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(54) Titre : ANTICORPS HUMAINS PRESENTANT UNE ACTIVITE DE LIAISON A MN ET DE NEUTRALISATION DE L'ADHESION CELLULAIRE

(54) Title: HUMAN ANTIBODIES THAT HAVE MN BINDING AND CELL ADHESION-NEUTRALIZING ACTIVITY

(57) Abrégé/Abstract:
The invention is composed of monoclonal human MN antibodies or MN antibody fragments that target the GEEDLP repeat within the proteoglycan domain. The proteoglycan domain of the MN cell surface protein contains four of these identical GEEDLP repeats. Binding to the desired epitope is verified by competition ELISA, where ELISA signal can be attenuated by co-incubation with a peptide containing this repeat (PGEEDLPGEEDLP). This inhibition of binding can also be verified using Biacore assays, where binding of desired antibodies to immobilized MN or proteoglycan peptides can be inhibited by the peptide repeat. In addition to binding to the peptide repeat, human anti-MN antibodies can inhibit the cell adhesion of CGL-1 cells to MN coated plastic plates. Human anti-MN antibodies have been used to diagnose and quantify MN expression in cancer cells and tumors using FACS and immunohistochemical methods. An example is also provided where a human anti-MN IgG1 mediates tumor cell lysis though antibody-dependent cell-mediated cytotoxicity. Therefore, these antibodies will be useful for the treatment of cancers in which MN is upregulated or can be useful for the diagnosis of cancers in which MN is upregulated.

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HUMAN ANTIBODIES THAT HAVE MN BINDING AND CELL ADHESION-NEUTRALIZING ACTIVITY

This application claims priority to and incorporates by reference co-pending provisional application Serial Number 60/343657 filed October 18, 2001, and co-pending provisional application Serial Number 60/377716 filed May 3, 2002.

This application incorporates by reference the sequence listing contained on a compact disc, which is part of this application. The sequence listing is a 1.44 MB ASCII file named " Human Antibodies That Have Mn Binding And Cell Adhesion-Neutralizing Activity", created on October 3, 2002

FIELD OF THE INVENTION

This invention relates to MN binding human antibodies

BACKGROUND OF THE INVENTION

MN is a cell surface protein that is detected in a number of clinical carcinoma samples but is absent in the normal tissue of the corresponding organs. The MN cDNA has been cloned (Pastorek, J. et al, *Oncogene* (1994), 9, 2877-2888) and the predicted protein consists of a signal peptide, a proteoglycan-related sequence, a carbonic anhydrase domain, a transmembrane segment, and a short intracellular tail. MN is normally expressed in stomach and bile duct mucosa (Liao, S.Y., et al, *Am J Pathol* (1994), 145, 598-609) and in highly proliferative normal cells located in the small intestine (Saarnio, J. et al, *J Histochem Cytochem* (1998) 46, 497-504). However, MN is ectopically expressed in 100% renal cell carcinomas (Liao, S.Y., *Cancer Res* (1997) 57, 2827-2831), 100% of carcinomas of the esophagus (Turner, J.R. *Hum Pathol*, (1997) 28, 740-744), greater than 90% of cervical carcinomas (Liao, S.Y., et al, *Am J Pathol* (1994), 145, 598-609), 76% of malignant colon carcinomas, (Saarnio, J. et al, *Am J Pathol* (1998) 153, 279-285), 80% of non-small cell lung carcinomas (Vermylen, P. et al, *Eur Respir J* (1999), 14, 806-811), and in 48% of breast cancers (Chia, S. K. et al, *J. Clin. Oncol.* (2001) 19, 3660-3668).

Antibodies against MN have been described. Mouse monoclonal antibody G250 is effective in the reduction of renal cell carcinoma tumor size in a mouse model (van Dijk, J. et al, *Int. J. Cancer* (1994) 56, 262-268). This antibody was

subsequently made into a chimeric antibody containing human Fc regions and the mouse variable regions. The chimeric G250 antibody is only 66% human, leading to a greater chance of immunogenicity in humans compared to a comparable fully human antibody. Therefore, treatment with the 33% mouse antibody may lead to a human anti-mouse immunogenic response, rendering the anti-cancer treatment ineffective. These problems with chimeric antibodies clearly raise the need for fully human antibodies against MN.

BRIEF SUMMARY OF THE INVENTION

The invention is composed of monoclonal human MN antibodies or MN antibody fragments that target the GEEDLP repeat within the proteoglycan domain. The proteoglycan domain of the MN cell surface protein contains four of these identical GEEDLP repeats. Binding to the desired epitope is verified by competition ELISA, where ELISA signal can be attenuated by co-incubation with a peptide containing this repeat (PGEEDLPGEEDLP). This inhibition of binding can also be verified using Biacore assays, where binding of desired antibodies to immobilized MN or proteoglycan peptides can be inhibited by the peptide repeat. In addition to binding to the peptide repeat, human anti-MN antibodies can inhibit the cell adhesion of CGL-1 cells to MN coated plastic plates. Human anti-MN antibodies have been used to diagnose and quantify MN expression in cancer cells and tumors using FACS and immunohistochemical methods. An example is also provided where a human anti-MN IgG1 mediates tumor cell lysis though antibody-dependent cell-mediated cytotoxicity. Therefore, these antibodies will be useful for the treatment of cancers in which MN is upregulated or can be useful for the diagnosis of cancers in which MN is upregulated.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 PC3mm2 human prostate cancer cells express MN as assayed by FACS.

Figure 2 Sequence identifications for SEQ ID #1 through SEQ ID #83

Figure 3 Fab display vector pMORPH18 Fab 1

Figure 4 Vector map of pMORPHx9_Fab1_FS

Figure 5 Images of Blocking of Cell Adhesion with Anti-MN Antibody MN-3

Figure 6 Antibody binding pairs for MN antibodies 1 through 39. BIAcore binding affinity is displayed.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides human antibodies that bind to MN. These antibodies are useful for a variety of therapeutic and diagnostic purposes. These purposes include:

Characteristics of Human MN Antibodies

"Antibody" as used herein includes intact immunoglobulin molecules (*e.g.*, IgG1, IgG2a, IgG2b, IgG3, IgM, IgD, IgE, IgA), as well as fragments thereof, such as Fab, $F(ab')_2$, scFv, and Fv, which are capable of specific binding to an epitope of a human MN protein. Antibodies that specifically bind to MN provide a detection signal at least 5-,10-, or 20-fold higher than a detection signal provided with other proteins when used in an immunochemical assay. Preferably, antibodies that specifically bind to human MN do not detect other proteins in immunochemical assays and can immunoprecipitate the MN from solution.

References to VL2 and/or VL3 in this specification are intended to denote the lambda (λ) class of light chain.

The K_d of human antibody binding to MN can be assayed using any method known in the art, including technologies such as real-time Bimolecular Interaction Analysis (BIA) (Sjolander & Urbaniczky, *Anal. Chem. 63*, 2338-2345, 1991, and Szabo *et al.*, *Curr. Opin. Struct. Biol. 5*, 699-705, 1995). BIA is a technology for studying biospecific interactions in real time, without labeling any of the interactants (*e.g.*, BIAcore™). Changes in the optical phenomenon surface plasmon resonance (SPR) can be used as an indication of real-time reactions between biological molecules.

In a *BIAcore*™ assay, human antibodies of the present invention specifically bind to human MN with a K_d in the range from about 0.6 nM (6×10^{-10} nM) to about 1800 nM (1.8×10^{-6} nM) see Figure 6. More preferred human antibodies of the present invention specifically bind to human MN with a K_d of approximately 0.6 nM to about 90 nM, with the most preferred antibodies of this invention binding human MN protein with a K_d of approximately 4nM.

Preferably, antibodies of this invention as envisioned will bind to the GEEDLP repeat within the proteoglycan domain, which contains four of these identical repeats. Binding to the desired epitope can be verified using any method known in the art, including techniques like competition ELISA (Zavada et al, *Br. J. of Cancer 82, 1808-1813,* 2000), where ELISA signal can be attenuated by co-incubation with a peptide containing this repeat (PGEEDLPGEEDLP), but not inhibited by a similar peptide (PSEEDSPREEDP), which is also within the proteoglycan domain. This pattern of binding inhibition also can be verified using BIAcore™ technologies, where binding of desired antibodies to immobilized MN or proteoglycan peptides can be inhibited by incubation with the peptide repeat. Preferably antibodies of this invention also can inhibit the cell adhesion of MN expressing cells to MN coated plastic plates ELISA (Zavada et al, *Br. J. of Cancer 82, 1808-1813,* 2000).

This invention uses Morphosys phage-antibody technology to generate fully human antibodies against the MN protein. The Morphosys library is based upon human backbones, greatly reducing the probability of immunogenicity.

A number of human antibodies having the MN binding and cell adhesion neutralizing characteristics described above have been identified by screening the MorphoSys HuCAL Fab library. The CDR cassettes assembled for the HuCAL library were designed to achieve a length distribution ranging from 5 to 28 amino acid residues, covering the stretch from position 95 to 102. Knappik *et al.*, *J. Mol. Biol. 296*, 57-86, 2000. A number of human antibodies having the MN binding and cell adhesion neutralizing characteristics described above have been identified by screening the MorphoSys HuCAL Fab library. The CDR cassettes assembled for the HuCAL library were designed to achieve a length distribution ranging from 5 to 28 amino acid residues, covering the stretch from position 95 to 102. Knappik *et al.*, *J. Mol. Biol. 296*, 57-86, 2000. In some embodiments of the invention, the VH3-CDR3 region of a human antibody has an amino acid sequence shown in Figure 2 in SEQ ID NOS: 61-80. In other embodiments of the invention, the VLλ1-CDR3, VLλ2-CDR3, and VLλ2-CDR1 regions of a human MN antibody has amino acid sequences as shown shown in Figure 2 in SEQ ID NOS: 81-89 with optimized VH3-CDR1

sequences as shown in SEQ ID NOS: 48-60, both are shown in Figure 2. Human antibodies that have MN binding and cell adhesion-neutralizing activity are as shown in Tables 1 and 2; the variable regions within these antibodies (the CDR3 loops) are shown in Tables 1 & 2.

Obtaining human antibodies

Human antibodies with the MN binding and cell adhesion-neutralizing activity described above can be identified from the MorphoSys HuCAL library as follows. Human MN is coated on a microtiter plate and incubated with the MorphoSys HuCAL-Fab phage library (see: Example 1). Those phage-linked Fabs not binding to MN can be washed away from the plate, leaving only phage that tightly bind to MN. The bound phage can be eluted by a change in pH and amplified by infection of *E. coli* hosts. This panning process can be repeated once or twice to enrich for a population of phage-linked antibodies that tightly bind to MN. The Fabs from the enriched pool are then expressed, purified, and screened in an ELISA assay. The identified hits are then tested for binding using a BIAcore™ assay, and these hits can be further screened in the cell adhesion assay as described above.

The initial panning of the HuCAL-Fab library also can be performed with MN as the antigen in round one, followed in round 2 by MN peptides fused to carrier proteins, such as BSA or transferrin, and in round 3 by MN antigen again. Human MN peptides that can be used for panning include human MN SEQ I.D. 45-47. These peptide sequences are derived from the MN proteoglycan sequence, which are thought to be involved in cell adhesion.

Alternatively, panning could be performed using MN expressing cells as antigen. For example, cells transfected with MN antigen can be labeled with biotin. These transfected cells are then mixed with unlabled, non-MN transfected cells at a labeled: unlabeled ratio of 1:10. The phage library is added to the cells, and the biotinylated, MN-bearing cells are captured with streptavidin-bound magnetic beads that are bound to a magnet. Non-specific phage are washed away, and the MN-bearing cells are specifically eluted by removing the magnetic field. These specifically bound phage can be amplifed for further rounds of cell panning or can be alternated with peptide and/or protein panning.

Details of the screening process are described in the specific examples, below. Other selection methods for highly active specific antibodies or antibody fragments can be envisioned by those skilled in the art and used to identify human MN antibodies.

Human antibodies with the characteristics described above also can be purified from any cell that expresses the antibodies, including host cells that have been transfected with antibody-encoding expression constructs. The host cells are cultured under conditions whereby the human antibodies are expressed. A purified human antibody is separated from other compounds that normally associate with the antibody in the cell, such as certain proteins, carbohydrates, or lipids, using methods well known in the art. Such methods include, but are not limited to, size exclusion chromatography, ammonium sulfate fractionation, ion exchange chromatography, affinity chromatography, and preparative gel electrophoresis. A preparation of purified human antibodies is at least 80% pure; preferably, the preparations are 90%, 95%, or 99% pure. Purity of the preparations can be assessed by any means known in the art, such as SDS-polyacrylamide gel electrophoresis. A preparation of purified human antibodies of the invention can contain more than one type of human antibody with the MN binding and neutralizing characteristics described above.

Alternatively, human antibodies can be produced using chemical methods to synthesize its amino acid sequence, such as by direct peptide synthesis using solid-phase techniques (Merrifield, *J. Am. Chem. Soc. 85*, 2149-2154, 1963; Roberge *et al.*, *Science 269*, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Optionally, fragments of human antibodies can be separately synthesized and combined using chemical methods to produce a full-length molecule.

The newly synthesized molecules can be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic polypeptide can be confirmed by amino acid analysis or sequencing (*e.g.*, using Edman degradation).

Assessment of therapeutic utility of human antibodies

To assess the ability of a particular antibody to be therapeutically useful to treat cancer, as an example, the antibody can be tested *in vivo* in a mouse xenograft tumor model. If desired, human Fab MN antibodies can be converted into IgG_1 antibodies before therapeutic assessment. This conversion is described in Example 5, and an example of a therapeutic model is detailed in Example 9. Utility also can be tested using an antibody dependent cell-mediated cytotoxicity assay as described in Example 13.

Polynucleotides encoding human MN antibodies

The invention also provides polynucleotides encoding human MN antibodies. These polynucleotides can be used, for example, to produce quantities of the antibodies for therapeutic or diagnostic use.

Polynucleotides that can be used to encode the VH-CDR3 regions shown in SEQ ID NOS: 14-33. Polynucleotides that can be used to encode the VL-CDR3 regions shown are shown in SEQ ID NOS: 34-44. Polynucleotides that encode heavy chains and light chains of human antibodies of the invention that have been isolated from the MorphoSys HuCAL library are shown in Figure 2. Additional optimized VH3-CDR1 sequences are shown in SEQ ID NOS: 1-13.

Polynucleotides of the invention present in a host cell can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, or synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or by using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated polynucleotides encoding antibodies of the invention. For example, restriction enzymes and probes can be used to isolate polynucleotides which encode the antibodies. Isolated polynucleotides are in preparations that are free or at least 70, 80, or 90% free of other molecules.

Human antibody-encoding cDNA molecules of the invention can be made with standard molecular biology techniques, using mRNA as a template. Thereafter, cDNA molecules can be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.* (1989). An amplification technique, such as PCR, can be used to obtain additional copies of the polynucleotides.

Alternatively, synthetic chemistry techniques can be used to synthesize polynucleotides encoding antibodies of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized that will encode an antibody having, for example one of the VH-CDR3, VH-CDR1 or VL-CDR3, light chain or heavy chain amino acid sequences shown in SEQ ID NOS: 48-89 respectively.

Expression of polynucleotides

To express a polynucleotide encoding a human antibody of the invention, the polynucleotide can be inserted into an expression vector that contains the necessary

elements for the transcription and translation of the inserted coding sequence. Methods that are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding human antibodies and appropriate transcriptional and translational control elements. These methods include *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. Such techniques are described, for example, in Sambrook *et al.* (1989) and in Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1995. See also Examples 1-3, below.

A variety of expression vector/host systems can be utilized to contain and express sequences encoding a human antibody of the invention. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (*e.g.*, baculovirus), plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids), or animal cell systems.

The control elements or regulatory sequences are those non-translated regions of the vector -- enhancers, promoters, 5′ and 3′ untranslated regions -- which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (*e.g.*, heat shock, RUBISCO, and storage protein genes) or from plant viruses (*e.g.*, viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a human antibody, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Pharmaceutical compositions

Any of the human MN antibodies described above can be provided in a pharmaceutical composition comprising a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier preferably is non-pyrogenic. The compositions

can be administered alone or in combination with at least one other agent, such as stabilizing compound, which can be administered in any sterile, biocompatible pharmaceutical carrier, including, but not limited to, saline, buffered saline, dextrose, and water. A variety of aqueous carriers may be employed, *e.g.*, 0.4% saline, 0.3% glycine, and the like. These solutions are sterile and generally free of particulate matter. These solutions may be sterilized by conventional, well known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, etc. The concentration of the antibody of the invention in such pharmaceutical formulation can vary widely, *i.e.*, from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., according to the particular mode of administration selected. See U.S. Patent 5,851,525. If desired, more than one type of human antibody, for example with different K_d for MN binding, can be included in a pharmaceutical composition.

The compositions can be administered to a patient alone, or in combination with other agents, drugs or hormones. In addition to the active ingredients, these pharmaceutical compositions can contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that facilitate processing of the active compounds into preparations which can be used pharmaceutically. Pharmaceutical compositions of the invention can be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, parenteral, topical, sublingual, or rectal means.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. Such labeling would include amount, frequency, and method of administration.

Diagnostic methods

The invention also provides diagnostic methods, with which human MN can be detected in a test preparation, including without limitation a sample of serum, lung, liver, heart, breast, kidney, colon, a cell culture system, or a cell-free system (*e.g.*, a tissue homogenate). Such diagnostic methods can be used, for example, to diagnose disorders in which MN is elevated. Such disorders include, but are not limited to carcinomas of the kidney, esophagus, breast, cervix, colon, and lung. When used for diagnosis, detection of an amount of the antibody-MN complex in a test sample from a patient which is greater than an amount of the complex in a

normal sample identifies the patient as likely to have the disorder. An immunohistochemical method for the detection of MN in cancer tissues is described in Example 12.

The test preparation is contacted with a human antibody of the invention, and the test preparation is then assayed for the presence of an antibody-MN complex. If desired, the human antibody can comprise a detectable label, such as a fluorescent, radioisotopic, chemiluminescent, or enzymatic label, such as horseradish peroxidase, alkaline phosphatase, or luciferase. A fluorescence-based assay for the detection of MN expressing tumor cells is shown in Example 11.

Optionally, the antibody can be bound to a solid support, which can accommodate automation of the assay. Suitable solid supports include, but are not limited to, glass or plastic slides, tissue culture plates, microtiter wells, tubes, silicon chips, or particles such as beads (including, but not limited to, latex, polystyrene, or glass beads). Any method known in the art can be used to attach the antibody to the solid support, including use of covalent and non-covalent linkages, passive absorption, or pairs of binding moieties attached to the antibody and the solid support. Binding of MN and the antibody can be accomplished in any vessel suitable for containing the reactants. Examples of such vessels include microtiter plates, test tubes, and microcentrifuge tubes.

Therapeutic methods

The invention also provides methods of ameliorating symptoms of a disorder in which MN is elevated. These disorders include, without limitation, carcinomas of the kidney, esophagus, breast, cervix, colon, and lung. *See, e.g.*, (Liao, S.Y., *Cancer Res* (1997) 57, 2827-2831), (Turner, J.R. *Hum Pathol*, (1997) 28, 740-744), (Liao, S.Y., et al, *Am J Pathol* (1994), 145, 598-609), (Saarnio, J. et al, *Am J Pathol* (1998) 153, 279-285), and (Vermylen, P. et al, *Eur Respir J* (1999), 14, 806-811).

In one embodiment of the invention, a therapeutically effective dose of a human antibody of the invention is administered to a patient having a disorder in which MN is elevated, such as those cancers described above.

Determination of a Therapeutically Effective Dose

The determination of a therapeutically effective dose is well within the capability of those skilled in the art. A therapeutically effective dose refers to the amount of human antibody that is used to effectively treat a cancer compared with the efficacy that is evident in the absence of the therapeutically effective dose.

The therapeutically effective dose can be estimated initially in animal models, usually rats, mice, rabbits, dogs, or pigs. The animal model also can be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. A subcutaneous mouse xenograft model is described in Example 9.

Therapeutic efficacy and toxicity, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population) of a human antibody, can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}.

Pharmaceutical compositions that exhibit large therapeutic indices are preferred. The data obtained from animal studies is used in formulating a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the patient who requires treatment. Dosage and administration are adjusted to provide sufficient levels of the human antibody or to maintain the desired effect. Factors that can be taken into account include the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long-acting pharmaceutical compositions can be administered every 3 to 4 days, every week, or once every two weeks depending on the half-life and clearance rate of the particular formulation.

Polynucleotides encoding human antibodies of the invention can be constructed and introduced into a cell either *ex vivo* or *in vivo* using well-established techniques including, but not limited to, transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated cellular fusion, intracellular transportation of DNA-coated latex beads, protoplast fusion, viral infection, electroporation, "gene gun," and DEAE- or calcium phosphate-mediated transfection.

Effective *in vivo* dosages of an antibody are in the range of about 5 μg to about 50 μg/kg, about 50 μg to about 5 mg/kg, about 100 μg to about 500 μg/kg of patient body weight, and about 200 to about 250 μg/kg of patient body weight. For administration of polynucleotides encoding the antibodies, effective *in vivo* dosages

are in the range of about 100 ng to about 200 ng, 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA.

The mode of administration of human antibody-containing pharmaceutical compositions of the invention can be any suitable route which delivers the antibody to the host. Pharmaceutical compositions of the invention are particularly useful for parenteral administration, *i.e.*, subcutaneous, intramuscular, intravenous, or intranasal administration.

All patents and patent applications cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples, which are provided for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLE 1

Construction of a Human Combinatorial Antibody Library (HuCAL-Fab 1)

Cloning of HuCAL-Fab 1. HuCAL-Fab 1 is a fully synthetic, modular human antibody library in the Fab antibody fragment format. HuCAL-Fab 1 was assembled starting from an antibody library in the single-chain format (HuCAL-scFv; Knappik *et al.*, *J. Mol. Biol. 296* (2000) 55). HuCAL-Fab 1 was cloned into a phagemid expression vector pMORPH18 Fab1 (FIG. 3). This vector comprises the Fd fragment with a phoA signal sequence fused at the C-terminus to a truncated gene III protein of filamentous phage, and further comprises the light chain VL-CL with an ompA signal sequence. Both chains are under the control of the lac operon. The constant domains Cλ, Ck, and CH are synthetic genes fully compatible with the modular system of HuCAL (Knappik *et al.*, 2000).

First, the Vλ and Vκ libraries were isolated from HuCAL-scFv by restriction digest using EcoRV/DraIII and EcoRV/BsiWI, respectively. These Vλ and Vκ libraries were cloned into pMORPH18 Fab1 cut with EcoRV/DraIII and EcoRV/BsiWI, respectively. After ligation and transformation in *E. coli* TG-1, library sizes of 4.14 x 10^8 and 1.6 x 10^8, respectively, were obtained, in both cases exceeding the VL diversity of HuCAL-scFv.

Similarly, the VH library was isolated from HuCAL-scFv by restriction digest using StyI/MunI. This VH library was cloned into the pMORPH18-Vλ and Vκ libraries cut with StyI/MunI. After ligation and transformation in *E. coli* TG-1, a total library size

of 2.09 x 10^{10} was obtained, with 67% correct clones (as identified by sequencing of 207 clones).

Phagemid rescue, phage amplification and purification. HuCAL-Fab was amplified in 2 x TY medium containing 34 µg/ml chloramphenicol and 1 % glucose (2 x TY-CG). After helper phage infection (VCSM13) at 37°C at an OD600 of about 0.5, centrifugation and resuspension in 2 x TY / 34 µg/ml chloramphenicol/ 50 µg/ml kanamycin, cells were grown overnight at 30°C. Phage were PEG-precipitated from the supernatant (Ausubel *et al.*, 1998), resuspended in PBS/20% glycerol, and stored at –80°C. Phage amplification between two panning rounds was conducted as follows: mid-log phase TG1-cells were infected with eluted phage and plated onto LB-agar supplemented with 1% of glucose and 34 µg/ml of chloramphenicol. After overnight incubation at 30°C, colonies were scraped off and adjusted to an OD600 of 0.5. Helper phage were added as described above.

EXAMPLE 2

Solid phase panning

Wells of MaxiSorp™ microtiter plates (Nunc) were coated with human MN protein in PBS (2 µg/well). After blocking with 5% non-fat dried milk in PBS, 1–5 x 1012 HuCAL-Fab phage purified as above were added for 1h at 20°C. After several washing steps, bound phage were eluted by pH-elution with 100 mM triethylamine and subsequent neutralization with 1M TRIS-Cl pH 7.0. Three rounds of panning were performed with phage amplification conducted between each round as described above.

EXAMPLE 3

Subcloning of selected Fab fragments for expression

The Fab-encoding inserts of the selected HuCAL Fab fragments were subcloned into the expression vector pMORPHx7_FS to facilitate rapid expression of soluble Fab. The DNA preparation of the selected HuCAL Fab clones was digested with *XbaI / EcoRI*, thus cutting out the Fab encoding insert (ompA-VL and phoA-Fd). Subcloning of the purified inserts into the *XbaI / EcoRI* cut vector pMORPHx7, previously carrying a scFv insert, leads to a Fab expression vector designated pMORPHx9_Fab1_FS (FIG. 4). Fabs expressed in this vector carry two C-terminal tags (FLAG and Strep) for detection and purification.

EXAMPLE 4

Identification of MN-binding Fab fragments by ELISA

The wells of a Maxisorp ELISA plates were coated with 100 µl/well solutions of human MN at a concentration of 5 µg/ml diluted in coating buffer. Expression of individual Fab was induced with 0.5 mM IPTG for 12 h at 30°C. Soluble Fab was extracted from the periplasm by osmotic shock (Ausubel *et al.*, 1998) and used in an ELISA. The Fab fragment was detected with an anti-Fab antibody (Dianova). Values at 370 nm were read out after addition of horseradish peroxidase-conjugated anti-mouse IgG antibody and POD soluble substrate (Roche Diagnostics).

EXAMPLE 5

Construction of HuCAL immunoglobulin expression vectors

Heavy chain cloning. The multiple cloning site of pcDNA3.1+ (Invitrogen) was removed (NheI / ApaI), and a stuffer compatible with the restriction sites used for HuCAL design was inserted for the ligation of the leader sequences (NheI / EcoRI), VH-domains (EcoRI / BlpI), and the immunoglobulin constant regions (BlpI / ApaI). The leader sequence (EMBL M83133) was equipped with a Kozak sequence (Kozak, 1987). The constant regions of human IgG1 (PIR J00228), IgG4 (EMBL K01316), and serum IgA1 (EMBL J00220) were dissected into overlapping oligonucleotides with lengths of about 70 bases. Silent mutations were introduced to remove restriction sites non-compatible with the HuCAL design. The oligonucleotides were spliced by overlap extension-PCR.

Light chain cloning. The multiple cloning site of pcDNA3.1/Zeo+ (Invitrogen) was replaced by two different stuffers. The κ-stuffer provided restriction sites for insertion of a κ-leader (NheI / EcoRV), HuCAL-scFv Vk-domains (EcoRV / BsiWI,) and the κ-chain constant region (BsiWI / ApaI). The corresponding restriction sites in the λ-stuffer were NheI / EcoRV (l-leader), EcoRV / HpaI (Vl- domains), and HpaI / ApaI (λ-chain constant region). The κ-leader (EMBL Z00022) as well as the λ-leader (EMBL L27692) were both equipped with Kozak sequences. The constant regions of the human κ- (EMBL J00241) and λ-chain (EMBL M18645) were assembled by overlap extension-PCR as described above.

Generation of IgG-expressing CHO-cells. CHO-K1 cells were co-transfected with an equimolar mixture of IgG heavy and light chain expression vectors. Double-

resistant transfectants were selected with 600 µg/ml G418 and 300 µg/ml Zeocin (Invitrogen) followed by limiting dilution. The supernatant of single clones was assessed for IgG expression by capture-ELISA (see below). Positive clones were expanded in RPMI-1640 medium supplemented with 10% ultra-low IgG-FCS (Life Technologies). After adjusting the pH of the supernatant to 8.0 and sterile filtration, the solution was subjected to standard protein A column chromatography (Poros 20 A, PE Biosystems).

EXAMPLE 6

Design of the CDR3 libraries

Vλ positions 1 and 2. The original HuCAL master genes were constructed with their authentic N-termini: VLλ1: QS (CAGAGC), VLλ2: QS (CAGAGC), and VLλ3: SY (AGCTAT). Sequences containing these amino acids are shown in WO 97/08320. During HuCAL library construction, the first two amino acids were changed to DI to facilitate library cloning (*Eco*RI site). All HuCAL libraries contain VLλ genes with the *Eco*RV site GATATC (DI) at the 5'-end. All HuCAL kappa genes (master genes and all genes in the library) contain DI at the 5'-end.

VH position 1. The original HuCAL master genes were constructed with their authentic N-termini: VH1A, VH1B, VH2, VH4, and VH6 with Q (=CAG) as the first amino acid and VH3 and VH5 with E (=GAA) as the first amino acid. Sequences containing these amino acids are shown in WO 97/08320. In the HuCAL Fab 1 library, all VH chains contain Q (=CAG) at the first position.

Vκ1/Vκ3 position 85. Because of the cassette mutagenesis procedure used to introduce the CDR3 library (Knappik *et al.*, *J. Mol. Biol. 296*, 57-86, 2000), position 85 of Vκ1 and Vκ3 can be either T or V. Thus, during HuCAL scFv 1 library construction, position 85 of Vκ1 and Vκ3 was varied as follows: V*k*1 original, 85T (codon ACC); Vκ1 library, 85T or 85V (TRIM codons ACT or GTT); Vκ3 original, 85V (codon GTG); Vκ3 library, 85T or 85V (TRIM codons ACT or GTT); the same applies to HuCAL Fab1.

CDR3 design. All CDR3 residues which were kept constant are indicated in Tables 1 & 2.

CDR3 length. The designed CDR3 length distribution is as follows. Residues which were varied are shown in the Sequence Listing as shown in Figure 2. V kappa CDR3, 8 amino acid residues (position 89 to 96) (occasionally 7 residues), with Q90 fixed; V lambda CDR3, 8 to 10 amino acid residues (position 89 to 96) (occasionally

7-10 residues), with Q89, S90, and D92 fixed; and VH CDR3, 5 to 28 amino acid residues (position 95 to 102) (occasionally 4-28), with D101 fixed.

EXAMPLE 7

Competition ELISA for Epitope Mapping

Nunc Maxisorb microtiter plates were coated overnight at 4 °C with 100 µL of MN or MN-peptide-coupled BSA in PBS at a concentration of 5 µg/mL. Each well is blocked with 5% non-fat milk in PBS for 2 hours at RT on a microtiter plate shaker. The plate is washed with PBS with 0.05% Tween-20. 200 µL per well of antibody or antibody + proteoglycan peptide A, B, or C (SEQ ID 20 - 22) is added to the well. Antibody and proteoglycan peptide concentrations were optimized to yield greatest ease in determining the 50% end point. These antibody/peptide mixtures were incubated for 1.5 hours at RT on a microtiter plate shaker. The ELISA plates are washed 5x quickly with TBS containing 0.05% Tween-20. Bound antibody was tested using peroxidase conjugated goat anti-Fab IgG (Sigma). After further washing with TBS-Tween, 100 µL of BM Blue POD Substrate (Roche) is added. After 30 minutes of incubation, the absorbance is read at 370 nm.

EXAMPLE 8

Cell Adhesion Assay

1 µg/mL of purified MN in 50 mM bicarbonate buffer pH 9.2 was adsorbed in 30 µL drops on the bottom of bacteriological 5 cm Petri dishes for 1.5 hours. The drops were removed and rinsed 3 times with PBS. Subsequently the drops were blocked with 50% fetal calf serum in DMEM. The drops were further treated with 30 mL of 20-100 µg/mL anti-MN IgGs or with PBS and irrelevant antibodies as a control. After washing the drops with PBS, the spots were incubated with 30 µL of CGL-1 cell suspension (10^5 cells/mL) and incubated overnight. The ability of anti-MN antibodies to block adhesion of CGL-1 cells to MN coated plates was assessed after washing the drops with PBS. An example of this experiment in shown in Figure 5 where 20 µg/ml of Anti-MN antibody MN-3 (Figure 5A) inhibits cell adhesion compared to control gamma globulin (Figure 5B) and to no antibody treatment (Figure 5C).

EXAMPLE 9

Subcutaneous Xenograft Cancer Model

Antitumor effects of anti-MN antibodies were evaluated using subcutaneous xenograft models in immunodeficient mice. HT-29 cells were maintained as adherent cultures in DMEM supplemented with 10% FBS. SCID mice of 6-7 weeks age were inoculated subcutaneously in the right flank with 1 x 10^7 cells in 0.1 mL of medium. Monoclonal antibodies were administered i.p. daily at a dose of 500 µg. Control mice were treated with PBS or an irrelevant monoclonal antibody. Tumors were measured twice a week with a sliding caliper. Anti-tumor efficacy was evaluated by comparing the tumor size of anti-MN antibody treatment versus control treatment.

EXAMPLE 10

Subcutaneous Xenograft Cancer Model with Immunoconjugate

Anti-MN antibodies were conjugated to cytotoxic small molecules using protocols that are known in the art (e.g. C. Liu et al., *Proc. Natl. Acad. Sci.* (1996), **93**, 8618-8623.) HT-29 cells were maintained as adherent cultures in DMEM supplemented with 10% FBS. Female CB-17 SCID mice, 6-7 weeks of age were inoculated subcutaneously at the right flank with 1 x 10e7 tumor cells in 0.1 mL of medium. After tumor sizes reach from 65 mm^3, animals were injected daily with 0.5 mg of antibody conjugate for five consecutive days. Control mice were treated with PBS, an irrelevant monoclonal antibody, or free unconjugated drug. Tumors were measured twice a week with a sliding caliper. Anti-tumor efficacy was evaluated by comparing the tumor size of anti-MN antibody treatment versus control treatment.

EXAMPLE 11

Fluorescence-activated cell sorting assay (FACS assay)

Cells can be assayed for MN expression as a diagnostic tool. For adherent cell lines, detach cells from their flask by first removing their culture medium, rinsing them once with ice cold PBS, and treating them with 1mM EDTA in PBS for 5 to 10 min depending on the cell line (encourage by periodically tapping the flask). Spin the cells

down (1500 rpm, 5 min) and wash the cells once with ice cold Staining Buffer (10% FBS, 0.1% sodium azide, PBS). Resuspend the cells in ice-cold Staining Buffer at 1 million cells in 200 ul. Add the primary antibody at 3.2 E-11 to 3.2 E-8 M and incubate on ice for 1 h. Wash the unbound antibody with the ice-cold Staining Buffer. Resuspend the cell pellet in 200 ul of ice cold Staining Buffer and add 20 ul per 200 ul of cells of FITC-conjugated anti-human secondary antibody (Pharmingen). Incubate on ice for 1 h. Wash the unbound antibody and resuspend the cells in 200 ul of 2.5 ug/ml Propidium Iodide (PI) (Sigma) in the Staining Buffer (to gate for dead cells). Proceed with the FACS analysis gating out the cells that take up PI . PC3mm2 human prostate cancer cells express MN as assayed by FACS as shown in Figure 7. The red line represents staining with human anti-MN antibody, while the black line represents a control, isotype-matched human antibody.

Example 12

Immunohistochemical Analysis of Tumor Samples

Tumor sections can be tested for MN expression. Since MN is highly expressed in cancer and low expression levels are present in normal tissue, analyzing MN expression is of utility for the diagnosis and detection of cancer in patient samples. For analysis of tissue sections, standard immunohistochemical techniques can be used. Tissue sections containing a PC-3 prostate carcinoma were implanted in SCID mice. 20 micrograms/mL of anti-MN antibody was incubated with the dewaxed paraffin section and the slide was developed using a peroxidase conjugated secondary antibody, and developed using DAB chromogen. A strong membrane-associated signal was readily observed and is characteristic of high MN expression in the prostate cancer cells.

Example 13

Antibody-dependent cell mediated cytotoxicity assays (ADCC assays)

Anti-tumor activity of anti-MN IgGgs can be mediated by ADCC activity. MN-expressing PC-3mm2 cells and non-MN expressing HCT-116 cells are incubated with 250 ng/mL, 1000 ng/mL or 2000 ng/mL human anti-MN IgG1 or control human

IgG1 anti-digoxin antibody. Human PBMCs are added to these cells at effector: target ratios of 50: 1, 25: 1, and 5: 1 ratios. A chromium-51 release assay is performed to determine the level of target cell lysis. A small amount of lysis is observed upon incubation of control antibody or no antibody in the presence of HCT-116 or PC-3mm2 cells. This spontaneous level of lysis is 10-15% , 5-10%, or 2-3% for 50:1, 25:1, and 5:1 target effector ratios respectively. Similarly, lysis of non-MN expressing HCT-116 cells was in the 0-10% range when incubated with the anti-MN antibodies. However, lysis of PC-3mm2 cells when incubated with the human anti-MN IgGs was significantly higher than the controls. Lysis of 40, 50, and 60% was observed when using 250 ng/mL, 1000 ng/mL and 2000 ng/mL at 50:1 target:effector ratios. Similarly, 30, 33, and 38% lysis was observed at 25:1 ratios, and finally, 8, 10, and 15% lysis was observed at 5:1 target:effector ratios. These experiments show that human anti-MN antibodies mediate anti-tumor ADCC activity and may be used for the therapeutic treatment of cancer.

MSB-7289.txt

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Takeuchi, Toshi

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<213> Homo sapiens

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<210> 83
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<212> DNA
<213> Homo sapiens

<400> 83
ggatttacct ttagcgggag catgatgacg 30

We claim:

1. A purified preparation of a human antibody, wherein the antibody binds MN protein.

2. The purified preparation of Claim 1 wherein the antibody binds to the MN protein's proteoglycan domain.

3. The purified preparation of Claim 1 wherein the antibody binds to a GEEDLP repeat region within the MN protein's proteoglycan domain.

4. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 1800 nM..

5. The preparation of claim 3 wherein the antibody binds to the human MN protein with a K_d of about 0.6 nM to about 90 nM.

6. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 61-80.

7. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 48-60.

8. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 region comprising the amino acid of SEQ ID NO: 64.

9. The purified preparation of Claim 1 wherein the human antibody comprises a VLλ1-CDR3 region comprising the amino acid sequence of SEQ ID NOS: 81.

10. The purified preparation of Claim 1 comprising a VLλ2-CDR1 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 82-83.

11. The purified preparation of Claim 1 wherein the human antibody comprises a VLλ2-CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 84-89.

12. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 84, SEQ ID NOS: 62 and 87, SEQ ID NOS: 63 and 89, SEQ ID NOS: 64 and 84, SEQ ID NOS: 65 and 84, SEQ ID NOS: 66 and 85, SEQ ID NOS: 67 and 88.

13. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 86, SEQ ID NOS: 61 and 85, SEQ ID NOS: 61 and 87, SEQ ID NOS: 61 and 88, SEQ ID NOS: 61 and 89, SEQ ID NOS: 63 and 86, SEQ ID NOS: 63 and 85, SEQ ID NOS: 63 and 87, SEQ ID NOS: 63 and 88, and SEQ ID NOS: 63 and 84.

14. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL2-CDR3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 71 and 87, SEQ ID NOS: 61 and 87, SEQ ID NOS: 72 and 87, SEQ ID NOS: 73 and 87, SEQ ID NOS: 74 and 87, SEQ ID NOS: 75 and 87, SEQ ID NOS: 76 and 87, SEQ ID NOS: 77 and 87, SEQ ID NOS: 78 and 87, SEQ ID NOS: 79 and 87, and SEQ ID NOS: 80 and 87.

15. The purified preparation of Claim 1 wherein the human antibody comprises a VH3-CDR3 and VL1-CDR-3 amino acid sequence pair selected from the group consisting of SEQ ID NOS: 61 and 81, SEQ ID NOS:69 and 81, and SEQ ID NOS:70 and 81.

16. The purified preparation of Claim 1 wherein the human antibody comprises VH3-CDR3, VL2-CDR3, and VH3-CDR1 amino acid sequences selected from the group consisting of SEQ ID NOS: 61 and 86 and 48, SEQ ID NOS: 61 and 86 and 49, SEQ ID NOS: 61 and 86 and 50, SEQ ID NOS: 61 and 86 and 51, SEQ ID NOS: 61 and 86 and 52, SEQ ID NOS: 61 and 86 and 53, SEQ ID NOS: 61 and 86 and 54, SEQ ID NOS: 61 and 86 and 55, SEQ ID NOS: 61 and 86 and 56, and SEQ ID NOS: 61 and 86 and 57.

17. A nucleotide sequence that codes for a purified preparation of a human antibody, wherein the antibody binds MN protein.

18. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 1-13.

19. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VH3-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 14-33.

20. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VLλ1-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 34-36.

21. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VL2-CDR1 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 37-38.

22. The purified preparation of Claim 17 wherein the nucleotide sequence comprises a VLλ2-CDR3 region comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS: 39-44.

23. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR1, VH3-CDR3 and VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 1 and 14 and 41, SEQ ID: 2 and 14 and 41, SEQ ID: 3 and 14 and 41, and SEQ ID: 4 and 14 and 41.

24. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR1, and VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 37 and 41, and SEQ ID: 14 and 38 and 41.

25. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 and VH3-CDR1 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 41 and 1, SEQ ID: 14

and 41 and 2, SEQ ID: 14 and 41 and 3, SEQ ID: 14 and 41 and 4, SEQ ID: 14 and 41 and 5, SEQ ID: 14 and 41 and 6, SEQ ID: 14 and 41 and 7, SEQ ID: 14 and 41 and 8, SEQ ID: 14 and 41 and 9, and SEQ ID: 14 and 41 and 10.

26. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 39, SEQ ID: 15 and 42, SEQ ID: 16 and 44, SEQ ID: 17 and 39, SEQ ID: 18 and 39, SEQ ID: 19 and 40, and SEQ ID: 20 and 43.

27. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL1-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 34 , SEQ ID: 22 and 34, SEQ ID: 22 and 35, SEQ ID: 22 and 36, and SEQ ID: 23 and 34.

28. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 14 and 41, SEQ ID: 14 and 40, SEQ ID: 14 and 42, SEQ ID: 14 and 43, SEQ ID: 14 and 44, SEQ ID: 16 and 41, SEQ ID: 16 and 40, SEQ ID: 16 and 42, SEQ ID: 16 and 43, and SEQ ID: 16 and 39.

29. The purified preparation of Claim 17 wherein the nucleotide sequence comprises VH3-CDR3, VL2-CDR3 nucleotide sequences selected from the group consisting of SEQ ID: 24 and 42, SEQ ID: 14 and 42, SEQ ID: 25 and 42, SEQ ID: 26 and 42, SEQ ID: 27 and 42, SEQ ID: 28, and 42, SEQ ID: 29 and 42, SEQ ID: 30 and 42, SEQ ID: 31 and 42, SEQ ID: 32 and 42 and SEQ ID: 33 and 42.

30. An expression vector comprising the polynucleotide of claim 17, wherein said vector codes for MN binding antibody.

31. An expression vector comprising the polynucleotide of claim 18, wherein said vector codes for MN binding antibody.

32. An expression vector comprising the polynucleotide of claim 19, wherein said vector codes for MN binding antibody.

33. An expression vector comprising the polynucleotide of claim 20, wherein said vector codes for MN binding antibody.

34. An expression vector comprising the polynucleotide of claim 21, wherein said vector codes for MN binding antibody.

35. An expression vector comprising the polynucleotide of claim 22, wherein said vector codes for MN binding antibody.

36. An expression vector comprising the polynucleotide of claim 23, wherein said vector codes for MN binding antibody.

37. An expression vector comprising the polynucleotide of claim 24, wherein said vector codes for MN binding antibody.

38. An expression vector comprising the polynucleotide of claim 25, wherein said vector codes for MN binding antibody.

39. An expression vector comprising the polynucleotide of claim 26, wherein said vector codes for MN binding antibody.

40. An expression vector comprising the polynucleotide of claim 27, wherein said vector codes for MN binding antibody.

41. An expression vector comprising the polynucleotide of claim 28, wherein said vector codes for MN binding antibody.

42. An expression vector comprising the polynucleotide of claim 29, wherein said vector codes for MN binding antibody.

43. A host cell comprising the expression vector of claim 30, wherein said cell expresses MN binding antibody.

44. A host cell comprising the expression vector of claim 31, wherein said cell expresses MN binding antibody.

45. A host cell comprising the expression vector of claim 32, wherein said cell expresses MN binding antibody.

46. A host cell comprising the expression vector of claim 33, wherein said cell expresses MN binding antibody.

47. A host cell comprising the expression vector of claim 34, wherein said cell expresses MN binding antibody.

48. A host cell comprising the expression vector of claim 35, wherein said cell expresses MN binding antibody.

49. A host cell comprising the expression vector of claim 36, wherein said cell expresses MN binding antibody.

50. A host cell comprising the expression vector of claim 37, wherein said cell expresses MN binding antibody.

51. A host cell comprising the expression vector of claim 38, wherein said cell expresses MN binding antibody.

52. A host cell comprising the expression vector of claim 39, wherein said cell expresses MN binding antibody.

53. A host cell comprising the expression vector of claim 40, wherein said cell expresses MN binding antibody.

54. A host cell comprising the expression vector of claim 41, wherein said cell expresses MN binding antibody.

55. A host cell comprising the expression vector of claim 42, wherein said cell expresses MN binding antibody.

56. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 31 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

57. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 32 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

58. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 33 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

59. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 34 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

60. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 35 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

61. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 36 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

62. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 37 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

63. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 38 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

64. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 39 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

65. A method of making a human antibody, comprising the steps of: culturing the host cell of claim 40 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

66. A method of making a human antibody, comprising the steps of:

culturing the host cell of claim 41 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

67. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 42 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

68. A method of making a human antibody, comprising the steps of:
culturing the host cell of claim 43 under conditions whereby the antibody is expressed; and purifying the human antibody from the host cell culture.

69. A method of treating a human disorder in which MN protein is expressed in certain cells, comprising the step of:
a) Providing a human having a condition in which MN protein is expressed in certain cells; and
b) Administering to said human an effective amount of a human MN antibody compound whereby, said compound includes an MN antibody and a cytotoxic agent, wherein said cytotoxic agent is capable of inducing cell death in said MN expressing cells.

70. The method of claim 69 wherein the disorder is selected from the group consisting of renal cell carcinomas, esophagus carcinomas, cervical carcinomas, malignant colon carcinomas, and non-small cell lung carcinomas.

71. The method of claim 69 wherein the antibody comprises a VH3-CDR3 region selected from the group consisting of SEQ ID NOS: 61-80.

72. The method of claim 69 wherein the antibody comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 48-60.

73. The method of claim 69 wherein the antibody comprises a VL1-CDR3 region consisting of SEQ ID NOS: 81.

74. The method of claim 69 wherein the antibody comprises a VL2-CDR1 region selected from the group consisting of SEQ ID NOS: 82-83.

75. The method of claim 69 wherein the antibody comprises a VL2-CDR3 region selected from the group consisting of SEQ ID NOS: 84-89.

76. A method of detecting MN antigen in a test preparation comprising the steps of:

a) contacting the test preparation with an antibody that specifically binds to MN antigen, and

b) assaying the test preparation for the presence of the antibody - MN antigen complex.

77. The method of Claim 76 wherein the antibody comprises a detectable label.

78. The method of Claim 76 wherein the antibody is bound to a solid support.

79. A method to aid in diagnosing a disorder in which MN protein level is elevated, comprising the steps of:

a) contacting a sample from a patient suspected of having the disorder with a human antibody that binds to MN; and

b) assaying for the presence of an antibody-MN complex, whereby detection of an amount of the complex which is greater than an amount of the complex in a normal sample identifies the patient as likely to have the disorder.

80. The method of claim 79 wherein the antibody comprises a detectable label.

81. The method of claim 79 wherein the antibody is bound to a solid support.

82. The method of claim 79 wherein the antibody comprises a VH3-CDR3 region selected from the group consisting of SEQ ID NOS: 61-80.

83. The method of claim 79 wherein the antibody comprises a VH3-CDR1 region selected from the group consisting of SEQ ID NOS: 48-60.

84. The method of claim 79 wherein the antibody comprises a VL1-CDR3 region consisting of SEQ ID NOS: 81.

85. The method of claim 79 wherein the antibody comprises a VL2-CDR1 region selected from the group consisting of SEQ ID NOS: 82-83.

86. The method of claim 79 wherein the antibody comprises a VL2-CDR3 region selected from the group consisting of SEQ ID NOS: 84-89.

87. A pharmaceutical composition comprising a human antibody that binds to MN protein and a pharmaceutically acceptable carrier.

Tables 1 and 2
Remarks

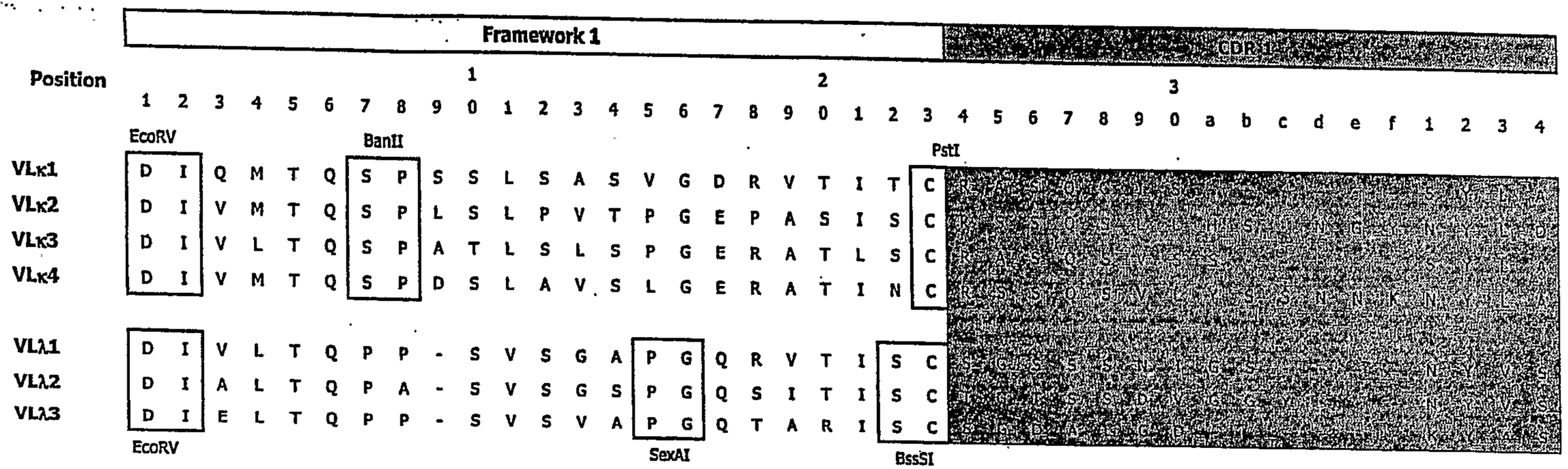
Remarks concerning the HuCAL sequence summary (HuCAL Libraries scFv1, scFv2, scFv3 and Fab1)

1. Numbering:	The numbering is according to VBASE except the gap in Vλ position 9. In VBASE the gap is set at position 10. See also Chothia *et al.* (1992) J. Mol. Biol., **227**, 776-798, Tomlinson *et al.* (1995) EMBO J., **14**, 4628-4638 and Williams *et al.* (1996) J. Mol. Biol., **264**, 220-232).
2. Restriction sites:	The position of the sites are given only approximately in the protein sequence summary. For detailed positions use the DNA sequence summary or the VectorNTI Master Gene sequence files (can be found in the Shared Database).
3. V lambda position 1&2:	The original HuCAL master genes have been constructed with their authentic N-termini: VLλ1: QS (CAGAGC) VLλ2: QS (CAGAGC) VLλ3: SY (AGCTAT) Sequences containing this amino acids can be found i.e. in the patent application and in the PDB structure model files During HuCAL library construction this first two amino acids have been changed to DI to facilitate library cloning (EvoRV site) **All HuCAL libraries contain VLλ genes with the EcoRV site GATATC (DI) at the 5'-end** **All HuCAL kappa genes (master genes and all genes in the library) contain DI at the 5'-end.**
4. VH position 1:	The original HuCAL master genes have been constructed with their authentic N-termini: VH1A, VH1B, VH2, VH4 and VH6 with Q (=CAG) as the first amino acid. VH3 and VH5 with E (=GAA) as the first amino acid. All HuCAL VH chains fused to the short FLAG sequence (DYKD) contain E (=GAA) at the first position (HuCAL libraries scFv1, 2 and 3) **In the HuCAL Fab 1 library all VH chains contain Q (=CAG) at the first position**
5. Vκ1/Vκ3 position 85:	During HuCAL scFv 1 library construction position 85 of Vκ1 and Vκ3 was varied: Vκ1 original : 85T (codon ACC) **Vκ1 library: 85T or 85V (TRIM codons ACT or GTT)** Vκ3 original: 85V (codon GTG) **Vκ3 library: 85T or 85V (TRIM codons ACT or GTT)** **HuCAL scFv2 and 3 as well as HuCAL Fab1 are as HuCAL scFv1 with respect to this position**
6. CDR3 design:	In the sequence summary all CDR3 residues which were kept constant are indicated
7. CDR3 length:	Here is the designed CDR3 length distribution. In the sequence summary the residues which were varied are given in brackets (x) V kappa CDR3: 8 amino acid residues (position 89 to 96) (occasionally 7 residues); Q90 fixed V lambda CDR3: 8 to 10 amino acid residues (position 89 to 96) (occasionally 7-10 residues); Q89, S90, D92 fixed VH CDR3: 5 to 28 amino acid residues (position 95 to 102) (occasionally 4-28); D101 fixed

Table 1

Sequence Summary HuCAL Libraries scFv1, scFv2, scFv3 and Fab1

VL



VH

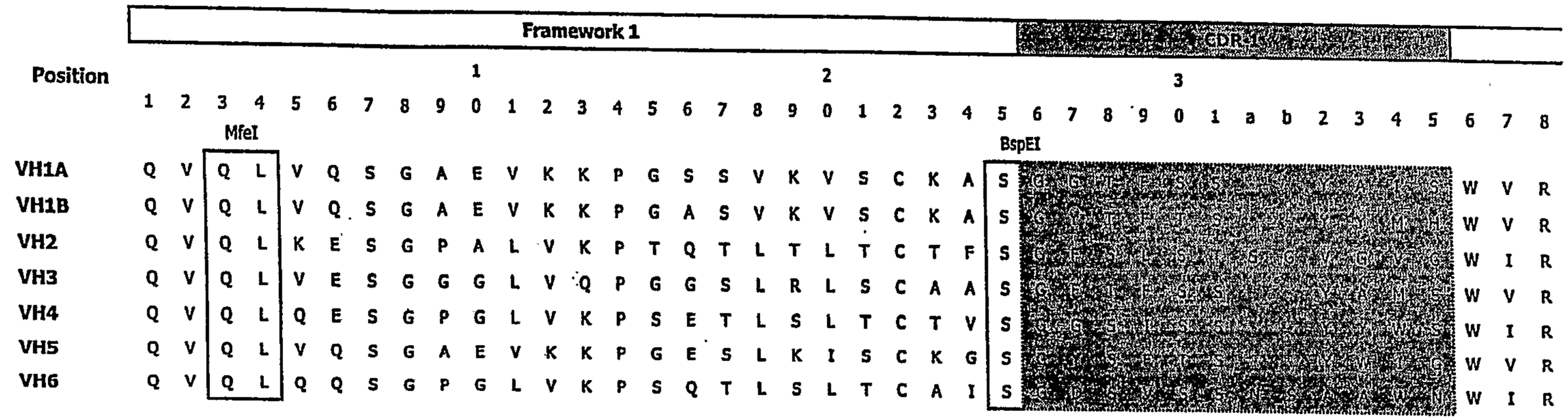


Table 1

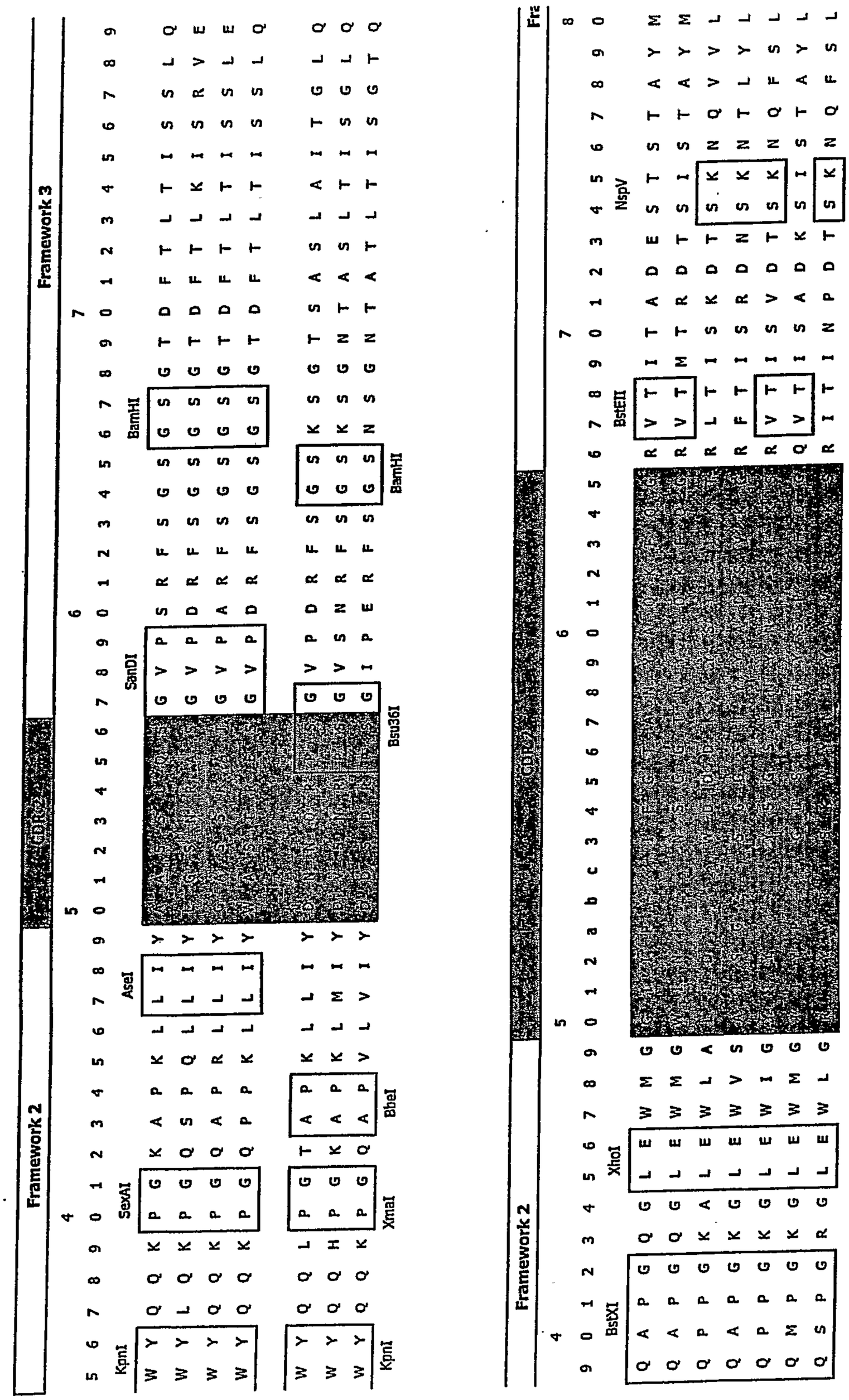

Framework 3
Framework 2
CDR2
BamHI
SanDI
AseI
Bsu36I
BbeI
SexAI
XmaI
KpnI
BstEII
NspV
XhoI
BstXI

Table 1

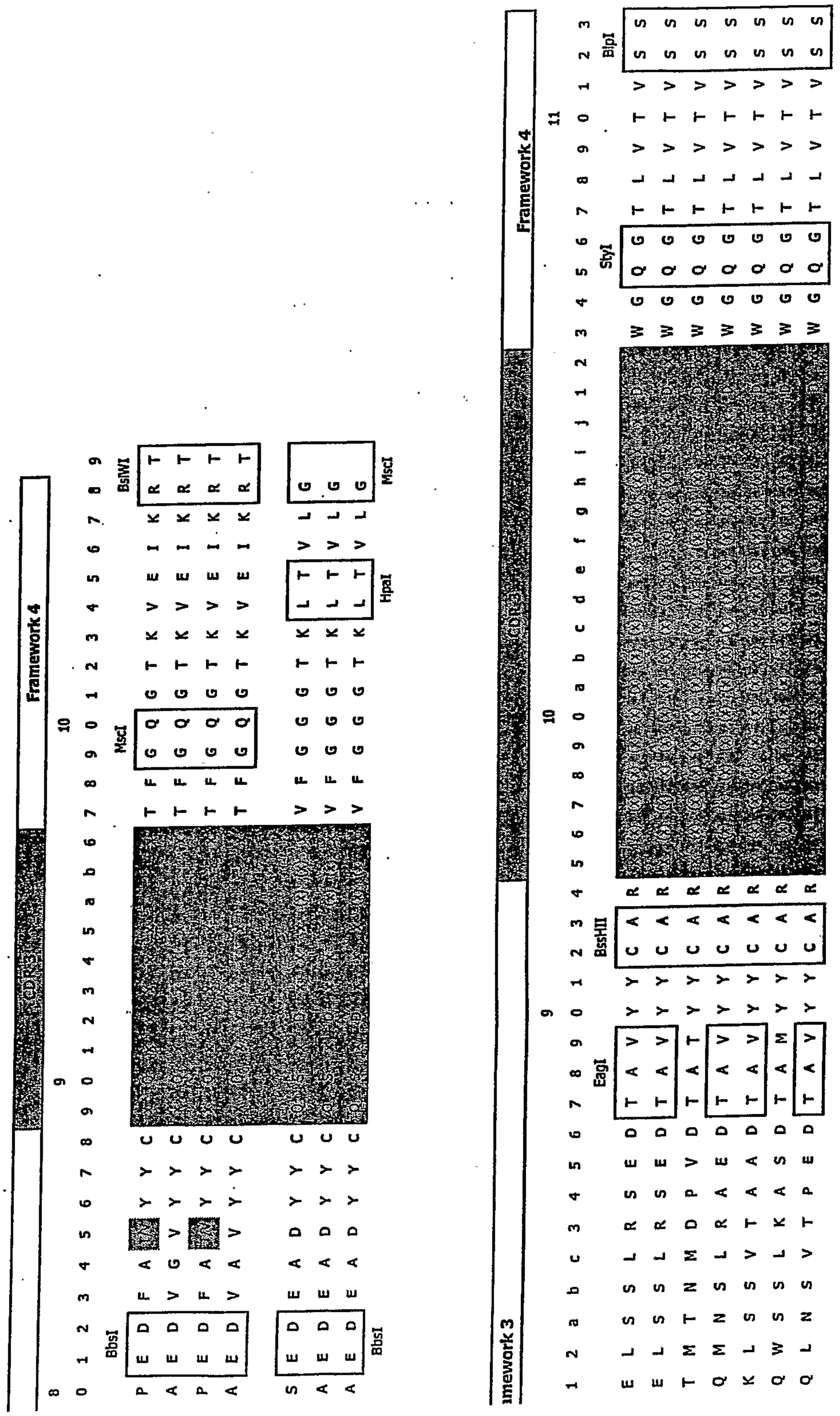
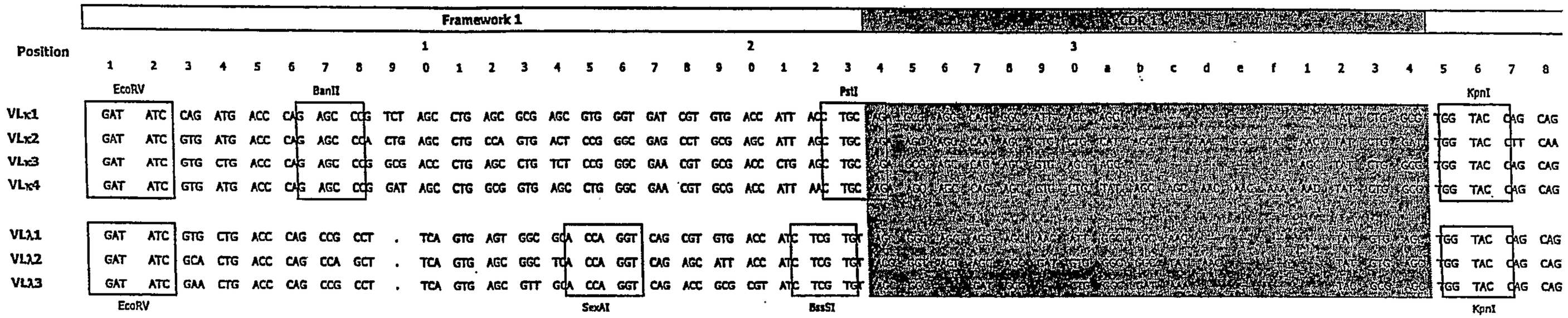


Table 2

Sequence Summary HuCAL Libraries scFv1, scFv2, scFv3 and Fab1

VL



VH

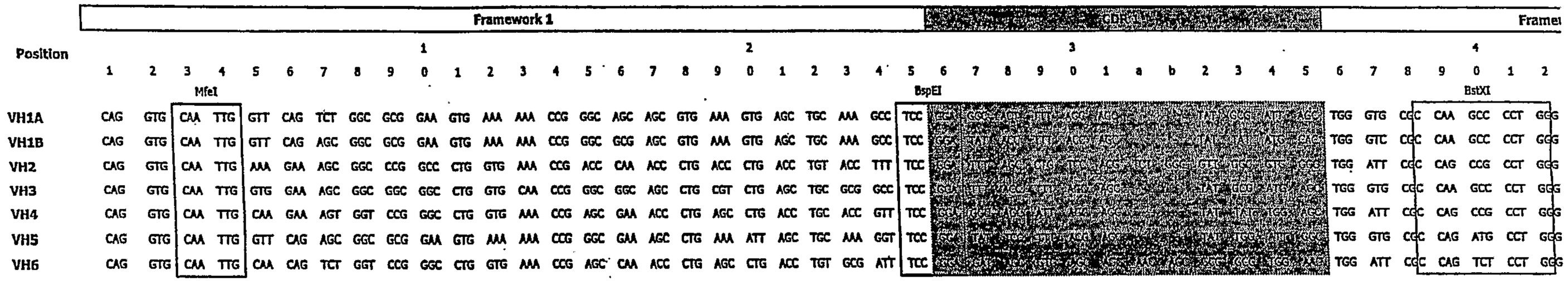


Table 2

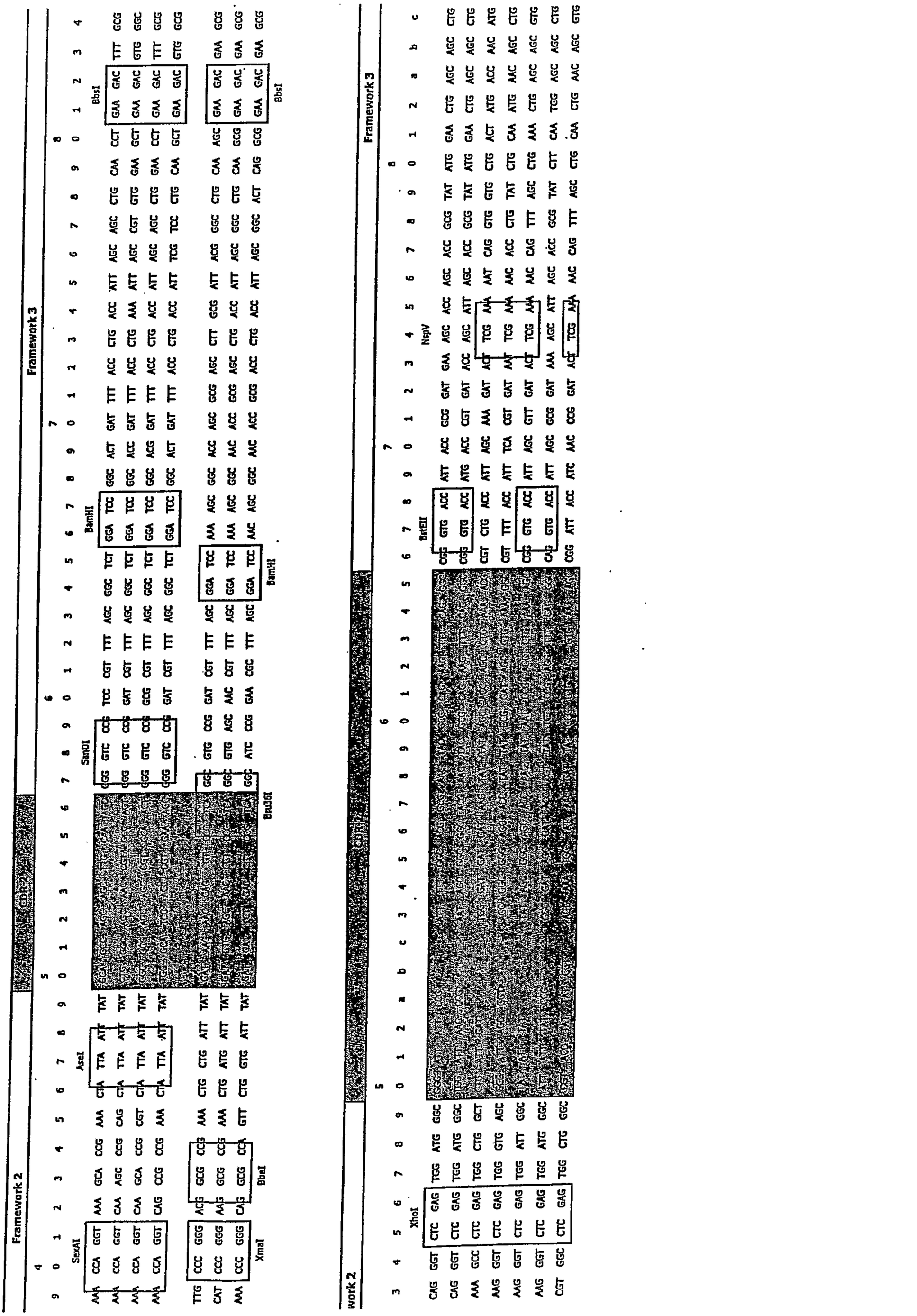


Table 2

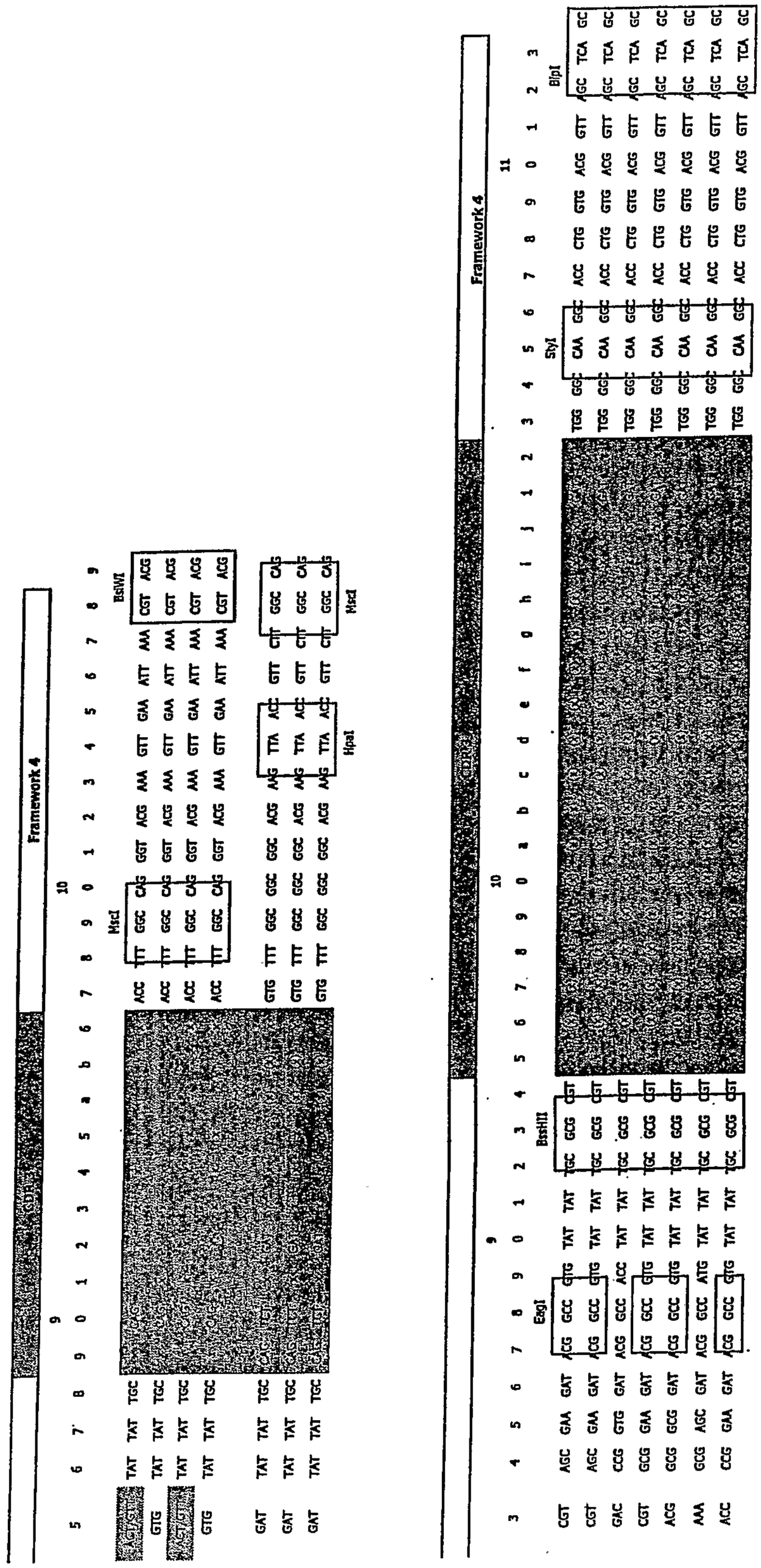


FIGURE 1

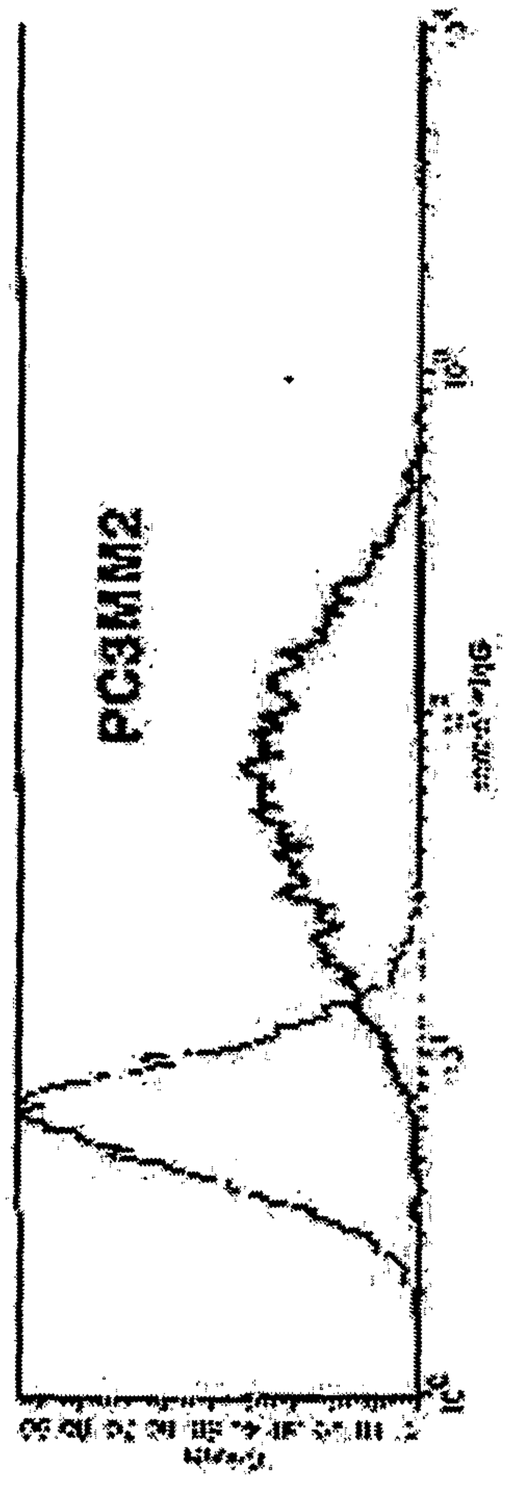


FIGURE 2

Sequence Information

SEQ I.D. #	Sequence Information	DNA Sequence
	VH3-CDR1	
1	1	GGATTTACCTTTAGCGAGAGGGCCATGACC
2	2	GGATTTACCTTTAGCGCGGCCATGATGACG
3	3	GGATTTACCTTTAGCGGGAGCATGATGGCC
4	4	GGATTTACCTTTAGCGACTGGGCGATGACG
5	5	GGATTTACCTTTGTGAAGAGCATGGTGGTG
6	6	GGATTTACCTTTAGCAGGAACCTGATGACC
7	7	GGATTTACCTTTGAGCGGTGGATGGGGGCG
8	8	GGATTTACCTTTAGCAGGAGGATGATGGTC
9	9	GGATTTACCTTTAGCAGGTGGATGATGGTC
10	10	GGATTTACCTTTAGCGAGAGCATGATGACG
11	11	GGATTTACCTTTAGCTGGCACATGATGACG
12	12	GGATTTACCTTTAGCTCCGTGATGATGACG
13	13	GGATTTACCTTTAGCGGGAGCATGATGACG
	VH3-CDR3	
14	1	TCTGCTACTCGTTTTGATTAT
15	2	AATGGTACTCGTATGGATGTT
16	3	GGTATTGTTCGTGGTATGGATCAT
17	4	GGTGGTTCTCGTTATGATGTT
18	5	AATATTACTAAGTCTGATGTT
19	6	GGTGGTACTCGTTTTGATTAT
20	7	AATGGTCGTAATCTTGATTAT
21	8	ACTGCTACTCGTTTTGATTAT
22	9	AAGCCTTTTACTGGTAAGTATTGGGGTCATACTGGTTTTGATATT
23	10	AAACCTTTTACTGGTAAGTATTGGGGTCATACTGGTTTTGATATT
24	11	AATGGCCTGCGTATGGATGTT
25	12	AATCTGCTGCGTATGGATGTT
26	13	AATGCGGTGCGTATGGATGTT
27	14	AATGCGATGCGTATGGATGTT
28	15	AATGCCCTCCGTATGGATGTT
29	16	AATGTGCTGCGTATGGATGTT
30	17	GGGGGGACGCGTATGGATGTT
31	18	CAGGGCACCCGTATGGATGTT
32	19	AATGGCGTGCGTATGGATGTT
33	20	AATGGCATCCGTATGGATGTT

FIGURE 2

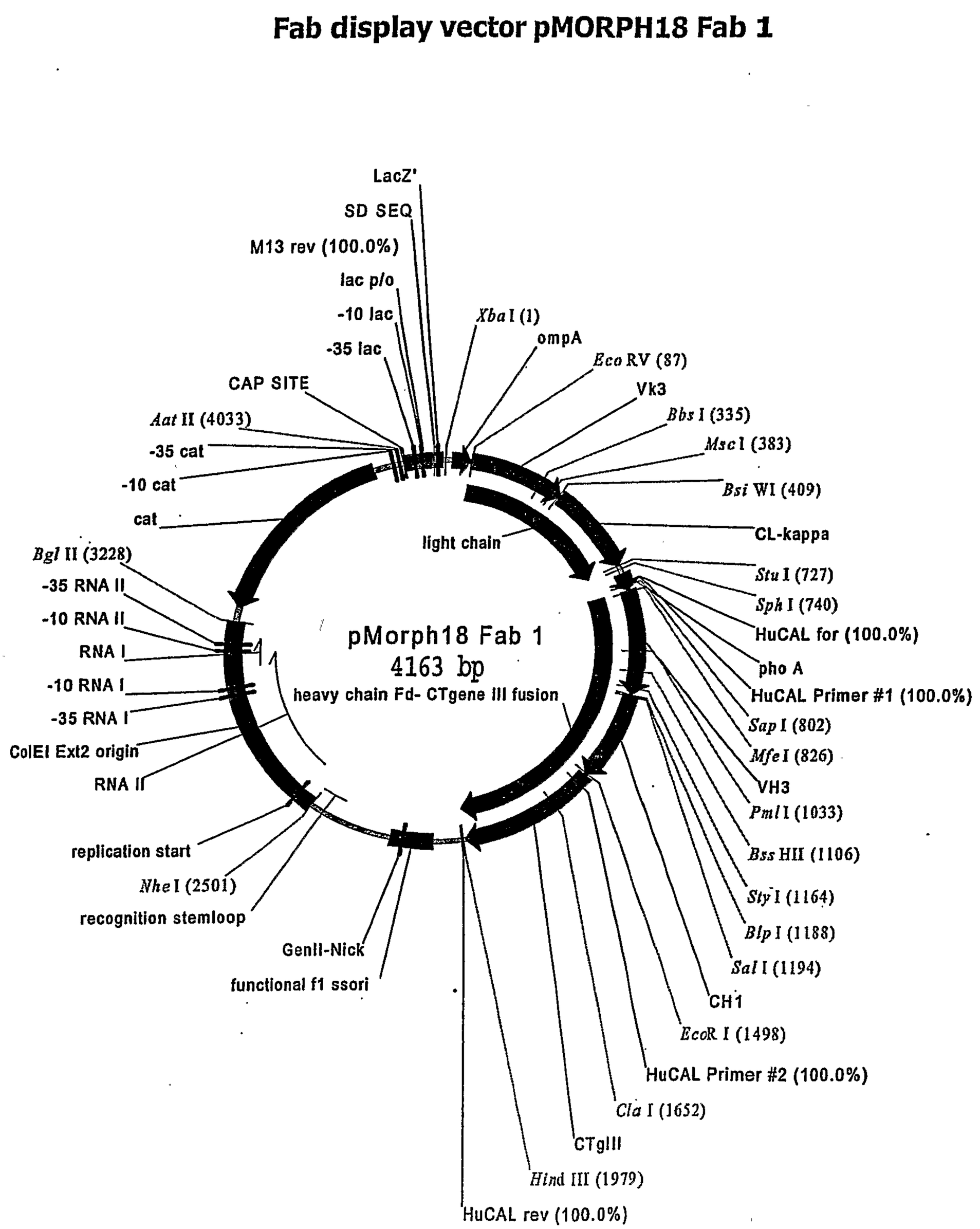
Sequence Information

	VL1-CDR3	
34	1	CAGAGCCGTGACTATGAGAAGCCTATGATT
35	2	CAGAGCCGAGACTATGAGAAGCCTATGATT
36	3	CAGAGCCGCGACTATGAGAAGCCTATGATT
	VL2-CDR1	
37	1	ACGGGTACTAGCAGCGATAGGACGCGCCCGCCGAAGTACGCC
38	2	ACGGGTACTAGCAGCGATGTGTCCGGCCTCAACATCGTGTCC
	VL2-CDR3	
39	1	CAGAGCTATGACCGTGCTTTTAAGTCTGTT
40	2	CAGAGCTATGACCATAAGAAGACTGAG
41	3	CAGAGCTATGACATGTTTGCTCGTGTTATT
42	4	CAGAGCTATGACCGTCTTTATAAGAAGCTT
43	5	CAGAGCTATGACCGGGCTTATCGACTTCTT
44	6	CAGAGCTATGACCGTTCTCGTTATGCT
45	proteoglycan A	GEEDLPSEEDSPREEDPPGEEDLPGEEDLP
46	proteoglycan B	PGEEDLPGEEDLP
47	proteoglycan C	PSEEDSPREEDP
	VH3-CDR1	
48	1	GFTFSERAMT
49	2	GFTFSAAMMT
50	3	GFTFSGSMMA
51	4	GFTFSDWAMT
52	5	GFTFVKSMVV
53	6	GFTFSRNLMT
54	7	GFTFERWMGA
55	8	GFTFSRRMMV
56	9	GFTFSRWMMV
57	10	GFTFSESMMT
58	11	GFTFSWHMMT
59	12	GFTFSSVMMT
60	13	GFTFSGSMMT

FIGURE 2
Sequence Information

	VH3-CDR3	Amino Acid Sequence
61	1	SATRFDY
62	2	NGTRMDV
63	3	GIVRGMDH
64	4	GGSRYDV
65	5	NITKSDV
66	6	GGTRFDY
67	7	NGRNLDY
68	8	TATRFDY
69	9	KPFTGKYWGHTGFDI
70	10	KPFTGKYWGHTGFDI
71	11	NGLRMDV
72	12	NLLRMDV
73	13	NAVRMDV
74	14	NAMRMDV
75	15	NALRMDV
76	16	NVLRMDV
77	17	GGTRMDV
78	18	QGTRMDV
79	19	NGVRMDV
80	20	NGIRMDV
	VL1-CDR3	
81	1	QSRDYEKPMI
	VL2-CDR1	
82	1	TGTSSDRTRPPKYA
83	2	TGTSSDVSGLNIVS
	VL2-CDR3	
84	1	QSYDRAFKSV
85	2	QSYGHKKTE
86	3	QSYDMFARVI
87	4	QSYDRLYKKL
88	5	QSYDRAYRLL
89	6	QSYDRSRYA

FIGURE 3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SD SEQ
M13 rev (100.0%)
lac p/o
-10 lac
-35 lac
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Nhe I (2501)
recognition stemloop
GenII-Nick
functional f1 ssori
Xba I (1)
ompA
Eco RV (87)
Vk3
Bbs I (335)
Msc I (383)
Bsi WI (409)
light chain
CL-kappa
Stu I (727)
Sph I (740)
HuCAL for (100.0%)
pho A
HuCAL Primer #1 (100.0%)
Sap I (802)
Mfe I (826)
VH3
Pml I (1033)
Bss HII (1106)
Sty I (1164)
Blp I (1188)
Sal I (1194)
CH1
EcoR I (1498)
HuCAL Primer #2 (100.0%)
Cla I (1652)
CTgIII
Hind III (1979)
HuCAL rev (100.0%)
pMorph18 Fab 1
4163 bp
heavy chain Fd- CTgene III fusion

FIGURE 4

Vector map of pMORPHx9_Fab1_FS

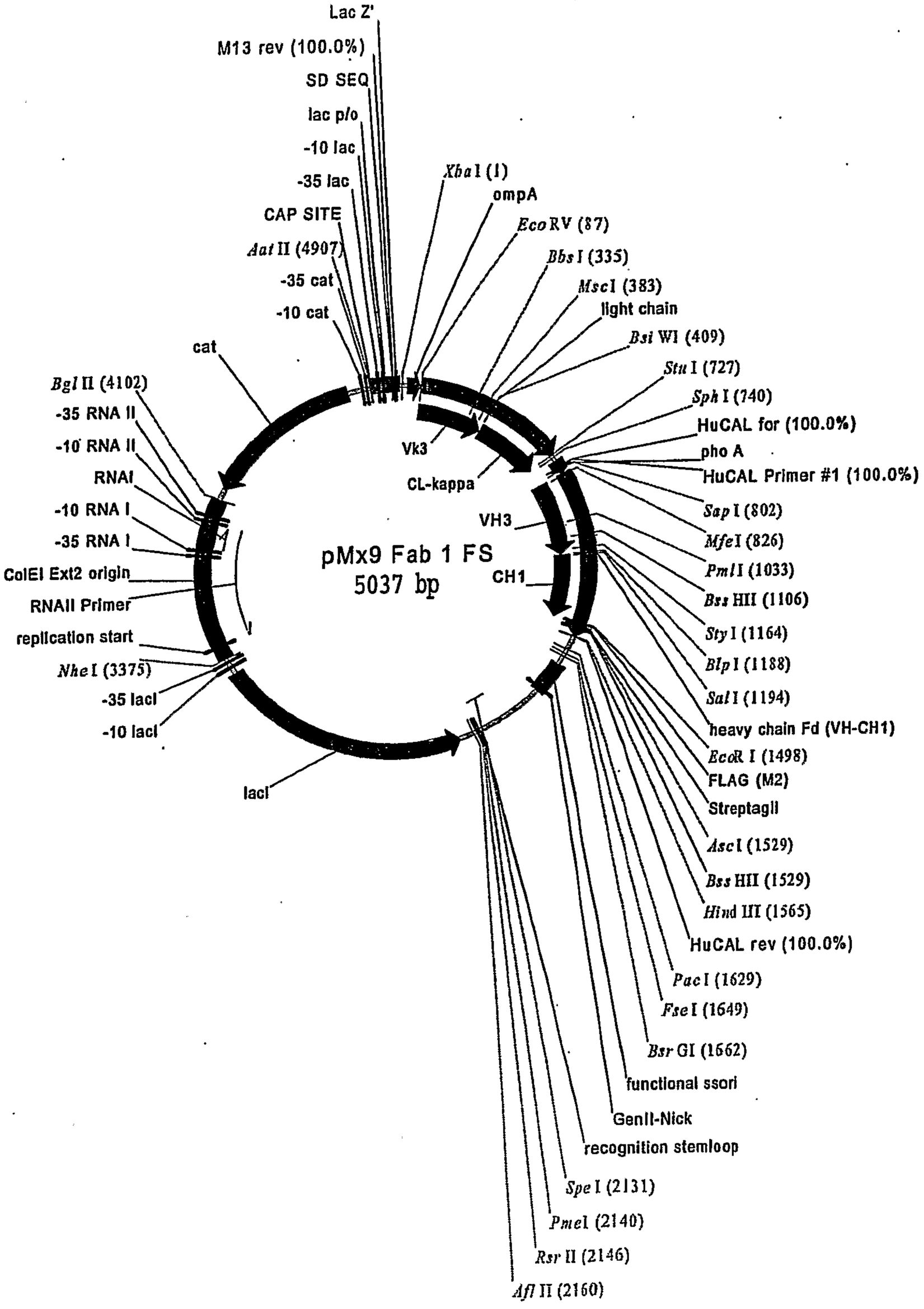
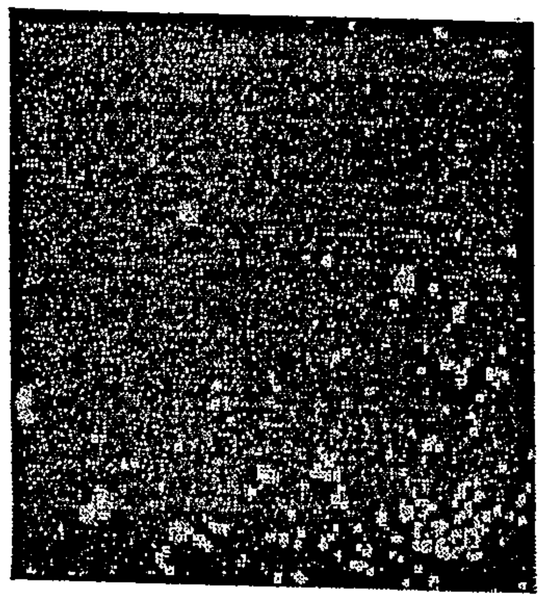
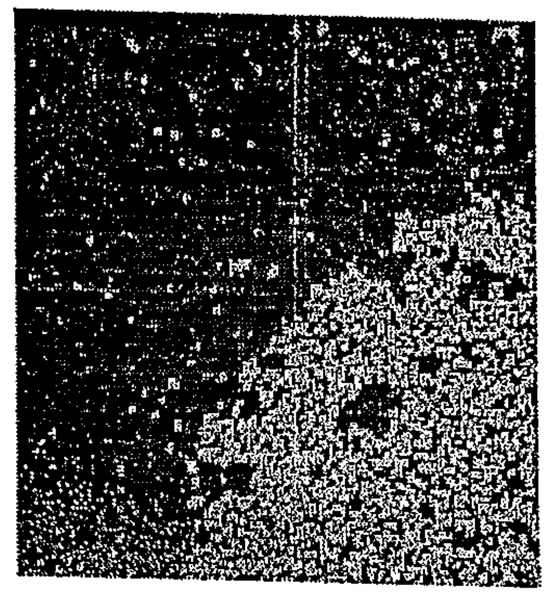


Figure 5

Blocking of Cell Adhesion with Anti-MN Antibody MN-3

A. 

B. 

C. 

A. 20 ug/mL Ab MN-3
B. 20 ug/mL Human gamma globulin
C. No antibody treatment

FIGURE 6

Heavy/Light Chain Pairs

Antibody	Heavy chain/Light chain	Approximate affinity in BIAcore to human MN proteoglycan peptide labeled BSA	Inhibition of ELISA signal with proteoglycan peptide B (1mg/ml)	Inhibition in MN cell binding assay (100 ug/ml Fab)
1	VH3-CDR3-1/VL2-CDR3-1	1.82E-06	75.00%	>95%
2	VH3-CDR3-2/VL2-CDR3-4	3.96E-08	93.00%	>95%
3	VH3-CDR3-3/VL2-CDR3-6	4.65E-09	74.00%	>95%
4	VH3-CDR3-4/VL2-CDR3-1	6.40E-06	75.00%	ND
5	VH3-CDR3-5/VL2-CDR3-1	8.56E-07	32.00%	ND
6	VH3-CDR3-6/VL2-CDR3-2	ND	40.00%	ND
7	VH3-CDR3-7/VL2-CDR3-5	ND	51.00%	ND
8	VH3-CDR3-1/VL1-CDR3-1	ND	73.00%	ND
9	VH3-CDR3-9/VL1-CDR3-1	ND	ND	ND
10	VH3-CDR3-9/VL1-CDR3-2	ND	ND	ND
11	VH3-CDR3-9/VL1-CDR3-3	ND	ND	ND
12	VH3-CDR3-10/VL1-CDR3-1	ND	ND	ND
13	VH3-CDR3-1/VL2-CDR3-3	6.03E-08	ND	ND
14	VH3-CDR3-1/VL2-CDR3-2	ND	ND	ND
15	VH3-CDR3-1/VL2-CDR3-4	9.00E-08	ND	ND
16	VH3-CDR3-1/VL2-CDR3-5	9.90E-08	ND	ND
17	VH3-CDR3-1/VL2-CDR3-6	ND	ND	ND
18	VH3-CDR3-3/VL2-CDR3-3	ND	ND	ND
19	VH3-CDR3-3/VL2-CDR3-2	ND	ND	ND
20	VH3-CDR3-3/VL2-CDR3-4	ND	ND	ND
21	VH3-CDR3-3/VL2-CDR3-5	ND	ND	ND
22	VH3-CDR3-3/VL2-CDR3-1	ND	ND	ND
	ND = not determined			

Figure 6

Heavy/Light Chain Pairs

Antibody	Heavy chain/Light chain pairs	Approximate affinity in BIAcore to human MN proteoglycan peptide-labeled BSA	Inhibition of ELISA signal with proteoglycan peptide B(1 mg/ml)	Inhibition in MN cell binding assay (100 ug/ml Fab)
23	VH3-CDR1-1/VH3-CDR3-1/VL2-CDR3-3	5.84E-09	ND	ND
24	VH3-CDR1-2/VH3-CDR3-1/VL2-CDR3-3	3.31E-09	ND	ND
25	VH3-CDR1-3/VH3-CDR3-1/VL2-CDR3-3	4.89E-09	ND	ND
26	VH3-CDR1-4/VH3-CDR3-1/VL2-CDR3-3	3.57E-09	ND	ND
27	VH3-CDR3-1/VL2-CDR1-1/VL2-CDR3-3	9.47E-09	ND	ND
28	VH3-CDR3-1/VL2-CDR1-2/VL2-CDR3-3	3.95E-09	ND	ND
29	VH3-CDR3-11/VL2-CDR3-4	8.19E-09	ND	ND
30	VH3-CDR3-1/VL2-CDR3-4	3.91E-09	ND	ND
31	VH3-CDR3-12/VL2-CDR3-4	1.23E-09	ND	ND
32	VH3-CDR3-13/VL2-CDR3-4	1.34E-09	ND	ND
33	VH3-CDR3-14/VL2-CDR3-4	1.23E-09	ND	ND
34	VH3-CDR3-15/VL2-CDR3-4	1.61E-09	ND	ND
35	VH3-CDR3-16/VL2-CDR3-4	1.31E-09	ND	ND
36	VH3-CDR3-17/VL2-CDR3-4	2.87E-09	ND	ND
37	VH3-CDR3-18/VL2-CDR3-4	2.82E-09	ND	ND
38	VH3-CDR3-19/VL2-CDR3-4	1.43E-09	ND	ND
39	VH3-CDR3-20/VL2-CDR3-4	1.55E-09	ND	ND
40	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-1	1.82E-08	ND	ND
41	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-2	5.70E-09	ND	ND
42	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-3	6.00E-10	ND	ND
43	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-4	N.D.	ND	ND
44	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-5	2.00E-09	ND	ND
45	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-6	6.00E-10	ND	ND
46	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-7	8.00E-10	ND	ND
47	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-8	4.10E-09	ND	ND
48	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-9	1.20E-09	ND	ND
49	VH3-CDR3-1/VL2-CDR3-3/VH3-CDR1-10	1.00E-09	ND	ND