Methods of producing immunoglobulins, vectors and transformed host cells for use therein

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ABSTRACT

The invention relates to processes for producing an immunoglobulin or an immunologically functional immunoglobulin fragment containing at least the variable domains of the immunoglobulin heavy and light chains. The processes can use one or more vectors which produce both the heavy and light chains or fragments thereof in a single cell. The invention also relates to the vectors used to produce the immunoglobulin or fragment, and to cells transformed with the vectors.

IMAGES (19)

DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 06/483,457, filed Apr. 8, 1983, now U.S. Pat. No. 4,816,567, issued Mar. 28, 1989.

BACKGROUND OF THE INVENTION

This invention relates to the field of immunoglobulin production and to modification of naturally occurring immunoglobulin amino acid sequences. Specifically, the invention relates to using recombinant techniques to produce both immunoglobulins which are analogous to those normally found in vertebrate systems and to take advantage of these gene modification techniques to construct chimeric or other modified forms.

A. Immunoglobulins and Antibodies

Antibodies are specific immunoglobulin polypeptides produced by the vertebrate immune system in response to challenge by foreign proteins, glycoproteins, cells, or other antigenic foreign substances. The sequence of events which permits the organism to overcome invasion by foreign cells or to rid the system of foreign substances is at least partially understood. An important part of this process is the manufacture of antibodies which bind specifically to a particular foreign substance. The binding specificity of such polypeptides to a particular antigen is highly refined, and the multitude of specificities capable of being generated by the individual vertebrate is remarkable in its complexity and variability. Thousands of antigens are capable of eliciting responses, each almost exclusively directed to the particular antigen which elicited it.

Immunoglobulins include both antibodies, as above described, and analogous

CLAIMS (36)

What is claimed is:

1. A process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising at least the variable domains of the immunoglobulin heavy and light chains, in a single host cell, comprising the steps of:

(i) transforming said single host cell with a first DNA sequence encoding at least the variable domain of the immunoglobulin heavy chain and a second DNA sequence encoding at least the variable domain of the immunoglobulin light chain, and

(ii) independently expressing said first DNA sequence and said second DNA sequence so that said immunoglobulin heavy and light chains are produced as separate molecules in said transformed single host cell.

2. The process according to claim **1** wherein said first and second DNA sequences are present in different vectors.

3. The process according to claim **1** wherein said first and second DNA sequences are present in a single vector.

4. A process according to claim **3** wherein the vector is a plasmid.

5. The process according to claim **4** wherein the plasmid is pBR322.

6. The process according to claim **1** wherein the host cell is a bacterium or yeast.

7. The process according to claim **6** wherein the host cell is *E. coli* or *S.*

protein substances which lack antigen specificity. The latter are produced at low levels by the lymph system and in increased levels by myelomas.

A.1 Source and Utility

Two major sources of vertebrate antibodies are presently utilized—generation in situ by the mammalian B lymphocytes and in cell culture by B-cell hybrids. Antibodies are made in situ as a result of the differentiation of immature B lymphocytes into plasma cells, which occurs in response to stimulation by specific antigens. In the undifferentiated B cell, the portions of DNA coding for the various regions on the immunoglobulin chains are separated in the genomic DNA. The sequences are reassembled sequentially prior to transcription. A review of this process has been given by Gough, *Trends in Biochem Sci*, 6: 203 (1981). The resulting rearranged genome is capable of expression in the mature B lymphocyte to produce the desired antibody. Even when only a single antigen is introduced into the sphere of the immune system for a particular mammal, however, a uniform population of antibodies does not result. The in situ immune response to any particular antigen is defined by the mosaic of responses to the various determinants which are present on the antigen. Each subset of homologous antibody is contributed by a single population of B-cells—hence in situ generation of antibodies is “polyclonal”.

This limited but inherent heterogeneity has been overcome in numerous particular cases by use of hybridoma technology to create “monoclonal” antibodies (Kohler, et al., *Eur. J. Immunol.*, 6: 511 (1976)). In this process, splenocytes or lymphocytes from a mammal which has been injected with antigen are fused with a tumor cell line, thus producing hybrid cells or “hybridomas” which are both immortal and capable of producing the genetically coded antibody of the B cell. The hybrids thus formed we segregated into single genetic strains by selection, dilution, and regrowth, and each strain thus represents a single genetic line. They therefore produce immunoreactive antibodies against a desired antigen which are assured to be homogenous, and which antibodies, referencing their pure genetic parentage, are called “monoclonal”. Hybridoma technology has to this time been focused largely on the fusion of murine lines, but human-human hybridomas (Olsson, L. et al., *Proc. Natl. Acad. Sci.* (*USA*), 77: 5429 (1980)); human-murine hybridomas (Schlom, J. , et al. (ibid) 77: 6841 (1980)) and several other xenogenic hybrid combinations have been prepared as well. Alternatively, primary, antibody producing, B cells have been immortalized in vitro by transformation with viral DNA.

Polyclonal, or, much more preferably monoclonal, antibodies have a variety of useful properties similar to those of the present invention. For example, they can be used as specific immunoprecipitating reagents to detect the presence of the antigen which elicited the initial processing of the B cell genome by coupling this antigen-antibody reaction with suitable detection techniques such as labeling with radioisotopes or with enzymes capable of assay (RIA, EMIT, and ELISA). Antibodies are thus the foundation of immuno diagnostic tests for many antigenic substances. In another important use, antibodies can be directly injected into subjects suffering from an attack by a substance or organism containing the antigen in question to combat this attack. This process is currently in its experimental stages, but its potential is clearly seen. Third, whole body diagnosis and treatment is made possible because injected antibodies are directed to specific target disease tissues, and thus can be used either to determine the presence of the disease by carrying with them a suitable label, or to attack the diseased tissue by carrying a suitable drug.

Monoclonal antibodies produced by hybridomas, while theoretically effective as suggested above and clearly preferable to polyclonal antibodies because of their specificity, suffer from certain disadvantages. First, they tend to be contaminated with other proteins and cellular materials of hybridoma, (and, therefore, mammalian) origin. These cells contain additional materials, notably nucleic acid fragments, but protein fragments as well, which are capable of enhancing, causing, or mediating carcinogic responses. Second, hybridoma lines producing

cerevisiae.

8. A process according to claim **7** wherein the host cell is *E. coli* strain X1776 (ATCC No. 31537).

9. A process according to claim **1** wherein the immunoglobulin heavy and light chains are expressed in the host cell and secreted therefrom as an immunologically functional immunoglobulin molecule or immunoglobulin fragment.

10. A process according to claim **1** wherein the immunoglobulin heavy and light chains are produced in insoluble form and are solubilized and allowed to refold in solution to form an immunologically functional immunoglobulin molecule or immunoglobulin fragment.

11. A process according to claim **1** wherein the DNA sequences code for the complete immunoglobulin heavy and light chains.

12. The process according to claim **1** wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from the same source as the variable domain to which it is attached.

13. The process according to claim **1** wherein said first or said second DNA sequence further encodes at least one constant domain, wherein the constant domain is derived from a species or class different from that from which the variable domain to which it is attached is derived.

14. The process according to claim **1** wherein said first and second DNA sequences are derived from one or more monoclonal antibody producing hybridomas.

15. A vector comprising a first DNA sequence encoding at least a variable domain of an immunoglobulin heavy chain and a second DNA sequence encoding at least a variable domain of an immunoglobulin light chain wherein said first DNA sequence and said second DNA sequence are located in said vector at different insertion sites.

16. A vector according to claim **15** which is a plasmid.

17. A host cell transformed with a vector according to claim **15**.

18. A transformed host cell comprising at least two vectors, at least one of said vectors comprising a DNA sequence encoding at least a variable domain of an immunoglobulin heavy chain and at least another one of said vectors comprising a DNA sequence encoding at least the variable domain of an immunoglobulin light chain.

19. The process of claim **1** wherein the host cell is a mammalian cell.

20. The transformed host cell of claim **18** wherein the host cell is a mammalian cell.

21. A method comprising

a) preparing a DNA sequence consisting essentially of DNA encoding an immunoglobulin consisting of an immunoglobulin heavy chain and light chain or Fab region, said immunoglobulin having specificity for a particular known antigen;

b) inserting the DNA sequence of step a) into a replicable expression vector operably linked to a suitable promoter;

c) transforming a prokaryotic or eukaryotic microbial host cell culture with the vector of step b);

d) culturing the host cell; and

monoclonal antibodies tend to be unstable and may alter the structure of antibody produced or stop producing antibody altogether (Kohler, G., et al. *Proc. Natl. Acad. Sci* (*USA*) 77: 2197 (1980); Morrison, S. L., *J. Immunol*. 123: 793 (1979)). The cell line genome appears to alter itself in response to stimuli whose nature is not currently known, and this alteration may result in production of incorrect sequences. Third, both hybridoma and B cells inevitably produce certain antibodies in glycosylated form (Melchers, F., *Biochemistry*, 10: 653 (1971)) which, under some circumstances, may be undesirable. Fourth, production of both monoclonal and polyclonal antibodies is relatively expensive. Fifth, and perhaps most important, production by current techniques (either by hybridoma or by B cell response) does not permit manipulation of the genome so as to produce antibodies with more effective design components than those normally elicited in response to antigens from the mature B cell in situ. The antibodies of the present invention do not suffer from the foregoing drawbacks, and, furthermore, offer the opportunity to provide molecules of superior design.

Even those immunoglobulins which lack the specificity of antibodies are useful, although over a smaller spectrum of potential uses than the antibodies themselves. In presently understood applications, such immunoglobulins are helpful in proteins replacement therapy for globulin related anemia. In this context, an inability to bind to antigen is in fact helpful, as the therapeutic value of these proteins would be impaired by such functionality. At present, such non-specific antibodies are derivable in quantity only from myeloma cell cultures suitably induced. The present invention offers an alternative, more economical source. It also offers the opportunity of canceling out specificity by manipulating the four chains of the tetramer separately.

A.2 General Structure Characteristics

The basic immunoglobin structural unit in vertebrate systems is now well understood (Edelman, G. M., *Ann. N.Y. Acad. Sci*., 190: 5 (1971)). The units are composed of two identical light polypeptide chains of molecular weight approximately 23,000 daltons, and two identical heavy chains of molecular weight 53,000-70,000. The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket-the heavy-chains starting at the mouth of the Y and continuing through the divergent region as shown in FIG. 1. The "branch" portion, as there indicated, is designated the Fab region. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, with some subclasses among them, and the nature of this chain, as it has a long constant region, determines the "class"of the antibody as IgG, IgM, IgA, IgD, or IgE. Light chains are classified-as either kappa or lambda. Each heavy chain class can be prepared with either kappa or lambda light chain. The light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages when the immunoglobulins are generated either by hybridomas or by B cells. However, if non-covalent association of the chains can be effected in the correct geometry, the aggregate will still be capable of reaction with antigen, or of utility as a protein supplement as a non-specific immunoglobulin.

The amino acid sequence runs from the N-terminal end at the top of the Y to the C-terminal end at the bottom of each chain. At the N-terminal end is a variable region which is specific for the antigen which elicited it, and is approximately 100 amino acids in length, there being slight variations between light and heavy chain and from antibody to antibody. The variable region is linked in each chain to a constant region which extends the remaining length of the chain. Linkage is seen, at the genomic level, as occurring through a linking sequence known currently as the "J" region in the light chain gene, which encodes about 12 amino acids, and as a combination of "D" region and "J" region in the heavy chain gene, which together encode approximately 25 amino acids.

The remaining portions of the chain are referred to as constant regions and within a particular class do not to vary with the specificity of the antibody (i.e., the antigen eliciting it),

As stated above, there are five known major classes of constant regions which determine the class of the immunoglobulin

e) recovering the immunoglobulin from the host cell culture, said immunoglobulin being capable of binding to a known antigen.

22. The method of claim **21** wherein the heavy and light chain are the heavy and light chains of anti-CEA antibody.

23. The method of claim **21** wherein the heavy chain is of the gamma family.

24. The method of claim **21** wherein the light chain is of the kappa family.

25. The method of claim **21** wherein the vector contains DNA encoding both a heavy chain and a light chain.

26. The method of claim **21** wherein the host cell is *E. coli* or yeast.

27. The method of claim **26** wherein the heavy chain and light chains or Fab region are deposited within the cells as insoluble particles.

28. The method of claim **27** wherein the heavy and light chains are recovered from the particles by cell lysis followed by solubilization in denaturant.

29. The method of claim **21** wherein the heavy and light chains are secreted into the medium.

30. The method of claim **21** wherein the host cell is a gram negative bacterium and the heavy and light chains are secreted into the periplasmic space of the host cell bacterium.

31. The method of claim **21** further comprising recovering both heavy and light chain and reconstituting light chain and heavy chain to form an immunoglobulin having specific affinity for a particular known antigen.

32. The insoluble particles of heavy chain and light chains or Fab region produced by the method of claim **27**.

33. A process for producing an immunoglobulin molecule or an immunologically functional immunoglobulin fragment comprising at least the variable domains of the immunoglobulin heavy and light chains, in a single host cell, comprising:

independently expressing a first DNA sequence encoding at least the variable domain of the immunoglobulin heavy chain and a second DNA sequence encoding at least the variable domain of the immunoglobulin light chain so that said immunoglobulin heavy and light chains are produced as separate molecules in said single host cell transformed with said first and second DNA sequences.

34. The process of claim **9**, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

35. The process of claim **10**, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

36. The process of claim **33**, further comprising the step of attaching the immunoglobulin molecule or immunoglobulin fragment to a label or drug.

molecule (IgG, IgM, IgA, IgD, and IgE corresponding to γ, μ, α, δ, and ε heavy chain constant regions). The constant region or class determines subsequent effector function of the antibody, including activation of complement (Kabat, E. A., *Structural Concepts in Immunology and Immunochemistry*, 2nd Ed., p. 413-436, Holt, Rinehart Winston (1976)), and other cellular responses (Andrews, D. W., et al., *Clinical Immunobiology* pp 1-18, W. B. Sanders (1980); Kohl, S., et al., *Immunology*, 48: 187 (1983)); while the variable region determines the antigen with which it will react.

B. Recombinant DNA Technology

Recombinant DNA technology has reached sufficient sophistication that it includes a repertoire of techniques for cloning and expression of gene sequences. Various DNA sequences can be recombined with some facility, creating new DNA entities capable of producing heterologous protein product in transformed microbes and cell cultures. The general means and methods for the in vitro ligation of various blunt ended or "sticky" ended fragments of DNA, for producing expression vectors, and for transforming organisms are now in hand.

DNA recombination of the essential elements (i.e., an origin of replication, one or more phenotypic selection characteristics, expression control sequence, heterologous gene insert and remainder vector) generally is performed outside the host cell. The resulting recombinant replicable expression vector, or plasmid, is introduced into cells by transformation and large quantities of the recombinant vehicle is obtained by growing the transformants. Where the gene is properly inserted with reference to portions which govern the transcription and translation of the encoded DNA message, the resulting expression vector is useful to produce the polypeptide sequence for which the inserted gene codes, a process referred to as "expression." The resulting product may be obtained by lysis, if necessary, of the host cell and recovery of the product by appropriate purifications from other proteins.

In practice, the use of recombinant DNA technology can express entirely heterologous polypeptides—so-called direct expression—or alternatively may express a heterologous polypeptide fused to a portion of the amino acid sequence of a homologous polypeptide. In the latter cases, the intended bioactive product is sometimes rendered bioinactive within the fused, homologous/heterologous polypeptide until it is cleaved in an extracellular environment.

The art of maintaining cell or tissue cultures as well as microbial systems for studying genetics and cell physiology is well established. Means and methods are available for maintaining permanent cell lines, prepared by successive serial transfers from isolated cells. For use in research, such cell lines are maintained on a solid support in liquid medium, or by growth in suspension containing support nutriments. Scale-up for large preparations seems to pose only mechanical problems.

SUMMARY OF THE INVENTION

The invention relates to antibodies and to non-specific immunoglobulins (NSIs) formed by recombinant techniques using suitable host cell cultures. These antibodies and NSIs can be readily prepared in pure "monoclonal" form. They can be manipulated at the genomic level to produce chimeras of variants which draw their homology from species which differ from each other. They can also be manipulated at the protein level, since all four chains do not need to be produced by the same cell. Thus, there are a number of "types" of immunoglobulins encompassed by the invention.

First, immunoglobulins, particularly antibodies, are produced using recombinant techniques which mimic the amino acid sequence of naturally occurring antibodies produced by either mammalian B cells in situ, or by B cells fused with suitable immortalizing tumor lines, i.e., hybridomas. Second, the methods of this invention produce, and the invention is directed to, immunoglobulins which comprise polypeptides not hitherto found associated with each other in nature. Such reassembly is particularly useful in producing "hybrid" antibodies capable of binding more than one antigen; and in producing "composite" immunoglobulins wherein heavy and light chains of different origins essentially damp out specificity. Third, by genetic manipulation, "chimeric" antibodies can be formed wherein, for example, the variable regions correspond to the amino acid sequence from one mammalian model system, whereas the constant region mimics the amino acid sequence of another. Again, the derivation of these two mimicked sequences may be from different species. Fourth, also by genetic manipulation, "altered" antibodies with improved specificity and other characteristics can be formed.

Two other types of immunoglobulin-like moieties may be produced: "univalent" antibodies, which are useful as homing carriers to target tissues, and "Fab proteins" which include only the "Fab" region of an immunoglobulin molecule i.e., the branches of the "Y". These univalent antibodies and Fab fragments may also be "mammalian" i.e., mimic mammalian amino acid sequences; novel assemblies of mammalian chains, or chimeric, where for example, the constant and variable sequence patterns may be of different origin. Finally, either the light chain or heavy chain alone, or portions, thereof, produced by recombinant techniques are included in the invention and may be mammalian or chimeric.

In other aspects, the invention is directed to DNA which encodes the aforementioned NSIs, antibodies, and portions thereof, as well as expression vectors or plasmids capable of effecting the production of such immunoglobulins in suitable host cells. It includes the host cells and cell cultures which result from transformation with these vectors. Finally, the invention is directed to methods of producing these NSIs and antibodies, and the DNA sequences, plasmids, and transformed cells intermediate to them.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a representation of the general structure of immunoglobulins.

FIGS. 2A-B shows the detailed sequence of the cDNA insert of pK17G4 which encodes kappa anti CEA chain.

FIG. 3 shows the coding sequence of the fragment shown in FIG. 2, along with the corresponding amino acid sequence.

FIGS. 4A-C shows the combined detailed sequence of the cDNA inserts of pγ298 and pγ11 which encode gamma anti CEA chain.

FIGS. 5A-B shows the corresponding amino acid sequence encoded by the fragment in FIG. **4**.

FIGS. 6 and 7 outline the construction of expression vectors for kappa and gamma anti-CEA chains respectively.

FIGS. 8A, **8**B, and **8**C show the results of sizing gels run on, extracts of *E. coli* expressing the genes for gamma chain, kappa chain, and both kappa and gamma chains respectively.

FIG. 9 shows the results of western blots of extracts of cells transformed as those in FIGS. **8**.

FIG. 10 shows a standard curve for ELISA assay of anti CEA activity.

FIGS. 11 and 12 show the construction of a plasmid for expression of the gene encoding a chimeric heavy chain.

FIG. 13 shows the construction of a plasmid for expression of the gene encoding the Fab region of heavy chain.

DETAILED DESCRIPTION

A. Definitions

As used herein, “antibodies” refers to tetramers or aggregates thereof which have specific immunoreactive activity, comprising light and heavy chains usually aggregated in the “Y” configuration of FIG. 1, with or without covalent linkage between them; “immunoglobulins” refers to such assemblies whether or not specific immunoreactive activity is a property. “Non-specific immunoglobulin” (“NSI”) means those immunoglobulins which do not possess specificity—i.e., those which are not antibodies.

“Mammalian antibodies” refers to antibodies wherein the amino acid sequences of the chains are homologous with those sequences found in antibodies produced by mammalian systems, either in situ, or in hybridomas. These antibodies mimic antibodies which are otherwise capable of being generated, although in impure form, in these traditional systems.

“Hybrid antibodies” refers to antibodies wherein chains are separately homologous with referenced mammalian antibody chains and represent novel assemblies of them, so that two different antigens are precipitable by the tetramer. In hybrid antibodies, one pair of heavy and light chain is homologous to antibodies raised against one antigen, while the other pair of heavy and light chain is homologous to those raised against another antigen. This results in the property of “divalence” i.e., ability to bind two antigens simultaneously. Such hybrids may, of course, also be formed using chimeric chains, as set forth below.

“Composite” immunoglobulins means those wherein the heavy and light chains mimic those of different species origins or specificities, and the resultant is thus likely to be a non-specific immunoglobulin (NSI), i.e.—lacking in antibody character.

“Chimeric antibodies” refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chains is homologous to corresponding sequences in another. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals while the constant portions are homologous to the sequences in antibodies derived from another. One clear advantage to such chimeric forms is that, for example, the variable regions can conveniently be derived from presently known sources using readily available hybridomas or B cells from non human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation, and the specificity is not affected by its source, the constant region being human, is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non-human source.

However, the definition is not limited to this particular example. It includes any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, differing antigen responses, or differing species of origin and whether or not the fusion point is At the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose constant region can elicit enhanced

complement fixation or to make other improvements in properties possessed by a particular constant region.

"Altered antibodies" means antibodies wherein the amino acid sequence has been varied from that of a mammalian or other vertebrate antibody. Because of the relevance of recombinant DNA techniques to this invention, one need not be confined to the sequences of amino acids found in natural antibodies; antibodies can be redesigned to obtain desired characteristics. The possible variations are many and range from the changing of just one or a few amino acids to the complete redesign of, for example, the constant region. Changes in the constant region will, in general, be made in order to improve the cellular process characteristics, such as complement fixation, interaction with membranes, and other effector functions. Changes in the variable region will be made in order to improve the antigen binding characteristics. The antibody can also be engineered so as to aid the specific delivery of a toxic agent according to the "magic bullet" concept. Alterations, can be made by standard recombinant techniques and also by oligonucleotide-directed mutagenesis techniques (Dalbadie-McFarland, et al *Proc. Natl. Acad. Sci.* (*USA*), 79:6.409 (1982)).

"Univalent antibodies" refers to aggregations which comprise a heavy chain/light chain dimer bound to the Fc (or stem) region of a second heavy chain. Such antibodies are specific for antigen but have the additional desirable property of targeting tissues with specific antigenic surfaces, without causing its antigenic effectiveness to be impaired—i.e., there is no antigenic modulation. This phenomenon and the property of univalent antibodies in this regard is set forth in Glennie, M. J., et al., *Nature*, 295: 712 (1982). Univalent antibodies have heretofore been formed by proteolysis.

"Fab" region refers to those portions of the chains which are roughly equivalent, or analogous, to the sequences which comprise the Y branch portions of the heavy chain and to the light chain in its entirety, and which collectively (in aggregates) have been shown to exhibit antibody activity. "Fab protein", which protein is one of the aspects of the invention, includes aggregates of one heavy and one light chain (commonly known as Fab′), as well as tetramers which correspond to the two branch segments of the antibody Y, (commonly known as $F(ab)_2$), whether any of the above are covalently or non-covalently aggregated, so long as the aggregation is capable of selectively reacting with a particular antigen or antigen family. Fab antibodies have, as have univalent ones, been formed heretofore by proteolysis, and share the property of not eliciting antigen modulation on target tissues. However, as they lack the "effector" Fc: portion they cannot effect, for example, lysis of the target cell by macrophages.

"Fab protein" has similar subsets according to the definition of the present invention as does the general term "antibodies" or "immunoglobulins". Thus, "mammalian" Fab protein, "hybrid" Fab protein "chimeric" Fab and "altered" Fab protein are defined analogously to the corresponding definitions set forth in the previous paragraphs for the various types of antibodies.

Individual heavy or light chains may of course be "mammalian", "chimeric" or "altered" in accordance with the above. As will become apparent from the detailed description of the invention, it is possible, using the techniques disclosed to prepare other combinations of the four-peptide chain aggregates, besides those specifically defined, such as hybrid antibodies containing chimeric light and mammalian heavy chains, hybrid Fab proteins containing chimeric Fab proteins of heavy chains associated with mammalian light chains, and so forth.

"Expression vector" includes vectors which are capable of expressing DNA sequences contained therein, i.e., the coding sequences are operably linked to other sequences capable of effecting their expression. It is implied, although not always explicitly stated, that these expression vectors must be replicable in the host organisms either as episomes or as an integral part of the chromosomal DNA. Clearly a lack of replicability would render them effectively inoperable. A useful, but not a necessary, element of an effective expression vector is a marker encoding sequence—i.e. a sequence encoding a protein which results in a phenotypic property (e.g. tetracycline resistance) of the cells containing the protein which permits those cells to be readily identified. In sum, "expression vector" is given a functional definition, and any DNA sequence which is capable of effecting expression of a specified contained DNA code is included in this term, as it is applied to the specified sequence. As at present, such vectors are frequently in the form of plasmids, thus "plasmid" and "expression vector" are often used interchangeably. However, the invention is intended to include such other forms of expression vectors which serve equivalent functions and which may, from time to time become known in the art.

"Recombinant host cells" refers to cells which have been transformed with vectors constructed using recombinant DNA techniques. As defined herein, the antibody or modification thereof produced by a recombinant host cell is by virtue of this transformation, rather than in such lesser amounts, or more commonly, in such less than detectable amounts, as would be produced by the untransformed host.

In descriptions of processes for isolation of antibodies from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of antibody from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

B. Host Cell Cultures and Vectors

The vectors and methods disclosed herein are suitable for use in host cells over a wide range of prokaryotic and eukaryotic organisms.

In-general, of course, prokaryotes are preferred for cloning of DNA sequences in constructing the vectors useful in the invention. For example, *E. coli* K12 strain 294 (ATCC No. 31446) is particularly useful. Other microbial strains which may be used include *E. coli* strains such as *E. coli* B, and *E. coli* X1776 (ATTC No. 31537). These examples are, of course, intended to be illustrative rather than limiting.

Prokaryotes may also be used for expression. The aforementioned strains, as well as *E. coli* W3110 (F^-, λ^-, prototrophic, ATTC No. 27325), bacilli such as *Bacillus subtilus*, and other enterobacteriaceae such as *Salmonella typhimurium* or *Serratia marcesans*, and various Pseudomonas species may be used.

In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. For example, *E. coli* is typically transformed using pBR322, a plasmid derived from an *E. coli* species (Bolivar, et al., *Gene* 2: 95 (1977)). PBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR322 plasmid, or other microbial plasmid must also contain, or be modified to contain, promoters which can be used by the microbial organism for expression of its own proteins. Those promoters most commonly used in recombinant DNA construction include the β-lactamase (penicillinase) and lactose promoter systems Chang et al, *Nature* 275: 615 (1978); Itakura, et al, *Science* 198: 1056 (1977); (Goeddel et al, *Nature* 281: 544 (1979)) and a tryptophan (trp) promoter system (Goeddel, et al, *Nucleic kids Res.*, 8: 4057 (1980); EPO Appl Publ No. 0036776). While these are the most commonly used, other microbial promoters have been discovered and utilized., and details concerning their nucleotide sequences have been published, enabling a skilled worker to ligate them functionally with plasmid vectors (Siebenlist, et al, Cell 20: 269 (1980)).

In addition to prokaryates, eukaryotic microbes, such as yeast cultures may also be used. *Saccharomyces cerevisiae*, or common baker's yeast is the most commonly used among eukaryotic microorganisms, although a number of other strains are commonly available. For expression in Saccharomyces, the plasmid YRp7, for example, (Stinchcomb et al, *Nature*, 282: 39 (1979); Kingsman et al, *Gene*, 7: 141 (1979); Tschemper, et al, *Gene*, 10: 157 (1980)) is commonly used. This plasmid already contains the trp1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics*, 85: 12 (1977)). The presence of the trp1 lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable promoting sequences in yeast vectors include the promoters for 3-phosphoglycerate kinase (Hitzeman, et al., *J. Biol. Chem.*, 255: 2073 (1980)) or other glycolytic enzymes (Hess, et al, *J. Adv. Enzyme Reg.*, 7: 149 (1968); Holland, et al, *Biochemistry*, 17: 4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. In constructing suitable expression plasmids, the termination sequences associated with these genes are also ligated into the expression vector 3′ of the sequence desired to be expressed to provide polyadenylation of the mRNA and termination. Other promoters, which have the additional advantage of transcription controlled by growth conditions are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, and the aforementioned glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization (Holland, ibid.). Any plasmid vector containing yeast-compatible promoter, origin of replication and termination sequences is suitable.

In addition to microorganisms cultures of cells derived from multicellular organisms may also be used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. However interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure in recent years (*Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973)). Examples of such useful host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cell lines, and W138, BHK, COS-7 and MDCK cell lines. Expression vectors for such cells ordinarily include (if necessary) an origin of replication, a promoter located in front of the gene to be expressed, along with any necessary ribosome binding sites, RNA splice sites, polyadenylation site, and transcriptional terminator sequences.

For use in mammalian cells, the control functions on the expression vectors are often provided by viral material. For example, commonly used promoters are derived from polyoma, Adenovirus 2, and most frequently Simian Virus 40 (SV40). The early and late promoters of SV40 virus are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers, et al, *Nature*, 273: 113 (1978)) incorporated herein by reference. Smaller or larger SV40 fragments may also be used, provided there is included the approximately 250 bp sequence extending from the Hind III site toward the Bgl I site located in the viral origin of replication. Further, it is also possible, and often desirable, to utilize promoter or control sequences normally associated with the desired gene sequence, provided such control sequences are compatible with the host cell systems.

An origin of replication may be provided either by construction of the vector to include an exogenous origin, such as may be derived from SV40 or other viral (e.g. Polyoma, Adeno, VSV, BPV, etc.) source, or may be provided by the host cell chromosomal replication mechanism. If the vector is integrated into the host cell chromosome, the latter is often sufficient.

It will be understood that this invention, although described herein in terms of a preferred embodiment, should not be construed as limited to those host cells, vectors and expression systems exemplified.

C. Methods Employed

C.1 Transformation

If cells without formidable cell wall barriers are used as host cells, transfection is carried out by the calcium phosphate precipitation method as described by Graham and Van der Eb, *Virology*, 52: 546 (1978). However, other methods for introducing DNA into cells such as by nuclear injection or by protoplast fusion may also be used.

If prokaryotic cells or cells which contain substantial cell wall constructions are used, the preferred method of transfection is calcium treatment using calcium chloride as described by Cohen, F. N. et al *Proc. Natl. Acad. Sci.* (*USA*) 69: 2110 (1972).

C.2 Vector Construction

Construction of suitable vectors containing the desired coding and control sequences employ standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and religated in the form desired to form the plasmids required. The methods employed are not dependent on the DNA source, or intended host.

Cleavage is performed by treating with restriction enzyme (or enzymes) in suitable buffer. In general, about 1 µg plasmid or DNA fragments is used with about 1 unit of enzyme in about 20 µl of buffer solution. (Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer.) Incubation times of about 1 hour at 37° C. are workable. After incubations, protein is removed by extraction with phenol and chloroform, and the nucleic acid is recovered from the aqueous fraction by precipitation with ethanol.

If blunt ends are required, the preparation is treated for 15 minutes at 15° with 10 units of *E. coli* DNA Polymerase I (Klenow), phenol-chloroform extracted, and ethanol precipitated.

Size separation of the cleaved fragments is performed using 6 percent polyacrylamide gel described by Goeddel, D., et al, *Nucleic Acids Res.,* 8: 4057 (1980) incorporated herein by reference.

For ligation, approximately equimolar amounts of the desired components, suitably end tailored to provide correct matching are treated with about 10 units T4 DNA ligase per 0.5 µg DNA. (When cleaved vectors are used as components, it may be useful to prevent religation of the cleaved vector by pretreatment with bacterial alkaline phosphatase.)

In the examples described below correct ligations for plasmid construction are confirmed by transforming *E. coli* K12 strain 294 (ATCC 31446) with the ligation mixture. Successful transformants were selected by ampicillin or tetracycline resistance depending on the mode of plasmid construction. Plasmids from the transformants were then prepared, analyzed by restriction and/or sequenced by the method of Messing, et al, *Nucleic Acids Res.,* 9:309 (1981) or by the method of Maxam, et al, *Methods in Enzymology,* 65:499 (1980).

D. Outline of Procedures

D.1 Mammalian Antibodies

The first type of antibody which forms a part of this invention, and is prepared by the methods thereof, is “mammalian antibody”-one wherein the heavy and light chains mimic the amino acid sequences of an antibody otherwise produced by a mature mammalian B lymphocyte either in situ or when fused with an immortalized cell as part of a hybridoma culture. In outline, these antibodies are produced as follows:

Messenger RNA coding for heavy or light chain is isolated from a suitable source, either mature B cells or a hybridoma culture, employing standard techniques of RNA isolation, and the use of oligo-dT cellulose chromatography to segregate the poly-A mRNA. The poly-A mRNA may, further, be fractionated to obtain sequences of sufficient size to code for the amino acid sequences in the light or heavy chain of the desired antibody as the case may be.

A cDNA library is then prepared from the mixture of mRNA using a suitable primer, preferably a nucleic acid sequence which is characteristic of the desired cDNA. Such a primer may be hypothesized and synthesized based on the amino acid sequence of the antibody if the sequence is known. In the alternative cDNA from unfractionated poly-A mRNA from a cell line producing the desired antibody or poly-dT may also be used. The resulting cDNA is optionally size fractionated on polyacrylamide gel and then extended with, for example, dC residues for annealing with pBR322 or other suitable cloning vector which has been cleaved by a suitable restriction enzyme, such as Pst I, and extended with dG residues. Alternative means of forming cloning vectors containing the cDNA using other tails and other cloning vector remainder may, of course, also be used but the foregoing is a standard and preferable choice. A suitable host cell strain, typically *E. coli,* is transformed with the annealed cloning vectors, and the successful transformants identified by means of, for example, tetracycline resistance or other phenotypic characteristic residing on the cloning vector plasmid.

Successful transformants are picked and transferred to microtiter dishes or other support for further growth and preservation. Nitrocellulose filter imprints of these growing cultures are then probed with suitable nucleotide sequences containing bases known to be complementary to desired sequences in the cDNA. Several types of probe may be used, preferably synthetic single stranded DNA sequences labeled by kinasing with ATP^{32}. The cells fixed to the nitrocellulose filter are lysed, the DNA denatured, and then fixed before reaction with kinased probe. Clones which successfully hybridize are detected by contact with a photoplate, then plasmids from the growing colonies isolated and sequenced by means known in the art to verify that the desired portions of the gene are present.

The desired gene fragments are excised and tailored to assure appropriate reading frame with the control segments when inserted into suitable expression vectors. Typically, nucleotides are added to the 5′ end to include a start signal and a suitably positioned restriction endonuclease site.

The tailored gene sequence is then positioned in a vector which contains a promoter in reading frame with the gene and compatible with the proposed host cell. A number of plasmids such as those described in U.S. patent application Ser. Nos. 307,473; 291,892; and 305,657 (EPO Publ. Nos. 0036776; 0048970 and 0051873) have been described which already contain the appropriate promoters, control sequences, ribosome binding sites, and transcription termination sites, as well as convenient markers.

In the present invention, the gene coding for the light chain and that coding for the heaving chain are recovered separately by the procedures outlined above. Thus then may be inserted into separate expression plasmids, or together in the same plasmid, so long as each is under suitable promoter and translation control.

The expression vectors constructed above are then used to transform suitable cells. The light and heavy chains may be transformed into separate cell cultures, either of the same or of differing species; separate plasmids for light and heavy chain may be used to co-transform a single cell culture, or, finally, a single expression plasmid containing both genes and capable of expressing the genes for both light and heavy chain may be transformed into a single cell culture.

Regardless of which of the three foregoing options is chosen, the cells are grown under conditions appropriate to the production of the desired protein. Such conditions are primarily mandated by the type of promoter and control systems used in the expression vector, rather than by the nature of the desired protein. The protein thus produced is then recovered from the cell culture by methods known in the art, but choice of which is necessarily dependent on the form in which the protein is expressed. For example, it is common for mature heterologous proteins expressed in *E. coli* to be deposited within the cells as insoluble particles which require cell lysis and solubilization in denaturant to permit recovery. On the other hand, proteins under proper synthesis circumstances, in yeast and bacterial strains, can be secreted into the medium (yeast and gram positive bacteria) or into the periplasmic space (gram negative bacteria) allowing recovery by less drastic procedures. Tissue culture cells as hosts also appear, in general, to permit reasonably facile recovery of heterologous proteins.

When heavy and light chain are coexpressed in the same host, the isolation procedure is designed so as to recover reconstituted antibody. This can be accomplished in vitro as described below, or might be possible in vivo in a microorganism which secretes the IgG chains out of the reducing environment of the cytoplasm. A more detailed description is given in D.2, below.

D.2 Chain Recombination Techniques

The ability of the method of the invention to produce heavy and light chains or portions thereof, in isolation from each other offers the opportunity to obtain unique and unprecedented assemblies of immunoglobulins, Fab regions, and univalent antibodies. Such preparations require the use of techniques to reassemble isolated chains. Such means are known in the art, and it is, thus, appropriate to review them here.

While single chain disulfide bond containing proteins have been reduced and reoxidized to regenerate in high yield native structure and activity (Freedman, R. B., et al. In *Enzymology of Post Translational Modification of Proteins,* I: 157-212 (1980) Academic Press, NY.), proteins which consist of discontinuous polypeptide chains held together by disulfide bonds are more difficult to reconstruct in vitro after reductive cleavage. Insulin, a cameo case, has received much experimental attention over the years, and can now be reconstructed so efficiently that an industrial process has been built around it (Chance, R. E., et al., In *Peptides: Proceedings of the Seventh Annual American Peptide Symposium* (Rich, D. H. and Gross. E., eds.) 721-728, Pierce Chemical Co., Rockford, Ill. (1981)).

Immunoglobulin has proved a more difficult problem than insulin. The tetramer is stabilized intra and intermolecularly by 15 or more disulfide bonds. It has been possible to recombine heavy and light chains, disrupted by cleavage of only the interchain disulfides, to regain antibody activity even without restoration of the inter-chain disulfides (Edelman, G. M., et al., *Proc. Natl. Acad. Sci. (USA)* 50: 753 (1963)). In addition, active fragments of IgG formed by proteolysis (Fab fragments of ˜50,000 MW) can be split into their fully reduced heavy chain and light chain components and fairly efficiently reconstructed to give active antibody (Haber, E., *Proc. Natl. Acad. Sci. (USA)* 52: 1099 (1964); Whitney, P. L., et al., *Proc. Natl. Acad. Sci. (USA)* 53: 524 (1965)). Attempts to reconstitute active antibody from fully reduced native IgG have been largely unsuccessful, presumably due to insolubility of the reduced chains and of side products or intermediates in the refolding

pathway (see discussion in Freedman, M. H., et al., *J. Biol. Chem.* 241: 5225 (1966)). If, however, the immunoglobulin is randomly modified by polyalanylation of its lysines before complete reduction, the separated chains have the ability to recover antigen-combining activity upon reoxidation (ibid).

A particularly suitable method for immunoglobulin reconstitution is derivable from the now classical insulin recombination studies, wherein starting material was prepared by oxidative sulfitolysis, thus generating thiol-labile S-sulfonate groups at all cysteines in the protein, non-reductively breaking disulfides (Chance et al. (supra)). Oxidative sulfitolysis is a mild disulfide cleavage reaction (Means, G. E., et al., *Chemical Modification of Proteins,* Holden-Day, San Francisco (1971)) which is sometimes more gentle than reduction, and which generates derivatives which are stable until exposed to mild reducing agent at which time disulfide reformation can occur via thiol-disulfide interchange. In the present invention the heavy and light chain S-sulfonates generated by oxidative sulfitolysis were reconstituted utilizing both air oxidation and thiol-disulfide interchange to drive disulfide bond formation. The general procedure is set forth in detail in U.S. Ser. No. 452,187, filed Dec. 22, 1982 (EPO Appln. No. 83.307840.5), incorporated herein by reference.

D.3 Variants Permitted by Recombinant Technology

Using the techniques described in paragraphs D.1 and D.2, additional operations which were utilized to gain efficient production of mammalian antibody can be varied in quite straightforward and simple ways to produce a great variety of modifications of this basic antibody form. These variations are inherent in the use of recombinant technology, which permits modification at a genetic level of amino acid sequences in normally encountered mammalian immunoglobulin chains, and the great power of this approach lies in its ability to achieve these variations, as well as in its potential for economic and specific production of desired scarce, and often contaminated, molecules. The variations also inhere in the ability to isolate production of individual chains, and thus create novel assemblies.

Briefly, since genetic manipulations permit reconstruction of genomic material in the process of construction of expression vectors, such reconstruction can be manipulated to produce new coding sequences for the components of “natural” antibodies or immunoglobulins. As discussed in further detail below, the coding sequence for a mammalian heavy chain may not be derived entirely from a single source or single species, but portions of a sequence can be recovered by the techniques described in D.1 from differing pools of mRNA, such as murine-murine hybridomas, human-murine hybridomas, or B cells differentiated in response to a series of antigen challenges. The desired portions of the sequences in each case can be recovered using the probe and analysis techniques described in D.1, and recombined in an expression vector using the same ligation procedures as would be employed for portions of the same model sequence. Such chimeric chains can be constructed of any desired length; hence, for example, a complete heavy chain can be constructed, or only sequence for the Fab region thereof.

The additional area of flexibility which arises from the use of recombinant techniques results from the power to produce heavy and light chains or fragments thereof in separate cultures or of unique combinations of heavy and light chain in the same culture, and to prevent reconstitution of the antibody or immunoglobulin aggregation until the suitable components are assembled. Thus, while normal antibody production results automatically in the formation of “mammalian antibodies” because the light and heavy chain portions are constructed in response to a particular determinant in the same cell, the methods of the present invention present the opportunity to assemble entirely new mixtures. Somewhat limited quantities of “hybrid” antibodies have been produced by “quadromas” i.e., fusions of two hybridoma cell cultures which permit random assemblies of the heavy and light chains so produced.

The present invention permits a more controlled assembly of desired chains, either by mixing the desired chains in vitro, or by transforming the same culture with the coding sequences for the desired chains.

D.4 Composite Immunoglobulins

The foregoing procedure, which describes in detail the recombinant production of mammalian antibodies is employed with some modifications to construct the remaining types of antibodies or NSIs encompassed by the present invention. To prepare the particular embodiment of composite non-specific immunoglobulin wherein the homology of the chains corresponds to the sequences of immunoglobulins of different specificities, it is of course, only necessary to prepare the heavy and light chains in separate cultures and reassemble them as desired.

For example, in order to make an anti-CEA light chain/antihepatitis heavy chain composite antibody, a suitable source for the mRNA used as a template for the light chain clone would comprise, for instance, the anti CEA producing cell line of paragraph E.1. The mRNA corresponding to heavy chain would be derived from B cells raised in response to hepatitis infection or from hybridoma in which the B cell was of this origin. It is clear that such composites can be assembled using the methods of the invention almost at will, and are limited only by available sources of mRNA suitable for use as templates for the respective chains. All other features of the process are similar to those described above.

D.5 Hybrid Antibodies

Hybrid antibodies are particularly useful as they are capable of simultaneous reaction with more than one antigen. Pairs of

heavy and light chains corresponding to chains of antibodies for different antigens, such as those set forth in paragraph D.4 are prepared in four separate cultures, thus preventing premature assembly of the tetramer. Subsequent mixing of the four separately prepared peptides then permits assembly into the desired tetramers. While random aggregation may lead to the formation of considerable undesired product, that portion of the product in which homologous light and heavy chains are bound to each other and mismatched to another pair gives the desired hybrid antibody.

D.6 Chimeric Antibodies

For construction of chimeric antibodies (wherein, for example, the variable sequences are separately derived from the constant sequences) the procedures of paragraph C.1 and D.2 are again applicable with appropriate additions and modifications. A preferred procedure is to recover desired portions of the genes encoding for parts of the heavy and light chains from suitable, differing, sources and then to religate these fragments using restriction endonucleases to reconstruct the gene coding for each chain.

For example, in a particularly preferred chimeric construction, portions of the heavy chain gene and of the light chain gene which encode the variable sequences of antibodies produced by a murine hybridoma culture are recovered and closed from this culture and gene fragments encoding the constant regions of the heavy and light chains for human antibodies recovered and cloned from, for example, human myeloma cells. Suitable restriction enzymes may then be used to ligate the variable portions of the mouse gene to the constant regions of the human gene for each of the two chains. The chimeric chains are produced as set forth in D.1, aggregated as set forth in D.2 and used in the same manner as the non-chimeric forms. Of course, any splice point in the chains can be chosen.

D.7 Altered Antibodies

Altered antibodies present, in essence, an extension of chimeric ones. Again, the techniques of D.1 and D.2 are applicable; however, rather than splicing portions of the chain(s), suitable amino acid alterations, deletions or additions are made using available techniques such as mutagenesis (supra). For example, genes which encode antibodies having diminished complement fixation properties, or which have enhanced metal binding capacities are prepared using such techniques. The latter type may, for example, take advantage of the known gene sequence encoding metalothionein II (Karin, M., et al., *Nature,* 299: 797 (1982)). The chelating properties of this molecular fragment are useful in carrying heavy metals to tumor sites as an aid in tumor imaging (Scheinberg, D. A., et al., *Science,* 215: 19 (1982).

D.8 Univalent Antibodies

In another preferred embodiment, antibodies are formed which comprise one heavy and light chain pair coupled with the Fc region of a third (heavy) chain. These antibodies have a particularly useful property. They can, like ordinary antibodies, be used to target antigenic surfaces of tissues, such as tumors, but, unlike ordinary antibodies, they do not cause the antigenic surfaces of the target tissue to retreat and become non-receptive. Ordinary antibody use results in aggregation and subsequent inactivation, for several hours, of such surface antigens.

The method of construction of univalent antibodies is a straightforward application of the invention. The gene for heavy chain of the desired Fc region is cleaved by restriction enzymes, and only that portion coding for the desired Fc region expressed. This portion is then bound using the technique of D.2 to separately produced heavy chain the desired pairs separated from heavy/heavy and Fc/Fc combinations, and separately produced light chain added. Pre-binding of the two heavy chain portions thus diminishes the probability of formation of ordinary antibody.

D.9 Fab Protein

Similarly, it is not necessary to include the entire gene for the heavy chain portion. All of the aforementioned variations can be superimposed on a procedure for Fab protein production and the overall procedure differs only in that the portion of the heavy chain coding for the amino terminal **220** amino acids is employed in the appropriate expression vector.

E. Specific Examples of Preferred Embodiments

The invention has been described above in general terms and there follow several specific examples of embodiments which set forth details of experimental procedure in producing the desired antibodies. Example E.1 sets forth the general procedure for preparing anti CEA antibody components, i.e. for a “mammalian antibody”. Example E.3 sets forth the procedure for reconstitution and thus is applicable to preparation of mammalian, composite, hybrid and chimeric immunoglobulins, and Fab proteins and univalent antibodies. Example E.4 sets forth the procedure for tailoring the heavy or light chain so that the variable and constant regions may be derived from different sources. Example E.5 sets forth the method of obtaining a shortened heavy chain genome which permits the production of the Fab regions and, in an analogous manner, Fc region.

The examples set forth below are included for illustrative purposes and do not limit the scope of the invention.

E.1 Construction of Expression Vectors for Murine anti-CEA Antibody Chains and Peptide Synthesis

Carcinoembryonic antigen (CEA) is associated with the surface of certain tumor cells of human origin (Gold, P., et al., *J.*

Exp. Med., 122: 467 (1965)). Antibodies which bind to CEA (anti-CEA antibodies) are useful in early detection of these tumors (Van Nagell, T. R., et al., *Cancer Res.* 40: 502 (1980)), and have the potential for use in treatment of those human tumors which appear to support CEA at their surfaces. A mouse hybridoma cell line which secretes anti-CEA antibodies of the IgG_1 class, CEA.66-E3, has been prepared as described by Wagener, C, et al., *J. Immunol.* (in press) which is incorporated herein by reference, and was used as mRNA source. The production of anti CEA antibodies by this cell line was determined. The N-terminal sequences of the antibodies produced by these cells was compared with those of monoclonal anti CEA as follows. Purified IgG was treated with PCAse (Podell, D. N., et al., BBRC 81: 176 (1978)), and then dissociated in 6M guanidine hydrochloride, 10 mM 2-mercaptoethanol (1.0 mg of immunoglobulin, 5 min, 100° C. water bath). The dissociated chains were separated on a Waters Associates alkyl phenyl column using a linear gradient from 100 percent A (0.1 percent TFA-water) to 90 percent B (TFA/H_2O/MeCN 0.1/9.9/90) at a flow rate of 0.8 ml/min. Three major peaks were eluted and analyzed on SDS gels by silver staining. The first two peaks were pure light chain (MW 25,000 daltons), the third peak showed a (7:3) mixture of heavy and light chain. 1.2 nmoles of light chain were sequenced by the method of Shively, J. E., *Methods in Enzymology,* 79: 31 (1981), with an NH_2-terminal yield of 0.4 nmoles. A mixture of heavy and light chains (3 nmoles) was also sequenced, and sequence of light chain was deducted from the double sequence to yield the sequence of the heavy chain.

In the description which follows, isolation and expression of the genes for the heavy and light chains for anti CEA antibody produced by CEA.66-E3 are described. As the constant regions of these chains belong to the gamma and kappa families, respectively, “light chain” and “kappa chain”, and “heavy chain” and “gamma chain”, respectively, are used interchangeably below.

E.1.1 Isolation of Messenger RNA for Anti CEA Light and Heavy (Kappa and Gamma) Chains

Total RNA from CEA.66-E3 cells was extracted essentially as reported by Lynch et al, *Virology,* 98: 251 (1979). Cells were pelleted by centrifugation and approximately 1 g portions of pellet resuspended in 10 ml of 10 mM NaCl, 10 mM Tris HCl (pH 7.4), 1.5 mM $MgCl_2$. The resuspended cells were lysed by addition of non-ionic detergent NP-40 to a final concentration of 1 percent, and nuclei removed by centrifugation. After addition of SDS (pH 7.4) to 1 percent final concentration, the supernatant was extracted twice with 3 ml portions of phenol (redistilled)/chloroform: isoamyl alcohol 25:1 at 4° C. The aqueous phase was made 0.2 M in NaCl and total RNA was precipitated by addition of two volumes of 100 percent ethanol and overnight storage at −20° C. After centrifugation, polyA mRNA was purified from total RNA by oligo-dT cellulose chromatography as described by Aviv and Leder, *Proc. Nat'l, Acad. Sci. (USA),* 69: 1408 (19672), 142 µg of polyA mRNA was obtained from 1 g cells.

E.1.2 Preparation of *E. coli* Colony Library Containing Plasmids with Heavy and Light DNA Sequence Inserts

5 µg of the unfractionated polyA mRNA prepared in paragraph E.1.1 was used as template for oligo-dT primed preparation of double-stranded (ds) cDNA by standard procedures as described by Goeddel et al., *Nature* 281: 544 (1979) and Wickens et al., *J. Biol. Chem.* 253: 2483 (1978) incorporated herein by reference. The cDNA was size fractionated by 6 percent polyacrylamide gel electrophoresis and 124 ng of ds cDNA greater than 600 base pairs in length was recovered by electroelution. A 20 ng portion of ds cDNA was extended with deoxy C residues using terminal deoxynucleotidyl transferase as described in Chang et al., *Nature* 275: 617 (1978) incorporated herein by reference, and annealed with 200 ng of the plasmid pBR322 (Bolivar et al., *Gene* 2: 95 (1977)) which had been cleaved with Pst I and tailed with deoxy G. Each annealed mixture was then transformed into *E. coli* K12 strain 294 (ATCC No. 31446). Approximately 8500 ampicillin sensitive, tetracycline resistant transformants were obtained.

E.1.3 Preparation of Synthetic Probes

The 14mer, 5′ GGTGGGAAGATGGA 3′ complementary to the coding sequence of constant region for mouse MOPC21 kappa chain which begins 25 basepairs 3′ of the variable region DNA sequence was used as kappa chain probe. A 15 mer, 5′ GACCAGGCATCCCAG 3′, complementary to a coding sequence located 72 basepairs 3′ of the variable region DNA sequence for mouse MOPC21 gamma chain was used to probe gamma chain gene.

Both probes were synthesized by the phosphotriester method described in German Offenlegungschrift 2644432, incorporated herein by reference, and made radioactive by kinasing as follows: 250 ng of deoxyoligonucleotide were combined in 25 µl of 60 mM Tris HCl (pH 8), 10 mM $MgCl_2$, 15 mM beta-mercaptoethanol, and 100 µCi (γ-^{32}P) ATP (Amersham, 5000 Ci/mMole). 5 units of T4 polynucleotide kinase were added and the reduction was allowed to proceed at 37° C. for 30 minutes and terminated by addition of EDTA to 20 mM.

E.1.4 Screening of Colony Library for Kappa or Gamma Chain Sequences

˜2000 colonies prepared as described in paragraph E.1.2 were individually inoculated into wells of microtitre dishes containing LB (Miller, Experiments in Molecular Genetics, p. 431-3, Cold Spring Harbor, Lab., Cold Spring Harbor, N.Y. (1972))+5 µg/ml tetracycline and stored at −20° C. after addition of DMSO to 7 percent. Individual colonies from this library were transferred to duplicate sets of Schleicher and Schuell BA85/20 nitrocellulose filters and grown on agar plates

containing LB+5 μg/ml tetracycline. After ˜10 hours growth at 37° C. the colony filters were transferred to agar plates containing LB+5 μg/ml tetracycline and 12.5 μg/ml chloramphenicol and reincubated overnight at 37° C. The DNA from each colony was then denatured and fixed to the filter by a modification of the Grunstein-Hogness procedure as described in Grunstein et al., *Proc. Natl. Acad. Sci. (USA)* 72: 3961 (1975), incorporated herein by reference. Each filter was floated for 3 minutes on 0.5 N NaOH, 1.5 M NaCl to lyse the colonies and denature the DNA then neutralized by floating for 15 minutes on 3 M NaCl, 0.5 M Tris HCl (pH 7.5). The filters were then floated for an additional 15 minutes on 2XSSC, and subsequently baked for 2 hours in an 80° C. vacuum oven. The filters were prehybridized for ˜2 hours at room temperature in 0.9 M NaCl, 1X Denhardts, 100 mM Tris HCl (pH 7.5), 5 mM Na-EDTA, 1 mM ATP, 1 M sodium phosphate (dibasic), 1 mM sodium pyrophosphate, 0.5 percent NP-40, and 200 μg/ml *E. coli* t-RNA, and hybridized in the same solution overnight, essentially as described by Wallace et al. *Nucleic Acids Research* 9: 879 (1981) using ˜40×10^6 cpm of either the kinased kappa or gamma probe described above.

After extensive washing at 37° C. in 6X SSC, 0.1 percent SDS, the filters were exposed to Kodak XR-5 X-ray film with DuPont Lightning-Plus intensifying screens for 16-24 hours at −80° C. Approximately 20 colonies which hybridized with kappa chain probe and 20 which hybridized with gamma chain probe were characterized.

E.1.5 Characterization of Colonies which Hybridize to Kappa DNA Sequence Probe

Plasmid DNAs isolated from several different transformants which hybridized to kappa chain probe were cleaved with Pst I and fractionated by polyacrylamide gel electrophoresis (PAGE). This analysis demonstrated that a number of plasmid DNAs contained cDNA inserts large enough to encode full length kappa chain. The complete nucleotide sequence of the cDNA insert of one of these plasmids was determined by the dideoxynucleotide chain termination method as described by Smith, *Methods Enzymol.* 65, 560 (1980) incorporated herein by reference after subcloning restriction endonuclease cleavage fragments into M13 vectors (Messing et al., *Nucleic Acids Research* 9: 309 (1981). FIG. 2 shows the nucleotide sequence of the cDNA insert of pK17G4 and FIG. 3 shows the gene sequence with the corresponding amino acid sequence. Thus, the entire coding region of mouse anti-CEA kappa chain was isolated on this one large DNA fragment. The amino acid sequence of kappa chain, deduced from the nucleotide sequence of the pK17G4 cDNA insert, corresponds perfectly with the first 23 N-terminal amino acids of mature mouse anti-CEA kappa chain as determined by amino acid sequence analysis of purified mouse anti-CEA kappa chain. The coding region of pK17G4 contains 27 basepairs or 9 amino acids of the presequence and 642 basepairs or 214 amino acids of the mature protein. The mature unglycosylated protein (MW 24,553) has a variable region of 119 amino acids, including the J1 joining region of 12 amino acids, and a constant region of 107 amino acids. After the stop codon behind amino acids 215 begins 212 basepairs of 3′ untranslated sequence up to the polyA addition. The kappa chain probe used to identify pK17G4 hybridizes to nucleotides 374-388 (FIG. **2**).

E.1.6 Characterization of Colonies which Hybridize to Gamma 1 DNA Probe

Plasmid DNA isolated from several transformants positive for hybridization with the heavy chain gamma 1 probe was subjected to Pst I restriction endonuclease analysis as described in E.1.5. Plasmid DNAs demonstrating the largest cDNA insert fragments were selected for further study. Nucleotide sequence coding for mouse heavy (gamma-1) chain, shows an NcoI restriction endonuclease cleavage site near the junction between variable and constant region. Selected plasmid DNAs were digested with both PstI and NcoI and sized on polyacrylamide. This analysis allowed identification of a number of plasmid DNAs that contain NcoI restriction endonuclease sites, although none that demonstrate cDNA insert fragments large enough to encode the entire coding region of mouse anti-CEA heavy chain.

In one plasmid isolated, p γ298 the cDNA insert of about 1300 bp contains sequence information for the 5′ untranslated region, the signal sequence and the N-terminal portion of heavy chain. Because pγ298 did not encode the C-terminal sequence for mouse anti-CEA gamma 1 chain, plasmid DNA was isolated from other colonies and screened with PstI and NcoI. The C-terminal region of the cDNA insert of pγ11 was sequence and shown to contain the stop codon, 3′ untranslated sequence and that portion of the coding sequence missing from p γ298.

FIG. 4 presents the entire nucleotide sequence of mouse anti-CEA heavy chain (as determined by the dideoxynucleotide chain termination method of Smith, *Methods Enzymol.,* 65: 560 (1980)) and FIG. 5 includes the translated sequence.

The amino acid sequence of gamma 1 (heavy chain) deduced from the nucleotide sequence of the pγ298 cDNA insert corresponds perfectly to the first 23 N-terminal amino acids of mature mouse anti-CEA gamma 1 chain as determined by amino acid sequence analysis of purified mouse anti-CEA gamma-1 chain. The coding region consists of 57 basepairs or 19 amino acids of presequences and 1346 basepairs or 447 amino acids of mature protein. The mature unglycosolated protein (MW 52,258) has a variable region of 135 amino acids, including a D region of 12 amino acids, and a J4 joining region of 13 amino acids. The constant region is 324 amino acids. After the stop codon behind amino acid 447 begins 96 bp of 3′ untranslated sequences up to the polyA addition. The probe used to identify pγ298 and pγ11 hybridized to nucleotides 528-542 (FIG. **4**).

E.1.7 Construction of a Plasmid for Direct Expression of Mouse Mature Anti-CEA Kappa Chain Gene, pKCEAtrp207-1*

FIG. 6 illustrated the construction of pKCEAtrp207-1*

First, an intermediate plasmid pHGH207-1*, having a single trp promoter, was prepared as follows:

The plasmid pHGH 207 (described in U.S. patent application Ser. No. 307,473, filed Oct. 1, 1981 (EPO Publn. No. 0036776)) has a double lac promoter followed by the trp promoter, flanked by EcoR I sites and was used to prepare pHGH207-1. pHGH207 was digested with BamH 1, followed by partial digestion with EcoR I. The largest fragment, which contains the entire trp promoter, was isolated and ligated to the largest EcoR I- BamH I fragment from pBR322, and the ligation mixture used to transform *E. coli* 294. Tet^R Amp^R colonies were isolated, and most of them contained pHGH207-1. pHGH207-1* which lacks the EcoR1 site between the amp^R gene and the trp promoter, was obtained by partial digestion of pHGH207-1 with EcoR I, filling in the ends with Klenow and dNTPs, and religation.

5 μg of pHGH207-1* was digested with EcoRI, and the ends extended to blunt ends using 12 units of DNA Polymerase I in a 50 μl reaction containing 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4) and 1 mM in each dNTP at 37° C. for 1 hour, followed by extraction with phenol/$CHCl_3$ and precipitation with ethanol. The precipitated DNA was digested with BamH I, and the large vector fragment (fragment 1) purified using 5 percent polyacrylamide gel electrophoresis, electroelution, phenol/$CHCl_3$ extraction and ethanol precipitation.

The DNA was resuspended in 50 μl of 10 mM Tris pH 8, 1 mM EDTA and treated with 500 units Bacterial Alkaline Phosphatase (BAP) for 30′ at 65° followed by phenol/$CHCl_3$ extraction and ethanol precipitation.

A DNA fragment containing part of the light chain sequence was prepared as follows: 7 μg of pH17G4 DNA was digested with Pst I and the kappa chain containing cDNA insert was isolated by 6 percent gel electrophoresis, and electroelution. After phenol/$CHCl_3$ extraction, ethanol precipitation and resuspension in water, this fragment was digested with Ava II. The 333 bp Pst I-Ava II DNA fragment was isolated and purified from a 6 percent polyacrylamide gel.

A 15 nucleotide DNA primer was synthesized by the phosphotriester method G. O. 2,644,432 (supra) and has the following sequence:

Met Asp Ile Val Met
5′ ATG GAC ATT GTT ATG 3′

The 5′ methionine serves as the initiation codon. 500 ng of this primer was phosphorylated at the 5′ end with 10 units T4 DNA kinase in 20 μl reaction containing 0.5 mM ATP. ˜200 ng of the Pst I-Ava II DNA fragment was mixed with the 20 μl of the phosphorylated primer, heated to 95° C. for 3 minutes and quick frozen in a dry-ice ethanol bath. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C. this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, and digested to completion with Sau 3A. The reaction mixture was then electrophoresed on a 6 percent polyacrylamide gel and ˜50 ng of the 182 basepair amino-terminal blunt-end to Sau 3A fragment (fragment 2) was obtained after electroelution.

100 ng of fragment 1 (supra) and 50 ng of fragment 2 were combined in 20 μl of 20 mM Tris HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM DTT, 2.5 mM ATP and 1 unit of T4 DNA ligase. After overnight ligation at 14° C. the reaction was transformed into *E. coli* K12 strain 294. Restriction endonuclease digestion of plasmid DNA from a number of ampicillin resistant transformants indicated the proper construction and DNA sequence analysis proved the desired nucleotide sequence through the initiation codon of this new plasmid, pKCEAInt1 (FIG. **6**).

The remainder of the coding sequence of the kappa light chain gene was prepared as follows:

The Pst I cDNA insert fragment from 7 μg of K17G4 DNA was partially digested with Ava II and the Ava II cohesive ends were extended to blunt ends in a DNA Polymerase I large fragment reaction. Following 6 percent polyacrylamide gel electrophoresis the 686 basepair Pst I to blunt ended Ava II DNA fragment was isolated, purified and subjected to Hpa II restriction endonuclease digestion. The 497 basepair Hpa II to blunt ended Ava II DNA fragment (fragment 3) was isolated and purified after gel electrophoresis.

10 μg of pKCEAInt1 DNA was digested with Ava I, extended with DNA polymerase I large fragment, and digested with Xba I. Both the large blunt ended Ava I to Xba I vector fragment and the small blunt ended Ava I to Xba I fragment were isolated and purified from a 6 percent polyacrylamide gel after electrophoresis. The large vector fragment (fragment 4) was treated with Bacterial Alkaline Phosphatase (BAP), and the small fragment was digested with Hpa II, electrophoresed on a 6 percent polyacrylamide and the 169 basepair Xba I-Hpa II DNA fragment (fragment 5) was purified. ˜75 ng of fragment 4, ˜50 ng of fragment 3 and ˜50 ng of fragment 5 were combined in a T4 DNA ligase reaction and incubated overnight at 14°, and the reaction mixture transformed into *E. coli* K12 strain 294. Plasmid DNA from six ampicillin resistant transformants were analyzed by restriction endonuclease digestion. One plasmid DNA demonstrated the proper construction and was designated pKCEAInt2.

Final construction was effected by ligating the K-CEA fragment, including the trp promoter from pKCEAInt2 into pBR322(XAP). (pBR322(XAP) is prepared as described in U.S. application Ser. No. 452,227, filed Dec. 22, 1982; from pBR322 by deletion of the AvaI-PvuII fragment followed by ligation.)

The K-CEA fragment was prepared by treating pKCEAInt2 with Ava I, blunt ending with DNA polymerase I (Klenow fragment) in the presence of DNTPs, digestion with Pst I and isolation of the desired fragment by gel electrophoresis and electroelution.

The large vector fragment from pBR322(XAP) was prepared by successive treatment with EcoR I, blunt ending with polymerase, and redigestion with Pst I, followed by isolation of the large vector fragment by electrophoresis and electroelution.

The K-CEA and large vector fragments as prepared in the preceding paragraphs were ligated with T4 DNA ligase, and the ligation mixture transformed into *E. coli* as above. Plasmid DNA from several ampicillin resistant transformants were selected for analysis, and one plasmid DNA demonstrated the proper construction, and was designated pKCEAtrp207-I*.

E.1.8 Construction of a Plasmid Vector for Direct Expression of Mouse Mature Anti-CEA Heavy (Gamma 1) Chain Gene, pγCEAtrp207-1*

FIG. 7 illustrates the construction of pγCEAtrp207-1*. This plasmid was constructed in two parts beginning with construction of the C-terminal region of the gamma 1 gene.

5 μg of plasmid pHGH207-1* was digested with Ava I, extended to blunt ends with DNA polymerase I large fragment (Klenow fragment), extracted with phenol/$CHCl_3$, and ethanol precipitated. The DNA was digested with BamH I treated with BAP and the large fragment (fragment A) was purified by 6 percent polyacrylamide gel electrophoresis and electroelution.

˜5 μg of pγ11 was digested with Pst I and the gamma chain cDNA insert fragment containing the C-terminal portion of the gene was purified, digested with Ava II followed by extension of the Ava II cohesive ends with Klenow, followed by Taq I digestion. The 375 basepair blunt ended Ava II to Taq I fragment (fragment B) was isolated and purified by gel electrophoresis and electroelution.

9 μg of pγ298 was digested with Taq I and BamH I for isolation of the 496 basepair fragment (fragment C).

Approximately equimolar amounts of fragments A, B, and C were ligated overnight at 14° in 20 μl reaction mixture, then transformed into *E. coli* strain 294. The plasmid DNA from six ampicillin resistant transformants was committed to restriction endonuclease analysis and one plasmid DNA, named pγCEAInt, demonstrated the correct construction of the C-terminal portion of gamma 1 (FIG. **5**).

To obtain the N-terminal sequences, 30 μg of pγ298 was digested with Pst I and the 628 basepair DNA fragment encoding the N-terminal region of mouse anti-CEA gamma chain was isolated and purified. This fragment was further digested with Alu I and Rsa I for isolation of the 280 basepair fragment. A 15 nucleotide DNA primer

met glu val met leu
5′ ATG GAA GTG ATG CTG 3′

was synthesized by the phosphotriester method (supra).

The 5′ methionine serves as the initiation codon. 500 ng of this synthetic oligomer primer was phosphorylated at the 5′ end in a reaction with 10 units T4 DNA kinase containing 0.5 mM ATP in 20 μl reaction mixture. ˜500 ng of the 280 basepair Alu I-Rsa I DNA fragment was mixed with the phosphorylated primer. The mixture was heat denatured for 3 minutes at 95° and quenched in dry-ice ethanol. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C., this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, and digested to completion with HpaII. ˜50 ng of the expected 125 basepair blunt-end to Hpa II DNA fragment (fragment D) was purified from the gel.

A second aliquot of pγ298 DNA was digested with Pst I, the 628 basepair DNA fragment purified by polyacrylamide gel electrophoresis, and further digested with BamH I and Hpa II. The resulting 380 basepair fragment (fragment E) was purified by gel electrophoresis.

˜5 μg of pγCEAIntI was digested with EcoR I, the cohesive ends were made flush with DNA polymerase I (Klenow), further digested with BamH I, treated with BAP and electrophoresed on a 6 percent polyacrylamide gel. The large vector fragment (fragment F) was isolated and purified.

In a three fragment ligation, 50 ng fragment D, 100 ng fragment E, and 100 ng fragment F were ligated overnight at 4° in a 20 μl reaction mixture and used to transform *E. coli* K12 strain 294. The plasmid DNAs from 12 ampicillin resistant transformants were analyzed for the correct construction and the nucleotide sequence surrounding the initiation codon was

verified to be correct for the plasmid named pγCEAInt2.

The expression plasmid, pγCEAtrp207-l* used for expression of the heavy chain gene is prepared by a 3-way ligation using the large vector fragment from pBR322(XAP) (supra) and two fragments prepared from pγCEAInt2.

pBR322(XAP) was treated as above by digestion with EcoR1, blunt ending with DNA polymerase (Klenow) in the presence of dNTPs, followed by digestion with Pst I, and isolation of the large vector fragment by gel electrophoresis. A 1543 base pair fragment from pγCEAInt2 containing trp promoter linked with the N-terminal coding region of the heavy chain gene was isolated by treating pγCEAInt2 with Pst I followed by BamH I, and isolation of the desired fragment using PAGE. The 869 base pair fragment containing the C-terminal coding portion of the gene was prepared by partial digestion of pγCEAInt2 with Ava I, blunt ending with Klenow, and subsequent digestion with BamH I, followed by purification of the desired fragment by gel electrophoresis.

The aforementioned three fragments were then ligated under standard conditions using T4 DNA ligase, and a ligation mixture used to transform *E. coli* strain 294. Plasmid DNAs from several tetracycline resistant transformants were analyzed; one plasmid DNA demonstrated the proper construction and was designated pγCEAtrp207-1*.

E.1.9 Production of Immunoglobulin Chains by *E. coli*

E. coli strain W3110 (ATTC No. 27325) was transformed with pγCEAtrp207-1* or pKCEAtrp207-1* using standard techniques.

To obtain double transformants, *E. coli* strain W3110 cells were transformed with a modified pKCEAtrp207-1*, pKCEAtrp207-1*Δ, which had been modified by cleaving a Pst I-Pvu I fragment from the amp^R gene and religating. Cells transformed with pKCEAtrp207-1*Δ are thus sensitive to ampicillin but still resistant to tetracycline. Successful transformants were retransformed using pγCEAInt2 which confers resistance to ampicillin but not tetracycline. Cells containing both pKCEAtrp207-1*Δ and pγCEAInt2 thus identified by growth in a medium containing both ampicillin and tetracycline.

To confirm the production of heavy and/or light chains in the transformed cells, the cell samples were inoculated into M9 tryptophan free medium containing 10 μg/ml tetracycline, and induced with indoleacrylic acid (IAA) when the OD 550 reads 0.5. The induced cells were grown at 37° C. during various time periods and then spun down, and suspended in TE buffer containing 2 percent SDS and 0.1 M β-mercaptoethanol and boiled for 5 minutes. A 10×volume of acetone was added and the cells kept at 22° C. for 10 minutes, then centrifuged at 12,000 rpm. The precipitate was suspended in O'Farrell SDS sample buffer (O'Farrell, P. H., *J. Biol. Chem.,* 250: 4007 (1975)); boiled 3 minutes, recentrifuged, and fractionated using SDS PAGE (10 percent), and stained with silver stain (Goldman, D. et al., *Science* 211: 1437 (1981)); or subjected to Western blot using rabbit anti-mouse IgG (Burnett, W. N., et al., *Anal. Biochem.* 112: 195 (1981)), for identification light chain and heavy chain.

Cells transformed with pγCEAtrp207-1* showed bands upon SDS PAGE corresponding to heavy chain molecular weight as developed by silver stain. Cells transformed with pKCEAtrp207-1* showed the proper molecular weight band for light chain as identified by Western blot; double transformed cells showed bands for both heavy and light chain molecular weight proteins when developed using rabbit anti-mouse IgG by Western blot. These results are shown in FIGS. 8A, **8**B, and **8**C.

FIG. 8A shows results developed by silver stain from cells transformed with pγCEAtrp207-1*. Lane 1 is monoclonal anti-CEA heavy chain (standard) from CEA.66-E3. Lanes 2b-5b are timed samples 2 hrs, 4 hrs, 6 hrs, and 24 hrs after IAA addition. Lanes 2a-5a are corresponding untransformed controls; Lanes 2c-5c are corresponding uninduced transformants.

FIG. 8B shows results developed by Western blot from cells transformed with pKCEAtrp207-1*. Lanes 1b-6b are extracts from induced cell immediately, 1 hr, 3.5 hrs, 5 hrs, 8 hrs, and 24 hrs after IAA addition, and 1a-6a corresponding uninduced controls. Lane 7 is an extract from a pγCEAtrp207-1* control, lanes 8, 9, and 10 are varying amounts of anti CEA-kappa chain from CEA.66-E3 cells.

FIG. 8C shows results developed by Western blot from four colonies of double transformed cells 24 hours after IAA addition (lanes 4-7). Lanes 1-3 are varying amounts of monoclonal gamma chain controls, lanes 8 and 9 are untransformed and pγCEAtrp207-1* transformed cell extracts, respectively.

In another quantitative assay, frozen, transformed *E. coli* cells grown according to E.1.10 (below) were lysed by heating in sodium dodecyl sulfate (SDS)/β-mercaptoethanol cell lysis buffer at 100°. Aliquots were loaded on an SDS polyacrylamide gel next to lanes loaded with various amounts of hybridoma anti-CEA. The gel was developed by the Western blot, Burnett (supra), using ^{125}I-labeled sheep anti-mouse IgG antibody from New England Nuclear. The results are shown in FIG. **9**. The figure shows that the *E. coli* products co-migrate with the authentic hybridoma chains, indicating no detectable proteolytic degradation in *E. coli.* Heavy chain from mammalian cells is expected to be slightly heavier than *E. coli* material due to glycosylation in the former. Using the hybridoma lanes as a standard, the following estimates of heavy and light chain production were made:

	(Per gram of cells)
E. coli (W3110/pγCEAtrp207-1*)	5 mg γ
E. coli (W3110/pKCEAtrp207-1*)	1.5 mg K
E. coli (W3110/pKCEAtrp207-1*A, pγCEAInt2)	0.5 mg K, 1.0 mg γ

E.1.10 Reconstitution of Antibody from Recombinant K and Gamma Chains

In order to obtain heavy and light chain preparations for reconstitution, transformed cells were grown in larger batches, harvested and frozen. Conditions of growth of the variously transformed cells were as follows:

E. coli (W3110/pγCEAtrp207-1*) were inoculated into 500 ml LB medium containing 5 μg/ml tetracycline and grown on a rotary shaker for 8 hours. The culture was then transferred to 10 liters of fermentation medium containing yeast nutrients, salts, glucose, and 2 μg/ml tetracycline. Additional glucose was added during growth and at OD 550=20, indoleacrylic (IAA), a trp derepressor, was added to a concentration of 50 μg/ml. The cells were fed additional glucose to a final OD 550=40, achieved approximately 6 hours from the IAA addition.

E. coli (W3110) cells transformed with pKCEA trp 207-1* and double transformed (with pKCEAtrp207-1*Δ and pγCEAInt2) were grown in a manner analogous to that described above except that the OD 550 six hours after IAA addition at harvest was 25-30.

The cells were then harvested by centrifugation, and frozen.

E.2 Assay Method for Reconstituted Antibody

Anti-CEA activity was determined by ELISA as a criterion for successful reconstitution. Wells of microtiter plates (Dynatech Immulon) were saturated with CEA by incubating 100 μl of 2-5 μg CEA/ml solution in 0.1M carbonate buffer, pH 9.3 for 12 hours at room temperature. The wells were then washed 4 times with phosphate buffered saline (PBS), and then saturated with BSA by incubating 200 μl of 0.5 percent BSA in PBS for 2 hours at 37° C., followed by washing 4 times with PBS. Fifty microliters of each sample was applied to each well. A standard curve (shown in FIG. **10**), was run, which consisted of 50 μl samples of 10 μg, 5 μg, 1 μg, 500 ng, 100 ng, 50 ng, 10 ng, 5 ng and 1 ng anti-CEA/ml in 0.5 percent BSA in PBS, plus 50 μl of 0.5 percent BSA in PBS alone as a blank. All of the samples were incubated in the plate for 90 minutes at 37° C.

The plates were then washed 4 times with PBS, and sheep anti-mouse IgG-alkaline phosphate (TAGO, Inc.) was applied to each well by adding 100 μl of an enzyme concentration of 24 units/ml in 0.5 percent BSA in PBS. The solution was incubated at 37° C. for 90 minutes. The plates were washed 4 times with PBS before adding the substrate, 100 μl of a 0.4 mg/ml solution of p-nitrophenylphosphate (Sigma) in ethanolamine buffered saline, pH 9.5. The substrate was incubated 90 minutes at 37° C. for color development.

The A_{450} of each well was read by the Microelisa Auto Reader (Dynatech) set to a threshold of 1.5, calibration of 1.0 and the 0.5 percent BSA in PBS (Blank) well set to 0.000. The A_{450} data was tabulated in RS-1 on the VAX system, and the standard curve data fitted to a four-parameter logistic model. The unknown samples' concentration were calculated based on the A_{450} data.

E.3 Reconstitution of Recombinant Antibody and Assay

Frozen cells prepared as described in paragraph E.1.10 were thawed in cold lysis buffer [10 mM Tris HCl, pH 7.5, 1 mM EDTA, 0.1M NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF)] and lysed by sonication. The lysate was partially clarified by centrifugation for 20 mins at 30,000 rpm. The supernatant was protected from proteolytic enzymes by an additional 1 mM PMSF, and used immediately or stored frozen at −80° C.; frozen lysates were never thawed more than once.

The S-sulfonate of *E. coli* produced anti-CEA heavy chain (γ) was prepared as follows: Recombinant *E. coli* cells transformed with pγCEAtrp207-1* which contained heavy chain as insoluble bodies, were lysed and centrifuged as above; the pellet was resuspended in the same buffer, sonicated and re-centrifuged. This pellet was washed once with buffer, then suspended in 6M guanidine HCl, 0.1M Tris HCl, pH 8, 1 mM EDTA, 20 mg/ml sodium sulfite and 10 mg/ml sodium tetrathionate and allowed to react at 25° for about 16 hrs. The reaction mixture was dialyzed against 8M urea, 0.1M Tris HCl, pH 8, and stored at 4°, to give a 3 mg/ml solution of γ-SSO_3.

650 μl of cell lysate from cells of various *E. coli* strains producing various IgG chains, was added to 500 mg urea. To this was added β-mercaptoethanol to 20 mM, Tris-HCl, pH 8.5 to 50 mM and EDTA to 1 mM, and in some experiments, γ-SSO_3 was added to 0.1 mg/ml. After standing at 25° for 30-90 mins., the reaction mixtures were dialyzed at 4° against a buffer composed of 0.1M sodium glycinate, pH 10.8, 0.5M urea, 10 mM glycine ethyl ester, 5 mM reduced glutathione, 0.1 mM oxidized glutathione. This buffer was prepared from N_2-saturated water and the dialysis was performed in a capped Wheaton

bottle. After 16-48 hours, dialysis bags were transferred to 4° phosphate buffered saline containing 1 mM PMSF and dialysis continued another 16-24 hrs. Dialysates were assayed by ELISA as described in paragraph E.2 for ability to bind CEA. The results below show the values obtained by comparison with the standard curve in x ng/ml anti-CEA. Also shown are the reconstitution efficiencies calculated from the ELISA responses, minus the background (108 ng/ml) of cells producing K chain only, and from estimates of the levels of γ and K chains in the reaction mixtures.

	ng/ml anti-CEA	Percent recombination
E. coli W3110 producing IFN-αA (control)	0	—
E. coli (W3110/pKCEAtrp207-1*)	108	—
E. coli (W3110/pKCEAtrp207-1*), plus γ-SSO_3	848	0.33
E. coli (W3110/pKCEAtrp207-1*Δ, pγCEAInt2)	1580	0.76
Hybridoma anti-CEA K-SSO_3 and γ-SSO_3	540	0.40

E.4 Preparation of Chimeric Antibody

FIGS. 11 and 12 show the construction of an expression vector for a chimeric heavy (gamma) chain which comprises the murine anti CEA variable region and human γ-2 constant region.

A DNA sequence encoding the human gamma-2 heavy chain is prepared as follows: the cDNA library obtained by standard techniques from a human multiple myeloma cell line is probed with 5′ GGGCACTCGACACAA 3 ′ to obtain the plasmid containing the cDNA insert for human gamma-2 chain (Takahashi, et al., *Cell,* 29: 671 (1982), incorporated herein by reference), and analyzed to verify its identity with the known sequence in human gamma-2 (Ellison, J., et al., *Proc. Natl. Acad. Sci.* (*USA*), 79: 1984 (1982) incorporated herein by reference).

As shown in FIG. 11, two fragments are obtained from this cloned human gamma 2 plasmid (pγ2). The first fragment is formed by digestion with PvuII followed by digestion with Ava III, and purification of the smaller DNA fragment, which contains a portion of the constant region, using 6 percent PAGE. The second fragment is obtained by digesting the pγ2 with any restriction enzyme which cleaves in the 3′ untranslated region of γ2, as deduced from the nucleotide sequence, filling in the Klenow and dNTPs, cleaving with Ava III, and isolating the smaller fragment using 6 percent PAGE. (The choice of a two step, two fragment composition to supply the PvuII-3′ untranslated fragment provides a cleaner path to product due to the proximity of the AvaIII site to the 3 terminal end thus avoiding additional restriction sites in the gene sequence matching the 3′ untranslated region site.) pγCEA207-1* is digested with EcoR 1, treated with Klenow and dNTPs to fill in the cohesive end, and digested with Pvu II, the large vector fragment containing promoter isolated by 6 percent PAGE.

The location and DNA sequence surrounding the PvuII site in the mouse gamma-1 gene are identical to the location and DNA sequence surrounding the PvuII site in the human gamma-2 gene.

The plasmid resulting from a three way ligation of the foregoing fragments, pChim1, contains, under the influence of trp promoter, the variable and part of the constant region of murine anti-CEA gamma 1 chain, and a portion of the gamma 2 human chain. pChim1 will, in fact, express a chimeric heavy chain when transformed into *E. coli,* but one wherein the change from mouse to human does not take place at the variable to constant junction.

FIG. 12 shows modification of pChim1 to construct pChim2 so that the resulting protein from expression will contain variable region from murine anti CEA antibody and constant region from the human γ-2 chain. First, a fragment is prepared from pChim1 by treating with Nco I, blunt ending with Klenow and dNTPs, cleaving with Pvu II, and isolating the large vector fragment which is almost the complete plasmid except for short segment in the constant coding region for mouse anti CEA. A second fragment is prepared from the previously described pγ2 by treating with Pvu II, followed by treating with any restriction enzyme which cleaves in the variable region, blunt ending with Klenow and dNTPs and isolating the short fragment which comprises the junction between variable and constant regions of this chain.

Ligation of the foregoing two fragments produces an intermediate plasmid which is correct except for an extraneous DNA fragment which contains a small portion of the constant region of the murine anti CEA antigen, and a small portion of the variable region of the human gamma chain. This repair can be made by excising the Xba I to Pvu II fragment and cloning into M13 phage as described by Messing et al., *Nucleic Acids Res.* 9: 309 (1981), followed by in vitro site directed deletion mutagenesis as described by Adelman, et al., *DNA,* in press (1983) which is incorporated herein by reference. The Xba I-Pvu II fragment thus modified is ligated back into the intermediate plasmid to form pChim2. This plasmid then is capable of expressing in a suitable host a cleanly constructed murine variable/human constant chimeric heavy chain.

In an analogous fashion, but using mRNA templates for cDNA construction for human kappa rather than γ chain, the

expression plasmid for chimeric light chain is prepared.

The foregoing two plasmids are then double transformed into *E. coli* W3110, the cells grown and the chains reconstituted as set forth in paragraph E.1-E.3 supra.

E.5 Preparation of Altered Murine Anti-CEA Antibody

E.5.1 Construction of Plasmid Vectors for Direct Expression of Altered Murine Anti-CEA Heavy Chain Gene

The cysteine residues, and the resultant disulfide bonds in the region of amino acids 216-230 in the constant region of murine anti-CEA heavy chain are suspected to be important for complement fixation (Klein, et al., *Proc. Natl. Acad. Sci.,* (*USA*), 78: 524 (1981)) but not for the antigen binding property of the resulting antibody. To decrease the probability of incorrect disulfide bond formation during reconstitution according to the process of the invention herein, the nucleotides encoding the amino acid residues 226-232 which includes codons for three cysteines, are deleted as follows:

A “deleter” deoxyoligonucelotide, 5′ CTAACACCATGTCAGGGT is used to delete the relevant portions of the gene from pγCEAtrp207-1* by the procedure of Wallace, et al., *Science,* 209: 1396 (1980) or of Adelman, et al., *DNA* 2, 183 (1983). Briefly, the “deleter” deoxyoligonucelotide is annealed with denatured pγCEAtrp207-1* DNA, and primer repair synthesis carried out in vitro, followed by screening by hybridization of presumptive deletion clones with P^{32} labelled deleter sequence.

E.5.2 Production of Cysteine Deficient Altered Antibody

The plasmid prepared in E.5.1 is transformed into an *E. coli* strain previously transformed with pKCEAtrp207-1* as described above. The cells are grown, extracted for recombinant antibody chains, and the altered antibody reconstituted as described in E.1.10.

E.6 Preparation of Fab

E.6.1 Construction of a Plasmid Vector for Direct Expression of Murine Anti-CEA Gamma 1 Fab Fragment Gene pγCEAFabtrp207-1*

FIG. 13 presents the construction of pγCEAFabtrp207-1*. 5 μg of pBR322 was digested with Hind III, the cohesive ends made flush by treating with Klenow and dNTPs; digested with Pst I, and treated with BAP. The large vector fragment, fragment I, was recovered using 6 percent PAGE followed by electroelution.

5 μg of pγCEAtrp207-1* was digested with both BamH I and Pst I and the ˜1570 bp DNA fragment (fragment II) containing the trp promoter and the gene sequence encoding the variable region continuing into constant region and further into the anti-CEA gamma 1 chain hinge region, was isolated and purified after electrophoresis.

Expression of the anti-CEA gamma 1 chain Fab fragment rather than complete heavy chain requires that a termination codon be constructed at the appropriate location in the gene. For this, the 260 bp Nco I-Nde I DNA fragment from 20 μg of the pγ298 was isolated and purified. A 13 nucleotide DNA primer, the complement of which encodes the last 3 C-terminal amino acids of the Fab gene and 2 bases of the 3 needed for the stop codon, was synthesized by the phosphotriester method (supra). The probe hybridizes to nucleotides 754 to 767 (FIG. 4) which has the following sequence:

```
          AspCysGlyStop
5′ GGGATTGTGGTTG    3′
```

The third base of the stop codon is provided by the terminal nucleotide of the filled-in Hind III site from pBR322 cleavage described above. 500 ng of this primer was used in a primer repair reaction by phosphorylation at the 5′ end in a reaction with 10 units T4 DNA kinase containing 0.5 mM ATP in 20 μl, and mixing with ˜200 ng of the Nco I-Nde I DNA fragment. The mixture was heat denatured for 3 minutes at 95° and quenched in dry-ice ethanol. The denatured DNA solution was made 60 mM NaCl, 7 mM $MgCl_2$, 7 mM Tris HCl (pH 7.4), 12 mM in each dNTP and 12 units DNA Polymerase I-Large Fragment was added. After 2 hours incubation at 37° C., this primer repair reaction was phenol/$CHCl_3$ extracted, ethanol precipitated, digested with BamH I and the reaction electrophoresed through a 6 percent polyacrylamide gel. ˜50 ng of the 181 bp blunt end to BamH I DNA fragment, fragment III, was isolated and purified.

˜100 ng of fragment I, ˜100 ng each of fragments II and III were ligated overnight and transformed into *E. coli* K12 strain 294. Plasmid DNA from several tetracycline resistant transformants was analyzed for the proper construction and the nucleotide sequence through the repair blunt end filled-in Hind III junction was determined for verification of the TGA stop codon.

E.6.2 Production of Fab Protein

The plasmid prepared in E.6.1 is transformed into an *E. coli* strain previously transformed with pKCEAtrp207-1* as described above. The cells are grown, extracted for recombinant antibody chains and the Fab protein reconstituted as described in E.1.10.

PATENT CITATIONS

Cited Patent	Filing date	Publication date	Applicant	Title
US4179337	Jul 28, 1977	Dec 18, 1979	Davis Frank F	Non-immunogenic polypeptides
US4237224	Jan 4, 1979	Dec 2, 1980	Board Of Trustees Of The Leland Stanford Jr. University	Process for producing biologically functional molecular chimeras
US4338397	Apr 11, 1980	Jul 6, 1982	President And Fellows Of Harvard College	Mature protein synthesis
US4342832	Jul 5, 1979	Aug 3, 1982	Genentech, Inc.	Method of constructing a replicable cloning vehicle having quasi-synthetic genes
US4403036	Dec 2, 1980	Sep 6, 1983	University Of Iowa Research Foundation	Genetic reagents for generating plasmids containing multiple copies of DNA segments
US4444878	Dec 21, 1981	Apr 24, 1984	Boston Biomedical Research Institute, Inc.	Bispecific antibody determinants
US4510244	Sep 22, 1982	Apr 9, 1985	The Board Of Trustees Of The Leland Stanford Jr. University	Cell labeling with antigen-coupled microspheres
US4512922	Jun 1, 1984	Apr 23, 1985	Genentech, Inc.	Purification and activity assurance of precipitated heterologous proteins
US4518584	Dec 20, 1983	May 21, 1985	Cetus Corporation	Human recombinant interleukin-2 muteins
US4642334	Dec 5, 1983	Feb 10, 1987	Dnax Research Institute Of Molecular And Cellular Biology, Inc.	Hybrid DNA prepared binding composition
US4704362	Nov 5, 1979	Nov 3, 1987	Genentech, Inc.	Recombinant cloning vehicle microbial polypeptide expression
US4816397	Mar 23, 1984	Mar 28, 1989	Celltech, Limited	Multichain polypeptides or proteins and processes for their production
US4816567	Apr 8, 1983	Mar 28, 1989	Genentech, Inc.	Recombinant immunoglobin preparations
US5225539	Oct 25, 1991	Jul 6, 1993	Medical Research Council	Recombinant altered antibodies and methods of making altered antibodies
US5545403	Nov 23, 1993	Aug 13, 1996	Burroughs Wellcome Co.	Method for treating a mammal by administering a CHO-glycosylated antibody
US5545404	Nov 3, 1994	Aug 13, 1996	Burroughs Wellcome Co.	Method for treating a mammal suffering from a T-cell medicated disorder with a CHO-Glycosylated antibody
US5545405	Nov 3, 1994	Aug 13, 1996	Burroughs Wellcome Co.	Method for treating a mammal suffering from cancer with a cho-glycosylated antibody
AU1241783A				*Title not available*
AU2642984A				*Title not available*
AU4655685A				*Title not available*
AU6598186A				*Title not available*
EP0037723A2	Apr 3, 1981	Oct 14, 1981	The Regents Of The University Of California	Expression of hormone genomic clones
EP0041313A2	Apr 1, 1981	Dec 9, 1981	Biogen, Inc.	DNA sequences, recombinant DNA molecules and processes for producing human fibroblast interferon
EP0041767A2	Apr 1, 1981	Dec 16, 1981	Biogen, Inc.	Improved vectors and methods for making such vectors and for expressing cloned genes
EP0044722A1	Jul 16, 1981	Jan 27, 1982	The Board Of Trustees Of The Leland Stanford Junior University	Human hybridomas, precursors and products
EP0055945A2	Dec 31, 1981	Jul 14, 1982	Genentech, Inc.	Human proinsulin and analogs thereof and method of preparation by microbial polypeptide expression and conversion thereof to human insulin
EP0057107A2	Jan 27, 1982	Aug 4, 1982	Coats Patons Plc	Method of manufacturing monoclonal antibodies and cells capable of manufacturing such antibodies
EP0068763A2	Jun 18, 1982	Jan 5, 1983	Board Of Regents The University Of Texas System	Recombinant monoclonal antibodies
EP0073656A2	Aug 26, 1982	Mar 9, 1983	Genentech, Inc.	Preparation of polypeptides in vertebrate cell culture
EP0075444B1	Sep 16, 1982	Dec 23, 1992	Genentech, Inc.	Methods and products for facile microbial expression of dna sequences
EP0088994B1	Mar 9, 1983	Jun 19, 1991	Schering Corporation	Hybrid dna, binding composition prepared thereby and processes therefor
EP0093619A1	May 4, 1983	Nov 9, 1983	Genentech, Inc.	Human tissue plasminogen activator, pharmaceutical compositions containing it, processes for making it, and DNA and transformed cell intermediates therefor
EP0120694A2	Mar 23, 1984	Oct 3, 1984	Celltech Limited	Processes for the production of multichain polypeptides or proteins
EP0125023B1	Apr 6, 1984	Jun 5, 1991	Genentech, Inc.	Recombinant immunoglobulin preparations, methods for their preparation, dna sequences, expression vectors and recombinant host cells therefor

EP0194276B1	Sep 3, 1985	Aug 11, 1993	Celltech Therapeutics Limited	Production of chimeric antibodies
EP0196864A2	Mar 25, 1986	Oct 8, 1986	Cetus Corporation	Alkaline phosphatase-mediated processing and secretion of recombinant proteins, DNA sequences for use therein and cells transformed using such sequences
EP0234592A1	Feb 27, 1987	Sep 2, 1987	Teijin Limited	Plasmid containing DNA fragment coding for human immunoglobulin G Fc region protein and use thereof for production of said protein
EP0255694A1	Jul 29, 1987	Feb 10, 1988	Teijin Limited	Mouse-human chimera antibody and its components and gene therefor
EP0324162B1	Dec 24, 1988	Mar 9, 1994	Arne Skerra	Method of producing antibodies by genetic engineering
EP0550400A2	Jul 25, 1988	Jul 7, 1993	Xoma Corporation	Modular assembly of antibody genes, antibodies prepared thereby and use
JPS62201581A				*Title not available*
WO1986001533A1	Sep 3, 1985	Mar 13, 1986	Celltech Limited	Production of chimeric antibodies

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US6841154	Jun 17, 2002	Jan 11, 2005	Inhibitex, Inc.	Cross-reactive monoclonal and polyclonal antibodies which recognize surface proteins from coagulase-negative staphylococci and Staphylococcus aureus

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US7838266		Nov 23, 2010	University Of Victoria Innovation And Development Corporation	Proaerolysin containing protease activation sequences and methods of use for treatment of prostate cancer
US7847070	Jan 31, 2006	Dec 7, 2010	Raven Biotechnologies, Inc.	LUCA2 and antibodies that bind thereto

US7851181		Dec 14, 2010	Schering Corporation	Neutralizing human anti-IGFR antibody
US7858359	Nov 9, 2006	Dec 28, 2010	Stratagene	Method for tapping the immunological repertoire
US7858559	Nov 14, 2001	Dec 28, 2010	University Of Rochester	In vitro methods of producing and identifying immunoglobulin molecules in eukaryotic cells
US7858752	Dec 5, 2006	Dec 28, 2010	Abbott Laboratories	Recombinant antibodies against hepatitis C virus and methods of obtaining and using same
US7879322		Feb 1, 2011	Novartis Ag	Compositions and methods for use for antibodies against sclerostin
US7888480		Feb 15, 2011	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in leukemia signaling pathways
US7919079		Apr 5, 2011	Biosante Pharmaceuticals, Inc.	Cancer immunotherapy compositions and methods of use
US7923221 *	Apr 13, 1995	Apr 12, 2011	Genentech, Inc	Methods of making antibody heavy and light chains having specificity for a desired antigen
US7927594		Apr 19, 2011	Rinat Neuroscience Corp.	Antibodies directed against amyloid-beta peptide
US7935790	Aug 11, 2006	May 3, 2011	Cell Singaling Technology, Inc.	Reagents for the detection of protein phosphorylation in T-cell receptor signaling pathways
US7939077	Jun 28, 2002	May 10, 2011	Kyowa Hakko Kirin Co., Ltd.	Humanized antibody against fibroblast growth factor-8 and antibody fragment thereof
US7939636		May 10, 2011	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in c-Src signaling pathways
US7947509	May 7, 2007	May 24, 2011	Massachusetts Institute Of Technology	Optoelectronic detection system
US7960517	Oct 31, 2008	Jun 14, 2011	Epotimics, Inc.	Methods for antibody engineering
US7964190		Jun 21, 2011	Human Genome Sciences, Inc.	Methods and compositions for decreasing T-cell activity
US7968094	Jul 31, 2008	Jun 28, 2011	Genentech, Inc.	Use of anti-tissue factor antibodies for treating thromboses
US7968690	Dec 23, 2004	Jun 28, 2011	Rinat Neuroscience Corp.	Agonist anti-trkC antibodies and methods using same
US7973134		Jul 5, 2011	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in anaplastic large cell lymphoma signaling pathways
US7973136	Oct 6, 2006	Jul 5, 2011	Xencor, Inc.	Optimized anti-CD30 antibodies
US7977462	Apr 18, 2008	Jul 12, 2011	Cell Signaling Technology, Inc.	Tyrosine phosphorylation sites
US7982012	Mar 10, 2009	Jul 19, 2011	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of cytomegalovirus
US7982017		Jul 19, 2011	Bioalliance C.V.	Antibodies recognizing a carbohydrate containing epitope on CD-43 and CEA expressed on cancer cells and methods using same
US8007795		Aug 30, 2011	Genentech, Inc.	Anti-tissue factor antibodies and methods of use thereof
US8007800	Mar 16, 2009	Aug 30, 2011	Pfizer Inc.	Methods for treating bone cancer pain by administering a nerve growth factor antagonist antibody
US8034346	Dec 22, 2009	Oct 11, 2011	Rinat Neuroscience Corp.	Methods for treating pain by administering a nerve growth factor antagonist and an NSAID and compositions containing the same
US8039592	Oct 31, 2007	Oct 18, 2011	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8052974	May 11, 2006	Nov 8, 2011	Crucell Holland B.V.	Host cell specific binding molecules capable of neutralizing viruses and uses thereof
US8057796	Nov 12, 2008	Nov 15, 2011	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of influenza
US8058511		Nov 15, 2011	Fraunhofer Usa, Inc.	System for expression of genes in plants
US8067184		Nov 29, 2011	Massachusetts Institute Of Technology	Optoelectronic detection system
US8084582		Dec 27, 2011	Xencor, Inc.	Optimized anti-CD20 monoclonal antibodies having Fc variants
US8088384		Jan 3, 2012	Rinat Neuroscience Corp.	Anti-NGF antibodies and methods using same
US8093357	Jul 7, 2006	Jan 10, 2012	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8093359	Oct 31, 2007	Jan 10, 2012	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8097425		Jan 17, 2012	Tethys Bioscience, Inc.	Multiplex protein fractionation
US8101720		Jan 24, 2012	Xencor, Inc.	Immunoglobulin insertions, deletions and substitutions
US8114402	Mar 17, 2010	Feb 14, 2012	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of influenza
US8114404	May 10, 2004	Feb 14, 2012	Mmrglobal, Inc.	Method and composition for altering a B cell mediated pathology
US8124731	Oct 29, 2007	Feb 28, 2012	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8133486	May 10, 2004	Mar 13, 2012	Mmrglobal, Inc.	Method and composition for altering a B cell mediated pathology
US8148088	Jul 17, 2009	Apr 3, 2012	Abgent	Regulation of autophagy pathway phosphorylation and uses

				thereof
US8148608	Feb 18, 2005	Apr 3, 2012	Fraunhofer Usa, Inc	Systems and methods for clonal expression in plants
US8183357		May 22, 2012	Macrogenics, Inc.	Antibodies that bind to EphA2 and methods of use thereof
US8188231	May 5, 2005	May 29, 2012	Xencor, Inc.	Optimized FC variants
US8193321		Jun 5, 2012	Genentech, Inc.	Multispecific antibodies
US8198046	Jun 21, 2007	Jun 12, 2012	Danisco Us Inc.	KEX2 cleavage regions of recombinant fusion proteins
US8207130		Jun 26, 2012	University Of Southern California	Combination cancer immunotherapy with co-stimulatory molecules
US8211431	Jun 5, 2007	Jul 3, 2012	Crucell Holland B.V.	Human binding molecules having killing activity against staphylococci and uses thereof
US8211648	Nov 14, 2005	Jul 3, 2012	Kalobios Pharmaceuticals, Inc.	Secretion of antibodies without signal peptides from bacteria
US8216570		Jul 10, 2012	Macrogenics, Inc.	TES7 and antibodies that bind thereto
US8216578		Jul 10, 2012	Macrogenics, Inc.	Antibodies to oncostatin M receptor
US8216797	Nov 30, 2006	Jul 10, 2012	Massachusetts Institute Of Technology	Pathogen detection biosensor
US8222188	Jun 29, 2009	Jul 17, 2012	Catalent Pharma Solutions, Llc	Antibody libraries
US8226951	Jun 6, 2011	Jul 24, 2012	Pfizer Inc.	Methods for treating bone cancer by administering a nerve growth factor antagonist antibody
US8246953		Aug 21, 2012	Novartis Ag	Compositions and methods for use for antibodies against sclerostin
US8257714		Sep 4, 2012	Michigan State University	Compositions and methods for immunotherapy of cancer and infectious diseases
US8268309		Sep 18, 2012	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of cytomegalovirus
US8268593	Apr 5, 2010	Sep 18, 2012	Rinat Neuroscience Corp.	Polynucleotides encoding antibodies directed against amyloid-beta peptide
US8268788		Sep 18, 2012	University Of Southern California	Combination cancer immunotherapy with co-stimulatory molecules
US8277816		Oct 2, 2012	Fraunhofer Usa, Inc.	Bacillus anthracis antigens, vaccine compositions, and related methods
US8283122	Aug 3, 2005	Oct 9, 2012	The United States Of America, As Represented By The Secretary Of The Department Of Health And Human Services	Prediction of clinical outcome using gene expression profiling and artificial neural networks for patients with neuroblastoma
US8313916		Nov 20, 2012	Macrogenics West, Inc.	KID31 and antibodies that bind thereto
US8318907		Nov 27, 2012	Xencor, Inc.	Fc variants with altered binding to FcRn
US8324351		Dec 4, 2012	Xencor, Inc.	Fc variants with altered binding to FcRn
US8338107	Nov 16, 2006	Dec 25, 2012	Scripps Research Institute	Method for producing polymers having a preselected activity
US8338574		Dec 25, 2012	Xencor, Inc.	FC variants with altered binding to FCRN
US8361475		Jan 29, 2013	Macrogenics West, Inc.	ADAM-9 modulators
US8367805	Dec 22, 2008	Feb 5, 2013	Xencor, Inc.	Fc variants with altered binding to FcRn
US8383109	Jan 9, 2012	Feb 26, 2013	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8388955	Jun 15, 2007	Mar 5, 2013	Xencor, Inc.	Fc variants
US8389688	Mar 6, 2007	Mar 5, 2013	Aeres Biomedical, Ltd.	Humanized anti-CD22 antibodies and their use in treatment of oncology, transplantation and autoimmune disease
US8394374	Sep 18, 2007	Mar 12, 2013	Xencor, Inc.	Optimized antibodies that target HM1.24
US8398978		Mar 19, 2013	Rinat Neuroscience Corp.	Antibodies directed against amyloid-beta peptide and methods using same
US8404816	May 3, 2011	Mar 26, 2013	Epitomics, Inc.	Methods for antibody engineering
US8426187		Apr 23, 2013	Research Development Foundation	Immunoglobulin libraries
US8440197	Jul 7, 2010	May 14, 2013	Macrogenics, Inc.	KID3 and KID3 antibodies that bind thereto
US8444983	Mar 23, 2010	May 21, 2013	Quark Pharmaceuticals, Inc.	Composition of anti-ENDO180 antibodies and methods of use for the treatment of cancer and fibrotic diseases
US8460666		Jun 11, 2013	Crucell Holland B.V.	Human binding molecules having killing activity against staphylococci and uses thereof
US8460671		Jun 11, 2013	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of influenza
US8486661	Jul 12, 2012	Jul 16, 2013	Novartis Ag	Polynucleotides encoding antibodies against sclerostin and uses therefor
US8491893	Mar 26, 2010	Jul 23, 2013	Quantum Immunologics, Inc.	Oncofetal antigen/immature laminin receptor antibodies for diagnostic and clinical applications
US8491901	Nov 18, 2011	Jul 23, 2013	Toshio Imai	Neutralizing anti-CCL20 antibodies

US8507426	May 26, 2010	Aug 13, 2013	Amgen, Inc.	Peptides and related molecules that bind to TALL-1
US8518667	May 15, 2012	Aug 27, 2013	Danisco Us Inc.	KEX2 cleavage regions of recombinant fusion proteins
US8524867	Aug 14, 2007	Sep 3, 2013	Xencor, Inc.	Optimized antibodies that target CD19
US8545838	Feb 13, 2007	Oct 1, 2013	University Of Southern California	Compositions and methods for cancer immunotherapy
US8546543	Oct 12, 2011	Oct 1, 2013	Xencor, Inc.	Fc variants that extend antibody half-life
US8551486	Dec 4, 2008	Oct 8, 2013	Savoy Pharmaceuticals, Inc.	Monoclonal antibodies to human thymidine kinase to treat cancer
US8568718	Jun 8, 2011	Oct 29, 2013	Bioalliance C.V.	Antibodies recognizing a carbohydrate containing epitope on CD-43 and CEA expressed on cancer cells and methods using same
US8597942	Sep 23, 2011	Dec 3, 2013	Ibio, Inc.	System for expression of genes in plants
US8603950	Feb 20, 2008	Dec 10, 2013	Anaptysbio, Inc.	Methods of generating libraries and uses thereof
US8609101	Apr 23, 2010	Dec 17, 2013	Theraclone Sciences, Inc.	Granulocyte-macrophage colony-stimulating factor (GM-CSF) neutralizing antibodies
US8629245	Apr 30, 2008	Jan 14, 2014	Research Development Foundation	Immunoglobulin Fc libraries
US8637638	Nov 10, 2011	Jan 28, 2014	Mmrglobal, Inc.	Method and composition for altering a B cell mediated pathology
US8664363	Feb 28, 2013	Mar 4, 2014	Medimmune, Llc	Humanized anti-CD22 antibodies and their use in treatment of oncology, transplantation and autoimmune disease
US8669349	Mar 25, 2009	Mar 11, 2014	Macrogenics, Inc.	BCR-complex-specific antibodies and methods of using same
US8679493	Jun 30, 2010	Mar 25, 2014	Research Development Foundation	Immunoglobulin Fc polypeptides
US8685666	Jan 31, 2011	Apr 1, 2014	The Board Of Trustees Of Southern Illinois University	ARL-1 specific antibodies and uses thereof
US8685897	May 17, 2011	Apr 1, 2014	Anaptysbio, Inc.	Methods of generating libraries and uses thereof
US8716194	Sep 29, 2011	May 6, 2014	4-Antibody Ag	Identification of antigen or ligand-specific binding proteins
US8722347	Feb 27, 2009	May 13, 2014	Massachusetts Institute Of Technology	Optoelectronic sensor
US8734791	Feb 27, 2012	May 27, 2014	Xencor, Inc.	Optimized fc variants and methods for their generation
US8734792	May 9, 2012	May 27, 2014	Duke University	Reagents and treatment methods for autoimmune diseases
US8734793	Nov 22, 2010	May 27, 2014	Abbott Laboratories Inc.	Recombinant antibodies against hepatitis C virus and methods of obtaining and using same
US8734798	Oct 27, 2010	May 27, 2014	Ucb Pharma S.A.	Function modifying NAv 1.7 antibodies
US8735545	Jun 19, 2007	May 27, 2014	Xencor, Inc.	Fc variants having increased affinity for fcyrIIc
US8735547	Apr 25, 2013	May 27, 2014	Xencor, Inc.	Optimized Fc Variants
US8741810 *	Aug 17, 2007	Jun 3, 2014	University Of Rochester	In vitro methods of producing and identifying immunoglobulin molecules in eukaryotic cells
US8748353	Mar 4, 2009	Jun 10, 2014	4-Antibody Ag	Identification of antigen or ligand-specific binding proteins
US8753628	Jun 11, 2013	Jun 17, 2014	Xencor, Inc.	Optimized Fc variants
US8753629	Jun 14, 2013	Jun 17, 2014	Xencor, Inc.	Optimized Fc variants
US8779098	Mar 27, 2009	Jul 15, 2014	Macrogenics West, Inc.	B7-H3L cell surface antigen and antibodies that bind thereto
US8795672	Feb 13, 2004	Aug 5, 2014	University Of Southern California	Compositions and methods for cancer immunotherapy
US8802091	May 4, 2012	Aug 12, 2014	Macrogenics, Inc.	Antibodies reactive with B7-H3 and uses thereof
US8802820	Oct 31, 2007	Aug 12, 2014	Xencor, Inc.	Fc variants with altered binding to FcRn
US8802823	Nov 12, 2013	Aug 12, 2014	Xencor, Inc.	Optimized Fc variants
US8809503	May 20, 2013	Aug 19, 2014	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8815242	May 27, 2010	Aug 26, 2014	Synageva Biopharma Corp.	Avian derived antibodies
US8822651	Aug 28, 2012	Sep 2, 2014	Theraclone Sciences, Inc.	Human rhinovirus (HRV) antibodies
US8828381	Sep 15, 2009	Sep 9, 2014	Wilex Ag	Co-administration of CG250 and IL-2 or IFN-alpha for treating cancer such as renal cell carcinomas
US8835127	Oct 6, 2011	Sep 16, 2014	Massachusetts Institute Of Technology	Optoelectronic detection system
US8840889	Aug 13, 2010	Sep 23, 2014	The Johns Hopkins University	Methods of modulating immune function
US8852586	Feb 1, 2010	Oct 7, 2014	Xencor, Inc.	Fc variants with altered binding to FcRn
US8852594	Jun 1, 2012	Oct 7, 2014	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of cytomegalovirus infections
US8858937	Feb 21, 2013	Oct 14, 2014	Xencor, Inc.	Optimized Fc variants and methods for their generation
US8858948	May 20, 2010	Oct 14, 2014	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of influenza

US8883973	Feb 1, 2010	Nov 11, 2014	Xencor, Inc.	Fc variants with altered binding to FcRn
US8900590	Aug 12, 2011	Dec 2, 2014	Theraclone Sciences, Inc.	Anti-hemagglutinin antibody compositions and methods of use thereof
US8911738	Aug 25, 2011	Dec 16, 2014	Crucell Holland B.V.	Host cell specific binding molecules capable of neutralizing viruses and uses thereof
US8912385	Nov 17, 2009	Dec 16, 2014	University Of Georgia Research Foundation, Inc.	Rapid production of monoclonal antibodies
US8916160	Feb 14, 2012	Dec 23, 2014	Theraclone Sciences, Inc.	Compositions and methods for the therapy and diagnosis of influenza
US8916161	Jun 14, 2006	Dec 23, 2014	Sophiris Bio Inc.	Method of treating or preventing benign prostatic hyperplasia using modified pore-forming proteins
US8921281	May 20, 2010	Dec 30, 2014	Novimmune S.A.	Synthetic polypeptide libraries and methods for generating naturally diversified polypeptide variants
US8926977	Oct 27, 2010	Jan 6, 2015	Ucb Pharma S.A.	Antibodies to the E1 extracellular loop of ion channels
US8927694	Oct 14, 2009	Jan 6, 2015	Merrimack Pharmaceuticals, Inc.	Human serum albumin linkers and conjugates thereof
US8936917	Jul 3, 2013	Jan 20, 2015	Danisco Us Inc.	KEX2 cleavage regions of recombinant fusion proteins
US8945580	Feb 25, 2013	Feb 3, 2015	Ibio Inc.	Yersinia pestis antigens, vaccine compositions, and related methods
US8951791	Dec 2, 2013	Feb 10, 2015	Ibio, Inc.	System for expression of genes in plants
US8952132	Feb 6, 2012	Feb 10, 2015	Research Development Foundation	Engineered immunoglobulin FC polypeptides
US8962278	Aug 3, 2006	Feb 24, 2015	Ibio Inc.	Compositions and methods for production of immunoglobulins
US8969013	Jan 23, 2013	Mar 3, 2015	Epitomics, Inc.	Method for identifying lineage-related antibodies
US8975377	Aug 11, 2008	Mar 10, 2015	Vasgene Therapeutics, Inc	Cancer treatment using humanized antibodies that bind to EphB4
US8986694	Aug 29, 2014	Mar 24, 2015	Kymab Limited	Targeting human nav1.7 variants for treatment of pain
US8986954	May 23, 2014	Mar 24, 2015	Ucb Pharma S.A.	DNA encoding function modifying Nav1.7 antibodies
US8992927	Aug 29, 2014	Mar 31, 2015	Kymab Limited	Targeting human NAV1.7 variants for treatment of pain
US8993730	Sep 23, 2013	Mar 31, 2015	Macrogenics, Inc.	BCR-complex-specific antibodies and methods of using same
US9005989	Mar 3, 2011	Apr 14, 2015	Massachusetts Institute Of Technology	Optoelectronic detection system
US9012155	Jul 22, 2013	Apr 21, 2015	Benovus Bio, Inc.	Oncofetal antigen/immature laminin receptor antibodies for diagnostic and clinical applications
US9017686	Feb 18, 2011	Apr 28, 2015	Genentech, Inc.	Multispecific antibodies
US9023356	Mar 14, 2008	May 5, 2015	Ludwig Institute For Cancer Research Ltd	Treatment method using EGFR antibodies and SRC inhibitors and related formulations
US9040041	Jun 4, 2010	May 26, 2015	Xencor, Inc.	Modified FC molecules
US9040042	Mar 11, 2013	May 26, 2015	Xencor, Inc.	Optimized antibodies that target HM1.24
US9045529	Mar 25, 2011	Jun 2, 2015	Ucb Pharma S.A.	Disulfide stabilized antibodies and fragments thereof
US9045545	Nov 7, 2014	Jun 2, 2015	Kymab Limited	Precision medicine by targeting PD-L1 variants for treatment of cancer
US9051373	Feb 11, 2013	Jun 9, 2015	Xencor, Inc.	Optimized Fc variants
US9052322	Feb 10, 2014	Jun 9, 2015	Epitomics, Inc.	Method for identifying lineage-related antibodies
US9062105	Nov 7, 2014	Jun 23, 2015	Kymab Limited	Precision Medicine by targeting VEGF-A variants for treatment of retinopathy
US9067995	Oct 27, 2010	Jun 30, 2015	Ucb Pharma S.A.	Method to generate antibodies to ion channels
US9067998	Nov 10, 2014	Jun 30, 2015	Kymab Limited	Targeting PD-1 variants for treatment of cancer
US9072798	Feb 17, 2010	Jul 7, 2015	Ludwig Institute For Cancer Research Ltd.	Specific binding proteins and uses thereof
US9079942	Jan 28, 2010	Jul 14, 2015	Epitomics, Inc.	CDR-anchored amplification method
US9090690	Jun 16, 2010	Jul 28, 2015	Pfizer Inc.	Anti Notch-1 antibodies
US9090693	Jan 24, 2008	Jul 28, 2015	Dana-Farber Cancer Institute	Use of anti-EGFR antibodies in treatment of EGFR mutant mediated disease
US9127046	May 7, 2012	Sep 8, 2015	Kalobios Pharmaceuticals, Inc.	Secretion of antibodies without signal peptides from bacteria
US9127057	Jul 20, 2011	Sep 8, 2015	Teva Pharmaceuticals Ausralia Pty Ltd	Anti-IL-23 heterodimer specific antibodies
US9127060	Dec 9, 2011	Sep 8, 2015	Wyeth Llc	Anti-Notch1 antibodies
US9133273	Jun 21, 2013	Sep 15, 2015	Eisai R&D Management Co., Ltd.	Nucleic acids encoding neutralizing anti-CCL20 antibodies
US9139645	Jul 9, 2013	Sep 22, 2015	Amgen Inc.	Peptides and related molecules that bind to TALL-1
US9150656	Mar 1, 2011	Oct 6, 2015	Macrogenics, Inc.	Antibodies reactive with B7-H3, immunologically active fragments thereof and uses thereof
US9156915	Apr 26, 2013	Oct 13, 2015	Thomas Jefferson	Anti-GCC antibody molecules

			University	
US9193794	Jan 20, 2010	Nov 24, 2015	Bioalliance C.V.	Antibodies recognizing a carbohydrate containing epitope on CD-43 and CEA expressed on cancer cells and methods using same
US9193798	Nov 21, 2014	Nov 24, 2015	Xencor, Inc.	Optimized Fc variants and methods for their generation
US9200079	Nov 14, 2005	Dec 1, 2015	Xencor, Inc.	Fc variants with altered binding to FcRn
US9234037	Jun 14, 2012	Jan 12, 2016	Ucb Biopharma Sprl	Method to generate antibodies to ion channels
US9244070	Apr 3, 2013	Jan 26, 2016	Research Development Foundation	Immunoglobulin libraries
US9250244	Feb 18, 2014	Feb 2, 2016	Epitomics, Inc.	Method for identifying lineage-related antibodies
US9260524	Dec 11, 2012	Feb 16, 2016	Ludwig Institute For Cancer Research	Specific binding proteins and uses thereof
US9260533	Feb 14, 2014	Feb 16, 2016	Anaptysbio, Inc.	Methods of generating libraries and uses thereof
US9267948	Dec 30, 2010	Feb 23, 2016	Brigham Young University	Compositions and methods for cancer management using antibodies binding to nucleotide salvage pathway enzymes and complexes thereof
US9272002	Oct 26, 2012	Mar 1, 2016	The Trustees Of The University Of Pennsylvania	Fully human, anti-mesothelin specific chimeric immune receptor for redirected mesothelin-expressing cell targeting
US9279008	Feb 17, 2014	Mar 8, 2016	The Trustees Of The University Of Pennsylvania	Isolated B7-H4 specific compositions and methods of use thereof
US9283276	Aug 14, 2008	Mar 15, 2016	Ludwig Institute For Cancer Research Ltd.	Monoclonal antibody 175 targeting the EGF receptor and derivatives and uses thereof
US9291549	Apr 24, 2012	Mar 22, 2016	Massachusetts Institute Of Technology	Pathogen detection biosensor
US9315843	Nov 14, 2013	Apr 19, 2016	Regeneron Pharmaceuticals, Inc.	Methods of producing hybrid antibodies
US20020123057 *	Nov 14, 2001	Sep 5, 2002	University Of Rochester	In vitro methods of producing and identifying immunoglobulin molecules in eukaryotic cells
US20020147312 *	Jan 30, 2002	Oct 10, 2002	O'keefe Theresa	Hybrid antibodies and uses thereof
US20030012782 *	Aug 10, 2001	Jan 16, 2003	Gold Daniel P.	Method and composition for altering a T cell mediated pathology
US20030035799 *	May 16, 2002	Feb 20, 2003	Glaxo Wellcome Inc.	Glycosylated antibody
US20030064069 *	Sep 13, 2002	Apr 3, 2003	Thompson Julia Elizabeth	Specific binding members for TGFbeta1
US20030068320 *	Mar 4, 2002	Apr 10, 2003	Christine Dingivan	Methods of administering/dosing CD2 antagonists for the prevention and treatment of autoimmune disorders or inflammatory disorders
US20030077739 *	Aug 26, 2002	Apr 24, 2003	Genentech, Inc.	System for antibody expression and assembly
US20030091566 *	Sep 13, 2002	May 15, 2003	Thompson Julia Elizabeth	Specific binding members for TGFbeta1
US20030096343 *	Jan 9, 2002	May 22, 2003	Xoma Technology Ltd.	Modular assembly of antibody genes, antibodies prepared thereby and use
US20030108966 *	Oct 16, 2002	Jun 12, 2003	Mather Jennie P.	Antibodies that bind to cancer-associated antigen CD46 and methods of use thereof
US20030109680 *	Nov 21, 2001	Jun 12, 2003	Sunol Molecular Corporation	Antibodies for inhibiting blood coagulation and methods of use thereof
US20030153039 *	Sep 17, 2002	Aug 14, 2003	Wyeth	Method for producing monoclonal antibodies
US20030157092 *	Mar 11, 2003	Aug 21, 2003	Research Development Foundation	Immunotoxins directed against CD33 related surface antigens
US20030176664 *	Dec 4, 2002	Sep 18, 2003	Jin-An Jiao	Use of anti-tissue factor antibodies for treating thromboses
US20030185833 *	Jun 17, 2002	Oct 2, 2003	Timothy Foster	Cross-reactive monoclonal and polyclonal antibodies which recognize surface proteins from coagulase-negative staphylococci and staphylococcus aureus
US20030190705 *	Aug 29, 2002	Oct 9, 2003	Sunol Molecular Corporation	Method of humanizing immune system molecules
US20030195156 *	May 13, 2002	Oct 16, 2003	Amgen Inc.	Peptides and related molecules that bind to TALL-1
US20030202975 *	Feb 21, 2003	Oct 30, 2003	Tedder Thomas F.	Reagents and treatment methods for autoimmune diseases
US20030219873 *	Feb 21, 2003	Nov 27, 2003	Lowy Douglas R.	Self-assembling recombinant papillomavirus capsid proteins
US20030224415 *	Mar 26, 2003	Dec 4, 2003	Gala Design, Inc.	Selection free growth of host cells containing multiple integrating vectors
US20030233197 *	Mar 19, 2003	Dec 18, 2003	Padilla Carlos E.	Discrete bayesian analysis of data
US20040002062 *	Mar 26, 2003	Jan 1, 2004	Gala Design, Inc.	Retrovirus-based genomic screening
US20040009147 *	Feb 28, 2003	Jan 15, 2004	Human Genome Sciences, Inc.	Apoptosis inducing molecule II and methods of use
US20040022869 *	Dec 2, 2002	Feb 5, 2004	Chen Lan Bo	Methods and compositions for modulating the immune system and uses thereof
US20040037833 *	Jun 19, 2003	Feb 26, 2004	Mather Jennie P.	Novel RAAG10 cell surface target and a family of antibodies

				recognizing that target
US20040038304 *	Mar 27, 2003	Feb 26, 2004	Gala Design, Inc.	Antibody libraries
US20040048312 *	Apr 14, 2003	Mar 11, 2004	Ronghao Li	Antibodies that bind to integrin alpha-v-beta-6 and methods of use thereof
US20040048319 *	May 2, 2003	Mar 11, 2004	Mather Jennie P.	ALCAM and ALCAM modulators
US20040086979 *	Aug 7, 2003	May 6, 2004	Dongxiao Zhang	Humanized rabbit antibodies
US20040091480 *	May 9, 2003	May 13, 2004	Kyowa Hakko Kogyo Co., Ltd.	Anti-fibroblast growth factor-8 monoclonal antibody
US20040093643 *	Nov 12, 2002	May 13, 2004	Burt Ensley	Production of pharmaceutically active proteins in sprouted seedlings
US20040110930 *	Oct 3, 2003	Jun 10, 2004	Reinl Stephen J.	Multimeric protein engineering
US20040157214 *	Mar 18, 2004	Aug 12, 2004	Cambridge Antibody Technology Limited	Methods for producing members of specific binding pairs
US20040157215 *	Mar 18, 2004	Aug 12, 2004	Cambridge Antibody Technology Limited	Methods for producing members of specific binding pairs
US20040171814 *	Nov 13, 2003	Sep 2, 2004	Mather Jennie P.	Antigen PIPA and antibodies that bind thereto
US20040197629 *	Jan 20, 2004	Oct 7, 2004	Yasuo Arishima	Electric power generating element for fuel cell and fuel cell using the same
US20040228836 *	Feb 13, 2004	Nov 18, 2004	University Of Southern California	Compositions and methods for cancer immunotherapy
US20040228862 *	Oct 8, 2003	Nov 18, 2004	Shelton David L.	Methods for treating post-surgical pain by administering a nerve growth factor antagonist and compositions containing the same
US20040235095 *	Aug 23, 2002	Nov 25, 2004	Denmeade Samuel R.	Proaerolysin containing protease activation sequences and methods of use for treatment of prostate cancer
US20040235173 *	Jan 16, 2004	Nov 25, 2004	Gala Design, Inc.	Production of host cells containing multiple integrating vectors by serial transduction
US20040265955 *	Jun 3, 2003	Dec 30, 2004	Jianmin Fang	Compositions and methods for generating multiple polypeptides from a single vector using a virus derived peptide cleavage site, and uses thereof
US20050003482 *	Apr 26, 2004	Jan 6, 2005	Jianmin Fang	Compositions and methods for enhanced expression of immunoglobulins from a single vector using a peptide cleavage site
US20050031617 *	Nov 26, 2003	Feb 10, 2005	Jing Ma	Antibodies specific for cancer associated antigen SM5-1 and uses thereof
US20050042664 *	Aug 20, 2004	Feb 24, 2005	Medimmune, Inc.	Humanization of antibodies
US20050042721 *	Apr 26, 2004	Feb 24, 2005	Jianmin Fang	Compositions and methods for enhanced expression of recombinant polypeptides from a single vector using a peptide cleavage site
US20050048572 *	Oct 30, 2003	Mar 3, 2005	Genentech, Inc.	Methods and compositions for increasing antibody production
US20050049403 *	Jul 23, 2003	Mar 3, 2005	Cambridge Antibody Technology Limited	Specific binding members for human transforming growth factor beta; materials and methods
US20050058658 *	Jul 15, 2004	Mar 17, 2005	Barros Research Institute	Compositions and methods for immunotherapy of human immunodeficiency virus (HIV)
US20050060762 *	Sep 23, 2004	Mar 17, 2005	Bleck Gregory T.	Expression vectors
US20050084495 *	Sep 23, 2004	Apr 21, 2005	Favrille, Inc.	Altering A B cell pathology using self-derived antigens in conjunction with specific-binding cytoreductive agent
US20050089521 *	Dec 23, 2003	Apr 28, 2005	Shelton David L.	Methods for treating taxol-induced sensory neuropathy
US20050089929 *	Jul 11, 2003	Apr 28, 2005	Sunol Molecular Corporation	Antibodies for inhibiting blood coagulation and methods of use thereof
US20050100952 *	Dec 21, 2004	May 12, 2005	Bremel Robert D.	Host cells containing multiple integrating vectors
US20050131219 *	Aug 18, 2004	Jun 16, 2005	Urdea Michael S.	Methods for reducing complexity of a sample using small epitope antibodies
US20050152894 *	Sep 3, 2004	Jul 14, 2005	Genentech, Inc.	Antibodies with altered effector functions
US20050152896 *	Dec 10, 2004	Jul 14, 2005	Amgen Inc.	Anti-galanin antibodies and uses thereof
US20050152907 *	Sep 17, 2004	Jul 14, 2005	Liang Tony W.	KID3 and KID3 antibodies that bind thereto
US20050169935 *	Jul 15, 2004	Aug 4, 2005	Charles Aylsworth	Compositions and methods for immunotherapy of cancer and infectious diseases
US20050170464 *	Mar 2, 2005	Aug 4, 2005	Genentech, Inc.	Prokaryotically produced antibodies and use thereof
US20050196755 *	Jun 20, 2003	Sep 8, 2005	Maurice Zauderer	In vitro methods of producing and identifying immunoglobulin molecules in eukaryotic cells
US20050202004 *	May 10, 2004	Sep 15, 2005	Favrille, Inc.	Method and composition for altering a B cell mediated pathology
US20050207977 *	May 17, 2005	Sep 22, 2005	Reinl Stephen J	Multimeric protein engineering
US20050221429 *	Jan 14, 2005	Oct 6, 2005	Cardinal Health Pts, Llc	Host cells containing multiple integrating vectors comprising an amplifiable marker
US20050227324 *	Dec 17, 2004	Oct 13, 2005	Genentech, Inc.	Monovalent antibody fragments useful as therapeutics

US20050232926 *	Dec 2, 2004	Oct 20, 2005	Oncomax Acquisition Corp.	Antibodies specific for cancer associated antigen SM5-1 and uses thereof
US20050244403 *	Mar 24, 2005	Nov 3, 2005	Xencor, Inc.	Immunoglobulin variants outside the Fc region
US20050260710 *	Mar 30, 2005	Nov 24, 2005	Sekisui Chemical Co. Ltd.	Methods for producing recombinant polyclonal immunoglobulins
US20050271664 *	Mar 22, 2005	Dec 8, 2005	Tanox, Inc.	Antibodies for inhibiting blood coagulation and methods of use thereof
US20060010906 *	Jul 15, 2004	Jan 19, 2006	Taras Michael F	Economized dehumidification system
US20060019260 *	Aug 2, 2004	Jan 26, 2006	Lerner Richard A	Method for tapping the immunological repertoire
US20060024311 *	Apr 28, 2005	Feb 2, 2006	Nathaniel Lallatin	Anti-viral activity of an anti-thymidine kinase monoclonal antibody
US20060034805 *	Jul 13, 2005	Feb 16, 2006	Jianmin Fang	AAV vector compositions and methods for enhanced expression of immunoglobulins using the same
US20060039901 *	May 5, 2005	Feb 23, 2006	Tanox, Inc.	Antibodies for inhibiting blood coagulation and methods of use thereof
US20060039908 *	Jun 7, 2005	Feb 23, 2006	Mather Jennie P	Transferrin receptor antibodies
US20060039914 *	May 20, 2005	Feb 23, 2006	Nathaniel Lallatin	Anti-cancer activity of an anti-thymidine kinase monoclonal antibody
US20060074225 *	Sep 14, 2005	Apr 6, 2006	Xencor, Inc.	Monomeric immunoglobulin Fc domains
US20060085871 *	Feb 18, 2005	Apr 20, 2006	Fraunhofer Usa, Inc.	Systems and methods for clonal expression in plants
US20060099204 *	Nov 8, 2004	May 11, 2006	Couto Fernando Jose R D	Methods for antibody engineering
US20060134104 *	Aug 4, 2005	Jun 22, 2006	Genentech, Inc.	Humanized anti-cmet antagonists
US20060135431 *	Nov 10, 2005	Jun 22, 2006	Amgen Inc.	Peptides and related molecules that bind to TALL-1
US20060135459 *	Nov 8, 2005	Jun 22, 2006	Epstein Alan L	Targeted innate immunity
US20060141574 *	Jan 4, 2006	Jun 29, 2006	Wyeth	Method for producing monoclonal antibodies
US20060147450 *	Oct 6, 2003	Jul 6, 2006	Shelton David L	Methods for treating cardiac arrhythmia and preventing death due to cardiac arrhythmia using ngf antagonists
US20060159675 *	Dec 19, 2005	Jul 20, 2006	Jin-An Jiao	Compositions and methods for treating coagulation related disorders
US20060166291 *	Jan 12, 2006	Jul 27, 2006	Mather Jennie P	KID31 and antibodies that bind thereto
US20060171949 *	Oct 28, 2005	Aug 3, 2006	Alan Epstein	Combination cancer immunotherapy with co-stimulatory molecules
US20060171952 *	Feb 2, 2006	Aug 3, 2006	Mather Jennie P	JAM-3 and antibodies that bind thereto
US20060172349 *	Jan 31, 2006	Aug 3, 2006	Mather Jennie P	LUCA2 and antibodies that bind thereto
US20060172350 *	Feb 2, 2006	Aug 3, 2006	Mather Jennie P	ADAM-9 modulators
US20060177453 *	Feb 6, 2006	Aug 10, 2006	Mather Jennie P	Antibodies that bind to EphA2 and methods of use thereof
US20060204493 *	Sep 1, 2005	Sep 14, 2006	Genentech, Inc.	Heteromultimeric molecules
US20060235208 *	Mar 31, 2006	Oct 19, 2006	Xencor, Inc.	Fc variants with optimized properties
US20060235209 *	Dec 4, 2002	Oct 19, 2006	Jin-An Jiao	Use of anti-tissue factor antibodies for treating thromboses
US20060239990 *	Oct 31, 2005	Oct 26, 2006	Nabel Elizabeth G	Protein Arginine N-Methyltransferase 2 (PRMT-2)
US20060246542 *	Feb 9, 2006	Nov 2, 2006	Genentech, Inc.	System for antibody expression and assembly
US20060269954 *	Jun 9, 2006	Nov 30, 2006	Lowy Douglas R	Self-assembling recombinant papillomavirus capsid proteins
US20070003546 *	Jul 7, 2006	Jan 4, 2007	Xencor, Inc.	Optimized Fc variants and methods for their generation
US20070014786 *	Mar 22, 2004	Jan 18, 2007	Rinat Neuroscience Corp.	Methods for treating taxol-induced gut disorder
US20070014802 *	Jul 1, 2004	Jan 18, 2007	Celltech R & D Limited	Modified antibody fragments
US20070015244 *	May 22, 2006	Jan 18, 2007	Genentech, Inc.	System for antibody expression and assembly
US20070020685 *	Nov 14, 2005	Jan 25, 2007	Kalobios Pharmaceuticals, Inc.	Secretion of antibodies without signal peptides from bacteria
US20070020725 *	May 22, 2006	Jan 25, 2007	Genentech, Inc.	Prokaryotically produced antibodies and uses thereof
US20070025990 *	Sep 29, 2006	Feb 1, 2007	Medimmune, Inc.	Methods of administering/dosing CD2 antagonists for the prevention and treatment of autoimmune disorders or inflammatory disorders
US20070031418 *	Apr 26, 2006	Feb 8, 2007	Lucia Tabares	Methods for treating lower motor neuron diseases and compositions containing the same
US20070042359 *	Aug 28, 2006	Feb 22, 2007	Crucell Holland B.V.	Binding molecules capable of neutralizing west nile virus and uses thereof
US20070042431 *	Oct 23, 2006	Feb 22, 2007	Tethys Bioscience, Inc.	Methods for reducing complexity of a sample using small epitope antibodies
US20070059301 *	Jul 1, 2004	Mar 15, 2007	Celltech R & D Limited	Modified antibody fragments
US20070059820 *	Jul 18, 2006	Mar 15, 2007	Jianmin Fang	Methods to express recombinant proteins from lentiviral vectors
US20070059845 *	Aug 11, 2006	Mar 15, 2007	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in T-cell receptor signaling pathways
US20070065909 *	Feb 9, 2006	Mar 22, 2007	Genentech, Inc.	Prokaryotically produced antibodies and uses thereof

US20070065912 *	Jul 21, 2006	Mar 22, 2007	Abbott Laboratories	Multiple Gene Expression including sORF Constructs and Methods with Polyproteins, Pro-Proteins, and Proteolysis
US20070072235 *	Aug 11, 2006	Mar 29, 2007	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in anaplastic large cell lymphoma signaling pathways
US20070128111 *	May 22, 2006	Jun 7, 2007	Genentech, Inc.	Methods and compositions for increasing antibody production
US20070128202 *	Sep 19, 2006	Jun 7, 2007	Mather Jennie P	Antibodies that bind to cancer-associated antigen CD46 and methods of use thereof
US20070141025 *	Feb 13, 2007	Jun 21, 2007	University Of Southern California	Compositions and methods for cancer immunotherapy
US20070148774 *	Nov 1, 2006	Jun 28, 2007	Medical Research Council	Methods for producing members of specific binding pairs
US20070160605 *	Oct 8, 2003	Jul 12, 2007	Shelton David L	Methods for treating post-surgical pain by administering an anti-nerve growth factor antagonist antibody
US20070207475 *	Nov 16, 2006	Sep 6, 2007	Scripps Research Institute	Method for producing polymers having a preselected activity
US20070224664 *	Jan 25, 2007	Sep 27, 2007	Genentech, Inc.	System for Antibody Expression and Assembly
US20070237766 *	Jun 19, 2007	Oct 11, 2007	Xencor, Inc.	Fc Variants Having Increased Affinity for FcyRIIIa
US20070243188 *	Jun 21, 2007	Oct 18, 2007	Xencor, Inc.	Fc Variants Having Decreased Affinity for FcyRIIa
US20070269371 *	Feb 28, 2007	Nov 22, 2007	Genentech, Inc.	Antibodies with altered effector functions
US20070275460 *	Oct 3, 2006	Nov 29, 2007	Xencor.Inc.	Fc Variants With Optimized Fc Receptor Binding Properties
US20070292922 *	Mar 28, 2007	Dec 20, 2007	Cell Genesys, Inc.	Regulated expression of recombinant proteins from adeno-associated viral vectors
US20080004434 *	Nov 9, 2006	Jan 3, 2008	Stratagene	method for tapping the immunological repertoire
US20080009017 *	May 7, 2007	Jan 10, 2008	Harper James D	Optoelectronic detection system
US20080014197 *	Aug 8, 2007	Jan 17, 2008	Yan Wang	Neutralizing human anti-igfr antibody
US20080014222 *	Mar 28, 2007	Jan 17, 2008	Cell Genesys, Inc.	Cancer immunotherapy compositions and methods of use
US20080026376 *	Jul 11, 2006	Jan 31, 2008	Huaming Wang	KEX2 cleavage regions of recombinant fusion proteins
US20080038752 *	Aug 11, 2006	Feb 14, 2008	Albrecht Moritz	Reagents for the detection of protein phosphorylation in c-Src signaling pathways
US20080050359 *	Jan 22, 2007	Feb 28, 2008	Medical Research Council	Production of anti-self antibodies from antibody segment repertoires and displayed on phage
US20080057056 *	Aug 20, 2007	Mar 6, 2008	Xencor, Inc.	Fc Variants with Increased Affinity for FcyRIIC
US20080069820 *	Aug 17, 2007	Mar 20, 2008	Genentech, Inc.	Multispecific antibodies
US20080089869 *	Sep 17, 2007	Apr 17, 2008	University of Victoria Innovation	Proaerolysin containing protease activation sequences and methods of use for treatment of prostate cancer
US20080089898 *	Oct 8, 2007	Apr 17, 2008	Rosenblum Michael G	Immunotoxins directed against CD33 related surface antigens
US20080108795 *	Jun 21, 2007	May 8, 2008	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in EGFR-signaling pathways
US20080131912 *	Dec 5, 2006	Jun 5, 2008	Bailin Tu	Recombinant antibodies against hepatitis C virus and methods of obtaining and using same
US20080167193 *	Aug 17, 2007	Jul 10, 2008	University Of Rochester	In Vitro methods of producing and identifying immunoglobulin molecules in eukaryotic cells
US20080171043 *	Jun 7, 2007	Jul 17, 2008	Rong-Hwa Lin	Antibodies recognizing a carbohydrate containing epitope on CD-43 and CEA expressed on cancer cells and methods using same
US20080176246 *	Mar 12, 2007	Jul 24, 2008	Urdea Michael S	Multiplex protein fractionation
US20080187966 *	Oct 30, 2007	Aug 7, 2008	Genentech, Inc.	Antibodies and methods for making and using them
US20080206754 *	Jun 29, 2007	Aug 28, 2008	Wyeth	Method for producing monoclonal antibodies
US20080206867 *	Oct 31, 2007	Aug 28, 2008	Desjarlais John R	Fc variants with optimized Fc receptor binding properties
US20080226634 *	Nov 8, 2007	Sep 18, 2008	Mather Jennie P	Tes7 and antibodies that bind thereto
US20080238709 *	Mar 25, 2008	Oct 2, 2008	Faramarz Vaziri	One-way communication apparatus with dynamic key generation
US20080241934 *	Aug 7, 2007	Oct 2, 2008	Urdea Michael S	Methods for reducing complexity of a sample using small epitope antibodies
US20080248490 *	Feb 29, 2008	Oct 9, 2008	Cell Signaling Technologies, Inc.	Reagents for the detection of protein phosphorylation in Leukemia signaling pathways
US20080279852 *	Jul 28, 2008	Nov 13, 2008	Smithkline Beecham Corporation	Methods of treatment with glycosylated antibodies
US20080280356 *	Oct 26, 2007	Nov 13, 2008	Cell Genesys, Inc.	Compositions and methods for enhanced expression of recombinant polypeptides from a single vector using a peptide cleavage site
US20080305516 *	Nov 30, 2005	Dec 11, 2008	Kristian Kjaergaard	Method of Producing Antibodies
US20080313379 *	Jun 15, 2007	Dec 18, 2008	United Memories, Inc.	Multiple bus charge sharing
US20090017068 *	Jul 29, 2008	Jan 15, 2009	Crucell Holland B.V.	Vaccines against West Nile Virus
US20090041766 *	Jun 10, 2008	Feb 12, 2009	Wong Hing C	Antibodies for inhibiting blood coagulation and methods of use thereof
US20090061459 *	Feb 29, 2008	Mar 5, 2009	Cell Signaling Technolgy, Inc.	Reagents for the detection of protein phosphorylation in carcinoma signaling pathways

US20090068684 *	Mar 26, 2008	Mar 12, 2009	Cell Signaling Technology, Inc.	Serine and threoninephosphorylation sites
US20090092602 *	Jul 31, 2008	Apr 9, 2009	Jin-An Jiao	Use of anti-tissue factor antibodies for treating thromboses
US20090099340 *	Oct 12, 2007	Apr 16, 2009	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in carcinoma signaling pathways
US20090104204 *	Jun 5, 2007	Apr 23, 2009	Mark Throsby	Human Binding Molecules Having Killing Activity Against Staphylococci and Uses Thereof
US20090130113 *	Oct 10, 2008	May 21, 2009	Michaela Kneissel	Compositions and methods for use for antibodies against sclerostin
US20090130652 *	Jun 22, 2006	May 21, 2009	Crucell Holland B.V.	Optimization of West Nile Virus Antibodies
US20090136501 *	Feb 22, 2008	May 28, 2009	Jin-An Jiao	Compositions and methods for treating coagulation related disorders
US20090136936 *	Apr 30, 2008	May 28, 2009	George Georgiou	Immunoglobulin fc libraries
US20090136999 *	Apr 14, 2008	May 28, 2009	Michigan State University	Compositions and methods for immunotherapy of cancer and infectious diseases
US20090142340 *	Oct 29, 2007	Jun 4, 2009	Xencor, Inc.	Optimized Fc Variants and Methods for Their Generation
US20090155810 *	Nov 1, 2006	Jun 18, 2009	Medical Research Council	Methods for producing members of specific binding pairs
US20090155824 *	Oct 31, 2008	Jun 18, 2009	Fernando Jose Rebelo Do Couto	Methods for antibody engineering
US20090196880 *	Aug 11, 2008	Aug 6, 2009	Vasgene Therapeutics, Inc.	Cancer treatment using humanized antibodies that bind to EphB4
US20090202526 *	Dec 23, 2004	Aug 13, 2009	Rinat Neuroscience Corporation	Agonist anti-trkc antibodies and methods using same
US20090202561 *	Mar 27, 2009	Aug 13, 2009	Mather Jennie P	Novel raag10 cell surface target and a family of antibodies recognizing that target
US20090215033 *	Aug 3, 2005	Aug 27, 2009	Javed Khan	Prediction of Clinical Outcome Using Gene Expression Profiling and Artificial Neural Networks for Patients with Neuroblastoma
US20090215119 *	Feb 13, 2009	Aug 27, 2009	Dyax Corp.	Methods for producing specific binding pairs
US20090220510 *	Dec 23, 2008	Sep 3, 2009	Ludwig Institute For Cancer Research	Specific binding proteins and uses thereof
US20090220991 *	Feb 29, 2008	Sep 3, 2009	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in leukemia signaling pathways
US20090226433 *	Nov 12, 2008	Sep 10, 2009	Spaltudaq Corp.	Compositions and methods for the therapy and diagnosis of influenza
US20090226455 *	Mar 6, 2009	Sep 10, 2009	Genentech, Inc.	Combination therapy with c-met and her antagonists
US20090226922 *	Mar 4, 2009	Sep 10, 2009	4-Antibody Ag	Identification of antigen or ligand-specific binding proteins
US20090252726 *	Mar 13, 2009	Oct 8, 2009	Jin-An Jiao	Antibodies for inhibiting blood coagulation and methods of use thereof
US20090252746 *	May 14, 2008	Oct 8, 2009	Abbott Laboratories	Erythropoietin receptor binding antibodies
US20090258442 *	Feb 29, 2008	Oct 15, 2009	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in carcinoma signaling pathways
US20090269340 *	Jul 2, 2009	Oct 29, 2009	Mather Jennie P	Antibodies to oncostatin m receptor
US20090269786 *		Oct 29, 2009	The Board Of Trustees Of The University Of Illinois	RHO1-Gamma Amino Butyric Acid C Receptor-Specific Antibodies
US20090270485 *	Jul 24, 2008	Oct 29, 2009	Cell Genesys, Inc.	Cell specific replication-competent viral vectors comprising a self processing peptide cleavage site
US20090274620 *	Dec 23, 2008	Nov 5, 2009	Wilex Ag	Hybridoma Cell Line G250 and its use for Producing Monoclonal Antibodies
US20090298093 *	Apr 27, 2007	Dec 3, 2009	Roberto Polakiewicz	Reagents for the Detection of Protein Phosphorylation in ATM & ATR Kinase Signaling Pathways
US20090324613 *	Mar 10, 2009	Dec 31, 2009	Spaltudaq Corporation	Compositions and Methods for the Therapy and Diagnosis of Cytomegalovirus
US20090325815 *		Dec 31, 2009	Medical Research Council	Production of anti-self antibodies from antibody segment repertoires and displayed on phage
US20100003700 *	Feb 27, 2009	Jan 7, 2010	Massachusetts Institute Of Technology	Optoelectronic sensor
US20100008888 *	Sep 15, 2009	Jan 14, 2010	Wilex Ag	Co-administration of cg250 and il-2 or ifn-alpha for treating cancer such as renal cell carcinomas
US20100009869 *		Jan 14, 2010	Bremel Robert D	Antibody libraries
US20100041031 *	Jul 3, 2008	Feb 18, 2010	Schwoebel Eric D	Optoelectronic detection system
US20100055731 *	Jun 21, 2007	Mar 4, 2010	Huaming Wang	KEX2 Cleavage Regions OF Recombinant Fusion Proteins
US20100062415 *	Nov 30, 2006	Mar 11, 2010	Eric Schwoebel	Pathogen Detection Biosensor
US20100086541 *		Apr 8, 2010	Chun Wu	Regulation of autophagy pathway phosphorylation and uses thereof
US20100086969 *		Apr 8, 2010	Macrogenics, Inc.	Antibodies That Bind to EphA2 and Methods of Use Thereof
US20100092458 *	Jul 20, 2009	Apr 15, 2010	Chun Wu	Regulation of apg8 phosphorylation and uses thereof

US20100143244 *	Dec 4, 2008	Jun 10, 2010	Lallatin Nathaniel C	Monoclonal antibodies to human thymidine kinase to treat cancer
US20100151495 *	Feb 29, 2008	Jun 17, 2010	Cell Signaling Technolgy, Inc.	Reagents for the detection of protein phosphorylation in carcinoma signaling pathways
US20100151523 *	Jun 30, 2009	Jun 17, 2010	Cell Genesys, Inc.	Regulated expression of recombinant proteins from adeno-associated viral vectors
US20100183605 *		Jul 22, 2010	Macrogenics, Inc.	Tes7 and antibodies that bind thereto
US20100204059 *	Jan 28, 2010	Aug 12, 2010	Epitomics, Inc.	CDR-Anchored Amplification Method
US20100239594 *	Aug 3, 2006	Sep 23, 2010	Vidadi Yusibov	Compositions and methods for production of immunoglobulins
US20100240590 *	May 26, 2010	Sep 23, 2010	Amgen Inc.	Peptides and related molecules that bind to tall-1
US20100247536 *	Mar 26, 2010	Sep 30, 2010	Quantum Immunologics, Inc.	Oncofetal antigen/immature laminin receptor antibodies for diagnostic and clinical applications
US20100266495 *	Apr 26, 2010	Oct 21, 2010	Brigham Young University	Anti-Cancer Activity of an Anti-Thymidine Kinase Monoclonal Antibody
US20100287632 *	Nov 17, 2009	Nov 11, 2010	Meagher Richard B	Rapid Production of Monoclonal Antibodies
US20100291075 *		Nov 18, 2010	Theraclone Sciences, Inc.	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) Neutralizing Antibodies
US20100303801 *	May 11, 2006	Dec 2, 2010	Mark Throsby	Host cell specific binding molecules capable of neutralizing viruses and uses thereof
US20100316574 *	Mar 26, 2010	Dec 16, 2010	Quantum Immunologics, Inc.	Oncofetal Antigen/Immature Laminin Receptor Peptides for the Sensitization of Dendritic Cells for Cancer Therapy
US20100317539 *		Dec 16, 2010	Guo-Liang Yu	Library of Engineered-Antibody Producing Cells
US20100322851 *	Jul 7, 2010	Dec 23, 2010	Macrogenics, Inc.	KID3 and KID3 Antibodies That Bind Thereto
US20100322946 *	Sep 1, 2009	Dec 23, 2010	Genentech, Inc.	Multispecific antibodies
US20100330076 *	Jun 30, 2010	Dec 30, 2010	George Georgiou	Immunoglobulin fc polypeptides
US20110002920 *	Mar 25, 2010	Jan 6, 2011	University Of Southern California	Combination cancer immunotherapy with co-stimulatory molecules
US20110002921 *	Mar 25, 2010	Jan 6, 2011	University Of Southern California	Combination cancer immunotherapy with co-stimulatory molecules
US20110033476 *	Jun 7, 2010	Feb 10, 2011	Theraclone Sciences Inc.	Compositions and methods for the therapy and diagnosis of influenza
US20110045006 *		Feb 24, 2011	Macrogenics West, Inc.	LUCA2 and Antibodies That Bind Thereto
US20110052592 *	Nov 11, 2010	Mar 3, 2011	Michaela Kneissel	Compositions and Methods for Use for Antibodies Against Sclerostin
US20110065610 *		Mar 17, 2011	Nicolas Fischer	Synthetic Polypeptide Libraries and Methods for Generating Naturally Diversified Polypeptide Variants
US20110135662 *	Oct 27, 2010	Jun 9, 2011	Helene Margaret Finney	FUNCTION MODIFYING NAv 1.7 ANTIBODIES
US20110150861 *		Jun 23, 2011	Abbott Laboratories	Sorf constructs and multiple gene expression
US20110165161 *		Jul 7, 2011	Shih-Yao Lin	Anti-epcam antibodies that induce apoptosis of cancer cells and methods using same
US20110176996 *	Dec 30, 2010	Jul 21, 2011	Brigham Young University	Compositions and methods for cancer management using antibodies binding to nucleotide salvage pathway enzymes and complexes thereof
US20110182887 *	Oct 25, 2010	Jul 28, 2011	Medimmune, Llc.	Humanized anti-cd22 antibodies and their use in treatment of oncology, transplantation and autoimmune disease
US20110195411 *		Aug 11, 2011	The Board Of Trustees Of Southern Illinois University	Arl-1 specific antibodies and uses thereof
US20110200596 *		Aug 18, 2011	Genentech, Inc.	Monovalent antibody fragments useful as therapeutics
US20110229909 *		Sep 22, 2011	The Board Of Trustees Of The University Of Illinois	RHO1-Gamma Amino Butyric Acid C Receptor-Specific Antibodies
US20140017238	Sep 25, 2013	Jan 16, 2014	Regeneron Pharmaceuticals, Inc.	Methods of Modifying Eukaryotic Cells
EP1972639A2	Mar 7, 2008	Sep 24, 2008	Cell Signaling Technology, Inc.	Reagents for the detection of protein phosphorylation in carcinoma signaling pathways
EP1975184A2	Mar 26, 2008	Oct 1, 2008	Albrecht Moritz	Serine or threonine phosphorylation sites
EP1983002A2	Apr 18, 2008	Oct 22, 2008	Peter Hornbeck	Tyrosine phosphorylation sites and antibodies specific for them
EP1983003A2	Apr 18, 2008	Oct 22, 2008	Peter Hornbeck	Tyrosine phosphorylation sites and antibodies specific for them
EP2062920A2	Nov 21, 2008	May 27, 2009	Peter Hornbeck	Protein phosphorylation by basophilic serine/threonine kinases in insulin signalling pathways
EP2100902A1	Oct 8, 2003	Sep 16, 2009	Rinat Neuroscience Corp.	Methods for treating pain by administering an antagonist antibody against the nerve growth factor and an opioid analgesic, and compositions containing the same
EP2123679A2	May 1, 2008	Nov 25, 2009	Peter Hornbeck	Tyrosine phosphorylation sites
EP2128270A1	Aug 9, 2004	Dec 2, 2009	Genenews Inc.	Osteoarthritis biomarkers and uses thereof

EP2145902A2	Apr 18, 2008	Jan 20, 2010	Peter Hornbeck	Tyrosine phosphorylation sites and antibodies specific for them
EP2163563A1	Mar 29, 2007	Mar 17, 2010	Massachusetts Institute of Technology	Treatment of tumors expressing mutant EGF receptors
EP2191846A1	Feb 19, 2004	Jun 2, 2010	Rinat Neuroscience Corp.	Method for treating pain by administering a nerve growth factor antagonist and an NSAID and composition containing the same
EP2206728A1	Apr 7, 2005	Jul 14, 2010	Rinat Neuroscience Corp.	Methods for treating bone cancer pain by administering a nerve growth factor antagonistic antibody
EP2221316A1	May 5, 2006	Aug 25, 2010	Duke University	Anti-CD19 antibody therapy for autoimmune disease
EP2258724A1	Nov 21, 2003	Dec 8, 2010	Celltech R & D, Inc.	Modulating immune responses using multimerized anti-CD83 antibodies
EP2263692A1	Dec 24, 2003	Dec 22, 2010	Rinat Neuroscience Corp.	Anti-NGF antibodies and methods using same
EP2270048A2	Dec 24, 2003	Jan 5, 2011	Rinat Neuroscience Corp.	Anti-NGF antibodies and methods using same
EP2277917A2	Jun 19, 2003	Jan 26, 2011	Raven Biotechnologies, Inc.	B7-H3L cell surface target and a family of antibodies recognizing that target
EP2298807A2	Aug 1, 2005	Mar 23, 2011	Rinat Neuroscience Corp.	Antibodies directed against amyloid-beta peptide and methods using same
EP2305711A2	Apr 11, 2006	Apr 6, 2011	Rinat Neuroscience Corp.	Methods for treating osteoarthitis pain by administering a nerve growth factor antagonist and compositions containing the same
EP2319941A2	Oct 23, 2006	May 11, 2011	GeneNews Inc.	Method and apparatus for correlating levels of biomarker products with disease
EP2320233A1	Jun 17, 2002	May 11, 2011	Inhibitex, Inc.	Cross-reactive monoclonal and polyclonal antibodies which recognize surface proteins from coagulase-negative staphylococci and staphylococcus aureus
EP2322214A1	Apr 16, 2004	May 18, 2011	Altropus Gmbh	Immunogenic recombinant antibody
EP2339344A1	Jun 17, 2002	Jun 29, 2011	Inhibitex, Inc.	Cross-reactive monoclonal and polyclonal antibodies which recognize surface proteins from coagulase-negative staphylococci and staphylococcus aureus
EP2354159A1	Feb 5, 2010	Aug 10, 2011	RWTH Aachen	CCL17 inhibitors for use in T helper cell-driven diseases
EP2359834A1	Mar 15, 2007	Aug 24, 2011	Alexion Pharmaceuticals, Inc.	Treatment of paroxysmal nocturnal hemoglobinuria patients by an inhibitor of complement
EP2365062A1	Feb 20, 2002	Sep 14, 2011	University Of Georgia Research Foundation, Inc.	Rapid production of monoclonal antibodies
EP2366717A2	Oct 28, 2005	Sep 21, 2011	University of Southern California	Combination Cancer Immunotherapy with Co-Stimulatory Molecules
EP2380592A2	Nov 2, 2006	Oct 26, 2011	Rinat Neuroscience Corp.	Antagonist antibodies directed against calcitonin gene-related peptide and methods using same
EP2380907A1	Sep 5, 2007	Oct 26, 2011	Alexion Pharmaceuticals, Inc.	Methods and compositions for the treatment of antibody mediated neuropathies
EP2384767A1	Mar 10, 2006	Nov 9, 2011	Millennium Pharmaceuticals, Inc.	Antibodies that bind OV064 and methods of use therefor
EP2402756A2	Dec 23, 2004	Jan 4, 2012	Rinat Neuroscience Corp.	Agonist anti-trkC antibodies and methods using same
EP2420246A1	Apr 20, 2007	Feb 22, 2012	The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.	Methods and compositions based on shiga toxin type 1 protein
EP2468768A2	Jul 21, 2006	Jun 27, 2012	Abbott Laboratories	Multiple gene expression including sorf contructs and methods with polyproteins, pro-proteins, and proteolysis
EP2468881A2	Jul 21, 2006	Jun 27, 2012	Abbott Laboratories	Multiple gene expression including sorf contructs and methods with polyproteins, pro-proteins, and proteolysis
EP2471554A1	Dec 28, 2010	Jul 4, 2012	Hexal AG	Pharmaceutical formulation comprising a biopharmaceutical drug
EP2484696A1	Aug 24, 2007	Aug 8, 2012	Kyowa Hakko Kirin Co., Ltd.	Antagonistic human light-specific human monoclonal antibodies
EP2484774A2	Jul 21, 2006	Aug 8, 2012	Abbott Laboratories	Multiple gene expression including sorf contructs and methods with polyproteins, pro-proteins, and proteolysis
EP2495252A2	Jul 7, 2005	Sep 5, 2012	The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.	Soluble forms of hendra and nipah virus G glycoprotein
EP2511293A1	Apr 13, 2011	Oct 17, 2012	LEK Pharmaceuticals d.d.	A method for controlling the main complex N-glycan structures and the acidic variants and variability in bioprocesses producing recombinant proteins
EP2520669A2	Feb 6, 2006	Nov 7, 2012	GeneNews Inc.	Mild osteoathritis biomarkers and uses thereof
EP2535349A1	Sep 26, 2008	Dec 19, 2012	UCB Pharma S.A.	Dual specificity antibody fusions
EP2535350A1	Sep 26, 2008	Dec 19, 2012	UCB Pharma S.A.	Dual specificity antibody fusions
EP2535351A2	Sep 26, 2008	Dec 19, 2012	UCB Pharma S.A.	Dual specificity antibody fusions
				Humanized anti-CD22 antibodies and their use in treatment of

EP2540741A1	Mar 6, 2007	Jan 2, 2013	Aeres Biomedical Limited	oncology, transplantation and autoimmune disease
EP2543389A2	Aug 1, 2008	Jan 9, 2013	Gilead Biologics, Inc.	Methods and compositions inhibiting a lysyl oxidase (-like) protein for treatment and diagnosis of fibrosis, tumor invasion, angiogenesis, and metastasis
EP2548575A1	Feb 15, 2006	Jan 23, 2013	Duke University	Anti-CD19 antibodies that mediate ADCC for use in treating autoimmune diseases
EP2607485A1	Jun 21, 2007	Jun 26, 2013	Danisco US Inc.	KEX2 cleavage regions of recombinant fusion proteins
EP2607486A1	Jun 21, 2007	Jun 26, 2013	Danisco US Inc.	KEX2 cleavage regions of recombinant fusion proteins
EP2626371A1	Jul 30, 2008	Aug 14, 2013	MedImmune, LLC	Multispecific epitope binding proteins and uses thereof
EP2662091A2	Nov 30, 2007	Nov 13, 2013	Selexys Pharmaceuticals Corporation	Anti-P-selectin antibodies and methods of using the same to treat inflammatory diseases
EP2703011A2	May 7, 2008	Mar 5, 2014	MedImmune, LLC	Anti-icos antibodies and their use in treatment of oncology, transplantation and autoimmune disease
EP2724728A1	Jun 27, 2008	Apr 30, 2014	The United States of America, as Represented by The Secretary, Department of Health and Human Services	Complexes of IL-15 and IL-15R alpha and uses thereof
EP2728017A1	Nov 19, 2008	May 7, 2014	Celera Corporation	Lung cancer markers and uses thereof
EP2737907A2	May 7, 2008	Jun 4, 2014	MedImmune, LLC	Anti-icos antibodies and their use in treatment of oncology, transplantation and autoimmune disease
EP2762496A1	Feb 5, 2013	Aug 6, 2014	EngMab AG	Method for the selection of antibodies against BCMA
EP2762497A1	Feb 5, 2013	Aug 6, 2014	EngMab AG	Bispecific antibodies against CD3epsilon and BCMA
EP2772262A1	Mar 29, 2007	Sep 3, 2014	Aduro GVAX Inc.	Cancer Immunotherapy Compositions and Methods of Use
EP2789630A1	Apr 9, 2013	Oct 15, 2014	EngMab AG	Bispecific antibodies against CD3e and ROR1
EP2815764A1	Jun 14, 2007	Dec 24, 2014	Macrogenics, Inc.	Methods for the treatment of autoimmune disorders using monoclonal antibodies with reduced toxicity
EP2891666A1	Oct 15, 2003	Jul 8, 2015	Purdue Pharma L.P.	Antibodies that bind cell-associated CA 125/O722P and methods of use thereof
EP2913342A1	Sep 5, 2007	Sep 2, 2015	Alexion Pharmaceuticals, Inc.	Compositions for use in the treatment of antibody mediated neuropathies
EP2926830A2	Aug 31, 2011	Oct 7, 2015	Theraclone Sciences, Inc.	Human immunodeficiency virus (HIV)-neutralizing antibodies
EP2982380A1	Mar 1, 2011	Feb 10, 2016	MacroGenics, Inc.	Antibodies reactive with b7-h3, immunologically active fragments thereof and uses thereof
EP2982692A1	Aug 4, 2014	Feb 10, 2016	EngMab AG	Bispecific antibodies against CD3epsilon and BCMA
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EP2998405A1	May 13, 2010	Mar 23, 2016	Genzyme Corporation	Anti-human cd52 immunoglobulins
EP3009448A1	Jul 24, 2007	Apr 20, 2016	UCB Biopharma SPRL	Single chain fc polypeptides
WO2002066618A1	Feb 20, 2002	Aug 29, 2002	University Of Georgia Research Foundation, Inc.	Rapid production of monoclonal antibodies
WO2002102829A2	Jun 17, 2002	Dec 27, 2002	Inhibitex, Inc.	Cross-reactive monoclonal and polyclonal antibodies which recognize surface proteins from coagulase-negative staphylococci and staphylococcus aureus
WO2004032870A2	Oct 8, 2003	Apr 22, 2004	Rinat Neuroscience Corp.	Methods for treating post-surgical pain by admisnistering a nerve growth factor antagonist and compositions containing the same
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WO2004113493A2	Apr 26, 2004	Dec 29, 2004	Cell Genesys, Inc.	Compositions and methods for enhanced expression of immunoglobulins from a single vector using a peptide cleavage site
WO2005070962A1 *	Jan 20, 2005	Aug 4, 2005	Novozymes A/S	Production of a monoclonal antibody in a heterokaryon fungus or in a fungal host cell
WO2005121179A2	Jun 7, 2005	Dec 22, 2005	Raven Biotechnologies, Inc.	Transferrin receptor antibodies
WO2006084226A2	Feb 6, 2006	Aug 10, 2006	Raven Biotechnologies, Inc.	Antibodies that bind to epha2 and methods of use thereof
WO2006089133A2	Feb 15, 2006	Aug 24, 2006	Duke University	Anti-cd19 antibodies and uses in oncology
WO2006136601A1 *	Jun 22, 2006	Dec 28, 2006	Crucell Holland B.V.	Optimization of west nile virus antibodies
WO2007119623A1	Mar 30, 2007	Oct 25, 2007	Riken	Novel use of g-protein-conjugated receptor and ligand thereof
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WO2008018472A1	Aug 7, 2007	Feb 14, 2008	Kyoto University	Novel monoclonal antibody and use of the same
WO2008038024A1	Sep 28, 2007	Apr 3, 2008	Ucb Pharma S.A.	Altered antibodies
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WO2009030936A1	Sep 4, 2008	Mar 12, 2009	Ucb Pharma S.A.	Method for the treatment of glomerulonephritis
WO2009064805A1	Nov 12, 2008	May 22, 2009	Spaltudaq Corporation	Compositions and methods for the therapy and diagnosis of influenza
WO2009079649A1	Dec 18, 2008	Jun 25, 2009	Bioalliance C.V.	Antibodies recognizing a carbohydrate containing epitope on cd-43 and cea expressed on cancer cells and methods using same
WO2010029513A2	Sep 11, 2009	Mar 18, 2010	Rinat Neuroscience Corporation	Pcsk9 antagonists
WO2010056804A1	Nov 12, 2009	May 20, 2010	Medimmune, Llc	Antibody formulation
WO2010079850A2	Jan 12, 2010	Jul 15, 2010	Takeda Pharmaceutical Company Limited	Agent for prophylaxis or treatment of cancer
WO2010086828A2	Jan 29, 2010	Aug 5, 2010	Rinat Neuroscience Corporation	Agonist anti-trkb monoclonal antibodies
WO2010096036A2	May 14, 2009	Aug 26, 2010	Millennium Pharmaceuticals, Inc.	Methods and kits for monitoring the effects of immunomodulators on adaptive immunity
WO2010098166A1	Jan 28, 2010	Sep 2, 2010	University Of Miyazaki	Cell adhesion inhibitor and use thereof
WO2010111671A1	Mar 26, 2010	Sep 30, 2010	Quantum Immunologics, Inc.	Oncofetal antigen/immature laminin receptor antibodies for diagnostic and clinical applications
WO2010146511A1	Jun 11, 2010	Dec 23, 2010	Pfizer Limited	Treatment of overactive bladder
WO2010146550A1	Jun 16, 2010	Dec 23, 2010	Pfizer Inc.	Anti notch-1 antibodies
WO2011014438A1	Jul 26, 2010	Feb 3, 2011	N.V. Organon	Fully human antibodies to btla
WO2011020024A2	Aug 13, 2010	Feb 17, 2011	The Johns Hopkins University	Methods of modulating immune function
WO2011036460A1	Sep 24, 2010	Mar 31, 2011	Ucb Pharma S.A.	Disulfide stabilised multivalent antibodies
WO2011050242A1	Oct 22, 2010	Apr 28, 2011	Millennium Pharmaceuticals, Inc.	Anti-gcc antibody molecules and related compositions and methods
WO2011051349A1	Oct 27, 2010	May 5, 2011	Ucb Pharma S.A.	Antibodies to ion channels
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WO2011053699A1	Oct 28, 2010	May 5, 2011	Abbott Laboratories	Sorf constructs and multiple gene expression
WO2011063277A1	Nov 19, 2010	May 26, 2011	Amgen Inc.	Anti-orai1 antigen binding proteins and uses thereof
WO2011071957A1	Dec 7, 2010	Jun 16, 2011	Sea Lane Biotechnologies, Llc	Conjugates comprising an antibody surrogate scaffold with improved pharmacokinetic properties
WO2011104687A1	Feb 24, 2011	Sep 1, 2011	Rinat Neuroscience Corporation	Antagonist anti-il-7 receptor antibodies and methods
WO2011107520A1	Mar 2, 2011	Sep 9, 2011	Cilian Ag	Expression of monoclonal antibodies in ciliate host cells
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WO2011117648A2	Mar 25, 2011	Sep 29, 2011	Ucb Pharma S.A.	Disulfide stabilised antibodies and fragments thereof
WO2011117653A1	Mar 25, 2011	Sep 29, 2011	Ucb Pharma S.A.	Disulfide stabilized dvd-lg molecules
WO2011133886A2	Apr 22, 2011	Oct 27, 2011	Genentech, Inc.	Production of heteromultimeric proteins
WO2012015758A2	Jul 25, 2011	Feb 2, 2012	Saint Louis University	Methods of treating pain
WO2012021648A1	Aug 10, 2011	Feb 16, 2012	Amgen Inc.	Dual function in vitro target binding assay for the detection of neutralizing antibodies against target antibodies
WO2012022982A2	Aug 19, 2011	Feb 23, 2012	Ucb Pharma S.A.	Improved antibodies of the class igg4
WO2012040518A2	Sep 22, 2011	Mar 29, 2012	Amgen Inc.	Carrier immunoglobulins and uses thereof
WO2012045085A1	Oct 3, 2011	Apr 5, 2012	Oxford Biotherapeutics Ltd.	Anti-rori antibodies
WO2012067188A1	Nov 17, 2011	May 24, 2012	National University Corporation Okayama University	Method for producing b-cell capable of producing human-type antibody
WO2012089778A1	Dec 28, 2011	Jul 5, 2012	Hexal Ag	Pharmaceutical formulation comprising a biopharmaceutical drug
WO2012090895A1	Dec 26, 2011	Jul 5, 2012	Japan Health Sciences Foundation	Monoclonal antibody capable of recognizing human papilloma virus (hpv) l2 protein, and method for measuring hpv-neutralizing antibody titer using same
WO2012092376A3 *	Dec 28, 2011	Dec 6, 2012	Short Jay M	Comprehensive monoclonal antibody generation
WO2012094252A1	Dec 30, 2011	Jul 12, 2012	The Terasaki Family Foundation	Anti-hla-e antibodies, therapeutic immunomodulatory antibodies to human hla-e heavy chain, useful as ivig mimetics and methods of their use
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WO2012122484A1	Mar 9, 2012	Sep 13, 2012	Roberto Polakiewicz	Methods and reagents for creating monoclonal antibodies
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WO2012140138A1	Apr 12, 2012	Oct 18, 2012	Lek Pharmaceuticals D.D.	A method for controlling the main complex n-glycan structures and the acidic variants and variability in bioprocesses producing recombinant proteins
WO2013014538A2	Jul 25, 2012	Jan 31, 2013	American University In Cairo	Single-domain antibodies and graphene coated magnetic metal nanoparticles conjugate and methods for using the same
WO2013033069A1	Aug 28, 2012	Mar 7, 2013	Theraclone Sciences, Inc.	Human rhinovirus (hrv) antibodies
WO2013068571A1	Nov 9, 2012	May 16, 2013	Ucb Pharma S.A.	Albumin binding antibodies and binding fragments thereof
WO2013068946A2	Nov 7, 2012	May 16, 2013	Rinat Neuroscience Corp.	Antibodies specific for trop-2 and their uses
WO2013093379A1	Dec 21, 2012	Jun 27, 2013	Lfb Biotechnologies	Novel pharmaceutical compositions comprising an antibody which binds the human anti-mullerian hormone receptor type ii
WO2013093693A1	Dec 7, 2012	Jun 27, 2013	Rinat Neuroscience Corp.	Staphylococcus aureus specific antibodies and uses thereof
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WO2013106586A2	Jan 10, 2013	Jul 18, 2013	The Terasaki Family Foundation	Anti-hla class-ib antibodies mimic immunoreactivity and immunomodulatory functions of intravenous immunoglobulin (ivig) useful as therapeutic ivig mimetics and methods of their use
WO2013124450A1	Feb 22, 2013	Aug 29, 2013	Ucb Pharma S.A.	Sequence symmetric modified igg4 bispecific antibodies
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WO2013126813A1	Feb 22, 2013	Aug 29, 2013	Amgen Inc.	Autologous mammalian models derived from induced pluripotent stem cells and related methods
WO2013156534A1	Apr 17, 2013	Oct 24, 2013	Arsanis Biosciences Gmbh	Cross-reactive staphylococcus aureus antibody
WO2014003008A1	Jun 25, 2013	Jan 3, 2014	Hiroshima University	Novel histamine liberator contained in human sweat
WO2014027626A1	Aug 9, 2013	Feb 20, 2014	Hiroshima University	MONOCLONAL IgE ANTIBODY BONDING TO SWEAT ALLERGY ANTIGEN PROTEIN
WO2014031694A2	Aug 20, 2013	Feb 27, 2014	The Institute Of Molecular Medicine	Anti-tau antibodies and methods of making and using in treatment of tauopathies
WO2014034700A1	Aug 28, 2013	Mar 6, 2014	Hiroshima University	CELL PROLIFERATION REGULATION COMPOSITION CONTAINING PEPTIDE COMPRISING PARTIAL AMINO ACID SEQUENCE FOR BBF2H7 (BBF2 human homologue on chromosome7) OR ANTIBODY CAPABLE OF BINDING TO SAID PEPTIDE
WO2014068079A1	Nov 1, 2013	May 8, 2014	Max-Delbrück-Centrum für Molekulare Medizin	An antibody that binds cd269 (bcma) suitable for use in the treatment of plasma cell diseases such as multiple myeloma and autoimmune diseases
WO2014072876A1	Oct 28, 2013	May 15, 2014	Pfizer Inc.	Platelet-derived growth factor b specific antibodies and compositions and uses thereof
WO2014096672A1	Dec 17, 2013	Jun 26, 2014	Laboratoire Francais Du Fractionnement Et Des Biotechnologies	Use of monoclonal antibodies for the treatment of inflammation and bacterial infections
WO2014100079A1	Dec 18, 2013	Jun 26, 2014	Merck Sharp & Dohme Corp.	Antibodies that bind to human programmed death ligand 1 (pd-l1)
WO2014100823A1	Dec 23, 2013	Jun 26, 2014	Amplimmune, Inc.	Anti-h7cr antibodies
WO2014111516A1	Jan 17, 2014	Jul 24, 2014	Arsanis Biosciences Gmbh	Mdr e. coli specific antibody
WO2014122143A1	Feb 5, 2014	Aug 14, 2014	Engmab Ag	Method for the selection of antibodies against bcma
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WO2014130635A1	Feb 20, 2014	Aug 28, 2014	Novartis Ag	Effective targeting of primary human leukemia using anti-cd123 chimeric antigen receptor engineered t cells
WO2014130657A1	Feb 20, 2014	Aug 28, 2014	The Trustees Of The University Of Pennsylvania	Treatment of cancer using humanized anti-egfrviii chimeric antigen receptor
WO2014151644A2	Mar 13, 2014	Sep 25, 2014	Genzyme Corporation	Anti-cd52 antibodies
WO2014153270A1	Mar 15, 2014	Sep 25, 2014	Novartis Ag	Treatment of cancer using humanized anti-cd19 chimeric antigen receptor
WO2014181229A2	May 2, 2014	Nov 13, 2014	Rinat Neuroscience Corp.	Anti-glucagon receptor antibodies and methods of use thereof
WO2014187746A2	May 16, 2014	Nov 27, 2014	Arsanis Biosciences Gmbh	Generation of highly potent antibodies neutralizing the lukgh (lukab) toxin of staphylococcus aureus
WO2014190356A2	May 27, 2014	Nov 27, 2014	Amplimmune, Inc.	Anti-b7-h5 antibodies and their uses
WO2015015401A2	Jul 28, 2014	Feb 5, 2015	Pfizer Inc.	Anti-cxcr4 antibodies and antibody-drug conjugates
WO2015055814A1	Oct 17, 2014	Apr 23, 2015	Arsanis Biosciences Gmbh	Cross-reactive staphylococcus aureus antibody sequences

WO2015073580A1	Nov 12, 2014	May 21, 2015	Pfizer Inc.	Tumor necrosis factor-like ligand 1a specific antibodies and compositions and uses thereof
WO2015087187A1	Nov 26, 2014	Jun 18, 2015	Rinat Neuroscience Corp.	Anti-sclerostin antibodies
WO2015109212A1	Jan 16, 2015	Jul 23, 2015	Pfizer Inc.	Anti-il-2 antibodies and compositions and uses thereof
WO2015112626A1	Jan 21, 2015	Jul 30, 2015	June Carl H	Enhanced antigen presenting ability of car t cells by co-introduction of costimulatory molecules
WO2015142675A2	Mar 13, 2015	Sep 24, 2015	Novartis Ag	Treatment of cancer using chimeric antigen receptor
WO2015157252A1	Apr 7, 2015	Oct 15, 2015	BROGDON, Jennifer	Treatment of cancer using anti-cd19 chimeric antigen receptor
WO2015164330A1	Apr 21, 2015	Oct 29, 2015	Millennium Pharmaceuticals, Inc.	Anti-psyk antibody molecules and use of same for syk-targeted therapy
WO2015168019A2	Apr 27, 2015	Nov 5, 2015	Pfizer Inc.	Anti-ptk7 antibody-drug conjugates
WO2015184099A1	May 28, 2015	Dec 3, 2015	4-Antibody Ag	Anti-gitr antibodies and methods of use thereof
WO2016014530A1	Jul 21, 2015	Jan 28, 2016	Novartis Ag	Combinations of low, immune enhancing, doses of mtor inhibitors and cars
WO2016014553A1	Jul 21, 2015	Jan 28, 2016	Novartis Ag	Sortase synthesized chimeric antigen receptors
WO2016014565A2	Jul 21, 2015	Jan 28, 2016	Novartis Ag	Treatment of cancer using humanized anti-bcma chimeric antigen receptor
WO2016020332A1	Aug 3, 2015	Feb 11, 2016	Engmab Ag	Bispecific antibodies against cd3epsilon and bcma
WO2016025880A1	Aug 14, 2015	Feb 18, 2016	Novartis Ag	Treatment of cancer using gfr alpha-4 chimeric antigen receptor
WO2016028656A1	Aug 17, 2015	Feb 25, 2016	Merck Sharp & Dohme Corp.	Anti-tigit antibodies
WO2016028672A1	Aug 17, 2015	Feb 25, 2016	Merck Sharp & Dohme Corp.	Anti-lag3 antibodies and antigen-binding fragments
WO2016044605A1	Sep 17, 2015	Mar 24, 2016	Beatty, Gregory	Targeting cytotoxic cells with chimeric receptors for adoptive immunotherapy
WO2016055592A1	Oct 8, 2015	Apr 14, 2016	Engmab Ag	Bispecific antibodies against cd3epsilon and ror1

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CLASSIFICATIONS

U.S. Classification	435/69.6, 435/485, 435/471, 435/252.3, 435/320.1, 435/455, 435/254.21, 435/71.2, 435/254.11, 435/69.7, 435/252.33, 435/69.1, 435/252.1, 435/70.1, 435/70.21, 435/254.2, 435/483, 435/71.1
International Classification	C12N5/10, C12P21/08, C07K19/00, G01N33/531, C12N15/81, C07K14/00, C12N1/00, C12P21/02, C12N1/15, C12N1/21, C07K16/30, C12N15/63, G01N33/577, C12N1/19, C07K16/46, C07K16/00, A61K39/395, C07K7/06, C12N15/09, C12N15/13, C12R1/19, C07K16/18
Cooperative Classification	Y10S530/866, Y10S930/30, Y10S530/867, C07K2319/02, C07K2317/24, C07K2319/30, C12N15/63, C07K16/462, C07K16/3007, C12N15/81, C07K2319/00
European Classification	C07K16/46B1, C12N15/81, C07K16/30A, C12N15/63

LEGAL EVENTS

Date	Code	Event	Description
Jun 25, 2002	CC	Certificate of correction	
May 26, 2005	FPAY	Fee payment	**Year of fee payment:** 4
Jun 21, 2005	RR	Request for reexamination filed	**Effective date:** 20050513
Jun 6, 2006	RR	Request for reexamination filed	**Effective date:** 20051223
May 12, 2009	FPAY	Fee payment	**Year of fee payment:** 8
May 19, 2009	B1	Reexamination certificate first reexamination	**Free format text:** THE PATENTABILITY OF CLAIMS 1-20 AND 33-36 IS CONFIRMED. CLAIMS 21, 27 AND 32 ARE DETERMINED TO BE PATENTABLE AS AMENDED. CLAIMS 22-26 AND 28-31, DEPENDENT ON AN AMENDED CLAIM, ARE DETERMINED TO BE PATENTABLE.
Mar 18, 2013	FPAY	Fee payment	**Year of fee payment:** 12
Sep 1, 2015	IPR	Aia trial proceeding filed before the patent and appeal board: inter partes review	**Free format text:** TRIAL NO: IPR2015-01624 **Opponent name:** SANOFI-AVENTIS U.S. LLC ANDREGENERON PHARMACEUTICA **Effective date:** 20150727
Feb 9, 2016	IPR	Aia trial proceeding filed before the patent and appeal board: inter partes review	**Free format text:** TRIAL NO: IPR2016-00383 **Opponent name:** GENZYME CORPORATION **Effective date:** 20151230
Feb 23, 2016	IPR	Aia trial proceeding filed before the patent and appeal board: inter partes review	**Free format text:** TRIAL NO: IPR2016-00460 **Opponent name:** GENZYME CORPORATION **Effective date:** 20160115
		Aia trial proceeding filed before the patent and	**Free format text:** TRIAL NO: IPR2016-00710

Apr 5, 2016	IPR	appeal board: inter partes review	**Opponent name:** MYLAN PHARMACEUTICALS INC.,MYLAN INC.,MYLAN GMBH,M **Effective date:** 20160303