

# United States Patent [19]

## Tsuchiya et al.

## [54] RESHAPED HUMAN TO HUMAN INTERLEUKIN-6 RECEPTOR

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   Japan
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   Feb. 19, 1992
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   Japan
   4-032084
- [51] Int. Cl.<sup>6</sup> ...... C07K 16/00
- [52] U.S. Cl. ...... 530/387.3; 530/388.24;
- 530/388.73
- [58] Field of Search ...... 530/387.3, 388.22. 530/388.73

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# [11] Patent Number: 5,795,965

## [45] Date of Patent: Aug. 18, 1998

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Primary Examiner—Lila Feisee Assistant Examiner—Geetha P. Bansal Attorney, Agent, or Firm—Foley & Lardner

## [57] ABSTRACT

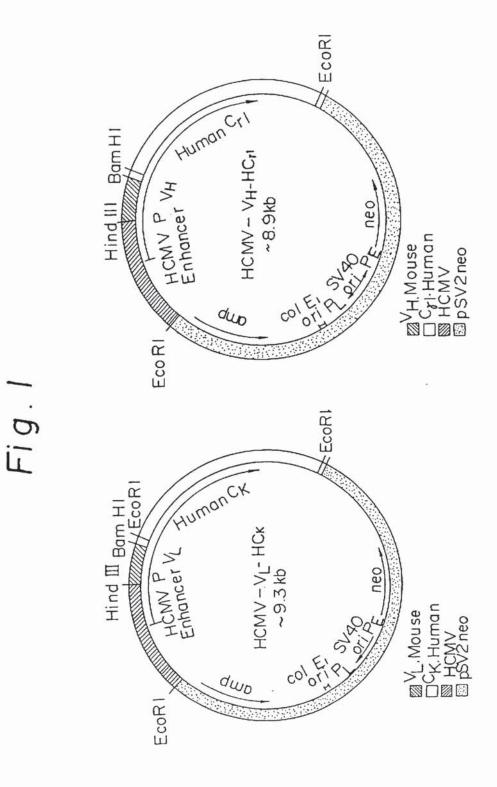
A reshaped human antibody to the human IL-6R, comprising:

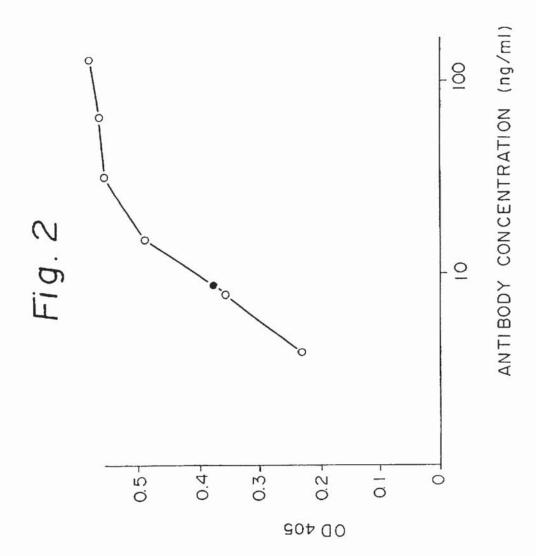
(A) an L chain comprising.

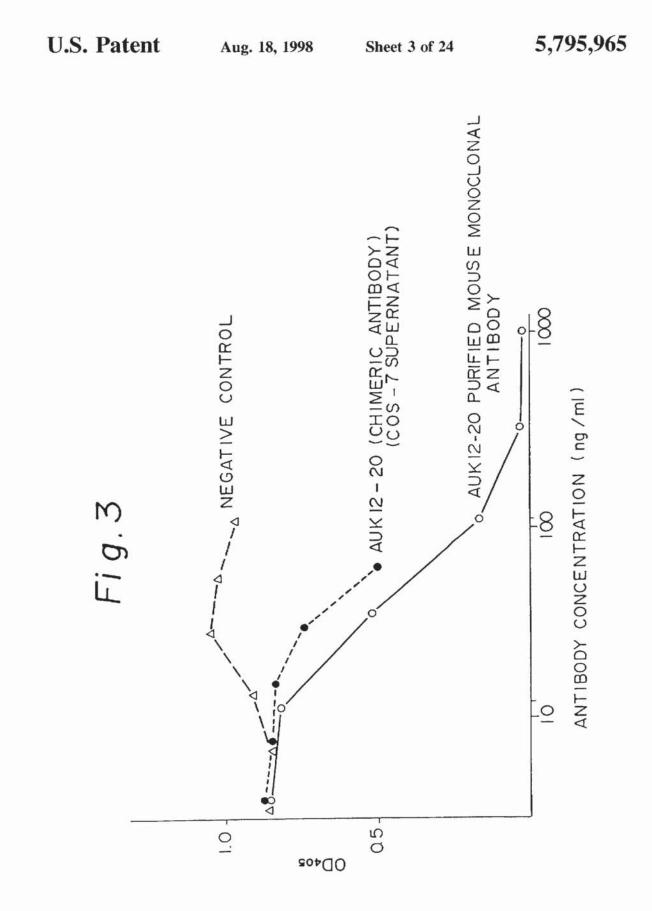
- (1) a human L chain C region. and
- (2) an L chain V region comprising human L chain framework regions (FRs), and mouse L chain complementary determination regions (CDRS) of a momoclonal antibody to the IL-6 receptor (IL-6R); and
- (B) an H chain comprising,
  - (1) a human H chain C region, and
  - (2) an H chain V region comprising human H chain FRs. and mouse H chain CDRs of a monoclonal antibody to the IL-6R.

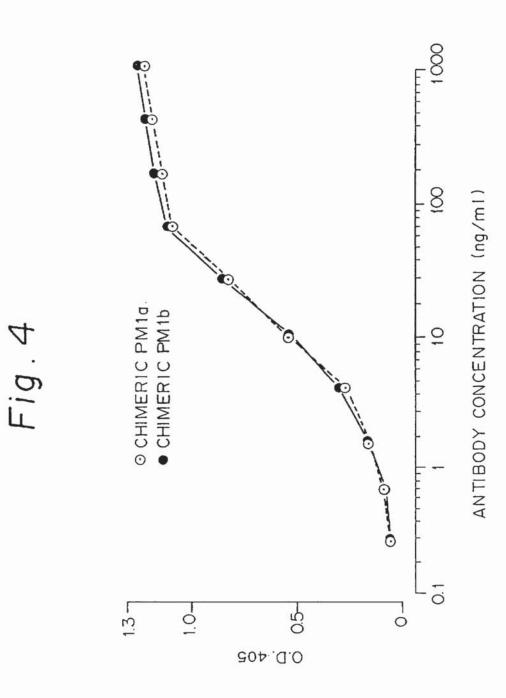
Since major portion of the reshaped human antibody is derived from a human antibody and the mouse CDRs which are less immunogenic, the present reshaped human antibody is less immunogenic to human, and therefor is promised for therapeutic uses.

## 6 Claims, 24 Drawing Sheets

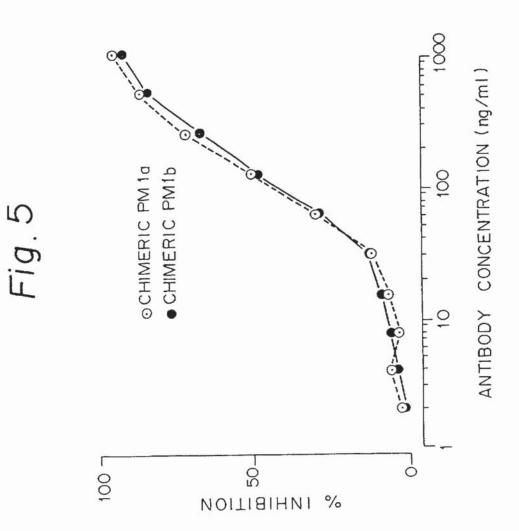












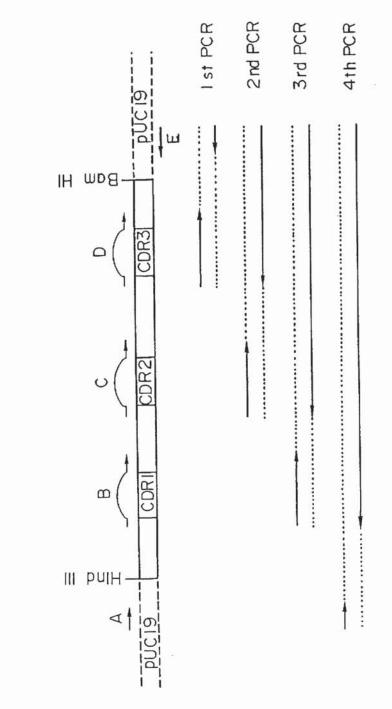
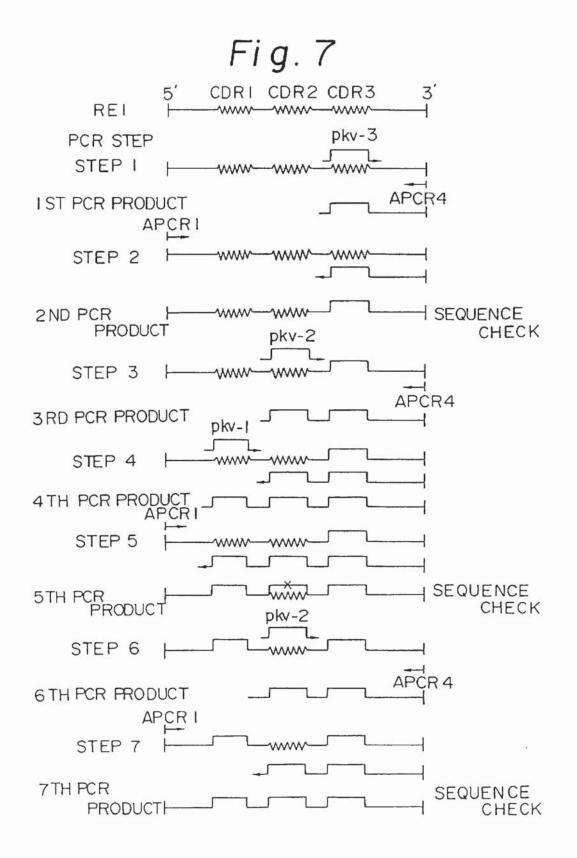
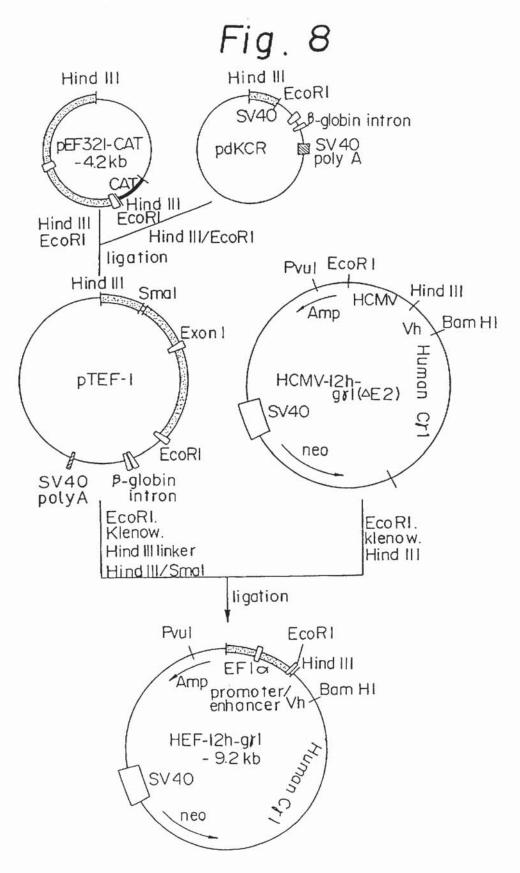
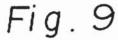
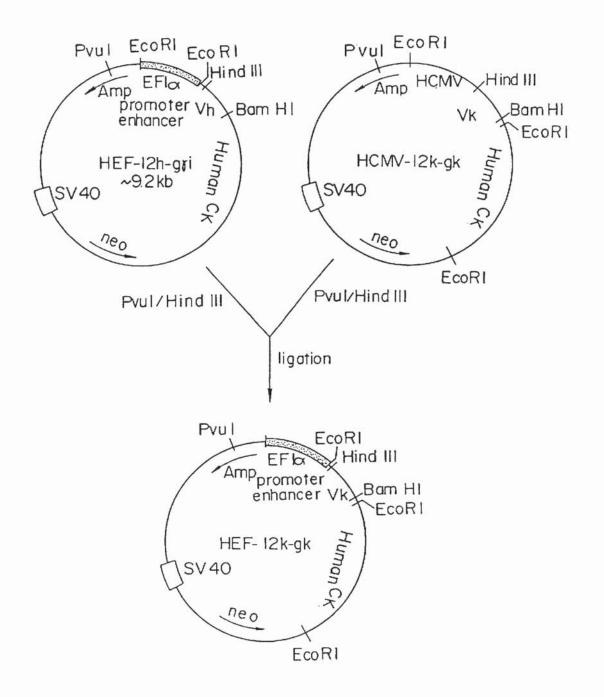


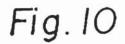
Fig. 6

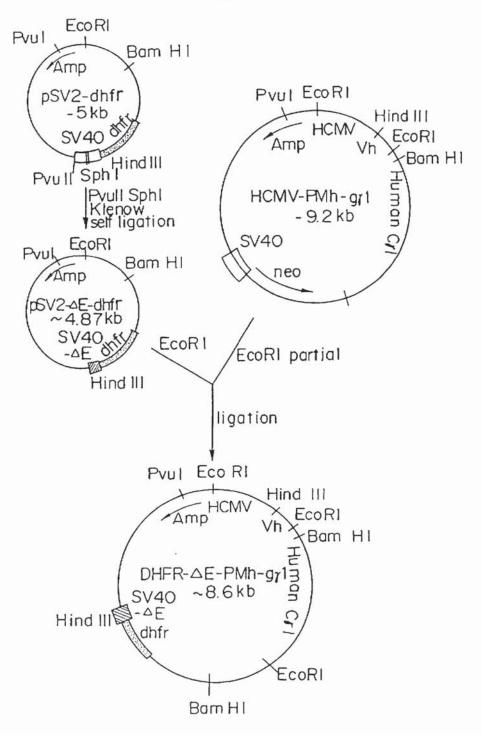




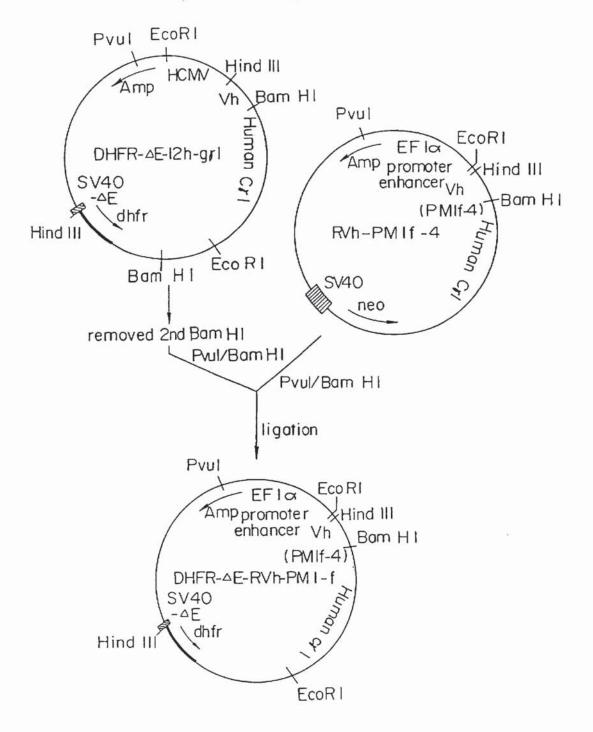












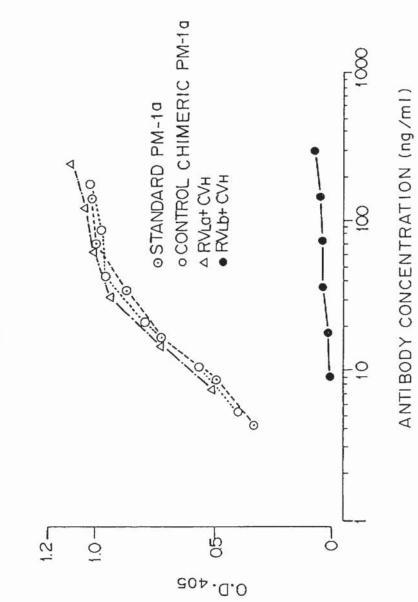
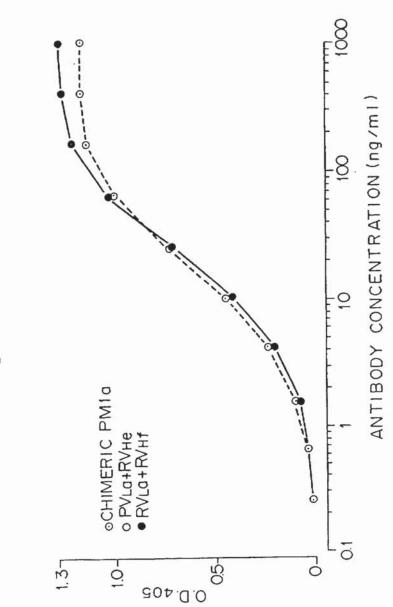
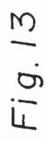


Fig. 12





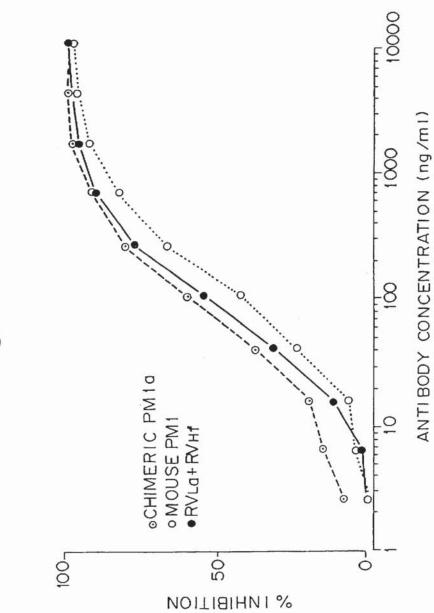


Fig . 14

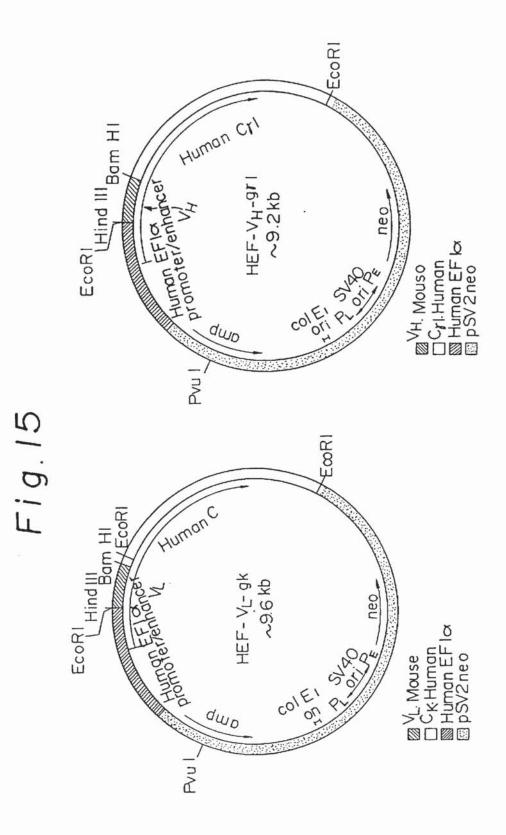
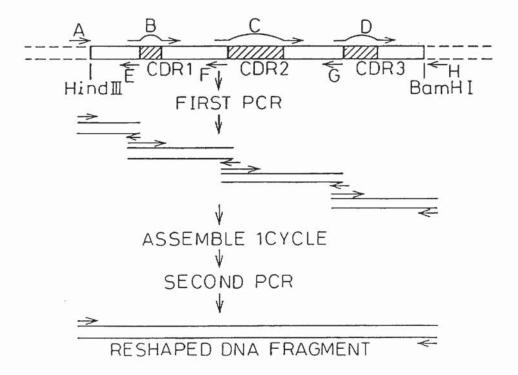
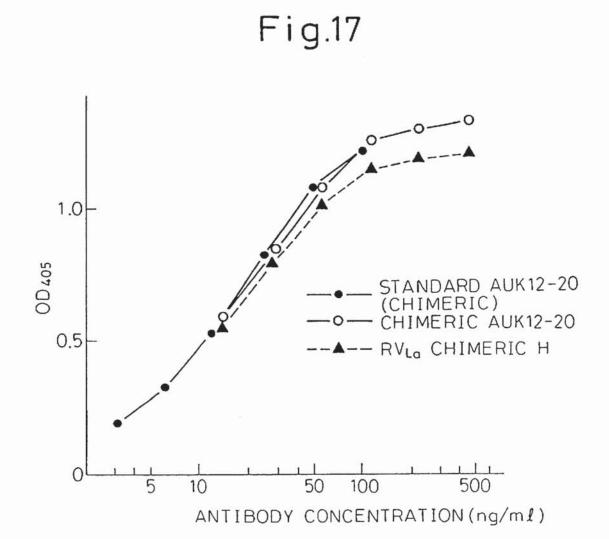
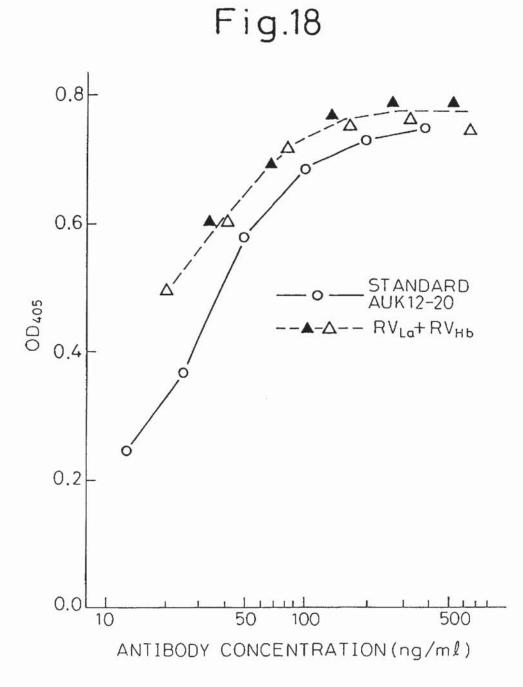


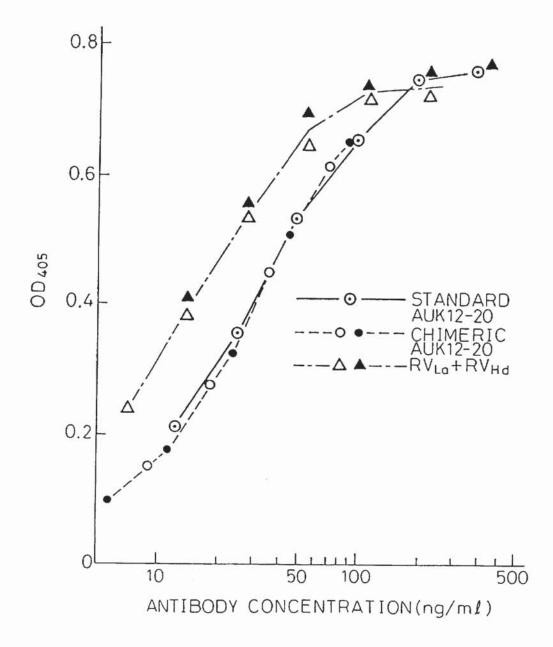
Fig.16



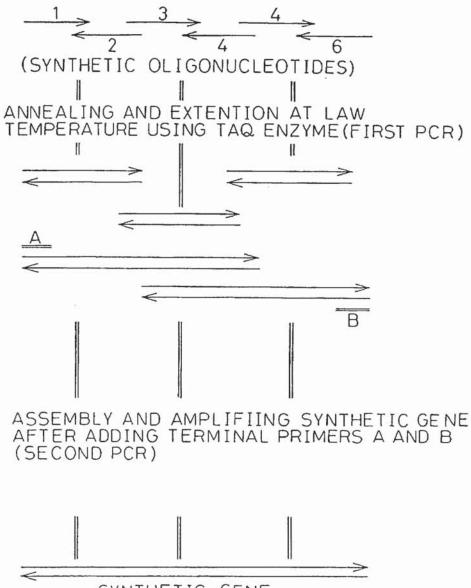




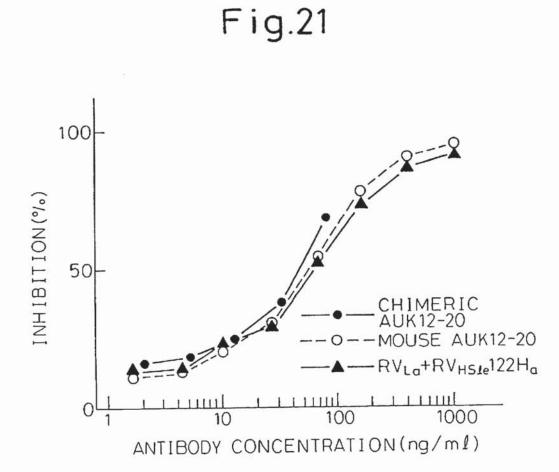


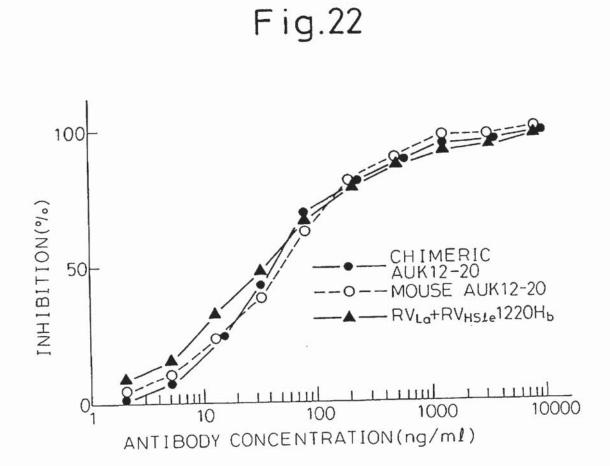


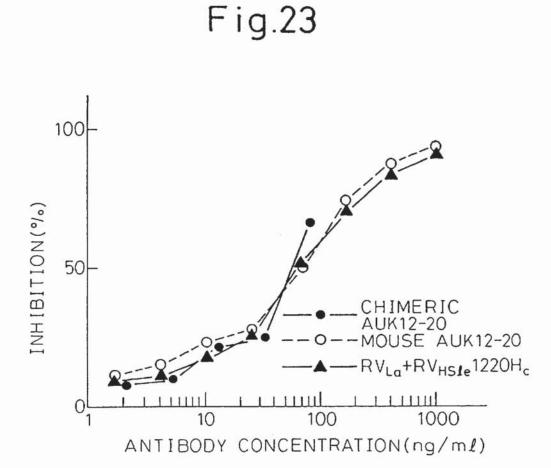


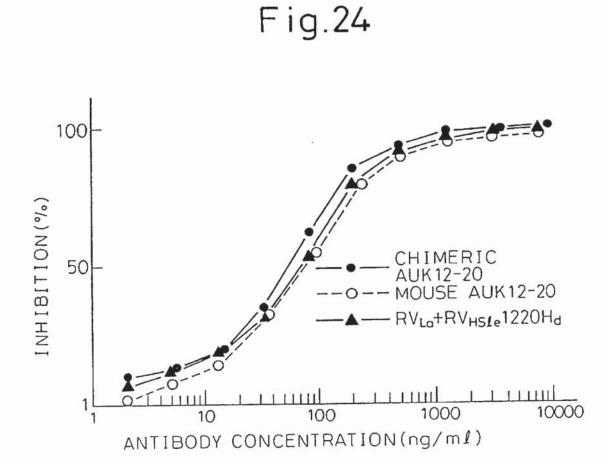


SYNTHETIC GENE









### RESHAPED HUMAN TO HUMAN INTERLEUKIN-6 RECEPTOR

#### TECHNICAL FIELD

The present invention relates to variable regions (V region) of a mouse monoclonal antibody to the human interleukin-6 receptor (IL-6R), human/mouse chimeric antibody to the human IL-6R, and reshaped human antibody comprising a human antibody wherein the complementarity determining regions (CDRs) of the human light chain (L  $^{10}$ chain) V region and of the human heavy chain (H chain) V region are grafted with the CDRs of a mouse monoclonal antibody to the human IL-6R. Moreover, the present invention provides DNA coding for the above-mentioned antibodies or part thereof. The present invention further pro-15 vides vectors, especially expression vectors comprising said DNA, and host cells transformed or transfected with said vector. The present invention still more provides a process for production of a chimeric antibody to the human IL-6R, and process for production of a reshaped human antibody to 20 the human IL-6R.

#### BACKGROUND ART

Interleukin-6 (IL-6) is a multi-function cytokine that is 25 produced by a range of cells. It regulates immune responses, acute phase reactions, and hematopoiesis, and may play a central role in host defense mechanisms. It acts on a wide range of tissues, exerting growth-inducing, growth inhibitory, and differentiation-inducing effects, depending 30 on the nature of the target cells. The specific receptor for IL-6 (IL-6R) is expressed on lymphoid as well as nonlymphoid cells in accordance with the multifunctional properties of IL-6. Abnormal expression of the IL-6 gene has been suggested to be involved in the pathogenesis of a 35 variety of diseases, especially autoimmune diseases, mesangial proliferative glomerulonephritis, and plasmacytoma/ myeloma (see review by Hirano et al., Immunol. Today 11, 443-449, 1990). Human myeloma cells are observed to produce IL-6 and express IL-6R. In experiments, antibody 40 against IL-6 inhibited the in vitro growth of myeloma cells thus indicating that an autocrine regulatory loop is operating in oncogenesis of human myelomas (Kawano et al., Nature, 332, 83, 1988).

The IL-6R is present on the surface of various animal 45 cells. and specifically binds to IL-6, and the number of IL-6R molecules on the cell surface has been reported (Taga et al., J. Exp. Med. 196, 967, 1987). Further, cDNA coding for a human IL-6R was cloned and a primary structure of the IL-6R was reported (Yamasaki et al., Science, 241, 825, 50 1988).

Mouse antibodies are highly immunogenic in humans and, for this reason, their therapeutic value in humans is limited. The half-life of mouse antibodies in vivo in human is relatively short. In addition, mouse antibodies can not be 55 administered in multiple doses without generating an immune response which not only interferes with the planned efficacy but also risks an adverse allergic response in the patient.

To resolve these problems methods of producing humanized mouse antibodies were developed. Mouse antibodies can be humanized in two ways. The more simple method is to construct chimeric antibodies where the V regions are derived from the original mouse monoclonal antibody and the C regions are derived from suitable human antibodies. 65 The resulting chimeric antibody contains the entire V domains of the original mouse antibody and can be expected 2

to bind antigen with the same specificity as the original mouse antibody. In addition, chimeric antibodies have a substantial reduction in the percent of the protein sequence derived from a non-human source and, therefore, are expected to be less immunogenic than the original mouse antibody. Although chimeric antibodies are predicted to bind antigen well and to be less immunogenic, an immune response to the mouse V regions can still occur (LoBuglio et al., Proc. Natl. Acad. Sci. USA 84, 4220–4224, 1989).

The second method for humanizing mouse antibodies is more complicated but more extensively reduces the potential immunogenicity of the mouse antibody. In this method, the complementarity determining regions (CDRs) from the V regions of the mouse antibody are grafted into human V regions to create "reshaped" human V regions. These reshaped human V regions are then joined to human C regions. The only portions of the final reshaped human antibody derived from non-human protein sequences are the CDRs. CDRs consist of highly variable protein sequences. They do not show species-specific sequences. For these reasons, a reshaped human antibody carrying murine CDRs should not be any more immunogenic than a natural human antibody containing human CDRs.

As seen from the above, it is supposed that reshaped human antibodies are useful for therapeutic purposes, but reshaped human antibodies to the human IL-6R are not known. Moreover, there is no process for construction of a reshaped human antibodies, universally applicable to any particular antibody. Therefore to construct a fully active reshaped human antibody to a particular antigen, various devices are necessary. Even though mouse monoclonal antibodies to the human IL-6R, i.e., PM1 and MT18, were prepared (Japanese Patent Application No. 2-189420), and the present inventors prepared mouse monoclonal antibodies to the human IL-6R, i.e., AUK12-20, AUK64-7 and AUK146-15, the present inventors are not aware of publications which suggest construction of reshaped human antibodies to the human IL-6R.

The present inventors also found that, when the mouse monoclonal antibodies to the human IL-6R were injected into nude mice transplanted with a human myeloma cell line, the growth of the tumor was remarkably inhibited. This suggests that the anti-human IL-6 receptor antibody is useful as a therapeutic agent for the treatment of myeloma.

#### DISCLOSURE OF INVENTION

Therefore, the present invention is intended to provide a less immunogenic antibody to the human IL-6R. Accordingly, the present invention provides reshaped human antibodies to the human IL-6R. The present invention also provides human/mouse chimeric antibodies useful during the construction of the reshaped human antibody. The present invention further provides a part of reshaped human antibody, as well as the expression systems for production of the reshaped human antibody and a part thereof, and of the chimeric antibody.

More specifically, the present invention provides L chain V region of mouse monoclonal antibody to the human IL-6R; and H chain V region of a mouse monoclonal antibody to the human IL-6R.

The present invention also provides a chimeric antibody to the human IL-6R, comprising:

- an L chain comprising a human L chain C region and an L chain V region of a mouse monoclonal antibody to the IL-6R; and
- (2) an H chain comprising a human H chain C region and an H chain V region of a mouse monoclonal antibody to the human IL-6R.

The present invention also provides CDR of an L chain V region of a mouse monoclonal antibody to the human IL-6R; and CDR of an H chain V region of a mouse monoclonal antibody to the human IL-6R.

The present invention moreover provides a reshaped 5 human L chain V region of an antibody to the human IL-6R, comprising:

- (1) framework regions (FRs) of a human L chain V region, and
- (2) CDRs of an L chain V region of a mouse monoclonal <sup>10</sup> antibody to the human IL-6R; and
  - a reshaped human H chain V region of an antibody to the human IL-6R comprising:
    - (1) FRs of a human H chain V region, and
  - (2) CDRs of an H chain V region of a mouse <sup>15</sup> monoclonal antibody to the human IL-6R.

The present invention also provides a reshaped human L chain of an antibody to the human IL-6R, comprising:

- (1) a human L chain C region; and
- (2) an L chain V region comprising human FRs, and CDRs of a mouse monoclonal antibody to the human IL-6R; and
  - a reshaped human H chain of an antibody to the human IL-6R, comprising:
    - (1) a human H chain C region, and
    - (2) an H chain V region comprising a human FRs. and CDRs of a mouse monoclonal antibody to the human IL-6R.

The present invention still more provides a reshaped 30 human antibody to the human IL-6R, comprising:

- (A) an L chain comprising,
  - (1) a human L chain C region, and
  - (2) an L chain V region comprising human L chain FRs, and L chain CDRs of a mouse monoclonal antibody 35 to the human IL-6R; and
- (B) an H chain comprising,
  - (1) a human H chain C region, and
  - (2) an H chain V region comprising human H chain FRs, and H chain CDRs of a mouse monoclonal, <sup>40</sup> antibody to the human IL-6R.

The present invention further provides DNA coding for any one of the above-mentioned antibody polypeptides or parts thereof.

The present invention also provides vectors, for example, <sup>45</sup> for reshaped human AUK 12-20 antibody L chain V region. expression vectors comprising said DNA. FIG. 17 is a graph showing results of an ELISA for

The present invention further provides host cells transformed or transfected with the said vector.

The present invention still more provide a process for production of a chimeric antibody to the human IL-6R, and <sup>50</sup> a process for production of reshaped human antibody to the human IL-6R.

#### BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 represents expression vectors comprising human <sup>55</sup> cytomegalo virus (HCMV) promoter/enhancer system, useful for the expression of the present antibody peptide.

FIG. 2 is a graph showing a result of ELISA for confirmation of an ability of the present chimeric antibody  $_{60}$  AUK12-20 to bind to the human IL-6R.

FIG. 3 is a graph showing a result of measurement of an ability of the present chimeric antibody AUK12-20 to inhibit the binding of IL-6 to the human IL-6R.

FIG. 4 is a graph showing a result of ELISA for binding 65 of the present chimeric antibodies PM1a and PM1b to human IL-6R.

FIG. 5 is a graph showing a result of ELISA testing the ability of the present chimeric antibodies PM1a and PMIb to inhibit IL-6 from binding to the human IL-6R.

FIG. 6 is a diagram of the construction of the first version of a reshaped human PM-1 H chain V region.

FIG. 7 is a diagram of the construction of the first version of a reshaped human PM-1 L chain V region.

FIG. 8 represents a process for construction of an expression plasmid HEF-12h-gyl comprising a human elongation factor  $1\alpha$  (HEF-1 $\alpha$ ) promoter/enhancer. useful for the expression of an H chain.

FIG. 9 represents a process for construction of an expression plasmid HEF-12k-gk comprising the HEF-1 $\alpha$ promoter/enhancer system, useful for the expression of an L chain.

FIG. 10 represents a process for construction of an expression plasmid DHFR-PMh-gy1 comprising HCMV promoter/enhancer and the dihydrofolate reductase (dhfr) gene linked to a defective SV40 promoter/enhancer sequence for amplification, useful for expression of an H chain.

FIG. 11 represents a process for the construction of an expression plasmid DHFR-ΔE-RVh-PM1-f comprising EFla promoter/enhancer and dhfr gene linked to a defective SV40 promoter/enhancer sequence for amplification, useful for expression of an H chain.

FIG. 12 is a graph showing an ability of version "a" and "b" of the reshaped human PM-1 L chain V region for binding to the human IL-6R.

FIG. 13 is a graph showing an ability of version "f" of the reshaped human PM-1 H chain V region plus version "a" of the reshaped PM-1 L chain L chain V region for binding to the human IL-6R.

FIG. 14 is a graph showing an ability of vergion "f" of the reshaped PM-1 H chain V region plus version "a" of the reshaped PM-1 L chain V region to inhibit the binding of IL-6 to the human IL-6R.

FIG. 15 represents expression plasmids HEF-V<sub>L</sub>-gk and HEF-V<sub>H</sub>-gyl comprising a human EFl  $-\alpha$  promoter/ enhancer, useful for expression of an L chain and H chain respectively.

FIG. 16 shows a process for construction of DNA coding for reshaped human AUK 12-20 antibody L chain V region.

FIG. 17 is a graph showing results of an ELISA for confirm of an ability of a reshaped human AUK 12-20 antibody L chain V region to bind to human IL-6R. In the Figure, "Standard AUK 12-20 (chimera) means a result for chimeric AUK 12-20 antibody produced by CHO cells and purified in a large amount.

FIG. 18 is a graph showing a result of an ELISA for an ability of a reshaped human AUK 12-20 antibody (L chain version "a"+H chain version "b") to bind to human IL-6R.

FIG. 19 is a graph showing a result of an ELISA for an ability of a reshaped human AUK 12-20 antibody (L chain version "a"+H chain version "d") to bind to the human IL-6R.

FIG. 20 shows a process for chemical synthesis of a reshaped human sle 1220 H antibody H chain V region.

FIG. 21 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a"+H chain version "a") to inhibit the binding of IL-6 to the human IL-6R.

FIG. 22 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain

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version "a"+H chain version "b") to inhibit the binding of IL-6 to the human IL-6R.

FIG. 23 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a"+H chain version "c") to inhibit the binding of <sup>5</sup> IL-6 to the human IL-6R.

FIG. 24 is a graph showing a result of an ELISA for an ability of a reshaped human sle 1220 antibody (L chain version "a"+H chain version "d") inhibit the binding of IL-6 to the human LI-6R.

## Best Mode for Carrying Out the Invention Cloning of DNA coding for mouse V regions

More specifically, to clone DNA coding for V regions of 15 a mouse monoclonal antibody to a human IL-6R, the construction of hybridoma, which produces a monoclonal antibody to the human IL-6R, is necessary as a gene source. As such a hybridoma. Japanese Patent Application No. 2-189420 describes a mouse hybridoma PM-1 which produces a monoclonal antibody PM1 and the properties 20 thereof. Reference Examples 1 and 2 of the present specification describe the construction process of the hybridoma PM1. The present inventors have constructed hybridomas AUK12-20, AUK64-7, and AUK146-15, each producing a 25 mouse monoclonal antibody to the human IL-6R. The construction process of these hybridomas is described in the Reference Examples 3 of this specification.

To clone desired DNAs coding for V regions, of a mouse monoclonal antibody, hybridoma cells are homogenized and a total RNA is obtained according to a conventional procedure described by Chirgwin et al., Biochemistry 18, 5294, 1977. Next, the total RNA is used to synthesize singlestranded cDNAs according to the method described by J. W. Larrick et al., Biotechnology, 7, 934, 1989.

Next, a specific amplification of a relevant portion of the cDNA is carried out by a polymerase chain reaction (PCR) method. For amplification of a K L chain V region of a mouse monoclonal antibody, 11 groups of oligonucleotide primers (Mouse Kappa Variable; MKV) represented in SEQ ID NO: 40 1 to 11, and an oligonucleotide primer (Mouse Kappa Constant; MKC) represented in SEQ ID NO: 12 are used as 5'-terminal primers and a 3'-terminal primer respectively. The MKV primers hybridize with the DNA sequence coding for the mouse  $\kappa$  L chain leader sequence, and the MKC 45 primer hybridizes with the DNA sequence coding for the mouse K L chain constant region. For amplification of the H chain V region of a mouse monoclonal antibody, 10 groups of oligonucleotide primers (Mouse Heavy Variable; MHV) represented in SEQ ID NO: 13 to 22, and a oligonucleotide 50 primer (Mouse Heavy Constant MHC) represented in SEQ ID NO: 23 are used as 5'-terminal primers and a 3'-terminal primer, respectively.

Note, the 5'-terminal primers contain the nucleotide sequence GTCGAC near the 5'-end thereof, which sequence 55 provides a restriction enzyme Sal I cleavage site; and the 3'-terminal primer contains the nucleotide sequence CCCGGG near the 5-end thereof, which sequence provides a restriction enzyme Xma I cleavage site. These restriction enzyme cleavage sites are used to subclone the DNA frag- 60 ments coding for a variable region into cloning vectors.

Next, the amplification product is cleaved with restriction enzymes Sal I and Xma I to obtain a DNA fragment coding for a desired V region of a mouse monoclonal antibody. On the other hand, an appropriate cloning vector such as plasmid pUCl9 is cleaved with the same restriction enzymes Sal I and Xma I and the above DNA fragment is ligated with the

cleaved pUC19 to obtain a plasmid incorporating a DNA fragment coding for a desired V region of a mouse monoclonal antibody.

The sequencing of the cloned DNA can be carried out by any conventional procedure.

The cloning of the desired DNA, and the sequencing thereof, are described in detail in Examples 1 to 3.

## Complementarity Determining Regions (CDRs)

The present invention provides hypervariable or complementarity determining regions (CDRs) of each V region of the present invention. The V domains of each pair of L and H chains from the antigen binding site. The domains on the L and H chains have the same general structure and each domain comprises four framework regions (FRs), whose sequences are relatively conserved, connected by three CDRs (see Kabat, E. A., Wu, T. T., Bilofsky, H., Reid-Miller, M. and Perry, H., in "Sequences of Proteins of Immunological Interest". US Dept. Health and Human Services 1983). The four FRs largely adopt a B-sheet conformation and the CDRs form loops connecting FRs, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs are held in close proximity by FRs and, with the CDRs from the other domain, contribute to the formation of the antigen binding site. The CDRs are described in Example 4.

#### Construction of Chimeric Antibody

Prior to designing reshaped human V regions of an antibody to the human IL-6R, it is necessary to confirm that the CDRs to be used actually form an effective antigen binding region. For this purpose, chimeric antibodies were constructed. In addition the amino acid sequences of V regions of mouse anti human IL-6R antibodies predicted from the nucleotide sequences of cloned DNAs of the 4 35 mouse monoclonal antibodies described in Example 1 and 2 were compared to each other and to V regions from known mouse and human antibodies. For each of the 4 mouse monoclonal antibodies, a set of typical, functional mouse L and H chain V regions had been cloned. All four mouse anti-IL-6R antibodies, however, had relatively distinct V regions. The 4 antibodies were not simply minor variations of each other. Using the cloned mouse V regions, 4 chimeric anti-IL-6R antibodies were constructed.

The basic method for constructing chimeric antibodies comprises joining the mouse leader and V region sequences, as found in the PCR-cloned cDNAs, to human C regionscoding sequence already present in mammalian cell expression vectors. Among said 4 monoclonal antibodies, construction of a chimeric antibody from the monoclonal antibody AUK12-20 is described in Example 5.

Construction of a chimeric antibody from the monoclonal antibody PM-1 is described in Example 6. The cDNA coding for the mouse PM-1 K L chain leader and V region was PCR-subcloned into an expression vector containing a genomic DNA coding for the human kappa C region. The cDNA coding for the mouse PM-1 H chain leader and V regions was PCR-subcloned into an expression vector containing a genomic DNA coding for the human gamma-1 C region. Using specially designed PCR primers, the cDNA coding for the mouse PM-1 V region were adapted at their 5'- and 3'-ends (1) so that they would be easy to insert into the expression vectors and (2) so that they would function properly in these expression vectors. The PCR-modified mouse PM-1 V regions were then inserted into HCMV expression vectors already containing the desired human C regions (FIG. 1). These vectors are suitable for either

transient or stable expression of genetically-engineered antibodies in a variety of mammalian cell lines.

In addition to constructing a chimeric PM-1 antibody with V regions identical to the V regions present in mouse PM-1 antibody (version a). a second version of chimeric PM-1 5 antibody was constructed (version b). In chimeric PM-1 antibody (version b), the amino acid at position 107 in the L chain V region was changed from asparagine to lysine. In comparing the L chain V region from mouse PM-1 antibody to other mouse L chain V regions, it was noticed that the 10 occurrence of an asparagine at position 107 was an unusual event. In mouse K L chain V regions, the most typical amino acid at position 107 is a lysine. In order to evaluate the importance of having the atypical amino acid asparagine at position 107 in the L chain V region of mouse PM-1 15 antibody, position 107 was changed to the typical amino acid lysine at this position. This change was achieved using a PCR-mutagenesis method (M. Kamman et al., Nucl. Acids Res. (1989) 17:5404) to make the necessary changes in the 20 DNA sequences coding for the L chain V region.

The chimeric PM-1 antibody version (a) exhibited an activity to bind to the human IL-6R. The chimeric PM-1 antibody version (b) also binds to the human IL-6R as well as version (a). Similarly, from other 2 monoclonal antibodies AUK64-7 and AUK146-15, chimeric antibodies were constructed. All 4 chimeric antibodies bound well to the human IL-6R thus indicating in a functional assay that the correct mouse V regions had been cloned and sequenced.

From the 4 mouse anti-IL-6R antibodies, PM-1 antibody 30 was selected as the first candidate for the design and construction of a reshaped human antibody to the human 1L-6R. The selection of mouse PM-1 antibody was based largely on results obtained studying the effect of the mouse anti-IL-6R antibodies on human myeloma tumor cells transplanted into nude mice. Of the 4 mouse anti-IL-6R antibodies. PM-1 antibody showed the strongest anti-tumor cell activity.

### Comparison of the V Regions from Mouse Monoclonal Antibody PM-1 to V Regions from known Mouse and Human Antibodies

To construct a reshaped human antibody wherein the CDRs of a mouse monoclonal antibody are grafted into a human monoclonal antibody, it is desired that there is high 45 similar to the FRs from the consensus sequence for subgroup homology between FRs of the mouse monoclonal antibody and FRs of the human monoclonal antibody. Therefore, the amino acid sequences of the L and H chain V regions from mouse PM-1 antibody were compared to all known mouse and mouse V regions as found in the OWL (or Leeds) 50 database of protein sequences.

With respect to V regions from mouse antibodies, the L chain V region of PM-1 antibody was most similar to the L chain V region of mouse antibody musigkcko (Chen. H. T. et al., J. Biol. Chem. (1987) 262:13579-13583) with a 55 93.5% identity. The H chain V region of PM-1 antibody was most similar to the H chain V region of mouse antibody musigvhr2 (F. J. Grant et al., Nucl. Acids Res. (1987) 15:5496) with a 84.0% identity. The mouse PM-1 V regions show high percents of identity to known mouse V regions 60 thus indicating that the mouse PM-1 V regions are typical mouse V regions. This provides further indirect evidence that the cloned DNA sequences are correct. There is generally a higher percent identity between the L chain V regions than between the H chain V regions. This is probably due to 65 the lower amount of diversity generally observed in L chain V regions as compared to H chain V regions.

With respect to V regions from human antibodies, the L chain V region of PM-1 antibody was most similar to the L chain V region of human antibody klhure, also referred to as REI (W. Palm et al., Physiol. Chem. (1975) 356:167-191) with a 72.2% identity. The H chain V region of PM-1 antibody was most similar to the H chain V region of human antibody humighvap (VAP) (H.W. Schroeder et al., Science (1987) 238:791-793) with a 71.8% identity. The comparisons to human V regions are most important for considering how to design reshaped human antibodies from mouse PM-1 antibody. The percent identities to human V regions are less than the percent identities to mouse V regions. This is indirect evidence that the mouse PM-1 V regions do look like mouse V regions and not like human V regions. This evidence also indicates that it will be best to humanize mouse PM-1 V regions in order to avoid problems of immunogenicity in human patients.

The V regions from mouse PM-1 antibody were also compared to the consensus sequences for the different subgroups of human V regions as defined by E. A. Kabat et al. ((1987) Sequences of Proteins of Immunological Interest, Forth Edition. U.S. Department of Health and Human servides, U.S. Government Printing Office). The comparisons were made between the FRs of the V regions. The 25 results are shown in Table 1.

TABLE 1

PM	4-1 V regions and puences <sup>(1)</sup> for the	ween the FRs from the FRs from the different subgroup V regions.	consensus
A. FRs in th	e L chain V regio	ns	
HSGI	HSGI	HSGIII	HSGIV
70.1 B. FRs in th	53.3 e H chain V regio	60.7	59.8
HSGI	HSGII	HSGIII	

<sup>(1)</sup>The consensus sequences were taken from the subgroups of human V regions as described in Kabat et al., (1987).

The FRs of mouse PM-1 L chain V region are most I (HSGI) of human L chain V regions with 70.1% identity. The FRs of mouse PM-1 H chain V region are most similar to the FRs from the consensus sequence for subgroup II (HSGII) of human H chain V regions with 52.9% identity. These results support the results obtained from the comparisons to known human antibodies. The L chain V region in human REI belongs to subgroup I of human L chain V regions and the H chain V region in human VAP belongs to subgroup II of human H chain V regions.

From these comparisons to the V regions in human antibodies, it is possible to select human V regions that will be the basis for the design of reshaped human PM-1 V regions. It would be best to use a human L chain V region that belongs to subgroup I (SGII) for the design of reshaped human PM-1 L chain V region and a human H chain V region that belongs to subgroup II (SGII) for the design of reshaped human PM-1 H chain V region.

## Design of Reshaped Human PM-1 Variable Regions

The first step in designing the reshaped human PM-1 V regions was to select the human V regions that would be the

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basis of the design. The FRs in the mouse PM-1 L chain V region were most similar to the FRs in human L chain V regions belonging to subgroup I (Table 1). As discussed above, in comparing the mouse PM-1 L chain V region to known human L chain V regions, it was most similar to the human L chain V regions. In designing reshaped human PM-1 L chain V regions, the FRs from REI were used. Moreover the REI FRs were used as starting material for the construction of reshaped human PM-1 L chain V region.

In these human FRs based on REI, there were five differences from the FRs in the original human REI (positions 39, 71, 104, 105, and 107 according to Kabat et al., 1987; see Table 2). The three changes in FR4 (positions 104, 105, and 107) were based on a J region from another human kappa L chain and, therefore, do not constitute a 10

amino acid at this position is predicted to directly influence the structure of the CDR1 loop of the L chain V region and, therefore, may well influence antigen binding. In the mouse PM-1 L chain V region, position 71 is a tyrosine. In the modified REI FRs used in the design of version "a" of reshaped human PM-1 L chain V region, position 71 was a phenylalanine. In version "b" of reshaped human PM-1 L chain V region, the phenylalanine at position 71 was
10 changed to a tyrosine as found in mouse PM-1 L chain V region. Table 2 shows the amino acid sequences of mouse PM-1 L chain V region, the FRs of REI as modified for use in reshaped human CAMPATH-1H antibody (Riechmann et al., 1988), and the two versions of reshaped human PM-1 L chain V region.

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The FRs given for REI are those found in the reshaped human CAMPATH-1H antibody (Reichmann et al., 1988). The five underlined amino acid residues in the REI FRs are those that differ from the amino acid sequence of human REI (Palm et al., 1975; O. Epp et al., Biochemistry (1975) 14:4943-4952).

deviation from human (L. Riechmann et al., Nature (1988) 322:21–25). The two changes at positions 39 and 71 were changes back to the amino acids that occurred in the FRs of rat CAMPATH-1 L chain V region (Riechmann et al., 1988). The FRs in the mouse PM-1 H chain V regions belonging to subgroup II (Table 1). As discussed above, in comparing the mouse PM-1 H chain V region to known human H chain V

Two versions of reshaped human PM-1 L chain V region were designed. In the first version (version "a"), the human FRs were identical to the REI-based FRs present in reshaped human CAMPATH-1H (Riechmann et al., 1988) and the mouse CDRs were identical to the CDRs in mouse PM-1 L chain V region. The second version (version "b") was based on version "a" with only one amino acid change at position 71 in human FR3. Residue 71 is part of the canonical 55 structure for CDR1 of the L chain V region as defined by C. Chothia et al., (J. Mol. Biol (1987) 196:901–917). The

The FRs in the mouse PM-1 H chain V region were most similar to the FRs in human H chain V regions belonging to subgroup II (Table 1). As discussed above, in comparing the mouse PM-1 H chain V region to known human H chain V regions, it was most similar to the human H chain V region VAP, a member of subgroup II of human H chain V regions. DNA sequences coding for the FRs in human H chain V region NEW, another member of subgroup II of human H chain V regions, were used as starting material for the construction of reshaped human PM-1 H chain V region, and as a base for designing the reshaped human PM-1 H chain V region.

Six versions of reshaped human PM-1 H chain V region were designed. In all six versions, the human FRs were based on the NEW FRs present in reshaped human CAMPATH-1H (Riechmann et al., 1988) and the mouse CDRs were identical to the CDRs in mouse PM-1 H chain V region. Seven amino acid residues in the human FRs (positions 1, 27, 28, 29, 30, 48, and 71, see Table 3) were 5 identified as having a possible adverse influence on antigen binding. In the model of mouse PM-1 V regions, residue 1

the overall structure of the V region and its antigen-binding site. Residue 71 is part of the canonical structure for CDR2 of the H chain V region as predicted by Chothia et al., (1989). The six versions of reshaped human PM-1 antibody incorporate different combinations of amino acid changes at these seven positions in the human NEW FRs (see Table 3).

TABLE 3

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Note:

The FRs given for NEW are those found in the first version of reshaped human CAMPATH-1H antibody (Riechmann et al., 1988).

in the H chain V region is a surface residue that is located close to the CDR loops. Residues 27, 28, 29, and 30 are either part of the canonical structure for CDR1 of the H chain V region, as predicted by C. Chothia et al., Nature (1989) 34:877–883, and/or are observed in the model of the mouse PM-1 V regions to form part of the first structural loop of the H chain V region (Chothia, 1987). Residue 48 65 was observed in the model of the mouse PM-1 V regions to be a buried residue. Changes in a buried residue can disrupt

#### Construction of Reshaped Human PM-1 V Regions

The first versions of the reshaped human PM-1 L and H chain V regions were each constructed using a novel PCRbased method. Essentially, a plasmid DNA coding for reshaped human V region that already contained suitable human FRs was modified using PCR primers to replace the CDRs present in the starting reshaped human V region with the CDRs from mouse PM-1 antibody. The starting material for the construction of the reshaped human PM-1 L chain V region was a plasmid DNA containing the reshaped human D1.3 L chain V region. The reshaped human D1.3 L chain V region was constructed based on the FRs present in the human L chain V region of REI. The starting material for the construction of the reshaped human PM-1 H chain V region was a plasmid DNA containing the reshaped human D1.3 H chain V region. The reshaped human D1.3 H chain V region The reshaped numan D1.3 H chain V region of NEW (M. Verhoeyen et al., Science (1988) 239:1534–1536).

Once the starting plasmid DNAs containing the desired 10 human FRs were selected. PCR primers were designed to enable the substitution of the mouse PM-1 CDRs in place of the mouse D1.3 CDRs. For each reshaped human PM-1 V region, three primers containing the DNA sequences coding for the mouse PM-1 CDRs and two primers flanking the 15 entire DNA sequence coding for the reshaped human V region were designated and synthesized. Using the five PCR primers in a series of PCR reactions yielded a PCR product that consisted of the human FRs present in the starting reshaped human V region and the CDRs present in mouse PM-1 V region (see Example 7, and FIGS. 7 and 8). The <sup>20</sup> PCR products were cloned and sequenced to ensure that the entire DNA sequence of version "a" of reshaped human PM-1 L and H chain V region coded for correct amino acid sequence (SEQ ID NO 55).

The remaining versions of the reshaped human PM-1 V 25 regions were constructed using slight modifications of published PCR-mutagenesis techniques (Kamman et al., 1989). As described for the design of the reshaped human PM-1 V regions, one additional version (version "b") of the reshaped human PM-1 L chain V region was constructed and five 30 reshaped human V regions contained deletions. additional versions (versions "b", "c", "d", "e", and "f") of the reshaped human PM-1 H chain V region were constructed. These additional versions contain a series of minor changes from the first versions. These minor changes in the amino acid sequences were achieved using PCR mutagenesis to make minor changes in the DNA sequences. PCR primers were designed that would introduce the necessary changes into the DNA sequence. Following a series of PCR reactions, a PCR product was cloned and sequenced to ensure that the changes in the DNA sequence had occurred as planned. Sequence of the reshaped human PM-1 antibody H chain V region version "f" is shown in SEQ ID NO 54).

Once the DNA sequences of the different versions of reshaped human PM-1 V regions were confirmed by sequencing, the reshaped human PM-1 V regions were subcloned into mammalian cell expression vectors already containing human C regions. Reshaped human PM-1 L chain V regions were joined to DNA sequences coding for human  $\kappa$  C region. Reshaped human PM-1 H chain V regions were joined to DNA sequences coding for human  $\kappa$  C region. Reshaped human PM-1 H chain V regions were joined to DNA sequences coding for human gamma-1 C region. In order to achieve higher levels of expression of the reshaped human PM-1 antibodies, the HCMV expression vectors, as shown in FIG. 1, were modified to replace the HCMV promoter-enhancer region with the human elongation factor (HEF-1 $\alpha$ ) promoter-enhancer (see FIG. 15).

Next, all combinations of the reshaped human L chain versions (a) and (b) with the H chain V region versions (a) to (f) were tested for biding to human IL-6R, and as a result, a reshaped human antibody comprising the L chain version (a) and the H chain version (f) exhibited an ability to bind to IL-6R at a same level as that of chimeric PM-1 (a) (FIG. 13) as described in detail in Example 11.

#### Modifications in the DNA Sequences Coding for the Reshaped Human PM-1 V Regions to Improve the Levels of Expression.

In reviewing the levels of reshaped human PM-1 antibodies being produced in cos cells, it became apparent that 14

the levels of expression of the reshaped human H chains were always approximately 10-fold lower than the levels of expression of the reshaped human L chains or of the chimeric L or H chains. It appeared that there was a problem in DNA coding for the reshaped human H chain V region that caused low levels of expression. In order to identify whether the lower levels of protein expression were the result of lower levels of transcription. RNA was prepared from cos cells co-transfected with vectors expressing reshaped human PM-1 L and H chains. First-strand cDNA was synthesized as described for the PCR cloning of the mouse PM-1 V regions. Using PCR primers designed to flank the ends of DNA coding for the reshaped human L or H chain V regions. PCR products were generated from the cDNAs that corresponded to reshaped human L chain V region or to reshaped human H chain V region.

For the reshaped human L chain V region, there were two PCR products, one 408 bp long, as expected, and a shorter PCR product 299 bp long. The correct size PCR product made up approximately 90% of the total yield of PCR product and the shorter PCR product made up approximately 10% of the total yield. For the reshaped human H chain V region, there were also two PCR products, one 444 bp long, as expected, and a shorter PCR product 370 bp long. In this case, however, the incorrect, shorter PCR product, approximately 90%. The correct size PCR product made up only approximately 10% of the total yield of PCR product. These results indicated that some of the RNAs coding for the reshaped human V regions contained deletions.

In order to determine which sequences were being deleted, the shorter PCR products were cloned and sequenced. From the DNA sequences, it became clear that for both the L and H chain V regions specific sections of 35 DNA were being deleted. Examination of the DNA sequences flanking the deleted sequences revealed that these sequences corresponded to the consensus sequences for splice donor-acceptor sequences (Breathnach, R. et al., Ann. Rev. Biochem (1981) 50:349-383). The explanation for the low levels of expression of the reshaped human H chains was that the design of the reshaped human H chain V regions had inadvertently created a rather efficient set of splice donor-acceptor sites. It also appeared that the design of the reshaped human L chain V regions had inadvertently created 45 a rather inefficient set of splice donor-acceptor sites. In order to remove the splice donor-acceptor sites, minor modifications in the DNA sequences coding for versions "a" and "f". respectively, of the reshaped human PM-1 L and H chain V regions were made using the PCR-mutagenesis methods

Another possible cause of reduced levels of expression was thought to be the presence of introns in the leader sequences in both the reshaped human L and H chain V regions (SEQ ID NOs: 54 and 55). These introns were 55 originally derived from a mouse mu H chain leader sequence (M.S. Neuberger et al., Nature 1985 314:268-270) that was used in the construction of reshaped human D1.3 and V regions (Verhoeyen et al., 1988). Since the reshaped human D1.3 was expressed in a mammalian cell vector that employed a mouse immunoglobulin promoter, the presence of the mouse leader intron was important. The leader intron contains sequences that are important for expression from immunoglobulin promoters but not from viral promoters like HCMV (M. S. Neuberger et al., Nucl. Acids Res. (1988) 65 16:6713-6724). Where the reshaped human PM-1 L and H chains were being expressed in vectors employing nonimmunoglobulin promoters, the introns in the leader 10

15

20

sequences were deleted by PCR cloning cDNAs coding for the reshaped human V regions (see Example 12).

Another possible cause of reduced levels of expression was thought to be the presence of a stretch of approximately 5 190 bp of non-functional DNA within the intron between the reshaped human PM-1 H chain V region and the human gamma-1 C region. The reshaped human PM-1 H chain V region was constructed from DNA sequences derived originally from reshaped human B1-8 H chain V region (P. T. Jones et al., Nature (1986) 321:522-525). This first reshaped human V region was constructed from the mouse NP H chain V region (M. S. Neuberger et al., Nature (1985); M. S. Neuberger et al., EMBO J. (1983) 2:1373-1378). This stretch of approximately 190 bp occurring in the intron between the reshaped human H chain V region and the BamHI site for joining of the reshaped human V regions to the expression vector was removed during the PCR cloning of cDNAs coding for the reshaped human V regions.

The DNA and amino acid sequences of the final versions of reshaped human PM-1 L and H chain V regions, as altered to improve expression levels, are shown in SEQ ID NOs: 57 and 56. These DNA sequences code for version "a" of the reshaped human PM-1 L chain V region as shown in Table 2 and version "f" of the reshaped human PM-1 H chain V region as shown in Table 3. When inserted into the HEF-la expression vectors (FIG. 15), these vectors transiently produce approximately 2 µg/ml of antibody in transfected cos 30 cells. In order to stably produce larger amounts of reshaped human PM-1 antibody, a new HEF-la expression vector incorporating the dhfr gene was constructed (see Example 10, FIG. 11). The "crippled" dhfr gene was introduced into the HEF-10 vector expressing human gamma-1 H chains as 35 was described for the HCMV vector expressing human gamma-1 H chains. The HEF-1 vector expressing reshaped human PM-1 L chains and the HEF-1a-dhfr vector expressing reshaped human P-1 H chains were co-transfected into CHO dhfr(-) cells. Stably transformed CHO cell lines were 40 selected in Alpha-Minimum Essential Medium (a-MEM) without nucleosides and with 10% FCS and 500 µg/ml of G418. Prior to any gene amplification steps. CHO cell lines were observed that produced up to 10 µg/106 cells/day of reshaped human PM-1 antibody.

## Comparison of V Regions from Mouse Monoclonal Antibody AUK 12-20 to V Regions from Known Human Antibodies

The homology of FRs of KL chain V region of the mouse monoclonal antibody AUK 12-20 with FRs of human KL chain V region subgroup (HSG) I to IV. and the homology of FRs of H chain V region of the mouse monoclonal 55 antibody AUK 12-20 will FRs of human H chain V regions subgroup (HSG) I to III are shown in Table 4.

CARLE 4			
	DI	17	
	ю	H	4

A	UK 12-20 V regions as sequence for the differ	een FRs from the mouse nd FRS from the consensus rent subgroups of human egions	
FRs in the L	chain V regions		
HSG1	HSG2	HSG3	HSG4
65.8 FRs in the H	64.0 chain V regions	67.6	67.6
HSGI	HSGI	HSGIII	
58.6	53.6	49.1	

As seen from Table 4. the KL chain V region of the mouse monoclonal antibody AUK 12-20 is homologous in a similar extent (64 to 68%) with the human KL chain V region subgroups (HSG) I to IV. In a search of the Data base "LEEDS" for protein. L chain V region of human antibody Len (M. Schneider et al., Physiol. Chem. (1975) 366:507-557) belonging to the HSG-IV exhibits the highest homology 68%. On the other hand, the human antibody REI, used for construction of a reshaped human antibody from the mouse monoclonal antibody PM-1 belongs to the HSG I. exhibits a 62% homology with L chain V region of the mouse monoclonal antibody AUK 12-20. In addition, the CDRs in the AUK 12-20 antibody L chain V region particularly CDR2, corresponded better to canonical structures of the CDRs in REI rather than those in LEN.

Considering the above, it is not necessary to choose a human antibody used for humanization of the mouse monoclonal antibody AUK 12-20 L chain V region from those antibodies belonging to the HSG IV. Therefore, as in the case of the humanization of the mouse monoclonal antibody PM-1 L chain V region, the FRs of REI are used for humanization of the mouse monoclonal antibody AUK 12-20 L chain V region.

As shown in Table 4, H chain V region of the antibody AUK 12-20 exhibits the highest homology with the HSG L Moreover, in a search of Data base "LEEDS", human antibody HAX (Stollar, B. O. et al., J. Immunol. (1987) 139:2496-2501) also belonging to the HSG I exhibits an about 66% homology with the AUK 12-20 antibody H chain V region. Accordingly, to design reshaped human AUK 45 12-20 antibody H chain V region, the FRs of the human antibody HAX belonging to the HSG L and FRs of humanized 425 antibody H chain V region which has FRs consisting of HSGI consensus sequence (Ketteborough C. A. et al., Protein Engineering (1991) 4:773-783) are used. Note, the

AUK 12-20 antibody H chain V region exhibits an about 50 64% homology with version "a" of the humanized 425 antibody H chain V region.

## Design of Reshaped Human AUK 12-20 Antibody L Chain V Regions

According to the above reason, reshaped human AUK 12-20 antibody L chain V regions is designed as shown in Table 5 using FRs of the REL

	TABLE 5
	CDR1
	FR1 3
	1 2
	12345678901234567890123 45677778901234
	ABCD
V1AUK 12-20	DI VLTQSPASLGVSLGQRATISC RASKSVSTSGYSYMH
REI	DIQMTQSPSSLSASVGDRVTITC
RVL	DI QMTQSPSSLSASVGDRVTITC RASKSVSTSGYSYMH
	FR2 CDR2
	4 5
	567890123456789 0123456
V/AUK 12-20	WYQQKPGQTPKLLIY ASNLES
REI	WY Q Q K P G K A P K L L I Y
RVL	WYQQKPGKAPKLLIY ASNLES
	FR3 CDR3
	6 7 8 9
	78901234567890123456789012345678 9012345678 901234567
VLAUK 12-20	GVPARFSGSGSGTDFTLNIHPVEEEDAATYYC QHSRENPYT
REI	GVPSRFSGSGSGTDYTFTISSLQPEDIATYYC
RVL	GVPSRFSGSGSGTDETFTISSLQPEDIATYYC QHSRENPYT
	FR4
	10
	8 9 0 1 2 3 4 5 6 7
VLAUK 12-20	FGGGTKLEIk
REI	FGQGTKLQIT
RVL	FGQGTKVEIK

Note:

5 underlined nucleotides are those changed in the design of CAMPATH-1H antibody (see the note of Table 2).

## Design of Reshaped Human AUK 12-20 antibody H Chain V Regions

17

According to the above reason, reshaped human AUK 12-20 antibody H chain V regions are designed using FRS of the reshaped human VHa 425. It is found, however, that nucleotide sequence of DNA coding for reshaped human AUK 12-20 antibody H chain V region thus designed has a sequence well conforming to a splicing donor sequence. Therefore, as in the case of reshaped human PM-1 antibody

there is a possibility of an abnormal splicing in the reshaped human AUK 12-20 antibody. Therefore, the nucleotide sequence was partially modified to eliminate the splicing donor-like sequence. The modified sequence is designated as version "a".

In addition, version "b" to "d" of the reshaped human AUK 12-20 antibody H chain V region were designed. Amino acid sequences of the versions "a" to "d" are shown in Table 6.

TABLE 6

														FR	1																C	DF	15		
									1										2										1	3					
V <sub>H</sub> AUK 12-20 SGI	1 2	1 3	3 4	5	6	7	8	9	0	1	2	3	4	5	6 '	7	8	9	0	1	2	3	4	5	6	7	8	9	(	)		2			
RVHa	EI		)I	, C	00	S	G	P	E	L	M	K	P	G	A	S	v	K	I	s	C	ĸ	A	S	G	Y	S	F	1	Г	s	Y	Y	1	H
RVHb	21	1	δı	V	C	S	G	A	E	V	K	ĸ	P	G	X	S	v	X	v	S	С	ĸ	A	S	G	Y	Т	F	1	5					
RVHC	01	1	οī	V	10	S	G	A	E	V	K	K	P	G	A	S	V	K	v	S	C	ĸ	A	S	G	Y	S	F		Г	s	Y	Y	I	н
RVnd	1.			_	_	_	_	-	-	_	-	-	_			-	_	-	_	-	-	-	-	-	-	-	-			-	-	-	-	-	-
	-		1	1		-	_	_	-	-	-	_	_	-	-	-	-	-	-	-	_	-	-	-	_	-	-	÷	1.14	-	÷	-	-	-	-
				-	-	-	-	-	-	-	-	-	-			+	-	-	-	-	-	-	-	-	-	-	-	-	i a	-	÷	÷	-	+	-
							F	R2	2												c	D	R	2											
				4											5											6	5								
V <sub>H</sub> AUK 12-20 SGI	6 7	1 1	3 9	0	1	2	3	4	5	6	7	8	9		0	1	2	2 A	3	4	5	6	7	8	5	0 0	)	1 2	2 3	4	5				
RVHa	W	v	KO	) S	ł	IG	K	S	L	E	N	Л	G		Y	I	D	P	F	N	10	÷C	37	1 5	5 7	YI	N	QI	KF	K	G				
RV <sub>H</sub> b	W	VI	RO	A	F	G	X	G	L	E	N	TV	G																						
RVHC	W														Y	I	D	P	F	N	10	θC	31	1 5	5 3	YI	N	QI	KF	K	G				
RVHd					_	_	_	_	_	_	_	_	_		-	_	_	_	_	-	-	-								-	-				
						-	-	-	-	-	_	I	-		4	_	_	1	2		-		2	1		-	-	-	_	-	-				

	TA	BLE 6-con	ntinued
		FR3	
	7	8	9
V <sub>H</sub> AUK 12-20 SGI	67890123456	789012	2 2 2 2 3 4 5 6 7 8 9 0 1 2 3 4 ABC
RVHa	KATLTVDKSSS	TAYMHL	LSSLTSEDSAVYYCA R
RV <sub>H</sub> b			LSSLRSEDTAVYYCA R
RV <sub>H</sub> c			LSSLRSEDTAVYYCA R
RV <sub>H</sub> d	K V		
	K V		
	CDR3	FR	<b>t</b> 4
	10		11
V <sub>H</sub> AUK 12-20 SGI	5678900012 AB	345678	890123
RVHa	GGN-RFAY	WGOGTI	L VT VS A
RV <sub>H</sub> b	0.36536 2656 126770		LVTVSS
RVHC	GGN-RFAY		
RV <sub>H</sub> d			

Note:

Note: The position where one common amino acid residue is not identified in the HSG I  $V_H$  regions (SGI) is shown as "X". Two under lined amino acid residues are different from those in SGI consensus sequence. For  $RV_H b$ ,  $RV_H c$  and  $RV_H d$ , only amino acid residues different from those of  $RV_H a$  are shown.

Moreover. version "a" to "d" of reshaped human AUK 12-20 antibody H chain V region are designed as shown in Table 7, using FRs of the human antibody HAX (J. Immunology (1987) 139:2496-2501; an antibody produced by

hybridoma 21/28 cells derived from B cells of a SLE patient; its amino acid sequence is described in FIG. 6 and nucleotide sequence of DNA coding for the amino acid sequence is shown in FIGS. 4 and 6, of this literature).

TABLE 7

	FR1
	1 2 3
	1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 0 1 2 3 4 5 6 7 8 9 1 2 3 4 5 6 7 8 9
V <sub>H</sub> AUK 12-20	EIQLQQSGPELMKPGASVKISCKASGYSFT SYYI
SGI	Q V Q L V Q S G A E V K K P G A S V K V S C K A S G Y T F T
sle:	
1220Ha	QVQLVQSGAEVKKPGASVKVSCKASGYSFT SYYI H
1220Hb	
1220Hc	
1220Hd	S
	FR2 CDR2
	4 5 6
	67890123456789 0122223456789012345
	ABC
V <sub>H</sub> AUK 12-20	WVKQSHGKSLEWIG YIDPFNGGTSYNQKFKG
HAX	WVRQAPGQRLEWMG
sle:	
1220Ha	WVRQAPGQRLEWMG YIDP FNGGTSYNQKFKG
1220Hb	
1220Hc	
1220Hd	I
	FR3
	7 8 9
	67890123456789012222345678901234
VHAUK 12-20	KATLTVDKSSSTAYMHLSSLTSEDSAVYYCAR
HAX sle:	R V T I T R D T S A S T A Y ME L S S L R S E D T A V Y Y C A R
1220Ha	R VTI T VDTS AS TAYMELS SLRSEDT AVYYCAR
1220Hb	
1220Hc	KV
1220Hd	KV

	TAB	LE 7-continued	
	CDR3	FR4	
	10	11	
	578900012	3 4 5 6 7 8 9 0 1 2 3	
	AB		
VHAUK 12-20	GGN-RFAY	WGQGTLVTVSA	
HAX		WGQGTLVTVSS	
sle:			
1220Ha	G G N - R F A Y	WGQGTLVTVSA	
1220Hb			
1220Hc			
1220Hd			

Note:

The two underlined residues in sle1220Ha are changes from the HAX FRs. For Sle1220Hb, sle1220Hc, and sle1220Hd, only the amino acids in the FRs that differ from those in the HAX FRs are shown.

For the production of the present chimeric or reshaped human antibodies to the human IL-6R. any expression system, including eucaryotic cells, for example, animal cells, such as established mammalian cell lines, fungal cells. 20 and yeast cells, as well as procaryotic cells, for example, bacterial cells such as *E.coli* cells, may be used. Preferably the present chimeric or reshaped human antibodies are expressed in mammalian cells such as cos cells or CHO cells. 25

In such cases, a conventional promoter useful for the expression in mammalian cells can be used. For example, viral expression system such as human cytomegalovirus immediate early (HCMV) promoter is preferably used. Examples of the expression vector containing the HCMV promoter include HCMV- $V_{H}$ -HC $\gamma$ 1, HCMV- $V_L$ -HC $_K$ -HCMV-12h-g $\gamma$ 1, HCMV-12k-gk and the like derived from pSV2neo, as shown in FIG. 1.

Another embodiment of promoter useful for the present invention is the human elongation factor  $l\alpha$  (HEF- $l\alpha$ ) promoter. Expression vectors containing this promotor include HEF-l2h-gyl and HEF-l2h-gx (FIGS. 8 and 9), as well as HEF- $V_{F}$ gyl and HEF- $V_{L}$ -gx (FIG. 15).

For gene amplification dhfr in a host cell line, an expression vector may contain a dhfr gene. Expression vectors containing the dhfr gene, are for example, DHFR- $\Delta$ E-PMhgy1 (FIG. 10), DHFR- $\Delta$ E-RVh-PM1-f (FIG. 11) and the like.

In summary, the present invention first provides an L chain v region and an H chain V region of a mouse monoclonal antibody to the human IL-6R, as well as DNA coding for the L chain V region and DNA coding for the H <sup>45</sup> chain V region. These are useful for the construction of a human/mouse chimeric antibody and reshaped human antibody to the human IL-6R. The monoclonel antibodies are, for example, AUK12-20, PM-1, AUK64-7 and AUK146-15. The L chain V region has an amino acid sequence shown in. 50 for example, SEQ ID NOs: 24, 26, 28 or 30; and the H chain V region has an amino acid sequence shown in SEQ ID NOs: 25, 27, 29, or 31. These amino acid sequences are encoded by nucleotide sequences, for example, shown in SEQ ID NOs: 24 to 31 respectively. 55

The present invention also relates to a chimeric antibody to the human IL-6R, comprising:

- (1) an L chain comprising a human L chain C region and a mouse L chain V region; and
- (2) an H chain comprising a human H chain C region and 60 a mouse H chain V region. The mouse L chain V region and the mouse H chain V region and DNA encoding them are as described above. The human L chain C region may be any human L chain C region, and for example, is human  $C_{\kappa}$ . The human H chain C region 65 may be any human H chain C region, and for example human  $C_{\gamma 1}$ .

For the production of the chimeric antibody, two expression vectors, i.e., one comprising a DNA coding for a mouse L chain V region and a human L chain C region under the 20 control of an expression control region such as an enhancer/ promoter system, and another comprising a DNA coding for a mouse H chain V region and a human H chain C region under the expression control region such as an enhancer/ promotor system, are constructed. Next, the expression vectors are co-transfected to host cells such as mammalian cells, and the transfected cells are cultured in vitro or in vivo to produce a chimeric antibody.

Alternatively, a DNA coding for a mouse L chain V region and a human L chain C region and a DNA coding for a mouse H chain V region and a human H chain C region are introduced into a single expression vector, and the vector is used to transfect host cells, which are then cultured in-vivo or in-vitro to produce a desired chimeric antibody.

The present invention further provides a reshaped anti-35 body to the human IL-6R, comprising:

- (A) an L chain comprising.
  - (1) a human L chain C region, and
  - (2) an L chain V region comprising a human L chain FRs, and L chain CDRs of a mouse monoclonal antibody to the human IL-6R; and
- (B) an H chain comprising.
  - (1) a human H chain C region, and
  - (2) an H chain V region comprising human H chain FRs, and H chain CDRs of a mouse monoclonal antibody to the IL-6R.

In a preferred embodiment, the L chain CDRs have amino acid sequences shown in any one of SEQ ID NOS: 24, 26, 28 and 30 wherein the stretches of the amino acid sequences are defined in Table 9; the L chain CDRs have amino acid sequences shown in any one of SEQ ID NOS: 25, 27, 29 and 31 wherein the stretches of the amino acid sequences are defined in Table 9; human L chain FRs are derived from the REI; and human H chain FRs are derived from the NEW or HAX.

In the preferred embodiment, the L chain V region has an amino acid sequence shown in Table 2 as  $RV_La$ ; and the H chain V region has an amino acid sequence shown in Table 3 as  $RV_Ha$ .  $RV_Hb$ .  $RV_Hc$ .  $RV_Hd$ .  $RV_Hc$  or  $RV_Hf$ . The amino acid sequence  $RV_Hf$  is most preferable.

For the production of the reshaped human antibody, two expression vectors, i.e., one comprising a DNA coding for the reshaped L chain as defined above under the control of an expression control region such as an enhancer/promoter system, and another comprising a DNA coding for the reshaped human H chain as defined above under the expression control region such as an enhancer/promoter system, are constructed. Next, the expression vectors are co-transfected to host cells such as mammalian cells, and the transfected cells are cultured in vitro or in-vivo to produce a reshaped human antibody.

Alternatively, a DNA coding for the reshaped human L chain and a DNA coding for the reshaped H chain are 5 introduced into a single expression vector, and the vector is used to transfect host cells, which are then cultured in vivo or in vitro to produce a desired reshaped human antibody.

A chimeric antibody of a reshaped human antibody thus produced can be isolated and purified be a conventional 10 processes such as Protein A affinity chromatography, ion exchange chromatography, gel filtration and the like.

The present chimeric L chain or reshaped human L chain can be combined with an H chain to construct a whole antibody. Similarly, the present chimeric H chain or 15 reaction mixture was incubated at 37° C. for 60 minutes and reshaped human H chain can be combined with an L chain to construct a whole antibody.

The present mouse L chain V region, reshaped human L chain V region, mouse H chain V region and reshaped human H chain V region are intrinsically a region which 20 binds to an antigen. human IL-6R. and therefore considered to be useful as such or as a fused protein with other protein. for preparing pharmacenticals or diagnostic agents.

Moreover, the present L chain V region CDRs and H chain V region CDRs are intrinsically regions which bind to 25 Kappa Variable) primers represented in SEQ ID NO: 1 to 11. an antigen, human IL-6R, and therefore considered to be useful as such or as a fused protein with other protein, for preparing pharmacenticals or diagnostic agents.

DNA coding for a mouse L chain V region of the present invention is useful for construction of a DNA coding for a 30 chimeric L chain or a DNA coding for a reshaped human L chain.

Similarly, DNA coding for a mouse H chain V region of the present invention is useful for construction of a DNA coding for a chimeric H chain or a DNA coding for a 35 reshaped human H chain. Moreover, DNA coding for L chain V region CDR of the present invention is useful for construction of a DNA coding for a reshaped human L chain V region and a DNA coding for a reshaped human L chain. Similarly, DNA coding for H chain V region CDR of the 40 present invention is useful for construction of a DNA coding for a reshaped human H chain V region and a DNA coding for a reshaped human H chain.

#### EXAMPLES

The present invention will be further illustrated by, but is by no means limited to, the following Examples.

#### Example 1

Cloning of DNA coding for V region of mouse monoclonal 50 antibody to human IL-6R (1)

A DNA coding for the V region of a mouse monoclonal antibody to a human IL-6R was cloned as follows.

1. Preparation of total RNA

Total RNA from hybridoma AUK12-20 was prepared 55 according to a procedure described by Chirgwin et al., Biochemistry 18, 5294 (1979). Namely, 2.1×108 cells of the hybridoma AUK12-20 were completely homogenized in 20 ml of 4M guanidine thiocyanate (Fulka). The homogenate was layered over a 5.3M cesium chloride solution layer in a 60 ethanol precipitation. Next, the DNA precipitate was centrifuge tube, which was then centrifuged in a Beckman SW40 rotor at 31000 rpm at 20° C. for 24 hours to precipitate RNA. The RNA precipitate was washed with 80% ethanol and dissolved in 150 µl of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA and 0.5% SDS. and after 65 adding Protenase (Boehringer) thereon to 0.5 mg/ml, incubated at 37° C. for 20 minutes. The mixture was extracted

with phenol and chloroform. and RNA was precipitated with ethanol. Next, the RNA precipitate was dissolved in 200 µl of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA.

2. Synthesis of single stranded cDNA

To synthesize single stranded cDNA according to a procedure described by J. W. Larrick et al., Biotechnology, 7, 934 (1989), about 5 µg of the total RNA prepared as described above was dissolved in 10 µl of 50 mM Tris-HCl (pH 8.3) buffer solution containing 40 mM KC1. 6 MM MgCl<sub>2</sub>, 10 mM dithiothreitol, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dCTP. 0.5 mM dTTP. 35 µM oligo dT primer (Amersham), 48 units of RAV-2 reverse transcriptase (RAV-2: Rous associated virus 2; Amersham) and 25 units of human placenta ribonuclease inhibitor (Amersham), and the directly used for the subsequent polymerase chain reaction (PCR) method.

3. Amplification of cDNA coding for antibody V region by PCR method

The PCR method was carried out using a Thermal Cycler Model PHC-2 (Techne).

(1) Amplification of cDNA coding for mouse  $\kappa$  light ( $\kappa$  L) chain variable region

The primers used for the PCR method were MKV (Mouse which hybridize with a mouse K L chain reader sequence (S. T. Jones et al., Biotechnology, 9, 88, 1991), and an MKC (Mouse Kappa Constant) primer represented in SEQ ID NO: 12, which hybridizes with a mouse K L chain C region (S. T. Jones et al., Biotechnology, 9, 88, 1991).

First, 100 µl of a PCR solution comprising 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1 mM dATP, 0.1 mM dGTP, 0.1 mM dCTP, 0.1 mM dTTP, 1.5 mM MgCl, 2.5 units of DNA polymerase Ampli Taq (Perkin Elmer Cetus). 0.25 µM of each group of MKV primer. 3 µM MKC primer and 1 µl of the reaction mixture of the single-stranded cDNA synthesis was heated at an initial temperature of 94° C. for 1.5 minutes, and then at 94° C. for 1 minute, 50° C. for 1 minute and 72° C. for 1 minute, in this order. After this temperature cycle was repeated 25 times, the reaction mixture was further incubated at 72° C. for 10 minutes.

(2) Amplification of cDNA coding for mouse H chain V region

As primers for the PCR. MHV (Mouse Heavy Variable) 45 primers 1 to 10 represented in SEQ ID NO: 13 to 22 (S. T. Jones et al., Biotechnology, 9, 88, 1991), and an MHC (Mouse Heavy Constant) primer represented in SEQ ID NO: 23 (S. T. Jones et al., Biotechnology, 9, 88, 1991) were used. Amplification was carried out according to the same procedure as described for the amplification of the  $\kappa$  L chain V region gene in section 3. (1).

4. Purification and Digestion of PCR Product

The DNA fragments amplified by the PCR as described above were purified using a QIAGEN PCR product purification kit (QIAGEN Inc. US), and digested with 10 units of restriction enzyme Sal I (GIBCO BRL) in 100 mM Tris-HCl (pH 7.6) containing 10 mM MgCl<sub>2</sub> and 150 mM NaCl. at 37° C. for three hours. The digestion mixture was extracted with phenol and chloroform, and the DNA was recovered by digested with 10 units of restriction enzyme Xma I (New England Biolabs), at 37° C. for two hours, and resulting DNA fragments were separated by agarose gel electrophoresis using low melting agarose (FMC Bio Products USA).

An agarose piece containing DNA fragments of about 450 bp in length was excised and melted at 65° C. for 5 minutes. and an equal volume of 20 mM Tris-HCl (pH 7.5) containing

2 mM EDTA and 200 mM NaCl was added thereon. The mixture was extracted with phenol and chloroform, and the DNA fragment was recovered by ethanol precipitation and dissolved in 10 µl of 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA. In this manner, a DNA fragment comprising 5 a gene coding for a mouse K L chain V region, and a DNA fragment comprising a gene coding for a mouse H chain V region were obtained. Both of the above DNA fragments had a Sal I cohesive end at the 5'-end thereof and an Xma I cohesive end at the 3'-end thereof.

5. Ligation and Transformation

About 0.3 µg of the Sal I - Xma I DNA fragment comprising a gene coding for a mouse K L chain V region. prepared as described above, was ligated with about 0.1 µg of a pUC19 vector prepared by digesting plasmid pUC19 by 15 Sal I and Xma I, in a reaction mixture comprising 50 mM Tris-HCl (pH 7.4), 10 MM MgCl<sub>2</sub>, 10 mM dithiothreitol, 1 mM spermidine, 1 mM dATP, 0.1 µg/ml of bovine serum albumin and 2 units of T4 DNA ligase (New England Biolabs), at 16° C. for 16 hours. 20

Next, 7 µl of the above ligation mixture was added to 200 µl of competent cells of E. coli DH5a, and the cells were incubated for 30 minutes on ice, for one minute at 42° C., and again for one minute on ice. After adding 800 µl of SOC medium (Mlecular Cloning: A Laboratory Manual, Sam- 25 brook et al., Cold Spring Habor Laboratory Press, 1989), the cell suspension was incubated at 37° C. for one hour, and inoculated onto an 2×YT agar plate (Mlecular Cloning: A Laboratory Manual, Sambrook et al., Cold Spring Habor Laboratory Press, 1989), which was then incubated at 37° C. 30 overnight to obtain an E.coli transformant. The transformant was cultured in 5 ml of 2×YT medium containing 50 µg/ml ampicillin, at 37° C. overnight, and a plasmid DNA was prepared from the culture according to an alkaline method (Mlecular Cloning: A Laboratory Manual, Sambrook et al., 35 Cold Spring Habor Laboratory Press, 1989). The thusobtained plasmid containing a gene coding for a mouse K L chain V region derived from the hybridoma AUK12-20, was designated p12-k2.

According to the same procedure as described above, a 40 plasmid containing a gene coding for a mouse H chain V region derived from the hybridoma AUK12-20 was constructed from the Sal I - Xma I DNA fragment, and designated p12-h2.

#### Example 2

Cloning of DNA coding for V region of mouse monoclonal antibody (2)

Substantially the same procedure as described in Example 1 was applied to the hybridoma PM1, AUK64-7, and 50 AUK146-15, to obtain the following plasmids:

- a plasmid pPM-k3 containing a gene coding for a K L chain V region derived from the hybridoma PM1;
- a plasmid pPM-h1 containing a gene coding for an H chain V region derived from the hybridoma PM1:
- a plasmid p64-k4 containing a gene coding for a K L chain V region derived from the hybridoma AUK64-7;
- a plasmid p64-h2 containing a gene coding for an H chain V region derived from the hybridoma AUK64-7;
- a plasmid p146-k3 containing a gene coding for a K L chain V region derived from the hybridoma AUK146-15; and
- a plasmid pl46-h1 containing a gene coding for an H chain V region derived from the hybridoma AUK146-15. 65

Note E. coli strains containing the above-mentioned plasmid were deposited with the National Collections of Industrial and Marine Bacteria Limited under the Budapest Treaty on Feb. 11, 1991, and were given the accession number shown in Table 8.

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Plas	mid	SEQ ID NO	Accession No.
p12	- k2	24	NCIMB 40367
p12	- h2	25	NCIMB 40363
pPM	- k3	26	NCIMB 40366
pPM	- h1	27	NCIMB 40362
p64	- k4	28	NCIMB 40368
p64	- h2	29	NCIMB 40364
p146	5 - k3	30	NCIMB 40369
p146	5 - h1	31	NCIMB 40365

#### Example 3

Sequencing of DNA

Nucleotide sequences of a cDNA coding region in the above-mentioned plasmids were determined using a kit. Sequenase<sup>™</sup> Version 2.0 (U.S. Biochemical Corp. USA).

First, about 3 µg of plasmid DNA obtained as described above was denatured with 0.2N NaOH, annealed with a sequencing primer, and labeled with <sup>35</sup>S-dATP according to a protocol of the supplier. Next, the labeled DNA was applied to 6% polyacrylamide gel containing 8M urea, and. after electrophoresis, the gel was fixed with 10% methanol and 10% acetic acid, dried, and subjected to autoradiography to determine the nucleotide sequence.

The nucleotide sequence of cDNA coding region in each plasmid is shown in SEQ ID NOs 24 to 31.

#### Example 4

Determination of CDRs

General structures of L chain and H chain V regions are similar each other, wherein 4 frame works (FRs) are linked through 3 super variable regions, i.e., complementarity determining regions (CDRs). While amino acid sequences in the FRS are relatively well conserved, amino acid sequences in CDRs are very highly variable (Kabat, E. A. et al., "Sequences of Proteins of Immunological Interest", U.S. Dept. Heath and Human Services 1983).

On the basis of the above-determined amino acid sequences of V regions of mouse monoclonal antibodies to human IL-6R, and according to Kabat, E. A. et al., "Sequences of Proteins of Immunological Interest". US Dept. Health and Human Services 1983, CDRs of each V region of mouse monoclonal antibodies to the human IL-6R were determined as shown in Table 9.

TABLE 9

plasmid	SEQ ID NO	CDR(1)	CDR(2) amino acid No	CDR(3) 5.)
p12-K2	24	24-38	54-60	93-101
p12-h2	25	31-35	50-66	99-105
pPM-k3	26	24-34	50-56	89-97
pPM-h1	27	31-36	51-66	99-108
p64-k4	28	24-38	54-60	93-101
p64-h2	29	31-35	50-66	99-109
p146-k3	30	24-34	50-56	89-97
p146-h1	31	31-35	50-66	99-106

#### Example 5

Confirmation of expression of cloned cDNA(1) (Construction of Chimeric AUK12-20 antibody)

Construction of Expression Plasmid

A chimeric L chain/H chain was constructed from PCRcloned cDNAs coding for V regions K L chain and H chain

45

of AUK12-20. In order to easily join a cDNA coding for the mouse AUK12-20 V region to a DNA coding for a human C region in a mammalian expression vector containing an enhancer and promoter of human cytomegalovirus (HCMV) expression vector, it is necessary to introduce convenient 5 restriction enzyme cleavage sites to the 5'- and 3'- termini of the mouse cDNA.

This modification of the 5'- and 3'- termini was carried out by PCR method. Two sets of primers were designed and synthesized. An L chain V region backward primer (SEQ ID 10 NO: 32) and H chain V region backward primer (SEQ ID NO: 33) were designed so that the primers hybridize with a DNA coding for the beginning of the leader sequence. maintain a DNA sequence essential for efficient translation (Kozak, M., J. Mol. Biod. 196: 947-950, 1987) and form a 15 HindIII site for cloning into the HCMV expression vector. An L chain V region forward primer (SEQ ID NO: 34) and an H chain V region forward primer (SEQ ID NO: 35) were designed so that the primers hybridize with a DNA coding for the terminal portion of the J region. maintain a DNA 20 sequence essential for splicing into the C region and form a Bam HI site for joining to the human C region in the HCMV expression vector.

Following the amplification by the PCR, the PCR product was digested with Hind III and BamHI, cloned into the 25 HCMV vector containing the human  $\kappa$  and  $\gamma$  chain C regions DNA and sequenced to confirm that errors were not introduced during the PCR amplification. The resulting expression vectors are designated as HCMV-12k-gk and HCMV-12h-gy1. 30

The structures of the HCMV expression plasmids are shown in FIG. 1. In the plasmid HCMV- $V_L$ -HC<sub>K</sub>,  $V_L$  region may be any mouse L chain V region. In this example, AUK12-20 KL chain V region was inserted to obtain the HCMV-12k. In the plasmid HCMV- $V_{H}$ -HC $\gamma$ l,  $V_{H}$  region 35 may be any mouse H chain V region. In this example, AUK12-20 H chain V region was inserted to obtain HCMV-12h-g $\gamma$ l.

Transient expression in COS cells

To observe transient expression of a chimeric AUK12-20 40 antibody in COS cells, the expression vectors constructed as described above were tested in the COS cells. The vector DNAs were introduced into COS cells by electroporation using a Gene Pulsar apparatus (Bio Rad). Namely, COS cells were suspended in phosphate-buffered saline (PBS) to a cell 45 concentration of  $1 \times 10^7$  cells / ml. and to 0.8 ml aliquot of the suspension was added 10 µg (per each plasmid) of DNA. Pulses were applied at 1.900 V and 25 µF.

After recovery period of 10 minutes at a room temperature, the electroporated cells were added to 8 ml of 50 DMEM (GIBCO) containing 10% fetal bovine serum. After incubation for 72 hours, a culture supernatant is collected, centrifuged to eliminate cell debris, and aseptically stored for a short period at  $4^{\circ}$  C. or for a long period at  $-20^{\circ}$  C.

Quantification of chimeric antibody by ELISA 55 A culture supernatant of the transfected COS cells was assayed by ELISA to confirm the production of chimeric antibody. To detect the chimeric antibody, a plate was coated with goat anti-human IgG whole molecule (Sigma). The plate was blocked, and serially diluted supernatant from the COS cell culture was added to each well. After incubation and washing, alkaline phosphatase-linked goat anti-human IgG ( $\gamma$ -chain specific, Sigma) was added to each well. After incubation and washing, substrate buffer was added thereon. The reaction mixture was incubated, and after termination of the reaction, optical density at 405 mm was measured. As a standard, purified human IgG (Sigma) was used.

ELISA for confirmation of an ability to bind to human IL-6R

A culture supernatant of the transformed COS cells was assayed by ELISA to determine whether the produced antibody can bind to the antigen. To detect the binding to the antigen, a plate was coated with MT18 mouse monoclonal antibody (Reference Example 1). and after blocking with 1% bovine serum albumin (BSA) soluble recombinant human IL-6R (SR 344) was added thereon. After washing. a serially diluted culture supernatant from the COS cells was added to each well. After incubation and washing alkaline phosphatase-linked goat anti-human IgG was added. The reaction mixture was incubated, and after washing a substrate buffer was added. After incubation, the reaction was terminated, and optical density at 405 mm was measured.

A result is shown in FIG. 2. Transfection of gene coding for a chimeric antibody AUK12-20 into COS cells was twice repeated. Both the culture supernatant samples exhibited a strong binding to IL-6R, and optical density at 405 mm was changed in a sample dilution (monaclonal antibody concentration)—dependent manner as shown in FIG. 2 by open circles and closed circles revealing the presence of an antibody to IL-6R in the sample.

Determination of an ability to inhibit the binding to IL-6R with IL-6

To determine whether an antibody present in a medium inhibits the binding of IL-6R with IL-6, a plate was coated with MT18 monoclonal antibody (Reference Example 1). After blocking, soluble recombinant human IL-6R (SR 344) was added thereon. After washing, serially diluted sample from COS cell culture was added to each well with biotinated IL-6.

After washing, alkaline phosphatase-linked streptoavidin was added, and after incubation and washing, a substrate buffer was added. The reaction mixture was incubated, and after termination of the reaction, optical density at 405 mm was measured, purified mouse AUK12-20 monoclonal antibody was added as a positive control, and a culture medium from COS cells expressing a non-related antibody was used as a negative control.

A result is shown in FIG. 3. A culture supernatant of COS cells transfected with genes coding for chimeric antibody AUK 12-20 exhibited the binding of IL-6R with IL-6 at the highest and second highest concentrations. Namely, as shown by closed circles in FIG. 3, optical density at 405 mm changed in a sample dilution (antibody concentration) dependent manner, revealing the inhibition of the binding to IL-6R with IL-6 by an antibody in the sample. This is further confirmed by substantive conformity with antibody concentration dependent change of the positive control (open circles). Note, the negative control did not exhibit inhibition activity (open triangles).

#### Example 6

Confirmation of expression of cloned cDNA (2) 55 (Construction of chimeric PM-1 antibody)

(Construction of expression vectors)

In order to construct vectors expressing chimeric PM-1 antibody, the cDNA clones pPM-k3 and pPM-h1, coding for the mouse PM-1  $\kappa$ L chain and the H chain V regions, respectively, were modified by a PCR technique, and then introduced into the HCMV expression vectors (see FIG. 1). The backward primers pmk-s (SEQ NO: 38) for L chain V region and pmh-s (SEQ NO: 40) for H chain V region were designed to hybridize to the DNA sequences coding for the beginning of the leader sequences, and to have Kozak consensus sequence and a HindIII restriction site. The forward primers pmk-a (SEQ No: 36) for L chain V region and pmh-a (SEQ No: 39) for H chain V region were designed to hybridize to the DNA sequences coding for the ends of the J regions, and to have a splice donor sequence and a BamHI restriction site.

For the kappa L chain V region, two forward primers were 5 synthesized. Although in most kappa L chains lysine at position 107 is conserved, in mouse PM-1 kappa L chain position 107 is an asparagine. In order to investigate the effect of this change on the antigen-binding activity of the chimeric PM-1 antibody, the forward primer pmk-b (SEQ 10 NO: 37) was designed to mutate position 107 from an asparagine to a lysine. Following the PCR reaction, the PCR products were purified, digested with HindIII and BamHI, and subcloned into a pUC19 vector (Yanishe-Perron et al., Gene (1985) 33:103-109). After DNA sequencing, the 15 HindIII-BamHI fragments were excised and cloned into the expression vector HCMV-VH-HCy1 to obtain HCMV-pmhgy1 for the chimeric H chain, and into the expression vector HCMV-V<sub>L</sub>-HC<sub>k</sub> to obtain HCMV-pmka-gk and HCMVpmkb-gk for the chimric L chain.

Transfection of cos cells

The vectors were tested in cos cells to look for transient expression of chimeric human PM-1 antibodies. The HCMV-pmh-gy1. and either HCMV-pmka-gk or HCMVpmkb-gk were co-transfected into the cos cells by electroporation using the Gene Pulsar apparatus (BioRad). DNA (10  $\mu$ g of each plasmid) was added to a 0.8 ml aliquot of  $1 \times 10^7$ cells/ml in PBS. A pulse was delivered at 1,900 volts. 25 microfarads capacitance. After a 10 min recovery period at a room temperature, the electroporated cells were added to 30 20 ml of Dulbecco's Modified Eagle Medium (DMEM) (GIBCO) containing 10% gamma-globulin-free fetal calf serum. After 72 h incubation, the medium was collected, centrifuged to remove cellular debris, and stored under sterile conditions at 4° C. for short periods of time, or at 35 -20° C. for longer periods.

Expression and analysis of the chimeric PM-1 antibodies After 3 days of transient expression, medium from the cos cells was collected and tested for chimeric PM-1 antibody. The medium was first analyzed by ELISA to determine if 40 human-like antibody was being produced by the transfected cos cells. By using known amounts of purified human IgG as a standard in this assay, it is also possible to estimate an amount of human-like antibody (in this case, chimeric PM-1 antibody) present in the medium from the cos cells. For the 45 detection of human antibody, plates were coated with goat anti-human IgG (whole molecule, Sigma). Following blocking, the samples from cos cells were serially diluted and added to each well. After incubation and washing. alkaline phosphatase-conjugated goat anti-human IgG 50 (gamma chain specific, Sigma) was added. After incubation and washing, substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. Purified human IgG (Sigma) was used as a standard.

The medium from the cos cells transfected with the vectors carrying the chimeric PM-1 genes was positive for the expression of a human-like antibody and the approximate amounts were quantified as described.

Next, the same medium from the cos cells transfected 60 with the vectors carrying the chimeric PM-1 genes was assayed for a an ability to bind to human IL-6R. For the detection of binding to the antigen, plates were coated with MT18 mouse monoclonal antibody (Reference Example 1). an antibody to the human IL-6R. Following blocking. 65 soluble recombinant human IL-6R (SR344) was added. After washing, the samples were serially diluted and added

to each well. After incubation and washing, alkaline phosphatase-conjugated goat anti-human IgG (gamma chain specific sigma) was added. After incubation and washing, substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. There was no standard available for this assay.

Two samples were from transfection with genes coding for a chimeric antibody with V regions identical to those found in mouse PM-1 antibody (chimeric PM-1a antibody. FIG. 4). One sample was from transfection with genes coding for a chimeric antibody with a single amino acid change at position 107 in the L chain V region as described above (chimeric PM-1b antibody, FIG. 4). All samples showed strong binding to IL-6R that decreased with dilution of the sample. Thus, the chimeric PM-1 antibody, as constructed, is functional and can bind well to its antigen. Most importantly, the demonstration of a functional chimeric PM-1 is direct evidence that the correct mouse PM-1 V regions have been cloned and sequenced. The chimeric PM-1 antibody, with either amino acid at position 107 in the L chain V region, bound well to its antigen, IL-6R. It appears that position 107 in the mouse PM-1 L chain V region is not very critical in antigen-binding and that either an asparagine or a lysine at this position will function satisfactorily. Since the mouse PM-1 antibody has an asparagine at this position in its L chain V region, all future work with chimeric PM-1 antibody was done with version a, the version that has V regions identical to those found in mouse PM-1 antibody.

In order to stably produce larger amounts of chimeric PM-1 antibody, a new HCMV expression vector incorporating the dhfr gene was constructed. The first step in achieving higher levels of expression of the chimeric PM-1 antibody was to modify the vector HCMV-V<sub>H</sub>-HC<sub>v1</sub> (FIG. 1) so that this vector contained a dhfr gene being expressed by a "crippled" SV40 promoter-enhancer. The SV40 enhancer elements were deleted from the pSV2-dhfr vector (S. Subramani et al., Mol. Cell. Biol. (1981) 1:854-864) and the dhfr gene being expressed by the "crippled" SV40 promoter was inserted into the HCMV-V<sub>H</sub>-HC<sub>γ1</sub> vector in place of the neo gene being expressed by the SV40 promoter-enhancer. The mouse PM-1 V region was then inserted into this new HCMV-V<sub>H</sub>-HC<sub>Yl</sub>-dhfr vector. Construction of the improved expression vector is described in Example 10 in detail.

CHO dhfr(-) cells (G. Urlaub et al., Proc. Natl. Acad. Sci. USA (1980) 77:4216-4220) were co-transfected with two plasmid DNAs, the HCMV-VL-HCk vector for expressing chimeric PM-1a L chain (HCMV-pmka-gk) and the HCMV-V<sub>H</sub>-HC<sub>y1</sub>-dhfr vector for expressing chimeric PM-1 H chain (DHFR-AE PMh-gyl; Example 10). DNA (10 µg/ml of each plasmid) was added to a 0.8 ml aliquot of 1×107 cells/ml in PBS. A pulse was delivered at 1900 volts, 25 microfarads capacitance. After a 10 min recovery period at a room temperature, the electroporated cells were added to 10 ml of Alpha minimum essential medium (a-MEM) containing 55 nucleosides and 10% FCS. After overnight incubation, the medium was changed to  $\alpha$ -MEM without nucleosides and with 10% FCS and 500 µg/ml of G418 (GIBCO) for the selection of dhfr<sup>+</sup>and neo<sup>+</sup>transformed cells. After selection, the selected clones were used for gene amplification. After one round of amplification in 2×10<sup>-8</sup> M methotrexate (MTX), a cell line (PM1k3-7) was selected that produced approximately 3.9 µg/106 cells/day of chimeric PM-1a antibody.

ELISA assay for the ability of chimeric antibodies to inhibit IL-6 from binding to human IL-6R.

Antibodies produced in transfected cos cells or in stable CHO cell lines were assayed to determine whether the

antibodies could compete with biotinylated IL-6 for binding to IL-6R. Plates were coated with MT18 mouse monoclonal antibody. Following blocking, soluble recombinant human IL-6R (SR344) was added. After washing, the samples from the cos cells were serially diluted and added together with 5 biotinylated IL-6 to each well. After washing, alkaline phosphatase-conjugated streptavidin was added. After incubation and washing, substrate buffer was added. After incubation, the reaction was stopped and the optical density at 405 nm measured. The Results are shown in FIG. 5.

#### Example 7

Construction of reshaped human PM-1 antibodies

In order to achieve CDR-grafting more rapidly and efficiently, a method for sequential CDR-grafting by PCR was developed. This method is based on PCR-mutagenesis 15 repeated. The ramp time between the annealing and synthemethods (Kamman et al., 1989).

In order to prepare the template DNAs containing the selected human FRs for CDR-grafting, it was necessary to reclone suitable reshaped human V regions into convenient vectors. Plasmid DNAs alysll and F10 code for reshaped 20 human D1.3 L and H chains and contain the FRs from human REI and NEW, respectively. An approximately 500 bp NcoI-BamHI fragment containing DNA sequence coding for the reshaped human D1.3 L chain V region was excised from alysll and subcloned into HindIII-BamHI cleaved- 25 same manner, a 665 bp PCR product from the second PCR pBR327 to obtain a vector V1-lys-pBR327. HindIII-BamHI fragment from the V1-lys-pBR327 was inserted into HindIII-BamHI cleaved pUC19 to obtain a vector V1-lyspUC19. An approximately 700 bp NcoI-BamHI fragment containing DNA sequence coding for the reshaped human 30 D1.3 H chain V region was excised from F10 and subcloned into the HindIII-BamHI site of pBR327 vector, using a HindIII-NcoI adaptor, yielding Vh-lys-pBR327. A HindIII-BamHI fragment was then excised from this vector and subcloned into HindIII-BamHI cleaved pUC19 vector yield- 35 ing Vh-lys-pUC19.

Note the construction of the plasmid alysll and the DNA sequence coding for the reshaped human D1.3 L chain V region used in a template is described. The DNA sequence coding for the reshaped human D1.3 H chain V region in the 40 plasmid F10 used as a template is described in V. Verhoey et al., Science 237:1534-1536 (1988) FIG. 2.

FIG. 6 diagrams the primers and the PCR reactions used in the construction of the first version of reshaped human PM-1 H chain V region. A backward primer A (APCR1; 45 SEQ NO: 41) and a forward primer E (APCR4; SEQ NO: 42) hybridize to DNA sequences on the vector. Although APCR1 and APCR4 were specifically designed for pUC19 vector, universal M13 sequence primers could be used.

The CDR1-grafting/mutagenic primer B (phv-1; SEQ 50 NO: 43). CDR2-grafting primer C (phv-2; SEQ NO: 44) and CDR3-grafting primer D (phv-3; SEQ NO: 45) were 40-60 bp in length, consisting of DNA sequences coding for CDRs from the mouse PM-1 H chain V region and the human FRs in the template DNA that flank the CDR regions. In the first 55 PCR reaction, the forward primer APCR4 and the backward primer D were used. The first PCR product, which contains the mouse PM-1 CDR3 sequence, was purified and used in the second PCR reaction as a forward primer with primer C as the backward primer. In the same manner, the second and 60 third PCR products, which contain mouse PM-1 CDR2 and CDR3, and all three mouse PM-1 CDRs, respectively, were used as primers in the following PCR step. The fourth PCR product, which has the complete reshaped human PM-1 H chain V region, was purified, digested with HindIII and 65 PM-1 L chain V region. For the construction of the first BamHI, and subcloned into a pUC19 vector for further analysis.

Three mutagenic primers phv-1, phv-2, and phv-3 were synthesized for the construction of reshaped human PM-1 H chain V region. They were purified on 12% polyacrylamide gels containing 8M urea. The mutagenic primer phv-1 was designed not only for mouse PM-1 CDR1-grafting but also for mutations at positions 27 and 30 in human FR1. Ser to Tyr and Ser to Thr. respectively. Each 100 µl PCR reaction typically contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl. 1.5 mM MgCl<sub>2</sub>, 250 µM dNTPs, 50 ng of the template DNA (Vh-lys-pUC19). 2.5µ of AmpliTaq (Perkin Elmer Cetus) and the primers. The first PCR reaction containing 1 µM of each of the phv-3 and APCR4 primers was carried out, after an initial denaturation at 94° C. for 1.5 min, for 30 cycles of 94° C. for 1 min, 37° C. for 1 min and 72° C. for 1 min were sis steps was set for 2.5 min. The completion of the last cycle was followed by a final extension at 72° C. for 10 min. A 523 bp PCR product was purified using a 1.6% low melting temperature agarose gel and then used as a primer in the second PCR reaction.

In the second PCR reaction approximately 1 µg of the purified first PCR product and 25 pmoles of the mutagenic primer phv-2 were used as primers. The PCR conditions were the same as described for the first PCR reaction. In the reaction and a 737 bp PCR product from the third reaction were used as primers in the third PCR reaction with the primer phv-1, and in the fourth PCR reaction with the primer APCR1, respectively. A 1.172 kb PCR product from the fourth PCR reaction was purified. digested with HindIII and BamHI, and then an approximately 700 bp fragment containing the reshaped human PM-1 H chain V region was subcloned into a pUC19 vector. Two of four clones sequenced had the DNA sequence coding for the correct amino acid sequence and were designated pUC-RVh-PM1a.

In order to construct other versions of reshaped PM-1 H chain V region, five mutagenic PCR primers were synthesized. Each PCR reaction was essentially carried out under the same condition as described above. For version "b", mutagenic primer phv-m4 (Val-71 to Arg-71; the number is according to Kabat et al; see Table 3) (SEQ ID NO: 46) and APCR4 were used in the first PCR reaction with template DNA. pUC-RVh-PM1a. The PCR product from this first PCR reaction was purified and was used as a forward primer in the second PCR reaction with the primer APCR1. The PCR product from the second PCR reaction was purified using a 1.6% low melting temperature agarose gel, digested with HindIII and BamHI, and subcloned into a pUC19 vector yielding pUC-RVh-PM1b. In the same manner, version "c" (pUC-RVh-PM1c) was obtained using a mutagenic primer phv-nm (Asp-1 to Gln-1) (SEQ ID NO: 47) and a template pUC-RVh-PM1b; version "d" (pUC-RVh-PM1d) was obtained using a mutagenic primer phv-m6 (Ile-48 to Met-48) (SEQ ID NO: 48) and a template pUC-RVh-PM1b; version "e" (pUC-RVh-PM1e) was obtained using the mutagenic primer phv-m6 and a template pVC-RVh-PM1c; and "version f" (pUC-RVh-PM1f) was obtained using a mutagenic primer phv-m7 (Thr-28 to Ser-28, and Phe-29 to Ile-29) (SEQ ID NO: 49) and a template pUC-RVh-PM1b. Amino acid sequence of the version "f" of the reshaped human H chain V region, and a nucleotide sequence codin therefor is shown in SEQ ID NO: 54.

FIG. 7 diagrams the primers and the PCR reactions used in the construction of the first version of reshaped human version of reshaped human PM-1 L chain V region. CDR1grafting primer pkv-1 (SEQ ID NO: 50). CDR2-grafting

primer pkv-2 (SEQ ID NO: 51) and CDR3-grafting primer pkv-3 (SEQ ID NO: 52) were synthesized and purified on a 12% polyacrylamide gel containing 8M urea. PCR reactions were carried out as described above. The first PCR reaction contained 1 µM of each of the pkv-3 and APCR4 primers. A 350 bp PCR product from the first PCR reaction was purified using a 1.5% low melting temperature agarose gel and used as a forward primer in the second PCR reaction. The PCR product from the second PCR reaction was purified, digested with BamHI and HindIII. and the 500 bp fragment containing the CDR3-grafted DNA was subcloned into a pUC19 vector for DNA sequencing. A plasmid DNA having the correct sequence was identified and used as the template DNA in the following PCR reaction. In the third PCR reaction. 25 pmoles of mutagenic primers pkv-2 and APCR4 were used. The PCR product from the third PCR reaction was purified and used as a primer, with the primer pkv-1, in the fourth PCR reaction. In the same manner, the PCR product from the fourth PCR reaction was used as a primer. with the APCR1 primer. in the fifth PCR reaction.

A 972 bp PCR product from the fifth PCR reaction was purified, digested with BamHI and HindIII, and subcloned into a pUC19 vector for DNA sequencing. A problem was identified in the CDR2 region. Two additional PCR reactions were necessary. In the sixth and seventh PCR reactions, the PCR product from the fifth PCR reaction, as cloned into pUC19 vector, was used as template DNA. In the sixth PCR reaction, the primers were pkv-2 and APCR4. The PCR product from the sixth PCR reaction was purified and used as a primer, with the APCR1 primer, in the seventh PCR reaction. The PCR product of the seventh PCR reaction was purified, digested with BamHI and HindIII, and a 500 bp DNA fragment was subcloned into a pUC19 vector for DNA sequencing. Two of five clones sequenced had the correct DNA sequence. The clone was designated pUC-RV1-PM1a. 35 The sequence is shown in SEQ ID NO: 55.

For the construction of the other version of reshaped human PM-1 L chain V region, a mutagenic primer pvk-m1 (SEQ ID NO: 53) was synthesized. The PCR reactions were essentially as described above. In the first PCR reaction, the mutagenic primer pkv-m1 (Phe-71 to Tyr-71) and the APCR4 primer were used with the template DNA pUC-RV1-PM1a. The PCR product of the first PCR reaction was purified and used as a primer, with the APCR1 primer, in the second PCR reaction. The PCR product of the second PCR reaction was purified, digested with BamHI and HindIII, and subcloned into a pUC19 vector for DNA sequencing. The clone was designated pUC-RV1-PM1b.

#### Example 8

Construction of vectors that employ the human cytomega- 50 lovirus immediate early (HCMV) promoter to express genetically-engineered antibodies in mammalian cells (FIG. 1).

The DNA fragments coding for the chimeric PM-1 L and H chain V regions were initially inserted into HCMV vectors 55 (HCMV-V<sub>L</sub>-HC<sub>K</sub> and HCMV-V<sub>H</sub>-HC<sub>N</sub>) designed to express either human kappa L chains or human gamma-1 H chains in mammalian cells (see FIG. 1). A detailed description of the construction of the HCMV expression vectors is published in Maeda et al., Human Antibodies and Hybridomas 60 (1991) 2:124–134; C. A. Kettleborough et al., Protein Engeneering (1991) 4:773–783. Both vectors are based on pSV2neo (P. J. Southern et al., J. Mol. Appln. Genet. (1982) 1:327–341) and contain the human cytomegalovirus (HCMV) promoter and enhancer (M. Boshart et al., Cell 65 (1985) 41:521–530) for high level transcription of the immunoglobulin L and H chains. The L chain expression

vector contains genomic DNA coding for the human kappa C region (T. H. Rabbitts et al., Curr. Top. Microbiol. Immunol. (1984) 113:166–171) and the H chain expression vector contains genomic DNA coding for the human gamma-1 C region (N. Takahashi et al. Cell (1982) 29:671–679). The HCMV expression vectors are versatile and can be used for both transient and stable expression in a variety of mammalian cell types.

#### Example 9

10 Construction of vectors that employ the human elongation factor 1α (HEF-1α) promoter to express geneticallyengineered antibodies in mammalian cells (FIG. 8 and FIG. 9)

The human polypeptide chain elongation factor 1a (HEF-15 1 $\alpha$ ) is one of the most abundant proteins. It is expressed in most cells. The transcriptional activity of the human EF-1 $\alpha$ promoter-enhancer is about 100-fold stronger than that of the SV40 early promoter-enhancer (D. W. Kim et al., Gene (1990) 91:217-223, and T. Uetsuki et al., J. Biol. Chem. (1989) 264:5791-5798). The 2.5 kb HEF-1a promoter-20 enhancer region consists of approximately 1.5 kb of DNA flanking the 5'-end of the gene, 33 bp in the first exon, 943 bp in the first intron, and 10 bp of the first part of the 2nd exon. The approximately 2.5 kb HindIII-EcoRI fragment was excised from plasmid DNA pEF321-CAT (D. W. Kim et 25 al., Gene (1990) 91:217-223, and T. Uetsuki et al., J. Biol. Chem. (1989) 264:5791-5798) and cloned into pdKCR vector DNA (M. Tsuchiya et al., EMBO J. (1987) 6:611-616) (K. O'Hare et al., Proc. Natl. Acod. Sci USA Vol. 78, No. 3, 1527-1531, 1981) to replace an approximately 300 bp HindIII-EcoRI fragment containing the SV40 early promoter-enhancer sequence thus yielding pTEF-1. pTEF-1 was digested with EcoRI, filled-in with the Klenow polymerase, and ligated to HindIII linkers. An approximately 1.6 kb HindIII-SmaI fragment was then excised from

the modified pTEF-1 vector DNA. Plasmid DNA HCMV-12h-gy1 ( $\Delta$ E2) was constructed from the HCMV-12h-gy1 constructed in Example 5 by partially digesting HCMV-12h-gy1 with EcoRI. filling-in with klenow polymerase, and self-ligating.

The plasmid HCMV-12h-gy1 ( $\Delta$ E2) was digested with EcoRI, filled-in with Klenow polymerase, and digested with HindIII. The resulting approximately 7 kb fragment containing the DNA sequence coding for human gamma-I C region was ligated to the above-prepared 1.6 kb HindIII-SmaI fragment containing the HEF-1 $\alpha$  promoter-enhancer yielding HEF-12h-gy1. The HEF-1 $\alpha$  promoter-enhancer region in this vector was the same as that in pTEF-1 except for 380 bp of DNA flanking the 5'-region. The H chain V region, present as a HindIII-BamHI fragment, was easily interchangeable with other H chain V regions.

HindIII-BamHI DNA fragments containing the reshaped H chain V region were excised from the pUC-RVh-PM1a, pUC-RVh-PM1b, pUC-RVh-PM1c, pUC-RVh-PM1d, pUC-RVh-PM1e, and pUC-RVh-PM1f (Example 7), and inserted into the HindIII-BamHI portion of the HEF-12h-gy1 to obtain expression vectors RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e and RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e and RVh-PM1f, respectively. The expression vectors RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e and RVh-PM1f, as well as HEF-PMh-gy1 have the reshaped human PM-1 H chain V regions versions "a", "b", "c", "d", "e" and "f", as well as the mouse PM-1 H chain V region, respectively.

To construct the L chain expression vector. HEF-12k-gk, an approximately 3.0 kb PvuI-HindIII fragment containing the HEF-1 $\alpha$  promoter-enhancer region was excised from the HEF-12h-gyl and ligated to an approximately 7.7 kb PvuI-

HindIII fragment from the HCMV L chain expression vector HCMV-12k-gk constructed in Example 5 to obtain HEF-12k-gk. As for the H chain expression vector HEF-12h-gy1. the L chain V region in HEF-12k-gk, present as a HindIII-BamHI fragment, is easily interchangeable with other L 5 chain V regions.

HindIII-BamHI DNA fragments containing the reshaped human L chain V region were excised from the pUC-RV1-PM1a and pUC-RV1-PM1b (Example 7), and inserted into the HindIII-BamHI portion of the HEF-12k-gk to obtain 10 expression vectors RV1-PM1a and RV1-PM1b, respectively. The expression vectors RV1-PM1a, RV1-PM1b, and HEF-PMk-gk have the reshaped human L chain V regions "a", "b", and the mouse PM-1 L chain V region, respectively.

#### Example 10

Construction of vectors that employ the dihydrofolate reductase (dhfr) gene linked to a defective SV40 promoterenhancer sequence to achieve high levels of expression of genetically-engineered antibodies in CHO cells (FIG. 10 and FIG. 11).

In order to remove the enhancer sequence from the SV40 early promoter, the plasmid DNA pSV2-dhfr (S.Subramani et al., Mol. Cell. Biol. (1981) 1: 854-864) (ATCC 33694) was digested with SphI and PvuII, filled-in with Klenow polymerase, and self-ligated to yield pSV2-dhfr- $\Delta E$  (see FIG. 10). An approximately 3.7 kb EcoRI fragment containing the HCMV promoter, the H chain V region, and the human gamma-1 C region was excised from HCMV-PMhgyl by partially digesting with EcoRI. This fragment was ligated to EcoRI-digested pSV2-dhfr-AE to yield DHFR-ΔE-PMh-gyl.

A similar vector was constructed based on the H chain expression vector that employs the HEF-1a promoterenhancer (see FIG. 11). An approximately 3.7 kb EcoRI fragment derived from HCMV-12h-gyl was ligated with EcoRI-digested pSV2-dhfr- $\Delta E$  to yield DHFR- $\Delta E$ -12h-gy1. The BamHI site following the dhfr cDNA sequence in DHFR-AE-12h-gyl was removed by partially digesting with BamHI, filling-in with Klenow polymerase, and selfligating. An approximately 4 kb PvuI-BamHI fragment 40 containing the dhfr cDNA was excised from the modified DHFR- $\Delta E$ -12h-gy1 DNA and ligated to an approximately 3 kb PvuI-BamHI fragment from RVh-PM1f-4 (constructed in Example 12) to yield DHFR-AE-RVh-PM1f. 45

The improved expression plasmids as prepared above can be used for the production of the reshaped human PH-1 antibodies of the present invention.

#### Example 11

Expression and analysis of different versions of reshaped 50 human PM-1 antibody

The HEF-1a vectors expressing reshaped human PM-1 L and H chains were co-transfected into cos cells. As a standard control, HEF-10 vectors expressing chimeric PM-1 L and H chains were also co-transfected into cos cells. After 55 3 days the medium from the transfected cos cells was collected and analyzed by ELISA (1) for the amount of human IgG antibody present in the supernatant and (2) for the ability of that human IgG to bind to IL-6R. Later the same samples were also tested by ELISA for the ability of 60 coding for the leader sequences of the reshaped human the antibody to inhibit human IL-6 from binding to human IL-6R.

Evaluation of the two versions of reshaped human PM-1 L chain V regions were conducted by co-transfecting cos cells with one of the two vectors expressing reshaped human 65 After 48 hrs. total RNA was prepared (Chirgwin et al., PM-1 L chains (RV1-PM1a or RV1-PM1b) and the vector expressing chimeric PM-1 H chain (HCMV-PMh-gyl).

Cells were also co-transfected with vectors expressing chimeric PM-1 L and H chains (HCMV-PMka-gk and HCMV-PMh-gy1). Data using unpurified cos cell supernatants showed that version "a" of reshaped human PM-1 L chain was equivalent to chimeric PM-1 L chain in assays for binding to IL-6R. Version "b" of reshaped human PM-1 L chain, however, virtually abolished binding to IL-6R (FIG. 12). From these results, it was concluded that the change at position 71 in FR3 from phenylalanine (as present in the human REI as modified for CAMPATH-1H) to tyrosine (as present in natural human REI and in mouse PM-1) was very detrimental to the formation of a functional antigen-binding site.

Version "a" of the reshaped human PM-1 L chain V region was selected as the best version. In subsequent experiments evaluating the different versions of reshaped human PM-1 H chain V regions, version "a" of the reshaped human PM-1 L chain v region was always used.

Evaluation of the six versions of reshaped human PM-1 H chain V regions were conducted by co-transfecting cos cells 20 with one of the six vectors expressing reshaped human PM-1 H chains (RVh-PM1a, RVh-PM1b, RVh-PM1c, RVh-PM1d, RVh-PM1e or RVh-PM1f) and the vector expressing version "a" of the reshaped human PM-1 L chain (RV1-PM1a). Cells were also co-transfected with vectors expressing chimeric PM-1 L and H chains (HEF-PMK-gk and HEF-PMh-gy1). Preliminary data using unpurified cos cell supernatants showed that version "a" of reshaped human PM-1 L chain and version "f" of reshaped human PM-1 H chain were equivalent to chimeric PM-1 L and H chains in assays for binding to IL-6R.

To confirm this preliminary data, chimeric and reshaped human PM-1 antibodies were concentrated and purified from cos cell supernatants using Protein A. Namely the media from cos cells was concentrated using a 100 kd cut-off ultrafiltration device (Amicon). The concentrated media was purified using Protein A agarose (Affi-Gel Protein A MAPSII kit. BioRad). Briefly, the concentrated media was applied to a Protein A agarose column that was equilibrated with five bed volumes of binding buffer. The column was washed with 15 bed volumes of the binding buffer, followed by 5 bed volumes of the elution buffer. The eluate was concentrated and the buffer changed to PBS using a microconcentrator (Centricon 10, Amicon). The purified antibodies were used for further analysis.

The analysis of purified samples of chimeric PM-1 antibody, and reshaped human PM-1 antibodies with version "a" of the L chain V region and versions "a", "b", "c", "d", "e", and "f" of the reshaped human H chain V region was carried out. Version "a" of the L chain plus version "f" of the H chain is clearly the best reshaped human PM-1 antibody. It binds to IL-6R as well as chimeric PM-1 antibody does (FIG. 13). It also inhibits human IL-6 from binding to the IL-6R as well as both the mouse and chimeric PM-1 antibodies do (FIG. 14).

#### Example 12

Reconstruction of the reshaped human PM-1 V regions to improve the levels of expression.

In order to remove the introns within the DNA sequences PM-1 L and H chain V regions (see SEQ ID Nos: 54 and 55). the cDNAs coding for the V regions were recloned using the PCR primers. The L and H chain expression vectors RV1-PM1a and RVh-PM1f were co-transfected into cos cells.

Biochemistry (1979) 18:5294-5299) and 5 µg of total RNA was used for the first strand cDNA synthesis as described for the PCR cloning of mouse antibody V regions. Three PCR primers were designed and synthesized. LEV-P1 (SEQ ID NO: 60) and HEV-P1 (SEQ ID NO: 58) contain the splice donor sequence and the BamHI site and were used as forward primers for the L and H chain V regions. respec- 5 tively. HEV-P2 (SEQ ID BO: 59) contains the Kozak consensus sequence before the ATG initiation codon and the HindIII site and was used as a backward primer for both the L and H chain V regions. Each 100 µl PCR reaction contained 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM 10  $(NH_4)_2SO_4$ . 2 mM MgSO<sub>4</sub>. 0.1% Triton X-100, 0.1 µg BSA. 250 µM dNTPs. 2.5µ of Vent DNA polymerase (Biolabs. U.K.), 50% of the first-strand cDNA synthesis reaction and 100 pmoles each of the forward and backward primers. Each PCR tube was overlayed with 50 µl of mineral oil and then 15 cycled, after an initial melt at 94° C. for 1.5 min, at 94° C. for 1 min, 50° C. for 1 min, and 72° C. for 1 min, and then at 72° C. for 10 min. The 408 bp PCR product containing the L chain V region and the 444 bp PCR product containing the H chain V region were purified using 2.0% low melting 20 temperature agarose gels, digested with BamHI and HindIII, and subcloned into a pUC19 vector to obtain pUC-RV1-PM1a-3 and pUC-RVh-PM1f-3 respectively.

It was revealed that the DNA sequences of the reshaped human PM-1 L and H chain V regions contain inappropriate 25 splice donor and acceptor sites (see SEQ ID NOs: 54 and 55). The sites within the L chain V region are not frequently used (approximately 10% of the mRNA), but the sites within the H chain V region are used frequently (approximately 90% of the mRNA). This aberrant splicing resulted in low 30 levels of expression of the reshaped human PM-1 antibody. In order to avoid aberrant splicing in the V regions, the splice donor sites were removed using a PCR-based method. For the H chain V region, the backward primer NEW-SP1 (SEQ ID NO: 61) and the forward primer NEW-SP2 (SEQ 35 ID NO: 62) were synthesized, changing the DNA sequence TGG GTG AGA to the DNA sequence TGG GTT CGC. The conditions for the PCR reactions were as described above for cDNA cloning except that the template DNA was 50 ng of pUC-RVh-PM1f-3 and the primers were either HEV-P2 40 and NEW-SP2, or HEV-P1 and NEW-SP1.

The PCR products from the two PCR reactions were purified using a 2.0% low melting temperature agarose gel and used in a PCR joining reaction. A 98 µl PCR reaction containing 0.5 µg of each of the first PCR products and 5µ 45 of Vent DNA polymerase was incubated at 94° C. for 2 min. 50° C. for 2 min, and 72° C. for 5 min, and then 100 pmoles each of HEV-P1 and HEV-P2 primers were added. The PCR tube was overlayed with 30 µl of mineral oil and subjected to 25 cycles of PCR, at 94° C. for 1 min, 50° C. for 1 min, 50 and 72° C. for 1 min, and then incubated at 72° C. for 10 min.

In the same manner, the splice donor site in the reshaped human PM-1 L chain V region was removed using PCR primers REI-SP1 (SEQ ID NO: 63) and REI-SP2 (SEQ ID 55 containing 8M area prior to using them. NO: 64) that changed the DNA sequence CAG GTA AGG to the DNA sequence CAG GAA AGG (see). Both PCR products, a 408 bp DNA fragment for the L chain V region and a 444 bp DNA fragment for the H chain V region, were purified using a 2.0% low melting temperature agarose gel. 60 digested with HindIII and BamHI, and subcloned into a pUC19 vector to yield pUC-RV1-PM1a-4 and pUC-RVh-RM1f-4, respectively.

RVh-PM1f-4 was constructed by replacing the HindIII-BamHI fragment of RVh-PM1f with the HindIII-BamHI 65 followed by an incubation at 72° C. for 10 minutes. fragment excised from pUC-RVh-PM1f-4. Sequence of reshaped human PM-1 antibody L chain V region version

"a" wherein introns have been deleted is shown in SEQ ID NO: 57. and sequence of reshaped human PM-1 antibody H chain V region version "f" wherein have been deleted is shown in SEQ ID NO: 56.

#### Example 13

Construction of DNA coding for reshaped human AUK 12-20 antibody L chain V region

A process for construction of DNA coding for a reshaped human AUK 12-20 antibody L chain V region is shown in FIG. 16. A gene coding for a human antibody L chain V region is incorporated into pUC19 vector using restriction enzymes HindIII and BamHI. Eight PCR primers (A to H) are prepared, and in the first PCR 4 regions which form a gene coding for the V region are amplified. The primers A and H have homology to DNA sequences on the pUC19 vector. The primers B. C and D are primers of 40 to 60 bp length each having a gene sequence of CDR to be grafted. respectively. The primers E, F and G have homology to DNA sequence of 15 to 20 bp length of the 5'-terminus of the primers B. C and D. respectively. Four first PCR use pairs of primers A and E. B and F. C and G, as well as D and H. respectively.

The PCR product A-E encodes FR1, and the PCR product B-F encodes CDR1 and FR2. The 3'-terminal portion of the A-E fragment and the 5'-terminal portion of the B-F fragment have homology in their 15 to 20 bp length, allowing to join there fragments at latter stage. Similarly, the B-F fragment has a homology with the C-G fragment which encodes CDR2 and FR3. The C-G fragment further has a homology with the D-H fragment which encodes CDR3 and FR4. Thus, these 4 fragments can be joined by their mutual homology. After joining these 4 fragments in a PCR reaction mixture, primers A and H are added thereon in the second PCR to amplify a product formed by correct joining of the 4 fragment. The second PCR product thus obtained has three grafted CDRs, and after digestion with HindIII and BamHI.

is subcloned into pUC19 vector.

More specifically, as a template, plasmid pUC-RV1-PM1a-4 constructed by inserting a DNA encoding reshaped human PM-1 antibody L chain V region version "a" into plasmid pUC19 was used.

The above-mentioned primers A to H have the following sequences.

Backward Primer	SEQ ID NO.	Forward primer	SEQ ID NO.
A. REVERSE	83	1220-L16	66
B. 1220-L1	65	1220-L2b	68
C. 1220-L2	67	1220L3b	70
D. 1220-L3	69	UNIVERSAL	82

The backward primers 1220-L1, 1220-L2 and 1220L3 for CDR grafting were purified with 12% polyacrylamide gel

A 100 µl PCR reaction mixture contained 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgSO<sub>4</sub>, 0.1% Triton X-100, 1 µg BSA, 250 µm dNTPs, 5 units Vent DNA polymerase (BioLabs. U.K.). 50 ng pUC-RV1-PM1a-4 DNA, and 100 p moles each of the forward and backward primers. Each PCR tube was overlaid with 50 µl of mineral oil, and after an initial denaturation at 94° C. for 1.5 minutes. 30 cycles of reaction at 94° C. for 1 minute. 50° C. for 1 minute and 72° C. for 1 minute was carried out,

Each of the PCR products, 252 bp (A-E), 96 bp (B-F), 130 bp (C-G) and 123 bp (D-H) was purified with a 2.0% low

melting agarose (FMC, Bio. Products, USA). Namely, an agarose piece containing a DNA fragment was excised, melted at 65° C. for 5 minutes, and added to the same volume of 20 mM Tris-HCl (pH 7.5) containing 2 mM EDTA and 200 mM NaCl. The mixture was extracted with phenol and chloroform. The DNA fragment was recovered by an ethanol precipitation, dissolved in 10 mM Tris-HCl (pH 7.5) containing 1 mM EDTA, and used for PCR joining reaction.

Next. 98 µl of a PCR reaction mixture containing 0.2 µg 10 each of the first PCR products and 5 units of Vent DNA polymerase was incubated at 94° C. for 2 minutes, 50° C. for 2 minutes and 72° C. for 5 minutes for a joining reaction. Next, 100 p moles each of the primers A (REVERSE) and H(UNIVERSAL) were added to the reaction mixture to 15 make it to 100 µl volume, and the reaction mixture was overlaid with 50 µl of mineral oil and subjected to 30 cycles of a reaction at 94° C. for 1 minute, 50° C. for 1 minute and 72° C. for 1 minute, followed by an incublation at 72° C. for 10 minutes.

The second PCR product of 558 bp length containing an L chain V region into which CDRs of the mouse monoclonal antibody AUK 12-20 L chain had been grafted was purified by a 2.0% low melting agarose gel. and after digestion with BamHI and HindIII, subcloned into a pUC19 vector to 25 obtain pUC-RLL-1220a, and sequenced. A resulting amino acid sequence of the L chain V region and a nucleotide sequence encoding the amino acid sequence is shown in SEQ ID NO: 71.

Next, for construction of an L chain expression vector, a 30 HindIII-BamHI DNA fragment containing a reshaped human AUK 12-20 antibody L chain V region was excised from the above-mentioned plasmid pUC-RV,-1220a, and inserted to HindIII-BamHI site of an L chain expression vector HEF-12k-gk to obtain an expression vector  $RV_L^-$  35 1220a for reshaped human AUK 12-20 antibody L chain V at the reshaped human V ad25 containing HSC I region version "a".

#### Example 14.

Expression and analysis of reshaped human AUK 12-20 antibody L chain

Transient expression in COS cells

The expression vector RV<sub>L</sub>-1220a for reshaped human AUK 12-20 antibody L chain and the expression vector HEF-12h-gyl for chimeric 12-20 antibody H chain (Example 5) were cotransfected into COS cells to evaluate 45 the reshaped human AUK 1220 antibody L chain version "a". Namely, COS cells were suspended in a phosphatebuffeted saline (PBS) at a concentration of 1×107 cells 1 ml, and to 0.8 ml of the suspension were added the plasmid DNAs (10 µg for each plasmid). Pulses were applied to the 50 suspension at an electric capacity of 1,900 V, 25 µF using a Gene Pulser apparatus (Bio Rad).

After restoraction at a room temperature for 10 minutes, electroporated cells were added to 8 ml of DMEM medium (GIBCO) containing 10% bovine fetal serum. After incuba- 55 tion for 72 hours, supernatant was collected, centrifuged to eliminate cell debris, and stored in an aseptic condition at 4° C. for short period or at -20° C. for longer period.

Determination of human-like antibody by ELISA

A supernatant of the transfected COS cells was assaied by 60 ELISA and the production of chimeric antibody was confirmed. To detect human-like antibody, a plate was coated with a goat anti-human IgG (whole molecule) (Sigma). After blocking, the supernatant from COS cells was sequentially diluted and added to each well.

The plate was incubated and washed, and an alkaline phosphatase-conjugated goat anti-human IgG (α-chain

specific. Sigma) was added thereon. After incubation and washing, a substrate solution was added. After further incubation, the reaction was terminated and an optical density at 405 nm was measured. As a standard, purified IgG (Sigma) was used.

ELISA for confirmation of an ability to bing to human IL-6R

A supernatant from the transfected COS cells was assaied by ELISA to determine whether the produced human-like antibody can bind to the antigen, human IL-6R. A plate was coated with a mouse monoclonal antibody MT18 (Reference Example 1). After blocking with 1% BSA, soluble recombinant human IL-6R (SR 344) was added to the plate. After washing the plate, supernatant from COS cells was sequentially diluted and added to each well of the plate. After inclusion and washing, alkaline phosphatase-conjugated goat anti-human IgG was added to the wells, and after further incubation and washing, a substrate solution was added thereon. After incubation, the reaction was terminated and optical density at 405 nm was measured.

A result is shown in FIG. 17. The human-like antibody comprising a combination of a reshaped human AUK 12-20 antibody L chain version "a" and a chimeric 12-20 antibody H chain exhibited a binding ability to IL-6R as strong as chimeric 12-20 antibody. Optical density at 405 nm changed in a dilution rate-dependent manner, confirming that the sample contains an antibody to IL-6R. In addition, this result shows that the reshaped human AUK 12-20 antibody L chain version "a" has an antigen binding ability as high as chimeric AUK 12-20 antibody L chain.

#### Example 15.

Construction of gene coding for reshaped human AUK 12-20 antibody H chain using HSGI consensus sequence

According to the same procedure as described in Example grafted into the reshaped human V<sub>H</sub>a425 containing HSG I consensus sequences as its FRs (Kettleborough et al., Protein Engineering (1991) 4:773-783). Fist, a HindIII-BamHI DNA fragment encoding the reshaped human V<sub>H</sub>a425 (FIG. 3 in the literature) was excised from a plasmid HCMV-RV<sub>H</sub>a-425-yl and subcloned at HindIII-BamHI sites in pUC 19 vector to obtain pUC-RV<sub>H</sub>-425a, which was then used as a template. 8 PCR primers (A1 to H1) were synthesized. The primer 1220-H1 was designed to graft CDR1 and to induce a mutation from T-28 to S-28, and the primer 1220-H3 was designed to graft CDR3 and to induce a mutation from S-94 to R-94. The primers 1220-H1. 1220-H2 and 1220-H3 were purified using a 12% polyacrylamide gel containing 8 M urea prior to using them. Nucleotide sequence of each primer was as follow.

Backward primer	SEQ ID NO.	Forward primer	SEQ ID NO.	
A1. REVERSE	83	Е1. 1220-Н1Ь	73	
B1. 1220-H1	72	E1. 1220-H2b	75	
C1. 1220-H2	74	G1. 1220-H3b	77	
D1. 1220-H3	76	H1. UNIVERSAL	82	

Condition of PCR was the same as that described in Example 13, except that the pUC-RV<sub>H</sub>-425a was used as a template DNA and the above-mentioned primers were used for grafting H chain CDRs. Primer pairs of A1 and E1, B1 and F1, C1 and G1, as well as D1 and H1 were used to carry out first PCR reactions, and the respective first PCR products, 186 bp (A1-E1), 75 bp (B1-F1), 173 bp (C1-G1) and 105 bp (D1-H1) were purified with 2.0% low melting

agarose gel, and used in subsequent second PCR joining reaction. According to the condition described in Example 13, 0.2  $\mu$ g each of the first PCR products were used to carry out the second PCR reaction (including PCR joining reaction) to obtain a PCR product of 495 bp containing DNA 5 coding for a human H chain V region into which mouse AUK 12-20 antibody H chain V region CDRs had been grafted, and the PCR product was purified using 2.5% low melting agarose gel. After digesting the PCR product with BamHI and HindIII, resulting BamHI-HindIII DNA frag- 10 ment was subcloned into pUC19 and sequenced to obtain pUC-RV<sub>MC</sub>1220a.

It was revealed that DNA sequence coding for reshaped human AUK 12-20 antibody H chain V region contains a sequence well conforming to a splicing donor sequence. 15 which may cause an abnormal splicing which was troublesome in the production of the reshaped human PM-1 antibody. Therefore, this DNA sequence was modified by PCR. Mutagenetic primers, SGI-SP1 (SEQ ID NO: 97) and SGI-SP2 (SEQ ID NO: 98) were synthesized. These primers 20 convert the DNA sequence AAG GTG AGC to the DNA sequence AAA GTC AGC. Condition of PCR reaction was same as described above, except that 50 ng of pUC-RV<sub>H</sub>-1220a was used as a template DNA, and the SGI-SP1 and UNIVERSAL (SEQ ID NO: 82), or the SGI-SP2 and 25 REVERS (SEQ ID NO: 83) were used as primers.

PCR products from two PCR reactions were purified by 2% low melting agarose gel and used in a PCR joining reaction. 98 µl of PCR reaction mixture containing 0.2 µg each of the first PCR products and 5 units of Vent DNA 30 polymerase was incubated at 94° C. for 2 minutes, at 55° C. for 2 minutes and at 72° C. for 5 minutes for a joining reaction. Next, 100 pmoles each of UNIVERSAL and REVERSE primers were added to the reaction mixture, which was then overlaid with 50 µl of mineral oil and 35 subjected to 30 cycles of second PCR reaction consisting of incubations at 94° C. for 1 minutes, at 50° C. for 1 minute and at 72° C. for 1 minute, followed by an incubations at 72° C. for 10 minutes. DNA fragment of 495 bp obtained in the second PCR was purified by a 2.0% low melting agarose gel. 40 and subcloned into pUC19 vector and sequenced to obtain pUC-RV<sub>H</sub>-1220a-2.

Next, HindIII-BamHI DNA fragment containing DNA coding for reshaped human AUK 12-20 antibody H chain V region was excised from the pUC-RV<sub>H</sub>-1220a-2, and 45 inserted at HindIII-BamHI sites of an H chain expression vector HEF-12h-g $\gamma$ 1 to obtain an expression vector RV<sub>H</sub>-1220a for the reshaped human AUK 12-20 antibody H chain version "a".

For construction of genes coding for reshaped human 50 AUK 12-20 antibody H chain V region versions "b" to "d", two paires of mutagenic primers were synthesized. Each PCR reaction was carried out under substantially the same condition as described above. For construction of version "b", in two first PCR reactions, either UNIVERSAL primer 55 (SEQ ID NO: 82) and mutagenic primer 120H-ml (SEQ ID NO: 78), or REVERSE primer (SEQ ID NO: 83) and mutagenic primer 1220H-mlb (SEQ ID NO: 79), as well as pUC-RV<sub>H</sub>-1220a as a template were used. The first PCR products of 202 bp and 323 bp were purified by a 2.0% low 60 melting agarose gel, and used in second PCR (including PCR joining reaction) under the same condition as described above to obtain a 495 bp product (version "b"). The product was digested with HindIII and BamHI, and subcloned into pUC19 vector to obtain pUC-RV<sub>H</sub>1220b.

Similarly, mutagenic primer 1220H-m2 (SEQ ID NO: 80), 1220H-m2b (SEQ ID NO: 81) and a template pUC-

 $RV_{H}$ -1220a were used in a PCR to obtain a PCR product (version "c"). The product was digested with HindIII-BamHI and inserted at HindIII-BamHI sites of pUC19 vector to obtain pUC-R<sub>H</sub>V-1220c. Moreover, mutagenic primers 1220H-mla (SEQ ID NO: 78). 1220H-mlb (SEQ ID NO: 79), and a template pUC-RV<sub>H</sub>-1220c were used to obtain a PCR Product (version "d"). which was then digested with HindIII and BamHI and inserted into HindIII-BamHI sites of pUC19 vector to obtain pUC-RV<sub>H</sub>-1220d.

Note, an amino acid sequence of the reshaped human AUK 12-20 antibody H chain V region version "b" and a nucleotide sequence coding therefor in the plasmid pUC-RV<sub>H</sub>-1220b is shown in SEQ No. 84; and an amino acid sequence of the reshaped human AUK 12-20 antibody H chain V region version "d" and a nucleotide sequence coding therefor in the plasmid pUC-RV<sub>H</sub>-1220d is shown in SEQ ID NO: 85.

Next, to construct the expression vectors. HindIII-BamHI fragments containing a reshaped human AUK 12-20 antibody H chain V region were excised from pUC-RV<sub>H</sub>-1220b, pUC-RV<sub>H</sub>-1220c and pUC-RV<sub>H</sub>-1220d and inserted into HindIII-BamHI sites of H chain expression vector HEF-12h-gyl to obtain RV<sub>H</sub>-1220b, RV<sub>H</sub>-1220c and RV<sub>H</sub>-1220d respectively.

#### Example 16.

Expression and analysis of various versions of reshaped human AUK 12-20 antibody.

COS cells were cotransfected with one of 4 expression vectors for reshaped human AUK 12-20 antibody H chain  $(RV_{H}-1220a, RV_{H}-1220b, RV_{H}-1220c \text{ or } RV_{H}-1220d)$  and an expression vector  $VR_{H}-1^{220}a$  to evaluate 4 versions of the reshaped human AUK 12-20 antibody H chain V region. For reference, COS cells were cotransfected with expression vectors for chimeric 12-20 antibody L chain and H chain (HEF-12h-gyl and FEF-12-gk). In an assay for binding to the human IL-6R, a reshaped human AUK 12-20 antibody L chain and reshaped human AUK 12-20 antibody L chain of reshaped human AUK 12-20 antibody L chain of reshaped human AUK 12-20 antibody L chain and reshaped human AUK 12-20 antibody H chain version "d" shows good binding as well as chimeric 12-20 antibody. These results are shown in FIGS. 18 and 19.

#### Example 17.

Construction of gene coding for reshaped human sle 1220 antibody H chain using human antibody HAX

A human antibody having the highest homology with the mouse monoclonal antibody AUK 12-20 H chain V region is HAX (J. Immunology (1987) 139:2496-2501; an antibody produced by hybridoma 21/28 derived from B cells of an SLE patient; its amino acid sequence is shown in FIG. 6. and nucleotide sequence therefor is shown in FIGS. 4 and 5 of this literature), according to a protein data base "Leeds". Reshaped human sle 1220H antibody H chain V region was constructed using FRs of the antibody HAX and CDRs of mouse monoclonal antibody AUK 12-20 H chain V region.

An entire DNA coding for a reshaped human sle 1220 H antibody H chain V region version "a" was chemically synthesized. DNA coding for sle 1220 H antibody H chain V region of an entire length 439 bp was designed by dividing the DNA into 6 oligonucleotides of 90 to 94 bp length overlapping each other by 21 bp (sle 1220 h 1 to 6; SEQ ID NOs: 86 to 91, respectively). In designing the oligonucleotides, secondary structure was tested and for sites having structural problems the third nucleotide in a codon was changed without change of amino acid encoded

thereby. The relationship of these oligonucleotides and a process for construction of double-stranded synthetic DNA are shown in FIG. 20.

The reaction shown in FIG. 20 is carried out using PCR. technique. Namely, 6 synthetic oligonucleotides were added to a single PCR reaction tube to carry out the first PCR reaction, thereby two oligonucleotides are anealed and extended, and further 4 oligonucleotides or an entire oligonucleotide are obtained.

Next, terminal primers A (SEQ ID NO: 92) and B (SEQ 10 ID NO: 93) are added to carry out the second PCR reaction. wherein only a correct oligonucleotide having an entire length can be amplified. The resulting product is digested with BamHI and HindIII, and subcloned into pUC19 vector, followed by sequencing.

More specifically, 98 µl of a reaction mixture containing 15 100 mM tris-HCl (pH 8.5). 50 mM KCl. 0.1 mM dATP, 0.1 mM dGTP, 0.1 mM dCTP, 0.1 mM dTTP, 1.5 mM MgCl<sub>2</sub> and 2.5 U of DNA polymerase AmpliTaq (Perkin Elmer Cetus) as well as 5 pmoles each of the oligonucleotides was denaturated at 94° C. for 1.5 minutes and subjected to 3 20 cycles of reaction by incubation at 92° C. for 3 minutes, 50° C. for 2 minutes and 72° C. for 5 minutes, followed by an incubation at 72° C. for 10 minutes. One µl each of 50 mM terminal primers A and B were added to the reaction mixture, which was then overlaid with 80 µl of mineral oil, 25 and after denaturation of 94° C. for 1.5 minutes, subjected to 30 cycles of reaction by incubation at 94° C. for 1 minute, 50° C. for 1 minute and at 72° C. for 1 minute, followed by an incubation at 72° C. for 10 minutes. The PCR product of 439 bp was purified by a 1.5% low melting agarose gel. 30 digested with restriction enzymes BamHI HindIII, and subcloned into pUC19 vector. followed by confirmation of sequence. A clone thus obtained was designated pUC-RV sle 1220Ha. An amino acid sequence of reshaped human sle 1220H antibody H chain V region version "a" and a nucle- 35 otide coding therefor in the plasmid pUC-RV<sub>H</sub>-sle 1220Ha are shown in SEQ ID NO: 94.

Next, HindIII-BamHI DNA fragment containing a gene coding for reshaped human 12-20 (sle 1220H) antibody H chain V region was excised from the pUC-RV<sub>H</sub>-sle 1220Ha 40 and inserted at HindIII-BamHI sites of an H chain expression vector HEF-12h-gyl to obtain RV<sub>H</sub>-sle 1220Ha.

For construction of version "b" to "d" of reshaped human sle 1220H antibody H chain V region, two mutagenic primers sle 1220Hml (SEQ ID NO 95) and sle 1220Hm2 45 Construction of Hybridoma MT18 (SEQ ID NO: 96) were synthesized. In each PCR, Vent DNA polymerase and reaction mixture composition described in Example 13 were use. In each PCR reaction, a reaction mixture containing pUC-RV<sub>H</sub>-sle 1220Ha as template, 50 pmoles of a mutagenic primer sle 1220Hm1 or sle 50 1220Hm2, and 50 pmoles of the terminal primer B was denaturated at 94° C. for 1.5 minutes, and subjected to 30 cycles of reaction by incubation at 94° C. for 1 minute, at 50° C. for 1 minute and at 72° C. for 1 minute, followed by an incubation at 72° C. for 10 minutes. The product of 235 55 bp or 178 bp was purified by a 1.5% low melting agarose gel to use as a primer in the second PCR reaction. Namely the second PCR reaction was carried out using 50 pmoles of the terminal primer A. 0.2 µg of the PCR product and pUC-RV<sub>H</sub>-sle 1220Ha as a template, and resulting product of 439 60 bp was purified by a 1.5% low melting agarose gel, digested with BamHI and HindIII, and subcloned into pUC19 vector to obtain pUC-RV<sub>H</sub>-sle 1220Hb or pUC-RVE-sle 1220Hc, which encodes reshaped human sle 1220 antibody H chain V region version "b" or "c", respectivity.

A DNA coding for reshaped human sle 1220 H antibody H chain V region version "d" was constructed also follow.

As a templete pUC-RVh-sle 1220Hb was used. 50 pmoles each of a mutagenic primer sle 1220Hm2 and the terminal primer B was used to carry out 30 cycles of the first PCR reaction. Resulting 176 bp PCR product was purified on a 1.6% low melting agarose gel to use as a primer in the second PCR. This primer and 50 p moles of the terminal primer A was used in the second PCR to obtain a 439 bp DNA fragment. The PCR product thus obtained was purified, digested with BamHI and HindIII, and subcloned into pUC 19 vector to obtain pUC-RV<sub>H</sub>-sle 1220Hd.

Next, to construct expression vectors for various versions of reshaped human sle 1220H antibody H chain V region. BamHI-HindIII fragments containing a DNA encoding reshaped human sle 1220 antibody H chain V region were excised from pUC-RV<sub>H</sub>-sle 1220Hb, pUC-RV<sub>H</sub>-sle 122Hc and pUC-RV.-sle 1220Hd, and inserted into HindIII-BamHI sites of the H chain expression vector HEF-12h-gy1 to obtain expression vectors RV<sub>H</sub>-sle 1220Hb, RV<sub>H</sub>-sle 1220Hc and RV<sub>H</sub>-sle 1220Hd respectively.

Each of four vectors expressing reshaped human sle 1220H antibody H chain (RV<sub>H</sub>-sle 1220Ha, RV<sub>H</sub>-sle 1220Hb and RV<sub>H</sub>-sle 1220Hc or RV<sub>H</sub>-sle 1220Hd) and the vector RV<sub>L</sub>-1220a expressing reshaped human AUK 12-20 antibody L chain were cotransfected to COS cells to evaluate the four versions of the reshaped human sle 1220H antibody H chain V region for an ability to inhibit the binding of IL-6 to IL-6R. Results is shown in FIGS. 21 to 24. Note, these result were obtained after purifying the produced antibodies by protein A.

As seen from the above, according to the present invention, in a chimeric L chain or a reshaped human L chain, or a chimeric H chain or a reshaped human H chain, and especially in RF, one or more than one amino acid can be replaced with other amino acid maintaining an ability to bind to human IL-6R. Therefore, the present invention includes chimeric antibody and reshaped human antibody, chimeric L chain and reshaped human L chain, chimeric H chain and reshaped human H chain, reshaped L chain V region. and reshaped H chain V region, wherein one or more than one amino acid is replaced with other as well as DNA coding therefor, as far as they maintain their native property.

Starting hybridomas used in the present invention were constructed as follows.

#### Reference Example 1

To construct a hybridoma producing monoclonal antibody to human IL-6R, as an immunogen, a mouse T cell line expressing human IL-6R on the cell surface was constructed as follows. Namely, a plasmid pBSF2R.236 disclosed in Japanese Patent Application No. H1-9774 and pSV2neo was transfected into a mouse T cell line CTLL-2 (ATCC TIB214) according to a conventional procedure, and the resultant transformant was screened using G418 according to a conventional procedure to obtain a cell line expressing about 30.000 IL-6Rs per cell. This cell line was designated CTBC3.

The CTBC3 cells were cultured in RPMI 1640 according to a conventional procedure, the cultured cells were washed four times with PBS buffer, and 1×107 cells were intraperitoneally injected to C57BL/6 mice for immunization. The immunization was carried out once a week for 6 weeks.

Spleen cells were obtained from the immunized mice and fused with myeloma P3U1 cells using polyethylene glycol according to a conventional procedure, and the fused cells were screened as follows. The IL-6R negative human T cell line JURKAT (ATCC CRL 8163) was co-transfected with the plasmids pBSF2R.236 and pSV2neo, and transformed

cells were screened to obtain a cell line expressing about 100.000 IL-6Rs per cell. The cell line was designated NJBC8. A hybridoma cell clone producing an antibody which recognized NP40-lysed NJBC8 but did not recognize NP40-lysed JURKAT was cloned and designated MT18. 5 The hybridoma MT18 was deposited with the Fermentation Research Institute Agency of Industrial Science and Technology (FRI), under the Budapest Treaty, as FERM BP-2999 on Jul. 10, 1990.

#### Reference Example 2

Construction of Hybridoma PM1

To construct a hybridoma producing monoclonal antibody to the IL-6R, as an antigen, human IL-6R was extracted as follows. 3×10<sup>9</sup> human myeloma U266 cells (IL-6R-15 producing cells) were lysed in 1 ml of 1% digitonin, 10 mM triethanolamine buffer (pH 7.4), 0.15 M Nacl and 1 mM PMSF (phenylmethylsulfonyl fluoride: Wako Pure Chemicals). On the other hand, an MT18 antibody produced by the MT18 hybridoma prepared in Reference Example 1 was bonded to cyanogen bromide-activated Sepharose 4B (Pharmacia) according to a conventional procedure. This MT18 antibody-conjugated Sepharose 4B was mixed with the above-prepared cell lysate to bind the solubilized IL-6R to the MT18 antibody on Sepharose 4B. Substances nonspecifically bonded to the Sepharose 4B were washed off, and the IL-6R bound to Sepharose 4B via the MT18 antibody was used as an immunogen.

BALB/c mice were intraperitoneally immunized with the above-prepared immunogen, once a week for 4 weeks. Next, spleen cells were obtained from the immunized mice, and fused with myeloma cells P3U1 using polyethylene glycol according to a conventional procedure. The fused cells were screened as follows. First, a culture supernatant and 0.01 ml of Protein G Sepharose (Pharmacia) were mixed to adsorb immunoglobulin in the supernatant to the Protein G Sepharose. On the other hand, 107 U266 cells internally labeled with <sup>35</sup>S-methionine were lysed, and the IL-6R was affinity-purified using the MT18-conjugated Sepharose 4B. Next, the <sup>35</sup>S-methionine-labeled IL-6R was immunoprecipitated with the above-prepared Protein G Sepharose on which an immunoglobulin had been bonded, and the precipitate was analyzed by SDS/PAGE. As a result, one hybridoma clone producing antibody which specifically bound to the IL-6R was isolated, and designated PM1. The hybridoma PM1 was deposited with the FRI under the Budapest Treaty as FERM BP-2998, on Jul. 10, 1990.

#### Reference Example 3

Construction of Hybridoma AUK12-20, AUK64-7 and AUK146-15

As an immunogen, a soluble IL-6R (SR 344) was prepared according to a procedure described by Yasukawa, K. et al., J. Biochem. 108, 673-676, 1990. Namely, a plasmid pECEdhfr 344 containing a cDNA coding for IL-6R wherein the 345th codon from the N-terminus had been replaced by 55 binding of SR344 with the IL-6; and a hybridoma clone a stop codon was transfected to CHO (5E27) cells, the transfected cells were cultured in a serum-free medium (SF-O medium, Sanko Junyaku), and a resulting supernatant was concentrated with an HF-Labl system (Tosoh), and purified by Blue-5PW column and Phenyl-5PW column. 60 body to the human IL-6R, comprising a human antibody The purified soluble IL-6R showed a single band in an SDS-PAGE.

A female BALB/cAnNCrj mouse (Nippon CREA) was subcutaneously injected with 10 µg/mouse of the immunogen in Freund's complete adjuvant (Bacto Adjuvant Com- 65 plete H 37 Ra, Difco), followed by the second and third injections of the same amount of the immunogen in Freund's

incomplete adjuvant (Bacto Adjuvant Incomplete Freund, Difco) two and three weeks after the first injection, respectively. A final immunization (the fourth injection) was carried out without adjuvant into a tail vein one week after the third injection. A serum sample was prepared from the immunized mice, serially diluted with a dilution buffer, and assayed by ELISA according to a procedure described by Goldsmith, P. K., Analytical Biochemistry, 117, 53-60, 1981. Namely. an SR344 (0.1 µ/ml)-coated plate was blocked with 1% BSA, and the diluted sample was added thereon. Mouse IgG bound to the SR344 was measured using goat anti-mouse IgG/alkaline phosphatase (A/P) (ZYMED) and a substrate for alkaline phosphatase (Sigma-104).

After confirming an increase of the anti-SR344 antibody in the serum. spleen cells were obtained from 5 BALB/c mice three days after the final immunization. The spleen cells and myeloma cells (P3U1) were mixed at a ratio of 25:1. fused using PEG1500, and cultured in 2000 wells at a cell concentration of 0.7 to 1.1×10<sup>6</sup> cells/well. Supernatants from the wells were screened for their ability to bind SR344 (the first screening designated as R344 recognition assay). and for their ability to inhibit a binding of SR344 with an interleukin-6 by a IL-6/sIL-6R binding inhibition assay (RBIA). The first screening provided 240 positive wells, and 25 the second screening provided 36 positive wells.

The above-mentioned R344 recognition assay was carried out as follows. Goat anti-mouse Ig (Cappel) (1 µg/ml)coated plate (MaxiSorp, Nunc) was blocked with 1% BSA, and 100 µl/well of hybridoma culture supernatant was added thereon, followed by an incubation at room temperature for one hour. After washing the plate, 20 µg/ml of SR344 was added to each well, and incubation was carried out at room temperature for one hour. The amount of SR344 captured by the immobilized antibody derived from the supernatant was 35 determined by addition of rabbit anti-SR344 IgG (#2, 5 µg/ml), goat anti-rabbit IgG-alkaline phosphatase (A/P) (1:3000, Tago), and of a substrate (1 mg/ml, Sigma-104), followed by measurement of the optical dencity at 405-600

The above-mentioned RBIA was carried out as follows. MT18 antibody-coated plate was filled with 100 µg/ml of SR344 (100 µl/well), and incubation was carried out at a room temperature for one hour. After washing the plate, 50 µl/well of hybridoma supernatant and 50 µg/well of biotin-45 interleukin-6 conjugate (20 µg/ml) were simultaneously added to each well, and the wells were incubated at room temperature for one hour. An amount of biotin-IL-6 bound to SR344 was measured by an addition of streptavidin-A/P (1:7000, PIERCE) and a corresponding substrate (Sigma-50 104), followed by a measurement of the optical density at 405-600 nm.

Finally, positive clones were purified by a twice-repeated limiting dilution method, and three hybridoma clones, i.e., AUK12-20. AUK145-15 and AUK64-7. which inhibit the AUK181-6, which does not inhibit the binding of SR344 with the IL-6, were obtained.

Industrial Applicability

The present invention provides a reshaped human antiwherein the CDRs of the human V regions are replaced with the CDRs of a mouse monoclonal antibody to the human IL-6R. Since major portion of the reshaped human antibody is derived from a human antibody and the mouse CDRs which are less antigenic. the present reshaped human antibody is less immunogenic to human, and therefore is promised for therapeutic uses.

### 5.795.965

FERM BP-2999 July 10, 1990

FERM BP-2998 July 10, 1990

Deposition Date

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Reference to Deposite 2 of	ed Microorganisms u Budapest Treaty	mder Rule 13-		Reference to Dep	osited Microorganisms u 2 of Budapest Treaty	nder Rule 13-
Identification of Microorganism	Deposition No.	Deposition Date		Identification of Microorganism	Deposition No.	Deposition
Industrial and Address: 23 St Ma	ority: National Colk d Marine Bacteria Li cher Drive, Aberdee TED KINGDOM	imited	10	Inst	Authority: Fermentation itute, Agency of industria icience and Technology Higashi 1-chome Tsukuba	1
E. Coli DH50, pPM-h1 E. Coli DH50, p12-h2	NCIMB 40362 NCIMB 40363	Feb. 12, 1991 Feb. 12, 1991	15	Address: 105, 1	Japan	I-SIII IOAFAKI
E. Coli DH5α, p64-h2 E. Coli DH5α, p146-h1 E. Coli DH5α, pPM-k3 E. Coli DH5α, p12-k2	NCIMB 40364 NCIMB 40365 MCIMB 40366 NCIMB 40367	Feb. 12, 1991 Feb. 12, 1991 Feb. 12, 1991 Feb. 12, 1991	20 _	MT 18 PM 1	FERM BP-2999 FERM BP-2998	July 10, 199 July 10, 199
E. Coli DH5α, p64-k4 E. Coli DH5α, p146-k3	NCIMB 40368 NCIMB 40369	Feb. 12, 1991 Feb. 12, 1991				
		SEQ	UENCE LIS	STING		
(A) (B) (C) (C) (D) (xi)SEQUENCE ACTAGTCGAC AT (2)INFORMATION FOR S (i)SEQUENCE (A) (B) (C) (D)	F SEQUENCES: 158 EQ ID NO:1: CHARACTERISTICS: LENGTH: 40 base pairs TYPE: nucleic acid STRANDEDNESS: sing TOPOLOGY: linear DESCRIPTION: SEQ G A A G T T G C C EQ ID NO:2: CHARACTERISTICS LENGTH: 39 base pair TYPE: nucleic acid STRANDEDNESS: sing TOPOLOGY: linear DESCRIPTION: SEQ	i ID NO:1: TGTTAGGCT : gle ID NO:2:				
(A) (B) (C) (D)	CHARACTERISTICS LENGTH: 40 base pair TYPE: nucleic acid STRANDEDNESS: sin TOPOLOGY: linear E DESCRIPTION: SEQ GAGTGTGC T	s gle ID NO:3:	сство	SGTTG		

#### -continued

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5.195.905

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(B) TTPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
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2 ) INFORMATION FOR SEQ ID NO:5:	
( i ) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 40 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:	
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2 ) INFORMATION FOR SEQ ID NO:6:	
( i ) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 37 base pairs	
(B) TYPE: nucleic acid	
(C) STRANDEDNESS: single	
(D) TOPOLOGY: linear	
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(B) TYPE: nucleic acid	
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( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 37 base pairs 5.795.965

51		52	
	-continued		
	***		
(B) TYPE: nucleic acid			
(C) STRANDEDNESS: single (D) TOPOLOGY: linear			
(D) IOFOCOTT linear			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:10:			
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2 ) INFORMATION FOR SEQ ID NO:11:			
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(A) LENGTH: 38 base pairs			
( B ) TYPE: nucleic acid			
( C ) STRANDEDNESS: single			
(D) TOPOLOGY: linear			
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2 ) INFORMATION FOR SEQ ID NO:12:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 27 base pairs			
(B) TYPE: nucleic acid			
( C ) STRANDEDNESS: single			
(D) TOPOLOGY: linear			
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GATCCCGGG TGGATGGTGG GAAGATG			27
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(B) TYPE: nucleic acid			
( C ) STRANDEDNESS: single			
( D ) TOPOLOGY: linear			
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(2) INFORMATION FOR SEQ ID NO:14:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 36 base pairs (B) TYPE: nucleic acid			
(C) STRANDEDNESS: single			
(D) TOPOLOGY: linear			
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:			
ACTAGTCGAC ATGGGATGGA GCTRTATCAT	SYTCTT		36
(2) INFORMATION FOR SEQ ID NO:15:			
(i) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 37 base pairs			
(B) TYPE: nucleic acid			
(C) STRANDEDNESS: single			
( D ) TOPOLOGY: linear			
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(2) INFORMATION FOR SEQ ID NO:16:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 35 base pairs			
( B ) TYPE: nucleic acid			
(C) STRANDEDNESS: single			

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	-continued		
( D ) TOPOLOGY: linear			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:16:			
ACTAGTEGAE ATGRACTITG GGYTEAGETT	GRTTT		3 5
(2) INFORMATION FOR SEQ ID NO:17:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 40 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:17:			
ACTAGICGAC ATGGACICCA GGCTCAATIT	AGTTTTCCTT		4 0
(2) INFORMATION FOR SEQ ID NO:18:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 37 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:18:			
ACTAGTEGAE ATGGETGTEY TRGSGETRET	CTTCTGC		37
(2) INFORMATION FOR SEQ ID NO:19:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 36 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> (x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:			3 6
ACTAGTCGAC ATGGRATGGA GCKGGRTCTT	TMTCTT		30
(2) INFORMATION FOR SEQ ID NO:20:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 33 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:20:			
ACTAGTEGAE ATGAGAGTGE TGATTETTT	GTG		33
(2) INFORMATION FOR SEQ ID NO:21:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 40 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:21:			
ACTAGTCGAC ATGGMITGGG TGTGGAMCTT	GCTATTCCTG		4 0
(2) INFORMATION FOR SEQ ID NO:22:			
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 37 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>			

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	(r ) -					-c	ontinu	ed						
( <b>xi</b> )	SEQUENCE DE	SCRIPTIC	ON: SEQ	ID NO:2	2:			001						
ACTAGICO	AC ATGG	GCAG	ас т	TACA	ттст	C AT	тсст	G						37
(2) INFORMAT	TION FOR SEQ	ID NO:23:												
(1)	SEQUENCE CH (A)LEN (B)TYP (C)STR (D)TOP	GTH: 28   E: nucleic ANDEDN	base pairs acid ESS: sing											
( <b>x</b> i)	SEQUENCE DE	SCRIPTIO	N: SEQ	ID NO:23	i:									
GGATCCCG	GG CCAG	TGGA	TA G	ACAG	ATG									28
(2) INFORMAT	TON FOR SEC 1	D NO.24												
	SEQUENCE CH (A)LEN (B)TYP (C)STR (D)TOP	GTH: 393 E: nucleic ANDEDNI	base pair acid ESS: sing										÷	
(1X)	FEATURE: (A)NAN (B)LOC													
	FEATURE: (A)NAM (B)LOC	ATION: 1.	.393											
	SEQUENCE DES		_											
ATG GAG Met Glu 1	TCA GAC Ser Asp	ACA Thr 5	CTC Leu	CTG Leu	CTA Leu	TGG Trp	GTA Val 10	CTG Leu	C T G L e u	CTC Leu	T G G T r p	GTT Val 15	CCA Pro	4 8
GGT TCC Jly Ser	ACT GGT Thr Gly 20	GAC Asp	ATT Ile	GTG Val	CTG Leu	ACA Thr 25	CAG Gla	TCT Ser	CCT Pro	GCT Ala	ТСС Sел 30	TTA Leu	GGT Gly	96
GTA TCT Val Ser	CTG GGG Leu Gly 35	CAG Gln	AGG Arg	GCC Ala	A C C T b r 4 0	ATC Ile	T C A S e r	TGC Cys	AGG Arg	GCC Ala 45	AGC Ser	AAA Lys	AGT Ser	144
AGT AGT	ACA TCT Thr Ser	G G C G 1 y	T A T T y r	AGT Ser 55	T A T T y r	ATG Met	CAC His	TGG Trp	T A C T y r 6 0	C A A G l n	CAG Gln	AAA Lys	CCA Pro	192
GA CAG Gly Gln 65	ACA CCC Thr Pro	AAA Lys	СТС Lец 70	CTC Leu	AT C 11 e	T A T T y r	C T T L e u	G C A A 1 a 7 5	TCC Ser	AAC Asn	CTA Leu	GAA Glu	T C T S e r 8 0	2 4 0
GGG GTC ( Jy Val	CCT GCC Pro Ala	AGG Arg 85	ТТС РЬе	AGT Ser	G G C G 1 y	AGT Ser	G G G G G I y 9 0	TCT Ser	6 G G G G 1 y	ACA Tbr	GAC Asp	ТТС Рће 95	ACC Tbr	288
CTC AAC . .eu Asp	ATC CAT 110 His 100	С С Т Р г о	GTG Val	GAG Glu	G A G G l u	G A G G 1 u 1 0 5	GAT Asp	GC T Ala	GCA Ala	ACC Thr	T A T T y r 1 1 0	Т А С Т у т	TGT Cys	336
AG CAC	AGT AGG Ser Arg 115	GAG Glu	AAT Asn	CCG Pro	T A C T y r 1 2 0	ACG Tbr	ТТС РЬс	G G A G 1 y	GGG G1y	G G G G 1 y 1 2 5	ACC Thr	AAG Lys	C T G L e u	384
AA ATA 2 1 u 1 1 c 1 1 3 0														393

( 2 ) INFORMATION FOR SEQ ID NO:25:

# ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 131 amino acids ( B ) TYPE: amino acid

- ( D ) TOPOLOGY: linear

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	(ii)	MOLECU	LE TYPE	E: protein						1						
	(xi)	SEQUEN	CE DESC	RIPTION	: SEQ ID	NO:25:										
Met 1	Glu	Ser	Asp.	Thr 5	Leu	Leu	Leu	Ттр	Val 10	Leu	Leu	Leu	Гтр	Val 15	Рго	
G 1 y	Ser	Тbг	G 1 y 2 0	Asp	lle	V a l	Leu	Tbr 25	Gln	Ser	Pro	Ala	Ser 30	Leu	G 1 y	
V a 1	Ser	Leu 35	G 1 y	Gln	Arg	Ala	ТЬг 40	1 l e	Ser	C y s	Arg	A1a 45	Ser	Lys	Ser	
V a 1	Ser 50	Thr	Ser	G 1 y	Туг	Se 1 5 5	T y r	Met	His	Тгр	Тут 60	Gln	Gln	L y s	Pro	
G 1 y 6 5	Gln	Тbг	Pro	L y s	Leu 70	Leu	Ile	Туг	Leu	A 1 a 7 5	Ser	As n	Leu	G 1 u	S c T 8 0	
G 1 y	V a l	Pro	Ala	Arg 85	РЬс	Ser	Gly	Seı	G 1 y 9 0	Sсг	G 1 y	Thr	Asp	Phe 95	ТЬг	
Leu	As n	110	His 100	Рго	V a l	Glu	Glu	G 1 u 1 0 5	Asp	Ala	Ala	Τbr	Tyr 110	Туг	C y s	
Gln	His	Sет 115	Aıg	Glu	Asn	Pro	Тут 120	ТЪг	Phe	G 1 y	Gly	G1 y 125	Thr	Lys	Leu	
Glu	II e 130	Lys														
(2)	NFORMA	TION FO	r seq ie	D NO:26:												
	(i)	( B ( C	) LENG ) TYPE ) STRA	TH: 405 I	base pairs acid SS: single											
	( i x )		) NAM	E/KEY: C												
	( i x )		) NAM	E/KEY: m	at_peptid .405	c										
	( <b>x</b> i	SEQUER	NCE DES	CRIPTIO	N: SEQ II	) NO:26:										
ATG Met	G G A G 1 y	TGG Trp	AGC Ser	GGG Gly 5	АТС 11 е	ΤΤΤ Ρhe	CTC Leu	ТТС РЬс	C T T L e u 1 0	C T G L e u	TCA Ser	G G A G 1 y	ACT Tbr	G C A A 1 a 1 5	GGT Gly	48
GTC Val	CAC His	T C T S e T	G A G G 1 u 2 0	ATC Ile	CAG Gln	C T G L e u	CAG Gln	CAG Gln 25	Ser	G G A G 1 y	CCT Pro	GAG Glu	CTG Leu 30	ATG Met	AAG Lys	96
C C T P r o	G G G G 1 y	GCT Ala 35	T C A S e r	GTG Val	A A G L y s	ATA Ile	TCC Ser 40	Cys	AAG Lys	GCT Ala	T C T S e r	G G T G I y 4 5	T A C T y r	T C A S e s	ТТС Рьс	144
ACT Thr	AGC Ser 50	тат	Т А С Т у г	АТА 11е	САС Ніs	TGG Trp 55	GTG Val	AAG Lys	CAG Gln	A G C Ser	CAT His 60	G G A G 1 y	AAG Lys	AGC Ser	CTT Leu	192
Glu	T G G T r p	ATT Ile	GGA G1y	TAT Tyr	ATT 11e 70	GAT	CCT Pro	T T C P h e	AAT Asn	G G T G 1 y 7 5	GGT Gly	ACT Tbr	A G C S e r	ТАС Тут	A A C A s n 8 0	240
65 CAG Gln	A A A Lys	ТТС РЬс	A A G L y s	G G C G 1 y 8 5	AAG Lys	GCC Ala	ACA Thr	TTG Leu	ACT Thr 90	GTT	GAC Asp	AAA Lys	TCT Ser	TCC Set 95	Ser	288
A C A T b r	GCC Ala	T A C T y r	Mct	САТ Ніs	CTC	AGC Ser	A G C S e 1	CTG Leu 105	ACA	T C T S e r	G A G G l u	GAC Asp	T C T S e 1 1 1 0	GCA Ala	GIC	336
TAI	TAC	TGT	100 GCA	AGG	GGG	GGT	AAC	CGC	TTT Phe	GCT	T A C T y r	TGG Trp	GGC	CAA	GGG Gly	384

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		_						-0	ontinu	ed						
		115					120	)				12	5			
	C T G L e u 1 3 0															4 0 5
(2)	INFORMA	TION F	or seq	ID NO:27	7:											
	(i)	(	A ) LEN B ) TYP													
	(ii)	MOLE	CULE TY	PE: prote	ain											
	( <b>x</b> i)	SEQUE	NCE DE	SCRIPTI	ON: SEQ	ID NO:27	<i>т</i> :									
Mic t 1	G 1 y	Ттр	Ser	G 1 y 5	Ile	Phe	Leu	РЬе	L e u 1 0		Ser	Gly	Thr	Ala 15		
Val	His	Ser	G I u 2 0	11 e	Gln	Leu	Gla	Gln 25		Gly	Pro	Glu	Leu 30	M e t	Ly s	
Pro	G 1 y	Ala 35	Ser	V a 1	L y s	[]e	Ser 40	C y s	Lys	Ala	Ser	G 1 y 4 5		Ser	РЬс	
ГЬт	Ser 50	Туг	Тут	116	Нis	Ттр 55	V a 1	L y s	Gln	Ser	His 60	Gly	L y s	S e r	Leu	
0 5	Тгр				70					75					8 0	
ali	Lys	РЬс	Lys	G 1 y 8 5	Lys	Ala	Тһг	Lcu	Thr 90	<b>V</b> a 1	Asp	Lys	S e r	Ser 95	Ser	
[b r	Ala	Туг	Met 100	His	Leu	Ser	Ser	L e u 1 0 5	Tbr	Seı	Glu	As p	Ser 110	Ala	Val	
Гуr	Туг	C y s 1 1 5	Ala	Arg	Gly	Gιy	Asn 120	Arg	Pbe	Al a	Туг	Тгр 125	Gly	Gln	G 1 y	
ſbr	Leu 130	Val	Thr	Val	Ser	Ala 135										
2)1	NFORMAI	ION FO	r seq i	D NO:28:	8											
	(i)	( 4 ( 1 ( 4	A)LENK B)TYPE C)STRA	3TH: 381	ESS: sing	5										
	(ix)	()	A) NAM	E/KEY: O												
	(ix)	FEATUR ( /	E: NAM		nat_pepti	de										
	(xi):				N: SEQ I	D NO:28:										
TG	GTG	тсс	TCA	GCT	CAG	ттс	стт	GGT	CIC	CTG	TTG	CTC	тот	ттт	C	48
1	Val	Ser	Ser	Ala 5	Gln	Phe	Leu	Gly	Leu 10	Leu	Leu	Leu	Cys	Phe 15	Gln	* 8
GТ 1 у	ACC Thr	AGA Aīg	TGT Cys 20	GAT Asp	ATC Ile	CAG Gln	ATG Mei	A C A T h r 2 5	CAG Gln	ACT Thr	ACA Tbr	TCC Ser	TCC Ser 30	CTG Leu	TCT Ser	96
СС 1 а	TCT Ser	CTG Leu 35	G G A G 1 y	GAC Asp	AGA Arg	GTC Val	A C C T b r 4 0	ATC Ile	AGT Ser	TGC Cys	AGG Arg	GCA Ala 45	AGT Ser	CAG Gln	GAC Asp	144
TT	AGC Ser	A G T S e r	ТАТ Тут	TTA Leu	AAC As a	TGG Trp	T A T T y r	C A G G l n	CAG	A A A	CCA	GAT	GGA	ACT	ATT	192

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								-co	ntinued	1						
	0.7.0	CTC.	ATC	TAC	TAC	ACA	TCA	AGA	TTA	CAC	TCA	GGA	GTC	CCA	TCA	240
AAA Lys	Leu	Leu	I le	Tyr	Туг	Tbr	Ser	Arg	Leu	His	Ser	Gly	V a 1	Pro	Ser 80	
65					70					75						2723
AGG	ттс	AGT	GGC	AGT	GGG	тст	GGA	ACA	GAT	TAT	TCT	C T C L e u	ACC	ATT	AAC As n	288
Arg	РЬс	Ser	Gly	Ser 85	Giy	Ser	GIY	101	A S P 90	1 y f	361	Leu	101	95		
	CTC	G 4 G	C	GAA	GAC	ATT	GCC	ACT	TAC	ттт	TGC	CAA	CAG	GGT	AAC	3 3 6
Asa	Leu	Glu	Gln	Glu	Asp	110	Ala	Thr	Тут	РЬс	C y s	Gln	G l n 1 l 0	Gly	Asn	
			100					105								2.6.1
ACG	CTT	CCG	TAC	ACG	TTC	GGA	GGG Glv	GGG G1v	ACC Tbr	AAG Lys	C T G L c u	GAA Glu	ATA	AAT		381
	LUU	1 1 5	- / -				120					1 2 5				
(2)[	NFORM/	TION FO	R SEQ I	D NO:29:												
	( i	) SEQUE														
			A)LENG B)TYPE			ids										
		(1	D) TOPO	DLOGY: L	incar											
	( i i	) MOLEC	CULE TY	PE: protei	n											
	( <b>x</b> i	) SEQUE	NCE DES	SCRIPTIC	N: SEQ I	D NO:29	:									
					Gla	Phe	Len	Glv	Leu	Leu	Leu	Leu	Cys	Pbc	Gln	
Met	vai	561	361	5	0.1	1 4 6	200	<i></i> ,	1 0					15		
Glv	Thr	Arg	Суs	Asp	Ile	Gln	Met	Тbт	Gln	Tbr	Thr	Ser	Ser	Leu	Ser	
,			2 0					2 5					30			
Ala	Ser	Leu	Gιy	Asp	Arg	V a l	Thr	1 l e	Ser	C y s	Arg	Ala	Ser	Gln	Asp	
		35					4 0					4 5			111.02010	
11 e	Ser 50		Туr	Leu	As n	Ттр 55	T y r	Gln	Gln	Lys	Pro 60	Asp	Gly	Thr	Ile	
												<b>6</b> 1	W - 1	Dee	S	
Lys 65		Leu	[ ] e	Tyr	T y r 7 0		Ser	Arg	Leu	H1 5 7 5	Ser	G 1 y	vai	r i o	80	
	D h a	S	61.8	S	61.	Ser	61.	Thr	Asp	Туг	Sет	Leu	Tbr	Ile	Asn	
Arg	Рцс	561	019	8 5		501	0.,		90	- , -				95		
Asn	Leu	Glu	Gln	Glu	Asp	Ile	Ala	Thr	Tyr	РЬс	Суs	Gln	Gln	G 1 y	Asn	
			100					105					110			
Thr	Leu			Thr	Phe	G 1 y	Gly	Gly	Thr	Lys	Lcu	G1u 125	11 c	Asn		
		115					120					125				
(2)	INFORM	ATION F	OR SEO	ID NO:30	н											
(2)						2										
	( 1	) SEQUI	A)LEN													
			B) TYP C) STR			ele										
			D)TOF													
	( i x	) FEATU	JRE:													
			A)NA B)LOO													
	(13		A ) NAI			tide										
		(	B)LO	CATION:	1411											
	( x i	) SEQU	ENCE DE	ESCRIPTI	ON: SEQ	ID NO:3	0:									
ATO	AGA	GTO	сто	AT 1	г сті	тт	G TGC	э сто	TTC	ACA	oco	TTT	CCT	GGT	ATC	4 8
Met		val	Let		e Leu 5	Lei	u Trp	Lei	1 Phe 10	Thr )	Ala	Phe	PIO	G I y 1 5	116	
			. GT/				G G 4 4	3 TC	3 664		GTO	ст ст с	GTG	AAG	сст	96
Let	s ici	Asp	va va	1 G 1 i	a Lei	G1	n Glu	s s e s	Gly	Pro	Val	Leu	V a 1 3 0	L y s	Pro	
			2 (					2 3								
TCT	CAG	з тст	сто	3 TC	с сто	C AC	C TG	C AC	T GTO	C AC1	690	TAC	TCA	ATC	ACC	144

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Ser	Gln	Ser	Leu	Ser	Leu	Thr		Thr	V a l	Тһт	Gly	Туг	Ser	11 e	Tbr	
		3 5					4 0					4 5				
GT	GAT	CAT	GCC	TGG	AGC	TGG	ATC	CGG	CAG	TTT	CCA	GGA	AAC	AAA	CTG	19
e r	Asp	His	Ala	Тгр	Ser	Тгр	1 l e	Arg	Gln	Phe	Рто	Gly	Asn	Lys	Leu	
	50					5 5					60					
AG	TGG	A T G	GGC	TAC	ΑΤΑ	AGT	TAC	AGT	GGT	ATC	ACT	ACC	TAC	AAC	CCA	24(
ilu	Тгр	Met	Gly	Тут	[ ] e	Ser	Туг	Ser	Gly	1 1 e	Thr	Thr	Туг	Asa	Pro	
65					70					75			100		8 0	
СT	стс	A A A	AGT	CGA	ATC	тст	ATC	ACT	CGA	GAC	ACA	тсс	AAG	AAC	CAG	285
e r	Leu	Lys	Ser	Arg	11 c	Ser	Ile	Thr	Arg	Asp	Thr	Ser	Lys	Asn	Gln	
				8 5					90	110071-50				95		
тс	ттс	СТА	CAG	TTG	ΑΑΤ	ТСТ	GTG	ACT	ACT	GGG	GAC	ACG	тсс	ACA	TAT	336
he	Phe	Leu	Gln	Leu	Asn	Ser	Val	Thr	Thr	Gly	Asp	Thr	Ser	Thr	Тут	
			100					105			000000000		110			
A C	TGT	GCA	AGA	тсс	CTA	GCT	CGG	ACT	ACG	GCT	ATG	GAC	TAC	TGG	GGT	384
y r	Cys	Ala	Arg	Ser	Leu	Ala	Arg	Thr	Thr	Ala	Met	Asp	Тут	Тгр	Glv	
		115					120					1 2 5		0.000		
A A	GGA	ACC	TCA	GTC	ACC	GIC	тсс	TCA								411
1 n	Gly	Tbr	Ser	Val	Thr	V a 1	Ser	Ser								47.
	130					1 3 5										

(2) INFORMATION FOR SEQ ID NO:31:

( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 137 amino acids (B) TYPE: amino acid ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met 1	Arg	Val	Leu lle 5	Leu Leu	Trp Leu	Phe Thr Ala 10	Phe Pro	GI y II c 15
Leu	Ser	Asp	Val Gln 20	Leu Gla	Glu Ser 25	Gly Pro Val	Leu Val 30	Lys Pro
Ser	Glm	Ser 35	Leu Ser	Leu Thr	CysTbr 40	Val Thr Gly	Tyr Ser 45	lle Thr
Ser	A s p 5 0	Нis	Ala Trp	Ser Trp 55	Ile Arg	Gla Phe Pro 60	Gly Asn	Lys Leu
G 1 u 6 5	Ттр	Μει	Gly Tyr	11e Ser 70	Туг Ѕег	Gly Ile Thr 75	Thr Tyr	Asn Pro 80
Ser	Leu	L y s	Ser Arg 85	lle Ser	lle Thr	Arg Asp Thr 90	Ser Lys	Asa Gla 95
Phe	Phe	Leu	Gln Leu 100	Asn Ser	Val Thr 105	Thr Gly Asp	Thr Ser 110	Thr Tyr
Тут	Суз	Ala 115	Arg Ser	Leu Ala	Arg Thr 120	Thr Ala Met	Азр Тут 125	Trp Gly
Gla	Gly 130	Thr	Ser Val	Thr Val 135	Ser Ser			

(2) INFORMATION FOR SEQ ID NO:32:

( i ) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 393 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear
- ( i x ) FEATURE:
- (A) NAME/KEY: CDS (B) LOCATION: 1..393

( i x ) FEATURE:

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								-co	ntinue	1						
				E/KEY: III	at_peptid .393	lc										
	(x i )	SEQUE	NCE DES	CRIPTIO	N: SEQ II	D NO:32:										
ATG	GAG	TCA	GAC	ACA	стс	CTG	CTA	TGG	GTG	CTG	стб	стс	TGG	GTT	CCA	
Met 1	Glu	Ser	A s p	Tbr 5	Leu	Leu	Leu	Тгр	Val 10	Leu	Leu	Leu	Тгр	Val 15	Pro	
GGT	тсс	ACA	GGT	GAC	ATT	GTG	TTG	ATC	CAA	тст	CCA	GCT	тст	TTG	GCT	
Gly	Ser	Thr	G I y 2 0	Asp	1 1 c	V a l	Leu	I 1 e 2 5	Gln	Ser	Pro	Ala	Ser 30	Leu	Ala	
GTG	тст	СТА	GGG	CAG	AGG	GCC	ACC	ATA	тсс	TGC	AGA	GCC	AGT	GAA	AGT	
Val	Ser	L e u 3 5	Gly	Gln	Arg	Ala	Thr 40	110	Ser	Суş	Arg	Ala 45	Ser	Glu	Ser	
GTT	GAT	AGT	TAT	GGC	AAT	AGT	TTT	ATG	CAC	TGG	TAC	CAG	CAG	AAA	CCA	
V a l	Asp 50	Ser	T y r	G 1 y	Asn	Ser 55	РЬе	M c t	His	Ттр	Тут 60	Gln	Gla	Lys	Ριο	
GGA	CAG	CCA	ccc	AAA	стс	стс	ATC	TAT	CGT	GCA	тсс	AAC	СТА	GAA	тст	
G 1 y 6 5	Gln	Pro	Pro	Lys	Leu 70	Leu	11 e	T y r	Агд	A 1 a 7 5	S e r	Asn	Leu	Glu	Ser 80	
GGG	ATC	сст	GCC	AGG	ттс	AGT	GGC	AGT	GGG	тст	AGG	ACA	GAC	ттс	ACC	
G 1 y	1 l e	Pro	Ala	Arg 85	Phe	Ser	Gly	Ser	G 1 y 9 0	Ser	Arg	ТЬг	Asp	Phe 95	Thr	
стс	ACC	ATT	AAT	сст	GIG	GAG	GCT	GAT	GAT	GTT	GCA	ACC	TAT	TAC	TGT	
Leu	Τ <b>h</b> r	I 1 c	Asn 100	Рго	Val	Glu	Ala	Asp 105	Asp	V a l	Ala	Τhτ	Туг 110	Туг	Суз	
CAG	CAA	AGT	AAT	GAG	GAT	сст	ccc	ACG	ттс	GGT	GCT	GGG	ACC	AAG	CTG	
Gln	Gln	Ser 115	Asn	Glu	A s p	Pro	Рто 120	Thr	Рһс	Gly	Ala	Gly 125	Thr	Lys	Leu	
GAG	CTG	A A A														
Glu	L e u 1 3 0	L y s														
(2)		ATION FO	or seq 1	D NO:33												

( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 131 amino acids (B) TYPE: amino acid ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:33:

м	c	t 1	G	1	u	3	s e	. 1		A	\$	p		г 1	1 1		L	c	U	L	c	u	L	¢	u	т	r	P		a 1		L	e	u	L	¢	u	L	c	u	9	<b>F</b> 1	p	Va 1	1	5	P	r (	D.
G	1	у	s	¢	r	i i	г		t	G		y O	3	A :	s [		1	1	c	v	a	1	L	e	u			e 5	G	1	n	s	c	r	P	r	0	A	1	a	2		s r 3 0	Le	1	1	A	1 .	a
v	a	1	s	¢	I			сı 3 1		G	1	у	8	G I	lı		A	r	g	A	1	a			r 0	1	1	e	s	¢	r	с	у	s	A	r	8	A		a 5	1	5 6	. 1	 G 1	ı	1	s	c	r
v	a	1	A		р 0		5	• 1	r	Т	у	r	1	G	1 3		A	s	n			r 5	P	h	c	М	¢	t	н	ì	\$	т	r	P		у б		G	1	n		G 1	l n	Ly		\$	P	I	D
		у 5	G	1	n		P	r (	Þ	P	I	0		L	y :			с 7		L	¢	u	I	1	¢	T	у	r	A	r	g		1 7		s	c	r	A	\$	n	Ì	L	e u	G 1		a		e 8	
G	1	у	1	1	c		P	r	D	A	1	a	ļ		r (		P	h	c	s	c	r	G	1	у	s	c	r		1 9		s	c	r	A	r	g	Т	h	r	ļ	A :	s p	P b 9		7. S	T	h	r
L	¢	u	т	ł	1 7		t	1 .	c	A 1		n 0		P	r (	,	v	a	1	G	1	u	A	1	a			р 5	A	s	р	v	a	1	A	1	a	т	h	r			y 1 1 0	т,	ŀ	r	с	y	s
G	1	n	G	; 1	n			e : 1 :		A	5	n		G	1	1	A	s	P	P	r	0			0 0	т	b	r	P	b	c	G	1	у	A	ı	a	G 1	12	у 5		т	h r	Ly	Y	s	L	c	u
G	1	u			- u		L	y	s																																								

4 8

96

144

192

240

2 8 8

3 3 6

384

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#### (2) INFORMATION FOR SEQ ID NO:34:

( i ) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 417 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: single
   (D) TOPOLOGY: linear
- ( i x ) FEATURE:

## (A) NAME/KEY: CDS (B) LOCATION: 1..417

- ( i x ) FEATURE:
  - (A) NAME/KEY: mat\_peptide (B) LOCATION: 1..417

#### ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:34:

ATG	GGA	TGG	AGC	GGG	GTC	TTT	ATC	TTC	CTC	CIG	TCA	GTA	ACT	GCA	GGT	4 8
Met	Gly	Trp	Ser	Gly	Val	Phe	Ile	Phe	Leu	Leu	Ser	Val	Тһт	ALA	Glv	
1				5					10					15	5.,	
GTC	CAC	TCC	CAG	GTT	CAA	TTG	CAG	CAG	TCT	GGA	GCT	GAG	TTG	ATG	AAG	96
Val	His	Ser	Gln	Val	Gln	Leu	G 1 n	Gln	Ser	Gly	Ala	Glu	Leu	Met	Lys	
			20					2 5					3 0		1999 - 19 <del>7</del> 0 - 198	
сст	GGG	GCC	TCA	GTC	AAG	ATC	TCC	тос	A A G	CCT	ACT	0.00	TAC		<b>TT</b> 0	
Pro	Glv	ALA	Ser	Val	Lva	Ile	Ser	Cv.	Lve	41.2	The	GL.	Tue	ALA	11C	144
2020		3 5			- , -		40	- , ,	2,3	A 1 4	1 0 1	4 5	1 y f	101	Рпс	
							40					4.5				
AGT	AGT	TAT	TGG	ATA	GTG	TGG	ATA	AAG	CAG	AGG	ССТ	GGA	CAT	GGC	СТТ	192
Ser	Ser	Туг	Тгр	Ile	Val	Trp	Ile	Lys	Gln	Arg	Pro	Gly	His	Gly	Leu	1.000
	50					5 5				740-124 <b>.</b> 789	6 0	1996				
C+C	TOO															
GAG	TGG	AII	GGA	GAG	ATT	TTA	CCT	GGA	ACC	GGT	AGT	ACT	AAC	TAC	AAT	240
6 5	110	116	GTY	GIU		Leu	Pro	GIY	Thr		Ser	Thr	Asn	Туг		
0.5					70					75					8 0	
GAG	AAA	TTC	AAG	GOC	AAG	GCC	ACA	TTC	ACT	GCA	GAT	ACA	тст	TCC	AAC	288
Glu	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Phe	Thr	Ala	Asp	Thr	Ser	Ser	Asn	200
				8 5					90		•			95		
2002000																
ACA	GCC	TAC	ATG	CAA	CTC	AGC	AGC	CTG	ACA	TCT	GAG	GAC	TCT	GCC	GTC	3 3 6
Thr	Ala	Tyr	Met	Gln	Leu	Ser	Ser		Thr	Ser	Glu	Asp	Ser	Ala	V a 1	
			100					105					1 1 0			
TAT	TAC	TGT	GCA	AGT	CTA	GAC	AGC	TCG	0.00	TAC	тат	GCT	ATG	6.4.6	TAT	2.0.4
Tyr	Туг	Cys	Ala	Ser	Leu	Asp	Ser	Ser	Gly	Tyr	Tvr	41.	MAI	AAR	Tur	384
		115					120		0.7	• • •	. , .	125	MCI	Asp	1 9 1	
						GTC										417
Trp	G 1 y	Gla	Gly	Thr	Ser	Val	Thr	Val	Ser	Ser						
	130					1 3 5										

(2) INFORMATION FOR SEQ ID NO:35:

#### ( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 139 amino acids (B) TYPE: amino acid ( D ) TOPOLOGY: linear

#### ( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met 1	Gly	Trp	Ser G	1 y Val 5	Pbe 11e	Phe Leu Leu 10	Ser Val	Thr Ala Gly 15
V a 1	His	Ser	G 1 n V 2 0	al Gln	Leu Gla	Gln Ser Gly 25	Ala Glu	Leu Met Lys 30
Рго	G 1 y	A 1 a 3 5	Ser V	al Lys	Ile Ser 40	Cys Lys Ala	Thr Gly 45	Tyr Tbr Phe
Sет	Ser 50	T y r	Trp 1	le Val	Trp Ile 55	Lys Gln Arg	Pro Gly 60	His Gly Leu
Glu	Тгр	1 1 e	Gly G	l v 11 e	Leu Pro	Gly Thr Gly	Ser Thr	Asn Tyr Asn

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65					70					75					80	
Glu	Lys	Pbe	Lys	G 1 y 8 5	Lys	Ala	Thr	Pbe	T b r 9 0	Ala	As p	Tbr	Scı	Ser 95	As n	
Thr	Ala	Туr	Met 100	Gln	Leu	Ser	Ser	Leu 105	Tbr	Seı	Glu	As p	Ser 110	Ala	V a l	
Тут	T y r	Cys 115	Ala	Ser	Leu	Asp	Ser 120	Ser	Gly	Туг	Тут	Ala 125	M e t	A s p	Туr	
Trp	G 1 y 1 3 0	Gln	G 1 y	ТЪг	Ser	V a 1 1 3 5	ТЬт	Val	Ser	Sет						
(2)[	FORMA	TION FO	r seq id	NO:36:												
	( i	(B) (C)	) LENG ) TYPE: ) STRAI	RACTERI TH: 381 b nucleic a NDEDNE LOGY: br	ase pairs cid SS: single											
	( i <b>x</b>		) NAME	E/KEY: CI												
	(ix		) NAME	E/KEY: m. 110N: 1		¢										
	( <b>x</b> i	) SEQUE	NCE DES	CRIPTION	1: SEQ II	) NO:36:										
ATG Met	GTG Val	T C C S e T	ACA Thr	CCT Pro 5	CAG Gln	TTC Pbe	CTT Leu	G G T G 1 y	CTC Leu 10	CTG Leu	TTG Leu	ATC Ile	Т <b>G T</b> С у s	ТТТ РЬе 15	CAA Gln	4 8
GGT	ACC	AGA	TGT	GAT	ATC	CAG	ATG	ACA	CAG	ACT	ACA	тсс	тсс	СТО	тст	96
G 1 y	Tbr	AT 8	C y s 2 0	Asp	1 l e	Gln	Me t	Tbr 25	Gln	Thr	Thr	Ser	Ser 30	Leu	Ser	
GCC Ala	T C T S e I	CTG Leu 35	GGA Gly	GAC Asp	AGA Arg	GTC Val	ACC Thr 40	ATC Ile	AGT Scr	T G C C y s	AGG Arg	G C A A 1 a 4 5	AGT Ser	CAG Gln	GAC Asp	144
ATT 11e	AGT Ser 50	AAT As n	T A T T y r	T T A L e u	AAC Asn	TGG Trp 55	TAT	CAA Gln	CAG Gln	AAA Lys	CCA Pro 60	GAT Asp	GGA Gly	ACT Thr	GTT Val	192
AAA Lys 65	CTC	C T G L e u	ATC I 1 e	T A C T y r	TAT Tyr 70	ACA	T C A S e r	AGA Aīg	TTA Leu	CAC His 75	TCA Ser	G G A G 1 y	GTC Val	C C A P r o	TCA Ser 80	240
100	ТТС РЪс	AGT Ser	G G C G l y	AGT Ser 85	G G G G 1 y	TCT Ser	G G A G 1 y	ACA Thr	GAT Asp 90	Tyr	T C T S e r	CTC Leu	ACC Tbr	АТТ I 1 е 9 5	Ser	288
A A C A s a	CTG Leu	GAG Glu	C A A G 1 n 1 0 0	GAA Glu	GAT Asp	ATT Ilc	GCC A 1 a	А G T S е т 1 0 5	T A C T y r	TTT Pbe	TGC Cys	CAA Gla	C A G G l n 1 1 0	GIY	T A T T y r	336
ACG Thr	ССТ Рто	C C G P r o 1 1 5	T G G T r p	ACG Thr	ТТС РЪс	GGT Gly	G G A G 1 y 1 2 0	GGC Gly	ACC Thr	AAG Lys	TTG Leu	G A A G 1 u 1 2 5	Ilc	AAA Lys		381
(2)		ć	NCE CH		RISTICS: amino ac acid											

( i i ) MOLECULE TYPE: protein

(  $\mathbf x$  i ) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Met Val Ser Thr Pro Gln Phe Leu Gly Leu Leu Leu lle Cys Phe Gln
1 5 10 15
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_	-		-		_	3		-c	ontinue	ed						
G 1 y	Thr	Ar g	C y s 2 0	As p	110	Gln	Met	Thr 25		Thr	Tbr	Ser	Ser 30		Ser	
Ala	Ser	Leu 35	G 1 y	Asp	Атд	V a 1	Tbr 40	11 e	S e r	C y s	A T g	Ala 45		Gln	Asp	
[] e	Ser 50	As n	Туг	Leu	Asn	Тгр 55	Туr	Gln	Gln	Lys	Рто 60		Gly	Thr	V a l	
L y s 6 5	Leu	Leu	Ile	Тут	Туг 70	ТЪг	Ser	Arg	Leu	His 75	Ser	G 1 y	V a l	Pro	Ser 80	
Атд	Phe	Ser	G·l y	Ser 85	Gly	Ser	G 1 y	Tbr	Asp 90	Туг	Ser	Leu	Thr	11e 95		
A s n	Leu	Glu	G 1 n 1 0 0	Glu	A s p	] l e	Ala	Ser 105	Тут	Phe	C y s	Gln	G l n 1 1 0	Gly	Туг	
Tbr	Ρτο	Pro 115	Ттр	Thr	РЪс	Gly	G 1 y 1 2 0	Gly	Thr	Lys	Leu	G 1 u 1 2 5	I l e	Lys		
(2)	INFORM	ATION FO	OR SEQ I	D NO:38												
	( i	(	NCE CHL A ) LENG B ) TYPE C ) STR/ D ) TOPO	3TH: 402 3: nucleic NDEDN	base pair acid ESS: sing	s										
	( i x		RE: A)NAM B)LOCA													
	( i <b>x</b>		RE: A)NAM B)LOC/			de										
	( <b>x</b> i	) SEQUE	NCE DES	CRIPTIC	IN: SEQ I	D NO:38:										
ATG Met 1	G A G G I u	CTG Leu	GAT Asp	CTT Leu 5	TAT Tyr	CTT Leu	ATT Ile	CTG Leu	TCA Ser 10	GTA Val	ACT Thr	TCA Sei	GGT Gly	GTC Val 15	T A C T y r	4 8
TCA Ser	CAG Gln	GTT Val	CAG G1n 20	C T C L e u	C A G G l n	CAG Gln	T C T S e r	G G G G G 1 y 2 5	GCT Ala	GAG Glu	CTG Leu	GCA Ala	AGA Arg 30	CCT Pro	GGG Gly	96
GCT Ala	T C A S e 1	G T G V a 1 3 5	AAG Lys	TTG Leu	TCC Ser	TGC Cys	AAG Lys 40	GCT Ala	TCT Ser	GGC Gly	T A C T y r	ACC Tbr 45	ТТТ РЬе	ACT Thr	AAC Asn	144
Т А С Т у г	Т G G Т т р 5 0	GTG Val	CAG Gln	ТСС Ттр	GTA Val	A A A L y s 5 5	CAG Gln	AGG Aīg	CCT Pro	G G A G 1 y	C A G G 1 n 6 0	GGT Gly	C T G L e u	G A A G l u	TGG Trp	192
ATT 11c 65	G G G G l y	TCT Ser	ATT Ile	T A T T y r	ССТ Рто 70	G G A G I y	GAT Asp	GGT G1y	GAT Asp	ACT Tbr 75	AGG Arg	AAC Asn	ACT Thr	CAG Gln	A A G L y s 8 0	240
ТТС Рће	AAG Lys	66C 61 y	AAG Lys	GCC A 1 a 8 5	ACA Tbr	T T G L e u	ACT Thr	GCA Ala	GAT Asp 90	AAA Lys	TCC Sei	TCC Ser	ATC Ile	A C A T b r 9 5	GCC Ala	288
T A C T y r	ATG Met	CAA Gln	CTC Leu 100	ACC Thr	A G C S e r	TTG Leu	GCA Ala	T C T S e r 1 0 5	GAG Glu	GAC Asp	T C T S e r	GCG Ala	GTC Val 110	T A T T y r	T A C T y r	336
Т G Т С у з	GCA Ala	AGA Arg 115	TCG Ser	ACT Thr	G G T G 1 y	AAC Asn	CAC His 120	TTT Pbe	GAC Asp	Т А С Т у з	TGG Trp	G G C G 1 y 1 2 5	CAA Gln	GGC Gly	ACC Tbr	384
		ACA Tbr														402

(2) INFORMATION FOR SEQ ID NO:39:

( i ) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 134 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
( i i ) MOLECULE TYPE: protein	
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:39:	
Met Glu Leu Asp Leu Tyr Leu Ile Leu Ser Val Thr Ser Gly Val Ty 1 5 10 15	r
Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gl 20 25 30	у
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr As 35 40 45	n
Tyr Trp Val Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Tr 50 55 60	P
Ile Gly Ser lie Tyr Pro Gly Asp Gly Asp Thr Arg Asn Thr Gln Ly 65 70 75	ys 80
Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ile Thr A 85 90 95	la
Tyr Met Gln Leu Thr Ser Leu Ala Ser Glu Asp Ser Ala Val Tyr Ty 100 105 110	/ T
Cys Ala Arg Ser Thr Gly Asn His Phe Asp Tyr Trp Gly Gln Gly T 115 120 125	a r
Thr Leu Thr Val Ser Ser 130	
(2) INFORMATION FOR SEQ ID NO:40:	
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENOTH: 35 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:40: ACAAAGCTTC CACCATGGAG TCAGACACAC TCCTG	
(2) INFORMATION FOR SEQ ID NO:41:	
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 36 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> (x i) SEQUENCE DESCRIPTION: SEQ ID NO:41:	
GGCTAAGCTT CCACCATGGG ATGGAGCGGG ATCTIT	
(2) INFORMATION FOR SEQ ID NO:42:	
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 35 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:42:	
CITGGATCCA CTCACGTTIT ATTICCAGCT TGGTC	
(2) INFORMATION FOR SEQ ID NO:43:	
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 36 base pairs</li> <li>( B ) TYPE: nucleic acid</li> </ul>	

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	17.	.705

75		76
	-continued	10
(C) STRANDEDNESS: single (D) TOPOLOGY: linear		
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:43:		
GTTGGATCCA CICACCIGCA GAGACAGITA	CCAGAG	3 6
( 2 ) INFORMATION FOR SEQ ID NO:44:		
( i ) SEQUENCE CHARACTERISTICS:		
<ul> <li>(A) LENGTH: 35 base pairs</li> <li>(B) TYPE: nucleic acid</li> </ul>		
(C) STRANDEDNESS: single (D) TOPOLOGY: linear		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:44:		
CTTGGATCCA CTCACGATTT ATTTCCAGCT	IGGIC	
	10010	3.5
(2) INFORMATION FOR SEQ ID NO:45:		
( i ) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 35 base pairs (B) TYPE: nucleic acid		
(C) STRANDEDNESS: single (D) TOPOLOGY: linear		
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:45:		
CTTGGATCCA CTCACGTTTT ATTICCAGCT		
erreduiter ereacorrit arriterager	IGGIC	3 5
(2) INFORMATION FOR SEQ ID NO:46:		
( i ) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 36 base pairs (B) TYPE: nucleic acid		
(C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:46:		
ACAAAGCTTC CACCATGGTG TCCTCAGCTC A	GTTCC	3 6
(2) INFORMATION FOR SEQ ID NO:47:		
( i ) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 39 base pairs (B) TYPE: nucleic acid		
( C ) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:47:		
TGTTAGATCT ACTCACCTGA GGAGACAGTG A	CTGAGGTT	3 9
(2) INFORMATION FOR SEQ ID NO:48:		
( i ) SEQUENCE CHARACTERISTICS:		
(A) LENGTH: 36 base pairs (B) TYPE: nucleic acid		
(C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:48:		
GTCTAAGCTT CCACCATGAG AGTGCTGATT C	TTTTG	3 6
(2) INFORMATION FOR SEQ ID NO:49:		
( i ) SEQUENCE CHARACTERISTICS:		
( A ) LENGTH: 17 base pairs		
(B) TYPE: nucleic acid (C) STRANDEDNESS: single		
(D) TOPOLOGY: linear		

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	5.795.905		78	
77	-continued		/0	
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:49:				
TACGCAAACC GCCTCTC				17
(2) INFORMATION FOR SEQ ID NO:50:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 18 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>				
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:50:				
GAGTGCACCA TATGCGGT				18
(2) INFORMATION FOR SEQ ID NO:51:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 55 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> (x i) SEQUENCE DESCRIPTION: SEQ ID NO:51:				
ACCGTGTCTG GCTACACCTT CACCAGCGAT	CATGCCTGGA	GCTGGGTGAG	ACAGC	5 5
(2) INFORMATION FOR SEQ ID NO:52:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 63 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>				
( $\mathbf{x}_{-}i_{-}$ ) SEQUENCE DESCRIPTION: SEQ ID NO:52:				
TGAGTGGATT GGATACATTA GTTATAGTGG	AATCACAACC	TATAATCCAT	CTCTCAAATC	6 0
CAG				63
(2) INFORMATION FOR SEQ ID NO:53:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 54 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>				
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:53:				
TATTATTGTG CAAGATCCCT AGCTCGGACT	ACGGCTATGG	ACTACTGGGG	TCAA	54
(2) INFORMATION FOR SEQ ID NO:54:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 27 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>				
( $\mathbf{x}$ i ) SEQUENCE DESCRIPTION: SEQ ID NO:54:				
GTGACAATGC TGAGAGACAC CAGCAAG				2 7
(2) INFORMATION FOR SEQ ID NO:55:				
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 24 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>				

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J.,	195	,905

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79		80	
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(x i) SEQUENCE DESCRIPTION: SEQ ID NO:55:			
GGTGTCCACT CCGATGTCCA ACTG			24
(2) INFORMATION FOR SEQ ID NO:56:			
( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 27 base pairs			
( B ) TYPE: nucleic acid			
( C ) STRANDEDNESS: single			
(D) TOPOLOGY: linear			
( $\mathbf{x}$ i ) SEQUENCE DESCRIPTION: SEQ ID NO:56:			
GGTCTTGAGT GGATGGGATA CATTAGT			2 7
(2) INFORMATION FOR SEQ ID NO:57:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 29 base pairs			
(B) TYPE: nucleic acid (C) STRANDEDNESS: single			
(D) TOPOLOGY: linear		1.5.1	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:57:			
GTGTCTGGCT ACTCAATTAC CAGCATCAT			2 9
(2) INFORMATION FOR SEQ ID NO-58:			
( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 48 base pairs			
(B) TYPE: nucleic acid			
( C ) STRANDEDNESS: single			
(D) TOPOLOGY: linear			
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:58:			
TGTAGAGCCA GCCAGGACAT CAGCAGTTAC	CTGAACTGGT	ACCAGCAG	4 8
(2) INFORMATION FOR SEQ ID NO:59:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 42 base pairs			
( B ) TYPE: nucleic acid			
(C) STRANDEDNESS: single (D) TOPOLOGY: linear			
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:59:			
ATCTACTACA CCTCCAGACT GCACTCTGGT	GTGCCAAGCA	G. 4	4 2
	GIOCCANGEA	UX .	42
(2) INFORMATION FOR SEQ ID NO:60:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 50 base pairs			
(B) TYPE: nucleic acid			
(C) STRANDEDNESS: single (D) TOPOLOGY: linear			
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:60:			
ACCTACTACT GCCAACAGGG TAACACGCTT	CCATACACGT	TCGGCCAAGG	5 0
(2) INFORMATION FOR SEQ ID NO:61:			
( i ) SEQUENCE CHARACTERISTICS:			
(A) LENGTH: 27 base pairs			
(B) TYPE: nucleic acid			
(C) STRANDEDNESS: single			
(D) TOPOLOGY: linear			

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:61:

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GCGC	TAC	CG A	СТАС	ACCT	T CA	CCAT	с									27
2) INF	ORMAT	ION FOR	SEQ ID	NO:62:												
	(i)	( B ( C	) LENGI ) TYPE: ) STRAN	ACTERIS TH: 706 ba nucleic act IDEDNES OGY: line	ise pairs id S: single											
		11000		001: mie	a											
	(ix)		) NAME	/KEY: CD 110N: 85												
	(i <b>x</b> )		) NAME	/KEY: ma 110N: 85												
	(ix)		) NAME	VKEY: CD TION: 135												
	(ix)		) NAME	VKEY: ma TION: 135												
	(1)	SEQUEN				NO:62:										
			004		100	тот	ATC Ile	ATC Ile	CTC Leu	ТТС РЪс	T T G L e u 1 0	GTA Val	GCA Ala	ACA Tbr	GCT Ala	4 9
СА Ьг 15	GGT/	AAGGO	oc 1	CACA	GTAC	C AC	GCT	T G A G G	э тст	GGA	CATA	TATA	TGGG	3 T G		10
	TGA	CAT	CACT	гттос	с т	тст	CTCC	A CA	GGT Gly 1	GTC Val	CAC His	TCC Ser	CAG Gln 5	GTC Val	С А А G 1 п	15
T G e u	CAG Gln	G A G G 1 u 1 0	AGC Ser	GGT Gly	C C A P r o	G G T G 1 y	CTT Leu 15	G T G V a l	AGA Arg	CCT Pro	AGC Sei	C A G G 1 n 2 0	ACC Tbr	CTG Leu	AGC Set	2 0
T G c u	ACC Thr 25	T G C C y s	ACC Tbr	GTG Val	T C T S e r	G G C G 1 y 3 0	Т А С Т у т	TCA Ser	ATT 11c	ACC Thr	AGC Ser 35	GAT Asp	CAT His	GCC Ala	T G G T r p	2 5
e r	T.0.0	GTG Val	AGA Arg	CAG Gln	CCA Pro 45	сст	G G A G I y	CGA Arg	G G T G 1 y	CTT Leu 50	G A G G 1 u	TGG Trp	ATT Ile	G G A G 1 y	T A C T y r 5 5	29
	AGT Ser	T A T T y r	AGT Ser	G G A G 1 y 6 0	ATC	A C A T b r	ACC Thr	T A T T y r	AAT Asn 65	C C A P r o	TCT Ser	CTC Leu	AAA Lys	ТСС Sет 70	Arg	34
TG al	ACA Thr	A T G M e t	CTG Leu 75	AGA Arg	GAC Asp	A C C T b r	A G C S e T	AAG Lys 80	Asn	CAG Gla	ТТС Рье	AGC Ser	CTG Leu 85	AGA Arg	CTC Leu	39
GC	AGC Sei	GTG Val 90		000	G C C A 1 a	GAC Asp	ACC Thr 95	Ala	GTT Val	T A T T y r	ТАТ Туг	T G T C y s 1 0 0	GCA Ala	AGA Arg	TCC Ser	4 4
CTA cu	G C T A 1 a 1 0 5	C G G A r g	ACT Tbr	A C G T b r	GCT Ala	ATG Met 110	Asp	ТАС Туг	T G G T r p	G G T G I y	CAA Gln 115	GIY	AGC Ser	CTC Leu	GTC Val	4 9
A C A [ b r 1 2 0	GTC Val	TCC Ser	TCA Ser	ØGT	GAGT	сст	TACA	ACCT	ст с	тстт	стат	т са	GCTT	<b>A A A</b> T	•	5 4
AGA	тттт	ACT	GCAT	TTGT	TG G	6666	GAAA	TGT	GTGT	ATCI	GAA	TTTC	AGG	TCAT	GAAGGA	6 (
				GGAG												6 (

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(2) INFORMATION FOR SEQ ID NO:63: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 15 amino acids ( B ) TYPE: amino acid ( D ) TOPOLOGY: linear ( i i ) MOLECULE TYPE: protein ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:63: Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr 10 15 (2) INFORMATION FOR SEQ ID NO:64: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 123 amino acids ( B ) TYPE: amino acid ( D ) TOPOLOGY: linear ( i i ) MOLECULE TYPE: protein ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:64: Gly Val His Ser Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val 1 5 10 15 Arg Pro Ser Gln Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser 20 25 30 Ile Thr Ser Asp His Ala Trp Ser Trp Val Arg Gln Pro Pro Gly Arg 35 40 45 Gly Leu Glu Trp lle Gly Tyr lle Ser Tyr Ser Gly lle Thr Thr Tyr 50 55 60 As a Pro Ser Leu Lys Ser Arg Val Thr Met Leu Arg Asp Thr Ser Lys 65 70 75 80 Gln Phe Ser Leu Arg Leu Ser Ser Val Thr Ala Ala Asp Thr Ala 85 90 95 Val Tyr Tyr Cys Ala Arg Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr 100 105 110 Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser 115 120 ( 2 ) INFORMATION FOR SEQ ID NO:65: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 506 base pairs ( B ) TYPE: nucleic acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( i x ) FEATURE: ( A ) NAME/KEY: CDS ( B ) LOCATION: 8.52 ( i x ) FEATURE: ( A ) NAME/KEY: mat\_peptide ( B ) LOCATION: 8.52 ( i x ) FEATURE: ( A ) NAME/KEY: CDS ( B ) LOCATION: 135..467 ( i x ) FEATURE: ( A ) NAME/KEY: mat\_peptide ( B ) LOCATION: 135.467 ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:65: AAGCTTC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala

5.795.965

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Thr	GGTA	AGGG	GC T	CACA	GTAG	C AC	GCTT	GAGO	; TCT	GGAC	ATA	ΤΑΤΑ	TGGG	TG	
1 5	0014														
ACAA	TGAC	ат с	CACT	TTGC	сті	тст	СТССА	C A	G G T G 1 y 1	GTC Val	CAC His	TCC Ser	GAC Asp 5	ATC CAG 11e Gln	
ATG Met	ACC Thr	Gln	AGC S¢r	CCA Pro	AGC Ser	AGC Ser	CTG Leu 15	A G C S e r	GCC Ala	AGC Ser	GTG Val	G G T G I y 2 0	GAC Asp	AGA GTG Arg Val	
ACC	ATC	10 ACC	TGT	AGA	GCC	AGC	C + G	GAC	ATC	AGC	A G T Ser	T A C T y r	.CTG Leu	AAT TGG Ass Trp	
	2 5					30					35				
ТАС Тут 40	C A G G l n	CAG Gln	AAG Lys	CCA Pro	G G T G 1 y 4 5	AAG Lys	GCT Ala	CCA Pro	AAG Lys	CTG Leu 50	CTG Leu	ATC 11c	Tyr	TAC ACC Tyr Thr 55	
T C C S e r	AGA Arg	C T G L e u	CAC His	ТСТ Sет 60	G G T G I y	GTG Val	CCA Pro	AGC Ser	AGA Arg 65	ТТС РЬс	AGC Set	G G T G 1 y	AGC Ser	GGT AGC Gly Ser 70	
G G T G 1 y	ACC Thr	GAC Asp	TTC Pbe 75	ACC Thr	ТТС РЬс	ACC Thr	ATC 11c	AGC Ser 80	AGC Ser	C T C L e u	CAG Gln	С С А Р т о	G A G G l u 8 5	GAC ATC Asp 11e	
GCT Ala	ACC Tbr	Туг	<b>T</b> 1 C	T G C C y s	CAA Gln	CAG Gln	G G T G 1 y 9 5	AAC Asn	ACG Thr	C T T L c u	CCA Pro	Т А С Т у т 1 0 0	ACG Thr	TTC GGC Phe Gly	
CAA	666	90 ACC	AAG	GTG	GAA	ATC		сбт	GAGT	AGA	ATTT		тт т	GCTTCCTC	A
Gin	G 1 y 1 0 5	Thr	Lys	V a l	Glu	11e 110	Lys								
GTT	GGAT	cc													
(2)	INFORMA	TION FO	R SEQ I	D NO:66:											
	(i	(4	A)LEN B)TYPE	ARACTER GTH: 15 a E: amino a OLOGY: li	mino aci cid										
	(11	) MOLEC	ULE TY	PE: protei	п										
				PE: protei SCRIPTIO		id no:6	6:								
Met 1	( x i G l y	) SEQUE	NCE DE	SCRIPTIO	N: SEQ			РЬс	L e u 1 0	V a l	Ala	ТЪт	Ala	Th r 15	
1	(xi Gly	) SEQUE	NCE DE	SCRIPTIO Cys	N: SEQ 11c			РЬс	Leu 10	Val	Ala	ТЬт	Ala	Thr 15	
1	(xi Gly INFORM	) SEQUE Trp ATION FO ) SEQUE ( (	NCE DE S e r DR SEQ I ENCE CH A ) LEN B ) TYP	C y s	N: SEQ I I e RISTICS amino a acid	I 1 •		РЬс	Leu 10	V a l	Ala	Tbr	A 1 a	Thr 15	
1	(xi Gly INFORM (i	) SEQUE Trp ATION FO ) SEQUE ( ( (	NCE DE S c T DR SEQ 1 ENCE CH A ) LEN B ) TYP D ) TOP	C y s C y s 5 ID NO:67: IARACTE IGTH: 111 E: amino :	N: SEQ I I c RISTICS amino a acid linear	I 1 •		РЬс	L e u 1 0	V a l	Ala	ТЪт	Ala	Tbr 15	
1	(xi Gly INFORM (i (i i (xi	) SEQUE T r p ATION FO ) SEQUE ( ( ) MOLEO ) SEQUE	NCE DE: S e T DR SEQ I ENCE CH A ) LEN B ) TYP D ) TOP CULE TY ENCE DE	SCRIPTIO C y s 5 ID NO:67: IARACTE IGTH: 111 E: amino n OLOGY: 1 (PE: prote SCRIPTIO	N: SEQ I I e RISTICS amino a acid linear in DN: SEQ	[]] ( cids	: Leu 57:		10						
1	(xi Gly INFORM (i (i (xi (xi Val	) SEQUE Trp ATION FC ) SEQUE ( ( ) MOLEC ) SEQUE His	NCE DE: S c r DR SEQ I ENCE CH A ) LEN B ) TYP D ) TOP CULE TY ENCE DE S c r	C y s 5 ID NO:67: IARACTE KTH: 111 E: amino : VOLOGY: I YPE: prote SCRIPTIC A s p 5	N: SEQ I I c RISTICS amino a acid linear in DN: SEQ I I c	Il cids	: Leu 57: п. Мет	ТЬ	10 r Gln 10	S e 1	r Pro	а Sет	Ser	Leu Ser 15	
1 (2) G1y 1 A1a	(xi Gly INFORM (i (xi (xi Val	) SEQUE Trp ATION FC ) SEQUE ( ( ) MOLE ) SEQUE H i s V a l	NCE DE S e r DR SEQ I NCE CH A ) LEN B ) TYP D ) TOP CULE TY ENCE DE S e r G 1 y 2 0	C y s 5 ID NO:67: IGTH: 111 E: annico VOLOGY: I (PE: prote SSCRIPTIC A s p 5 ( A s p )	N: SEQ Ile RISTICS acid tinear in DN: SEQ Ile Arg	Il cids DNO:0 Gl g Va	: Leu 57: п Мет 1 Тhr	T b I 1 2	r Gln 10 e Thr	Ser Cys	Pro	Ser Ala	Ser Ser 30	Leu Ser 15 Gln Asy	
1 (2) G1y 1 A1a 11c	(xi Gly INFORM (i (xi (xi val Ser Ser	) SEQUE Trp ATION FO ) SEQUE ( ( ( ) MOLEO ) SEQUE His Val Ser 35	NCE DE S e r DR SEQ 1 ENCE CH A ) LEN B ) TYP D ) TOP CULE TY ENCE DE S e r G 1 y 2 0 T y r	SCRIPTIO C y s 5 ID NO:67: IARACTE IARACTE IARACTE IARACTE SCRIPTIO A s p 5 A s p 5 A s p 5 L c u	N: SEQ Ile RISTICS amino a acid linear in DN: SEQ Ile Arg Arg	Il cids cids Gl g Va a Tr	57: n Met 1 Thr p Tyr 40	T b I 1 2 G 1	r Gln 10 e Thr n Gln	Ser Cys Lys	s Pro	Ser Ala Gly 45	Ser Ser 30	E Leu Ser 15 Gln Asy Ala Pro	e O
1 (2) Gly 1 Ala 11c Lys	(xi Gly INFORM (i (xi (xi Ser Ser Ser 50	) SEQUE Trp ATION FO ) SEQUE ( ( ( ) MOLEO ) SEQUE H i s V a l S e r 3 5 L e u	NCE DE S e r DR SEQ 1 ENCE CH A ) LEN B ) TYP D ) TOP CULE TY ENCE DE S e r G 1 y 2 0 T y r 1 1 e	SCRIPTIO C y s 5 ID NO:67: IARACTE KITH: 111 E: amino OLOGY: 1 YPE: prote SCRIPTIC A s p 5 , A s p 5 L c u s T y r	N: SEQ Ile RISTICS amino a acid linear in DN: SEQ Ile Arg Ass Tys	Ile cids IDNO: Gl gVa hTr rTh 5	57: n Met 1 Thr p Tyr 40 r Ser 5	Tb Ill2 GJ Ar	r Gln 10 e Thr 5 n Gln g Leu	Ser Cys Lys His	r Pro s Arg s Pro s Ses 6	Ala Ala Gly 45 Gly	Ser Ser 30 Lys Val	Leu Ser 15 Gln Asy	i E

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b r	L	c u	Р	1	D	Т 1	у п 0 (	5	т	h r		PI	h e		G	l y		G	n		G   1 (	) y	1.00	ГЬ	r	L	у	s	v	al		G	lu			1 e 1 0		Ly	's						
2)	INFC	RM	ATIC	ON I	POR	s	EQ	ID	NO	:68	•																																		
		( 1	) 51	(	A B C	))))))	LEN FYP	E:	RAC TH: nuc IDE	438 leic DN	ac ac	id S: 1	pair	rs																															
	¢	i x	) FE	(	A	)1			/KE																																				
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	()	t i	) SE	QUI	ENC	ЗE	DE	sc	RIP	пс	N:	SE	QI	D	NO	:68:																													
AG	ст	C	C A	с	c		vi e	G t 1	G	G 1	A y	T T	G r	G P	AS	G e	C r	T C	у	T s 5	A I	Т ( 1 с	2	A I	т (		CI Le	C U	I	ГТ	C e	I L	T c 1	u	G V	Ta	A 1	G A	с. 1	A a	A T	CA br			5
CT 1 a	A C T b	СА 1 Т 1 5	G G	G T I y		9 1 V 4		1	СА Ні	C s	1	С с	C 1	0	31	G n 0	1	GT /a	C 1	0	A 1	A n	C L	T c	G u	C G	AG 1 n		G 1	G u 2 5		A G S e	C r		3 G 3 I	T y	¢ F	с с	A 0	G G	G 1	T y			9
T T e u 3 0	G T V a	G 1	A A	G A r g	1	20	C T 0		A G S e	C T	0	A 1 3	n	A I	с с ь	C r	C I	C T	Gu	AS	¢	C 1	C L	T e	G u	Т	C C 6 r 4 0		тс су	C s	1	С Г b	r C	0	ΞT /a	G 1	л s	c e	T r	G G	G ( 1 ) 4 :	Y		1	4
A C y r	T C S e	A T	A I	ΓT le	1	с в	C T	1	Se	C r 0	G A	A . s	T P	C H	C A	T s	¢ A	с 1	C a	T T	G	G p	S	Gi c 5	r	T ( T	G G F P		G T V a	т 1		G	C g	0	A 1	G n	C P	C r 6	o	C P	C i	r >		1	9
GA 1y	C G A r	8 8	G ( G (	ЭТ 1 у	1	. c	T u 5		3 A 3 1	G u	T	G r	G P	A I	I T	T c	0	G 1	A y	T T	A y 7	I	A 1	T' I d	T c	A G	G T		ГА Гу	r	s	e	T r	G	10	у	A I	Т 1	C ¢	A T	C /	•		2 -	4
СС br	ТА Ту	T r	A :	A T B B B O	P	r	•	1	ГС Se	T r	C L	T c	C u	AL	A y	A s	ı s	с е 8	r	A	G 1	A 8	G V	T ( a l	9 1	A ( T )	CA r	N	A T A c	G t	C L	с 9	u	A	G r	A g	G A	A   \$ ]	C P	A T	C C h r			2 9	9 (
G C c r	Ly	G s 5	A A	A C	G	1	G n	J	ъ	c e	A S	G ¢	C r	L	T c O	u	A	G	A 8	C L	T e	C u	A S	G ( c 1	5	A C S e	GC I	1	5 T / a   0	1	A	с ь	A r	G A	с 1	C a	G A	C ( 1 4	C 1	G A	A C s p			33	3 8
C C h r 1 0	G C A 1	G a	G 1 V a	Т 1	T T	A y	T T	ן ד	r A T y	T r	C	G y 1	s	G A	с 1	A a	A A	G . r	A B	T S	C (	C r	C L	T A c u	4	G C A 1 1 2	а	C A	G	G g	A T	C b	T	A T	C b	G 1	G A	C 1	Г L	M	T C e t 2 5			38	8 (
AC sp	ТА Ту	C t	Т	P P	G G	G 1	T y	0	A	n	G G	<b>G</b> (	C y	A S	G e	C T	C L	T (	C 1	G V	T ( a l	L	TI	CA hr 35		G 1 V a	C 1	ז s	c e	C r	T S	с. ¢	A r	G	G	тс	A G	G	G	G.	A			43	3 5
сс																																												43	8
2)11	FOR	MAT	ION	FO	RS	EQ	2 ID	N	0:6	9:																																			
	(	i )	SEQ	( / ( E	4) 3)		NG PE:	11H : ar	CT 1: 13 ninc GY:	8 a	min id	10 4		b																															
	( i	i )	MOI																																										
	( x	i ):	SEQ	UEN	ICE	D	ESC	CRI	РП	ON	: S	EQ	D	N	0:6	9:																													
с т 1																	L	e u		PI	a e	1	Le	: u	,	/ a	1	A	1	a	т	hr		A	l a		т	а т		G 1	y				

V a 1								-conti	nued							
	Hìs	Ser	G 1 n 2 0	Val	Gln	Leu	Gln (	3 lu S 2 5	er G	Iу	Pro C	ily L	eu 1 30	Val	Arg	
Pro	Ser	Gln 35	Гһт	Leu	Seı	Leu	Thr 9 40	Cys T	hr V	a 1	Ser (	45	yr !	Ser	[ ] e	
Thr	Ser 50	Asp	His	Ala	Ттр	Ser 55	Тгр	Val A	rg G	1 n	Рто Р 60	10 C	ily	Arg	G 1 y	
Leu 65	Glu	Ттр	1 <b>] c</b>	Gly	Tyr 70	lle	Ser	Гут S	er G	1 y 7 5	[]e]	`br 1	br	Tyr	Asa 80	
Pro	Ser	Leu	Lys	Ser. 85	Arg	Val	Thrl	Met L	е <b>ц А</b> 90	гg	Asp 7	Chr S	er	Lys 95	Asn	
Gln	Phe	Ser	L e u 1 0 0	Arg	Leu	Ser	Set	Val T 105	br A	1 a	Ala	Asp 7	br. 10	Ala	Val	
Туг	T y r	Cys 115	Ala	Arg	Ser	Leu	Ala 120	Arg T	br T	b r	Ala M	Alet 2 125	sp	Тут	Тгр	
G 1 y	Gln 130	Gly	Ser	Leu	Val	T b r 135	V a l	Ser S	6 c r							
(2)[	NFORM/	TION FOR	t SEQ ID	NO:70:												
	( i	(A (B (C	) LENG ) TYPE: ) STRAI	RACTERI TH: 402 b : nucleic as NDEDNES LOGY: lin	ase pairs cid SS: single											
	( i x	) FEATUR	E: ) NAME	E/KEY: CI ITION: 12.	DS											
	a	( B	) NAMI ) LOCA	E/KEY: ma ATION: 12. CRIPTION												
AAG	14		ATC Mei		TGO	3 40	C TGT Cys 5	110	ATC []e	CT ( Leu	с ттс РЪс	TTG Leu 10	GTA Val	GC/ Ala	ACA Thr	5 0
GCT Ala	ACA Tbr 15	G G T G 1 y	GTC Val	CAC His	TCC Ser	GAC Asp 20	ATC Ile	CAG Gln 1	ATG A Met 7	ACC [b]	CAG Gln 25	A G C S e t	CCA Pro	AGC Ser	AGC Set	98
CTG Leu 30	100	GCC Ala	AGC Ser	G T G V a l	G G T G 1 y 3 5	GAC Asp	AGA Arg	GTG Val	ACC Thr	ATC 11 c 40	ACC Thr	TGT Cys	AGA Arg	GCC Ala	AGC Ser 45	146
	GAC					CTG								001	AAG	
Gln	Asp	ATC Ile	AGC Set	AGT Ser 50	ТАС Тут	Leu	Asn	Trp	TAC Tyr 55	CAG Gln	CAG Gln	AAG Lys	CCA Pro	G1 y 60	Lys	194
Gln	Asp	Ile	Ser	Ser	Тут	Leu	A s n	Ттр	Tyr 55	Gln	Gla	Lys	Рто	GIY 60 GGT	L y s GTG	
Gln GCT Ala	Asp CCA Pro	Ile AAG Lys	Ser CTG Leu 65	Ser 50	Tyr ATC 11e	Leu TAC Tyr AGC	Asn TAC Tyr GGT	Trp ACC Tbr 70 AGC	Tyr 55 TCC Ser GGT	GI n AGA Arg ACC	Gln CTG Leu GAC	Lys CAC His TTC	Pro TCT Ser 75 ACC	GIY 60 GGT GIY TTC	Lys GTG Val ACC	242
Gln GCT Ala CCA Pro	Asp CCA Pro AGC Ser	Ile AAG Lys AGA Arg 80 AGC Ser	Ser CTG Leu 65 TTC Pbe	Ser 50 CTG Leu	Tyr ATC 11c GGT G1y CCA	Leu TAC Tyr AGC Ser GAG	Asn TAC Tyr GGT G1y 85 GAC	Trp ACC Tbr 70 AGC Ser ATC	Tyr 55 TCC Ser GGT G1y GCT	GIN AGA Arg ACC Thr ACC	Gln CTG Leu GAC Asp TAC	Lys CAC His TTC Pbe 90 TAC	TCT Ser 75 ACC Thr TGC	GIY 60 GGT GIY TTC Phe CAA	GTG Val ACC Tbr CAG	2 4 2 2 9 0
Gln GCT Ala CCA Pro ATC Ile	Asp CCA Pro AGC Ser 95 AAC Asp	Ile AAG Lys AGA Arg 80 AGC Ser	Ser CTG Leu 65 TTC Pbe CTC Leu	Ser 50 CTG Leu AGC Ser CAG	Tyr ATC 11c GGT G1y CCA Pro TAC	Leu TAC Tyr AGC Ser GAG Glu 100 ACG Thr	Asn TAC Tyr GGT G1y 85 GAC Asp TTC	Trp ACC Tbr 70 AGC Ser ATC []]e GGC	Tyr SS TCC Scr GGT GIY GCT Ala CAA GIn	GIN AGA Arg ACC Thr ACC Thr GGG	Gla CTG Leu GAC Asp TAC Tyr 105 ACC	Lys CAC His TTC Phe 90 TAC Tyr AAG	Pro TCT Ser 75 ACC Thr TGC Cys GTG	Gly 60 GGT Gly TTC Pbe CAA Gln GAA	GTG Val ACC Tbr CAG Glp ATC	194 242 290 338 386

(2) INFORMATION FOR SEQ ID NO:71:

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	(i	(	A)LEN B)TYP	IARACTE IGTH: 126 E: amino a	amino ac acid											
	(11			'OLOGY: I												
				SCRIPTIO		D NO:71:										
Mer				C y s				Phe	Lcu	Val	Ala	Трт	Ala	The	GLV	
1				5					10					15		
			20					2 5					3 0			
		35		Arg			40					4 5				
Ser	Ser 50	Туг	Leu	Asn	Ттр	Туг 55	Gln	Gln	Lys	Рго	G 1 y 6 0	Lys	Ala	Рто	L y s	
L e u 6 5	Leu	lle	Туr	Туг	Tbr 70	Ser	Arg	Leu	His	Ser 75	Gly	V a l	Рго	Ser	Arg 80	
РЪс	Ser	G 1 y	Ser	G 1 y 8 5	Ser	Gly	Thr	Asp	Р h е 9 0	Thr	РЬе	Thr	Il e	Ser 95	Ser	
Lcu	Gln	Pro	G 1 u 1 0 0	A s p	[] e	Ala	Τbr	Tyr 105	Τyr	C y s	Gln	Gln	G 1 y 1 1 0	As n	Tbr	
Leu	Pro	Туг 115	Τbr	РЬс	G 1 y	Gln	G 1 y 1 2 0	ТЪт	L y s	V a l	Glu	11e 125	L y s			
(2)	NFORMA	TION FO	r seq II	D NO:72:												
	(i)	SEQUEN	CE CH	ARACTER	ISTICS:											
				GTH: 36 bi E: nucleic a												
		(0	) STRA	NDEDNE	SS: single	-										
	( <b>x</b> i)			CRIPTION		) NO:72:										
ΤΑΑΟ				сстви	12		GTGA	CG	AGGC							
(2)11	FORMA	TION FOR	R SEQ II	D NO:73:												
	(i)	SEQUEN	CE CHA	RACTER	ISTICS:											
		1.000		TTH: 32 be : nucleic a	2.2.2											
		( C	) STRA	NDEDNE	SS: single	E.										
	(xi)			CRIPTION		NO:71-										
ATCA				ATGGG			TGTA	тс								
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				: nucleic a												
				LOGY: lin												
	( <b>x</b> i)	SEQUEN	CE DES	CRIPTION	I: SEQ ID	NO:74:										
AATG	GATC	CA C	TCAC	COTTT	G AT	TTCC	ACCT									
(2) IN	FORMAT	ION FOR	SEQ ID	NO:75:												
	(i)			RACTERI												
				TH: 33 bas nucleic ad												
		( C	) STRA	NDEDNES	SS: single											

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93	94	
	-continued	
(D) TOPOLOGY: linear		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:75:		33
CATGCCTGGA GCIGGGIICG CCAGCCACCT	GGA	5 5
(2) INFORMATION FOR SEQ ID NO:76:		
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 33 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>		
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:76:		
TCCAGGTGGC TGGCGAACCC AGCTCCAGGC	ATG	3 3
(2) INFORMATION FOR SEQ ID NO:77:		
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 30 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:77:		
CAGCAGAAGC CAGGAAAGGC TCCAAAGCTG		3 0
(2) INFORMATION FOR SEQ ID NO:78:		
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 30 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:78:		
CAGCTTTGGA GCCTTTCCTG GCTTCTGCTG		3 0
(2) INFORMATION FOR SEQ ID NO:79:		
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 66 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>		
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:79:		
ACCTGTAGAG CCAGCAAGAG TGTTAGTACA	TCTGGCTATA GITATATGCA CTGGTACCAG	6 0
CAGAAG		66
(2) INFORMATION FOR SEQ ID NO:80:		
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 15 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>		
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:80:		15
GCTGGCTCTA CAGGT		
(2) INFORMATION FOR SEQ ID NO.81:		
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 48 base pairs</li> <li>( B ) TYPE: nucleic acid</li> </ul>		

( B ) TYPE: nucleic acid ( C ) STRANDEDNESS: single

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95	5,175,705	07	
	-continued	96	
(D) TOPOLOGY: linear			
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:81:			
AAGCTGCTGA TCTACCTTCC ATCCACCCTG	GAATCTGGTG	TGCCAAGC	48
2 ) INFORMATION FOR SEQ ID NO:82:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 15 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:82:			
TAGATCAGC AGCTT			15
2 ) INFORMATION FOR SEQ ID NO:83:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 48 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:83:			
CTACCTACT ACTGCCAGCA CAGTAGGGAG	ACCCCATACA	CGTTCGGC	48
2 ) INFORMATION FOR SEQ ID NO:84:			
( i ) SEQUENCE CHARACTERISTICS:			
<ul> <li>(A) LENGTH: 15 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:84:			
TGGCAGTAG GTAGC			15
2 ) INFORMATION FOR SEQ ID NO:85:			
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 414 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>			
( i x ) FEATURE: ( A ) NAME/KEY: CDS ( B ) LOCATION: 12,401			
( i x ) FEATURE: ( A ) NAME/KEY: mat_peptide ( B ) LOCATION: 12.401			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:85:			
AGCTTCCAC C ATG GGA TGG AGC TGT Met Gly Trp Ser Cys 1 5	ATC ATC CTC Ile Ile Leu	TTC TTG GTA GCA ACA Phe Leu Val Ala Thr 10	5 0
CT ACA GGT GTC CAC TCC GAC ATC C a Thr Gly Val His Ser Asp Ile G 15 20	AG ATG ACC In Met Thr		98
G AGC GCC AGC GTG GGT GAC AGA G u Ser Ala Ser Val Gly Asp Arg V 0 35	TG ACC ATC al Tbr Ile 40	ACC TGT AGA CCC 400	146
AG AGT GTT AGT ACA TCT GGC TAT A s Ser Val Ser Thr Ser Gly Tyr S 50	GT TAT ATG o cr Tyr Met 1 55		194

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140	CCA	GGA	AAG	GCT	CCA	AAG	CTG	CTG	ATC	TAC	CTT	GCA	тсс	AAC	CTG	2 4
Lys	Pro	GLy	Lys	Ala	Pro	Lys	Leu	Leu	Ile	Туг	Leu	Ala	Ser	Asn	Leu	
		2	6 5					70					75			
	тот	COT	GTG	CCA	AGC	AGA	TTC	AGC	GGT	AGC	GGT	AGC	GGT	ACC	GAC	29
Glu	Ser	Gly	Val	Pro	Ser	AIR	Phe	Ser	G 1 y	Ser	Gly	Ser	Gly	Thr	Asp	
5.55		8 0					8 5					90				
				ATC			CTC	C + G	CCA	646	GAC	ATC	GCT	ACC	TAC	33
TTC	ACC	Phe	Thr	I l e	Set	Ser	Leu	Gln	Pro	Glu	Asp	110	Ala	Thr	Tyr	
1 4 6	95					100	1.000				105					
				253522	0.02220	02002	100					000	C 4 4	000	100	38
TAC	TGC	CAG	CAC	AGT Ser	AGG	GAG	AAC	PTO	TAC	Thr	Phe	Glv	Gla	Gly	Thr	50
1 y r 1 1 0	Cys	011	піз	561	115	010		110	. , .	120		,			125	
202020																
AAG	GTG	GAA	ATC	AAA	CGT	GAGT	GGA	тсс								4 1
Lys	Val	Glu	[]e	Lys 130												
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	NEODM	ATION FO	OR SEO I	D NO:86:												
(2)																
	(1			ARACTE												
				GTH: 130 E: amino a		105										
				OLOGY:												
			0.000/000000													
	( i i	) MOLE	CULE TY	PE: prote	LN.											
	( x i	) SEQUE	ENCE DE	SCRIPTIC	N: SEQ	D NO:86										
				Cys				Pha	Lev	V a 1	A 1 a	Thr	A 1 a	Thr	Glv	
Met 1	GTY	1 r p	561	Cys 5		110	Leu	1 1 0	10					15		
														0 <u>1</u> .2001.12	20.203	
V a 1	His	Ser		Il e	Gln	Mct	Thr	Gln	Ser	Pro	Ser	Ser	Leu 30	Ser	Ala	
			2 0					2 5					50			
Ser	Val	Gly	Asp	Arg	Val	Thr	I 1 e	Thr	C y s	Атд	Ala	Ser	Lys	Ser	V a l	
		3 5					4 0					4 5				
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Ser	Th r 50		GIY	lyr	Sei	5 5	Met	nıs	119	1 9 1	60	014	2,3		0.)	
		Ριο	L y s	Lcu			Туг	Leu	Ala	Ser	Asn	Leu	Glu	Ser	G 1 y 8 0	
65					70					75					80	
Val	Рго	Ser	Агя	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	
				8 5					9 0	6				95		
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Thr	1 1 e	Ser	Ser 100	Leu	GIn	Pro	GIU	A S P	110	ATA	101	TÀI	110	0,3	011	
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His	Ser			a Asn	Pro	Туг	Thr	Pbe	Gly	Gln	Gly	Thr	Lys	V a l	Glu	
		115					120					1 2 5				
11.	Lys															
	130															
(2)	INFORM	ATION F	OR SEO	ID NO:87												
( - )	and only				ui											
	( i			ARACTE												
				VGTH: 45 PE: nucleio		5										
		(	10 1 1 11	Con minerest	e count											

(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GGTTATTCAT TCACTAGTTA TTACATACAC TGGGTTAGAC AGGCC

4 5

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid

- (C) STRANDEDNESS: single (D) TOPOLOGY: linear

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( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:88:					
AGTGAATGAA TAACCGCTAG CTTTACA				2 7	
(2) INFORMATION FOR SEQ ID NO:89:					
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 69 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>					
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:89:					
GAGIGGGTGG GCTATATIGA TCCTTTCAAT	GGTGGTACTA	GCTATAATCA	GAAGTTCAAG	60	
GGCAGGGTT				69	
(2) INFORMATION FOR SEQ ID NO:90:					
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 15 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>					
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:90:					
ATAGCCCACC CACTC				15	
(2) INFORMATION FOR SEQ ID NO:91:					
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 36 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>					
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:91:					
GGGGGTAACC GCTITGCTTA CTGGGGACAG	GGTACC			36	
(2) INFORMATION FOR SEQ ID NO.92:					
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 36 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>					
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO.92:					
AGCAAAGCGG TTACCCCCTC TGGCGCAGTA	GTAGAC			36	
(2) INFORMATION FOR SEQ ID NO:93:					
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 30 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>					
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:93:					
CAAGGTTACC ATGACCGTGG ACACCTCTAC				3 0	
(2) INFORMATION FOR SEQ ID NO:54:					
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 30 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>					

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-continued (i i ) SEQUENCE DESCRIPTION SEQ D NOSH: CACGOTCATG GTAACCTIGC CCTTGAACTT () () SEQUENCE CHAACTERETCS: () SEQUENCE CHAACTERETCS: () SEQUENCE CHAACTERETCS: () SEQUENCE CHAACTERETCS: () SEQUENCE DESCRIPTION SEQ D NOSH: () SEQUENCE DESCRIPTION SEQUENCES () SEQUENCE D	101						10	2		
ACCOTCATE GIAACCTIECE CETTOAACTI 12 JINGUMATION FOR SEQ ID NOSS: (1) SEQUENCE CHAACTERSTICS: (A) LENGTH & Margin (A) LENGTH & Margin (A) JINGUENCE CHAACTERSTICS: (A) JENGTH & Dama pair (A) JINGUENCE CHAACTERSTICS: (A) JENGTH & Dama pair (B) TYPE maker and (C) JINGUENCE CHAACTERSTICS: (A) JENGTH & Dama pair (B) TYPE maker and (C) JINGUENCE CHAACTERSTICS: (A) JENGTH & Dama pair (B) TYPE maker and (C) JINGUENCE DESCREPTION: SEQ D NOS9: (I) SEQUENCE CHAACTERSTICS: (A) JENGTH & DAMA PAIR (C) JINGUENCE DESCREPTION: SEQ D NOS9: (I) SEQUENCE DESCREPTION: SEQ D NOS9:	101	-conti	nued							
ACCOTCATG GTAACCTTUCE CETTURACTI ACCOTCATG GTAACCTTUCE CETTURACTI (1) SEQUENCE CHAACTERISTICS: (1) SEQUENCE CHAACTERISTICS: (1) TONOLOOV: News (1) TONOLOV: News (1) SEQUENCE CHAACTERISTICS: (1) SEQUENCE DESCRIPTION: SEQ D NOSP: (1) SEQUENCE DESCRIPTION: SEQ D NOSP: (2) TONOLOOV: NEWS (3) LOCATOR: NEWS (3) LOCATOR: NEWS (3) LOCATOR: NEWS (4) NAMERET SEC SCONCE SEQUENCE DESCRIPTION: SEQ D NOSP: (3) LOCATOR: NEWS	(x i) SEQUENCE DESCRIPTION: SEQ ID NO:94:									
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LENGTH 30 Note Star (C) STRANEDENSES imple (D) TOPOLOGY Liner (i) SEQUENCE DESCRIPTION: SEQ D NOSE: 300 (1) DEFORMATION FOR SEQ D NOSE: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH 30 Note and (C) STRANEDENSES imple (C) D TOPOLOGY Liner (i) SEQUENCE DESCRIPTION: SEQ D NOSE: ADGATCAATA TAGCCAATCC ATTCGAGCCC (2) INFORMATION FOR SEQ D NOSE: (i) SEQUENCE DESCRIPTION: SEQ D NOSE: (ii) SEQUENCE DESCRIPTION: SEQ D NOSE: (i</pre>	CACGGTCATG GTAACCTTGC CCTTGAACTT									3 0
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LENGTH 30 Note Star (C) STRANEDENSES imple (D) TOPOLOGY Liner (i) SEQUENCE DESCRIPTION: SEQ D NOSE: 300 (1) DEFORMATION FOR SEQ D NOSE: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH 30 Note and (C) STRANEDENSES imple (C) D TOPOLOGY Liner (i) SEQUENCE DESCRIPTION: SEQ D NOSE: ADGATCAATA TAGCCAATCC ATTCGAGCCC (2) INFORMATION FOR SEQ D NOSE: (i) SEQUENCE DESCRIPTION: SEQ D NOSE: (ii) SEQUENCE DESCRIPTION: SEQ D NOSE: (i</pre>	A NUMBER ATTION FOR SEC ID NO.95									
( A ) LENGTH: 50 Most pån ( A ) LENGTH: 50 Most pån ( C ) TTWE mode said ( C ) TTWE mode said ( C ) STRANEDNESS: imge ( I ) SEQUENCE DESCRIPTION: SEQ D NO%: ( I ) SEQUENCE DESCRIPTION: SEQ D NO%? ( I ) SEQUENCE DESCRIPTION: SEQ D NO%? ( I ) SEQUENCE DESCRIPTION: SEQ D NO%? ( I ) SEQUENCE DESCRIPTION: SEQ D NO%? ACCOCT C C C C C C O C O C C O C O C C O C O										
<pre>( c) STRANEDENESS: might ( c) JIOPCOOVE Have ( c) JIOPCOOVE HAVE</pre>										
<pre>(i) ) SEQUENCE DESCRUTION: SEQ D NO98: DEGOCICCOAAT GGATTGGCTA TATTGATCCT 3: () ) SEQUENCE CHARACTERISTICS: () ) SEQUENCE CHARACTERISTICS: () ) SEQUENCE DESCRUTION: SEQ D NO98: () ) DOPOLODY: Hear () ) SEQUENCE DESCRUTION: SEQ D NO99: () ) SEQUENCE DESCRUTION: SEQ D NO97: () ) DOPOLODY: Hear () ) DOPOLODY: Hear ()</pre>										
<pre>(i) SEQUENCE DESCRIPTION: SEQ ID NOSE: DEGETECGAAT GGATTGGCTA TATTGATECT 30 (2) INFORMATION FOR SEQ ID NOSE: (i) SEQUENCE CHARACTERISTICS: (A) LENTH: DO BE part (c) STRUNDEUNSS: might (c) STRUNDEUNSS: might (c) D TOPOLODY: Here (c) D TOPOLODY: Here (</pre>										
DEGUCTECGAAT GGATTOGETA TATIGATECT     3.0       (1) DEGUAATO FOR SEQ ID NOSS:     (1) SEQUENCE CHARACTERSTEST:     (1) SEQUENCE DESCRIPTION SEQ ID NOSS:       (1) DI TYPE INNER     (1) SEQUENCE DESCRIPTION SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (3) IDECCIDENCES: Index add     1.0       (2) INFORMATION FOR SEQ ID NOSS:     3.0       (1) SEQUENCE DESCRIPTION: SEQ ID NOSS:     3.0 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>										
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LEMOTE: 30 base pairs (B) TYPE: models and (C) STRANDELMESS: single (D) D) TOPOLOGY: Hear (i) D) TOPOLOGY: Hear (i) SEQUENCE DESCRIPTION: SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENOTE: Ho base pairs (c) TYPE: models and (C) STRANDEDMESS: indule (C) STRANDESS INDULE (C) STRANDE</pre>	GGGCTCGAAT GGATTGGCTA TATTGATCCT	5								3 0
<pre>(A) LENGTH: 50 base pairs (B) TTPE: unders add (C) STRANDEDNESS: single (D) TOPOLOOY: Inset (1) SEQUENCE DESCRIPTION: SEQ ID NO:90: (1) SEQUENCE CHARACTERISTICS: (1) SEQUENCE CHARACTERISTICS: (2) INFORMATION FOR SEQ ID NO:97: (2) INFORMATION FOR SEQ ID NO:97: (3) SEQUENCE DESCRIPTION: SEQ D NO:97: (3) SEQUENCE DESCRIPTION: SEQ D NO:97: (3) SEQUENCE DESCRIPTION: SEQ D NO:97: (4) LENGTH: 70 base pairs (5) STANDEDNESS: single (5) STANDEDNESS: single (6) TTPE: undeix add (2) INFORMATION FOR SEQ ID NO:98: (3) SEQUENCE DESCRIPTION: SEQ D NO:97: (4) SEQUENCE DESCRIPTION: SEQ D NO:97: (5) SEQUENCE DESCRIPTION: SEQ D NO:97: (5) SEQUENCE DESCRIPTION: SEQ D NO:97: (6) STANDEDNESS: single (7) TOPOLOOY: Inset (1) SEQUENCE DESCRIPTION: SEQ D NO:99: (1) SEQUE</pre>	(2) INFORMATION FOR SEQ ID NO:96:									
<pre>( B) TTPE: mode: said ( ) TTPE: mode: said ( ) TOPOLOGY: inset ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:96: ( x i ) SEQUENCE CHARACTERISTICS:</pre>	( i ) SEQUENCE CHARACTERISTICS:									
<pre>( c) STRANDEDNESS: image ( i i ) SEQUENCE DESCRIPTION: SEQ ID NO.95: AGGATCAATA TAGCCAATCC ATTCGAGGCC 3( ( ) INFORMATION FOR SEQ ID NO.97:     ( i ) SEQUENCE CHARACTERISTICS:         ( i ) SEQUENCE CHARACTERISTICS:         ( c) ITANDEDNESS: image         ( c) ITANDEDNESS: image</pre>										
<pre>( D ) TOPOLOGY: Bases ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO.99: AGGATCAATA TAGCCAATCC ATTCOAGCCC 30 ( 2 ) INFORMATION FOR SEQ ID NO.97: ( i ) SEQUENCE CHARACTERISTICS:</pre>										
AGGATCAATA TAGCCAATCC ATTCGAGCCC 30 (1)INFORMATION FOR SEQ ID NO97: (i)SEQUENCE CHARACTERISTICS: (B)TYPE: mucleic add (C)STANDEDNESS: imgle (D)TOFOLOGY: linear (xi)SEQUENCE DESCRIPTION: SEQ ID NO97: GTAAAACGAG GCCAGT 11 (2)INFORMATION FOR SEQ ID NO98: (i)SEQUENCE CHARACTERISTICS: (A)LENGTH: IT base pairs (C)STANDEDNESS: imgle (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (A)LENGTH: 43 base pairs (B)TYPE: mucleic add (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (B)TYPE: mucleic add (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (B)TYPE: mucleic add (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (B)TYPE: mucleic add (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (B)TYPE: mucleic add (C)STANDEDNESS: imgle (I)SEQUENCE CHARACTERISTICS: (A)LENGTH: 43 base pairs (B)TYPE: mucleic add (C)STANDENESS: imgle (I)SEQUENCE CHARACTERISTICS: (A)LENGTH: 540 base pairs (B)TYPE: mucleic add (C)STANDENESS: imgle (I)SEQUENCE CHARACTERISTICS: (A)LENGTH: 540 base pairs (I)SEQUENCE DESCRIPTION: 540 base pairs (I)SEQUENCE DESCRIPTION: 540 base pairs (I)SEQUENCE DESCRIPTION: 540 base pairs (I)SEQUENCE DESCRIPTIO										
<pre>(1 ) INFORMATION FOR SEQ ID NO 97: (1 ) SEQUENCE CHARACTERISTICS: (A ) LENGTH: 16 has puis (B ) TYPE: incluid said (C ) STRANDEDNESS: single (D ) TOPOLOGY: linear (x i ) SEQUENCE DESCRIPTION: SEQ ID NO 97: GTAAAACGAG GCCAGT (1) (2 ) INFORMATION FOR SEQ ID NO 98: (A ) SEQUENCE CHARACTERISTICS: (A ) LENGTH: 17 has puin (B ) TYPE: models and (C ) STRANDEDNESS: ingle (C ) STRANEDNESS (S ) LEXCATOR : IS AND SAUCTOR : IS</pre>	( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:96:									
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 16 base pairs (B) TYPE: melcic acid (C) STEANDEDNESS: single (D) TOPOLOGY: lines (x i) SEQUENCE DESCRIPTION: SEQ ID NO-97: GTAAAACGAG GCCAGT 11 (2) INFORMATION FOR SEQ ID NO-98: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (C) STEANDEDNESS: single (C) STEANDEDNESS: single (C) TOPOLOGY: lines (x i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (x i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (x i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (x i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (C) STEANDEDNESS: single (C) STEANDENESS: single (C) STEANDE</pre>	AGGATCAATA TAGCCAATCC ATTCGAGCCC	2								3 0
<pre>(A ) LENGTH: 16 base pairs (B ) TYPE: meloic acid (C ) STRANDEDNESS: single (D ) TOPOLOGY: linear (x i ) SEQUENCE DESCRIPTION: SEQ ID NO97: GTAAAACGAG GCCAGT 11 (2 ) INFORMATION FOR SEQ ID NO98: (i ) SEQUENCE CHARACTERISTICS: (A ) LENGTH: 17 base pairs (B ) TYPE: meloic acid (C ) STRANDEDNESS: single (D ) TOPOLOGY: linear (x i ) SEQUENCE DESCRIPTION: SEQ ID NO98: AACAGCT AT G ACCATGA 1 (2 ) INFORMATION FOR SEQ ID NO99: (i ) SEQUENCE CHARACTERISTICS: (A ) LENGTH: 433 base pairs (B ) TYPE: meloic acid (C ) STRANDEDNESS: single (C ) STRANDEDNESS: single (D ) TOPOLOGY: linear (i x ) FEATURE: (A ) INAMERKET: CDS (B ) LOCATION: 16.420 (i x ) FEATURE: (A ) NAMERKET: CDS (B ) LOCATION: 16.420 (i x ) SEQUENCE DESCRIPTION: SEQ ID NO99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC MC t A: p Trp Thr Trp Arg Val Pho Cy: Low Low Ala</pre>	(2) INFORMATION FOR SEQ ID NO:97:									
( B ) TYPE: making and ( C ) STRANDEDNESS: imple ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO99: GTAAAACGAG OCCAGT 11 ( 2 ) INFORMATION FOR SEQ ID NO99: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENDTH: 17 bare pints ( B ) TYPE: making and ( C ) STRANDEDNESS: imple ( D ) TOPOLOGY: Inset ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO99: ( i ) SEQUENCE DESCRIPTION: SEQ ID NO99: ( i ) SEQUENCE DESCRIPTION: SEQ ID NO99: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENDTH: 17 base pints ( A ) LENDTH: 17 base pints ( B ) TYPE: making and ( C ) STRANDEDNESS: imple ( I ) SEQUENCE CHARACTERISTICS: ( A ) LENDTH: 43 base pints ( B ) TYPE: making and ( C ) STRANDENSES; imple ( D ) TOPOLOGY: Inset ( A ) NAME/REY: CDS ( A ) NAME/REY: CDS ( B ) LOCATION: 16.420 ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO99: AAGGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TIT TGC CTG CTC GCC M G t A SP TTP Thr TTP ATB VAI P be CYS Lee Lee Alla	( i ) SEQUENCE CHARACTERISTICS:									
<pre>( c) STRANDEDNESS: imgle ( b) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO-97: GTAAAACGAG GCCAGT 11 ( 2 ) INFORMATION FOR SEQ ID NO-98: ( A ) LENGTH: (7 base pairs ( B ) TYPE: making and ( C ) STRANDEDNESS: imgle ( C ) STRANDEDNESS: imgle ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO-98: AACAGCTATG ACCATGA 1 ( 2 ) INFORMATION FOR SEQ ID NO-99: ( A ) LENGTH: 43 base pairs ( B ) TYPE: making and ( C ) STRANDEDNESS: imgle ( C ) STRANDEDNESS: imgle ( D ) TOPOLOGY: linear ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 43 base pairs ( B ) TYPE: making and ( C ) STRANDEDNESS: imgle ( D ) TOPOLOGY: linear ( i x ) FEATURE: ( A ) NAME/KEY: CDS ( B ) LOCATION: 16.420 ( i x ) FEATURE: ( A ) NAME/KEY: CDS ( B ) LOCATION: 16.420 ( i x ) SEQUENCE DESCRIPTION: SEQ ID NO-99: AAGCTIGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TIT TGC CTG CTC GCC Met A is p Tr p Th t Tr p Art g Val Phe Cys Lee Lee Alla</pre>										
(D) TOPOLOGY: Baser (x i ) SEQUENCE DESCRIPTION: SEQ ID NO97: GTAAAACGAG GCCAGT II (2) INFORMATION FOR SEQ ID NO99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (C) STRANDEDNESS: single (C) STRANDEDNESS: single (X i) SEQUENCE DESCRIPTION: SEQ ID NO99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TTPE: moleic add (C) STRANDEDNESS: single (D) TOPOLOGY: Baser (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TTPE: moleic add (C) STRANDEDNESS: single (D) TOPOLOGY: Baser (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TTPE: moleic add (C) STRANDEDNESS: single (D) TOPOLOGY: Baser (i) SEQUENCE CHARACTERISTICS: (A) NAME/KEY: CDS (B) DCCATION: 16.420 (i) NAME/KEY: max_peptide (B) LOCATION: 16.420 (x i) SEQUENCE DESCRIPTION: SEQ ID NO99: AAGGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC MC t A: p Tr p Th t Tr p Art g Val Pho Cy; Loo Loo Alla										
GTAAAACGAG GCCAGT       11         (2)INFORMATION FOR SEQ ID N0:98:       (i)SEQUENCE CHARACTERISTICS:         (A)LENOTH: 17 base pairs       (B)TYPE: muches add         (C)STRANDEDNESS: single       (D) TOFOLOGY: Inser         (xi)SEQUENCE DESCRIPTION: SEQ ID N0:98:       (ACCAGCTATG ACCATGA         (2)INFORMATION FOR SEQ ID N0:99:       (ACCAGCTATG ACCATGA         (2)INFORMATION FOR SEQ ID N0:99:       (i)SEQUENCE CHARACTERISTICS:         (a)LENOTH: 435 base pairs       (b)TYPE: muches add         (c)STRANDEDNESS: single       (C)STRANDEDNESS: single         (c)STRANDEDNESS: single       (C)STRANDEDNESS: single         (d)DOPOLOGY: linear       (c)STRANDEDNESS: single         (c)STRANDENCESS: single       (C)DOPOLOGY: linear         (ix)FEATURE:       (A)NAMEREY: CDS         (ix)FEATURE:       (A)NAMEREY: cDS         (ix)SEQUENCE DESCRIPTION: 56.420       (ix)SEQUENCE DESCRIPTION: 56.420         (ixi)SEQUENCE DESCRIPTION: 56.420       (xi)SEQUENCE DESCRIPTION: 56.420         (xi)SEQUENCE DESCRIPTION: 56.420       (xi)SEQUENCE DESCRIPTION: 56.420										
(2) INFORMATION FOR SEQ ID NO:98: (i) SEQUENCE CHARACTERISTICS: (A) LENOTH: (7 base pairs (B) TYPE: muchic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACCAG CTATG ACCATGA (2) INFORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENOTH: 435 base pairs (B) TYPE: muchic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTT GCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC MCT ASP TTP ThY TTP ATB Val Pho Cys Low Low Ala	( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:97:									
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 base pairs (B) TYPE: muchics acid (C) D) TOPOLOGY: linear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACCAGCTATG ACCATGA (2) INFORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 435 base pairs (B) TYPE: muchics acid (C) STRANDEDHESS: single (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/REY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/REY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTIGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TIT TGC CTG CTC GCC Met Asp Trp Tbr Trp Arg Val Pbe Cys Leu Leu Ala</pre>	GTAAAACGAG GCCAGT									1 6
<pre>(A) LENGTH: 17 base pairs (B) TYPE: mucleic acid (C) STRANDEUNESS: single (D) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACCAGCTATG ACCATGA (2) INFORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TYPE: mucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (x i) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Lev Lev Ala</pre>	(2) INFORMATION FOR SEQ ID NO.98:									
(B) TYPE: nucleic axid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACAGCTATG ACCATGA (2) INPORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TYPE: nucleic axid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ix) FFATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FFATURE: (A) NAME/KEY: mai_pepide (B) LOCATION: 16.420 (x i) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Tbr Trp Arg Val Pbe Cys Leu Leu Ala	( i ) SEQUENCE CHARACTERISTICS:									
(C) STRANDEDNESS: ingle (D) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACAGCTATG ACCATGA I (2) INFORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: ingle (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_pepide (B) LOCATION: 16.420 (x i) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Tbr Trp Arg Val Pbe Cys Leu Leu Ala										
(D) TOPOLOGY: Inear (xi) SEQUENCE DESCRIPTION: SEQ ID NO:98: AACAGCTATG ACCATGA [] (2) INFORMATION FOR SEQ ID NO:99: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: Inear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_pepide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Lev Ala										
AACAGCTATG ACCATGA 1 (2)INFORMATION FOR SEQ ID NO:99: (i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 433 base pairs (B)TYPE: nucleic acid (C)STRANDEDNESS: single (D)TOPOLOGY: linear (ix)FEATURE: (A)NAME/KEY: CDS (B)LOCATION: 16.420 (ix)FEATURE: (A)NAME/KEY: mail_peptide (B)LOCATION: 16.420 (xi)SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
<pre>AACAGCTATG ACCATOA (2)INFORMATION FOR SEQ ID NO:99:     (i)SEQUENCE CHARACTERISTICS:         (A)LENGTH: 433 base pairs         (B)TYPE: nucleic acid         (C)STRANDEDNESS: single         (D)TOPOLOGY: linear         (ix)FEATURE:         (A)NAME/KEY: CDS         (B)LOCATION: 16.420         (ix)FEATURE:         (A)NAME/KEY: mat_pepide         (B)LOCATION: 16.420         (xi)SEQUENCE DESCRIPTION: SEQ ID NO:99:         AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5         Mot Asp Trp Tht Trp Atg Val Pho Cys Lou Lou Ala</pre>	( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:98:									
<pre>(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 433 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (i x) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (i x) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (x i) SEQUENCE DESCRIPTION: SEQ ID NO:59: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Mct Asp Trp Tht Trp Atg Val Phe Cys Leu Leu Ala</pre>	AACAGCTATG ACCATGA									1
<ul> <li>(A) LENGTH: 433 base pairs</li> <li>(B) TTYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul> (ix) FEATURE: <ul> <li>(A) NAME/KEY: CDS</li> <li>(B) LOCATION: 16.420</li> </ul> (ix) FEATURE: <ul> <li>(A) NAME/KEY: mat_pepide</li> <li>(B) LOCATION: 16.420</li> </ul> (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 <ul> <li>Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala</li> </ul>	(2) INFORMATION FOR SEQ ID NO.99:									
(B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
(C) STRANDEDNESS: single (D) TOPOLOGY: linear (ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
(ix) FEATURE: (A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_pepide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
(A) NAME/KEY: CDS (B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala	(D) TOPOLOGY: linear									
(B) LOCATION: 16.420 (ix) FEATURE: (A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:59: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala	( i x ) FEATURE:									
(A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Tbr Trp Arg Val Pbe Cys Leu Leu Ala										
(A) NAME/KEY: mat_peptide (B) LOCATION: 16.420 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:99: AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Tbr Trp Arg Val Pbe Cys Leu Leu Ala										
AAGCTTGCCG CCACC ATG GAC TGG ACC TGG CGC GTG TTT TGC CTG CTC GCC 5 Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
Met Asp Trp Thr Trp Arg Val Phe Cys Leu Leu Ala										
	AAGCTTGCCG CCACC ATG GAC TGG AC	C TGG	CGC	GTG	TTT	TGC	CTG	стс	GCC	5
				val	рре	Cys	10	Lea	A I A	

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GTG Val	GCT Ala	ССТ Рго 15	Gly	G C C A l a	CAC His	AGC Ser	CAG G1 n 2 0	GTG Val	C A A G l n	C T A L e u	GTG Val	CAG G1n 25	TCC Ser	6 G C G 1 y	GCC Ala	99
G A A G l u	G T G V a 1 3 0	AAG Lys	AAA Lys	CCC Pro	G G T G 1 y	G C T A 1 a 3 5	TCC Ser	GTG Val	A A A L y s	GTC Val	AGC Ser 40	TGT Cys	AAA Lys	GCT Ala	AGC Ser	147
G G T G I y 4 5	Т <b>А</b> Т Т у г	TCA Ser	TTC Pbe	ACT Tbr	AGT Ser 50	T A T T y r	Т А С Т у т	ATA Ile	CAC His	TGG Trp 55	GTT Val	AGA Aig	CAG GIn	GCC Ala	С С А Р г о 6 0	195
3 G C 3 1 y	CAA Gln	G G G G 1 y	C T C L e u	G A G G 1 u 6 5	TGG Trp	GTG Val	GGC Gly	T A T T y r	ATT [le 70	GAT Asp	CCT Pro	TTC Phe	AAT Asn	G G T G 1 y 7 5	GGT Gly	243
АСТ ГЪт	AGC Ser	T A T T y r	AAT Asn 80	CAG Gln	AAG Lys	TTC Pbe	AAG Lys	G G C G 1 y 8 5	AAG Lys	GTT Val	ACC Thr	ATG Met	A C C T h r 9 0	GTG Val	GAC Asp	291
ACC Fbr	T C T S e r	A C A T b r 9 5	AAC Asn	ACC Tbr	GCC Ala	Т А С Т у т	A T G M e t 1 0 0	G A A G l u	C T G L e u	TCC Ser	AGC Ser	C T G L e u 1 0 5	CGC Arg	ТСС S е т	GAG Glu	339
GAC Asp	A C T T b r 1 1 0	GCA Ala	TGC Cys	ТАС Тут	T A C T y r	Т G C С у s 1 1 5	GCC Ala	AGA Arg	GGG Gly	G G T G 1 y	AAC Asn 120	CGC Arg	ТТТ РЬе	GCT Ala	T A C T y r	387
GG 7 p 1 2 5	GGA Gly	CAG Gln	GGT Gly	ACC Thr	C T T L e u 1 3 0	GTC Val	ACC Thr	GTC Val	AGT Ser	T C A S e r 1 3 5	GGTC	GAGTO	GA '	rcc		433
	( , )	SEQUE														
		( 1	A) LENG B) TYPE: D) TOPO	TH: 135 anino as	bic	İs										
	(ii)	(H (I	A) LENG B) TYPE:	TH: 135 a annino as LOGY: lin	amino acia id near	is										
		( I ( I MOLEC	A) LENG B) TYPE: D) TOPO	TH: 135 a annino ao LOGY: liu E: protein	amino acia hid near											
I	(xi) Asp	(E (I MOLEC SEQUEN Trp	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r	TH: 135 a annino as LOGY: lin E: protein CRIPTION T r p 5	amino acio near N: SEQ ID A 1 8	• NO:100: V a 1	РЬе		10					1 5		
l	(xi) Asp His	(E (I MOLEC SEQUEN Trp Ser	A) LENG B) TYPE D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0	TH: 135 / armino as LOGY: liu E: protein CRIPTIOP T r p 5 V a 1	amino acid near N: SEQ ID A r g G 1 n	→ NO:100: Val Leu	Phe Val	Gln 25	10 Ser	Gly	Ala	Glu	Val 30	15 Lys	Ly s	
l la ro	(xi) Asp His Gly	(E (I MOLEC SEQUEN Trp Ser Ala 35	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0 S c r	TH: 135 a amino as LOGY: hi E: protein CRIPTION Trp 5 V a 1 V a 1	amino acia hear N:SEQID Arg Gln Lys	) NO:100: Val Leu Val	Phe Val Ser 40	Gln 25 Cys	10 Ser Lys	G 1 y A 1 a	Ala Ser	G l u G l y 4 5	Val 30 Tyr	15 Lys Ser	Lys Phe	
l la ro	(xi) Asp His Gly	(E (I MOLEC SEQUEN Trp Ser Ala 35	A) LENG B) TYPE D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0	TH: 135 a amino as LOGY: hi E: protein CRIPTION Trp 5 V a 1 V a 1	amino acia hear N:SEQID Arg Gln Lys	) NO:100: Val Leu Val	Phe Val Ser 40	Gln 25 Cys	10 Ser Lys	G 1 y A 1 a	Ala Ser	G l u G l y 4 5	Val 30 Tyr	15 Lys Ser	Lys Phe	
1 a 7 o h 7 1 u 6 5	(xi) Asp His Gly Ser 50 Trp	( E ( I MOLEC SEQUEN Trp Ser Ala 35 Tyr Val	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0 S c r T y r G 1 y	TH: 135 a amino as LOGY: hi E: protein Trp 5 V a 1 V a 1 I 1 c Tyr	amino acia near N:SEQ ID Arg Gln Lys His Ile 70	Val Leu Val Trp 55 Asp	Phe Val Ser 40 Val Pro	Gln 25 Cys Arg Phe	10 Ser Lys Gln Asn	G 1 y A 1 a A 1 a G 1 y 7 5	Ala Ser Pro 60 Gly	Glu Gly 45 Gly Thr	Val 30 Tyr Gln Ser	15 Lys Ser Gly Tyr	Lys Phe Leu Asn 80	
1 1 a 7 o h r 1 u 6 5 1 n	(xi) Asp His Gly Ser 50 Trp Lys	( E ( I MOLEC SEQUEN Trp Ser Ala 35 Tyr Val Phe	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r G 1 m 2 0 S c r T y r G 1 y L y s	TH: 135 a annino ac LOGY: hi E: protein Trp 5 Val Val 11 e Tyr G 1 y 8 5	amino acid near N:SEQID Arg Gln Lys His 1 le 70 Lys	Val Leu Val Trp 55 Asp Val	Phe Val Ser 40 Val Pro Thr	Gln 25 Cys Arg Phe Met	10 Ser Lys Gln Asn Thr 90	Gly Ala Ala Gly 75 Val	Ala Ser Pro 60 Gly Asp	Glu Gly 45 Gly Thr Thr	Val 30 Tyr Gln Ser Ser	15 Lys Ser Gly Tyr Thr 95	Lys Phe Leu Asn 80 Asn	
1 70 hr 10 65 1 n hr	(xi) Asp His Gly Ser 50 Trp Lys Ala	( E ( I MOLEC SEQUEN Trp Ser Ala 35 Tyr Val Phe Tyr	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0 S c r T y r G 1 y L y s Mc t 1 0 0	TH: 135 a amino a LOGY: hi E: protein Trp 5 Val Val 11e Tyr Gly 85 Glu	amino acid near N: SEQ III Arg Gln Lys His 1 le 70 Lys Leu	Val Leu Val Trp 55 Asp Val Ser	Phe Val Ser 40 Val Pro Thr Ser	Gln 25 Cys Arg Phe Met Leu 105	10 Ser Lys Gln Asn Thr 90 Arg	Gly Ala Ala Gly 75 Val Ser	Ala Ser Pro 60 Gly Asp Glu	Glu Gly 45 Gly Thr Thr Asp	Val 30 Tyr Glm Ser Ser Thr 110	15 Lys Ser Gly Tyr Thr 95 Ala	Lys Phe Leu Asn 80 Asn Cys	
i i i i i i i i i i i i i i i i i i i	(xi) Asp His Gly Ser 50 Trp Lys Ala Tyr	( E ( I MOLEC SEQUEN Trp Ser Ala 35 Tyr Val Phe Tyr Cys 115	A) LENG B) TYPE: D) TOPO ULE TYP NCE DESC T b r G 1 n 2 0 S c r T y r G 1 y L y s Mc t	TH: 135 a annino as LOGY: hi E: protein Trp 5 Val Val 1 1 e Tyr G 1 y 8 5 G 1 u Arg	amino acid near N:SEQID Arg Gln Lys His Ile 70 Lys Leu Gly	Vo:100: Val Leu Val Trp 55 Asp Val Ser Gly	Phe Val Ser 40 Val Pro Thr Ser	Gln 25 Cys Arg Phe Met Leu 105	10 Ser Lys Gln Asn Thr 90 Arg	Gly Ala Ala Gly 75 Val Ser	Ala Ser Pro 60 Gly Asp Glu Tyr	Glu Gly 45 Gly Thr Thr Asp	Val 30 Tyr Glm Ser Ser Thr 110	15 Lys Ser Gly Tyr Thr 95 Ala	Lys Phe Leu Asn 80 Asn Cys	

(2) INFORMATION FOR SEQ ID NO:101:

( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 433 base pairs ( B ) TYPE: nucleic acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear

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			A ) NAME B ) LOCAT												
	2010/01/2012		ENCE DESC												
AAG	сттб	ССG	C C A C C	ATG Met 1	Asp	т G G Т т р	АСС ТЬг	TGG Trp 5	Arg	GTG Val	5 ТТТ РЪс	TGC CT Cys Le 1	G CTC u Leu 0	GCC Ala	5 1
GTG Val	G C T A l a	ССТ Рго 15	G 1 y	GCC Ala	CAC His	AGC Ser	C A G G 1 a 2 0	GTG Val	C A A G 1 n	C T A L e u	GTG C Val G	AG TCC 1 a Ser 25	G G C G 1 y	GCC Ala	99
G A A G l u	GTG Val 30	Lys	AAA Lys	CCC Pro	G G Т G 1 у	G C T A 1 a 3 5	TCC Ser	GTG Val	AAA Lys	GTC Val	AGC T Set C 40	GT AAA Ys Lys	GCT Ala	AGC Set	147
G G T G 1 y 4 5	TAT Tyr	TCA Ser	ТТС РЬс	ACT Thr	AGT Ser 50	T A T T y r	T A C T y r	АТА 11с	CAC His	TGG Trp 55	GTT A Val A	GA CAG rg Gln	GCC Ala	ССА Рто 60	195
6 G C 6 1 y	CAA Glm	G G G G L S	GCTC Leu	G A A G 1 u 6 5	TGG Trp	A T T 1 1 c	G G C G 1 у	T A T T y r	ATT 11e 70	GAT Asp	CCT 7 Pro F	TTC AAD Phe Asn	GGT G1y 75	GGT Gly	2 4 3
ACT Tbr	A G C S e r	TAT Typ	FAAT Asn 80	CAG Gln	AAG Lys	ТТС РЬе	AAG Lys	G G C G 1 y 8 5	AAG Lys	GTT Val	ACC / Thr M	ATG ACC Act Thr 90	Val	GAC Asp	291
ACC Thi	T C T S e 1	Г АС / Т Б   9	r Asn	ACC Thr	GCC Ala	ТАС Тут	A T G M e t 1 0 0	G A A G 1 u	C T G L e u	TCC Ser	Ser 1	CTG CGC Leu Arg 105	C TCC Ser	GAG Glu	339
GAC Asp	A C 1 T b 1 1 1 C	AL.	A GTC a Val	T A C T y r	T A C T y r	т G C С у s 1 1 5	GCC Ala	AGA Arg	0 0 0 G 1 y	G G T G l y	AAC ( Asn / 120	CGC TT Arg Pbo	GCT Ala	ТАС Туг	387
T G G T r p 1 2 5	G1 ;	A CAG	G GGT n Gly	ACC Thr	C T T L e u 1 3 0	GTC Val	ACC Thr	GTC Val	AGT Ser	T C A S e r 1 3 5	GGTG	AGTGGA	тсс		433
(2)	INFORM	NOITAN	FOR SEQ [	D NO:102											
	(		JENCE CHL (A)LENG (B)TYPE (D)TOPO	GTH: 135 E: amino a	amino aci cid	ids									
	( i	i) MOL	ECULE TY	PE: protei	п										
	( *	i) SEQ	UENCE DE	SCRIPTIC	N: SEQ I	D NO:102	2:								
Me t				5					10			Val Al	15		
Al a	Hi	s Se	r Gla 20		Gla	Leu	Val	Gln 25	Ser	Gly	Ala	Glu Va 3	1 Lys 0	Lys	
		3	5				4 0					G1y Ty 45			
	5	0				55					60	Gly Gl			
6 :	5				70					75		Thr Se		80	
				8 5					90			Thr Se	95		
Tb	r Al	а Ту	r M.e.t 100		Leu	Ser	Ser	L e u 1 0 5	Атд	Ser	Glu	Asp Th 11	r Ala 0	Val	
Ту	r Ty	r Cy	s Ala	Arg	G 1 y	G 1 y	Asn	Arg	РЬс	Ala	Tyr	Trp Gl	y Gla	Gly	

5	70	5 (	65
.).	.15	5.5	0.0

107		108	
	-continued		
115	1 2 0	1 2 5	
Thr Leu Val Thr Val Ser Ser 130 135			
(2) INFORMATION FOR SEQ ID NO:103:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 90 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
$(\mathbf{x}, \mathbf{i})$ SEQUENCE DESCRIPTION: SEQ ID NO:103:			
GATAAGCTTG CCGCCACCAT GGACTG	GACC TGGAGGGTCT	TCTICTTGCT GGCTGTAGCT	6 0
CCAGGTGCTC ACTCCCAGGT GCAGCT	TGTG		9 0
(2) INFORMATION FOR SEQ ID NO:104:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 90 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:104:			
CACTCCCAGG TGCAGCTTGT GCAGTCT	TGGA GCTGAGGTGA	AGAAGCCTGG GGCCTCAGTG	6 0
AAGGTTTCCT GCAAGGCTTC TGGATAC	CTCA		9 0
(2) INFORMATION FOR SEQ ID NO:105:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 90 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( $\mathbf x \ i$ ) sequence description: seq id no:105:			
TGCAAGGCTT CTGGATACTC ATTCACT	AGT TATTACATAC	ACTGGGTGCG CCAGGCCCCC	60
GGACAAAGGC TTGAGTGGAT GGGATAT	TATT		90
(2) INFORMATION FOR SEQ ID NO:106:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 90 base pairs</li> <li>(B) TYPE: sucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:106:			
CTIGAGIGGA IGGGATATAT IGACCCI	TTC AATGGTGGTA	CTAGCTATAA TCAGAAGTTC	6 0
AAGGGCAGAG TCACCATTAC CGTAGAC	ACA		90
(2) INFORMATION FOR SEQ ID NO:107:			
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 90 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>			
( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:107:			
GTCACCATTA CCGTAGACAC ATCCGCG		IGGAGCTGAG CAGCCTGAGA	6 0
TCTGAAGACA CGGCTGTGTA TTACTGT	GCG		90

5	70	)5	0	65
.).	12		2	0.)

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INFORMATION FOR SEQ ID NO:108:

(2) INFORMATION FOR SEQ ID NO:108:	
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 94 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:108:	
ACGGCTGTGT ATTACTGTGC GAGAGGGGGT AACCGCTTTG CTTACTGGGG CCAGGGAACC	60
CTGGTCACCG TCTCCTCAGG TGAGTGGATC CGAC	94
(2) INFORMATION FOR SEQ ID NO:109:	
<ul> <li>( i ) SEQUENCE CHARACTERISTICS:</li> <li>( A ) LENGTH: 15 base pairs</li> <li>( B ) TYPE: nucleic acid</li> <li>( C ) STRANDEDNESS: single</li> <li>( D ) TOPOLOGY: linear</li> </ul>	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:109:	
GATAAGCTTG CCGCC	1 5
(2) INFORMATION FOR SEQ ID NO:110:	
<ul> <li>(i) SEQUENCE CHARACTERISTICS:</li> <li>(A) LENGTH: 15 base pairs</li> <li>(B) TYPE: nucleic acid</li> <li>(C) STRANDEDNESS: single</li> <li>(D) TOPOLOGY: linear</li> </ul>	
$(\mathbf{x}, \mathbf{i})$ SEQUENCE DESCRIPTION: SEQ ID NO:110:	
GTCGGATCCA CTCAC	1 5
(2) INFORMATION FOR SEQ ID NO:111:	
( i ) SEQUENCE CHARACTERISTICS:	
(A) LENGTH: 433 base pairs (B) TYPE: nucleic acid	
(C) STRANDEDNESS: single (D) TOPOLOGY: linear	
(ix) FEATURE:	
(A) NAME/KEY: CDS (B) LOCATION: 16.420	
( i x ) FEATURE: ( A ) NAME/KEY: mat_peptide ( B ) LOCATION: 16.420	
(x i) SEQUENCE DESCRIPTION: SEQ ID NO:111:	
AAGCTTGCCG CCACC ATG GAC TGG ACC TGG AGG GTC TTC TTC TTG CTG GCT Met Asp Trp Tbr Trp Arg Val Phe Phe Leu Leu Ala	5 1
	99
GTA GCT CCA GGT GCT CAC TCC CAG GTG CAG CTT GTG CAG TCT GGA GCT Val Ala Pro Gly Ala His Ser Gln Val Gln Leu Val Gin Ser Gly Ala 15 20 25	
GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTT TCC TGC AAG GCT TCT Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser	147
30 35 40	
GGA TAC TCA TTC ACT AGT TAT TAC ATA CAC TGG GTG CGC CAG GCC CCC Gly Tyr Ser Phe Thr Ser Tyr Tyr Ile His Trp Val Arg Gln Ala Pro 45 50 55 60	195
GGA CAA AGG CTT GAG TGG ATG GGA TAT ATT GAC CCT TTC AAT GGT GGT Gly Gln Arg Leu Glu Trp Met Gly Tyr lle Asp Pro Phe Asn Gly Gly	243
65 70 75	

#### 112

_	2.03.02				27			-co	ontinue	d						
ACT	AGC	TAT	AAT	CAG	AAG	TTC	AAG	GGC	AGA	GTC	ACC	ATT	ACC	GTA	GAC	2 9
Ihr	Ser	Туг	Asn 80	Gln	Lys	РЬе	Lys	G 1 y 8 5	Агд	Val	Tbr	I l e	ТЬ 1 90	V a l	Asp	
ACA	тсс	GCG	AGC	ACA	GCC	TAC	ATG	GAG	CTG	AGC	AGT	CTG	AGA	тст	GAA	33
ТЬг	Ser	Ala 95	Ser	Thr	Ala	Tyr	Мет 100	Glu	Leu	Ser	Ser	L e u 1 0 5	Arg	S e r	Glu	
GAC	ACG	GCT	GTG	ΤΑΤ	TAC	TGT	GCG	AGA	GGG	GGT	AAC	CGC	ттт	GCT	TAC	38
Asp	Tbr 110	Ala	Val	Туг	Туг	C y s 115	Ala	Arg	Gly	Gly	Asn 120	Arg	Phe	Ala	Туr	
TGG	GGC	CAG	GGA	ACC	СТС	GTC	ACC	GTC	тсс	TCA	GGT	GAGT	GA	тсс		433
Ттр 125	Gly	Gln	Gly	Thr	Leu 130	Val	Tbr	Val	Ser	Ser 135						
(2)]	NFORMA	TION FO	R SEQ II	D NO:112	:											
	( i )	( / ( I	A) LENG B) TYPE	RACTER TH: 135 : amino a LOGY: li	amino aci cid	ds										
	(11)			E: protein												
	(xi)	SEQUE	ICE DES	CRIPTIO	N: SEQ II	D NO:112:										
vîet 1	Asp	Ттр	ТЬт	Trp 5	Arg	Val	Phe	Pbe	L e u 1 0	Leu	Ala	Val	Ala	Рго 15	G 1 y	
A l a	His	Ser	G 1 n 2 0	V a 1	Gln	Leu	V a ]	Gln 25	Ser	G 1 y	Ala	Glu	V a 1 3 0	L y s	L y s	
Pr o	Gly	A 1 a 3 5	Ser	Val	Lys	Val	Ser 40	Суs	L y s	Ala	Ser	Gly 45	Туг	Ser	Phe	
[ b r	Ser 50	Туr	Туг	1 l c	His	Trp 55	Val	Arg	Gln	Ala	Pro 60	G 1 y	Gln	Arg	Leu	
3 l u	Trp	Mct	Gly	T y r	11e	Asp	Pro	Phe	As n	Gly	Gly	Tbr	Ser	Туг	Asn	
65					70					75					8 0	
Jla	Lys	РЬс	Lys	G 1 y 8 5	Arg	V a 1	Τbr	lle	ТЬг 90	V a 1	Asp	Тbг	Ser	Ala 95	Ser	
		T	Met	Glu	Lcu	Ser	Ser	Leu 105	Arg	Sет	Glu	As p	Tbr 110	Ala	Val	
br	Ala	. , .	100													
			100		Gly	G 1 y	Asn 120	Arg	Phe	Ala	Тут	Trp 125	G 1 y	Gln	G 1 y	

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 27 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: single (D) TOPOLOGY: incer

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:113:

AGGCTTGAGT GGATTGGATA TATTGAC

(2) INFORMATION FOR SEQ ID NO:114:

#### ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 27 base pairs
- ( B ) TYPE: nucleic acid
- (C) STRANDEDNESS: single (D) TOPOLOGY: linear
- ( 5 ) 101 010001. 11124

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:114:

AAGTTCAAGG GCAAGGTCAC CATTACC

27

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(2) INFORMATION FOR SEQ ID NO:115:

( i ) SEQUENCE CHARACTERISTICS:

( A ) LENGTH: 30 base pairs

- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear
- ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:115:

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GGTGCTTCCG TGAAAGTCAG CTGTAAAGCT
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(2) INFORMATION FOR SEQ ID NO:116:

( i ) SEQUENCE CHARACTERISTICS:

( A ) LENGTH: 30 base pairs

- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

(x i ) SEQUENCE DESCRIPTION: SEQ ID NO:116:

AGCTTTACAG CTGACTITCA CGGAAGCACC

(2) INFORMATION FOR SEQ ID NO:117:

( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 11 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:117: Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn 10 1 5 (2) INFORMATION FOR SEQ ID NO:118: ( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:118: Tyr Thr Ser Arg Leu His Ser 1 5 (2) INFORMATION FOR SEQ ID NO:119: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 9 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:119: Gln Gln Gly Asn Thr Leu Pro Tyr Thr 5 1

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:120:

-continued Asp Ile Gin Met Thr Gin Ser Pro Ser Ser Leu Ser Ala Ser Val Giy i 10 5 15 Asp Arg Val Thr Ile Thr Cys 20 (2) INFORMATION FOR SEQ ID NO:121: ( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 15 amino acids (B) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:121: Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu 11e Tyr 1 5 10 15 (2) INFORMATION FOR SEQ ID NO:122: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:122: Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr 1 5 10 15 Phe Thr lie Ser Ser Leu Gin Pro Glu Asp lie Ala Thr Tyr Tyr Cys 20 2 5 30 (2) INFORMATION FOR SEQ ID NO:123: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 10 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:123: Phe Gly Gln Gly Thr Lys Val Glu Ile Lys 1 5 10 (2) INFORMATION FOR SEQ ID NO:124: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids (B) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:124: Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr 1 5 10 15 Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys 20 25 30 ( 2 ) INFORMATION FOR SEQ ID NO:125: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 6 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:125:

						1.1.1	
	1	5					
(2)IN	FORMATION FOR S	EQ ID NO:126:					
	(A) (B) (C)	E CHARACTERISTICS: LENGTH: 16 amino acids TYPE: amino acid STRANDEDNESS: single TOPOLOGY: linear					
	(xi) SEQUENCE	E DESCRIPTION: SEQ ID N	NO:126:				
	Tyr Ile 1	Ser Tyr Ser 5	Gly Ile	Thr Thr	Tyr Asn 10	Pro Ser	Leu Lys Ser 15
(2)11	FORMATION FOR S	SEQ ID NO:127:					
	(A) (B) (C)	E CHARACTERISTICS: LENGTH: 10 amino acids TYPE: amino acid STRANDEDNESS: single TOPOLOGY: linear					
		E DESCRIPTION: SEQ ID 1					
	Ser Leu 1	Ala Arg Thr 5	Thr Ala	Met Asp	Tyr 10		
(2)	FORMATION FOR S	SEQ ID NO:128:					
	(A) (B) (C) (D)	E CHARACTERISTICS: LENGTH: 30 amino acids TYPE: amino acid STRANDEDNESS: single TOPOLOGY: linear					
		E DESCRIPTION: SEQ ID 1					
	Gln Val 1	Gln Leu Gln 5	Glu Ser	Gly Pro	Gly Leu 10	Val Arg	Pro Ser Gin 15
	Thr Leu	Ser Leu Thr 20	Cys Thr	Val Ser 25	Gly Tyr	ТЬг РЬс	T b r 3 0
(2)1	NFORMATION FOR S	SEQ ID NO:129:					
	(A) (B) (C)	E CHARACTERISTICS: 1 LENGTH: 14 amino acids 1 TYPE: amino acid 2 STRANDEDNESS: single 1 TOPOLOGY: linear					
	(xi) SEQUENC	E DESCRIPTION: SEQ ID	NO:129:				
	Trp Val 1	Arg Gla Pro 5	Pro Gly	Arg Gly	Leu Glu 10	Ттр ІІе	G 1 y
(2)	NFORMATION FOR :	SEQ ID NO:130:					
	(A) (B) (C)	TE CHARACTERISTICS: ) LENGTH: 32 amino acids ) TYPE: amino acid ) STRANDEDNESS: single ) TOPOLOGY: linear					
	( x i ) SEQUENC	E DESCRIPTION: SEQ ID	NO:130:				
	Arg Val 1	Thr Met Leu 5	Val Asp	Thr Ser	Lys Asn 10	Gln Phe	Ser Leu Arg 15
	Leu Ser	Ser Val Thr 20	Ala Ala	Asp Thr 25	Ala Val	Туг Туг	Cys Ala Arg 30

(2) INFORMATION FOR SEQ ID NO:131:

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( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 11 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:131: Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser 1 5 10 (2) INFORMATION FOR SEQ ID NO:132: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:132: Arg ValThrMetLeuArgAspThrSerLysAsnGlnPheSerLeuArg151015 Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg 20 25 30 (2) INFORMATION FOR SEQ ID NO:133: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 30 amino acids (B) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear (x i ) SEQUENCE DESCRIPTION: SEQ ID NO:133: Asp Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln 1 5 10 15 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Thr Phe Thr 20 25 30 (2) INFORMATION FOR SEQ ID NO:134: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:134: Trp Val Arg Gln Pro Pro Gly Arg Gly Leu Glu Trp Met Gly 1 5 10 ( 2 ) INFORMATION FOR SEQ ID NO:135: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 30 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:135: Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Arg Pro Ser Gln 1 5 10 15 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Tyr Ser Ile Thr 20 25 30

( 2 ) INFORMATION FOR SEQ ID NO:136:

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5.795.965
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-continued ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 15 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:136: Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met His 1 5 10 15 5 1 (2) INFORMATION FOR SEQ ID NO:137: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 6 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:137: Ala Ser Asn Leu Glu Ser 5 1 (2) INFORMATION FOR SEQ ID NO:138: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 9 amino acids (B) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:138: Gln His Ser Arg Glu Asn Pro Tyr Thr 5 1 (2) INFORMATION FOR SEQ ID NO:139: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 23 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:139: Asp lie Gin Met Thr Gin Ser Pro Ser Ser Leu Ser Ala Ser Val Gly 15 10 1 Asp Arg Val Thr Ile Thr Cys 20 (2) INFORMATION FOR SEQ ID NO:140: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 15 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:140: Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu lle Tyr 1 5 10 15 (2) INFORMATION FOR SEQ ID NO:141: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:141:

5	70	15	0	65
.).	13	7.)	.7	0.9

-continued Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr 1 5 10 15 1 5 Thr lle Ser Ser Leu Gla Pro Glu Asp Ile Ala Thr Tyr Tyr Cys 2 5 20 30 (2) INFORMATION FOR SEQ ID NO:142: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 10 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:142: Phe Gly Gla Gly Thr Lys Val Glu Ile Lys ' 5 10 (2) INFORMATION FOR SEQ ID NO:143: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 5 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:143: Ser Tyr Tyr lle His 1 5 (2) INFORMATION FOR SEQ ID NO:144: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 17 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:144: Tyr Ile Asp Pro Phe Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys 1 5 10 15 Gly (2) INFORMATION FOR SEQ ID NO:145: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 7 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:145: Gly Gly Asa Arg Phe Ala Tyr 1 5 (2) INFORMATION FOR SEQ ID NO:146: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 30 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:146: Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala 1 5 10 15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr

5	70	)5 (	965
	12	2.	20.2

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20 (2) INFORMATION FOR SEQ ID NO:147: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:147: Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Val Gly 1 5 10 (2) INFORMATION FOR SEQ ID NO:148: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid (C) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:148: Arg Val Thr Met Thr Leu Asp Thr Ser Thr Asn Thr Ala Tyr Met Glu 1 5 10 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 20 25 30 (2) INFORMATION FOR SEQ ID NO:149: ( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 11 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:149: Trp Gly Gin Gly Thr Leu Val Thr Val Ser Ser 1 5 10 (2) INFORMATION FOR SEQ ID NO:150: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:150: Lys Val Thr Met Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr Met Glu 1 5 10 15 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 20 25 30 (2) INFORMATION FOR SEQ ID NO:151: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:151: Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly 1 10

(2) INFORMATION FOR SEQ ID NO:152:

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-continued
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( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 30 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:152: Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala 1 5 10 15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr 20 25 30 (2) INFORMATION FOR SEQ ID NO:153: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:153: Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Met Gly 1 5 10 (2) INFORMATION FOR SEQ ID NO:154: ( i ) SEQUENCE CHARACTERISTICS: (A) LENGTH: 32 amino acids (B) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:154: Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu 1 5 10 15 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 20 25 30 (2) INFORMATION FOR SEQ ID NO:155: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:155: Trp Val Arg Gin Ala Pro Gly Gin Arg Leu Giu Trp lie Gly 1 5 10 (2) INFORMATION FOR SEQ ID NO:156: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 32 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:156: Lys Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu 1 5 10 15 Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg 20 25 30

(2) INFORMATION FOR SEQ ID NO:157:

5	795.965	
.).	19.3.90.)	

-continued ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 30 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO:157: Gla Val Gla Leu Val Gla Ser Gly Ala Glu Val Lys Lys Pro Gly Ala 10 15 5 10 1 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr 30 25 20 (2) INFORMATION FOR SEQ ID NO:158: ( i ) SEQUENCE CHARACTERISTICS: ( A ) LENGTH: 14 amino acids ( B ) TYPE: amino acid ( C ) STRANDEDNESS: single ( D ) TOPOLOGY: linear (x i) SEQUENCE DESCRIPTION: SEQ ID NO:158: Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile Gly

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We claim:

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1. A variable (V) region of a heavy (H) chain of an antibody to the human IL-6 receptor having the following structure:

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FR1<sup>2</sup>-CDR1<sup>2</sup>-FR2<sup>2</sup>-CDR2<sup>2</sup>-FR3<sup>2</sup>-CDR3<sup>2</sup>-FR4<sup>2</sup> wherein CDR1<sup>2</sup>, CDR2<sup>2</sup> and CDR3<sup>2</sup> represent a set of three complementarity determining regions comprising a set of the following amino acid sequences shown by:

130

SEQ ID No. 125 CDR12	Ser	Asp	His	Ala	Trp	Ser	-		
SEQ ID No. 126 CDR2 <sup>2</sup>	Tyr	Ile	Ser	Tyr	Ser	Gly	Ile	Thr	Thr
	Тут	Asn	Pro	Ser	Leu	Lys	Ser		
SEQ ID No. 127 CDR3 <sup>2</sup>	Ser Tyr;	Leu	Ala	Arg	Thr	Thr	Ala	Met	Asp

<sup>40</sup> and FR1<sup>2</sup>, FR2<sup>2</sup>, FR3<sup>2</sup> and FR4<sup>2</sup> comprise a set of the following amino acid sequences:

SEQ ID No. 135 FR12	Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Gly
	Leu	Val	Arg	Рто	Ser	Gln	Thr	Leu	Ser	Leu
	Thr	Cys	Thr	Val	Ser	Gly	Tyr	Ser	Ile	Thr
SEO ID No. 129 FR22	Trp	Val	Arg	Gln	Pro	Pro	Gly	Arg	Gly	Leu
	Glu	Trp	Ile	Gly						
SEQ ID No. 132 FR32	Arg	Val	Thr	Met	Leu	Arg	Asp	Thr	Ser	Lys
	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser	Ser	Val
	Thr	Ala	Ala	Asp	Thr	Ala	Val	Tyr	Тут	Cys
	Ala	Arg								
SEQ ID No. 131 FR42	Trp	Gly	Gln	Gły	Ser	Leu	Val	Thr	Val	Ser
	Ser.			1.12						
5 117 C 126 C										

2. An H chain of an antibody to the human IL-6 receptor, comprising:

(1) a V region of a heavy chain of an antibody to the human IL-6 receptor having the following structure: FR1<sup>2</sup>-CDR1<sup>2</sup>-FR2<sup>2</sup>-CDR2<sup>2</sup>-FR3<sup>2</sup>-CDR3<sup>2</sup>-FR4<sup>2</sup>

wherein CDR1<sup>2</sup>. CDR2<sup>2</sup> and CDR3<sup>2</sup> represent a set of three complementarity determining regions comprising a set of the following amino acid sequences:

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SEQ ID No. 125 CDR12	Ser	Asn	His	Ala	Тгр	Ser				_
SEQ ID No. 126 CDR22	Tyr	Ile	Ser			1000 A 100 A 100	Пе	Thr	Thr	Tyr
		Asn		Ser			Ser		1111	Lyi
SEQ ID No. 127 CDR32	Ser	Leu Тут;	Ala		Thr	Thr	Ala	Met	Asp	

and FR1<sup>2</sup>, FR2<sup>2</sup>, FR3<sup>2</sup> and FR4<sup>2</sup> comprise a set of the following amino acid sequences:

SEQ ID No. 135 FR12	Gh	Val	Gln	Leu	Gln	Glu	Ser	Gly	Рго	Gly
	Leu	Val	Arg	Pro	Ser	Gln	Thr	Leu	Ser	Leu
	Thr	Cys	Thr	Val	Ser	Gly	Tyr	Ser	Ile	Thr
SEQ ID No. 129 FR22	Trp	Val	Arg	Gh	Pro	Pro	Gly	Arg	Gly	Leu
	Glu	Trp	Ile	Gly						
SEQ ID No. 132 FR32	Arg	Val	Thr	Met	Leu	Arg	Asp	Thr	Ser	Lys
	Asn	Gln	Phe	Ser	Leu	Arg	Leu	Ser	Ser	Val
	Thr	Ala	Ala	Asp	Thr	Ala	Val	Тут	Tyr	Cys
	Ala	Arg								10
SEQ ID No. 131 FR42	Ттр	Gly	Gln	Gly	Ser	Leu	Val	Thr	Val	Ser
	Ser;									

and

(2) a C region of an H chain of a human antibody Cγ.
 3. A reshaped human antibody to the human IL-6 receptor, comprising

- (A) L chains of an antibody to said human IL-6 receptor. ach comprising:
- (1) a variable (V) region of a light (L) chain of an antibody to the human IL-6 receptor having the following structure:

FR1<sup>1</sup>-CDR1<sup>1</sup>-FR2<sup>1</sup>-CDR2<sup>1</sup>-FR3<sup>1</sup>-CDR3<sup>1</sup>-FR4<sup>1</sup> wherein CDR1<sub>1</sub>, CDR2<sup>1</sup> and CDR3<sup>1</sup> represent a set of three complementarity determining regions comprising a set of the following amino acid sequences:

- SEQ ID No. 117 CDR1<sup>1</sup> Arg Ala Ser Gln Asp Ile Ser Ser Tyr Leu Asn 40
- SEQ ID No. 118 CDR21 Tyr Thr Ser Arg Leu His Ser
- SEQ ID No. 119 CDR31 Gln Gln Gly Asn Thr Leu Pro Tyr Thr;

and

the FR1<sup>1</sup>, FR2<sup>1</sup>, FR3<sup>1</sup> and FR4<sup>1</sup> comprise a set of the following amino acid sequences:

SEQ ID No. 120 FR 1 <sup>1</sup>	Asp Ile Gin Met Thr Gin Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys	50
SEQ ID No. 121 FR2 <sup>1</sup>	Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr	
SEQ ID No. 122 FR3 <sup>1</sup>	Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys	55
SEQ ID No. 123 FR41	Phe Gly Gln Gly Thr Lys Val Glu Ile Lys;	
	or	60
SEQ ID No. 120 FR1 <sup>1</sup>	Asp Ile Gin Met Thr Gin Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys	
SEQ ID No. 121 FR21	Trp Tyr Gin Gin Lys Pro Giy Lys Ala Pro Lys Leu Leu Ile Tyr	65

SEQ ID No. 124 FR31

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#### -continued Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gin Pro Glu Asp Ile Ala Thr Tyr Tyr Cys

SEQ ID No. 123 FR4<sup>1</sup> Phe Gly Gln Gly Thr Lys Val Glu Ile Lys; and

(2) a C region of an L chain of a human antibody Cκ; and N

132

- (B) H chains of an antibody to the human IL-6 receptor, each comprising:
- (1) a V region of a heavy (H) chain of an antibody to the human IL-6 receptor having the following structure: FR1<sup>2</sup>-CDR1<sup>2</sup>-FR2<sup>2</sup> CDR2<sup>2</sup>-FR3<sup>2</sup>-CDR3<sup>2</sup>-FR4<sup>2</sup>

wherein  $CDR1_2$   $CDR2^2$  and  $CDR3^2$  represent a set of three complementarity determining regions comprising a set of the amino acid sequences shown by:

SEQ ID No. 125 CDR1<sup>2</sup> Ser Asp His Ala Trp Ser

- SEQ ID No. 126 CDR2<sup>2</sup> Tyr Ile Ser Tyr Ser Gly Ile Thr Thr Tyr Asn Pro Ser Leu Lys Ser
- SEQ ID No. 127 CDR3<sup>2</sup> Ser Leu Ala Arg Thr Thr Ala Met Asp Tyr; and

the FR1<sup>2</sup>, FR2<sup>2</sup>, FR3<sup>2</sup> and FR4<sup>2</sup> comprise a set of the following amino acid sequences:

SEQ ID No. 135 FR 12Gin Val Gin Leu Gin Giu Ser Giy Pro Giy<br/>Leu Val Arg Pro Ser Gin Thr Leu Ser Leu<br/>Thr Cys Thr Val Ser Giy Tyr Ser Ile ThrSEQ ID No. 129 FR 22Trp Val Arg Gin Pro Pro Giy Arg Giy Leu<br/>Giu Trp Ile GiySEQ ID No. 132 FR 32Arg Val Thr Met Leu Arg Asp Thr Ser Lys<br/>Asn Gin Phe Ser Leu Arg Leu Ser Ser Val<br/>Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys<br/>Ala ArgSEQ ID No. 131 FR 42Trp Giy Gin Giy Ser Leu Val Thr Val Ser<br/>Ser;

and

(2) a C region of an H chain of a human antibody Cy.

4. A V region of a heavy (H) chain of an antibody to the human IL-6 receptor having the following structure: FR14-CDR14-FR24-CDR24-FR34-CDR34-FR44

wherein CDR14, CDR24 and CDR34, represent a set of three complementarity determining regions compris-ing a set of the following amino acid sequences:

SEQ ID No. 143 CDR1 <sup>4</sup> SEQ ID No. 144 CDR2 <sup>4</sup>	Ser Tyr	Tyr Ile	Ile Pro			Gly Gly	Gly	Thr	Ser
SEQ ID No. 145 CDR34	Tyr Gly	Asn Gly		Phe	Ala	Tyr;			

and	
	the FR1 <sup>4</sup> , FR2 4, FR3 <sup>4</sup> and FR4 <sup>4</sup> comprise a set of the
	following amino acid sequences:

EQ ID No. 146 FR14	Gh	Val	Gin	Leu	Val	Gln	Ser	Gly	Ala	Glu
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
SEQ ID No. 147 FR24	Trp	Val	Arg	Gh	Ala	Pro	Gly	Gln	Gly	Leu
	Glu	Trp	Val	Gly						-
SEQ ID No. 150 FR34	Lys	Val	Thr	Met	Thr	Val	Asp	Thr	Ser	Thr
LQ ID NO. 100 THE	Asn	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Gly	Asp	Thr	Ala	Val	Tyr	Тут	Cys
	Ala	Arg								
SEQ ID No. 149 FR44	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
SEQ ID 110. 149 1 144	Ser:									
SEQ ID No. 146 FR 14	Gh	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Ghu
and in the state	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
	Ser	Cys	Lys	Ala	Ser	Gly	Тут	Ser	Phe	Thr
SEQ ID No. 151 FR24	Тгр	Val	Arg	Gh	Ala	Pro	Gly	Gln	Gly	Leu
SEQ 10 No. 151 PK2	Glu	Trp	Ile	Gly			÷.		- 25	
TO TO No. 150 EP 24	Lys	Val	Thr	Met	Thr	Val	Asp	Thr	Ser	Thr
SEQ ID No. 150 FR34	Asn	Thr	Ala	Тут	Met	Glu	Leu	Ser	Ser	Leu
		Ser	Ghu	Asp	Thr	Ala	Val	Tyr	Тут	Cys
	Arg		Olu	rish						
	Ala	Arg	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
SEQ ID No. 149 FR44	Trp	Gly	Сш	Oly	Im	Leu	Tas			
	Ser;			Gln	Ala	Pro	Gly	Gh	Arg	Leu
SEQ ID No. 152 FR14	Trp	Val	Arg		Ala	FIO	Oly	Om		2000
	Glu	Trp	Ile	Gly	Ala	Pro	Gly	Gln	Arg	Leu
SEQ ID No. 155 FR2*	Trp	Val	Arg	Gln	Ala	FIO	City	OIL1	100	2000
	Glu	Trp	Ile	Gly		Val	Asp	Thr	Ser	Ala
SEQ ID No. 154 FR34	Arg	Val	Thr	Ile	Thr		Leu	Ser	Ser	Leu
	Ser	Thr	Ala	Tyr	Met	Glu			Tyr	Cys
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	TÀI	Cys
	Ala	Arg			-	T	12-1	Thr	Val	Ser
SEQ ID No. 149 FR44	Trp	Gly	Gh	Gly	Thr	Leu	Val	Im	vai	Set
	Ser;					~	0	Ch	Ala	Glu
SEQ ID No. 152 FR14	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly		Val
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	
	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
SEQ ID No. 153 FR24	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Arg	Leu
	Glu	Trp	Met	Gly				-	Car	Ala
SEQ ID No. 156 FR34	Lys	Val	Thr	lle	Thr	Val	Asp	Thr	Ser	
	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cy
	Ala	Arg			- 02		122.20			<b>C</b>
SEQ ID No. 149 FR44	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
-	Ser;									
or									112	01
SEQ ID No. 152 FR14	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Gh
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Th
SEQ ID No. 155 FR24	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gh	Arg	Le
	Glu	Trp	Ile	Gly				152.3	1000	122
SEQ ID No. 156 FR34	Lys	Val	Thr	Ile	Thr	Val	Asp		Ser	Al
	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Le
	Arg		Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cy
	Ala	Arg								
SEQ ID No. 149 FR44	Ттр	Gly		Gly	Thr	Leu	Val	Thr	Val	Se
200 m 10. 14. 144	Ser.				12215					

5. An H chain of an antibody to the human IL-6 receptor. comprising:

 (1) a V region of heavy (H) chain of an antibody to the human IL-6 receptor having the following structure: FR1<sup>4</sup>-CDR1<sup>4</sup>-FR2<sup>4</sup>-CDR2<sup>4</sup>-FR3<sup>4</sup>-CDR3<sup>4</sup>-FR4<sup>4</sup> 5 where CDR1<sup>4</sup>, CDR2<sup>4</sup>, and CDR3<sup>4</sup> represent a set of three complementarity determining regions comprising a set of the following sequences shown by:

SEQ ID No. 143 CDR1 <sup>4</sup> SEQ ID No. 144 CDR2 <sup>4</sup>	Ser Tyr	Tyr Ile	Tyr Asp	Ile Pro	His Phe	Asn	Gly	Glv	Thr	Ser
SEQ ID No. 145 CDR34	Tyr Gly	Asn Gly	Gln Asn	10070-00	Phe Phe	Lys Ala	Gly Tyr;			

and

the FR1<sup>4</sup>, FR2<sup>4</sup>, FR3<sup>4</sup>, and FR4<sup>4</sup> comprise a set of the following amino acid sequences:

SEQ ID No. 146 FR14	Gh	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
SEQ ID No. 147 FR24	Ттр	Val	Arg	Gln	Ala	Pro	Gly	Gh	Gly	Leu
	Glu	Trp	Val	Gly						
SEQ ID No. 150 FR34	Lys	Val	Thr	Met	Thr	Val	Asp	Thr	Ser	Thr
	Asn	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Тут	Тут	Cys
	Ala	Arg								-,-
SEQ ID No. 149 FR44	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
SEO ID No. 146 ED 14	Ser;									
SEQ ID No. 146 FR14	Gh	Val	Gln	Leu	Val	Gh	Ser	Gly	Ala	Glu
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
SEO TO No. 151 ED 24	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
SEQ ID No. 151 FR24	Trp	Val	Arg	Gh	Ala	Pro	Gly	Gh	Gly	Leu
SEO ID No. 150 ED24	Glu	Ттр	Ile	Gly	100	1111				
SEQ ID No. 150 FR34	Lys	Val	Thr	Met	Thr	Val	Asp	Thr	Ser	Thr
	Asn	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Тут	Tyr	Cys
SEQ ID No. 149 FR44	Ala	Arg	~	~	1.1					
SEQ 10 10. 149 FR4	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
SEQ ID No. 157 FR14	Ser;		~							
SEQ ID NO. 157 FRI	Gh	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
TO TO N. 100 FD at	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
SEQ ID No. 158 FR2 <sup>4</sup>	Ттр	Val	Arg	Gh	Ala	Pro	Gly	Gln	Arg	Leu
TO TO M. I.C. Pood	Ghu	Trp	Ile	Gly						
SEQ ID No. 154 FR34	Arg	Val	Thr	Ile	Thr	Val	Asp	Thr	Ser	Ala
	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Тут	Tyr	Cys
	Ala	Arg								
SEQ ID No. 149 FR4 <sup>4</sup>	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
1.1.1	Ser;									
SEQ ID No. 152 FR14	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val
	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Thr
EQ ID No. 153 FR24	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gh	Arg	Leu
	Glu	Trp	Met	Gly				1.11.121	0	
EQ ID No. 156 FR34	Lys	Val	Thr	Ile	Thr	Val	Asp	Thr	Ser	Ala
	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Тут	Cys
	Ala	Arg						-).	, <i>.</i>	Cys
EQ ID No. 149 FR4 <sup>4</sup>	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser
	Ser;					2.00	Tui	****	Yaj	Ser
r										
EQ ID No. 152 FR14	Gln	Val	Gln	Leu	Val	Gln	Ser	Gla	A1-	01-
	Val	Lys	Lys	Pro	Gly	Ala	Ser	Gly	Ala	Glu
	Ser	Cys	Lys	Ala			Ser	Val	Lys	Val
EQ ID No. 155 FR24	Trp	Val	Arg	Gln	Ser Ala	Gly	Tyr	Ser	Phe	Thr
	Glu	Trp			Ala	Pro	Gly	Gln	Arg	Leu
EQ ID No. 156 FR34			Ile	Gly	-					
- 2 - 110. 100 I.K.S	Lys	Val	Thr	Пе	Thr	Val	Asp	Thr	Ser	Ala
	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu
	Arg	Ser	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
	Ala	Arg								

-continued										
SEQ ID No. 149 FR44	Trp Ser:	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser

## and

and			cro m N-			
6. A reshaped human an	chain of a human antibody Cγ. tibody to the human IL-6 receptor.	10	SEQ ID No			
	ibody to the human IL-6 receptor.	10	SEQ ID No			
antibody to the l		15	SEQ ID No			
wherein CDR1 <sup>3</sup> . ( of three complem	<sup>23</sup> -CDR2 <sup>3</sup> -FR3 <sup>3</sup> -CDR3 <sup>3</sup> -FR4 <sup>3</sup> CDR2 <sup>3</sup> and CDR3 <sup>3</sup> represent a set entarity determining regions com-		SEQ ID No			
•	e following amino acid sequences: Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr	20	SEQ ID No			
SEQ ID No. 137 CDR2 <sup>3</sup> Ala	fyr Met His Ser Asn Leu Glu Ser His Ser Arg Glu Asn Pro Tyr Thr,		SEQ ID No			
and FR1 <sup>3</sup> , FR2 <sup>3</sup> , FR3 <sup>3</sup> following amino	and FR4 <sup>3</sup> comprise a set of the acid sequences:	25	SEQ ID No			
Le	Leu Ser Ala Ser Val Gly Asp Arg Val Thr					
SEQ ID No. 140 FR23 Tr	e Thr Cys rp Tyr Gin Gin Lys Pro Gly Lys Ala Pro ys Leu Leu Ile Tyr		SEQ ID No			
S	ily Val Pro Ser Arg Phe Ser Gly Ser Gly er Gly Thr Asp Phe Thr Phe Thr Ile Ser er Leu Gln Pro Glu Asp Ile Ala Thr Tyr	35	SEQ ID N			
	yr Cys 1e Gly Gln Gly Thr Lys Val Glu Ile Lys;		SEQ ID N			
and						
(2) a C region of ar and	α L chain of a human antibody Cκ;	40	SEQ ID N			
(B) H chains of an an	tibody to the human IL-6 receptor.		SEQ ID N			
the human IL-6 r	heavy (H) chain of an antibody to ecceptor having the following struc-	45	SEQ ID N			
ture: FR 1 <sup>4</sup> -CDR 1 <sup>4</sup> -FR	24-CDR24-FR34-CDR34-FR44					
wherein CDR1 <sup>4</sup> , of three complem	CDR2 <sup>4</sup> and CDR3 <sup>4</sup> represent a set mentarity determining regions com- he following amino acid sequences:	50	SEQ ID N			
SEQ ID No. 143 CDR14 Ser	Tyr Tyr Ile His		SEQ ID N			
Asn	lle Asp Pro Phe Asn Gly Gly Thr Ser Tyr 1 Gly Lys Phe Lys Gly 7 Gly Asn Arg Phe Ala Tyr;	55	SEQ ID N			
and						
FR1 <sup>4</sup> , FR2 <sup>4</sup> , PR3 <sup>4</sup> following amino	<sup>4</sup> and FR4 <sup>4</sup> comprise a set of the acid sequences:	60	SEQ ID N			
1	Gin Val Gin Leu Val Gin Ser Gly Ala Giu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr		and			
	Frp Val Arg Gin Ala Pro Gly Gin Gly Leu Glu Trp Val Gly	65	(2) a (			

SEQ ID No. 150 FR34	-continued Lys Val Thr Met Thr Val Asp Thr Ser Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
SEQ ID No. 149 FR44	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser:
SEQ ID No. 146 FR 14	Gin Val Gin Leu Val Gin Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
SEQ ID No. 151 FR24	Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly
SEQ ID No. 150 FR34	Lys Val Thr Met Thr Val Asp Thr Ser Thr Asn Thr Asn Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
SEQ ID No. 149 FR44	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
SEQ ID No. 152 FR14	Gin Val Gin Leu Val Gin Ser Giy Ala Giu Val Lys Lys Pro Giy Ala Ser Val Lys Val Ser Cys Lys Ala Ser Giy Tyr Ser Phe Thr
SEQ ID No. 155 FR24	Trp Val Arg Gln Ala Pro Gły Gln Arg Leu Glu Trp Ile Gly
SEQ ID No. 154 FR34	Arg Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
SEQ ID No. 149 FR44	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser;
SEQ ID No. 152 FR 14	Gin Val Gin Leu Val Gin Ser Gly Ala Giu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
SEQ ID No. 153 FR24	Trp Val Arg Gln Ala Pro Gly Gin Arg Leu Glu Trp Met Gly
SEQ ID No. 156 FR34	Lys Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
SEQ ID No. 149 FR44	Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser, or
SEQ ID No. 152 FR 14	Gln Val Gln Leu Val Gln Ser Gly AlaGlu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr
SEQ ID No. 155 FR24	Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp Ile Gly
SEQ ID No. 156 FR34	Lys Val Thr Ile Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg
SEQ ID No. 149 FR44	Trp Gly Gin Gly Thr Leu Val Thr Val Ser Ser;
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(2) a C region of an H chain of a human antibody Cy.

\* \* \* \* \*

# UNITED STATES PATENT AND TRADEMARK OFFICE CERTIFICATE OF CORRECTION

Page 1 of 1

 PATENT NO.
 : 5,795,965

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 : August 18, 1998

 INVENTOR(S)
 : Masayuki et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Col. 132, claim 3, line 33, "and N" should read --and--.

Signed and Sealed this

Fifteenth Day of September, 2009

David J. Kgppos

David J. Kappos Director of the United States Patent and Trademark Office