1. Enrichment factors from phage-displayed Fab libraries

Round	Wash time (hours)	CDR-H1 YC265	CDR-H2 YC266	CDR-H2 YC103	CDR-H3 YC81	FR-H3 YC101	Control Y0192
1	0	8.2	1.7	1.3	3.3	4	1.5
2	1	1.6	25	0.7	10	110	90
3	2	340	880	100	570	2300	22000
4	18	6800	880	5200	3700	600	2700
5	37[a]	210	900	920	1300	480	32
6	47[a]	130	80	100	3500	30	20
7	63[a]	1	1	>3	>25	1	>8

Libraries are designated by CDR region and oligonucleotide label (see the text for details). Library Fab-phage (ampicillin-resistant) were mixed with non-displaying control phage (chloramphenicol-resistant) in each starting pool, and subjected to VEGF binding selection, washing, and elution as described in the text.

The enrichment factor for each library is reported here as the ratio of Amp/Cam colony-forming units in the eluted pool, divided by the ratio of Amp/Cam colony-forming units in the starting pool. Starting phage concentrations were about 10^{12}/ml, except 10^{13}/ml in round 1. The wild-type Fab-phage, Y0192 was included at each round for comparison of enrichment under the particular conditions used.

[a] In some cases, the wash-step included incubation at 37 °C.

Table 2. Anti-VEGF Fab variants selected from a CDR-H1 library (HL-265)

Variant	*n*	Y 27	T 28	T 30	N 31	Y 32	I 34[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
Y0243-1	5	Y	D	T	H	Y	M	3.1
Y0243-2	1	Y	E	Q	H	Y	M	
Y0243-3	1	Y	E	T	H	Y	M	
Y0243-4	1	Y	D	G	H	Y	M	
Y0243-5	1	Y	D	S	H	Y	M	
Y0243-6	1	Y	E	S	H	Y	M	
Consensus:		Y	D	T	H	Y	M	3.1

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n* indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987).

[a] Position 34 was not randomized, but was changed to Met (as in Fab-12) in this library. The consensus reported here, equivalent to clone Y0243-1, represents the most abundant amino acid residue at each position (including clones with multiple representation ($n > 1$)). K_d(Y0192)/K_d (variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

Clones from two independently constructed CDR-H2 libraries were remarkable in that all sequenced library clones conserved wild-type residues at virtually all positions mutated, except at position Y53, where all clones contained a Trp substitution (Table 3).

Because of the strong enrichment observed from the CDR-H3 library, a number of clones were sequenced from rounds 5 and 7 (Table 4). Of 39 sequenced clones, 37 retained the wild-type residue S100b, and all contained the mutation H97Y. The remaining positions showed greater diversity, even after seven cycles of selection. The dominant clone at round 7, Y0238-3, contained the mutation S100aT (in addition to H97Y), with wild-type residues Y99 and G100. Other substitutions observed included Lys or Arg for Y99 (in 18 of 39 clones), G100N (11 of 39 clones), and a variety of substitutions including Arg, Glu, Gln, and Asn at S100a. In this library, the consensus sequence is represented by the dominant clone, Y0238-1 (Table 4).

Clones from round 6 of the FR-H3 library (Table 5) showed conservation of wild-type residue S76, with wild-type residues or various substitutions at the remaining positions: Val or Ile substituting for L71, and Val or Lys substitutions at T73.

Binding affinity of selected variants

For measurements of binding affinity, we made use of an amber stop codon placed between the genes for the Fab heavy chain and the g3p C-terminal domain, and expressed soluble Fab variants from *E. coli* shake-flask or fermentation cultures. Fab variants purified from protein-G affinity chromatography were characterized for binding affinity using an SPR-based assay on a BIAcore™-2000 instrument. The binding-kinetics assay has been described (Muller *et al.*, 1998a).

Association kinetics (k_{on}) for the wild-type antibody binding to immobilized VEGF are slow (Presta *et al.*, 1997; Baca *et al.*, 1997; Muller *et al.*, 1998a), and none of the variants tested had significantly improved on-rates. On the other hand, dissociation kinetics varied over a range of 10^{-4} s^{-1} to $\leqslant 4 \times 10^{-6}$ s^{-1} at 25 °C (Table 6). Based on measurements of instrumental drift, we could not accurately measure k_{off} (and consequently K_d)

Table 3. Anti-VEGF Fab variants selected from CDR-H2 libraries (HL-266, YC103)

Variant	*n*	N 52[a]	T 52a	Y 53	T 54	G 55[a,b]	E 56[a]	K_d(Y0192)/ K_d(variant)
Round 6 (HCl)								
HL266-A[b]	6	N	T	W	T	G	E	1.3
HL266-E	1	N	T	W	T	G	T	
HL266-I	1	N	T	W	T	G	Q	
YC103-A[b]	7	N	T	W	T	G	E	1.3
YC103-C	1	N	T	W	D	G	E	
Consensus		N	T	W	T	G	E	1.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n* indicates the number of clones found with identical DNA sequence. The wild-type (Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clones HL266A and YC103A, represents the most abundant amino acid at each position (including clones with multiple representation; i.e. $n > 1$). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] Constant positions were position 52 in the HL-266 library and position 56 in the YC103 library.

[b] Equivalent clones are assumed to have equal affinity.

Table 4. Anti-VEGF Fab variants selected from a CDR-H3 library (YC81)

Variant	*n*	H 97	Y 99	G 100	S 100a	S 100b	K_d(Y0192)/K_d(variant)
Round 5 (VEGF)							
Y0228-21	1	Y	R	N	A	S	
Y0228-22	1	Y	T	T	R	S	
Y0228-23	1	Y	E	G	S	S	
Y0228-24	1	Y	R	Q	R	G	
Y0228-26	1	Y	T	G	R	S	
Y0228-27	1	Y	T	N	T	S	
Y0228-28	1	Y	R	K	G	S	
Y0228-29	1	Y	T	G	S	S	
Y0228-30	1	Y	R	S	G	S	
Round 5 (HCl)							
Y0229-20	1	Y	T	N	R	S	
Y0229-21	1	Y	R	N	S	S	
Y0229-22	1	Y	K	E	S	S	
Y0229-23	1	Y	R	D	A	S	
Y0229-24	1	Y	R	Q	K	G	
Y0229-25	1	Y	K	G	G	S	
Y0229-26	1	Y	Y	G	A	S	
Y0229-27	1	Y	R	G	E	S	
Y0229-28	1	Y	R	S	T	S	
Y0238-10[a]	1	Y	R	N	T	S	3.8
Round 7 (HCl)							
Y0238-3	6	Y	Y	G	T	S	≥9.4
Y0238-1	2	Y	R	G	T	S	7.3
Y0238-2	2	Y	I	N	K	S	
Y0238-10[a]	2	Y	R	N	T	S	3.8
Y0238-4	1	Y	Y	N	Q	S	
Y0238-5	1	Y	I	A	K	S	2.1
Y0238-6	1	Y	R	D	N	S	≥5.4
Y0238-7	1	Y	W	G	T	S	
Y0238-8	1	Y	R	Q	N	S	
Y0238-9	1	Y	R	Q	S	S	
Y0238-11	1	Y	K	N	T	S	
Y0238-12	1	Y	I	E	R	S	
Consensus		Y	R	G	T	S	7.3

All variants are in the background of Y0192 (Muller *et al.*, 1998a). The clones are grouped according to the round of selection (5 or 7) and the type of elution (VEGF competition or HCl elution) used for recovery of bound phage. *n*, indicates the number of clones found with identical DNA sequence within each group. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). The consensus reported here, equivalent to clone Y0238-1, represents the most abundant amino acid at each position (including clones with multiple representation (n > 1)). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One clone was identified at both rounds 5 and 7. Equivalent clones are assumed to have equal affinity.

under these conditions, but instead used the kinetics data to place an upper limit on K_d.

The phage-derived Fab variants tested showed a range of small (within experimental error of about twofold) to significant (>fivefold) improvements in binding affinity over the wild-type (parental phage) antibody Y0192 (Table 6). From the CDR-H1 library, the dominant clone (Y0243-1) showed threefold improved affinity. Variant Y0242-1, the dominant clone in each of three CDR-H2 libraries, showed an affinity equivalent to wild-type within experimental error, and two clones derived from the FR-H3 library (Y0244-1 and Y0244-4) were equivalent or slightly weaker in affinity. Small

Table 5. Anti-VEGF Fab variants selected from a FR-H3 library

Variant	*n*	L 71	T 73	S 76	K_d(Y0192)/K_d(variant)
Round 6 (HCl)					
Y0244-1	1	V	V	S	0.3
Y0244-2	1	L	K	S	
Y0244-3[a]	1	L	V	S	
Y0244-4	1	I	K	S	0.9

All variants are in the background of Y0192 (Muller *et al.*, 1998a). *n*, indicates the number of clones found with identical DNA sequence. The wild-type (Fab-12, or Y0192) residue is shown at the top of each column, and the sequence position number is indicated according to Kabat *et al.* (1987). K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity *versus* the wild-type humanized antibody Y0192 (see Table 6).

[a] One variant contained a spontaneous mutations, S74W.

Table 6. Binding kinetics of anti-VEGF Fab variants at 25 °C

Variant	$k_{on}/10^4$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Y0192)/K_d(variant)
Y0192[a]	4.1	1.2	2.9	1
A. *Library-derived*				
Y0238-1	2.6	0.09	0.4	7.3
Y0238-3	1.3	≤0.04[b]	≤0.3[b]	≥9.4[b]
Y0238-5	0.57	0.08	1.4	2.1
Y0238-7	1.1	≤0.06[b]	≤0.5[b]	≥5.4[b]
Y0238-10	1.2	0.09	0.8	3.8
Y0242-1	3.8	0.86	2.3	1.3
Y0243-1	4.8	0.45	0.9	3.1
Y0244-1	3.0	2.7	9.0	0.3
Y0244-4	5.2	1.7	3.3	0.9
B. *Engineered*				
Y0268-1	4.0	0.15	0.38	7.6
Y0313-1	3.5	≤0.05[b]	≤0.15[b]	≥20[b]
Y0192(T28D)	6.8	1.4	2.0	1.4
Y0192(N31H)	4.8	0.37	0.8	3.6
Y0192(H97Y)	2.5	0.045	0.2	14
Y0192(S100aT)	6.8	1.0	1.5	1.9
Y0317	3.6	≤0.05[b]	≤0.14[b]	≥20[b]

Kinetic constants were determined from measurements using a BIAcore™-2000 instrument with a biosensor chip containing immobilized VEGF(109). Measurements were performed at 25 °C. Fab concentrations were calculated from quantitative amino acid analysis. The equilibrium dissociation constant, K_d, is calculated form the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Y0192)/K_d(variant) indicates the fold increase in binding affinity versus the wild-type humanized antibody Y0192. Errors in K_d were approximately ±25%. Variant Y0242-1 corresponds to the point mutations Y53W in CDR-H2 of Fab Y0192; for descriptions of the other variants, see Tables 2, 3, 4, 5, and 8.

[a] Data for Y0192 is from Muller *et al.* (1998a,).

[b] In some cases, the dissociation rate constant observed was at or near the limit of detection; therefore, the reported k_{off} and K_d are upper limits, and the relative affinities are an upper limit.

improvements were seen in CDR-H3 variants Y0238-5 and Y0238-10. However, larger improvements (exceeding the limits of measurement (>fivefold to >ninefold)) were observed for the CDR-H3 variants Y0238-1, Y0238-3, and Y0238-7.

All tested variants (in fact all sequenced clones) from the CDR-H3 library contained the mutation H97Y. In the higher affinity group, Gly was conserved at position 100, while the lower affinity variant contained Ala (known to cause 1.8-fold reduction in Y0192 binding; Muller *et al.*, 1998a) or Asn (Table 4). The S100a position, while quite varied among sequenced clones, was changed to Thr in the higher affinity CDR-H3 variants, and Thr or Lys in the lower affinity ones. Substitutions at Y99, though mostly confined to basic or aromatic residues, apparently had little effect since Y0238-1 (representing the consensus CDR-H3 sequence with Y99R) was not significantly different in affinity from Y0238-3, which retained Y99.

Affinity improvements from combinations of CDR mutations

To improve affinity further, several combinations of the phage-selected CDR-H1, H2, and H3 mutations were made by site-directed mutagenesis (Table 7). Among these, the highest affinity was obtained with pY0313-1 (i.e. pY0192 with mutations CDR-H1 (T28D/N31H/I34M) and CDR-H3 (H97Y/S100aT); note I34M is a reversion to Fab-12 wild-type). From BIAcore™ kinetics measurements carried out at 25 °C, this Fab variant had ≥20-fold higher affinity than Y0192 (Table 6).

Addition of the Y53W mutation, which alone produced little or no improvement over Y0192, to Y0313-1 (producing variant Y0268-1) actually reduced binding affinity by >twofold (Table 6).

The final Fab version was constructed by removing the phage-expression enhancing mutations in CDR-L1 from pY0313-1 by site-directed mutagen-

Table 7. Anti-VEGF CDR combination variants

	CDR-L1					CDR-H1			CDR-H2	CDR-H3	
Y0192: Variant	R 24	N 26	E 27	Q 28	L 29	T 28	N 31	I 34	Y 53	H 97	S 100a
Y0313-1	-	-	-	-	-	D	H	M	-	Y	T
Y0268-1	-	-	-	-	-	D	H	M	W	Y	T
Y0317	S	S	Q	D	I	D	H	M	-	Y	T
Fab-12	S	S	Q	D	I	-	-	-	-	-	-

Substitutions are shown relative to Y0192. Fab-12 also contains T221 in the heavy chain. Dashes (-) indicate no substitution. Numbering is according to Kabat *et al.* (1987) for both the light chain (CDR-L1) and heavy chain (CDR-H1, H2, H3).

esis. The M4L substitution was identified during phage-humanization experiments (Baca *et al.*, 1997), and the Leu residue was retained so as to preclude possible oxidation of the Met side-chain. The first libraries were constructed from a Fab-12 phagemid derivative, pY0101, which contained a buried framework mutation, V_L(M4L), as well as a mutation (T221L) at the junction to g3p. Thus the final version, Y0317 (Table 7 and Figure 1) differs from Fab-12 by the following six mutations: V_L(M4L), V_H(T28D/N31H/H97Y/S100aT/T221L).

Each of the CDR mutations in H1 and H3 was tested for its effect on VEGF binding affinity by introducing the corresponding point mutation into the parental Y0192 Fab and measuring binding kinetics. The results (Table 6) show a 14-fold and 3.6-fold improvement with substitution of H97Y or N31H, respectively, into the parental Fab. However, T28D or S100aT had identical affinity to Y0192, within experimental error.

We compared Fab-12 and Y0317 Fab affinities in a solution binding assay, using VEGF competition with [^{125}I]VEGF for binding to Fab. The results showed Fab-12 having $K_d^{25°} = 433$ pM and Y0317 Fab having $K_d^{25°} = 20$ pM, a 22-fold improvement in binding affinity (Figure 2).

Because dissociation kinetics in surface plasmon resonance (SPR) experiments exceeded instrumental capabilities at 25 °C, and in order to assess binding affinity under more physiological conditions, we compared binding affinities of the original humanized antibody Fab-12 with the final variant Y0317 in kinetics experiments at 37 °C. k_{on} and k_{off} were faster for both antibodies than at 25 °C, and k_{off} was clearly measurable above background. Using either immobilized VEGF(109) or immobilized VEGF(165), Y0317 was 120-fold to 140-fold improved in affinity over Fab-12, with a $K_d^{37°}$ of 80-190 pM (Table 8).

VEGF Ala-scan of the Y0317 binding epitope

In order to understand how mutations in the Fab affected binding affinity to VEGF, we also tested VEGF variants for binding to the affinity-improved antibody. For these experiments, we made use of the full-length IgG forms of Fab-12 (known as rhuMab VEGF) and Y0317 (termed Y0317-IgG) produced in CHO cells (V. Chisholm, unpublished results). These VEGF variants were previously used for mapping the parental antibody's binding site on VEGF (Muller *et al.*, 1998a).

In this assay, carried out at 37 °C, VEGF competed with biotin-VEGF with an IC_{50} of 9 nM in binding rhuMab VEGF, compared with an IC_{50} of 1 nM for Y0317-IgG (Table 9). SPR measurements have shown similar affinity improvement of Y0317-IgG over rhuMAb VEGF (H. Lowman, unpublished results).

Alanine mutations of VEGF that affected rhuMab VEGF binding also affected Y0317-IgG. For example, M81A, G88A, and G92A all caused large (100 to >500-fold) losses in binding affinity. And smaller reductions (3 to 30-fold) in binding affinity for both antibodies were seen for I80A, K84A, I91A, E93A, and M94A.

However, significant differences in the magnitude of the effect were observed at certain sites, including Y45A, fourfold reduced in affinity for rhuMAb VEGF *versus* 26-fold for Y0317-IgG; Q89A, 19-fold *versus* sixfold; and M94A, 11-fold versus 25-fold. Most surprisingly, two mutations that led to loss of detectable binding affinity for rhumAb VEGF (>500-fold) had only modest effects (four- to ninefold) on binding to Y0317-IgG. These differences might suggest a shift in the binding epitope of the antibody, and this possibility was addressed with receptor-inhibition assays and structural analysis, both described below.

Inhibition of VEGF activity

Cell-proliferation assays have been described (Fairbrother *et al.*, 1998) for the measurement of VEGF mitogenic activity on human umbilical vein endothelial cells. Here, we compared the potency of Fab-12 and the affinity-improved variants Y0238-3 and Y0313-1.

The results (Figure 3) show both variants Y0238-3 and Y0313-1 inhibit VEGF activity more potently than Y0192 Fab. Comparing the Fab forms, variant Y0313-1 appeared at least 30-fold to 100-fold more potent than the wild-type Fab. In additional experiments, Y0317 activity was similar to that of Y0313-1 (data not shown). It should be noted that the amount of VEGF (0.2 nM) used in this assay is potentially limiting for determination of an accurate IC_{50} for the mutant. For example, if the bind-

Table 8. Binding kinetics of anti-VEGF Fab variants at 37 °C

Variant	Immobilized	$k_{on}/10^6$ (M^{-1} s^{-1})	$k_{off}/10^{-4}$ (s^{-1})	K_d (nM)	K_d(Fab-12)/K_d(variant)
Fab-12	VEGF(109)	5.1	6.6	13 ± 2.2	1
Y0317	VEGF(109)	5.4	0.059	0.11 ± 0.02	120
Fab-12	VEGF(165)	5.5	11	20 ± 3.8	1
Y0317	VEGF(165)	5.3	0.074	0.14 ± 0.05	140

Kinetic constants were determined by injecting Fab solutions onto a BIAcore™-2000 instrument with a biosensor chip containing approximately 190 RU of immobilized VEGF(109) or VEGF(165), as indicated. The equilibrium dissociation constant, K_d, is calculated from the ratio of the rate constants, k_{off}/k_{on}. The relative affinity, reported as K_d(Fab-12)/K_d(variant) indicates the fold increase in binding affinity *versus* the original humanized antibody (Fab-12; Presta *et al.*, 1997) under the specified conditions.

```
Light chain:

                1        10        20        30        40        50
Fab-12          DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF
Y0192           DIQLTQSPSSLSASVGDRVTITCRANEQLSNYLNWYQQKPGKAPKVLIYF
Y0317.          DIQLTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAPKVLIYF

                1        10        20        30        40        50
                        60        70        80        90       100
Fab-12          TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0192           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ
Y0317           TSSLHSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSTVPWTFGQ

                        60        70        80        90       100
                       110       120       130       140       150
Fab-12          GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0192           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
Y0317           GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV

                       110       120       130       140       150
                       160       170       180       190       200
Fab-12          DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0192           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
Y0317           DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG

                       160       170       180       190       200
                       210
Fab-12          LSSPVTKSFNRGEC
Y0192           LSSPVTKSFNRGEC
Y0317           LSSPVTKSFNRGEC

                       210
```

Figure 1 (*legend shown opposite*)

ing affinity (K_d) of the mutant is in fact <0.2 nM, then the IC_{50} in this experiment will appear higher than under conditions of lower VEGF concentration. The result therefore supports the conclusion that the affinity-improved variant is at least 30-fold improved in affinity for VEGF, and that it effectively blocks VEGF activity *in vitro*.

Structure of the complex

In order to compare the structure and binding site of the affinity-improved antibody with that of the parental antibody, we determined the complex structure by X-ray crystallography. Crystals of the complex between the receptor binding domain of VEGF (residues 8 to 109) and the affinity-matured Fab Y0317 were grown as described (see Materials and Methods) and diffracted to a maximum resolution of 2.4 Å. The structure was refined starting from the coordinates of the complex between VEGF and the parent of Fab Y0317, Fab-12 (Muller *et al.*, 1998a), and refined to an *R*-value of 19.9% (R_{free} = 27.4%) for the reflections between 20 Å and 2.4 Å resolution.

Heavy chain:

```
                1       10        20        30        40        50
Fab-12          EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPGKGLEWVGW
Y0192           EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGINWVRQAPGKGLEWVGW
Y0317           EVQLVESGGGLVQPGGSLRLSCAASGYDFTHYGMNWVRQAPGKGLEWVGW

                1       10        20        30        40        50
                        60        70        80        90        100
Fab-12          INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0192           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP
Y0317           INTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNSLRAEDTAVYYCAKYP

                  a     60        70        80  abc   90     96
                      110       120       130       140       150
Fab-12          HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0192           HYYGSSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC
Y0317           YYYGTSHWYFDVWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC

                100abcdef       110       120       130       140
                      160       170       180       190       200
Fab-12          LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0192           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG
Y0317           LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG

                      150       160       170       180       190
                      210       220       230
Fab-12          TQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
Y0192           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL
Y0317           TQTYICNVNHKPSNTKVDKKVEPKSCDKTHL

                      200       210       220
```

Figure 1. Sequence alignment of the original humanized antibody (Fab-12; Presta *et al.*, 1997), the phage-displayed antibody (Y0192; Muller *et al.*, 1998a) and the affinity-improved antibody (Y0317). Sequential numbering of each chain is shown above the sequences; numbering according to Kabat *et al.* (1987) is shown below. CDR regions are underlined. Positions at which Y0317 differs from Fab-12 are indicated with double underlining.

The final model consists of two Fab fragments bound to the symmetrical poles of the VEGF dimer. Only residues 14-107 of each VEGF monomer are well defined in the electron density, and therefore the six N-terminal and the two C-terminal residues of each monomer were omitted from the model. Each Fab light chain comprises residues 1 to 213, with the C-terminal residue disordered, whereas for each heavy chain residues 138 to 143 as well as the six C-terminal residues are absent from the model. As in the parental Fab complex, two out of 1050 residues, namely T51 in the V_L chain of each Fab fragment, are located in the "disallowed regions" (Laskowski *et al.*, 1993) of the Ramachandran plot; 85% of all residues have their main-chain torsion angles in the "most favored"

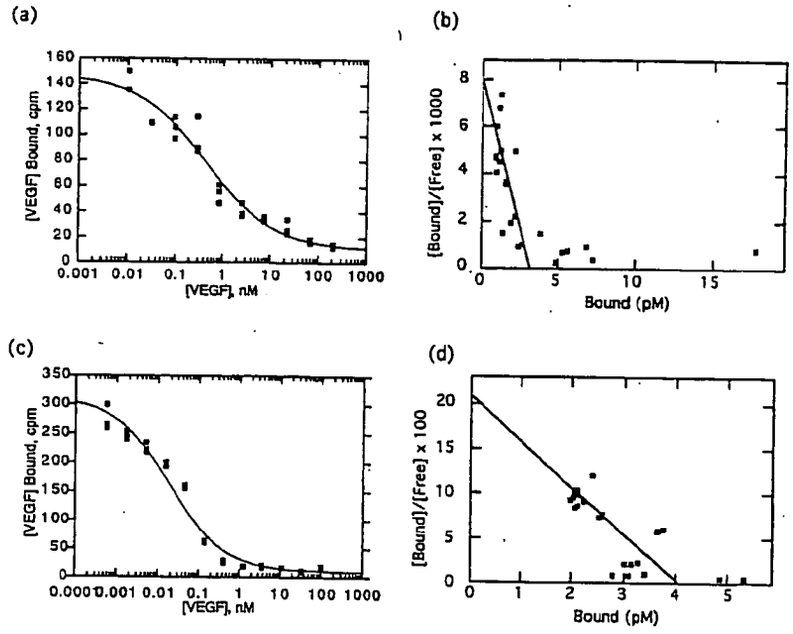


Figure 2. Radiolabeled VEGF binding assay. [^{125}I]VEGF was equilibrated (23 °C) with serial dilutions of unlabeled VEGF and (a) Fab-12 or (c) Y0317. Fabs were captured with an anti-Fab antibody-coated immunosorbant plate. Scatchard analysis (Munson & Rodbard, 1980) with a 1:1 binding model was used to calculate K_d of (b) 433 (±116) pM for Fab-12 and (d) 19.8(±4.3) pM for Y0317.

regions. The average *B*-factor of the model is 51.8 Å^2 and the mobility of the individual domains follows the pattern that was previously observed in the crystal structure of VEGF in complex with the Fab-12, with the constant domain dimer (C_L:C_H1) of one of the Fabs poorly ordered (Muller *et al.*, 1998a).

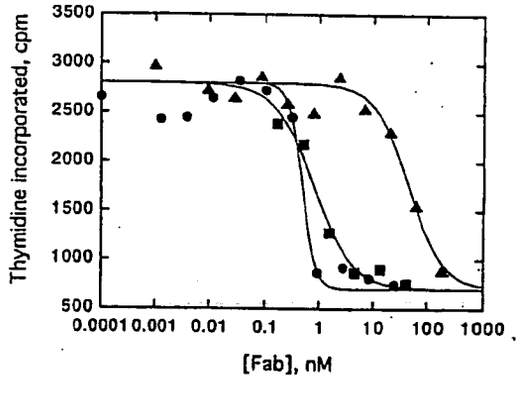


Figure 3. Human umbilical vein endothelial cell (HuVEC) assay of VEGF inhibition. Cells were cultured in the presence of 0.2 nM VEGF and serial dilutions of Fab Y0192 (triangles), Y0238-3 (squares), or Y0313-1 (circles). Cell proliferation was measured by incorporation of [^{3}H]thymidine. Curves show four-parameter fits to the data. Each point represents the mean of three treated wells.

Comparison of the final model with that of the parental Fab-VEGF complex (Muller *et al.*, 1998a) shows that the bound structures are very similar overall (Figure 4(a)) with Y0317 binding to the same site on VEGF as Fab-12 (Figure 4(b)). Side-chains show excellent overlap, and the main-chain structures show very little difference. The most prominent difference in contact residues is at H97Y (Figure 4(c); discussed below), where the tyrosine side-chain packs more favorably with VEGF and a buried water molecule from the parental Fab-VEGF complex is absent in the Y0317-Fab-VEGF complex.

Discussion

Antibody binding selections and affinity improvement

Here we made use of results from alanine-scanning and the previous structure of a humanized antibody-antigen complex to design Fab-phage libraries that randomized the three heavy-chain CDRs as well as one framework region (FR-H3) for improving the binding affinity of an anti-VEGF antibody. Affinity-improved Fab variants were obtained, with the largest effects seen in variants from the CDR-H3 library, although significant improvement was also obtained from mutation of CDR-H1. We therefore combined two mutations from H1 with two from H3, generating a further improved variant, Y0317. By making point mutations, we showed that the 20-fold (Figure 2)

Table 9. Alanine scan of VEGF by ELISA at 37 °C

VEGF(109) variant	IC_{50} (variant)/IC_{50} (VEGF)	
	Fab12-IgG	Y0317-IgG
VEGF(109)	1	1
F17A	1	1
Y21A	1	1
Y45A	4	26
K48A	2	1
Q79A	1	3
I80A	4	5
M81A	>500	930
R82A	>500	4
I83A	>500	9
K84A	3	10
H86A	1	1
Q87A	1	1
G88A	105	87
Q89A	19	6
H90A	1	1
I91A	2	6
G92A	>500	>900
E93A	4	7
M94A	11	25

ELISA assays were carried out using the full-length IgG form of Fab-12 or the IgG form of Y0317 and VEGF(109). Incubation of antibody with VEGF was at 37 °C for five hours. The IC_{50} for inhibition by each Ala mutant was evaluated using a four-parameter equation, and the relative affinities calculated as IC_{50}(mutant VEGF)/IC_{50}(wild-type VEGF). Under these conditions, Fab12-IgG and Y0317-IgG showed IC_{50} values of 9 nM and 1 nM, respectively.

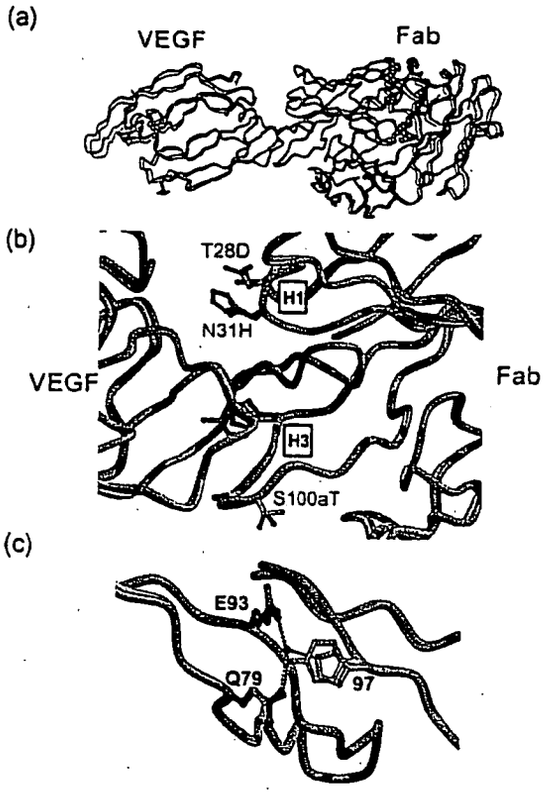


Figure 4. Structure of the affinity-improved Y0317 Fab in complex with VEGF. A superposition of the structure (Muller *et al.*, 1998a) of wild-type humanized antibody Fab-12 (gray) in complex with VEGF (gray) is shown with that of Fab Y0317 (green) in complex with VEGF (yellow). (a) Overall view of the complex, including one Fab molecule bound to one dimer of VEGF (a second Fab molecule is bound at left in the crystal) shows that the binding site for both antibody variants centers on the "80's loop" of VEGF. (b) A view of the four CDR changes between Fab-12 and Y0317 Fab shows that the new D28 and T100a side-chains do not directly contact antigen. However, H31 and Y97 form new contacts. (c) Interactions of H97 and an associated, buried water molecule in the Fab-12 complex, compared with those of Y97 in the Y0317 complex.

to >100-fold (Table 8) affinity improvement in Y0317 can be attributed to two CDR mutations: H97Y and N31H. In fact, H97Y alone improves binding affinty 14-fold.

Despite the relatively slow k_{on} and slow k_{off} of the parental antibody, binding selections described here yielded slower dissociation rates and improved equilibrium dissociation constants. Results of SPR measurements demonstrated that affinity is enhanced mainly through a slower dissociation rate (as opposed to faster association). These results are consistent with the idea of off-rate selection (Hawkins *et al.*, 1992) and with the progressively increased stringency in washing procedures used here (see Materials and Methods and Table 1). Previous binding-optimization efforts have also often yielded larger improvements in k_{off} than in k_{on} (see Lowman & Wells, 1993; Yang *et al.*, 1995; Schier *et al.*, 1996). This may suggest fundamental limitations to the improvements in k_{on} for a given binding site. Even if no conformational changes need occur between free and bound states, the on-rate is limited by the size of the binding interface and the translational and rotational diffusion rates of the binding components (reviewed by Delisi, 1983).

The association rate constants (k_{on}) for both the wild-type Y0192 and the final Y0317 antibodies are relatively slow (about 4×10^4 M^{-1} s^{-1} for both) compared to other antibodies of equal or weaker antigen binding affinity. In fact, the fastest k_{on} identified for any mutant was 6.8×10^4 M^{-1} s^{-1} (Table 6). Typically, k_{on} for antibodies binding to protein antigens, including affinity-matured antibodies, has fallen in the range of 3×10^4 to 1×10^6 M^{-1} s^{-1} (Karlsson *et al.*, 1991; Malmborg *et al.*, 1992; Barbas *et al.*, 1994; Yang *et al.*, 1995; Schier *et al.*, 1996; Wu *et al.*, 1998). In this particular protein-protein interaction, a likely explanation for the slow k_{on} is the high degree of flexibility associated with the binding site both on the Fab and on VEGF. In fact, crystallographic evidence suggests that the "80's loop" region is quite mobile (Muller *et al.*, 1997; Muller *et al.*, 1998b). We are pursuing other strategies to assess whether improvements to k_{on} can be obtained.

The contributions of point mutations in proteins to the free energy of binding or activation are often additive (Wells, 1990). This principle has been used to produce a variety of affinity-improved protein variants based on point or grouped mutations identified by phage display (Lowman & Wells, 1993; Yang *et al.*, 1995) or point-mutant screening (Wu *et al.*, 1998). Considering the initial library selectants Y0238-3 (>ninefold improved in affinity) and Y0243-1 (3.1-fold improved), we would have predicted an improvement of >27-fold for Y0313-1 or Y0317 (Table 7). In fact, a 22-fold improvement is observed (Figure 2) at 25 °C. Addition of the CDR-H1 mutation would be predicted to improve affinity slightly (1.3-fold), but in fact this mutation reduced affinity >twofold (Y0268-1 *versus* Y0313-1; Table 6). Certainly additivity does not always apply, particularly if interacting residues are involved (Wells, 1990). In this case, non-additivity probably results from steric interference between the new Trp in CDR-H2 and the new Tyr in CDR-H3.

To test the energetics of binding by the final Y0317 antibody to VEGF, we made use of a panel of alanine mutants that had been previously constructed for mapping the binding site of the original antibody (Muller *et al.*, 1998a). For these experiments, we made use of the full-length IgG forms of both antibodies. In view of the slow dissociation kinetics for both antibodies, ELISA assays were carried out at 37 °C with incubation for at least five hours to insure that equilibrium was reached. Under these conditions two dramatic differences appear in the Ala-scan of VEGF with respect to Y0317 *versus* Fab-12: both R82A and I83A have small effects on binding in Y0317, but result in large decreases in binding for Fab-12. The reasons for these differences are not clear, but R82 and I83 do have significant surface area (55 Å^2 and 32 Å^2, respectively) buried on binding to VEGF, and make contacts that include residues S100a of CDR-H3 and N52 of CDR-H2 in the wild-type antibody (Muller *et al.*, 1998a).

Structural analysis of the affinity-matured Fab

The structures of a number of antibodies derived from *in vivo* immunization and hybridoma techniques have been determined, in complex with their antigens (reviewed by Nezlin, 1998), and recently, crystallization and preliminary X-ray studies of a chain-shuffled anti-lysozyme scFv antibody in complex with antigen were reported (Küttner *et al.*, 1998). However, to our knowledge, the Y0317 Fab:VEGF structure is the first report of an *in vitro* affinity-matured Fab in complex with antigen. The structural basis of binding affinity improvement is therefore of interest

The Fab fragment of the affinity-matured anti-VEGF antibody Y0317 preserves the structure of the original humanized antibody, Fab-12. Superposition with Fab-12 results in an rmsd of only 0.38 Å for a total of 431 C^{α}-positions, demonstrating the absence of major structural changes between the two molecules. With a total of 1800 Å^2 of solvent-accessible surface buried in each VEGF-Fab interface, the contact area is about 50 Å^2 larger than in the Fab-12 complex. This small increase in buried surface area is mostly due to the exchange of H97 to a tyrosine residue. In the VEGF:Fab-12 complex, H97 buries a solvent-accessible area of 56 Å^2, while the larger tyrosine side-chain of the matured antibody accounts for 86 Å^2 of buried surface. The tyrosine side-chain also affects the hydrogen-bonding pattern and the number of ordered water molecules in the vicinity. In the parental antibody complex, a water molecule near H97 forms two hydrogen bonds to the side-chains of Q79 and E93 of VEGF (Figure 4(c)). In the complex with the affinity-matured Fab, this water molecule is replaced by the hydroxyl group of the newly introduced tyrosine side-chain at position 97. The H97Y mutation therefore not only increases the amount of buried surface area, but also introduces two additional hydrogen bonds between the ligand and Fab-0317 (Figure 4(c)). This is in good agreement with the observation that this single substitution improves VEGF binding affinity by 14-fold (Table 6). We therefore conclude that this single substiution is responsible for the majority of the improvement in binding affinity of Y0317 compared to the parent antibody.

In contrast, despite the availability of the crystal structures of both complexes, it remains uncertain what the structural basis is of the 3.6-fold enhanced binding caused by the N31H mutation. The side-chains of the asparagine and the histidine residues in this position adopt identical conformations in both crystal structures, and the amount of buried surface is not significantly increased in the VEGF:Fab-Y0317 complex. The only difference we can detect is a slight possible improvement in the hydrophobic interactions between the histidine side-chain and the phenyl group of VEGF residue F17, which has rotated slightly compared to the parent complex. It is unclear whether this could contribute to the increased affinity.

Neither of the remaining differences between Fab-12 and Fab-Y0317 has a significant effect on the binding affinity towards VEGF, and the structures show that these residues contribute only marginally to the interface. Some interactions are present between VEGF and the main-chain atoms of the serine and threonine residues in position 100a of the two Fabs, but the side-chains of these residues are not in contact with VEGF. Finally, no contact exist between VEGF and T28 (or D28) of the Fab fragments (the closest point on VEGF to this residue is more than 6 Å distant).

In summary, the analysis and comparison of the two crystal structures are in very good agreement with the results of the binding assays on the various single mutants of the Fab fragments. Although it is not possible to quantify the effects introduced by the amino acid exchanges solely based on the crystal structures, the detailed crystallographic

analysis supports and enables us to interpret the binding data.

Biological implications for antibody inhibition of VEGF

An inhibitory antibody of improved affinity may have improved potency or efficacy in treating diseases associated with VEGF expression. Preceding versions of the anti-VEGF antibody described here, including the murine A4.6.1 (Kim *et al.*, 1993), the humanized version Fab-12 (Presta *et al.*, 1997), as well as Y0192 (Muller *et al.*, 1998a), clearly demonstrated sufficient affinity to effect inhibition of VEGF activity. Here, we show that an affinity-improved variant, Fab Y0317, can inhibit endothelial cell proliferation *in vitro* with least 30-fold greater potency than the parental humanized Fab (Figure 3).

We have limited our optimization strategy to a subset of heavy-chain CDR residues implicated by alanine-scanning and crystallography (Muller *et al.*, 1998a). Furthermore, not all combinations of phage-derived mutations have been tested. One may therefore reasonably ask whether Y0317, with $K_d^{25°} = 20$ pM and $K_d^{37°} = 130$ pM, is the globally optimum variant for binding to this particular epitope (or others) on VEGF. Other affinity optimization efforts have resulted in protein-protein binding affinities in the low picomolar range, from $K_d = 6$ pM to 15 pM (see, e.g. Lowman & Wells, 1993; Schier *et al.*, 1996; Yang *et al.*, 1995). Indeed, we cannot exclude the possibility that higher affinity variants of the A4.6.1 antibody could be produced. However, it seems unlikely that further affinity improvement would greatly enhance biological potency or efficacy because for effective inhibition, the antibody must certainly occupy a significant fraction (perhaps >99 %) of the available (VEGF) binding sites. Serum VEGF concentrations of about 20 pM in normals, and of >300 pM in patients with metastatic carcinoma, have been observed (Kraft *et al.*, 1999). Local or effective concentrations are likely higher. If we conservatively assume the effective concentration of VEGF *in vivo* to be about 400 pM, then 400 pM of even an infinite-affinity Fab would be required to block all sites.

Other factors may limit the improvement in potency of a full-length IgG resulting from an improvement in intrinsic binding affinity of the Fab for antigen. The full-length IgG form of the antibody may benefit from an avidity effect *in vivo*, especially since VEGF is known to associate with proteoglycans on the cell surface (Gitay-Goren *et al.*, 1992). Even in cell-based assays, the IgG form of Fab-12 is a more effective inhibitor than the Fab form (data not shown). Finally, the half-life for dissociation of the affinity-improved antibody is already significant, even on the time-scale of the half-life of clearance for IgG's (days to weeks). The effect of an improved association rate constant for antibody in this system is unknown.

The fact that point (Ala) mutations in the antibody binding site on VEGF sometimes have lesser effects on the binding of Y0317 than on the binding of Fab-12 may suggest that the optimized binding site is more tolerant than the parental one of variations in the antigen. Indeed, Y0317 showed greatly enhanced affinity for murine VEGF over that of Fab-12 (data not shown), though still >100-fold weaker than its affinity for human VEGF. This could provide an advantage against naturally arising VEGF variants.

Materials and Methods

Construction of phage libraries and mutagenesis

A variant of the Fab-12 antibody (a humanized form of murine antibody A4.6.1) was previously identified from phage-displayed Fab libraries for improved expression on phage particles (Muller *et al.*, 1998a). We made use of the plasmid pY0192, a phagemid construct with ampicillin (or carbenicillin) resistance, as the parental ("wild-type") construct for libraries described here. To prevent contamination by wild-type sequence (Lowman *et al.*, 1991; Lowman, 1998), templates with the TAA stop codon at each residue targeted for randomization were prepared from CJ236 *E. coli* cells (Kunkel *et al.*, 1991). Libraries are designated according to the mutagenic oligonucleotides used for their construction: YC265, TCC TGT GCA GCT TCT GGC NNS NNS TTC NNS NNS NNS GGT ATG AAC TGG GTC CG, randomizing residues 27-28, 30-32 in CDR-H1; YC266, GAA TGG GTT GGA TGG ATT AAC NNS NNS NNS GGT NNS CCG ACC TAT GCT GCG G, randomizing residues 52a-54, 56 in CDR-H2; YC103, GAA TGG GTT GGA TGG ATT NNS NNS NNS NNS GGT GAA CCG ACC TAT G, randomizing residues 52-54 in CDR-H2; YC81, C TGT GCA AAG TAC CCG NNS TAT NNS NNS NNS NNS CAC TGG TAT TTC GAC, randomizing residues 97, 99-100b in CDR-H3; and YC101, CGT TTC ACT TTT TCT NNS GAC NNS TCC AAA NNS ACA GCA TAC CTG CAG , randomizing residues 71, 73, and 76 in the "FR-H3" region. An additional library in CDR-H2 was designed to insert three new residues: YC90, GA TGG ATT AAC ACC TAT NNS NNS NNS ACC GGT GAA CCG ACC.

The products of random mutagenesis reactions were electroporated into XL1-Blue *E. coli* cells (Stratagene) and amplified by growing 15-16 hours with M13KO7 helper phage. The complexity of each library, ranging from 2×10^7 to 1.5×10^8 , was estimated based on plating of the initial transformation onto LB plates containing carbenicillin.

Site-directed mutagenesis for point mutations was carried out as above, using appropriate codons to produce the respective mutations, and sequences were confirmed by single-strand DNA sequencing using Sequenase™ (USB).

Phage binding selections

For each round of selection, approximately 10^9-10^{10} phage were screened for binding to plates (Nunc Maxisorp 96-well) coated with 2 µg/ml VEGF(109) in 50 mM carbonate buffer (pH 9.6) and blocked with 5% (w/v) instant milk in 50 mM carbonate buffer, (pH 9.6). Also included were phage prepared from a non-displaying

control phagemid (pCAT), which confers chloramphenicol resistance, as a means of measuring background and enrichment (Lowman & Wells, 1993). Bound phage were eluted with 0.1 M HCl and immediately neutralized with one-third volume of 1 M Tris (pH 8.0). The eluted phage were propagated by infecting XL1 cells for the next selection cycle as described (Lowman, 1998).

In the first cycle, the VEGF plate was incubated with Fab-phage, then was briefly washed to remove bound phage. In the second cycle, binding and washing were followed by a one hour dissociative incubation at room temperature with binding buffer, after which the plate was again washed prior to acid elution. This process was repeated in rounds 3, 4 and 5, except that 1 μM VEGF was included in the dissociative incubation, and the incubation time was increased to 2, 18, and 37 hours, respectively. During these selections, Y0192 phage showed enrichments ranging from 1.5-fold (at the lowest stringency) to 22,000-fold (using a two hour dissociation incubation). However, further increases in stringency (rounds 4-5) resulted in decreasing enrichments for the control phage, with higher enrichments observed for certain libraries, especially the two CDR-H2 libraries and the CDR-H3 library (Table 1).

In cycle 6, a 17 hour dissociative incubation at room temperature was followed by an additional 30 hour incubation at 37 °C (also including VEGF in the buffer). Under these conditions, Y0192-phage showed only slight binding enrichment (20-fold), whereas the CDR-H3 library phage were enriched by 3500-fold. Cycle 7 was carried out with a 63 hour dissociative incubation, after which only small enrichment factors were observed. However, some libraries were continued through eight cycles (with 120 hours of dissociative incubation in the presence of VEGF), after which Fab-phage were still recoverable by acid elution (data not shown).

Purification of Fab

For small-scale preparations, Y0317 Fab and mutants were prepared from *E. coli* shake-flasks as described (Muller *et al.*, 1998a).

For large-scale preparation, whole cell broth was obtained from a ten liter *E. coli* fermentation. The cells were lysed with a Manton-Gaulin homogenizer (two passes at 6000 psi; lysate temperature maintained at 15-25 °C with a heat exchanger). A 5 % (v/v) solution of polyethylene imine (PEI), pH 6.0, was added to the lysate to give a final concentration of 0.25 % (v/v). The lysate was mixed for 30 minutes at room temperature. The suspension was centrifuged, and the supernatant (containing the Fab) was processed further. The pH of the supernatant was adjusted to 6.0 with 6 M HCl, followed by dilution to a conductivity of 5 mS/cm with purified water. The conditioned supernatant was loaded onto a BakerBond ABx ion-exchange column. Following a wash with the column equilibration buffer, the Fab was eluted with an increasing sodium chloride gradient in the equilibration buffer. Fractions containing the Fab were identified by SDS-PAGE. The BakerBond ABx column fractions were pooled, pH adjusted to 5.5 with 1 M Mes and diluted to a conductivity of 5 mS/cm with purified water. The conditioned BakerBond ABx pool was loaded onto a SP Sepharose HP cation exchange column (Pharmacia). Once again, the Fab was eluted with a sodium chloride-containing gradient. Fractions containing the Fab were identified by SDS-PAGE. The level of purity of Fab (as determined by SDS-PAGE) after this two column purification was >95 %.

BIAcore™ binding analysis

The VEGF-binding affinities of Fab fragments were calculated from association and dissociation rate constants measured using a BIAcore™ -2000 surface plasmon resonance system (BIAcore, Inc., Piscataway, NJ). A biosensor chip was activated for covalent coupling of VEGF using *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's (BIAcore, Inc., Piscataway, NJ) instructions. VEGF(109) or VEGF(165) was buffer-exchanged into 20 mM sodium acetate, pH 4.8 and diluted to approximately 50 μg/ml. Aliquots of VEGF were injected at a flow rate of 2 μl/minute to achieve approximately 700-1400 response units (RU) of coupled protein. A solution of 1 M ethanolamine was injected as a blocking agent.

For kinetics measurements, twofold serial dilutions of Fab were injected in PBS/Tween buffer (0.05 % Tween-20 in phosphate-buffered saline) at 25 °C or 37 °C at a flow rate of 10 μl/minute. Equilibrium dissociation constants, K_d values from SPR measurements were calculated as k_{off}/k_{on} (Tables 6 and 8).

Radiolabeled VEGF binding assay

Solution binding affinity of Fabs for VEGF was measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled VEGF(109) in the presence of a titration series of unlabeled VEGF, then capturing bound VEGF with an anti-Fab antibody-coated plate.

To establish conditions for the assay, microtiter plates (Dynex) were coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2 % (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C). In a non-adsorbant plate (Nunc #269620), 100 pM or 26 pM [^{125}I]VEGF(109) was mixed with serial dilutions of Fab-12 or Fab Y0317, respectively. Fab-12 was incubated overnight; however, the Fab Y0317 incubation was continued for 65 hours to insure that equilibrium was reached. Thereafter, the mixtures were transferred to the capture plate for incubation at room temperature for one hour. The solution was then removed and the plate washed eight times with 0.1 % Tween-20 in PBS. When the plates had dried, 150 μl/well of scintillant (MicroScint-20; Packard) was added, and the plates were counted on a Topcount gamma counter (Packard) for ten minutes. Concentrations of each Fab were chosen to give ≤20 % of maximal binding.

For competitive binding assays, Dynex plates were coated and blocked as above, and serial threefold dilutions of unlabeled VEGF(109) were made in PBS/Tween buffer in a Nunc plate. [^{125}I]VEGF(109) was added, followed by addition of a fixed concentration of Fab-12 or Fab Y0317. The final concentrations of Fab-12, and Fab Y0317 were 100 pM and 10 pM, respectively. After incubation (as above), bound VEGF was captured and quantified as described above. The binding data was analyzed using a computer program to perform Scatchard analysis (Munson & Rodbard, 1980) for determination of the dissociation binding constants, K_d, for Fab-12 and Fab Y0317.

ELISA assay of VEGF Ala mutants

The binding affinities of VEGF Ala mutants for full-length Fab-12-IgG (known as rhuMAb VEGF) and Y0317-IgG, a full-length IgG form of the improved antibody expressed in CHO cells (V. Chisholm, unpublished results) were measured as previously described (Muller *et al.*, 1997; Muller *et al.*, 1998a) for the murine antibody A4.6.1, except that the temperature was increased to 37 °C, and the incubation time increased to five hours, to insure that equilibrium was reached with the high-affinity antibody.

Cell-based assay of VEGF inhibition

Several versions of the anti-VEGF antibody were tested for their ability to antagonize VEGF(165) induction of the growth of HuVECs (human umbilical vein endothelial cells). The 96-well plates were seeded with 1000 HuVECs per well and fasted in assay medium (F12:DMEM 50:50 supplemented with 1.5 % (v/v) dia-filtered fetal bovine serum) for 24 hours.

The concentration of VEGF used for inducing the cells was determined by first titrating to identify the amount of VEGF that can stimulate 80 % of maximal DNA synthesis. Fresh assay medium containing fixed amounts of VEGF (0.2 nM final concentration), and increasing concentrations of anti-VEGF Fab or mab were then added. After 40 hours of incubation, DNA synthesis was measured by incorporation of tritiated thymidine. Cells were pulsed with 0.5 μCi per well of [^{3}H]thymidine for 24 hours and harvested for counting, using a TopCount gamma counter.

Crystallization and refinement

The complex between the Fab fragment of affinity-matured, humanized antibody Y0317 Fab and the receptor binding fragment of VEGF (VEGF(109)) was purified and crystallized as described for the analogous complex with the parental humanized Fab-12 fragment (Muller *et al.*, 1998a). The resulting crystals had symmetry consistent with space group $P2_1$ with cell parameters $a = 89.1$ Å, $b = 66.4$ Å, $c = 138.7$ Å, and $\beta = 94.7°$, and were isomorphous with the crystals obtained with the parent complex. A data set was collected from a single frozen crystal at beam line 5.0.2 at the Advanced Light Source, Berkeley, and processed using programs MOSFLM and SCALA (CCP4, 1994). The final data set (R_{merge} = 7.3 %) is described in Table 10. Starting with the model of Brookhaven Protein Data Bank entry 1bj1 (Muller *et al.*, 1998a), the structure was refined using the programs X-PLOR (Brünger *et al.*, 1987) and REFMAC (CCP4, 1994). The free *R*-value was monitored using the identical set of reflections sequestered before refinement of parent complex. The differences in the primary structure between Fab-12 and Fab-Y0317 were modeled using the program O (Jones *et al.*, 1991). After correction for anisotropy and application of a bulk solvent correction, the *R*-value reached its final value of 19.9 % for all reflections greater than 0.2σ (see Table 10; R_{free} = 27.4 %).

Table 10. Crystallographic data and refinement statistics

A. *Data collection*	Overall	Last shell
Resolution range (Å)	30-2.4	2.53-2.40
No. of observations	208,257	22,278
Unique reflections	61,742	8900
Completeness (%)	97.4	96.7
Mean $I/\sigma(I)$	13.6	2.7
R_{sym}	0.073	0.38
B. *Refinement*		
Resolution range (Å)	20-2.4	
No. of reflections	61,689	
No. of atoms	8577	
rmsd bond lengths (Å)	0.013	
rmsd angles (deg.)	1.9	
rmsd improper angles (deg.)	0.92	
rmsd *B*-factors for all bonded atoms, Å^2	3.5	
Number of main-chain torsion angles in disallowed regions of Ramachandran plot[a]	2	

[a] See Laskowski *et al.* (1993).

Protein Data Bank accession number

The coordinates for the VEGF:Y0317 Fab complex have been deposited in the Protein Data Bank, accession number 1cz8.

Acknowledgments

We thank Lyn Deguzman and Tom Zioncheck for providing ^{125}[I]VEGF; Alan Padua and Bill Henzel for quantitative amino acid analysis; James Bourell for mass spectrometry; Vanessa Chisholm and Lynne Krummen for construction of cell lines; and Manuel Baca, Napoleone Ferrara, Yves Muller, Leonard Presta, and James Wells for many helpful discussions.

References

Baca, M., Presta, L. G., O'Connor, S. J. & Wells, J. A. (1997). Antibody humanization using monovalent phage display. *J. Biol. Chem.* **272**, 10678-10684.

Barbas, C. F., III, Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R. M., Nara, P. L. & Burton, D. R. (1994). *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. *Proc. Natl Acad. Sci. USA*, **91**, 3809-3813.

Bass, S., Greene, R. & Wells, J. A. (1990). Hormone phage: an enrichment method for variant proteins with altered binding properties. *Proteins: Struct. Funct. Genet.* **8**, 309-314.

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). Crystallographic *R* factor refinement by molecular dynamics. *Science*, **235**, 458-460.

CCP4 (1994). Programs for protein crystallography. *Acta Crystallog. sect. D*, **50**, 760-763.

Chothia, C. & Lesk, A. M. (1987). Canonical structures for the hypervariable reigions of immunoglobulins. *J. Mol. Biol.* **196**, 901-917.

Delisi, C. (1983). Role of diffusion regulation in receptor-ligand interactions. *Methods Enzymol.* **93**, 95-109.

Fairbrother, W. J., Christinger, H. W., Cochran, A. G., Fuh, G., Keenan, C. J., Quan, C., Shriver, S. K., Tom, J. Y., Wells, J. A. & Cunningham, B. C. (1998). Novel peptides selected to bind vascular endothelial growth factor target the receptor-binding site. *Biochemistry*, **37**, 17754-17764.

Ferrara, N. (1995). The role of vascular endothelial growth factor in pathological angiogenesis. *Breast Cancer Res. Treat.* 36, 127-137.

Ferrara, N. (1999). Vascular endothelial growth factor: molecular and biological aspects. *Curr. Top. Microbiol. Immunol.* 237, 1-30.

Ferrara, N. & Henzel, W. J. (1989). Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 161, 851-858.

Folkman, J. (1995). Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nature Med.* 1, 27-31.

Folkman, J. & Klagsbrun, M. (1987). Angiogenic factors. *Science*, 235, 442-443.

Foote, J. & Winter, G. (1992). Antibody framework residues affecting the conformation of the hypervariable loops. *J. Mol. Biol.* 224, 487-499.

Gitay-Goren, H., Soker, S., Vlodavsky, I. & Neufeld, G. (1992). The binding of vascular endothelial growth factor to its receptors is dependent on cell-surface-associated heparin-like molecules. *J. Biol. Chem.* 267, 6093-6098.

Griffiths, A. D. & Duncan, A. R. (1998). Strategies for selection of antibodies by phage display. *Curr. Opin. Biotechnol.* 9, 102-108.

Hawkins, R. E., Russell, S. J. & Winter, G. (1992). Selection of phage antibodies by binding affinity mimicking affinity maturation. *J. Mol. Biol.* 226, 889-896.

Hawkins, R. E., Russell, S. J., Baier, M. & Winter, G. (1993). The contribution of contact and non-contact residues of antibody in the affinity of binding to antigen. *J. Mol. Biol.* 234, 958-964.

Iruela-Arispe, M. L. & Dvorak, H. F. (1997). Angiogenesis: a dynamic balance of stimulators and inhibitors. *Thromb. Haem.* 78, 672-677.

Jones, T. A., Zhou, J.-Y., Cowan, S. W. & Kjelgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallog. sect. A*, 47, 110-119.

Kabat, E. A., Wu, T. T., Redi-Miller, M., Perry, H. M. & Gottesman, K. S. (1987). *Sequences of Proteins of Immunological Interest*, 4th edit., National Institutes of Health, Bethesda, MD.

Karlsson, R., Michaelsson, A. & Mattsson, L. (1991). Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system. *J. Immunol. Methods*, 145, 229-240.

Kay, B. K. & Hoess, R. H. (1996). Principles and applications of phage display. In *Phage Display of Peptides and Proteins* (Kay, B. K., Winter, J. & McCafferty, J., eds), pp. 21-34, Academic Press, San Diego.

Kim, K. J., Li, B., Winer, J., Armanini, M., Gillett, N., Phillips, H. S. & Ferrara, N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth *in vivo*. *Nature*, 362, 841-844.

Kraft, A., Weindel, K., Ochs, A., Marth, C., Zmija, J., Schumacher, P., Unger, C., Marmé, D. & Gastl, G. (1999). Vascular endothelial growth factor in the sera and effusions of patients with malignant and nonmalignant disease. *Cancer*, 85, 178-187.

Kunkel, T. A., Bebenek, K. & McClary, J. (1991). Efficient site-directed mutagenesis using uracil-containing DNA. *Methods Enzymol.* 204, 125-139.

Küttner, G., Keitel, T., Gießmann, E., Wessner, H., Scholz, C. & Höhne, W. (1998). A phage library-derived single-chain Fv fragment in complex with turkey egg-white lysozyme: characterization, crystallization and preliminary X-ray analysis. *Mol. Immunol.* 35, 189-194.

Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993). Procheck: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallog.* 26, 283-291.

Lowman, H. B. (1998). Phage display of peptide libraries on protein scaffolds. In *Methods in Molecular Biology, Combinatorial Peptide Library Protocols* (Cabilly, S., ed.), vol. 87, pp. 249-264, Humana Press, Totowa, NJ.

Lowman, H. B. & Wells, J. A. (1993). Affinity maturation of human growth hormone by monovalent phage display. *J. Mol. Biol.* 234, 564-578.

Lowman, H. B., Bass, S. H., Simpson, N. & Wells, J. A. (1991). Selecting high-affinity binding proteins by monovalent phage display. *Biochemistry*, 30, 10832-10838.

Malmborg, A.-C., Michaelsson, A., Ohlin, M., Jansson, B. & Borrebaeck, C. A. K. (1992). Real time analysis of antibody-antigen reaction kinetics. *Scan. J. Immunol.* 35, 643-650.

Marks, J. D., Griffiths, A. D., Malmqvist, M., Clackson, T. P., Bye, J. M. & Winter, G. (1992). By-passing immunization: building high affinity human antibodies by chain shuffling. *Biotechnology*, 10, 779-783.

Mordenti, J., Thomsen, K., Licko, V., Chen, H., Meng, Y. G. & Ferrara, N. (1999). Efficacy and concentration-response of murine anti-VEGF monoclonal antibody in tumor-bearing mice and extrapolation to humans. *Toxicol. Pathol.* 27, 14-21.

Muller, Y. A., Li, B., Christinger, H. W., Wells, J. A., Cunningham, B. C. & de Vos, A. M. (1997). Vascular endothelial growth factor: crystal structure and functional mapping of the kinase domain receptor binding site. *Proc. Natl Acad. Sci. USA*, 94, 7192-7197.

Muller, Y. A., Chen, Y., Christinger, H. W., Li, B., Cunningham, B. C., Lowman, H. B. & de Vos, A. M. (1998a). VEGF and the Fab fragment of a humanized neutralizing antibody: crystal structure of the complex at 2.4 Å resolution and mutational analysis of the interface. *Structure*, 6, 1153-1167.

Muller, Y. A., Christinger, H. W., Keyt, B. A. & de Vos, A. M. (1998b). The crystal structure of vascular endothelial growth factor (VEGF) refined to 1.93 Å resolution: multiple copy flexibility and receptor binding. *Structure*, 5, 1325-1338.

Munson, P. & Rodbard, D. (1980). Ligand: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* 107, 220-239.

Nezlin, R. (1998). *The Immunoglobulins: Structure and Function*, pp. 151-204, Academic Press, San Diego.

Presta, L. G., Chen, H., O'Connor, S. J., Chisholm, V., Meng, Y. G., Krummen, L., Winkler, M. & Ferrara, N. (1997). Humanization of a vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. *Cancer Res.* 47, 4593-4599.

Rader, C. & Barbas, C. F., III (1997). Phage display of combinatorial antibody libraries. *Curr. Opin. Biotechnol.* 8, 503-508.

Schier, R., McCall, A., Adams, G. P., Marshall, K. W., Merritt, H., Yim, M., Crawford, R. S., Weiner, L. M., Marks, C. & Marks, J. D. (1996). Isolation of picomolar affinity anti-c-erbB-2 single-chain Fv by molecular evolution of the complementarity

determining regions in the center of the antibody binding site. *J. Mol. Biol.* **263**, 551-567.

Thompson, J., Pope, T., Tung, J.-S., Chan, C., Hollis, G., Mark, G. & Johnson, K. S. (1996). Affinity maturation of a high-affinity human monoclonal antibody against the third hypervariable loop of human immunodeficiency virus: use of phage display to improve affinity and broaden strain reactivity. *J. Mol. Biol.* **16**, 77-88.

Tramontano, A., Chothia, C. & Lesk, A. M. (1990). Framework residue 71 is a major determinant of the position and conformation of the second hypervariable region in the VH domains of immunoglobulins. *J. Mol. Biol.* **215**, 175-182.

Wells, J. A. (1990). Additivity of mutational effects in proteins. *Biochemistry*, **29**, 8509-8517.

Wu, H., Beuerlein, G., Nie, Y., Smith, H., Lee, B. A., Hensler, M., Huse, W. D. & Watkins, J. D. (1998). Stepwise *in vitro* affinity maturation of vitaxin, an $\alpha_v\beta_3$-specific humanized mAb. *Proc. Natl Acad. Sci. USA*, **95**, 6037-6042.

Xiang, J., Sha, Y., Jia, Z., Prasad, L. & Delbaere, L. T. (1995). Framework residues 71 and 93 of the chimeric B72.3 antibody are major determinants of the conformation of heavy-chain hypervariable loops. *J. Mol. Biol.* **253**, 385-390.

Yang, W.-P., Green, K., Pinz-Sweeney, S., Briones, A. T., Burton, D. R. & Barbas, C. F., III (1995). CDR walking mutagenesis for the affinity maturation of a potent human anti-HIV-1 antibody into the picomolar range. *J. Mol. Biol.* **254**, 392-403.

Edited by I. A. Wilson

(Received 19 July 1999; received in revised form 7 September 1999; accepted 13 September 1999)

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
1401 Rockville Pike
Rockville MD 20852-1448

rhuFab VEGF

Our Reference: BB-IND 8633

OCT 13 1999

21579

Genentech, Incorporated
Attention: Robert L. Garnick, Ph.D.
Vice President, Regulatory Affairs
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

The Center for Biologics Evaluation and Research has received your **Investigational New Drug Application (IND)**. The following product name and BB-IND number have been assigned to this application. They serve only to identify it and do not imply that this Center either endorses or does not endorse your application.

BB-IND #: 8633

SPONSOR: Genentech, Incorporated

PRODUCT NAME: Humanized Monoclonal Antibody Fragment (rhuFab V2) (E. coli, Genentech) to Vascular Endothelial Growth Factor (VEGF), Intravitreal

DATE OF SUBMISSION: October 6, 1999

DATE OF RECEIPT: October 7, 1999

This BB-IND number should be used to identify all future correspondence and submissions, as well as telephone inquiries concerning this IND. Please provide an **original and two copies of every submission to this file**. Please include three originals of all illustrations which do not reproduce well.

It is understood that studies in humans will not be initiated until 30 days after the date of receipt shown above. If this office notifies you, verbally or in writing, of serious deficiencies that require correction before human studies can begin, it is understood that you will continue to withhold such studies until you are notified that the material you have submitted to correct the deficiencies is satisfactory. If such a clinical hold is placed on this file, you will be notified in writing of the reasons for placing the IND on hold.

10-18-99 P02:54 IN
10-18-99 P

You are responsible for compliance with applicable portions of the Public Health Service Act, the Federal Food, Drug, and Cosmetic Act, and the Code of Federal Regulations (CFR). A copy of 21 CFR Part 312, pertaining to INDs, is enclosed. Copies of other pertinent regulations are available from this Center upon request. The following points regarding obligations of an IND sponsor are included for your information only, and are not intended to be comprehensive.

Progress reports are required at intervals not exceeding one year and are due within 60 days of the anniversary of the date that the IND went into effect [21 CFR 312.33]. Any unexpected, fatal or immediately life-threatening reaction associated with use of this product must be reported to this Division by telephone or facsimile transmission no later than seven calendar days after initial receipt of the information, and all serious, unexpected adverse experiences must be reported, in writing, to this Division and to all study centers within fifteen calendar days after initial receipt of this information [21 CFR 312.32].

Charging for an investigational product in a clinical trial under an IND is not permitted without the prior written approval of the FDA.

Prior to use of each new lot of the investigational biologic in clinical trials, please submit the lot number, the results of all tests performed on the lot, and the specifications when established (i.e., the range of acceptable results).

If not included in your submission, please provide copies of the consent forms for each clinical study. A copy of the requirements for and elements of informed consent are enclosed. Also, please provide documentation of the institutional review board approval(s) for each clinical study.

All laboratory or animal studies intended to support the safety of this product should be conducted in compliance with the regulations for "Good Laboratory Practice for Nonclinical Laboratory Studies" (21 CFR Part 58, copies available upon request). If such studies have not been conducted in compliance with these regulations, please provide a statement describing in detail all differences between the practices used and those required in the regulations.

Item 7a of form FDA 1571 requests that either an "environmental assessment," or a "claim for categorical exclusion" from the requirements for environmental assessment, be included in the IND. If you did not include a response to this item with your application, please submit one. See the enclosed information sheet for additional information on how these requirements may be addressed.

Sponsors of INDs for products used to treat life-threatening or severely debilitating diseases are encouraged to consider the interim rule outlined in 21 CFR 312.80 through 312.88.

Telephone inquiries concerning this IND should be made directly to me at (301) 827-5101. Correspondence regarding this file should be addressed as follows:

Center for Biologics Evaluation and Research
Attn: Office of Therapeutics Research and Review
HFM-99, Room 200N
1401 Rockville Pike
Rockville, MD 20852-1448

If we have any comments after we have reviewed this submission, we will contact you.

Sincerely yours,

Kay Schneider

Kay Schneider, M.S.
Consumer Safety Officer
Division of Application Review and Policy
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosures (3): 21 CFR Part 312
21 CFR 50.20, 50.25
Information sheet on 21 CFR 25.24

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

JAN 27 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality, and Compliance
1 DNA Way
South San Francisco, CA 94080-4990

Dear Dr. Garnick:

We have received your biologics license application (BLA) submitted under section 351 of the Public Health Service Act for the following biological product:

Our Submission Tracking Number (STN): BL #125156/0

Name of Biological Product: Lucentis™ (ranibizumab)

Indication: Treatment for patients with neovascular age-related macular degeneration

Date of Application: December 29, 2005

Date of Receipt: December 30, 2005

User Fee Goal Date: June 30, 2006

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We note that you have not fulfilled the requirement. We are waiving the requirement for pediatric studies for this application.

If you have not already done so, promptly submit the *content of labeling* (21 CFR 601.14(b)) in electronic format as described at the following website:
http://www.fda.gov/oc/datacouncil/spl.html.

We will notify you within 60 days of the receipt date if the application is sufficiently complete to permit a substantive review.

We request that you submit all future correspondence, supporting data, or labeling relating to this application in triplicate, citing the above STN number. Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions. Effective August 29, 2005, the new address for all submissions to this application is:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, please contact the Regulatory Project Manager, Lori Gorski, at (301) 796-0722.

Sincerely,

Maureen P. Dillon-Parker
Chief, Project Management Staff
Division of Anti-Infective and
Ophthalmology Products
Office of Antimicrobials
Center for Drug Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville, MD 20852

BLA 125156

MAR 14 2006

Genentech, Inc.
Attention: Robert L. Garnick, Ph.D.
Senior Vice President, Regulatory Affairs, Quality & Compliance
1 DNA Way
South San Francisco, California 94080-4990

Dear Dr. Garnick:

This letter is in regard to your biologics license application (BLA) submitted under section 351 of the Public Health Service Act.

We have completed an initial review of your application dated December 29, 2005, for Lucentis (ranibizumab injection) to determine its acceptability for filing. Under 21 CFR 601.2(a), your application was filed on February 28, 2006. The user fee goal date is June 30, 2006. This acknowledgment of filing does not mean that we have issued a license nor does it represent any evaluation of the adequacy of the data submitted.

At this time, we have not identified any potential review issues. Our filing review is only a preliminary review, and deficiencies may be identified during substantive review of your application. Following a review of the application, we shall advise you in writing of any action we have taken and request additional information if needed.

Please refer to http://www.fda.gov/cder/biologics/default.htm for important information regarding therapeutic biological products, including the addresses for submissions.

Please use the following address for any amendments to your application:

Food and Drug Administration
Center for Drug Evaluation and Research
Therapeutic Biological Products Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266

If you have any questions, call Lori M. Gorski, Project Manager, at (301) 796-0722.

Sincerely,

Maureen Dillon Parker
Chief, Project Management Staff
Division of Anti-Infective and Ophthalmology Products
Office of Antimicrobial Products
Center for Drug Evaluation and Research

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent of: Manuel Baca *et al.* – § 156	Docket No: 22338-80060
Patent No.: 7,060,269	Assignee: Genentech, Inc.
Issued: June 13, 2006	Unit: OPLA; Attn: K. Ferriter
Application No: 09/723,752	

For: ANTI-VEGF ANTIBODIES – Application for § 156 Patent Term Extenstion

Mail Stop: **Patent Ext.**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

POWER OF ATTORNEY BY ASSIGNEE

The assignee of the entire right, title, and interest in U.S. Patent No. 7,060,269 (granted on application serial no. 09/723,752), Genentech, Inc., hereby appoints the practitioners associated with

CUSTOMER NUMBER 33694

as its attorneys and agents to prosecute the captioned patent/application, and to transact all business in the U.S. Patent and Trademark Office connected therewith.

Pursuant to 37 C.F.R. § 3.73(b), the undersigned states that Genentech, Inc. is the assignee of the entire right, title, and interest in the captioned patent/application by virtue of an assignment by the inventors to Genentech Inc. recorded at Reel 008872/ Frame 0429.

The undersigned, whose title is supplied below, is authorized to act on behalf of the assignee.

Respectfully submitted,

GENENTECH, INC.

AK 08-15-06

Jeffrey S. Kubinec Date
Associate General Counsel – Patent Law

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
09/723,752	11/27/2000	Manuel Baca	P1093P1D1

CONFIRMATION NO. 6340

OC000000020339644

9157
GENENTECH, INC.
1 DNA WAY
SOUTH SAN FRANCISCO, CA 94080

Date Mailed: 09/07/2006

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/25/2006.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

SHARON KUANG
PTOSS (703) 305-3006

OFFICE COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
09/723,752	11/27/2000	Manuel Baca	22338-80060

CONFIRMATION NO. 6340

OC000000020339784

33694
SIDLEY AUSTIN LLP
ATTN: DC PATENT DOCKETING
1501 K STREET, N.W.
WASHINGTON, DC 20005

Date Mailed: 09/07/2006

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 08/25/2006.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

SHARON KUANG
PTOSS (703) 305-3006

OFFICE COPY

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

APR - 3 2007

Office of Regulatory Policy
HFD-7
5600 Fishers Lane (Rockwall II Rm 1101)
Rockville, MD 20857

Attention: Beverly Friedman

The attached application for patent term extension of U.S. Patent No. 7,060,269 was filed on August 25, 2006, under 35 U.S.C. § 156.

The assistance of your Office is requested in confirming that the product identified in the application, LUCENTIS® (ranibizumab), has been subject to a regulatory review period within the meaning of 35 U.S.C. § 156(g) before its first commercial marketing or use and that the application for patent term extension was filed within the sixty-day period set forth in 35 U.S.C. § 156(d)(1). Since a determination has not been made whether the patent in question claims a product which has been subject to the Federal Food, Drug and Cosmetic Act, or a method of manufacturing or use of such a product, this communication is NOT to be considered as notice which may be made in the future pursuant to 35 U.S.C. § 156(d)(2)(A).

Our review of the application to date indicates that the subject patent would be eligible for extension of the patent term under 35 U.S.C. § 156.

Applicant is advised that despite the statement regarding submission of a check in paragraph 14 on page 12 of the application of August 25, 2006, the Office has no record of having received or cashed such a check. Therefore, in accordance with the authorization provided in the same paragraph, the fee of $1120.00 prescribed in 37 CFR 1.20(j) is being charged to deposit account number 18-1260.

Inquiries regarding this communication should be directed to the undersigned at (571) 272-7754 (telephone) or (571) 273-7754 (facsimile).

Kathleen Kahler Fonda
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Jeffrey P. Kushan
Sidley Austin LLP
1501 K Street, NW
Washington DC 20005

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

JUL 24 2007

Food and Drug Administration
Rockville MD 20857

Re: Lucentis
Docket No. 2007E-0146

The Honorable Jon Dudas
Under Secretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the application for patent term extension for U.S. Patent No. 7,060,269 filed by Genentech, Inc. under 35 U.S.C. § 156. The human biological product claimed by the patent is Lucentis (ranibizumab), which was assigned biologics license application (BLA) No. 125156/0.

A review of the Food and Drug Administration's official records indicates that this product was subject to a regulatory review period before its commercial marketing or use, as required under 35 U.S.C. § 156(a)(4). Our records also indicate that it represents the first permitted commercial marketing or use of the product, as defined under 35 U.S.C. § 156(f)(1), and interpreted by the courts in *Glaxo Operations UK Ltd. v. Quigg*, 706 F. Supp. 1224 (E.D. Va. 1989), *aff'd*, 894 F. 2d 392 (Fed. Cir. 1990).

The BLA was approved on June 30, 2006, which makes the submission of the patent term extension application on August 25, 2006, timely within the meaning of 35 U.S.C. § 156(d)(1).

Should you conclude that the subject patent is eligible for patent term extension, please advise us accordingly. As required by 35 U.S.C. § 156(d)(2)(A) we will then determine the applicable regulatory review period, publish the determination in the *Federal Register*, and notify you of our determination.

Please let me know if we can be of further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

JAN - 8 2008

Office of Regulatory Policy
HFD - 7
5600 Fishers Lane (Rockwall II Rm. 1101)
Rockville, MD 20857

Attention: Beverly Friedman

Dear Ms. Axelrad:

Transmitted herewith is a copy of the application for patent term extension of U.S. Patent No. 7,060,269. The application was filed on August 25, 2006, under 35 U.S.C. § 156. It is noted that patent term extension applications for the same regulatory review period for the human biological product, LUCENTIS® (ranibizumab), have been filed in U.S. Patent Nos. 6,407,213 and 6,884,879.

The patent claims a product that was subject to regulatory review under the Federal Food, Drug and Cosmetic Act. Subject to final review, the subject patent is considered to be eligible for patent term extension. Thus, a determination by your office of the applicable regulatory review period is necessary. Accordingly, notice and a copy of the application are provided pursuant to 35 U.S.C. § 156(d)(2)(A).

Inquiries regarding this communication should be directed to the undersigned at (571)272-7755 (telephone) or (571) 273-7755 (facsimile).

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

RE: LUCENTIS® (ranibizumab)
FDA Docket No. 2007E-0146

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

APR 28 2008

Re: LUCENTIS - 6,407,213
Docket No.: 2007E-0424
LUCENTIS - 6,884,879
Docket No.: 2007E-0425
LUCENTIS - 7,060,269
Docket No.: 2007E-0146

The Honorable Jon Dudas
Undersecretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the applications for patent term extension for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269, filed by Genentech, Inc., under 35 U.S.C. section 156 et seq. We have reviewed the dates contained in the application and have determined the regulatory review period for LUCENTIS (ranibizumab), the human biological product claimed by the patents.

The total length of the regulatory review period for LUCENTIS is 2,430 days. Of this time, 2,247 days occurred during the testing phase and 183 days occurred during the approval phase. These periods of time were derived from the following dates:

1. The date an exemption under subsection 505(i) of the Federal Food, Drug, and Cosmetic Act involving this biologic product became effective: November 6, 1999.

   The applicant claims October 7, 1999, as the date the investigational new drug application (IND) became effective. However, FDA records indicate that the IND effective date was November 6, 1999, which was thirty days after FDA receipt of the IND.

2. The date the application was initially submitted with respect to the human biological product under section 351 of the Public Health Service Act: December 30, 2005.

   The applicant claims December 29, 2005, as the date the biologics license application (BLA) for LUCENTIS (BLA 125156/0) was initially submitted. However, FDA records indicate that BLA 125156/0 was submitted on December 30, 2005.

3. The date the application was approved: June 30, 2006.

   FDA has verified the applicant's claim that BLA 125156/0 was approved on June 30, 2006.

This determination of the regulatory review period by FDA does not take into account the effective date of the patents, nor does it exclude one-half of the testing phase as required by 35 U.S.C. section 156(c)(2).

Please let me know if we can be of further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

a person with Medicare could be identified because the sample is small enough to identify participants. CMS would make exceptions if the information is needed for one of the routine uses or if it's required by law.

POLICIES AND PRACTICES FOR STORING, RETRIEVING, ACCESSING, RETAINING, AND DISPOSING OF RECORDS IN THE SYSTEM:

STORAGE:

Records are stored on both tape cartridges (magnetic storage media) and in a DB2 relational database management environment (DASD data storage media).

RETRIEVABILITY:

Information is most frequently retrieved by HICN, provider number (facility, physician, IDs), service dates, and beneficiary state code.

SAFEGUARDS AND PROTECTIONS:

CMS has protections in place for authorized users to make sure they are properly using the data and there is no unauthorized use. Personnel having access to the system have been trained in the Privacy Act and information security requirements. Employees who maintain records in this system cannot use or disclose data until the recipient agrees to implement appropriate management, operational and technical safeguards that will protect the confidentiality, integrity, and availability of the information and information systems.

This system would follow all applicable Federal laws and regulations, and Federal, HHS, and CMS security and data privacy policies and standards. These laws and regulations include but are not limited to: the Privacy Act of 1974; the Federal Information Security Management Act of 2002 (when applicable); the Computer Fraud and Abuse Act of 1986; the Health Insurance Portability and Accountability Act of 1996; the E-Government Act of 2002, the Clinger-Cohen Act of 1996; the Medicare Modernization Act of 2003, and the corresponding implementing regulations. OMB Circular A–130, Management of Federal Resources, Appendix III, Security of Federal Automated Information Resources also applies. Federal, HHS, and CMS policies and standards include but are not limited to all pertinent National Institute of Standards and Technology publications, the HHS Information Systems Program Handbook, and the CMS Information Security Handbook.

RETENTION AND DISPOSAL:

Records are maintained with identifiers for all transactions after they are entered into the system for a period of 20 years. Records are housed in both active and archival files. All claims-related records are encompassed by the document preservation order and will be retained until notification is received from the Department of Justice.

SYSTEM MANAGER AND ADDRESS:

Director, Centers for Beneficiary Choices, CMS, Mail stop C5–19–07, 7500 Security Boulevard, Baltimore, Maryland 21244–1850.

NOTIFICATION PROCEDURE:

For purpose of notification, the subject individual should write to the system manager who will require the system name, and the retrieval selection criteria (e.g., HICN, facility/pharmacy number, service dates, etc.).

RECORD ACCESS PROCEDURE:

For purpose of access, use the same procedures outlined in Notification Procedures above. Requestors should also reasonably specify the record contents being sought. (These procedures are in accordance with Department regulation 45 CFR 5b.5 (a)(2).)

CONTESTING RECORD PROCEDURES:

The subject individual should contact the system manager named above, and reasonably identify the record and specify the information to be contested. State the corrective action sought and the reasons for the correction with supporting justification. (These procedures are in accordance with Department regulation 45 CFR 5b.7.)

RECORD SOURCE CATEGORIES:

Summary prescription drug claim information contained in this system is obtained from the Part D Sponsor daily and monthly drug event transaction reports, Medicare Beneficiary Database (09–70–0530), and other payer information to be provided by the TROOP Facilitator.

SYSTEMS EXEMPTED FROM CERTAIN PROVISIONS OF THE ACT:

None.

[FR Doc. E8–11949 Filed 5–28–08; 8:45 am]
BILLING CODE 4120–03–P

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket Nos. FDA–2007–E–0461 (formerly Docket No. 2007E–0424), FDA–2007–E–0165 (formerly Docket No. 2007E–0425), FDA–2007–E–0459 (formerly Docket No. 2007E–0146)]

Determination of Regulatory Review Period for Purposes of Patent Extension; LUCENTIS

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) has determined the regulatory review period for LUCENTIS and is publishing this notice of that determination as required by law. FDA has made the determination because of the submission of applications to the Director of Patents and Trademarks, Department of Commerce, for the extension of patents which claim that human biological product.

ADDRESSES: Submit written or electronic comments and petitions to the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Submit electronic comments to *http://www.regulations.gov*.

FOR FURTHER INFORMATION CONTACT: Beverly Friedman, Center for Drug Evaluation and Research, Food and Drug Administration, 10903 New Hampshire Ave., Bldg. 51, rm. 6222, Silver Spring, MD, 20993–0002, 301–796–3602.

SUPPLEMENTARY INFORMATION: The Drug Price Competition and Patent Term Restoration Act of 1984 (Public Law 98–417) and the Generic Animal Drug and Patent Term Restoration Act (Public Law 100–670) generally provide that a patent may be extended for a period of up to 5 years so long as the patented item (human drug product, animal drug product, medical device, food additive, or color additive) was subject to regulatory review by FDA before the item was marketed. Under these acts, a product's regulatory review period forms the basis for determining the amount of extension an applicant may receive.

A regulatory review period consists of two periods of time: A testing phase and an approval phase. For human biological products, the testing phase begins when the exemption to permit the clinical investigations of the biological product becomes effective

and runs until the approval phase begins. The approval phase starts with the initial submission of an application to market the human biological product and continues until FDA grants permission to market the biological product. Although only a portion of a regulatory review period may count toward the actual amount of extension that the Director of Patents and Trademarks may award (for example, half the testing phase must be subtracted as well as any time that may have occurred before the patent was issued), FDA's determination of the length of a regulatory review period for a human biological product will include all of the testing phase and approval phase as specified in 35 U.S.C. 156(g)(1)(B).

FDA recently approved for marketing the human biologic product LUCENTIS (ranibizumab). LUCENTIS is indicated for the treatment of patients with neovascular (wet) age-related macular degeneration. Subsequent to this approval, the Patent and Trademark Office received patent term restoration applications for LUCENTIS (U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269) from Genentech, Inc., and the Patent and Trademark Office requested FDA's assistance in determining this patent's eligibility for patent term restoration. In letters dated July 24, 2007, and November 21, 2007, FDA advised the Patent and Trademark Office that this human biological product had undergone a regulatory review period and that the approval of LUCENTIS represented the first permitted commercial marketing or use of the product. Shortly thereafter, the Patent and Trademark Office requested that FDA determine the product's regulatory review period.

FDA has determined that the applicable regulatory review period for LUCENTIS is 2,430 days. Of this time, 2,247 days occurred during the testing phase of the regulatory review period, while 183 days occurred during the approval phase. These periods of time were derived from the following dates:

1. *The date an exemption under section 505(i) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 355(i)) became effective*: November 6, 1999. The applicant claims October 7, 1999, as the date the investigational new drug application (IND) became effective. However, FDA records indicate that the IND effective date was November 6, 1999, which was 30 days after FDA receipt of the IND.

2. *The date the application was initially submitted with respect to the human biological product under section 351 of the Public Health Service Act (42 U.S.C. 262)*: December 30, 2005. The applicant claims December 29, 2005, as the date the biologics license application (BLA) for LUCENTIS (BLA 125156/0) was initially submitted. However, FDA records indicate that BLA 125156/0 was submitted on December 30, 2005.

3. *The date the application was approved*: June 30, 2006. FDA has verified the applicant's claim that BLA 125156/0 was approved on June 30, 2006.

This determination of the regulatory review period establishes the maximum potential length of a patent extension. However, the U.S. Patent and Trademark Office applies several statutory limitations in its calculations of the actual period for patent extension. In its applications for patent extension for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269, this applicant seeks 378 days; 307 days or 17 days, respectively, of patent term extension.

Anyone with knowledge that any of the dates as published are incorrect may submit to the Division of Dockets Management (see **ADDRESSES**) written or electronic comments and ask for a redetermination by July 28, 2008. Furthermore, any interested person may petition FDA for a determination regarding whether the applicant for extension acted with due diligence during the regulatory review period by November 25, 2008. To meet its burden, the petition must contain sufficient facts to merit an FDA investigation. (See H. Rept. 857, part 1, 98th Cong., 2d sess., pp. 41–42, 1984.) Petitions should be in the format specified in 21 CFR 10.30.

Comments and petitions should be submitted to the Division of Dockets Management. Three copies of any mailed information are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. Comments and petitions may be seen in the Division of Dockets Management between 9 a.m. and 4 p.m., Monday through Friday.

Please note that on January 15, 2008, the FDA Division of Dockets Management Web site transitioned to the Federal Dockets Management System (FDMS). FDMS is a Government-wide, electronic docket management system. Electronic comments or submissions will be accepted by FDA only through FDMS at *http://www.regulations.gov*.

Dated: May 8, 2008.

Jane A. Axelrad,

Associate Director for Policy, Center for Drug Evaluation and Research.

[FR Doc. E8–12007 Filed 5–28–08; 8:45 am]

BILLING CODE 4160–01–S

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

[Docket No. FDA–2007–M–0467] (formerly Docket No. 2007M–0408), [Docket No. FDA–2007–M–0481] (formerly Docket No. 2007M–0467), [Docket No. FDA–2007–M–0480] (formerly Docket No. 2007M–0409), [Docket No. FDA–2007–M–0472] (formerly Docket No. 2007M–0413), [Docket No. FDA–2007–M–0468] (formerly Docket No. 2007M–0446), [Docket No. FDA–2007–M–0494] (formerly Docket No. 2007M–0380), [Docket No. FDA–2007–M–0493] (formerly Docket No. 2007M–0411), [Docket No. FDA–2007–M–0492] (formerly Docket No. 2007M–0410), [Docket No. FDA–2007–M–0490] (formerly Docket No. 2007M–0415), [Docket No. FDA–2007–M–0491] (formerly Docket No. 2007M–0447]

Medical Devices; Availability of Safety and Effectiveness Summaries for Premarket Approval Applications

AGENCY: Food and Drug Administration, HHS.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is publishing a list of premarket approval applications (PMAs) that have been approved. This list is intended to inform the public of the availability of safety and effectiveness summaries of approved PMAs through the Internet and the agency's Division of Dockets Management.

ADDRESSES: Submit written requests for copies of summaries of safety and effectiveness data to the Division of Dockets Management (HFA–305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Please cite the appropriate docket number as listed in Table 1 of this document when submitting a written request. See the **SUPPLEMENTARY INFORMATION** section for electronic access to the summaries of safety and effectiveness.

FOR FURTHER INFORMATION CONTACT: Samie Allen, Center for Devices and Radiological Health (HFZ–402), Food and Drug Administration, 9200 Corporate Blvd., Rockville, MD 20850, 240–276–4013.

SUPPLEMENTARY INFORMATION:

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration
Rockville MD 20857

JAN 8 2009

Re: Lucentis
Docket Nos.: FDA-2007-E-0461
FDA-2007-E-0165
FDA-2007-E-0459

The Honorable Jon Dudas
Under Secretary of Commerce for Intellectual Property
Director of the United States Patent and Trademark Office
Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Director Dudas:

This is in regard to the patent term extension applications for U.S. Patent Nos. 6,407,213; 6,884,879; and 7,060,269 filed by Genentech, Inc., under 35 U.S.C. § 156. The patent claims Lucentis (ranibizumab), biologic license application (BLA) 125156/0.

In the May 29, 2008, issue of the Federal Register (73 Fed. Reg. 30949), the Food and Drug Administration published its determination of this product's regulatory review period, as required under 35 U.S.C. § 156(d)(2)(A). The notice provided that on or before November 25, 2008, 180 days after the publication of the determination, any interested person could file a petition with FDA under 35 U.S.C. § 156(d)(2)(B)(i) for a determination of whether the patent term extension applicant acted with due diligence during the regulatory review period.

The 180-day period for filing a due diligence petition pursuant to this notice has expired and FDA has received no such petition. Therefore, FDA considers the regulatory review period determination to be final.

Please let me know if we can provide further assistance.

Sincerely yours,

Jane A. Axelrad

Jane A. Axelrad
Associate Director for Policy
Center for Drug Evaluation and Research

cc: Jeffrey P. Kushan
SIDLEY AUSTIN LLP
1501 K Street, N.W.
Washington, DC 20005

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

MAR 26 2009

Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

In Re: Patent Term Extension
Application for
U.S. Patent No. 7,060,269

NOTICE OF FINAL DETERMINATION AND REQUIREMENT FOR ELECTION

A determination has been made that U.S. Patent No. 7,060,269, claims of which cover a method of using LUCENTIS® (ranibizumab), is eligible for patent term extension under 35 U.S.C. § 156. The period of extension has been determined to be 17 days.

A single request for reconsideration of this final determination as to the length of extension of the term of the patent may be made if filed within one month of the date of this notice. Extensions of time under 37 CFR § 1.136(a) are not applicable to this time period.

Applicant also has applied for patent term extension of U.S. Patent No. 6,407,213 and U.S. Patent No. 6,884,879 based on the regulatory review period for the human biologic drug product LUCENTIS® (ranibizumab).

When patent term extension applications are filed for extension of the terms of different patents based upon the same regulatory review period for a product, the certificate of extension is issued to the patent having the earliest date of issuance, unless applicant elects a different patent. In the absence of an election by applicant within ONE MONTH of the date of this notice, and in accordance with 37 CFR 1.785(b), the applications for patent term extension of U.S. Patent No. 6,884,879 and U.S. Patent No. 7,060,269 will be denied. Accordingly, the application for patent term extension of the patent having the earlier date of issuance will be granted, i.e., a certificate of extension will be issued to U.S. Patent No 6,407,213 for a period of 378 days.

In the absence of a request for reconsideration, and if U.S. Patent No. 7,060,269 is elected, the Director will issue to the applicant a certificate of extension, under seal, for a period of 17 days in U.S. Patent No. 7,060,269.

The period of extension has been calculated using the Food and Drug Administration determination of the length of the regulatory review period published in the Federal Register of May 29, 2008 (73 Fed. Reg. 30949). Under 35 U.S.C. § 156(c):

Period of Extension	=	½ (Testing Phase) + Approval Phase
	=	½ (2,247 days - 2,247 days) + (183 days - 166 days)
	=	17 days

Since the regulatory review period began November 6, 1999, before the patent issued June 13, 2006), only that portion of the regulatory review period occurring after the date the patent issued has been considered in the above determination of the length of the extension period 35 U.S.C. § 156(c). (From November 6, 1999, to and including, June 13, 2006, is 2,403 days (2,247 days in the testing phase and 166 days in the approval phase); this period is subtracted for the number of days occurring in the testing phase and approval phase according to the FDA's determination of the length of the regulatory review period.) No determination of a lack of due diligence under 35 U.S.C. § 156(c)(1) was made.

Neither the limitations of 35 U.S.C. § 156(g)(6) nor 35 U.S.C. § 156(c)(3) operate to reduce the period of extension determined above.

The limitations of 35 U.S.C. 156(g)(6) do not operate to further reduce the period of extension determined above.

Upon issuance of the certificate of extension, the following information will be published in the Official Gazette:

U.S. Patent No.:	7,060,269
Granted:	June 13, 2006
Original Expiration Date[1]:	July 4, 2019
Applicant:	Manuel Baca et al.
Owner of Record:	Genentech, Inc.
Title:	Anti-VEGF Antibodies
Product Trade Name:	LUCENTIS® (ranibizumab)
Term Extended:	17 days
Expiration Date of Extension:	July 21, 2019

Any correspondence with respect to this matter should be addressed as follows:

By mail: Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450

By FAX: (571) 273-7755

[1]Subject to the provisions of 35 U.S.C. § 41(b).

Alexandria, VA 22313-1450.

Telephone inquiries related to this determination should be directed to the undersigned at (571) 272-7755.

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Office of Regulatory Policy
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 51, Rm. 6222
Silver Spring, MD 20993-0002

Attention: Beverly Friedman

RE: LUCENTIS® (ranibizumab)
Docket No.: FDA-2007-E-0459

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. Patent No.	**7,060,269 – § 156**	Unit:	OPLA
Serial No.:	**09/ 723,752**		
Confirmation No.:	6340		
Filed:	25 August 2006		
First Inventor:	M. BACA		
Patent Owner:	Genentech, Inc.		
For:	Anti-VEGF antibodies Application for patent term extension under 35 U.S.C. § 156		

Mail Stop **Hatch-Waxman PTE**
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

ELECTION UNDER 37 C.F.R. § 1.785(b)

Sir:

This letter responds to the Notice of Final Determination and Requirement for Election mailed in the captioned application for patent term extension on 26 March 2009. The Notice observes that applicant filed applications to extend the terms of U.S. Patent Nos. 6,407,213, 6,884,879, and 7,060,269 based on the regulatory review period for LUCENTIS®. The Notice further states a requirement that applicant elect one of the patents to receive a term extension certificate within a period of one month of the date of the Notice. This election is filed within the stated period and is therefore timely.

Pursuant to § 1.785(b), **applicant elects U.S. Patent No. 6,407,213** to receive a certificate of extension under § 1.780 and 35 U.S.C. § 156(e)(1). Applicant requests that the Director proceed to issue a certificate of extension of that patent based on the regulatory review period for LUCENTIS®, as indicated in the Notice of Final Determination and Requirement for Election issued in the application for patent term extension in the record of U.S. Patent No. 6,407,213.

We believe that no fee is due in respect of this election. However, the Director is requested to debit any fee required for entry or consideration of this paper from our Deposit Account No. 18-1260.

Respectfully submitted,

/David L. Fitzgerald/

David L. Fitzgerald, Reg. No. 47,347
Attorney for Genentech, Inc.

24 April 2009

SIDLEY AUSTIN LLP
1501 K Street, NW
Washington, DC 20005

tel. (202) 736-8818
fax (202) 736-8711

Electronic Acknowledgement Receipt

EFS ID:	5212453
Application Number:	09723752
International Application Number:	
Confirmation Number:	6340
Title of Invention:	ANTI-VEGF ANTIBODIES
First Named Inventor/Applicant Name:	Manuel Baca
Customer Number:	33694
Filer:	David Laurence Fitzgerald
Filer Authorized By:	
Attorney Docket Number:	22338-80060
Receipt Date:	24-APR-2009
Filing Date:	27-NOV-2000
Time Stamp:	10:49:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Lucentis_269_PTE_election.pdf	77545 d8ae604426ae718af20de9d37a2a835a462d5141	no	2

Warnings:	
Information:	

Total Files Size (in bytes):	77545

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111
If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES PATENT AND TRADEMARK OFFICE

Commissioner for Patents
United States Patent and Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450
www.uspto.gov

NOV 18 2009

Jeffrey P. Kushan
Sidley Austin, LLP
1501 K Street, N.W.
Washington, DC 20005

Re: Patent Term Extension
Application for
U.S. Patent No. 7,060,269

WITHDRAWAL OF APPLICATION FOR PATENT TERM EXTENSION

This is in response to the "Election Under 37 C.F.R. § 1.785(b)" filed April 24, 2009, of the application for patent term extension for U.S. Patent No. 6,407,213, (LUCENTIS® (ranibizumab)). The present application for patent term extension for U.S. Patent No. 7,060,269 is withdrawn in favor of the elected patent, U.S. Patent No. 6,407,213.

The present application is hereby withdrawn from consideration and dismissed.

Any correspondence with respect to this matter should be addressed as follows:

By mail: Mail Stop Hatch-Waxman PTE
P.O. Box 1450
Alexandria, VA 22313-1450

Telephone inquiries related to this determination should be directed to the undersigned at (571) 272-7755. E-mail inquiries should be directed to mary.till@uspto.gov.

Mary C. Till
Legal Advisor
Office of Patent Legal Administration
Office of the Deputy Commissioner
for Patent Examination Policy

cc: Office of Regulatory Policy
Food and Drug Administration
10903 New Hampshire Ave., Bldg. 51, Rm. 6222
Silver Spring, MD 20993-0002

Attention: Beverly Friedman

RE: LUCENTIS® (ranibizumab)
FDA Docket No.: FDA-2007-E-0459