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(71) Applicant *(for all designated States except US)*: **LUDWIG INSTITUTE FOR CANCER RESEARCH** [US/US]; 605 Third Avenue, New York, NY 10158 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **RENNER, Christoph** [DE/DE]; Medical Department, Saarland University Medical School, 66421 Homburg (DE). **SCOTT, Andrew** [AU/AU]; Ludwig Institute for Cancer Research, P.O. Box 2008, Royal Melbourne Hospital, Parkville, Melbourne, AU 3050 (AU).

(74) Agent: **HANSON, Norman, D.**; 666 Fifth Avenue, New York, NY 10103 (US).

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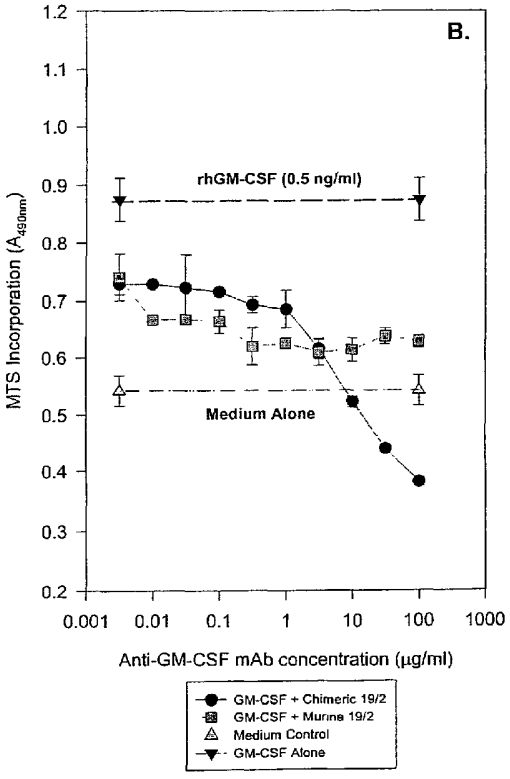

(57) Abstract: Chimeric antibodies, as well as fusion proteins which comprise chimeric antibodies, are disclosed. The antibodies bind to GM-CSF, CD-30, and G250 antigen. The fusion proteins include biologically active portions of tumor necrosis factor, or full length tumor necrosis factor. Expression vectors adapted for production of the antibodies, as well as methods for manufacturing these, are also disclosed.

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FUSION PROTEINS OF HUMANIZED G250 SPECIFIC ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

This invention relates to the field of molecular immunology, generally, and to vectors useful for expression of proteins, especially antibodies, such as fully human, humanized, and chimeric antibodies, as well as fusion proteins which incorporate the antibody and a protein or protein fragment, in eukaryotic cells, mammalian cells in particular. The resulting antibodies and fusion proteins are also a feature of the invention.

BACKGROUND AND PRIOR ART

One serious problem with using murine antibodies for therapeutic applications in humans is that they quickly raise a human anti-mouse response (HAMA) which reduces the efficacy of the antibody in patients, and prevents continued administration thereof. Parallel issues arise with the administration of antibodies from other, non-human species. One approach to overcoming this problem is to generate so-called "chimeric" antibodies. These can comprise murine variable regions, and human constant regions (Boulianne *et al.* (1984) *Nature* 312(5995): 643-646.; incorporated by reference herein in its entirety). Although chimeric antibodies contain murine sequences and can elicit an anti-mouse response in humans (LoBuglio *et al.* (1989) *Proc. Natl. Acad. Sci. U S A* **86(11):** 4220-4224 ; incorporated by reference herein in its entirety), trials with chimeric antibodies in the area of hematological disease (*e.g.,* Non-Hodgkin-Lymphoma; Witzig *et al.* (1999) *J. Clin. Oncol.* **17(12):** 3793-3803. ; incorporated by reference herein in its entirety) or autoimmune disease (*e.g.,* rheumatoid arthritis, chronic inflammatory bowel disease; Van den Bosch; et al, Lancet 356(9244):1821-2 (2000), incorporated by reference herein in its entirety) have led to FDA approval and demonstrate that these molecules have significant clinical potential and efficacy.

Recent studies have indicated that granulocyte-macrophage colony stimulating growth factor (GM-CSF) plays a role in the development of rheumatoid arthritis (RA) (Cook, et al., Arthritis Res. 2001, 3:293-298, incorporated by reference herein in its entirety) and possibly

other inflammatory diseases and conditions. Therefore, it would be of interest to develop a drug which would block GM-CSF and its effect on cells. The present invention provides a chimeric antibody, targeting the GM-CSF molecule, which has blocking capacity.

The increased use of chimeric antibodies in therapeutic applications has created the need for expression vectors that effectively and efficiently produce high yields of functional chimeric antibodies in eukaryotic cells, such as mammalian cells, which are preferred for production. The present invention provides novel expression vectors, transformed host cells and methods for producing chimeric antibodies in mammalian cells, as well as the antibodies themselves and fusion proteins containing them.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the binding of recombinant, chimeric anti GM-CSF antibody via Western Blotting.

Figure 2 shows the binding of the antibody via ELISA.

Figure 3 shows the blocking effect of the antibody on GM-CSF growth dependent TF-1 cells.

Figure 4 shows the blocking effect of the antibody on GM-CSF growth dependent AML-193 cells.

Figure 5 shows results of an assay testing the effect of increasing concentration of murine or chimeric 19/2 mAbs, on TF-1 cells grown in the presence of a constant amount of human GM-CSF.

Figure 6 parallels the experiment of figure 5, but uses the AML-153 cells.

Figure 7 shows a schematic map of the two expression vectors used to prepare the recombinant antibodies.

SUMMARY OF INVENTION

The present invention provides expression vectors which are useful in the expression of proteins, such as antibodies, especially fully human, humanized or chimerized antibodies, and fusion proteins containing these. Both light chains and heavy chains can be expressed. The expression vectors of the present invention comprise a human elongation factor 1 α

(EF1α) promoter/enhancer sequence, an internal ribosome entry site (IRES) sequence (U.S. Patent No. 4,937,190; incorporated herein in its entirety), a nucleotide sequence that confers neomycin resistance to a cell containing the expression vector, and a nucleotide sequence under control of a simian virus 40 promoter (SV40) that confers ampicillin resistance to a cell containing the expression vector. In a preferred embodiment, the EF1α promoter/enhancer sequence is upstream and adjacent to a nucleotide sequence encoding a chimeric light chain.

The expression vector of the present invention may contain a nucleotide sequence encoding any immunoglobulin light chain. In a preferred embodiment the light chain variable region is of murine origin, and the light chain constant region is either human kappa or human lambda. In a more preferred embodiment, the chimeric light chain variable region is derived from a murine antibody that binds to GM-CSF, CD-30, or G250 and in especially preferred embodiments, to the human forms of these molecules.

The present invention also provides a further expression vector useful in the expression of proteins, such as antibodies, especially fully human, humanized or chimeric antibodies, and fusion proteins containing these. This second embodiment differs from the first in that instead of the neomycin resistance sequence, described supra, it comprises a nucleotide sequence which encodes dihydrofolate reductase or "dhfr," which generates resistance against the well known selection marker methotrexate. Such an expression vector may contain nucleotide sequences encoding any antibody or portion thereof, such as heavy or light chains of fully human, humanized or chimerized antibodies. In a preferred embodiment, a heavy chain is expressed, where the variable region is of murine origin, and the heavy chain constant region is human IgG1. In a more preferred embodiment, the chimeric heavy chain variable region is derived from a murine antibody that binds CD-30, GM-CSF or G250, preferably the human forms of these.

In another embodiment, the present invention provides host cells transformed or transfected with any one of the expression vectors of the present invention. In a preferred embodiment, a host cell, preferably a eukaryotic cell, more preferably a mammalian cell, is transformed or transfected with an expression vector comprising a chimeric immunoglobulin light chain and an expression vector comprising a chimeric immunoglobulin heavy chain. The present invention contemplates prokaryotic and eukaryotic cells, such as mammalian cells, insect cells, bacterial or fungal cells. In a preferred embodiment, the host cell is a human or Chinese Hamster Ovary ("CHO") cell.

The present invention also provides methods for the recombinant production of a chimeric immunoglobulin light or heavy chain comprising the step of culturing a transformed or transfected host cell of the present invention. In one embodiment, the methods of the present invention further comprise the isolation of the chimeric immunoglobulin light or heavy chain.

The present invention also provides methods for the recombinant production of a fully human, humanized or chimeric immunoglobulin comprising culturing a host cell that has been transformed or transfected with an expression vector comprising a chimeric immunoglobulin light chain and an expression vector comprising a chimeric immunoglobulin heavy chain, or an expression vector encodes both chains. In one embodiment, the methods of the present invention further comprise the self-assembly of the chimeric heavy and light chain immunoglobulins and isolation of the chimeric immunoglobulin. Methods for accomplishing this are well known in the art.

The present invention also provides the chimeric immunoglobulin light chain, heavy chain or assembled chimeric immunoglobulin produced by the methods of the present invention. In another embodiment, the present invention provides compositions comprising the chimeric immunoglobulin light chain, heavy chain or assembled chimeric immunoglobulin of the present invention and a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION OF INVENTION

1. Definitions

As used herein "chimerized" refers to an immunoglobulin such as an antibody, wherein the heavy and light chains of the variable regions are not of human origin and wherein the constant regions of the heavy and light chains are of human origin.

"Humanized" refers to an immunoglobulin such as an antibody, wherein the amino acids directly involved in antigen binding, the so-called complementary determining regions (CDR), of the heavy and light chains are not of human origin, while the rest of the immunoglobulin molecule, the so-called framework regions of the variable heavy and light chains, and the constant regions of the heavy and light chains are of human origin.

"Fully human" refers to an immunoglobulin, such as an antibody, where the whole molecule is of human origin or consists of an amino acid sequence identical to a human form of the antibody.

"Immunoglobulin" or "antibody" refers to any member of a group of glycoproteins occurring in higher mammals that are major components of the immune system. As used herein, "immunoglobulins" and "antibodies" comprise four polypeptide chains—two identical light chains and two identical heavy chains that are linked together by disulfide bonds. An immunoglobulin molecule includes antigen binding domains, which each include the light chains and the end-terminal portion of the heavy chain, and the F_c region, which is necessary for a variety of functions, such as complement fixation. There are five classes of immunoglobulins wherein the primary structure of the heavy chain, in the F_c region, determines the immunoglobulin class. Specifically, the alpha, delta, epsilon, gamma, and mu chains correspond to IgA, IgD, IgE, IgG and IgM, respectively. As used herein "immunoglobulin" or "antibody" includes all subclasses of alpha, delta, epsilon, gamma, and mu and also refers to any natural (*e.g.*, IgA and IgM) or synthetic multimers of the four-chain immunoglobulin structure.

"Antigen-binding fragment", "antigen-binding domain" and "Fab fragment" all refer to the about 45 kDa fragment obtained by papain digestion of an immunoglobulin molecule and consists of one intact light chain linked by a disulfide bond to the N-terminal portion of the contiguous heavy chain. As used herein, "$F(ab)_2$ fragment" refers to the about 90 kDa protein produced by pepsin hydrolysis of an immunoglobulin molecule. It consists of the N-terminal pepsin cleavage product and contains both antigen binding fragments of a divalent

immunoglobulin, such as IgD, IgE, and IgG. Neither the "antigen-binding fragment" nor "$F(ab)_2$ fragment" contain the about 50 kDa F_c fragment produced by papain digestion of an immunoglobulin molecule that contains the C-terminal halves of the immunoglobulin heavy chains, which are linked by two disulfide bonds, and contain sites necessary for compliment fixation.

"Epitope" refers to an immunological determinant of an antigen that serves as an antibody-binding site. Epitopes can be structural or conformational.

"Hybridoma" refers to the product of a cell-fusion between a cultured neoplastic lymphocyte and a normal, primed B- or T-lymphocyte, which expresses the specific immune potential of the parent cell.

"Heavy chain" refers to the longer & heavier of the two types of polypeptide chain in immunoglobulin molecules that contain the antigenic determinants that differentiate the various Ig classes, *e.g.*, IgA, IgD, IgE, IgG, IgM, and the domains necessary for complement fixation, placental transfer, mucosal secretion, and interaction with F_c receptors.

"Light chain" refers to the shorter & lighter of the two types of polypeptide chain in an Ig molecule of any class. Light chains, like heavy chains, comprise variable and constant regions.

"Heavy chain variable region" refers to the amino-terminal domain of the heavy chain that is involved in antigen binding and combines with the light chain variable region to form the antigen-binding domain of the immunoglobulin.

"Heavy chain constant region" refers to one of the three heavy chain domains that are carboxy-terminal portions of the heavy chain.

"Light chain variable region" refers to the amino-terminal domain of the light chain and is involved in antigen binding and combines with the heavy chain to form the antigen-binding region.

"Light chain constant region" refers to the one constant domain of each light chain. The light chain constant region consists of either kappa or lambda chains.

"Murine anti-human-GM-CSF 19/2 antibody" refers to a murine monoclonal antibody that is specific for human GM-CSF. This antibody is well known and it has been studied in detail. See Dempsey, et al, Hybridoma 9:545-58 (1990); Nice, et al, Growth Factors 3:159-169 (1990), both incorporated by reference.

"Effective amount" refers to an amount necessary to produce a desired effect.

"Antibody" refers to any glycoprotein of the immunoglobulin family that non-covalently, specifically, and reversibly binds a corresponding antigen.

"Monoclonal antibody" refers to an immunoglobulin produced by a single clone of antibody-producing cells. Unlike polyclonal antiserum, monoclonal antibodies are monospecific (*e.g.*, specific for a single epitope of a single antigen).

"Granulocytes" include neutrophils, eosinophils, and basophils.

"GM-CSF" refers to a family of glycoprotein growth factors that control the production, differentiation, and function of granulocytes and monocytes-macrophages. Exemplary, but by no means the only form of such molecules, can be seen in U.S. Patent No. 5,602,007, incorporated by reference.

"Inflammatory condition" refers to immune reactions that are either specific or non-specific. For example, a specific reaction is an immune reaction to an antigen. Examples of specific reactions include antibody responses to antigens, such as viruses and allergens, including delayed-type hypersensitivity, including psoriasis, asthma, delayed type hypersensitivity, inflammatory bowel disease, multiple sclerosis, viral pneumonia, bacterial pneumonia, and the like. A non-specific reaction is an inflammatory response that is mediated by leukocytes such as macrophages, eosinophils and neutrophils. Examples of non-specific reactions include the immediate swelling after a bee sting, and the collection of polymorphonuclear (PMN) leukocytes at sites of bacterial infection. Other "inflammatory conditions" within the scope of this invention include, *e.g.*, autoimmune disorders such as psoriasis, rheumatoid arthritis, lupus, post-ischemic leukocyte mediated tissue damage (reperfusion injury), frost-bite injury or shock, acute leukocyte-mediated lung injury (acute respiratory distress syndrome or ARDS), asthma, traumatic shock, septic shock, nephritis, acute and chronic inflammation, and platelet-mediated pathologies such as ateriosclerosis and inappropriate blood clotting.

"Pharmaceutically acceptable carrier" refers to any carrier, solvent, diluent, vehicle, excipient, adjuvant, additive, preservative, and the like, including any combination thereof, that is routinely used in the art.

Physiological saline solution, for example, is a preferred carrier, but other pharmaceutically acceptable carriers are also contemplated by the present invention. The primary solvent in such a carrier may be either aqueous or non-aqueous. The carrier may

contain other pharmaceutically acceptable excipients for modifying or maintaining pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, and/or odor. Similarly, the carrier may contain still other pharmaceutically acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption or penetration across the blood-brain barrier.

The fully human, humanized or chimerized antibodies of the present invention may be administered orally, topically, parenterally, rectally or by inhalation spray in dosage unit formulations that contain conventional non-toxic pharmaceutically acceptable carriers, adjuvants and vehicles. As used herein, "parenterally" refers to subcutaneous, intravenous, intramuscular, intrasternal, intrathecal, and intracerebral injection, including infusion techniques.

The fully human, humanized or chimerized antibodies may be administered parenterally in a sterile medium. The antibodies, depending on the vehicle and concentration used, may be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle. The most preferred routes of administration of the pharmaceutical compositions of the invention are subcutaneous, intramuscular, intrathecal or intracerebral administration. Other embodiments of the present invention encompass administration of the composition in combination with one or more agents that are usually and customarily used to formulate dosages for parenteral administration in either unit dose or multi-dose form, or for direct infusion.

Active ingredient may be combined with the carrier materials in amounts necessary to produce single dosage forms. The amount of the active ingredient will vary, depending upon the type of antibody used, the host treated, the particular mode of administration, and the condition from which the subject suffers. Preferably, the amount of fully human, humanized or chimerized anti-GM-CSF immunoglobulin, for example, is a therapeutically effective amount which is sufficient to decrease an inflammatory response or ameliorate the symptoms of an inflammatory condition. It will be understood by those skilled in the art, however, that specific dosage levels for specific patients will depend upon a variety of factors, including the activity of the specific immunoglobulins utilized, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy. Administration of the fully human, humanized or chimerized immunoglobulins of the present invention may require either one or multiple dosings.

Regardless of the manner of administration, however, the specific dose is calculated according to approximate body weight or body surface area of the patient. Further refinement of the dosing calculations necessary to optimize dosing for each of the contemplated formulations is routinely conducted by those of ordinary skill in the art without undue experimentation, especially in view of the dosage information and assays disclosed herein.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the compounds of the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

EXAMPLES

Example 1: Cloning strategy for 19/2 heavy (H) and light (L) variable (V)-region genes.

Total RNA from the hybridoma producing murine 19/2 antibody was obtained by standard RNA isolation techniques (Chomczynski *et al.* (1987) *Anal. Biochem.* **162:** 156-159. ; incorporated by reference herein in its entirety). First strand cDNA was prepared using a commercially available, first strand cDNA synthesis kit and priming with d(T)18 for both the heavy and light chains (Renner *et al.* (1998) *Biotechniques* **24(5):** 720-722. ; incorporated by reference herein in its entirety). The resulting cDNA was subjected to PCR using combinations of primers for the heavy and light chains. The nucleotide sequences of the 5' primers for the heavy and light chains are shown in Tables 1 and 2 respectively. The 3' primers are shown in Table 3. The light chain primer hybridized within the mouse kappa constant region not far from the V-C junction. The heavy chain 3' primer hybridised within the CH-1 constant region of mouse heavy chain subgroup 1 not far from the V-CH1 junction.

TABLE 1: Oligonucleotide primers for the 5' region of Mouse Heavy Variable (MHV) domains.

		SEQ ID NO: 1
MHV-1:	5'ATGAAATGCAGCTGGGTCATSTTCTTC 3'	1
MHV-2:	5'ATGGGATGGAGCTRATCATSYTCTT 3'	2
MHV-3:	5'ATGAAGWTGTGGTTAAACTGGGTTTTT 3'	3
MHV-4:	5'ATGRACTTTGWYTCAGCTTGRTTT 3'	4
MHV-5:	5'ATGGACTCCAGGCTCAAMAGTTTTCCTT 3'	5
MHV-6:	5'ATGGCTGTCYTRGSGCTRCTCTTCTGC 3'	6
MHV-7:	5'ATGGRATGGAGCKGGRTCTTTMTCTT 3'	7
MHV-8:	5'ATGAGAGTGCTGATTCTTTTGTG 3'	8
MHV-9:	5'ATGGMTTGGGTGTGGAMCTTGCTATTCCTG 3'	9
MHV-10:	5'ATGGGCAGACTTACATTCTCATTCCTG 3'	10
MHV-11:	5'ATGGATTTTGGGCTGATTTTTTTTATTG 3'	11
MHV-12:	5'ATGATGGTGTTAAGTCTTCTGTACCTG 3'	12

NB KEY R=A/G, Y=T/C, W=A/T, K=T/G, M=A/C, S=C/G.

TABLE 2: Oligonucleotides primers for the 5' region of Mouse Kappa Variable (MKV) domains.

		SEQ ID NO: 1
MKV-1:	5'ATGAAGTTGCCTGTTAGGCTGTTGGTGCTG 3'	13
MKV-2:	5'ATGGAGWCAGACACACTCCTGYTATGGGT 3'	14
MKV-3:	5'ATGAGTGTGCTCACTCAGGTCCTGGSGTTG 3'	15
MKV-4:	5'ATGAGGRCCCCTGCTCAGWTTYTTGGMWTCTTG 3'	16
MKV-5:	5'ATGGATTTWCAGGTGCAGATTWTCAGCTTC 3'	17
MKV-6:	5'ATGAGGTKCYYTGYTSAGYTYCTGRGG 3'	18
MKV-7:	5'ATGGGCWTCAAGATGGAGTCACAKWYYCWGG 3'	19
MKV-8:	5'ATGTGGGGAYCTKTTTYCMMTTTTTCAATTG 3'	20
MKV-9:	5'ATGGTRTCCWCASCTCAGTTCCTTG 3'	21
MKV-10:	5'ATGTATATATGTTTGTTGTCTATTTCT 3'	22
MKV-11:	5'ATGGAAGCCCCAGCTCAGCTTCTCTTCC 3'	23
MKV-12:	5'ATGAAGTTTCCTTCTCAACTTCTGCTC 3'	24

NB KEY R=A/G, Y=T/C, W=A/T, K=T/G, M=A/C, S=C/G.

TABLE 3: Oligonucleotide primers for the 3' ends of mouse VH and VL genes.

Light chain (MKC):	5'TGGATGGTGGGAAGATG 3'	25
Heavy chain (MHC):	5'CCAGTGGATAGACAGATG 3'	26

Example 2. Ig sequences cloned from the 19/2 murine hybridoma.

Using the cloning strategy described, supra, PCR products for VH and VL of murine 19/2 were cloned using a commercially available product, and art recognized techniques. For the murine 19/2 VL region, PCR products were obtained using the mouse kappa constant region primer and primers MKV2 and MKV7 (SEQ ID NOS: 14 & 19). For the mouse 19/2 VH region, PCR products were obtained using the mouse gamma 1 constant region primer and primers MHV2, MHV5 and MHV7 (SEQ ID NOS: 2, 5 and 7). Extensive DNA sequencing of the cloned V-region inserts revealed two different light chain sequences and 2 different heavy chain sequences. Pseudogenes for heavy and light chain were amplified and were eliminated by standard sequence analyses. A novel immunoglobulin-coding sequence was determined for both the heavy and light chains. This is set forth at SEQ ID NOS: 27, 28, 29 & 30, which present the cDNA and amino acid sequences for the murine 19/2 heavy chain variable region (27 & 28), and the light chain variable region (29 & 30).

Example 3. Mouse 19/2 heavy chain leader sequence.

When comparing the DNA sequence of the leader sequence for 19/2 heavy chain obtained with the primers described supra, with the database, it appeared that the 19/2 HC

leader sequence is short (17 amino acids) and unique vis a vis public data bases. Specifically, amino acids 2, 3 and 5 were E, L & M, as compared to S, W & F in the data bases. As compared to the database, hydrophilic amino acids in the N-terminal region were separated by neutral or basic ones, respectively; however, since the influence of these changes on the secretory capability of the leader sequence is unclear, this sequence was unaltered in further experiments.

Example 4. Construction of mouse-human chimeric genes.

The chimeric 19/2 antibody was designed to have the mouse 19/2 VL and VH regions linked to human kappa and gamma-1 constant regions, respectively. PCR primers were used to modify the 5'- and 3'- sequences flanking the cDNA sequences coding for the mouse 19/2 VL and VH regions. PCR primers specific for 19/2 light chain V-region were designed using the sequence of the 19/2 light chain V-region gene obtained. These adapted mouse 19/2 variable regions were then subcloned into mammalian cell expression vectors already containing the human kappa (pREN-Neo vector) or the gamma-1 (pREN-DHFR vector) constant regions. The vectors employ parts of the human elongation factor 1α (EF1α) promoter/enhancer sequence to efficiently transcribe the light and heavy chains. The vectors also contain an IRES sequence following the multiple cloning site to allow for stringent, bicistronic expression and control of the individual selection marker in CHO cells. This pair of vectors was used in all of the recombinant work described herein, i.e., to manufacture all chimeric antibodies. The expression vectors were designed to have the variable regions inserted as PmeI-BamHI DNA fragments. PCR primers were designed to introduce these restrictions sites at the 5'- (PmeI) and 3'- (BamHI) ends of the cDNAs coding for the V-regions. In addition, the PCR primers were designed to introduce a standard Kozak sequence (Kozak (1987) *Nucleic Acids Res.* **15(20):** 8125-8148, incorporated by reference herein in its entirety) at the 5'-ends of both the light and heavy chain cDNAs to allow efficient translation, and to introduce splice donor sites at the 3'-ends of both the light and heavy chain cDNAs for the variable regions to be spliced to the constant regions. The PCR primers used for the construction of the chimeric 19/2 light and heavy chains were as follows: catgtttaaacgccfccaccatgggcttcaagatggagtca (5' end, light chain variable region, SEQ ID NO: 31); agaggatccactcacgtttcagttccacttggtcccag (3' end, SEQ ID NO: 32); catgtttaaacgccgccaccatggagctgatcatgctcttcct (primer for the 5' end of the heavy chain variable region, SEQ ID NO: 33); and agaggatccactcacctgaggagactctgagagtggt (primer for the 3' end of the heavy chain variable region, SEQ ID NO: 34). The DNA and amino acid sequences of

the mouse 19/2 VL and VH regions were adapted for use from the construction of chimeric 19/2 light and heavy chains. The entire DNA sequences of mouse 19/2 light and heavy chains cloned into the eukaryotic expression vectors pREN-Neo and pREN-DHFR, respectively, are set forth as SEQ ID NO: 35 & 36, with the resulting light and heavy chains resulting in chimerized molecules. Specifically, in SEQ ID NO: 35, nucleotides 1357-1756 encode the murine, light chain sequence, with nucleotides 1763-2206 encoding the human kappa region. Within this sequence (1763-2206), a 120 base pair region constituting an intron and splice acceptor site begins at nucleotide 1886. Within SEQ ID NO: 36, nucleotides 1357-1770 encode the murine 9/2 heavy chain constant sequence with a splice donor site. Nucleotides 1777-2833 encode the human IgG1 constant region. Within this sequence, there is a 60 base pair intron region and splice acceptor site which precedes the coding region.

Example 5.

The objective of the experiments described herein was to create stable cell lines expressing chimeric 19/2 (c19/2) anti-human GM-CSF monoclonal antibodies (mAb) in CHO (Chinese hamster ovary) DG44 cells and to test the secreted antibody for its binding properties. To do this, the DHFR negative CHO cell line DG044 was used. See Morris *et al.* (1990) *Gene* **94(2):** 289-294 ; incorporated by reference herein in its entirety). The CHO cells were cultured in RPMI, supplemented with 10% FCS and Hypoxanthine-Thymidine. DNA for transfection was purified from E. coli cells using a commercially available product, and the instructions provided therein. All DNA preparations were examined by restriction enzyme digestion. Sequences of chimeric 19/2 mAb variable regions in their respective vectors were confirmed using an ABI PRISM 310 or LICOR Sequencer.

Vectors encoding heavy and light chains of chimeric 19/2 mAbs were co-transfected simultaneously into CHO DG44 cells growing at log phase, using electroporation (270V, 975 uF). Cells were plated in 10 cm dishes and cultured with standard medium. Twenty-four hours later, medium was harvested and replaced by fresh RPMI medium supplemented with 10% dialyzed FCS and 500 ug/mL geneticin. After the initial phase of cell killing was over (7-10 days), GMP-grade methotrexate was added at a concentration of 5nM and gradually increased to 100nM over the following weeks. Out-growing colonies were picked and screened for antibody production.

Example 6. PCR amplification of variable chain DNA

CHO DG44 cells were centrifuged in an Eppendorf microcentrifuge, briefly, at full speed, washed once with PBS, and pelleted once again. Genomic DNA was prepared by ethanol precipitation after SDS lysis and Proteinase K treatment of the cell pellets.

A mixture containing one of the primer pairs described supra, dNTPs, buffer, and Pfu polymerase was used to amplify either the heavy or light chain variable region using genomic DNA as a template using methods well known in the art. The resulting PCR products were digested with the appropriate restriction enzyme and analysed by agarose gel electrophoresis to confirm their identity.

The primer pairs for the light chain were:

ttcttgaagt ctggtgatgc tgcc

(SEQ ID NO: 37), and

caagctagcc ctctaagactc ctcccctgtt

(SEQ ID NO: 38).

For the light chain and SEQ ID NO: 37 plus

gaactcgagt catttacccg gagacaggga gag

(SEQ ID NO: 39)

for the heavy chain.

The undigested heavy chain PCR product had a predicted size of 1200 base pairs, while the light chain PCR product had a predicted size of 800 base pairs. Identity was verified by restriction enzyme digest with BamHI.

Example 7. Dot-Blot method for measuring assembled IgG1/Kappa antibody in CHO cell supernatants.

CHO cell lines were transfected with the corresponding plasmids. Geneticin resistant cells were obtained and these cells were further selected for resistance to methotrexate. Single colonies were picked after amplification and transferred into 24-well plates. Culture supernatant was tested for chimeric IgG 3-4 days later by standard Dot Blot assays.

Any positive colonies were sub-cloned and cultured to achieve sufficient antibody production. The chimeric 19/2 antibody was purified from the supernatant on protein G columns and tested for its specific binding with recombinant GM-CSF by Western Blot (Figure 1) and ELISA (Figure 2).

Finally, the identity of producer cell lines were confirmed using PCR amplification of both their heavy and light chain variable regions. The DNA sequence of the heavy chain variable region PCR products for chimeric 19/2 mAb transfected cells was confirmed.

Example 8.

In order to optimize cell growth and antibody production, the CHODG44/pREN c19/2 cell line was first cultured in commercially available IMDM containing 10% FCS, at 37°C, in a 10% CO_2 atmosphere. The cells were then weaned into serum free medium, and cultured in a custom made medium, i.e., IMDM SFII, with the following additives, at 37°C, in a 10% CO_2 atmosphere.

Base IMDM Medium	**Final Concentration**
Pluronic F68	1.0mg/ml
Hypep 4601	1.0 mg/ml
Hypep 4605 DEV	0.5 mg/ml
HEPES	5.958 mg/ml
Na_2HCO_3	3.024 mg/ml
Additives	**Final Concentration**
Dextran sulfate	50.0 µg/ml
Putrescine	100.0 nM
Albumax I	2.0 mg/ml
Choline chloride	1.0 mg/ml
Trace elements	
$FeSO_4 \cdot 7H_2O$	0.8 µg/ml
$ZnSO_4 \cdot 7H_2O$	1.0 µg/ml
$CuSO_4 \cdot 5H_2O$	0.0025 µg/ml
$C_6H_5FeO_7 \cdot H_2O$	5.0 µg/ml
IGF-1	50.0 ng/ml
Transferrin	35.0 µg/ml
Ethanolamine	50.0 µM
Mercaptoethanol	50.0 µM

Culture supernatants were harvested asceptically, and then clarified by centrifugation. The antibodies were then purified by affinity chromatography on a 5 ml protein. A sepharose fast flow column that had been pre-equilibriated in 50 mM Tris-HCL, pH8, was used. The column was washed, 20 times, with this buffer, and any bound antibody was eluted using 50 mM sodium citrate, pH 3.0, and the eluate was then neutralized, immediately, using 1M Tris-HCl, pH8. Antibodies were concentrated with a centrifugal filter, and dialyzed overnight at 4°C in PBS. The yield was about 4-5 mg/liter. The purity of the antibodies was examined via SDS-PAGE, under both reducing and non-reducing conditions, using a 4-20% gradient on the SDS-PAGE.

Purified antibodies migrated as a single band under non-reducing conditions, and separated into the heavy and light chains, as expected, under reducing conditions.

The antibodies were also analyzed via size exclusion chromatography, (0.5 mg/ml), on a precalibrated HPLC column. Running buffer (5% n-propanol/PBS (0.5 M phosphate, 0/25 M NaCl, pH 7.4)) was used, at a flow rate of 0.2 ml/min at a temperature of 22°C, which is ambient column temperature.

The analysis demonstrated the integrity of the antibodies, which had calculated molecular weights of 179 kilodaltons.

Example 9.

The experiments described in this example were designed to determine the binding activity of the antibodies.

Biosensor analyses were carried out using a commercially available, BIAcore 2000, and a carboxymethyldetran coated sensor chip. The chip was derivatized with 1000, 300, or 100 RVs of recombinant human GM-CSF, on channels 1, 2, and 3 of the machine using standard amine coupling chemistry with channel 4 retained as the control blank channel.

Samples of the chimeric antibody were diluted in HBS buffer (10 mM HEPES, pH7.4, 150 mM NaCl, 3.4 mM di-NA-EDTA, 0.005% Tween-20), and aliquots were injected over the sensor chip at a flow rate of 1 µl/min. After injection, dissociation was monitored by allowing HBS buffer to flow over the chip surface for 5 minutes. Any bound antibody was then eluted, and the chip surface was regenerated, between samples, via injecting 40 µl of 100mM HCl, pH 2.7, at a rate of 5 µl/min. In order to carry out kinetic analyses of the binding of the chimeric antibody, varying concentrations, ranging from 1-10 nM, were injected over the chip surface, and both apparent association ("Ka") and dissociation ("Kd") rate constants were calculated, using a Langmuir 1:1 binding model, with global and local fitting for calculation of Rmax, using B1Aevaluation V3.1 software.

The results indicated that the chimeric antibody had slightly higher affinity for rhGM-CSF than the murine antibody. The calculated Ka for the chimeric antibody was $5.1x10^5M^{-1}s^{-1}$ using 100 RU of GM-CSF. No dissociation was observed, regardless of analyte concentration, precluding Kd determination and indicating very high affinity.

Global fitting of Rmax, using the software referred to, gave an off rate of $Kd=1.9x10^{-5}s^{-1}$ and a high affinity for the chimeric antibody of $2.69x10^{10}M^{-1}$.

Example 10.

These experiments were designed to determine both the binding activity of the antibodies, and if they cross-reacted with each other.

Nunc plates were coated with recombinant human GM-CSF (1 μg/ml), in carbonate buffer (pH 9.6, 0.05 M), 50 μl/well, and were incubated at 4°C, overnight, and were then blocked with 3% FCS/PBS at room temperature, for one hour.

Half-log, serially diluted triplicate 100 μl samples of either murine or chimeric antibody (10 μg/ml) were added to each well, to yield final concentrations of from 1.0 ng/ml to 10 μg/ml. Following incubation for 1 hour at room temperature, either goat antimouse IgG or antihuman IgG, labelled with horseradish peroxidase (10 ul/well Fc specific; 1:1000 dilution in 1% FCS/PBS) were used to detect bound antibody. After extensive washings, the bound antibodies were visualized by the addition of ABTS substrate (100 μl/well).

Optical density was read at 415 nm in a microplate reader.

The same protocol for binding antibody to the solid phase was used to determine if the antibodies competed with each other. As in the experiments, supra, half-log, serially diluted 100 μl samples, in triplicate, of 10 μg/ml of the murine or chimeric antibody were combined with 20 μg/ml of competing antibody, and then 100 ml of the mixture was added to the coated ELISA plates. Incubation was as above, and anti-murine or anti-human IgG labelled with horseradish peroxidase was used, also as described supra.

The results indicated that the antibodies did compete for binding for recombinant human GM-CSF. A shift in the binding curve was effected by addition of the excess, competing antibody. This indicated binding to, and competition for, a common epitope.

Example 11.

These experiments were designed to test the neutralizing activity of the anti-GM-CSF antibodies. Two human GM-CSF dependent cell lines, i.e., TF-1 and AML-193 were used. Growth curves were established, in the presence or absence of 0.5 ng/ml of recombinant human GM-CSF, and viable cell numbers were determined, via Trypan Blue exclusion, on day 0, 1, 2, 3, 5 and 7.

In a first bioassay, recombinant human GM-CSF, in amounts ranging from 0.0003 ng/ml up to 10 μg/ml, was mixed with anti-human GM-CSF antibodies, at a final concentration of 30 μg/ml, in 96 well, microtitre plates. Either TF-1 or AML-193 cells were added (10^3 cells/well), and plates were incubated at 37°C for 7 days.

After this incubation period, the DNA proliferation marker MTS was added, at 20 μl/well. Dye incorporation was measured after 2 hours, by measuring light absorbance at A_{490nm}.

Increased MTS dye incorporation was observed as the amount of rhGM-CSF in the medium increased. Total growth inhibition of both cell types was observed with the chimeric antibody when rhGM-CSF concentration was 0.1 ng/ml or less, and there was marked inhibition of cell growth at 0.3-10 ng/ml rhGM-CSF.

In contrast, while the murine antibody had a similar effect on AML-193 cells, it was less effective on TF-1 cells. These results are seen in figures 3 and 4.

In a second bioassay TF-1 and AML-193 cells were grown in the presence of 0.5 ng/mL rhGM-CSF and increasing amounts of murine or chimeric 19/2 mAbs (0.003-100 μg/mL) were added to the culture media and the neutralizing activity assessed after 7 days culture. Results are shown in Figure 5 and 6 for the TF-1 and AML-193 cells, respectively. In agreement with the initial bioassay, the chimeric 19/2 demonstrated marked neutralizing activity of GM-CSF stimulated cell growth. A direct correlation was observed between increasing ch19/2 concentration and GM-CSF neutralizing activity plateaued at 3 μg/mL for both cell lines, with higher concentrations unable to effect a greater reduction in TF-1 or AML-193 cell growth. These observations may be due to lower affinity of the murine mAb or steric hindrance at the binding site on GM-CSF.

Example 12

Additional experiments were carried out to produce a chimeric, HRS-3 antibody. The murine form of this antibody is described by Hombach, et al, Int. J. Cancer 55:830-836 (1993), incorporated by reference. The murine antibody binds to CD-30 molecules.

The protocols set forth for production of chimeric, anti GM-CSF antibody set forth supra were used. Since the antibodies were different, and sequences were known, however, different primers were used. These primers serve to introduce splice sites into the cDNA

sequences encoding the murine heavy chain and light chain variable regions, and are set forth at SEQ ID NOS: 44, 45, 46 & 47, with SEQ ID NOS: 44 & 45 the nucleotide and amino acid sequences of the heavy chain, and 46 & 47 comparable sequences for the light chain

The primers were:

gcgccatggc ccaggtgcaa ctgcagcagt ca
(SEQ ID NO: 40)

and

cagggatcca ctcacctgag gagacggtga ccgt
(SEQ ID NO: 41),

and for the light chain:

agcgccatgg acatcgagct cactcagtct cca
(SEQ ID NO: 42)

and

cagggatcca actcacgttg atttccagct tggt
(SEQ ID NO: 43).

Following amplification, the murine heavy and light chain variable regions were cloned into the pREN Neo and pREN-DHFR sequences, which are set forth at SEQ ID NOS: 48 & 49, respectively. The cloning was possible because the amplification introduced PmeI and BamHI restriction sites into SEQ ID NO: 44, at nucleotides 1-7, and the final 6 nucleotides. Comparable sites are found at nucleotides 1340-1348, and 1357-1362 of SEQ ID NO: 48. Similarly, PmeI and BamHI restriction sites were introduced at nucleotides 1-8, and the last 6 nucleotides of SEQ ID NO: 47, such that this nucleotide sequence could be cloned into SEQ ID NO: 49, at positions 1337-1344, and 1349-1354.

The chimeric HRS-3 antibody was designed to have murine HRS-3 VL and VH regions linked to human kappa and gamma-1 constant regions, respectively. PCR primers were used to modify the 5'- and 3'- sequences flanking the cDNA sequences coding for the murine HRS-3 VL and VH regions. Modification included the insertion of a NcoI site at the 5' primer end and a splice donor site followed by a BamHI restriction site at the 3'-end of

both the light and heavy chain cDNAs for the variable regions to be spliced to the constant regions. These adapted mouse HRS-3 variable regions were then subcloned through the NcoI/BamHI restriction sites into a prokaryotic vector harboring a 5'PmeI site followed by a 5' Kozak sequence and by a human antibody leader sequence. Sequences were cut from the prokaryotic vector by PmeI/BamHI digest and subcloned into mammalian cell expression vectors already containing the human kappa (pREN-Neo vector) or gamma-1 (pREN-DHFR vector) constant regions, described supra.

Example 13

Once the constructs were established, they were transfected into DGO44 cells, as described supra.

Positive colonies were sub-cloned, cultured to achieve sufficient antibody production, after which the antibodies were purified, on protein G columns via the Fc fragment.

The purified antibodies were analyzed via SDS-PAGE, following Laemmli, Nature 227:680-5 (1970), as modified by Renner, et al, Eur. J. Immunol 25:2027-35 (1995), incorporated by reference. Samples from different stages of purification were diluted, in either reducing or non-reducing buffer, and were separated on 10-12% polyacrylamide gel via electrophoreses followed by standard Coomassic staining.

The results were in accordance with production of a complete, chimeric antibody, as evidenced by the banding patterns found in both reducing and non-reducing solutions.

Example 14

The binding capacity of the chimeric HRS-3 antibody was determined via flow cytometry, in accordance with Renner, et al, supra. In brief, $1x10^6$ cells of a target tumor line which expressed CD-30 were washed, twice, in PBS, and then incubated with varying concentration of antibody, at 4°C, for 30 minutes. The cells were then washed, and incubated with a secondary antibody, which was directed to the light chain, conjugated to either FITC or PE.

The results indicated that there was weak binding from cell culture supernatant purified from transfected CHO cells, and string binding with purified antibody. No binding was found when CD-30 negative tumor cells were used.

Example 15

The antibody dependent cellular toxicity (ADCC), and the complement dependent toxicity of the chimeric HRS-3 antibody were determined using a europium released assay, as described by Hombach, et al, supra, and Renner, et al, supra.

In brief, for the ADCC assay, peripheral blood lymphocytes were isolated from tow healthy donors, and used at an effector:target ratio of 10:1, with 10, 000 europium labelled, CD-30 antigen positive L540CY tumor cells. Antibody was added at varying concentrations (10, 1, 0.1 and 0.01 μg/ml), as was a control of 0 μg/ml. The effect was compared to the murine antibody, a bispecific murine anti-CD16/CD30 antibody, and an irrelevant, chimeric IgG1 antibody. A CD30 negative line was also used. Maximum lysis was measured after 0.025% Triton was added, and all assays were carried out in triplicate.

The results indicated that the chimeric antibody performed better in the ADCC than the murine antibody.

In the CDC assays, 10,000 europium labelled cells (100 μg) (L540Y), were incubated, with 50, 5, 0.5, or 0.05 μg/ml antibody in a 50μl volume. Freshly isolated complement (50 μl) was added, and the mixture was incubated for 2 hours, at 37°C. The murine antibody was also tested, as was an anti CD-16 antibody and a chimeric anti IgG antibody, which served as controls, as did a CD-30 negative cell.

As in the ADCC assay the chimeric antibody was superior in terms of percent lysis to all other antibodies tested.

Example 16

This example details the production of a fusion protein of a chimeric, G250 specific antibody, and tumor necrosis factor ("TNF" hereafter).

G250 is an antigen also now as "carbonic anhydrase 9," or "CA9," or "MN." The G250 antigen and the corresponding antibody was described as being associated with renal cancer carcinoma by Oosterwijk, et al, PCT/US88/01511. The G250 antibody has also been the subject of several clinical trials (Oosterwijk, et al., Int. J. Cancer 1986: Oct. 15, 38(4):489-494; Divgi, et al., Clin. Cancer Res. 1998: Nov 4(11):2729-739.

Zavada, et al, have issued a series of patents in which the G250 antigen is referred to as "MN" or "MN/CAIX." See, e.g., U.S. Patent Nos. 6,051,226; 6,027,887; 5,995,075, and 5,981,711, all of which are incorporated by reference. These parents provide details on the antigen, and describe various tumors in which it is found, including cervical cancer, bladder cancer, mammary carcinoma, uterine, cervical, ovarian, and endometrial cancer.

Recently, Ivanov, et al, Am. Journal of Pathology 158(3):905-919 (2001), conducted investigations of CA9 and CA12 on tumor cells, and cell lines.

cDNA sequences for the light and heavy variable regions of a murine G250 specific antibody are known, and these include the endogenous antibody leader sequence. PCR primers were used to modify both the 5' and 3' regions, in order to introduce restriction sites necessary for the introduction of the coding sequences to the vectors employed, which were SEQ ID NOS: 48 & 49, supra. The cDNA sequence which encodes the murine G250 heavy chain variable region is set forth at SEQ ID NO: 50, with the amino acid sequence at SEQ ID NO: 51 and the light chain variable region, at SEQ ID NO: 52, with amino acid sequence at SEQ ID NO: 53. The first 8 nucleotides in each of SEQ ID NOS 50 & 52 represent a PmeI restriction site. The first 19 amino acids encoded by the nucleotide sequence represent the leader region, and the first 24 the leader sequence for the light chain. The last 6 nucleotides in each of SEQ ID NOS: 50 & 52 are a BamHI restriction site. The same protocol as was used for the HRS-3 chimera was used to splice these variable regions into SEQ ID NOS: 46 & 47.

To secure the cDNA encoding human TNF, a human leukocyte cDNA library was used. The peripheral blood lymphocytes were stimulated with PMA, and the cDNA for TNF was amplified, using standard methods. Restriction sites were introduced in the cDNA sequence, so that the cDNA for TNF was positioned right after the hinge region of the G250 heavy chain. A (Gly) Ser coding sequence linked the two. SEQ ID NOS: 54 & 55 set forth the nucleotide and amino acid sequences of a TNF fragment, and SEQ ID NO: 56, a construct wherein the human gamma-1 heavy chain is followed by the TNF coding sequence, right after the IgG1 hinge region.

Within SEQ ID NO: 56, nucleotides 1419-1754 encode a partial, human IgG1 constant region, containing the CH1 and hinge domain, preceded by a 60 base pair intron region and splice acceptor site. The linker, i.e., $(Gly)_4Ser$ is encoded by nucleotides 1755-

1769. The coding sequence for the human TNF fragment is set forth at nucleotides 1776-2296.

The resulting constructs were transfected into host cells, as described supra, and expressed. Note that SEQ ID NO: 56 contains a variant of the heavy chain vector noted supra, as it contains the human CH1 and hinge regions, followed by the TNF encoding sequence.

Cells were transfected and cultured as described supra for the HRS-3 chimera, and amplification was carried out using the primers of SEQ ID NOS: 40-43, described supra. The predicted size of the amplification product was 1100 base pairs, and this was in fact confirmed.

Positive colonies were then sub-cloned and cultured, as described supra. The chimeric G250-TNF fusion proteins were purified using anion exchanged chromatography on DEAE columns, using 5 ml samples, and increased salt concentrations in the elution buffer (NaCl, 0→0.5 M) (pH 8). The purity of the fusion proteins was determined, on SDS-PAGE, under reducing conditions. Two bands, of 45 and 28 kDa, respectively, appeared, consistent with the production of a chimeric fusion protein.

The purity of the chimeric fusion protein was confirmed in a sandwich ELISA. In brief, plates were coated with 1:6000 dilutions of affinity purified, goat anti-human IgG serum, and incubated overnight. They were then blocked with 2% gelatin. Either cell culture supernatant, or purified antibody was added, at varying concentrations, and then contacted with biotinylated goat anti-human TNFα specific serum, at 0.1 μg/ml, followed by visualization with a standard streptavidin peroxidase reagent.

The ELISA confirmed the purity of the antibody.

Example 17

FACS was carried out, as described supra for the chimeric HRS-3 antibodies, this time using the fusion protein, and G250 positive tumor cells. Two different purification runs were tested, with chimeric G250 antibody as a positive control, and an irrelevant chimeric IgG1 antibody as a negative control.

The results indicated that the chimeric fusion protein bound as well as the chimeric antibody did. No binding was detected when G250 negative cells were used.

Example 18

These experiments were designed to determine if the fusion proteins retained the ability of TNF to mediate cell death.

This was accomplished using an MTT assay as described by Renner, et al, Eur. J. Immunol 25:2027-2035 (1995), incorporated by reference, and TNF sensitive ("WEHI-R") cells. The WEHI cells were seeded at a density of 10,000 cells/well. Then, after 18 hours, sterile samples of the fusion protein, recombinant TNF, chimeric G250 antibody, or a negative control (plain medium), were added, at concentrations of $1.0x10^5$, $1.0x10^2$, 1, $1.0x10^{-2}$, $1.0x10^{-4}$, and $1.0x10^{-5}$ ng/ml, and the culture was incubated for additional period of from 48-72 hours. Any viable cells were detected, via standard methods, including Annexin V staining, and flow cytometry. To do this, $1x10^6$ WEHI cells were incubated, overnight, with varying antibody concentrations, and dye positive cells were counted. The effect of antibody loaded tumor cells in WEHI killing was determined by pre-staining with commercially available PKH-26GL dye.

The chimeric fusion proteins were found to be as effective as recombinant TNF in killing cells.

Example 19

It is known that TNF stimulates H_2O_2 release by human leukocytes. The chimeric fusion proteins were tested for this property.

Granulocytes were isolated from blood samples via standard methods, and were resuspended in reaction buffer (KRPG = 145 mM NaCl, 5 mM Na_2HPO_4, 4.8 mM KCl, 0.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 0.2 mM glucose, pH 7.35). This mix was added plates that had been precoated with fibronectin (1μg/ml, 2 hours, 37°C) to permit granulocyte adherence. Following this, 100μl of a dye solution (10 ml KRPG + 50μl A6550 + 10 μl horseradish-peroxidase) were added and incubated for 15 minutes at 37°C. Granulocytes were added, at 30,000 cells per well, and then either buffer (KRPG), PMA (5ng/ml), the chimeric fusion protein (1μg/ml) plus recombinant human IFN-γ (100μ/ml), or the fusion protein plus the

recombinant IFN-γ (at the indicated concentrations), were added. H_2O_2 release was measured for 3 hours, using standard methods.

The PMA served as a positive control. The chimeric fusion protein induced H_2O_2 release significantly higher than antibody alone, and the H_2O_2 release increases even more when IFN-γ was added.

WE CLAIM:

1. An isolated nucleic acid molecule which encodes a fusion protein, wherein said fusion protein comprises a chimerized antibodymolecule which specifically binds to a target of interest, and a tumor necrosis factor molecule or a fragment of a tumor necrosis factor molecule which possesses the cell killing properties of a full length tumor necrosis factor molecule.

2. The isolated nucleic acid molecule of claim 1, wherein said target is G250.

3. The isolated nucleic acid molecule of claim 1, wherein said tumor necrosis factor is full length tumor necrosis factor.

4. The isolated nucleic acid molecule of claim 1, wherein said fusion protein comprises the amino acid sequence encoded by nucleotides 1419-1754 of SEQ ID NO: 56, concatentated to the amino acid sequence encoded by nucleotides 1776-2296 of SEQ ID NO: 56.

5. A chimeric fusion protein encoded by the isolated nucleic acid molecule of claim 1.

6. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.

7. The expression vector of claim 1, comprising the nucleotide sequence of SEQ ID NO: 51.

8. Recombinant cell comprising the isolated nucleic acid molecule of claim 1 or the expression vector of claim 6.

9. The recombinant cell of claim 8, wherein said cell is mammalian.

10. The recombinant cell of claim 8, wherein said cell is a chinese hamster ovary cell.

11. An expression vector useful in manufacture of a recombinant antibody in a eukaryotic cell, said expression vector comprising (i) a human elongation factor 1α promoter enhancer sequence, (ii) an internal ribosome entry site, (iii) a nucleotide sequence which provides neomycin resistance, or encoded dihydrofolate reductase, (iv)a nucleotide sequence which encodes a light chain or heavy chain of an antibody molecule.

12. The expression vector of claim 11, consisting of the nucleotide sequence set forth in SEQ ID NO: 46 or SEQ ID NO: 47.

13. The expression vector of claim 11, wherein said recombinant antibody is a fully human, humanized , or chimeric antibody.

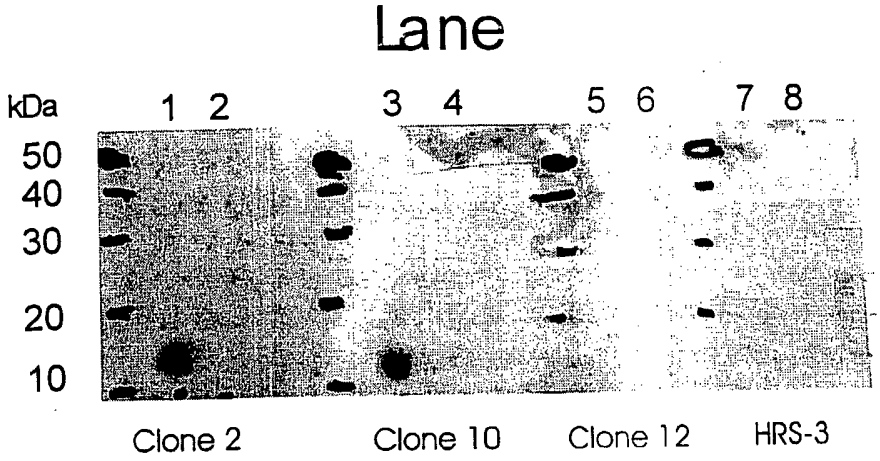


Fig 1

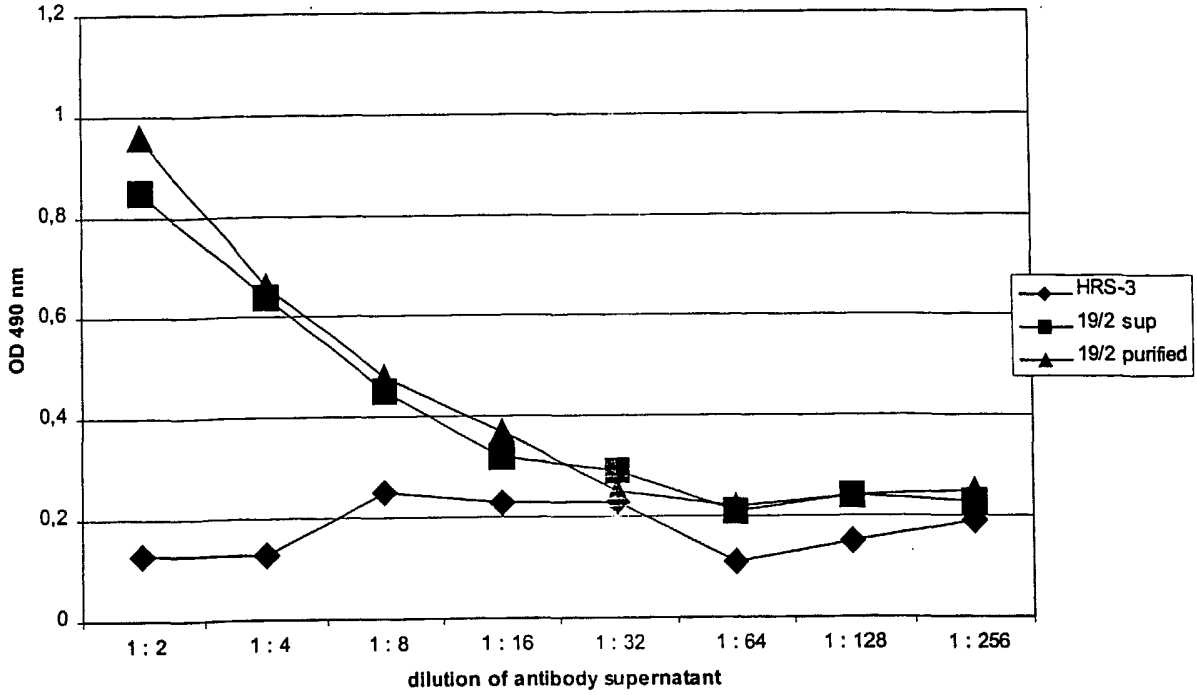

1,2
1
0,8
0,6
0,4
0,2
0
OD 490 nm
1 : 2
1 : 4
1 : 8
1 : 16
1 : 32
1 : 64
1 : 128
1 : 256
dilution of antibody supernatant
HRS-3
19/2 sup
19/2 purified

Fig 2

Fig 3

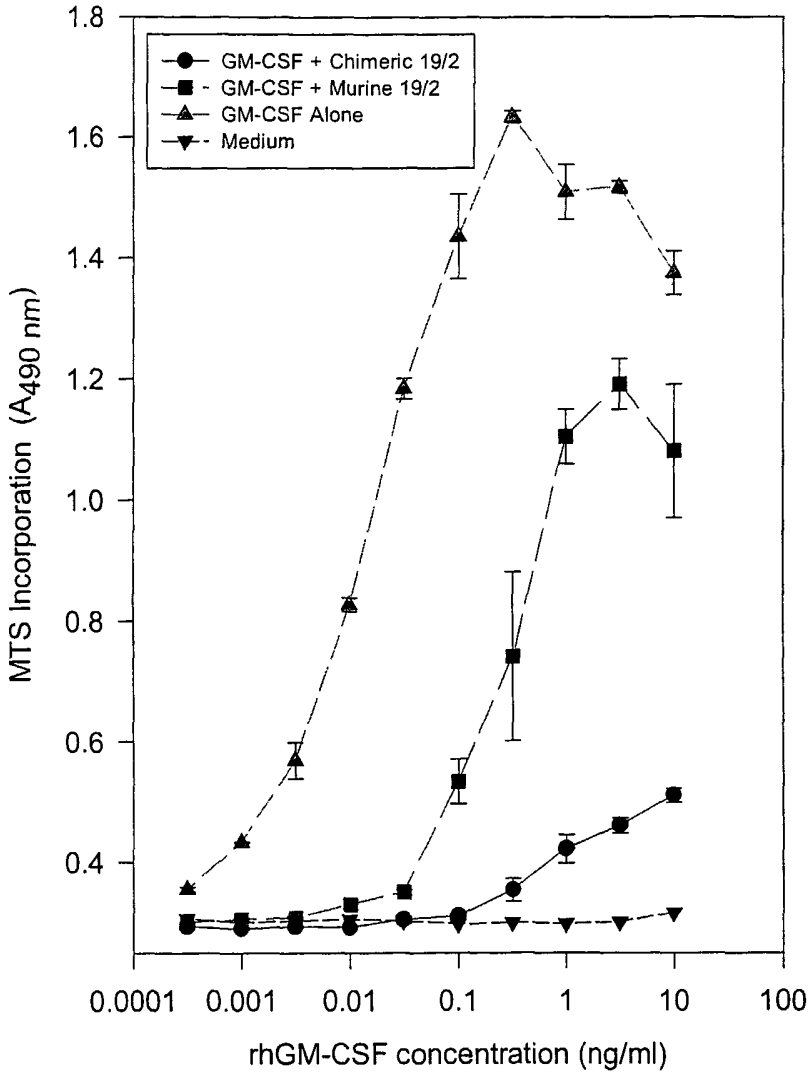
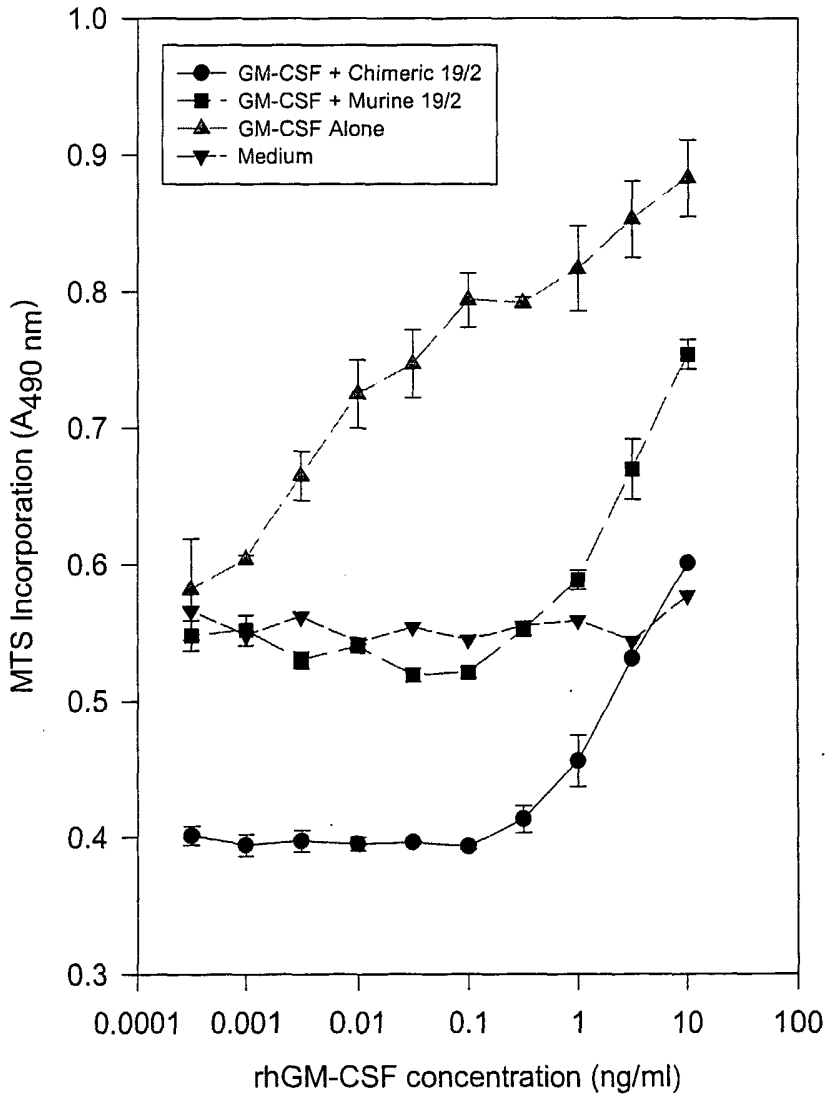

GM-CSF + Chimeric 19/2
GM-CSF + Murine 19/2
GM-CSF Alone
Medium
MTS Incorporation (A490 nm)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.0001
0.001
0.01
0.1
1
10
100
rhGM-CSF concentration (ng/ml)

Fig 4


GM-CSF + Chimeric 19/2
GM-CSF + Murine 19/2
GM-CSF Alone
Medium
MTS Incorporation (A_{490} nm)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.0001
0.001
0.01
0.1
1
10
100
rhGM-CSF concentration (ng/ml)

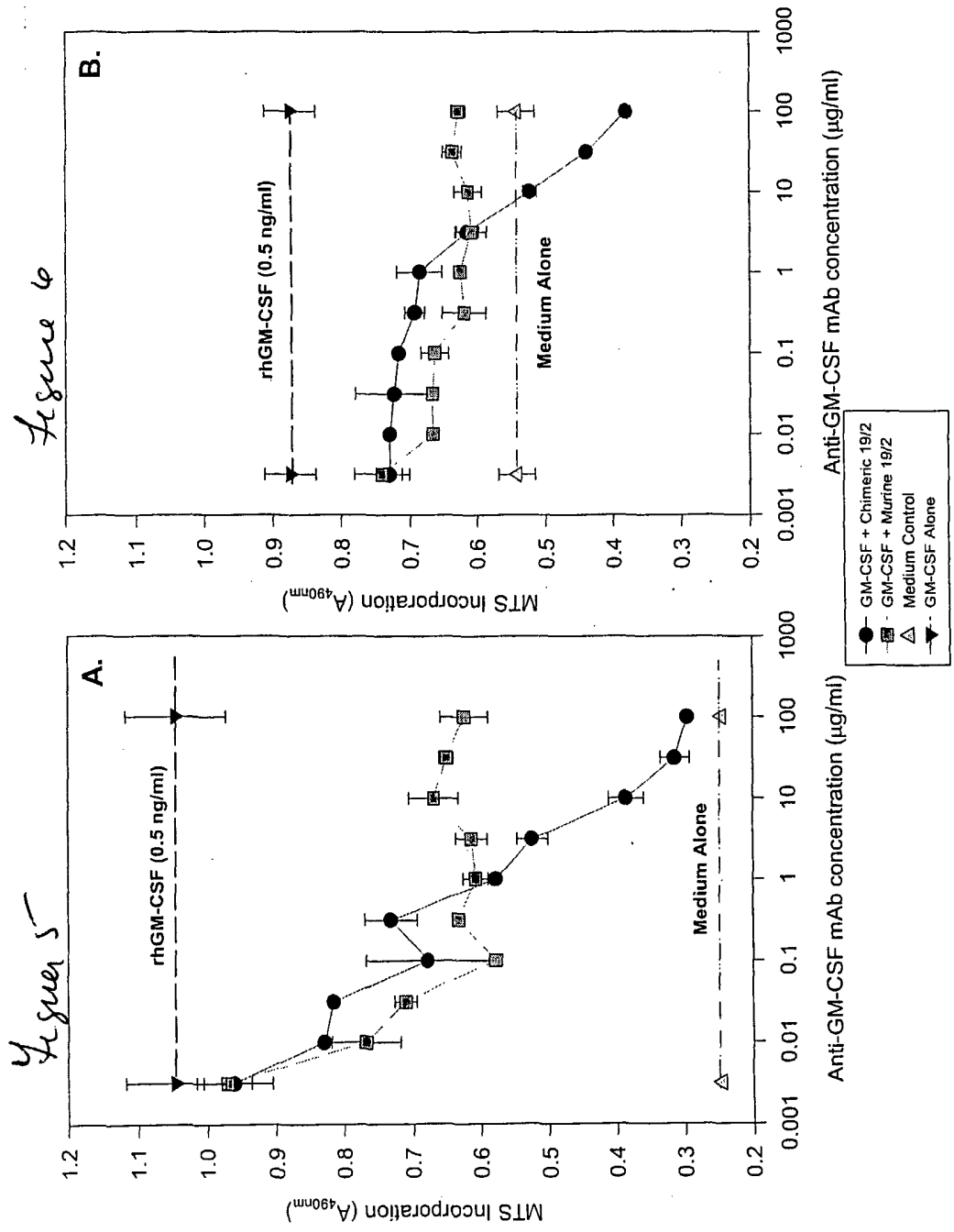
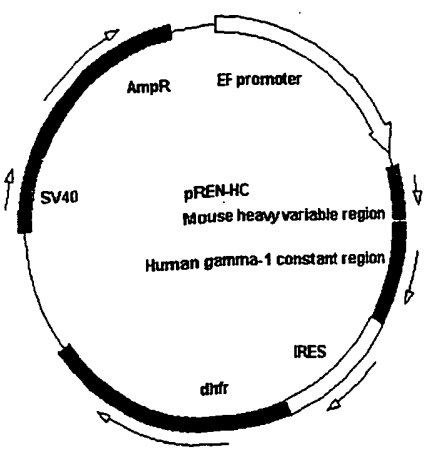


Figure 5
A.
rhGM-CSF (0.5 ng/ml)
Medium Alone
MTS Incorporation (A_{490nm})
Anti-GM-CSF mAb concentration (µg/ml)
Figure 6
B.
rhGM-CSF (0.5 ng/ml)
Medium Alone
MTS Incorporation (A_{490nm})
Anti-GM-CSF mAb concentration (µg/ml)
GM-CSF + Chimeric 19/2
GM-CSF + Murine 19/2
Medium Control
GM-CSF Alone


AmpR
EF promoter
SV40
pREN-HC
Mouse heavy variable region
Human gamma-1 constant region
IRES
dhfr

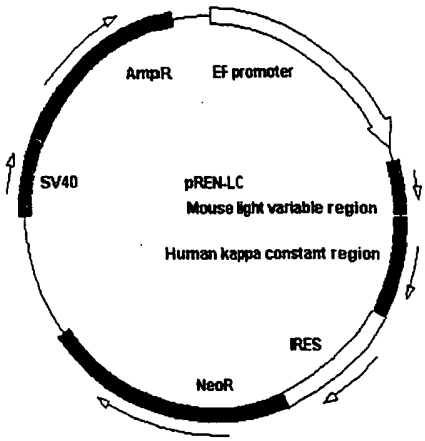

AmpR
EF promoter
SV40
pREN-LC
Mouse light variable region
Human kappa constant region
IRES
NeoR

FIGURE 7

1
ATGGAGCTGATCATGCTCTTCCTCCTGTCAGGAACTGCAGGCGTCCACTCTGAGGTCCAG

61
CTTCAGCAGTCAGGACCTGAACTGGTGAAACCTGGGGCCTCAGTGAAGATATCCTGCAAG

121
GCTTCTGGATACACTTTCACTGACTACAACATACACTGGGTGAAACAGAGCCATGGAAAG

181
AGCCTTGACTGGATTGGATATATTGCTCCTTACAGTGGTGGTACTGGTTACAACCAGGAG

241
TTCAAGAACAGGGCCACATTGACTGTAGACAAATCCTCCAGCACAGCCTACATGGAGCTC

301
CGCAGTCTGACATCTGATGACTCTGCAGTCTATTACTGTGCTAGACGAGACCGTTTCCCT

361
TATTACTTTGACTACTGGGGCCAAGGCACCCCTCTCACAGTCTCCTCAGCCAAAACGACA

421
CCCCCATCTGTCTATCCACTGGCAAGGGCGAATTCC

SEQ ID NO. 27

1
MELIMLFLLS GTAGVHSEVQ LQQSGPELVK PGASVKISCK ASGYTFTDYN

51
IHWVKQSHGK SLDWIGYIAP YSGGTGYNQE FKNRATLTVD KSSSTAYMEL

101 140
RSLTSDDSAV YYCARRDRFP YYFDYWGQGT TLRVSSVSGS

SEQ ID NO: 28

1 60
ATGGCTTCAAGATGGAGTCACAGATCCAGGTCTTTGTATACATGTTGCTGTGGTTGTCT

61

GGTGTTGATGGAGACATTGTGATGATCCAGTCTCAAAAATTCGTATCCACATCAGTAGGA

121

GACAGGGTCAATATCACCTGCAAGGCCAGTCAGAATGTGGGAAGTAATGTAGCCTGGTTG

181

CAACAGAAACCTGGACAATCTCCTAAAACGCTGATTTACTCGGCATCGTACCGGTCCGGT

241

CGAGTCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCATTCTTACCATCACT

301

ACTGTGCAGTCTGAAGACTTGGCAGAATATTTCTGTCAGCAATTTAACAGGTCTCCTCTC

361

ACGTTCGGTTCTGGGACCAAGTTGGAACTGAAACGGGCTGATGCTGCACCAACTGTATCC

421

[redacted]GTAAGGGCGAATTC

SEQ ID NO: 29

1 50

MGFKMESQIQ VFVYMLLWLS GVDGDIVMIQ SQKFVSTSVG DRVNITCKAS

51 110

QNVGSNVAWL QQKPGQSPKT LIYSASYRSG RVPDRFTGSG SGTDFILTIT TVQSEDLAEY

111 150

FCQQFNRSPL TFGSGTKLEL KRADAAPTVS IFPPSSKGEF

SEQ ID NO: 30

A) **SEQ ID NO.: 35.** pREN 19/2 LC Neo Vector

Xho I

1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC

50 ATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGT

EcoRI EF1α promoter

100 GGAGATTGTGAGCGGATAACAATTTCACACAGAATTCGTGAGGCTCCGGT

150 GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG

200 GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA

250 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

300 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA

350 CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGC

400 CTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG

450 CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

500 GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT

550 TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG

600 GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA

650 ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTA

700 AATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG

750 CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC

800 TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

850 CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC

900 GGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC

950 TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA

1000 GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTC

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1050  AGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACC

1100  TCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG

1150  GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT

1200  TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG

1251  AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTT
                              MluI        HindIII   PmeI
1300  TTTCTTCCATTTCAGGTGTACGCGTCTCGGGAAGCTTTAGTTTAAACGCC

1350  GCCACCATGGGCTTCAAGATGGAGTCACAGATCCAGGTCTTTGTATACAT
            M  G  F  K  M  E  S  Q  I  Q  V  F  V  Y  M

1401  GTTGCTGTGGTTGTCTGGTGTTGATGGAGACATTGTGATGATCCAGTCTC
       L  L  W  L  S  G  V  D  G  D  I  V  M  I  Q  S  Q

1451  AAAAATTCGTATCCACATCAGTAGGAGACAGGGTCAATATCACCTGCAAG
         K  F  V  S  T  S  V  G  D  R  V  N  I  T  C  K

1501  GCCAGTCAGAATGTGGGAAGTAATGTAGCCTGGTTGCAACAGAAACCTGG
      A  S  Q  N  V  G  S  N  V  A  W  L  Q  Q  K  P  G

1551  ACAATCTCCTAAAACGCTGATTTACTCGGCATCGTACCGGTCCGGTCGAG
       Q  S  P  K  T  L  I  Y  S  A  S  Y  R  S  G  R  V

1601  TCCCTGATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCATTCTTACC
         P  D  R  F  T  G  S  G  S  G  T  D  F  I  L  T

1651  ATCACTACTGTGCAGTCTGAAGACTTGGCAGAATATTTCTGTCAGCAATT
      I  T  T  V  Q  S  E  D  L  A  E  Y  F  C  Q  Q  F

1701  TAACAGGTCTCCTCTCACGTTCGGTTCTGGGACCAAGTTGGAACTGAAAC
       N  R  S  P  L  T  F  G  S  G  T  K  L  E  L  K  R
            BamHI
1751  GTGAGTGGATCCATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAAC

1801  ATGCCCTGTGATTATGCGCAAACAACACACCCAAGGGCAGAACTTTGTTA

1851  CTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCA
                                          T  V  A  A  P

1901  TCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC
      S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A

1951  CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
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          S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q

2001  AGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC
        W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V

2051  ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC
      T  E  Q  D  S  K  D  S  T  Y  S  L  S  S  T  L  T

2101  GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA
       L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T

2151  CCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAG
        H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E
             Nhe/Xba
2201  TGTTGAGCTAGAACTAACTAACTAAGCTAGCAACGGTTTCCCTCTAGCGG
      C  *
2251  GATCAATTCCGCCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAA

2301  TAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTC

2351  TTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCA

2401  TTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAAT

2451  GTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTC

2501  TGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCC

2551  TCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAA

2601  CCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCT

2651  CTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCC

2701  ATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACGTGTGTT

2751  TAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTT

2801  TTCCTTTGAAAAACACGATAATACCATGGTTGAACAAGATGGATTGCACG

2851  CAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCA

2901  CAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCA

2951  GGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATG

3001  AACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTT
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3051 CCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCT

3101 GCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTC

3151 CTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACG

3201 CTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGA

3251 GCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGG

3301 ACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAG

3351 GCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTG

3401 CTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACT

3451 GTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACC

3501 CGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGT

3551 GCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCC

blunt end SalI/SalI

3601 TTCTTGACGAGTTCTTCTGAGTCGATCGACCTGGCGTAATAGCGAAGAGG

3651 CCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGG

3701 GACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCG

3751 CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTT

3801 TCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTA

3851 AATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA

3901 CCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCT

3951 GATAGACGGTTTTTCGCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG

4001 GACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTTA

4051 TAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTA

4101 ACAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGT

4151 GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATATTTGTTTATTTTTC

4201 TAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT

4251 GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTG

4301 TCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTACTGTTTTTGCTCAC

4351 CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACG

4401 AGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT

4451 TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA

4501 TGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG

4551 CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAG

4601 AAAAGCATATTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC

4651 ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGG

4701 AGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA

4751 CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGAC

4801 GAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT

4851 ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACT

4901 GGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG

4951 GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCG

5001 CGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG

5051 TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAG

5101 ATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA

5151 AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTA

5201 AAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCT

5251 TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA

5301 AGGATGTTCTTGAGATCCTTTTTTTCTGCACGTAATCTGCTGCTTGCAAA

5351 CAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTAC

5401 CAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAAT

5451 ACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGT

5501 AGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG

5551 CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTA

5601 CCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCC

5651 CAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGC

5701 TATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG

5751 GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG

5801 AAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTG

5851 AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAAC

5901 GCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC

5951 TCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTA

6001 CCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGC

6051 AGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC

6101 TCTCCCCGCGCGTTGGCCGATTCATTAATGCAGGTATCACGAGGCCCTTT

6151 CGTCTTCAC

B) **SEQ ID NO.: 36.** pREN 19/2 HC DHFR Vector

Xho I

1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC

51 ATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGT

EcoRI EF1α promoter

101 GGAGATTGTGAGCGGATAACAATTTCACACAGAATTCGTGAGGCTCCGGT

151 GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG

201 GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA

251 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

301 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA

351 CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGC

401 CTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG

451 CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

501 GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT

551 TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG

601 GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA

651 ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTA

701 AATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG

751 CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC

801 TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

851 CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC

901 GGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC

951 TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA

1001 GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTC

1051 AGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACC

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1101  TCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG

1151  GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT

1201  TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG

1251  AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTT
                          MluI        HindIII   PmeI
1301  TTTCTTCCATTTCAGGTGTACGCGTCTCGGGAAGCTTTAGTTTAAACGCC

1351  GCCACCATGGAGCTGATCATGCTCTTCCTCCTGTCAGGAACTGCAGGCGT
            M  E  L  I  M  L  F  L  L  S  G  T  A  G  V

1401  CCACTCTGAGGTCCAGCTTCAGCAGTCAGGACCTGAACTGGTGAAACCTG
        H  S  E  V  Q  L  Q  Q  S  G  P  E  L  V  K  P  G

1451  GGGCCTCAGTGAAGATATCCTGCAAGGCTTCTGGATACACTTTCACTGAC
        A  S  V  K  I  S  C  K  A  S  G  Y  T  F  T  D

1501  TACAACATACACTGGGTGAAACAGAGCCATGGAAAGAGCCTTGACTGGAT
      Y  N  I  H  W  V  K  Q  S  H  G  K  S  L  D  W  I

1551  TGGATATATTGCTCCTTACAGTGGTGGTACTGGTTACAACCAGGAGTTCA
        G  Y  I  A  P  Y  S  G  G  T  G  Y  N  Q  E  F  K

1601  AGAACAGGGCCACATTGACTGTAGACAAATCCTCCAGCACAGCCTACATG
        N  R  A  T  L  T  V  D  K  S  S  S  T  A  Y  M

1651  GAGCTCCGCAGTCTGACATCTGATGACTCTGCAGTCTATTACTGTGCTAG
      E  L  R  S  L  T  S  D  D  S  A  V  Y  Y  C  A  R

1701  ACGAGACCGTTTCCCTTATTACTTTGACTACTGGGGCCAAGGCACCACTC
      R  D  R  F  P  Y  Y  F  D  Y  W  G  Q  G  T  T  L
                             BamHI
1751  TCAGAGTCTCCTCAGTGAGTGGATCCTCTGCGCCTGGGCCCAGCTCTGTC
        R  V  S  S

1801  CCACACCGCGGTCACATGGCACCACCTCTCTTGCAGCCTCCACCAAGGGC
                                                S  T  K  G
1851  CCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCAC
      P  S  V  F  P  L  A  P  S  S  K  S  T  S  G  G  T

1901  AGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGG
        A  A  L  G  C  L  V  K  D  Y  F  P  E  P  V  T  V

1951  TGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCT
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          S  W  N  S  G  A  L  T  S  G  V  H  T  F  P  A

2001  GTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCC
      V  L  Q  S  S  G  L  Y  S  L  S  S  V  Y  S  V  P

2051  CTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGC
       S  S  S  L  G  T  Q  T  Y  I  C  N  V  N  H  K  P

2101  CCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAA
        S  N  T  K  V  D  K  K  V  E  P  K  S  C  D  K

2151  ACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTC
      T  H  T  C  P  P  C  P  A  P  E  L  L  G  G  P  S

2201  AGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGA
       V  F  L  F  P  P  K  P  K  D  T  L  M  I  S  R  T

2251  CCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAG
        P  E  V  T  C  V  V  V  D  V  S  H  E  D  P  E

2301  GTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAACGCCAAGAC
      V  K  F  N  W  Y  V  D  G  V  E  V  H  N  A  K  T

2351  AAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGTCC
       K  P  R  E  E  Q  Y  N  S  T  Y  R  V  V  S  V  L

2401  TCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
        T  V  L  H  Q  D  W  L  N  G  K  E  Y  K  C  K

2451  GTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGC
      V  S  N  K  A  L  P  A  P  I  E  K  T  I  S  K  A

2501  CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGG
       K  G  Q  P  R  E  P  Q  V  Y  T  L  P  P  S  R  E

2551  AGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTC
        E  M  T  K  N  Q  V  S  L  T  C  L  V  K  G  F

2601  TATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
      Y  P  S  D  I  A  V  E  W  E  S  N  G  Q  P  E  N

2651  CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCC
       N  Y  K  T  T  P  P  V  L  D  S  D  G  S  F  F  L

2701  TCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTC
        Y  S  K  L  T  V  D  K  S  R  W  Q  Q  G  N  V
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2751  TTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
      F  S  C  S  V  M  H  E  A  L  H  N  H  Y  T  Q  K
                                        Nhe/Xba
2801  GAGCCTCTCCCTGTCTCCGGGTAAATGAGCTAGAAACTAACTAAGCTAGC
       S  L  S  L  S  P  G  K  *
2851  AACGGTTTCCCTCTAGCGGGATCAATTCCGCCCCCCCCCCCTAACGTTAC

2901  TGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTAT

2951  TTTCCACCATATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGC

3001  CCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCCTCTCGCCAAAGG

3051  AATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTT

3101  CTTGAAGACAAACAACGTCTGTAGCGACCCTTTGCAGGCAGCGGAACCCC

3151  CCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAAGATAC

3201  ACCTGCAAAGGCGGCACAACCCCAGTGCCACGTTGTGAGTTGGATAGTTG

3251  TGGAAAGAGTCAAATGGCTCTCCTCAAGCGTATTCAACAAGGGGCTGAAG

3301  GATGCCCAGAAGGTACCCCATTGTATGGGATCTGATCTGGGGCCTCGGTG

3351  CACATGCTTTACGTGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCC

3401  GAACCACGGGGACGTGGTTTTCCTTTGAAAAACACGATAATACCATGGTT

3451  CGACCATTGAACTGCATCGTCGCCGTGTCCCAAAATATGGGGATTGGCAA

3501  GAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTCAAGTACTTCC

3551  AAAGAATGACCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATT

3601  ATGGGTAGGAAAACCTGGTTCTCCATTCCTGAGAAGAATCGACCTTTAAA

3651  GGACAGAATTAATGGTTCGATATAGTTCTCAGTAGAGAACTCAAAGAACC

3701  ACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGATGATGCCTTAAGAC

3751  TTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTC

3801  GGAGGCAGTTCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTCAG

3851  ACTCTTTGTGACAAGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCC
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3901  CAGAAATTGATTTGGGGAAATATAAACTTCTCCCAGAATACCCAGGCGTC

3951  CTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAGTTTGAAGTCTA

4001  CGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCCTCC
                                                Blunt end SalI/SalI
4051  TAAAGCTATGCATTTTTATAAGACCATGGGACTTTTGCTGGTCGATCGAC

4101  CTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGC

4151  GCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAGCGCG

4201  GCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCT

4251  AGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCG

4301  GCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTT

4351  AGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTC

4401  ACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCTTTGACGTTGGA

4451  GTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCA

4501  ACCCTATCTCGGTCTATTTATAAGGGATTTTGCCGATTTCGGCCTATTGG

4551  TTAAAAAATGAGCTGATTTAACAAAATTTAACGCGAATTTTAACAAAATA

4601  TTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACC

4651  CCTATATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATG

4701  AGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTAT

4751  GAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTT

4801  GCCTTACTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCT

4851  GAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAG

4901  CGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGA

4951  GCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCC

5001  GGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGT

5051  TGAGTACTCACCAGTCACAGAAAAGCATATTACGGATGGCATGACAGTAA
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5101 GAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAAC

5151 TTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCA

5201 CAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGA

5251 ATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATG

5301 GCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTC

5351 CCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCAC

5401 TTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGA

5451 GCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGG

5501 TAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTA

5551 TGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAG

5601 CATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTT

5651 AAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATA

5701 ATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCA

5751 GACCCCGTAGAAAAGATCAAAGGATGTTCTTGAGATCCTTTTTTTCTGCA

5801 CGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTG

5851 TTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCA

5901 GCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGC

5951 CACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAAT

6001 CCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGT

6051 TGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACG

6101 GGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACT

6151 GAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGA

6201 GAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC

6251 ACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGG

6301 GTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGG

6351 GGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTG

6401 GCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGA

6451 TTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCC

6501 GCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAG

6551 CGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATG

6601 CAGGTATCACGAGGCCCTTTCGTCTTCAC

GT TTA AAC GCC GCC ACC ATG AAC TGG ACC TGG ACC GTG
TTT TGC CTG CTC GCT GTG GCT CCT GGG GCC CAC AGC GCC ATG GCC
CAG GTG CAA CTG CAG CAG TCA GGG GCT GAG CTG GCT AGA CCT GGG
GCT TCA GTG AAG ATG TCC TGC AAG GCT TCT GGC TAC ACC TTT ACT
ACC TAC ACA ATA CAC TGG GTA AGA CAG AGG CCT GGA CAC GAT CTG
GAA TGG ATT GGA TAC ATT AAT CCT AGC AGT GGA TAT TCT GAC TAC
AAT CAA AGC TTC AAG GGC AAG ACC ACA TTG ACT GCA GAC AAG TCC
TCC AAC ACA GCC TAC ATG CAA CTG AAC AGC CTG ACA TCT GAG GAC
TCT GCG GTC TAT TAC TGT GCA AGA AGA GCG GAC TAT GGT AAC TAC
GAA TAT ACC TGG TTT GCT TAC TGG GGC CAA GGG ACC ACG GTC ACC
GTC TCC TCA GGT GAG TGC ATC C

SEQ ID NO. 44

MNWTWTVFCLLAVAPGAHSAMAQVQLQQSGAELARPGASVKMSCKASGYTFTT

YTIHWVRQRPGHDLEWIGYINPSSGYSDYNQSFKGKTTLTADKSSNTAYMQLNS

LTSEDSAVYYCARRADYGNYEYTWFAYWGQGTTVTVSS

SEQ ID NO: 45

GT TTA AAC GCC GCC ACC ATG AAC TGG ACC TGG ACC GTG TTT TGC
CTG CTC GCT GTG GCT CCT GGG GCC CAC AGC GCC ATG GAC ATC GAG
CTC ACT CAG TCT CCA AAA TTC ATG TCC ACA TCA GTA GGA GAC AGG
GTC AAC GTC ACC TAC AAG GCC AGT CAG AAT GTG GGT ACT AAT GTA
GCC TGG TTT CAA CAA AAA CCA GGG CAA TCT CCT AAA GTT CTG ATT
TAC TCG GCA TCT TAC CGA TAC AGT GGA GTC CCT GAT CGC TTC ACA
GGC AGT GGA TCT GGA ACA GAT TTC ACT CTC ACC ATC AGC AAT GTG
CAG TCT GAA GAC TTG GCA GAG TAT TTC TGT CAG CAA TAT CAC ACC
TAT CCT CTC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATC AAA CGT
GAG TTG GAT CG

SEQ ID NO: 46

MNWTWTVFCLLAVAPGAHSAMDIELTQSPKFMSTSVGDRVNVTYKAS
QNVGTNVAWFQQKPGQSPKVLIYSASYRYSGVPDRFTGSGSGTDFTLTI
SNVQSEDLAEYFCQQYHTYPLTFGGGTKLEIKR

SEQ ID NO. 47

Figure 4A and B: *Mammalian cell expression vectors used to produce chimeric and reshaped human antibodies with human kappa light chains and human gamma-1 heavy chains.*

Figure 4A. *Light chain expression vector pREN-Neo.*

SEQ ID NO: 48

The components of the vector (5809bp) are:

1-6	= XhoI site
135-140	= EcoRI site
141-1324	= human elongation factor 1α promoter/enhancer
1325-1330	= MluI site
1333-1338	= HindIII site
1340-1348	= PmeI site
1357-1362	= BamHI site
1436-1806	= human kappa constant region, preceded by a 120bp intron region and splice acceptor site
1807-1812	= Ligation of NheI/XbaI sites
1813-3220	= IRES-Neo sequence
3221-3230	= Blunt end filled SalI/SalI sites
3299-3754	= F1 interregion
3880-4740	= beta-lactamase

Xho I

1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC

51 ATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGT

EcoRI EF1α promoter

101 GGAGATTGTGAGCGGATAACAATTTCACACAGAATTCGTGAGGCTCCGGT

151 GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG

201 GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA

251 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

301 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA

351 CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGC

401 CTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG

451 CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

501 GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT

551 TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG

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601   GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA

651   ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTA

701   AATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG

751   CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC

801   TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

851   CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC

901   GGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC

951   TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA

1001  GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTC

1051  AGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACC

1101  TCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG

1151  GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT

1201  TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG

1251  AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTT
                              MluI          HindIII    PmeI
1301  TTTCTTCCATTTCAGGTGTACGCGTCTCGGGAAGCTTTAGTTTAAACGCC
            BamHI
1351  GTGAGTGGATCCATCTGGGATAAGCATGCTGTTTTCTGTCTGTCCCTAAC

1401  ATGCCCTGTGATTATGCGCAAACAACACACCCAAGGGCAGAACTTTGTTA

1451  CTTAAACACCATCCTGTTTGCTTCTTTCCTCAGGAACTGTGGCTGCACCA
                                                T  V  A  A  P

1501  TCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGC
      S  V  F  I  F  P  P  S  D  E  Q  L  K  S  G  T  A

1551  CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTAC
       S  V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V  Q

1601  AGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTC
        W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S  V

1651  ACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGAC
      T  E  Q  D  S  K  D  S  T  Y  S  L  S  S  T  L  T

1701  GCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCA
       L  S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V  T
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1751  CCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAG
        H  Q  G  L  S  S  P  V  T  K  S  F  N  R  G  E
              Nhe/Xba
1801  TGTTGAGCTAGAACTAACTAACTAAGCTAGCAACGGTTTCCCTCTAGCGG
      C  *
1851  GATCAATTCCGCCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAA

1901  TAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTC

1951  TTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCA

2001  TTCCTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAAT

2051  GTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTC

2101  TGTAGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCC

2151  TCTGCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAA

2201  CCCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCT

2251  CTCCTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCC

2301  ATTGTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACGTGTGTT

2351  TAGTCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTT

2401  TTCCTTTGAAAAACACGATAATACCATGGTTGAACAAGATGGATTGCACG

2451  CAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCA

2501  CAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCA

2551  GGGGCGCCCGGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATG

2601  AACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTT

2651  CCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCT

2701  GCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTC

2751  CTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACG

2801  CTTGATCCGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGA

2851  GCGAGCACGTACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGG

2901  ACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAG

2951  GCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTG
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3001 CTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACT

3051 GTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACC

3101 CGTGATATTGCTGAAGAGCTTGGCGGCGAATGGGCTGACCGCTTCCTCGT

3151 GCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCATCGCCTTCTATCGCC

blunt end SalI/SalI

3201 TTCTTGACGAGTTCTTCTGAGTCGATCGACCTGGCGTAATAGCGAAGAGG

3251 CCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGG

3301 GACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCG

3351 CAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTT

3401 TCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTA

3451 AATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA

3501 CCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCT

3551 GATAGACGGTTTTTCGCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTG

3601 GACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTTA

3651 TAAGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTA

3701 ACAAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGT

3751 GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATATTTGTTTATTTTTC

3851 TAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAAT

3901 GCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTG

3951 TCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTACTGTTTTTGCTCAC

4001 CCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACG

4051 AGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTT

4101 TTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA

4151 TGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCG

4201 CCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAG

4251 AAAAGCATATTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCC

4301 ATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGG

4351 AGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAA

4401 CTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGAC

4451 GAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACT

4501 ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACT

4551 GGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCG

4601 GCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCG

4651 CGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAG

4701 TTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAG

4751 ATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA

4801 AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTA

4851 AAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCT

4901 TAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAA

4951 AGGATGTTCTTGAGATCCTTTTTTTCTGCACGTAATCTGCTGCTTGCAAA

5001 CAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTAC

5051 CAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAAT

5101 ACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGT

5151 AGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG

5201 CCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTA

5251 CCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCC

5301 CAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGC

5351 TATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCG

5401 GTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGG

5451 AAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTG

5501 AGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAAC

5551 GCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC

5601 TCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTA

5651 CCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGC

5701 AGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCC

5751 TCTCCCCGCGCGTTGGCCGATTCATTAATGCAGGTATCACGAGGCCCTTT

5801 CGTCTTCAC

Figure 4B: *Heavy chain expression vector pREN-DHFR*

The components of the vector (6257bp) are: SEQ ID NO: 49

1-7 = XhoI site
135-141 = EcoRI site
141-1325 = human elongation factor 1α promoter/enhancer
1317-1322 = MluI site
1329-1334 = HindIII site
1337-1343 = PmeI site
1349-1354 = BamHI site
1417-2406 = human IgG1 constant region, preceded by a 60bp intron region and splice acceptor site
2407-2412 = Ligation of NheI/XbaI sites
2413-3668 = IRES-DHFR sequence
3669-3678 = Blunt end filled SalI/SalI sites
3748-4203 = F1 interregion
4328-5188 = beta-lactamase

Xho I
1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC

51 ATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGT

EcoRI EF1α promoter
101 GGAGATTGTGAGCGGATAACAATTTCACACAGAATTCGTGAGGCTCCGGT

151 GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG

201 GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA

251 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

301 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA

351 CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGC

401 CTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG

451 CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

501 GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT

551 TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG

601 GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA

651 ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTA

701 AATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG

751 CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC

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801   TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

851   CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC

901   GGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC

951   TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA

1001  GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTC

1051  AGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACC

1101  TCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG

1151  GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT

1201  TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG

1251  AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTT
                          MluI          HindIII  PmeI
1301  CTTCCATTTCAGGTGTACGCGTCTCGGGAAGCTTTAGTTTAAACGCCTGG
      BamHI
1351  ATCCTCTGCGCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGCAC

1401  CACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCAC
                        S  T  K  G  P  S  V  F  P  L  A  P

1451  CCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC
        S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V

1501  AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCT
      K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A  L

1551  GACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCT
        T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L  Y

1601  ACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAG
        S  L  S  S  V  Y  S  V  P  S  S  S  L  G  T  Q

1651  ACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAA
      T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D  K

1701  GAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCC
        K  V  E  P  K  S  C  D  K  T  H  T  C  P  P  C  P

1751  CAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAA
        A  P  E  L  L  G  G  P  S  V  F  L  F  P  P  K

1801  CCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
      P  K  D  T  L  M  I  S  R  T  P  E  V  T  C  V  V
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1851  GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
        V  D  V  S  H  E  D  P  E  V  K  F  N  W  Y  V  D

1901  ACGGCGTGGAGGTGCATAACGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
        G  V  E  V  H  N  A  K  T  K  P  R  E  E  Q  Y

1951  AACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTG
        N  S  T  Y  R  V  V  S  V  L  T  V  L  H  Q  D  W

2001  GCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAG
        L  N  G  K  E  Y  K  C  K  V  S  N  K  A  L  P  A

2051  CCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCA
        P  I  E  K  T  I  S  K  A  K  G  Q  P  R  E  P

2101  CAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGT
        Q  V  Y  T  L  P  P  S  R  E  E  M  T  K  N  Q  V

2151  CAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGG
        S  L  T  C  L  V  K  G  F  Y  P  S  D  I  A  V  E

2201  AGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCC
        W  E  S  N  G  Q  P  E  N  N  Y  K  T  T  P  P

2251  GTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGA
        V  L  D  S  D  G  S  F  F  L  Y  S  K  L  T  V  D

2301  CAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATG
        K  S  R  W  Q  Q  G  N  V  F  S  C  S  V  M  H  E

2351  AGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT
        A  L  H  N  H  Y  T  Q  K  S  L  S  L  S  P  G
                Nhe/Xba
2401  AAATGAGCTAGAAACTAACTAAGCTAGCAACGGTTTCCCTCTAGCGGGAT
        K  *

2451  CAATTCCGCCCCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAA

2501  GGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTT

2551  TGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTC

2601  CTAGGGGTCTTTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTC

2651  GTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGT

2701  AGCGACCCTTTGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCT

2751  GCGGCCAAAAGCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCC

2801  CAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTC
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2851 CTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATT

2901 GTATGGGATCTGATCTGGGGCCTCGGTGCACATGCTTTACGTGTGTTTAG

2951 TCGAGGTTAAAAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTC

3001 CTTTGAAAAACACGATAATACCATGGTTCGACCATTGAACTGCATCGTCG

3051 CCGTGTCCCAAAATATGGGGATTGGCAAGAACGGAGACCTACCCTGGCCT

3101 CCGCTCAGGAACGAGTTCAAGTACTTCCAAAGAATGACCACAACCTCTTC

3151 AGTGGAAGGTAAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCT

3201 CCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATGGTTCGATA

3251 TAGTTCTCAGTAGAGAACTCAAAGAACCACCACGAGGAGCTCATTTTCTT

3301 GCCAAAAGTTTGGATGATGCCTTAAGACTTATTGAACAACCGGAATTGGC

3351 AAGTAAAGTAGACATGGTTTGGATAGTCGGAGGCAGTTCTGTTTACCAGG

3401 AAGCCATGAATCAACCAGGCCACCTCAGACTCTTTGTGACAAGGATCATG

3451 CAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATA

3501 TAAACTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAA

3551 AAGGCATCAAGTATAAGTTTGAAGTCTACGAGAAGAAAGACTAACAGGAA

3601 GATGCTTTCAAGTTCTCTGCTCCCCTCCTAAAGCTATGCATTTTTATAAG

Blunt end SalI/SalI

3651 ACCATGGGACTTTTGCTGGTCGATCGACCTGGCGTAATAGCGAAGAGGCC

3701 CGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGA

3751 CGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA

3801 GCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTC

3851 TTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAA

3901 TCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACC

3951 CCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGA

4001 TAGACGGTTTTTCGCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGA

4051 CTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTTATA

4151 AGGGATTTTGCCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAAC

4201 AAAATTTAACGCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGG

4251 CACTTTTCGGGGAAATGTGCGCGGAACCCCTATATTTGTTTATTTTTCTA

4301 AATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGC

4351 TTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTC

4401 GCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTACTGTTTTTGCTCACCC

4451 AGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAG

4501 TGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTT

4551 CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATG

4601 TGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCC

4651 GCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAA

4701 AAGCATATTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCAT

4751 AACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAG

4801 GACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACT

4851 CGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGA

4901 GCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTAT

4951 TAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGG

5001 ATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGC

5051 TGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCG

5101 GTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTT

5151 ATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGAT

5201 CGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAG

5251 TTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAA

5301 AGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTA

5351 ACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAG

5401 GATGTTCTTGAGATCCTTTTTTTCTGCACGTAATCTGCTGCTTGCAAACA

5451 AAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCA

5501 ACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATAC

5551 TGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAG

5601 CACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCC

5651 AGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACC

5701 GGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCA

5751 GCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTA

5801 TGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGT

5851 AAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAA

5901 ACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAG

5951 CGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGC

6001 CAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTC

6051 ACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACC

6101 GCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAG

6151 CGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTC

6201 TCCCCGCGCGTTGGCCGATTCATTAATGCAGGTATCACGAGGCCCTTTCG

6251 TCTTCAC

GT TTA AAC GCC GCC ACC ATG AAC TTC GGG CTC AGA TTG
ATT TTC CTT GTC CTG GTT TTA AAA GGT GTC CTG TGT GAC
GTG AAG CTC GTG GAG TCT GGG GCA GCC TTA GTG AAG CTT
GGA GGG TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC
ACT TTC AGT AAC TAT TAC ATG TCT TGG GTT CGC CAG ACT
CCA GAG AAG AGG CTG GAG TTG GTC GCA GCC ATT AAT AGT
GAT GGT GGT ATC ACC TAC TAT CTA GAC ACT GTG AAG GGC
CGA TTC ACC ATT TCA AGA GAC AAT GCC AAG AAC ACC CTG
TAC CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC
TTG TTT TAC TGT GCA AGA CAC CGC TCA GGC TAC TTT TCT
ATG GAC TAC TGG GGT CAA GGA ACC TCA GTC ACC GTC TCC
TCA GGT GAG TGC ATG G

SEQ ID NO: 50

MNFGLRLIFLVLVLKGVLCDVKLVESGAALVKLGGSLKLSCAASGFTFSNYYM

SWVRQTPEKRLELVAAINSDGGITYYLDTVKGRFTISRDNAKNTLYLQMSSLK

SEDTALFYCARHRSGYFSMDYWGQGTSVTVSSGE

SEQ ID NO. 51

GT TTA AAG GCC GCC ACC ATG GGC TTC AAG ATG GAG TTT CAT
ACT CAG GTC TTT GTA TTC GTG TTT CTC TGG TTG TCT GGT GTT
GAT GGA GAC ATT GTG ATG ACC CAG TCT CAA AGA TTC ATG TCC
ACA ACA GTA GGA GAC AGG GTC AGC ATC ACC TGC AAG GCC
AGT CAG AAT GTG GTT TCT GCT GTT GCC TGG TAT CAA CAG AAA
CCA GGA CAA TCT CCT AAA CTA CTG ATT TAC TCA GCA TCC AAT
CGG TAC ACT GGA GTC CCT GAT CGC TTC ACA GGC AGT GGA TCT
GGG ACA GAT TTC ACT CTC ACC ATT AGC AAT ATG CAG TCT GAA
GAC CTG GCT GAT TTT TTC TGT CAA CAA TAT AGC AAC TAT CCG
TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA CGT
GAG TGG ATC C

SEQ ID NO: 52

MGFKMEFHTQVFVFVFLWLSGVDGDIVMTQSQRFMSTTVGDRVSITCKASQNV

VSAVAWYQQKPGQSPKLLIYSASNRYTGVPDRFTGSGSGTDFTLTISNMQSED

LADFFCQQYSNYPWTFGGGTKLEIKRE

SEQ ID NO: 53

CG ATG GTC TCA TCT TCT CGA ACC CCG AGT GAC AAG CCT
GTA GCC CAT GTT GTA GCA AAC CCT CAA GCT GAG GGG CAG
CTC CAG TGG CTG AAC CGC CGG GCC AAT GCC CTC CTG GCC
AAT GGC GTG GAG CTG AGA GAT AAC CAG CTG GTG GTG CCA
TCA GAG GGC CTG TAC CTC ATC TAC TCC CAG GTC CTC TTC
AAG GGC CAA GGC TGC CCC TCC ACC CAT GTG CTC CTC ACC
CAC ACC ATC AGC CGC ATC GCC GTC TCC TAC CAG ACC AAG
GTC AAC CTC CTC TCT GCC ATC AAG AGC CCC TGC CAG AGG
GAG ACC CCA GAG GGG GCT GAG GCC AAG CCC TGG TAT GAG
CCC ATC TAT CTG GGA GGG GTC TTC CAG CTG GAG AAG GGT
GAC CGA CTC AGC GCT GAG ATC AAT CGG CCC GAC TAT CTC
GAC TTT GCC GAG TCT GGG CAG GTC TAC TTT GGG ATC ATT
GCC CTG **TGA** TGT AGA

SEQ ID NO. 54

MVSSSRTPSDKPVAHVVANPQAEGQLQWLNRRANALLANGVELRDNQLVVPSE

GLYLIYSQVLFKGQGCPSTHVLLTHTISRIAVSYQTKVNLLSAIKSPCQRETP

EGAEAKPWYEPIYLGGVFQLEKGDRLSAEINRPDYLDFAESGQVYFGIIAL*

SEQ ID NO: 57

Figure 5: *Mammalian cell expression vectors used to produce chimeric and reshaped human antibodies with parts of the human gamma-1 heavy chain followed by human TNF after the IgG1 hinge region. Heavy chain expression vector pREN-DHFR-TNF.*

The components of the vector (6147bp) are:

SEQ ID NO: 56

1-8 = XhoI site
135-142 = EcoRI site
141-1326 = human elongation factor 1α promoter/enhancer
1320-1325 = MluI site
1332-1337 = HindIII site
1340-1347 = PmeI site
1350-1355 = BamHI site
1419-1754 = partial human IgG1 constant region containing the CH1 and hinge domain, preceded by a 60bp intron region and splice acceptor site.
1755-1769 = five amino acid linker [$(Gly)_4Ser$]
1770-1775 = NcoI site
1776-2296 = human TNF, mature sequence
2297-2302 = XbaI site
2303-3559 = IRES-DHFR sequence
3560-3569 = Blunt end filled SalI/SalI sites
3639-4104 = F1 interregion
4229-5089 = beta-lactamase

Xho I
1 CTCGAGAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTC

51 ATTAGGCACCCCAGGCTTTACACTTTATGCTCCCGGCTCGTATGTTGTGT

EcoRI EF1α promoter
101 GGAGATTGTGAGCGGATAACAATTTCACACAGAATTCGTGAGGCTCCGGT

151 GCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGG

201 GGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAA

251 ACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGG

301 GGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAA

351 CGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCCCGCGGGC

401 CTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACG

451 CCCCTGGCTGCAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTG

501 GGTGGGAGAGTTCGAGGCCTTGCGCTTAAGGAGCCCCTTCGCCTCGTGCT

551 TGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAATCTGGTG

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601   GCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAA

651   ATTTTTGATGACCTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTA

701   AATGCGGGCCAAGATCTGCACACTGGTATTTCGGTTTTTGGGGCCGCGGG

751   CGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCGGGGCC

801   TGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGG

851   CCTGCTCTGGTGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGC

901   GGCAAGGCTGGCCCGGTCGGCACCAGTTGCGTGAGCGGAAAGATGGCCGC

951   TTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGGCGCTCGGGA

1001  GAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTC

1051  AGCCGTCGCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACC

1101  TCGATTAGTTCTCGAGCTTTTGGAGTACGTCGTCTTTAGGTTGGGGGGAG

1151  GGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGAGACTGAAGT

1201  TAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTG

1251  AGTTTGGATCTTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTT
                          MluI          HindIII    PmeI
1301  TTTCTTCCATTTCAGGTGTACGCGTCTCGGGAAGCTTTAGTTTAAACGCC
      BamHI
1351  GGATCCTCTGCGCCTGGGCCCAGCTCTGTCCCACACCGCGGTCACATGGC

1401  ACCACCTCTCTTGCAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGC
                            S  T  K  G  P  S  V  F  P  L  A

1451  ACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGG
       P  S  S  K  S  T  S  G  G  T  A  A  L  G  C  L  V

1501  TCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC
         K  D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A

1551  CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACT
      L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S  G  L

1601  CTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCC
       Y  S  L  S  S  V  Y  S  V  P  S  S  S  L  G  T  Q

1651  AGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGAC
         T  Y  I  C  N  V  N  H  K  P  S  N  T  K  V  D

1701  AAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTG
      K  K  V  E  P  K  S  C  D  K  T  H  T  C  P  P  C
```

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1751  CCCAGGTGGAGGTGGATCACCAATGGTCTCATCTTCTCGAACCCCGAGTG
        P  G  G  G  G  S  P  M  V  S  S  S  R  T  P  S  D

1801  ACAAGCCTGTAGCCCATGTTGTAGCAAACCCTCAAGCTGAGGGGCAGCTC
        K  P  V  A  H  V  V  A  N  P  Q  A  E  G  Q  L

1851  CAGTGGCTGAACCGCCGGGCCAATGCCCTCCTGGCCAATGGCGTGGAGCT
        Q  W  L  N  R  R  A  N  A  L  L  A  N  G  V  E  L

1901  GAGAGATAACCAGCTGGTGGTGCCATCAGAGGGCCTGTACCTCATCTACT
        R  D  N  Q  L  V  V  P  S  E  G  L  Y  L  I  Y  S

1951  CCCAGGTCCTCTTCAAGGGCCAAGGCTGCCCCTCCACCCATGTGCTCCTC
        Q  V  L  F  K  G  Q  G  C  P  S  T  H  V  L  L

2001  ACCCACACCATCAGCCGCATCGCCGTCTCCTACCAGACCAAGGTCAACCT
        T  H  T  I  S  R  I  A  V  S  Y  Q  T  K  V  N  L

2051  CCTCTCTGCCATCAAGAGCCCCTGCCAGAGGGAGACCCCAGAGGGGGCTG
        L  S  A  I  K  S  P  C  Q  R  E  T  P  E  G  A  E

2151  AGGCCAAGCCCTGGTATGAGCCCATCTATCTGGGAGGGGTCTTCCAGCTG
        A  K  P  W  Y  E  P  I  Y  L  G  G  V  F  Q  L

2201  GAGAAGGGTGACCGACTCAGCGCTGAGATCAATCGGCCCGACTATCTCGA
        E  K  G  D  R  L  S  A  E  I  N  R  P  D  Y  L  D
                                                      Xba
2251  CTTTGCCGAGTCTGGGCAGGTCTACTTTGGGATCATTGCCCTGTGATCTA
        F  A  E  S  G  Q  V  Y  F  G  I  I  A  L  *

2301  GAAACTAACTAAGCTAGCAACGGTTTCCCTCTAGCGGGATCAATTCCGCC

2351  CCCCCCCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTG

2401  CGTTTGTCTATATGTTATTTTCCACCATATTGCCGTCTTTTGGCAATGTG

2451  AGGGCCCGGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCT

2501  TTCCCCTCTCGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAG

2551  CAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGTCTGTAGCGACCCTT

2601  TGCAGGCAGCGGAACCCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAA

2651  GCCACGTGTATAAGATACACCTGCAAAGGCGGCACAACCCCAGTGCCACG

2701  TTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTCCTCAAGCGTA

2751  TTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCCATTGTATGGGATC

2801  TGATCTGGGGCCTCGGTGCACATGCTTTACGTGTGTTTAGTCGAGGTTAA
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2851 AAAACGTCTAGGCCCCCCGAACCACGGGGACGTGGTTTTCCTTTGAAAAA

2901 CACGATAATACCATGGTTCGACCATTGAACTGCATCGTCGCCGTGTCCCA

2951 AAATATGGGGATTGGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGA

3001 ACGAGTTCAAGTACTTCCAAAGAATGACCACAACCTCTTCAGTGGAAGGT

3051 AAACAGAATCTGGTGATTATGGGTAGGAAAACCTGGTTCTCCATTCCTGA

3101 GAAGAATCGACCTTTAAAGGACAGAATTAATGGTTCGATATAGTTCTCAG

3151 TAGAGAACTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTT

3201 TGGATGATGCCTTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTA

3251 GACATGGTTTGGATAGTCGGAGGCAGTTCTGTTTACCAGGAAGCCATGAA

3301 TCAACCAGGCCACCTCAGACTCTTTGTGACAAGGATCATGCAGGAATTTG

3351 AAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAACTTCTC

3401 CCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAA

3451 GTATAAGTTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCA

3501 AGTTCTCTGCTCCCCTCCTAAAGCTATGCATTTTTATAAGACCATGGGAC

Blunt end SalI/SalI

3551 TTTTGCTGGTCGATCGACCTGGCGTAATAGCGAAGAGGCCCGCACCGATC

3601 GCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGT

3651 AGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGC

3701 TACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCT

3751 TTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTC

3801 CCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACT

3851 TGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTT

3901 TTCGCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCC

3951 AAACTGGAACAACACTCAACCCTATCTCGGTCTATTTATAAGGGATTTTG

4001 CCGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAATTTAAC

4051 GCGAATTTTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGG

4101 GGAAATGTGCGCGGAACCCCTATATTTGTTTATTTTTCTAAATACATTCA

4151 AATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATA

4201 TTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTC

4251 CCTTTTTTGCGGCATTTTGCCTTACTGTTTTTGCTCACCCAGAAACGCTG

4301 GTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACAT

4351 CGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAG

4401 AACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTA

4501 TTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTA

4551 TTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATATTA

4601 CGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGT

4651 GATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGA

4701 GCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATC

4751 GTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACC

4801 ACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGA

4851 ACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGG

4901 ATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTT

4951 ATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGC

5001 AGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGA

5051 CGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATA

5101 GGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATA

5151 TATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGG

5201 TGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTT

5251 TCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATGTTCTTG

5301 AGATCCTTTTTTTCTGCACGTAATCTGCTGCTTGCAAACAAAAAACCACC

5351 GCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTC

5401 CGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTA

5451 GTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTAC

5501 ATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATA

5551 AGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG

5601 CAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCG

5651 AACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCG

5701 CCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGG

5751 GTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTA

5801 TCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTT

5851 TGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCG

5901 GCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTT

5951 TCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGT

6001 GAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTG

6051 AGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCG

6101 TTGGCCGATTCATTAATGCAGGTATCACGAGGCCCTTTCGTCTTCAC