(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 578 903 B1

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention of the grant of the patent:
23.11.2011 Bulletin 2011/47

(21) Application number: **02762148.1**

(22) Date of filing: **17.04.2002**

(51) Int Cl.:
C12N 15/00 *(2006.01)* ***C12P 21/06*** *(2006.01)*
C12N 15/09 *(2006.01)* ***C12N 15/63*** *(2006.01)*
C12N 15/70 *(2006.01)* ***C12N 15/74*** *(2006.01)*
C12P 21/04 *(2006.01)* ***C12Q 3/00*** *(2006.01)*
C12Q 1/70 *(2006.01)* ***G01N 33/53*** *(2006.01)*

(86) International application number:
PCT/US2002/012405

(87) International publication number:
WO 2002/083872 (24.10.2002 Gazette 2002/43)

(54) **NOVEL METHODS OF CONSTRUCTING LIBRARIES COMPRISING DISPLAYED AND/OR EXPRESSED MEMBERS OF A DIVERSE FAMILY OF PEPTIDES, POLYPEPTIDES OR PROTEINS AND THE NOVEL LIBRARIES**

NEUE VERFAHREN ZUM AUFBAU VON PRÄSENTIERTEN UND/ODER EXPRIMIERTEN MITGLIEDERN EINER WEITLÄUFIGEN FAMILIE VON PEPTIDEN, POLYPEPTIDEN ODER PROTEINEN UMFASSENDEN BIBLIOTHEKEN SOWIE DIE NEUEN BIBLIOTHEKEN

NOUVEAUX PROCEDES DE CONSTRUCTION DE BIBLIOTHEQUES COMPRENANT DES MEMBRES VISUALISES ET/OU EXPRIMES DE DIVERSES FAMILLES DE PEPTIDES, POLYPEPTIDES ET PROTEINES ET NOUVELLES BIBLIOTHEQUES OBTENUES

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **17.04.2001 US 837306**
24.10.2001 US 516
25.10.2001 US 45674

(43) Date of publication of application:
28.09.2005 Bulletin 2005/39

(73) Proprietor: **Dyax Corporation**
Cambridge, MA 02139 (US)

(72) Inventors:
- **LADNER, Robert C.**
  Ijamsville, MD 21754 (US)
- **COHEN, Edward H.**
  Belmont, MA 02478 (US)
- **NASTRI, Horacio G.**
  Needham, MA 02492 (US)
- **ROOKEY, Kristin L.**
  Revere, MA 02151 (US)
- **HOET, René**
  6226 CZ Maastricht (NL)
- **HOOGENBOOM, Hendricus R.J.M.**
  6214 AE Maastricht (NL)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4
81675 München (DE)

(56) References cited:
DE-A1- 19 624 562
US-A- 5 223 409
US-A- 5 380 833
US-A- 5 688 666
US-A- 5 798 208
US-A- 5 846 765
US-A- 5 858 671
US-A- 5 969 108

- **DAVIES J ET AL: "Affinity improvement of single antibody VH domains: residues in all three hypervariable regions affect antigen binding" IMMUNOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, NL, vol. 2, no. 3, September 1996 (1996-09), pages 169-179, XP004070292 ISSN: 1380-2933**
- **ARDEN B: "Conserved motifs in T-cell receptor CDR1 and CDR2: implications for ligand and CD8 co-receptor binding" CURRENT OPINION IN IMMUNOLOGY, CURRENT BIOLOGY LTD, vol. 10, no. 1, February 1998 (1998-02), pages 74-81, XP004313624 ISSN: 0952-7915**

EP 1 578 903 B1

- HOOGENBOOM H R ET AL: "By-passing Immunisation - Human Antibodies from Synthetic Repertoires of Germline VH Gene Segments Rearranged in Vitro" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 227, 1992, pages 381-388, XP002974448 ISSN: 0022-2836
- DE HAARD H J ET AL: "A large non-immunized human Fab fragment phage library that permits rapid isolation and kinetic analysis of high affinity antibodies" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOCHEMICAL BIOLOGISTS, BIRMINGHAM,, US, vol. 274, no. 26, 25 June 1999 (1999-06-25), pages 18218-18230, XP002128301 ISSN: 0021-9258
- TOMLINSON I M ET AL: "THE REPERTOIRE OF HUMAN GERMLINE VH SEQUENCES REVEALS ABOUT FIFTY GROUPS OF VH SEGMENTS WITH DIFFERENT HYPERVARIABLE LOOPS" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 227, 1992, pages 776-798, XP000990787 ISSN: 0022-2836
- BARBAS S M ET AL: "HUMAN AUTOANTIBODY RECOGNITION OF DNA" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 92, March 1995 (1995-03), pages 2529-2533, XP002927212 ISSN: 0027-8424

Description

[0001] This application is a continuation-in-part of United States provisional application 60/198,069, filed April 17, 2000, a continuation-in-part of United States patent application 09/837,306, filed on April 17, 2001, a continuation-in-part of PCT application PCT/US01/12454, filed on April 17, 2001, a continuation-in-part of United States application 10/000,516, filed on October 24, 2001 and a continuation-in-part of United States application 10/045,674, filed on October 25, 2001.

[0002] The present invention relates to the embodiments characterized in the claims.

[0003] Thus, it relates to a method for producing a population or library of immunoglobin genes that comprises the steps of:

(i) introducing synthetic diversity into at least one of VH CDR1 or VH CDR2 of those genes; and
(ii) combining the diversity from step (i) with VH CDR3 diversity captured from B cells.

[0004] The present invention also relates to a library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the family, the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising sequences encoding

(a) a VH CDR1 having an amino acid sequence according to the formula -X1-Y-X2-M-X3-, wherein X1, X2, and X3 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;
(b) a VH CDR2 having an amino acid sequence according to the formula X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G-, wherein X4 and X5 are independently selected from the group consisting of Y, R, W, V, G, and S, X6 is selected from the group consisting of P and S, and X7 and X8 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; and
(c) a sequence encoding a VH CDR3, wherein said VH CDR3 is a VH CDR3 captured from the VH CDR3 region of an immunoglobin gene from a B cell.

In a preferred embodiment, the genetic packages are filamentous phage or phagemids.

[0005] The present disclosure further relates to vectors for displaying and/or expressing a diverse family of peptides, polypeptides or proteins.

[0006] The present disclosure further relates to methods of screening the libraries of the invention and to the peptides, polypeptides and proteins identified by such screening.

BACKGROUND OF THE INVENTION

[0007] It is now common practice in the art to prepare libraries of genetic packages that display, express or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, express or comprise at least a portion of the diversity of the family. In many common libraries, the peptides, polypeptides or proteins are related to antibodies. Often, they are Fabs or single chain antibodies.

[0008] In general, the DNAs that encode members of the families to be displayed and/or expressed must be amplified before they are cloned and used to display and/or express the desired member. Such amplification typically makes use of forward and backward primers.

[0009] Such primers can be complementary to sequences native to the DNA to be amplified or complementary to oligonucleotides attached at the 5' or 3' ends of that DNA. Primers that are complementary to sequences native to the DNA to be amplified are disadvantaged in that they bias the members of the families to be displayed. Only those members that contain a sequence in the native DNA that is substantially complementary to the primer will be amplified. Those that do not will be absent from the family. For those members that are amplified, any diversity within the primer region will be suppressed.

[0010] For example, in European patent 368,684 B1, the primer that is used is at the 5' end of the V_H region of an antibody gene. It anneals to a sequence region in the native DNA that is said to be "sufficiently well conserved" within a single species. Such primer will bias the members amplified to those having this "conserved" region. Any diversity within this region is extinguished.

[0011] It is generally accepted that human antibody genes arise through a process that involves a combinatorial selection of V and J or V, D, and J followed by somatic mutations. Although most diversity occurs in the Complementary Determining Regions (CDRs), diversity also occurs in the more conserved Framework Regions (FRs) and at least some of this diversity confers or enhances specific binding to antigens (Ag). As a consequence, libraries should contain as much of the CDR and FR diversity as possible.

[0012] To clone the amplified DNAs of the peptides, polypeptides or proteins that they encode for display on a genetic

package and/or for expression, the DNAs must be cleaved to produce appropriate ends for ligation to a vector. Such cleavage is generally effected using restriction endonuclease recognition sites carried on the primers. When the primers are at the 5' end of DNA produced from reverse transcription of RNA, such restriction leaves deleterious 5' untranslated regions in the amplified DNA. These regions interfere with expression of the cloned genes and thus the display of the peptides, polypeptides and proteins coded for by them.

SUMMARY OF THE INTENTION

[0013] It is an object of this invention to provide a method for producing a population or library of immunoglobin genes that comprises the steps of:

(i) introducing synthetic diversity into at least one of VH CDR1 or VH CDR2 of those genes; and
(ii) combining the diversity from step (i) with VH CDR3 diversity captured from B cells.

[0014] These methods are not biased toward DNAs that contain native sequences that are complementary to the primers used for amplification. They also enable any sequences that may be deleterious to expression to be removed from the amplified DNA before cloning and displaying and/or expressing.

[0015] Additional objects of the invention are reflected in claims 3 to 14,Each of these claims is specifically incorporated by reference in this specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016]

FIG. 1 is a schematic of various methods that may be employed to amplify VH genes without using primers specific for VH sequences.
FIG. 2 is a schematic of various methods that may be employed to amplify VL genes without using primers specific for VL sequences.
FIG. 3 is a schematic of RACE amplification of antibody heavy and light chains.
FIG. 4 depicts gel analysis of amplification products obtained after the primary PCR reaction from 4 different patient samples.
FIG. 5 depicts gel analysis of cleaved kappa DNA from Example 2.
FIG. 6 depicts gel analysis of extender-cleaved kappa DNA from Example 2.
FIG. 7 depicts gel analysis of the PCR product from the extender-kappa amplification from Example 2.
FIG. 8 depicts gel analysis of purified PCR product from the extender-kappa amplification from Example 2.
FIG. 9 depicts gel analysis of cleaved and ligated kappa light chains from Example 2.
FIG. 10 is a schematic of the design for CDR1 and CDR2 synthetic diversity.
FIG. 11 is a schemaitc of the cloning schedule for construction of the heavy chain repertoire.
FIG. 12 is a schematic of the cleavage and ligation of the antibody light chain.
FIG. 13 depicts gel analysis of cleaved and ligated lambda light chains from Example 4.
FIG. 14 is a schematic of the cleavage and ligation of the antibody heavy chain.
FIG. 15 depicts gel analysis of cleaved and ligated lambda light chains from Example 5.
FIG. 16 is a schematic of a phage display vector.
FIG. 17 is a schematic of a Fab cassette.
FIG. 18 is a schematic of a process for incorporating fixed FR1 residues in an antibody lambda sequence.
FIG. 19 is a schematic of a process for incorporating fixed FR1 residues in an antibody kappa sequence.
FIG. 20 is a schematic of a process for incorporating fixed FR1 residues in an antibody heavy chain sequence.

TERMS

[0017] In this application, the following terms and abbreviations are used:

Sense strand	The upper strand of ds DNA as usually written. In the sense strand, 5'-ATG-3' codes for Met.
Antisense strand	The lower strand of ds DNA as usually written. In the antisense strand, 3'-TAC-5' would correspond to a Met codon in the sense strand.

Forward primer	A "forward" primer is complementary to a part of the sense strand and primes for synthesis of a new antisensestrand molecule. "Forward primer" and "lower-strand primer" are equivalent.
Backward primer	A "backward" primer is complementary to a part of the antisense strand and primes for synthesis of a new sensestrand molecule. "Backward primer" and "top-strand primer" are equivalent.
Bases	Bases are specified either by their position in a vector or gene as their position within a gene by codon and base. For example, "89.1" is the first base of codon 89, 89.2 is the second base of codon 89.
Sv	Streptavidin
Ap	Ampicillin
ap^R	A gene conferring ampicillin resistance.
RERS	Restriction endonuclease recognition site
RE	Restriction endonuclease - cleaves preferentially at RERS
URE	Universal restriction endonuclease
Functionally complementary	Two sequences are sufficiently complementary so as to anneal under the chosen conditions.
AA	Amino acid
PCR	Polymerization chain reaction
GLGs	Germline genes
Ab	Antibody: an immunoglobin. The term also covers any protein having a binding domain which is homologous to an immunoglobin binding domain. A few examples of antibodies within this definition are, *inter alia*, immunoglobin isotypes and the Fab, $F(ab^1)_2$, scfv, Fv, dAb and Fd fragments.
Fab	Two chain molecule comprising an Ab light chain and part of a heavy-chain.
scFv	A single-chain Ab comprising either VH::linker::VL or VL::linker::VH
w.t.	Wild type
HC	Heavy chain
LC	Light chain
VK	A variable domain of a Kappa light chain.
VH	A variable domain of a heavy chain.
VL	A variable domain of a lambda light chain.

[0018] In this application when it is said that nucleic acids are cleaved solely at the cleavage site of a restriction endonuclease, it should be understood that minor cleavage may occur at random, e.g., at non-specific sites other than the specific cleavage site that is characteristic of the restriction endonuclease. The skilled worker will recognize that such non-specific, random cleavage is the usual occurrence. Accordingly, "solely at the cleavage site" of a restriction

endonuclease means that cleavage occurs preferentially at the site characteristic of that endonuclease.

[0019] As used in this application and claims, the term "cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide" includes cleavage sites formed by the single-stranded portion of the partially double-stranded ologonucleotide duplexing with the single-stranded DNA, cleavage sites in the double-stranded portion of the partially double-stranded oligonucleotide, and cleavage sites introduced by the amplification primer used to amplify the single-stranded DNA-partially double-stranded oligonucleotide combination.

[0020] In the two methods of this invention for preparing single-stranded nucleic acid sequences, the first of those cleavage sites is preferred. In the methods of this invention for capturing diversity and cloning a family of diverse nucleic acid sequences, the latter two cleavage sites are preferred.

[0021] In this application, all references referred to are specifically incorporated by reference.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0022] The nucleic acid sequences that are useful in the methods of this invention, i.e., those that encode at least in part the individual peptides, polypeptides and proteins displayed, or expressed in or comprising the libraries of this invention, may be native, synthetic or a combination thereof. They may be mRNA, DNA or cDNA. In the preferred embodiment, the nucleic acids encode antibodies. Most preferably, they encode Fabs.

[0023] The nucleic acids useful in this invention may be naturally diverse, synthetic diversity may be introduced into those naturally diverse members, or the diversity may be entirely synthetic. For example, synthetic diversity can be introduced into one or more CDRs of antibody genes. Preferably, it is introduced into CDR1 and CDR2 of immunoglobulins. Preferably, natural diversity is captured in the CDR3 regions of the immunoglogin genes of this invention from B cells. Most preferably, the nucleic acids of this invention comprise a population of immunoglobin genes that comprise synthetic diversity in at least one, and more preferably both of the CDR1 and CDR2 and diversity in CDR3 captured from B cells.

[0024] Synthetic diversity may be created, for example, through the use of TRIM technology (U.S. 5,869,644). TRIM technology allows control over exactly which amino-acid types are allowed at variegated positions and in what proportions. In TRIM technology, codons to be diversified are synthesized using mixtures of trinucleotides. This allows any set of amino acid types to be included in any proportion.

[0025] Another alternative that may be used to generate diversified DNA is mixed oligonucleotide synthesis. With TRIM technology, one could allow Ala and Trp. With mixed oligonucleotide synthesis, a mixture that included Ala and Trp would also necessarily include Ser and Gly. The amino-acid types allowed at the variegated positions are picked with reference to the structure of antibodies, or other peptides, polypeptides or proteins of the family, the observed diversity in germline genes, the observed somatic mutations frequently observed, and the desired areas and types of variegation.

[0026] In a preferred embodiment of this invention, the nucleic acid sequences for at least one CDR or other region of the peptides, polypeptides or proteins of the family are cDNAs produced by reverse transcription from mRNA. More preferably, the mRNAs are obtained from peripheral blood cells, bone marrow cells, spleen cells or lymph node cells (such as B-lymphocytes or plasma cells) that express members of naturally diverse sets of related genes. More preferable, the mRNAs encode a diverse family of antibodies. Most preferably, the mRNAs are obtained from patients suffering from at least one autoimmune disorder or cancer. Preferably, mRNAs containing a high diversity of autoimmune diseases, such as systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome and vasculitis are used.

[0027] In a preferred embodiment of this invention, the cDNAs are produced from the mRNAs using reverse transcription. In this preferred embodiment, the mRNAs are separated from the cell and degraded using standard methods, such that only the full length (*i.e.*, capped) mRNAs remain. The cap is then removed and reverse transcription used to produce the cDNAs.

[0028] The reverse transcription of the first (antisense) strand can be done in any manner with any suitable primer. See, *e.g.*, HJ de Haard et al., Journal of Biological Chemistry, 279(26):18218-30 (1999). In the preferred embodiment of this invention where the mRNAs encode antibodies, primers that are complementary to the constant regions of antibody genes may be used. Those primers are useful because they do not generate bias toward subclasses of antibodies. In another embodiment, poly-dT primers may be used (and may be preferred for the heavy-chain genes). Alternatively, sequences complementary to the primer may be attached to the termini of the antisense strand.

[0029] In one preferred embodiment of this invention, the reverse transcriptase primer may be biotinylated, thus allowing the cDNA product to be immobilized on streptavidin (Sv) beads. Immobilization can also be effected using a primer labeled at the 5' end with one of a) free amine group, b) thiol, c) carboxylic acid, or d) another group not found in DNA that can react to form a strong bond to a known partner on an insoluble medium. If, for example, a free amine (preferably primary amine) is provided at the 5' end of a DNA primer, this amine can be reacted with carboxylic acid groups on a polymer bead using standard amide-forming chemistry. If such preferred immobilization is used during reverse transcription, the top strand RNA is degraded using well-known enzymes, such as a combination of RNAseH

and RNAseA, either before or after immobilization.

[0030] The nucleic acid sequences useful in the methods of this invention are generally amplified before being used to display and/or express the peptides, polypeptides or proteins that they encode. Prior to amplification, the single-stranded DNAs may be cleaved using either of the methods described before. Alternatively, the single-stranded DNAs may be amplified and then cleaved using one of those methods.

[0031] Any of the well known methods for amplifying nucleic acid sequences may be used for such amplification. Methods that maximize, and do not bias, diversity are preferred. In a preferred embodiment of this invention where the nucleic acid sequences are derived from antibody genes, the present invention preferably utilizes primers in the constant regions of the heavy and light chain genes and primers to a synthetic sequence that are attached at the 5' end of the sense strand. Priming at such synthetic sequence avoids the use of sequences within the variable regions of the antibody genes. Those variable region priming sites generate bias against V genes that are either of rare subclasses or that have been mutated at the priming sites. This bias is partly due to suppression of diversity within the primer region and partly due to lack of priming when many mutations are present in the region complementary to the primer. The methods disclosed in this invention have the advantage of not biasing the population of amplified antibody genes for particular V gene types.

[0032] The synthetic sequences may be attached to the 5' end of the DNA strand by various methods well known for ligating DNA sequences together. RT CapExtention is one preferred method.

[0033] In RT CapExtention (derived from Smart PCR(™)), a short overlap (5'-...GGG-3' in the upper-strand primer (USP-GGG) complements 3'-CCC....5' in the lower strand) and reverse transcriptases are used so that the reverse complement of the upper-strand primer is attached to the lower strand.

[0034] FIGs. 1 and 2 show schematics to amplify VH and VL genes using RT CapExtention. FIG. 1 shows a schematic of the amplification of VH genes. FIG. 1, Panel A shows a primer specific to the poly-dT region of the 3' UTR priming synthesis of the first, lower strand. Primers that bind in the constant region are also suitable. Panel B shows the lower strand extended at its 3' end by three Cs that are not complementary to the mRNA. Panel C shows the result of annealing a synthetic top-strand primer ending in three GGGs that hybridize to the 3' terminal CCCs and extending the reverse transcription extending the lower strand by the reverse complement of the synthetic primer sequence. Panel D shows the result of PCR amplification using a 5' biotinylated synthetic top-strand primer that replicates the 5' end of the synthetic primer of panel C and a bottom-strand primer complementary to part of the constant domain. Panel E shows immobilized double-stranded (ds) cDNA obtained by using a 5'-biotinylated top-strand primer.

[0035] FIG. 2 shows a similar schematic for amplification of VL genes. FIG. 2, Panel A shows a primer specific to the constant region at or near the 3' end priming synthesis of the first, lower strand. Primers that bind in the poly-dT region are also suitable. Panel B shows the lower strand extended at its 3' end by three Cs that are not complementary to the mRNA. Panel C shows the result of annealing a synthetic top-strand primer ending in three GGGs that hybridize to the 3' terminal CCCs and extending the reverse transcription extending the lower strand by the reverse complement of the synthetic primer sequence. Panel D shows the result of PCR amplification using a 5' biotinylated synthetic top-strand primer that replicates the 5' end of the synthetic primer of panel C and a bottom-strand primer complementary to part of the constant domain. The bottom-strand primer also contains a useful restriction endonuclease site, such as AscI. Panel E shows immobilized ds cDNA obtained by using a 5'-biotinylated top-strand primer.

[0036] In FIGs. 1 and 2, each V gene consists of a 5' untranslated region (UTR) and a secretion signal, followed by the variable region, followed by a constant region, followed by a 3' untranslated region (which typically ends in poly-A). An initial primer for reverse transcription may be complementary to the constant region or to the poly A segment of the 3'-UTR. For human heavy-chain genes, a primer of 15 T is preferred. Reverse transcriptases attach several C residues to the 3' end of the newly synthesized DNA. RT CapExtention exploits this feature. The reverse transcription reaction is first run with only a lower-strand primer. After about 1 hour, a primer ending in GGG (USP-GGG) and more RTase are added. This causes the lower-strand cDNA to be extended by the reverse complement of the USP-GGG up to the final GGG. Using one primer identical to part of the attached synthetic sequence and a second primer complementary to a region of known sequence at the 3' end of the sense strand, all the V genes are amplified irrespective of their V gene subclass.

[0037] In another preferred embodiment, synthetic sequences may be added by Rapid Amplification of cDNA Ends (RACE) (see Frohman, M.A., Dush, M.K., & Martin, G.R. (1988) Proc. Natl. Acad. Sci. USA (85): 8998-9002).

[0038] FIG. 1 shows a schematic of RACE amplification of antibody heavy and light chains. First, mRNA is selected by treating total or poly(A+) RNA with calf intestinal phosphatase (CIP) to remove the 5'-phosphate from all molecules that have them such as ribosomal RNA, fragmented mRNA, tRNA and genomic DNA. Full length mRNA (containing a protective 7-methyl cap structure) is uneffected. The RNA is then treated with tobacco acid pyrophosphatase (TAP) to remove the cap structure from full length mRNAs leaving a 5'-monophosphate group. Next, a synthetic RNA adaptor is ligated to the RNA population, only molecules which have a 5-phosphate (uncapped, full length mRNAs) will accept the adaptor. Reverse trascriptase reactions using an oligodT primer, and nested PCR (using one adaptor primer (located in the 5' synthetic adaptor) and one primer for the gene) are then used to amplify the desired transcript.

[0039] In a preferred embodiment of this invention, the upper strand or lower strand primer may be also biotinylated or labeled at the 5' end with one of a) free amino group, b) thiol, c) carboxylic acid and d) another group not found in DNA that can react to form a strong bond to a known partner as an insoluble medium. These can then be used to immobilize the labeled strand after amplification. The immobilized DNA can be either single or double-stranded.

[0040] After amplification (using *e.g.*, RT CapExtension or RACE), the DNAs of this invention are rendered single-stranded. For example, the strands can be separated by using a biotinylated primer, capturing the biotinylated product on streptavidin beads, denaturing the DNA, and washing away the complementary strand. Depending on which end of the captured DNA is wanted, one will choose to immobilize either the upper (sense) strand or the lower (antisense) strand.

[0041] To prepare the single-stranded amplified DNAs for cloning into genetic packages so as to effect display of, or for expression of, the peptides, polypeptides or proteins encoded, at least in part, by those DNAs, they must be manipulated to provide ends suitable for cloning and display and/or expression. In particular, any 5' untranslated regions and mammalian signal sequences must be removed and replaced, in frame, by a suitable signal sequence that functions in the display or expression host. Additionally, parts of the variable domains (in antibody genes) may be removed and replaced by synthetic segments containing synthetic diversity. The diversity of other gene families may likewise be expanded with synthetic diversity.

[0042] According to the methods of this invention, there are two ways to manipulate the single-stranded DNAs for display and/or expression. The first method comprises the steps of:

(i) contacting the nucleic acid with a single-stranded oligonucleotide, the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired and including a sequence that with its complement in the nucleic acid forms a restriction endonuclease recognition site that on restriction results in cleavage of the nucleic acid at the desired location; and

(ii) cleaving the nucleic acid solely at the recognition site formed by the complementation of the nucleic acid and the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

[0043] In this first method, short oligonucleotides are annealed to the single-stranded DNA so that restriction endonuclease recognition sites formed within the now locally double-stranded regions of the DNA can be cleaved. In particular, a recognition site that occurs at the same position in a substantial fraction of the single-stranded DNAs is identical.

[0044] For antibody genes, this can be done using a catalog of germline sequences. See, *e.g.*, "http://www.mrc-cpe.cam.ac.uk/imt-doc/restricted/ok.htm 1." Updates can be obtained from this site under the heading "Amino acid and nucleotide sequence alignments." For other families, similar comparisons exist and may be used to select appropriate regions for cleavage and to maintain diversity.

[0045] For example, Table 1 depicts the DNA sequences of the FR3 regions of the 51 known human VH germline genes. In this region, the genes contain restriction endonuclease recognition sites shown in Table 2. Restriction endonucleases that cleave a large fraction of germline genes at the same site are preferred over endonucleases that cut at a variety of sites. Furthermore, it is preferred that there be only one site for the restriction endonucleases within the region to which the short oligonucleotide binds on the single-stranded DNA, *e.g.*, about 10 bases on either side of the restriction endonuclease recognition site.

[0046] An enzyme that cleaves downstream in FR3 is also more preferable because it captures fewer mutations in the framework. This may be advantageous is some cases. However, it is well known that framework mutations exist and confer and enhance antibody binding. The present invention, by choice of appropriate restriction site, allows all or part of FR3 diversity to be captured. Hence, the method also allows extensive diversity to be captured.

[0047] Finally, in the methods of this invention restriction endonucleases that are active between about 37˚C and about 75˚C are used. Preferably, restriction endonucleases that are active between about 45˚C and about 75˚C may be used. More preferably, enzymes that are active above 50˚C, and most preferably active about 55˚C, are used. Such temperatures maintain the nucleic acid sequence to be cleaved in substantially single-stranded form.

[0048] Enzymes shown in Table 2 that cut many of the heavy chain FR3 germline genes at a single position include: *Mae*III(24@4), *Tsp*45I(21@4), *Hph*I(44@5), *Bsa*JI(23@65), *Alu*I(23@47), *Blp*I(21@48), *Dde*I(29@58), *Bgl*II(10@61), *Msl*I(44@72), *Bsi*EI(23@74), *Eae*I(23@74), *Eag*I(23@74), *Hae*III(25@75), *Bst*4CI(51@86), *Hpy*CH4III(51@86), *Hin*fI (38@2), *Mly*I(18@2), *Ple*I(18@2), *Mnl*I(31@67), *Hpy*CH4V(21@44), *Bsm*AI(16@11), *Bpm*I(19@12), *Xmn*I(12@30), and *Sac*I(11@51). (The notation used means, for example, that *Bsm*AI cuts 16 of the FR3 germline genes with a restriction endonuclease recognition site beginning at base 11 of FR3.)

[0049] For cleavage of human heavy chains in FR3, the preferred restriction endonucleases are: *Bst*4CI (or *Taa*I or

*Hpy*CH4III), *Blp*I, *Hpy*CH4V, and *Msl*I. Because ACNGT (the restriction endonuclease recognition site for *Bst*4CI, *Taa*I, and *Hpy*CH4III) is found at a consistent site in all the human FR3 germline genes, one of those enzymes is the most preferred for capture of heavy chain CDR3 diversity. *Blp*I and *Hpy*CH4V are complementary. *Blp*I cuts most members of the VH1 and VH4 families while *Hpy*CH4V cuts most members of the VH3, VH5, VH6, and VH7 families. Neither enzyme cuts VH2s, but this is a very small family, containing only three members. Thus, these enzymes may also be used in preferred embodiments of the methods of this invention.

[0050] The restriction endonucleases *Hpy*CH4III, *Bst*4CI, and *Taa*I all recognize 5'-ACnGT-3' and cut upper strand DNA after n and lower strand DNA before the base complementary to n. This is the most preferred restriction endonuclease recognition site for this method on human heavy chains because it is found in all germline genes. Furthermore, the restriction endonuclease recognition region (ACnGT) matches the second and third bases of a tyrosine codon (tay) and the following cysteine codon (tgy) as shown in Table 3. These codons are highly conserved, especially the cysteine in mature antibody genes.

[0051] Table 4 E shows the distinct oligonucleotides of length 22 (except the last one which is of length 20) bases. Table 5 C shows the analysis of 1617 actual heavy chain antibody genes. Of these, 1511 have the site and match one of the candidate oligonucleotides to within 4 mismatches. Eight oligonucleotides account for most of the matches and are given in Table 4 F.1. The 8 oligonucleotides are very similar so that it is likely that satisfactory cleavage will be achieved with only one oligonucleotide (such as H43.77.97.1-02#1) by adjusting temperature, pH, salinity, and the like. One or two oligonucleotides may likewise suffice whenever the germline gene sequences differ very little and especially if they differ very little close to the restriction endonuclease recognition region to be cleaved. Table 5 D shows a repeat analysis of 1617 actual heavy chain antibody genes using only the 8 chosen oligonucleotides. This shows that 1463 of the sequences match at least one of the oligonucleotides to within 4 mismatches and have the site as expected. Only 7 sequences have a second *Hpy*CH4III restriction endonuclease recognition region in this region.

[0052] Another illustration of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of human heavy chains. Cleavage in FR1 allows capture of the entire CDR diversity of the heavy chain.

[0053] The germline genes for human heavy chain FR1 are shown in Table 6. Table 7 shows the restriction endonuclease recognition sites found in human germline genes FR1s. The preferred sites are *Bsg*I(GTGCAG;39@4), *Bso*FI (GCngc:43@6,11@9,2@3,1@12), *Tse*I(Gcwgc;43@6,11@9,2@3,1@12), *Msp*A1I(CMGckg;46@7,2@1), *Pvu*II (CAGctg;46@7,2@1), *Alu*I(AGct;48@82@2), *Dde*I(Ctnag;22@52,9@48), *Hph*I(tcacc;22@80), *Bss*KI(Nccngg; 35@39,2@40), *Bsa*JI(Ccnngg;32@90,2@41), *Bst*NI(CCwgg;33@40), *Scr*FI(CCngg:35@40,2@41), *Eco*O109I (RGgnccy;22@46, 11@43), *Sau*96I(Ggncc;23@47,11@44), AvaII(Ggwcc;23@47,4@44), *Ppu*MI(RGgwccy; 22@96,9@43), *Bsm*FI(gtccc;20@48), *Hin*fI(Gantc:34@16,21@56,21@77), *Tfi*I(21@77), *Mly*I(GAGTC;34@16), *Mly*I (gactc;21@56), and *Alw*NI(CAGnnnctg:22@68). The more preferred sites are *MspAI* and *PvuII*. *Msp*AI and *Pvu*II have 46 sites at 7-12 and 2 at 1-6. To avoid cleavage at both sites, oligonucleotides are used that do not fully cover the site at 1-6. Thus, the DNA will not be cleaved at that site. We have shown that DNA that extends 3, 4, or 5 bases beyond a PvuII-site can be cleaved efficiently.

[0054] Another illustration of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of human kappa light chains. Table 8 shows the human kappa FR1 germline genes and Table 9 shows restriction endonuclease recognition sites that are found in a substantial number of human kappa FR1 germline genes at consistent locations. Of the restriction endonuclease recognition sites listed, *Bsm*AI and *Pfl*FI are the most preferred enzymes. *Bsm*AI sites are found at base 18 in 35 of 40 germline genes. *Pfl*FI sites are found in 35 of 40 germline genes at base 12.

[0055] Another example of choosing an appropriate restriction endonuclease recognition site involves cleavage in FR1 of the human lambda light chain. Table 10 shows the 31 known human lambda FR1 germline gene sequences. Table 11 shows restriction endonuclease recognition sites found in human lambda FR1'germline genes. *Hinf*I and *Dde*I are the most preferred restriction endonucleases for cutting human lambda chains in FR1.

[0056] After the appropriate site or sites for cleavage are chosen, one or more short oligonucleotides are prepared so as to functionally complement, alone or in combination, the chosen recognition site. The oligonucleotides also include sequences that flank the recognition site in the majority of the amplified genes. This flanking region allows the sequence to anneal to the single-stranded DNA sufficiently to allow cleavage by the restriction endonuclease specific for the site chosen.

[0057] The actual length and sequence of the oligonucleotide depends on the recognition site and the conditions to be used for contacting and cleavage. The length must be sufficient so that the oligonucleotide is functionally complementary to the single-stranded DNA over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location.

[0058] Typically, the oligonucleotides of this preferred method of the invention are about 17 to about 30 nucleotides in length. Below about 17 bases, annealing is too weak and above 30 bases there can be a loss of specificity. A preferred length is 18 to 24 bases.

[0059] Oligonucleotides of this length need not be identical complements of the germline genes. Rather, a few mismatches taken may be tolerated. Preferably, however, no more than 1-3 mismatches are allowed. Such mismatches

do not adversely affect annealing of the oligonucleotide to the single-stranded DNA. Hence, the two DNAs are said to be functionally complementary.

[0060] The second method to manipulate the single-stranded DNAs of this invention for display and/or expression comprises the steps of:

(i) contacting the nucleic acid with a partially double-stranded oligonucleotide, the single-stranded region of the oligonucleotide being functionally complementary to the nucleic acid in the region in which cleavage is desired, and the double-stranded region of the oligonucleotide having a restriction endonuclease recognition site; and
(ii) cleaving the nucleic acid solely at the cleavage site formed by the complementation of the nucleic acid and the single-stranded region of the oligonucleotide;

the contacting and the cleaving steps being performed at a temperature sufficient to maintain the nucleic acid in substantially single-stranded form, the oligonucleotide being functionally complementary to the nucleic acid over a large enough region to allow the two strands to associate such that cleavage may occur at the chosen temperature and at the desired location, and the cleavage being carried out using a restriction endonuclease that is active at the chosen temperature.

[0061] As explained above, the cleavage site may be formed by the single-stranded portion of the partially double-stranded oligonucleotide duplexing with the single-stranded DNA, the cleavage site may be carried in the double-stranded portion of the partially double-stranded oligonucleotide, or the cleavage site may be introduced by the amplification primer used to amplify the single-stranded DNA-partially double-stranded oligonucleotide combination. In this embodiment, the first is preferred. And, the restriction endonuclease recognition site may be located in either the double-stranded portion of the oligonucleotide or introduced by the amplification primer, which is complementary to that double-stranded region, as used to amplify the combination.

[0062] Preferably, the restriction endonuclease site is that of a Type II-S restriction endonuclease, whose cleavage site is located at a known distance from its recognition site.

[0063] This second method, preferably, employs Universal Restriction Endonucleases ("URE"). UREs are partially double-stranded oligonucleotides. The single-stranded portion or overlap of the URE consists of a DNA adapter that is functionally complementary to the sequence to be cleaved in the single-stranded DNA. The double-stranded portion consists of a restriction endonuclease recognition site, preferably type II-S.

[0064] The URE method of this invention is specific and precise and can tolerate some (*e.g.*, 1-3) mismatches in the complementary regions, *i.e.*, it is functionally complementary to that region. Further, conditions under which the URE is used can be adjusted so that most of the genes that are amplified can be cut, reducing bias in the library produced from those genes.

[0065] The sequence of the single-stranded DNA adapter or overlap portion of the URE typically consists of about 14-22 bases. However, longer or shorter adapters may be used. The size depends on the ability of the adapter to associate with its functional complement in the single-stranded DNA and the temperature used for contacting the URE and the single-stranded DNA at the temperature used for cleaving the DNA with the restriction enzyme. The adapter must be functionally complementary to the single-stranded DNA over a large enough region to allow the two strands to associate such that the cleavage may occur at the chosen temperature and at the desired location. We prefer singe-stranded or overlap portions of 14-17 bases in length, and more preferably 18-20 bases in length.

[0066] The site chosen for cleavage using the URE is preferably one that is substantially conserved in the family of amplified DNAs. As compared to the first cleavage method of this invention, these sites do not need to be endonuclease recognition sites. However, like the first method, the sites chosen can be synthetic rather than existing in the native DNA. Such sites may be chosen by references to the sequences of known antibodies or other families of genes. For example, the sequences of many germline genes are reported at http://www.mrc-cpe.cam.ac.uk/imt-doc/restricted/ok.html. For example, one preferred site occurs near the end of FR3 -- codon 89 through the second base of codon 93. CDR3 begins at codon 95.

[0067] The sequences of 79 human heavy-chain genes are also available at http://www.ncbi.nlm.nih.gov/entre2/nucleotide.html. This site can be used to identify appropriate sequences for URE cleavage according to the methods of this invention. *See*, *e.g.*, Table 12B.

[0068] Most preferably, one or more sequences are identified using these sites or other available sequence information. These sequences together are present in a substantial fraction of the amplified DNAs. For example, multiple sequences could be used to allow for known diversity in germline genes or for frequent somatic mutations. Synthetic degenerate sequences could also be used. Preferably, a sequence(s) that occurs in at least 65% of genes examined with no more than 2-3 mismatches is chosen

[0069] URE single-stranded adapters or overlaps are then made to be complementary to the chosen regions. Conditions for using the UREs are determined empirically. These conditions should allow cleavage of DNA that contains the functionally complementary sequences with no more than 2 or 3 mismatches but that do not allow cleavage of DNA

lacking such sequences.

[0070] As described above, the double-stranded portion of the URE includes an endonuclease recognition site, preferably a Type II-S recognition site. Any enzyme that is active at a temperature necessary to maintain the single-stranded DNA substantially in that form and to allow the single-stranded DNA adapter portion of the URE to anneal long enough to the single-stranded DNA to permit cleavage at the desired site may be used.

[0071] The preferred Type II-S enzymes for use in the URE methods of this invention provide asymmetrical cleavage of the single-stranded DNA. Among these are the enzymes listed in Table 13. The most preferred Type II-S enzyme is *Fok*I.

[0072] When the preferred *FokI* containing URE is used, several conditions are preferably used to effect cleavage:

1) Excess of the URE over target DNA should be present to activate the enzyme. URE present only in equimolar amounts to the target DNA would yield poor cleavage of ssDNA because the amount of active enzyme available would be limiting.

2) An activator may be used to activate part of the *Fok*I enzyme to dimerize without causing cleavage. Examples of appropriate activators are shown in Table 14.

3) The cleavage reaction is performed at a temperature between 45˚-75˚C, preferably above 50˚C and most preferably above 55˚C.

[0073] The UREs used in the prior art contained a 14-base single-stranded segment, a 10-base stem (containing a *FokI* site), followed by the palindrome of the 10-base stem. While such UREs may be used in the methods of this invention, the preferred UREs of this invention also include a segment of three to eight bases (a loop) between the *FokI* restriction endonuclease recognition site containing segments. In the preferred embodiment, the stem (containing the *FokI* site) and its palindrome are also longer than 10 bases. Preferably, they are 10-14 bases in length. Examples of these "lollipop" URE adapters are shown in Table 15.

[0074] One example of using a URE to cleave an single-stranded DNA involves the FR3 region of human heavy chain. Table 16 shows an analysis of 840 full-length mature human heavy chains with the URE recognition sequences shown. The vast majority (718/840=0.85) will be recognized with 2 or fewer mismatches using five UREs (VHS881-1.1, VHS881-1.2, VHS881-2.1, VHS881-4.1, and VHS881-9.1). Each has a 20-base adaptor sequence to complement the germline gene, a ten-base stem segment containing a *Fok*I site, a five base loop, and the reverse complement of the first stem segment. Annealing those adapters, alone or in combination, to single-stranded antisense heavy chain DNA and treating with *Fok*I in the presence of, *e.g.*, the activator FOKIact, will lead to cleavage of the antisense strand at the position indicated.

[0075] Another example of using a URE(s) to cleave a single-stranded DNA involves the FR1 region of the human Kappa light chains. Table 17 shows an analysis of 182 full-length human kappa chains for matching by the four 19-base probe sequences shown. Ninety-six percent of the sequences match one of the probes with 2 or fewer mismatches. The URE adapters shown in Table 17 are for cleavage of the sense strand of kappa chains. Thus, the adaptor sequences are the reverse complement of the germline gene sequences. The URE consists of a ten-base stem, a five base loop, the reverse complement of the stem and the complementation sequence. The loop shown here is TTGTT, but other sequences could be used. Its function is to interrupt the palindrome of the stems so that formation of a lollypop monomer is favored over dimerization. Table 17 also shows where the sense strand is cleaved.

[0076] Another example of using a URE to cleave a single-stranded DNA involves the human lambda light chain. Table 18 shows analysis of 128 human lambda light chains for matching the four 19-base probes shown. With three or fewer mismatches, 88 of 128 (69%) of the chains match one of the probes. Table 18 also shows URE adapters corresponding to these probes. Annealing these adapters to upper-strand ssDNA of lambda chains and treatment with *Fok*I in the presence of FOKIact at a temperature at or above 45˚C will lead to specific and precise cleavage of the chains.

[0077] The conditions under which the short oligonucleotide sequences of the first method and the UREs of the second method are contacted with the single-stranded DNAs may be empirically determined. The conditions must be such that the single-stranded DNA remains in substantially single-stranded form. More particularly, the conditions must be such that the single-stranded DNA does not form loops that may interfere with its association with the oligonucleotide sequence or the URE or that may themselves provide sites for cleavage by the chosen restriction endonuclease.

[0078] The effectiveness and specificity of short oligonucleotides (first method) and UREs (second method) can be adjusted by controlling the concentrations of the URE adapters/oligonucleotides and substrate DNA, the temperature, the pH, the concentration of metal ions, the ionic strength, the concentration of chaotropes (such as urea and formamide), the concentration of the restriction endonuclease(*e.g.*, *Fok*I), and the time of the digestion. These conditions can be optimized with synthetic oligonucleotides having: 1) target germline gene sequences, 2) mutated target gene sequences, or 3) somewhat related non-target sequences. The goal is to cleave most of the target sequences and minimal amounts of non-targets.

[0079] In accordance with this invention, the single-stranded DNA is maintained in substantially that form using a temperature between about 37˚C and about 75˚C. Preferably, a temperature between about 45˚C and about 75˚C is

used. More preferably, a temperature between 50˚C and 60˚C, most preferably between 55˚C and 60˚C, is used. These temperatures are employed both when contacting the DNA with the oligonucleotide or URE and when cleaving the DNA using the methods of this invention.

[0080] The two cleavage methods of this invention have several advantages. The first method allows the individual members of the family of single-stranded DNAs to be cleaved preferentially at one substantially conserved endonuclease recognition site. The method also does not require an endonuclease recognition site to be built into the reverse transcription or amplification primers. Any native or synthetic site in the family can be used.

[0081] The second method has both of these advantages. In addition, the preferred URE method allows the single-stranded DNAs to be cleaved at positions where no endonuclease recognition site naturally occurs or has been synthetically constructed.

[0082] Most importantly, both cleavage methods permit the use of 5' and 3' primers so as to maximize diversity and then cleavage to remove unwanted or deleterious sequences before cloning, display and/or expression.

[0083] After cleavage of the amplified DNAs using one of the methods of this invention, the DNA is prepared for cloning, display and/or expression. This is done by using a partially duplexed synthetic DNA adapter, whose terminal sequence is based on the specific cleavage site at which the amplified DNA has been cleaved.

[0084] The synthetic DNA is designed such that when it is ligated to the cleaved single-stranded DNA in proper reading frame so that the desired peptide, polypeptide or protein can be displayed on the surface of the genetic package and/or expressed. Preferably, the double-stranded portion of the adapter comprises the sequence of several codons that encoded the amino acid sequence characteristic of the family of peptides, polypeptides or proteins up to the cleavage site. For human heavy chains, the amino acids of the 3-23 framework are preferably used to provide the sequences required for expression of the cleaved DNA.

[0085] Preferably, the double-stranded portion of the adapter is about 12 to 100 bases in length. More preferably, about 20 to 100 bases are used. The double-standard region of the adapter also preferably contains at least one endonuclease recognition site useful for cloning the DNA into a suitable display and/or expression vector (or a recipient vector used to archive the diversity). This endonuclease restriction site may be native to the germline gene sequences used to extend the DNA sequence. It may be also constructed using degenerate sequences to the native germline gene sequences. Or, it may be wholly synthetic.

[0086] The single-stranded portion of the adapter is complementary to the region of the cleavage in the single-stranded DNA. The overlap can be from about 2 bases up to about 15 bases. The longer the overlap, the more efficient the ligation is likely to be. A preferred length for the overlap is 7 to 10. This allows some mismatches in the region so that diversity in this region may be captured.

[0087] The single-stranded region or overlap of the partially duplexed adapter is advantageous because it allows DNA cleaved at the chosen site, but not other fragments to be captured. Such fragments would contaminate the library with genes encoding sequences that will not fold into proper antibodies and are likely to be non-specifically sticky.

[0088] One illustration of the use of a partially duplexed adaptor in the methods of this invention involves ligating such adaptor to a human FR3 region that has been cleaved, as described above, at 5'-ACnGT-3' using HpyCH4III, Bst4CI or TaaI.

[0089] Table 4 F.2 shows the bottom strand of the double-stranded portion of the adaptor for ligation to the cleaved bottom-strand DNA. Since the HpyCH4III-Site is so far to the right (as shown in Table 3), a sequence that includes the *Afl*II-site as well as the *XbaI* site can be added. This bottom strand portion of the partially-duplexed adaptor, H43.XAExt, incorporates both *Xba*I and *Afl*II-sites. The top strand of the double-stranded portion of the adaptor has neither site (due to planned mismatches in the segments opposite the *XbaI* and *Afl*II-Sites of H43.XAExt), but will anneal very tightly to H43.XAExt. H43AExt contains only the *Afl*II-site and is to be used with the top strands H43.ABr1 and H43.ABr2 (which have intentional alterations to destroy the *Afl*II-site).

[0090] After ligation, the desired, captured DNA can be PCR amplified again, if desired, using in the preferred embodiment a primer to the downstream constant region of the antibody gene and a primer to part of the double-standard region of the adapter. The primers may also carry restriction endonuclease sites for use in cloning the amplified DNA.

[0091] After ligation, and perhaps amplification, of the partially double-stranded adapter to the single-stranded amplified DNA, the composite DNA is cleaved at chosen 5' and 3' endonuclease recognition sites.

[0092] The cleavage sites useful for cloning depend on the phage or phagemid or other vectors into which the cassette will be inserted and the available sites in the antibody genes. Table 19 provides restriction endonuclease data for 75 human light chains. Table 20 shows corresponding data for 79 human heavy chains. In each Table, the endonucleases are ordered by increasing frequency of cutting. In these Tables, Nch is the number of chains cut by the enzyme and Ns is the number of sites (some chains have more than one site).

[0093] From this analysis, *Sfi*I, *Not*I, *Afl*II, *Apa*LI, and *Asc*I are very suitable. *Sfi*I and *Not*I are preferably used in pCES1 to insert the heavy-chain display segment. *Apa*LI and *Asc*I are preferably used in pCES1 to insert the light-chain display segment.

[0094] *Bst*EII-sites occur in 97% of germ-line JH genes. In rearranged V genes, only 54/79 (68%) of heavy-chain

genes contain a *Bst*EII-Site and 7/61 of these contain two sites. Thus, 47/79 (59%) contain a single *Bst*EII-Site. An alternative to using *Bst*EII is to cleave via UREs at the end of JH and ligate to a synthetic oligonucleotide that encodes part of CH1.

[0095] One example of preparing a family of DNA sequences using the methods of this invention involves capturing human CDR 3 diversity. As described above, mRNAs from various autoimmune patients are reverse transcribed into lower strand cDNA. After the top strand RNA is degraded, the lower strand is immobilized and a short oligonucleotide used to cleave the cDNA upstream of CDR3. A partially duplexed synthetic DNA adapter is then annealed to the DNA and the DNA is amplified using a primer to the adapter and a primer to the constant region (after FR4). The DNA is then cleaved using *BstEII* (in FR4) and a restriction endonuclease appropriate to the partially double-stranded adapter (*e.g.*, *XbaI* and *AflII* (in FR3)). The DNA is then ligated into a synthetic VH skeleton such as 3-23.

[0096] One example of preparing a single-stranded DNA that was cleaved using the URE method involves the human Kappa chain. The cleavage site in the sense strand of this chain is depicted in Table 17. The oligonucleotide kapextURE is annealed to the oligonucleotides (kaBR01UR, kaBR02UR, kaBR03UR, and kaBR04UR) to form a partially duplex DNA. This DNA is then ligated to the cleaved soluble kappa chains. The ligation product is then amplified using primers kapextUREPCR and CKForeAsc (which inserts a AscI site after the end of C kappa). This product is then cleaved with *Apa*LI and *Asc*I and ligated to similarly cut recipient vector.

[0097] Another example involves the cleavage of lambda light chains, illustrated in Table 18. After cleavage, an extender (on_LamEx133) and four bridge oligonucleotides (ON_LamB1-133, ON-LamB2-133, ON_LamB3-133, and ON-LamB4-133) are annealed to form a partially duplex DNA. That DNA is ligated to the cleaved lambda-chain sense strands. After ligation, the DNA is amplified with ON_Lam133PCR and a forward primer specific to the lambda constant domain, such as CL2ForeAsc or CL7ForeAsc (Table 130).

[0098] In human heavy chains, one can cleave almost all genes in FR4 (downstream, *i.e.*, toward the 3' end of the sense strand, of CDR3) at a *Bst*EII-Site that occurs at a constant position in a very large fraction of human heavy-chain V genes. One then needs a site in FR3, if only CDR3 diversity is to be captured, in FR2, if CDR2 and CDR3 diversity is wanted, or in FR1, if all the CDR diversity is wanted. These sites are preferably inserted as part of the partially double-stranded adaptor.

[0099] The present disclosure also relates to recipient vectors (*e.g.*, for display and/or expression) having sites that allow cloning of either light or heavy chains. Such vectors are well known and widely used in the art. A preferred phage display vector in accordance with this invention is phage MALIA3. This displays in gene III. The sequence of the phage MALIA3 is shown in Table 21A (annotated) and Table 21B (condensed).

[0100] The DNA encoding the selected regions of the light or heavy chains can be transferred to the vectors using endonucleases that cut either light or heavy chains only very rarely. For example, light chains may be captured with *Apa*LI and *Asc*I. Heavy-chain genes are preferably cloned into a recipient vector having *Sfi*I, *Nco*I, *Xba*I, *Afl*II, *Bst*EII, *Apa*I, and *Not*I sites. The light chains are preferably moved into the library as *Apa*LI-*Asc*I fragments. The heavy chains are preferably moved into the library as *Sfi*I-*Not*I fragments.

[0101] Most preferably, the display is had on the surface of a derivative of M13 phage. The most preferred vector contains all the genes of M13, an antibiotic resistance gene, and the display cassette. The preferred vector is provided with restriction sites that allow introduction and excision of members of the diverse family of genes, as cassettes. The preferred vector is stable against rearrangement under the growth conditions used to amplify phage.

[0102] In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a phagemid vector (*e.g.*, pCES1) that displays and/or expresses the peptide, polypeptide or protein. Such vectors may also be used to store the diversity for subsequent display and/or expression using other vectors or phage.

[0103] In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed in a yeast vector.

[0104] In another embodiment, the mode of display may be through a short linker to anchor domains -- one possible anchor comprising the final portion of M13 III ("IIIstump") and a second possible anchor being the full length III mature protein.

[0105] The IIIstump fragment contains enough of M13 III to assemble into phage but not the domains involved in mediating infectivity. Because the w.t. III proteins are present the phage is unlikely to delete the antibody genes and phage that do delete these segments receive only a very small growth advantage. For each of the anchor domains, the DNA encodes the w.t. AA sequence, but differs from the w.t. DNA sequence to a very high extent. This will greatly reduce the potential for homologous recombination between the anchor and the w.t. gene that is also present (see Example 6).

[0106] Most preferably, the present invention uses a complete phage carrying an antibiotic-resistance gene (such as an ampicillin-resistance gene) and the display cassette. Because the w.t. *iii* and possibly *viii* genes are present, the w.t. proteins are also present. The display cassette is transcribed from a regulatable promoter (*e.g.*, P_{Lacz}). Use of a regulatable promoter allows control of the ratio of the fusion display gene to the corresponding w.t. coat protein. This ratio determines the average number of copies of the display fusion per phage (or phagemid) particle.

[0107] In another embodiment of the methods of this invention, the phage or phagemid can display and/or express proteins other than Fab, by replacing the Fab portions indicated above, with other protein genes.

[0108] Various hosts can be used the display and/or expression aspect of this invention. Such hosts are well known in the art. In the preferred embodiment, where Fabs are being displayed and/or expressed, the - preferred host should grow at 30˚C and be RecA- (to reduce unwanted genetic recombination) and EndA- (to make recovery of RF DNA easier). It is also preferred that the host strain be easily transformed by electroporation.

[0109] XL1-Blue MRF' satisfies most of these preferences, but does not grow well at 30˚C. XL1-Blue MRF' does grow slowly at 38˚C and thus is an acceptable host. TG-1 is also an acceptable host although it is RecA* and EndA*. XL1-Blue MRF' is more preferred for the intermediate host used to accumulate diversity prior to final construction of the library.

[0110] After display and/or expression, the libraries of this invention may be screened using well known and conventionally used techniques. The selected peptides, polypeptides or proteins may then be used to treat disease. Generally, the peptides, polypeptides or proteins for use in therapy or in pharmaceutical compositions are produced by isolating the DNA encoding the desired peptide, polypeptide or protein from the member of the library selected. That DNA is then used in conventional methods to produce the peptide, polypeptides or protein it encodes in appropriate host cells, preferably mammalian host cells, *e.g.*, CHO cells. After isolation, the peptide, polypeptide or protein is used alone or with pharmaceutically acceptable compositions in therapy to treat disease.

EXAMPLES

Example 1: RACE amplification of heavy and light chain antibody repertoires from autoimmune patients.

[0111] Total RNA was isolated from individual blood samples (50 ml) of 11 patients using a RNAzolTM kit (CINNA/Biotecx), as described by the manufacturer. The patients were diagnosed as follows:

1. SLE and phospholipid syndrome
2. limited systemic sclerosis
3. SLE and Sjogren syndrome
4. Limited Systemic sclerosis
5. Reumatoid Arthritis with active vasculitis
6. Limited systemic sclerosis and Sjogren Syndrome
7. Reumatoid Artritis and (not active) vasculitis
8. SLE and Sjogren syndrome
9. SLE
10. SLE and (active) glomerulonephritis
11. Polyarthritis/ Raynauds Phenomen

From these 11 samples of total RNA, Poly-A+ RNA was isolated using Promega PolyATtract® mRNA Isolation kit (Promega).

[0112] 250 ng of each poly-A+ RNA sample was used to amplify antibody heavy and light chains with the GeneRAacerTM kit (Invitrogen cat no. L1500-01). A schematic overview of the RACE procedure is shown in FIG. 3.

[0113] Using the general protocol of the GeneRAacer™ kit, an RNA adaptor was ligated to the 5'end of all mRNAs. Next, a reverse transcriptase reaction was performed in the presence of oligo(dT15) specific primer under conditions described by the manufacturer in the GeneRAacer™ kit.

[0114] 1/5 of the cDNA from the reverse transcriptase reaction was used in a 20 ul PCR reaction. For amplification of the heavy chain IgM repertoire, a forward primer based on the CH1 chain of IgM [HuCmFOR] and a backward primer based on the ligated synthetic adaptor sequence [5'A] were used. (See Table 22)

[0115] For amplification of the kappa and lambda light chains, a forward primer that contains the 3' coding-end of the cDNA [HuCkFor and HuCLFor2+HuCLfor7] and a backward primer based on the ligated synthetic adapter sequence [5'A] was used (See Table 22). Specific amplification products after 30 cycles of primary PCR were obtained.

[0116] FIG. 4 shows the amplification products obtained after the primary PCR reaction from 4 different patient samples. 8 ul primary PCR product from 4 different patients was analyzed on a agarose gel [labeled 1,2, 3 and 4]. For the heavy chain, a product of approximately 950 nt is obtained while for the kappa and lambda light chains the product is approximately 850 nt. M1-2 are molecular weight markers.

[0117] PCR products were also analyzed by DNA sequencing [10 clones from the lambda, kappa or heavy chain repertoires]. All sequenced antibody genes recovered contained the full coding sequence as well as the 5' leader sequence and the V gene diversity was the expected diversity (compared to literature data).

[0118] 50 ng of all samples from all 11 individual amplified samples were mixed for heavy, lambda light or kappa light chains and used in secondary PCR reactions.

[0119] In all secondary PCRs approximately 1 ng template DNA from the primary PCR mixture was used in multiple 50 ul PCR reactions [25 cycles].

[0120] For the heavy chain, a nested biotinylated forward primer [HuCm-Nested] was used, and a nested 5'end backward primer located in the synthetic adapter-sequence [5'NA] was used. The 5'end lower-strand of the heavy chain was biotinylated.

[0121] For the light chains, a 5'end biotinylated nested primer in the synthetic adapter was used [5'NA] in combination with a 3'end primer in the constant region of Ckappa and Clambda, extended with a sequence coding for the AscI restriction site [kappa: HuCkForAscI, Lambda: HuCL2-FOR-ASC + HuCL7-FOR-ASC]. [5'end Top strand DNA was biotinylated]. After gel-analysis the secondary PCR products were pooled and purified with Promega Wizzard PCR cleanup. Approximately 25 ug biotinylated heavy chain, lambda and kappa light chain DNA was isolated from the 11 patients.

Example 2: Capturing kappa chains with BsmAI.

[0122] A repertoire of human-kappa chain mRNAs was prepared using the RACE method of Example 1 from a collection of patients having various autoimmune diseases.

[0123] This Example followed the protocol of Example 1. Approximately 2 micrograms (ug) of human kappa-chain (Igkappa) gene RACE material with biotin attached to 5'-end of upper strand was immobilized as in Example 1 on 200 microliters (μL) of Seradyn magnetic beads. The lower strand was removed by washing the DNA with 2 aliquots 200 μL of 0.1 M NaOH (pH 13) for 3 minutes for the first aliquot followed by 30 seconds for the second aliquot. The beads were neutralized with 200 μL of 10 mM Tris (pH 7.5) 100 mM NaCl. The short oligonucleotides shown in Table 23 were added in 40 fold molar excess in 100 μL of NEB buffer 2 (50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol pH 7.9) to the dry beads. The mixture was incubated at 95˚C for 5 minutes then cooled down to 55˚C over 30 minutes. Excess oligonucleotide was washed away with 2 washes of NEB buffer 3 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol pH 7.9). Ten units of BsmAI (NEB) were added in NEB buffer 3 and incubated for 1 h at 55˚C. The cleaved downstream DNA was collected and purified over a Qiagen PCR purification column (FIGs. 5 and 6).

[0124] FIG. 5 shows an analysis of digested kappa single-stranded DNA. Approximately 151.5 pmol of adapter was annealed to 3.79 pmol of immobilized kappa single-stranded DNA followed by digestion with 15 U of *BsmAI*. The supernatant containing the desired DNA was removed and analyzed by 5% polyacrylamide gel along with the remaining beads which contained uncleaved full length kappa DNA. 189 pmol of cleaved single-stranded DNA was purified for further analysis. Five percent of the original full length ssDNA remained on the beads.

[0125] FIG. 6 shows an analysis of the extender - cleaved kappa ligation. 180 pmol of pre-annealed bridge/extender was ligated to 1.8 pmol of *BsmAI* digested single-stranded DNA. The ligated DNA was purified by Qiagen PCR purification column and analyzed on a 5% polyacrylamide gel. Results indicated that the ligation of extender to single-stranded DNA was 95% efficient.

[0126] A partially double-stranded adaptor was prepared using the oligonucleotide shown in Table 23. The adaptor was added to the single-stranded DNA in 100 fold molar excess along with 1000 units of T4 DNA ligase and incubated overnight at 16˚C. The excess oligonucleotide was removed with a Qiagen PCR purification column. The ligated material was amplified by PCR using the primers kapPCRt1 and kapfor shown in Table 23 for 10 cycles with the program shown in Table 24.

[0127] The soluble PCR product was run on a gel and showed a band of approximately 700 n, as expected (FIGs. 7 and 8). The DNA was cleaved with enzymes *Apa*LI and *Asc*I, gel purified, and ligated to similarly cleaved vector pCES1.

[0128] FIG. 7 shows an analysis of the PCR product from the extender-kappa amplification. Ligated extender-kappa single-stranded DNA was amplified with primers specific to the extender and to the constant region of the light chain. Two different template concentrations, 10 ng versus 50 ng, were used as template and 13 cycles were used to generate approximately 1.5 ug of dsDNA as shown by 0.8% agarose gel analysis.

[0129] FIG. 8 shows an analysis of the purified PCR product from the extender-kappa amplification. Approximately 5 ug of PCR amplified extender-kappa double-stranded DNA was run out on a 0.8% agarose gel, cut out, and extracted with a GFX gel purification column. By gel analysis, 3.5 ug of double-stranded DNA was prepared.

[0130] The assay for capturing kappa chains with BsmA1 was repeated and produced similar results. FIG 9A shows the DNA after it was cleaved and collected and purified over a Qiagen PCR purification column. FIG. 9B shows the partially double-stranded adaptor ligated to the single-stranded DNA. This ligated material was then amplified (FIG. 9C). The gel showed a band of approximately 700 n.

[0131] Table 25 shows the DNA sequence of a kappa light chain captured by this procedure. Table 26 shows a second sequence captured by this procedure. The closest bridge sequence was complementary to the sequence 5'-agccacc-3', but the sequence captured reads 5'-Tgccacc-3', showing that some mismatch in the overlapped region is tolerated.

Example 3: Construction of Synthetic CDR1 and CDR2 Diversity in V-3-23 VH Framework.

[0132] Synthetic diversity in Complementary Determinant Region (CDR) 1 and 2 was created in the 3-23 VH framework in a two step process: first, a vector containing the 3-23 VH framework was constructed; and then, a synthetic CDR 1 and 2 was assembled and cloned into this vector.

[0133] For construction of the 3-23 VH framework, 8 oligonucleotides and two PCR primers (long oligonucleotides - TOPFR1A, BOTFR1B, BOTFR2, BOTFR3, F06, BOTFR4, ON-vgCl, and ON-vgC2 and primers - SFPRMET and BOT-PCRPRIM, shown in Table 27) that overlap were designed based on the Genebank sequence of 3-23 VH framework region. The design incorporated at least one useful restriction site in each framework region, as shown in Table 27. In Table 27, the segments that were synthesized are shown as bold, the overlapping regions are underscored, and the PCR priming regions at each end are underscored.

[0134] A mixture of these 8 oligos was combined at a final concentration of 2.5uM in a 20ul PCR reaction. The PCR mixture contained 200uM dNTPs, 2.5mM $MgCl_2$, 0.02U *Pfu Turbo*™ DNA Polymerase, 1U Qiagen HotStart Taq DNA Polymerase, and 1X Qiagen PCR buffer. The PCR program consisted of 10 cycles of 94˚C for 30s, 55˚C for 30s, and 72˚C for 30s.

[0135] The assembled 3-23 VH DNA sequence was then amplified, using 2.5ul of a 10-fold dilution from the initial PCR in 100ul PCR reaction. The PCR reaction contained 200uM dNTPs, 2.5mM $MgCl_2$, 0.02U *Pfu Turbo*™ DNA Polymerase, 1U Qiagen HotStart Taq DNA Polymerase, 1X Qiagen PCR Buffer and 2 outside primers (SFPRMET and BOT-PCRPRIM) at a concentration of 1uM. The PCR program consisted of 23 cycles at 94˚C for 30s, 55˚C for 30s, and 72˚C for 60s. The 3-23 VH DNA sequence was digested and cloned into pCES1 (phagemid vector) using the *Sfi*I and *Bst*EII restriction endonuclease sites. All restriction enzymes mentioned herein were supplied by New England BioLabs, Beverly, MA and used as per the manufacturer's instructions.

[0136] Stuffer sequences (shown in Table 28 and Table 29) were introduced into pCES1 to replace CDR1/CDR2 sequences (900 bases between BspEI and *Xba*I RE sites) and CDR3 sequences (358 bases between *Afl*II and *Bst*EII) prior to cloning the CDR1/CDR2 diversity. This new vector was termed pCES5 and its sequence is given in Table 29.

[0137] Having stuffers in place of the CDRs avoids the risk that a parental sequence would be over-represented in the library. The stuffer sequences are fragments from the penicillase gene of *E. coli.* The CDR1-2 stuffer contains restriction sites for *Bg1*II, *Bsu*36I, *Bcl*I, *Xcm*I, *Mlu*I, *Pvu*II, *Hpa*I, and *Hinc*II, the underscored sites being unique within the vector pCES5. The stuffer that replaces CDR3 contains the unique restriction endonuclease site *Rsr*II.

[0138] A schematic representation of the design for CDR1 and CDR2 synthetic diversity is shown FIG. 10. The design was based on the presence of mutations in DP47/3-23 and related germline genes. Diversity was designed to be introduced at the positions within CDR1 and CDR2 indicated by the numbers in FIG. 10. The diversity at each position was chosen to be one of the three following schemes: 1 = ADEFGHIKLMNPQRSTVWY; 2 = YRWVGS; 3 = PS, in which letters encode equimolar mixes of the indicated amino acids.

[0139] For the construction of the CDR1 and CDR2 diversity, 4 overlapping oligonucleotides (ON-vgC1, ON_Br12, ON_CD2Xba, and ON-vgC2, shown in Table 27 and Table 30) encoding CDR1/2, plus flanking regions, were designed. A mixture of these 4 oligos was combined at a final concentration of 2.5uM in a 40ul PCR reaction. Two of the 4 oligos contained variegated sequences positioned at the CDR1 and the CDR2. The PCR mixture contained 200uM dNTPs, 2.5U Pwo DNA Polymerase (Roche), and 1X Pwo PCR buffer with 2mM $MgSO_4$. The PCR program consisted of 10 cycles at 94˚C for 30s, 60˚C for 30s, and 72˚C for 60s. This assembled CDR1/2 DNA sequence was amplified, using 2.5ul of the mixture in 100ul PCR reaction. The PCR reaction contained 200uM dNTPs, 2.5U Pwo DNA Polymerase, 1X Pwo PCR Buffer with 2mM $MgSO_4$ and 2 outside primers at a concentration of 1uM. The PCR program consisted of 10 cycles at 94˚C for 30s, 60˚C for 30s, and 72˚C for 60s. These variegated sequences were digested and cloned into the 3-23 VH framework in place of the CDR1/2 stuffer.

[0140] We obtained approximately 7 X 10^7 independent transformants. CDR3 diversity either from donor populations or from synthetic DNA can be cloned into the vector containing synthetic CDR1 and CDR 2 diversity.

[0141] A schematic representation of this procedure is shown in FIG. 11. A sequence encoding the FR-regions of the human V3-23 gene segment and CDR regions with synthetic diversity was made by oligonucleotide assembly and cloning via *BspE1* and *Xba1* sites into a vector that complements the FR1 and FR3 regions. Into this library of synthetic VH segments, the complementary VH-CDR3 sequence (top right) was cloned via XbaI an BstEII sites. The resulting cloned CH genes contain a combination of designed synthetic diversity and natural diversity (see FIG. 11).

Example 4: Cleavage and ligation of the lambda light chains with HinfI.

[0142] A schematic of the cleavage and ligation of antibody light chains is shown in FIGS. 12A and 12B. Approximately 2 ug of biotinylated human Lambda DNA prepared as described in Example 1 was immobilized on 200 ul Seradyn magnetic beads. The lower strand was removed by incubation of the DNA with 200 ul of 0.1 M NaOH (pH=13) for 3 minutes, the supernatant was removed and an additional washing of 30 seconds with 200 ul of 0.1 M NaOH was

performed. Supernatant was removed and the beads were neutralized with 200 ul of 10 mM Tris (pH=7.5), 100 mM NaCl. 2 additional washes with 200 ul NEB2 buffer 2, containing 10 mM Tris (pH=7.9), 50 mM NaCl, 10 mM MgCl2 and 1 mM dithiothreitol, were performed. After immobilization, the amount of ssDNA was estimated on a 5% PAGE-UREA gel.

[0143] About 0.8 ug ssDNA was recovered and incubated in 100 ul NEB2 buffer 2 containing 80 molar fold excess of an equimolar mix of ON_LamlaB7, ON_Lam2aB7, ON Lam31B7 and ON_Lam3rB7 [each oligo in 20 fold molar excess] (see Table 31).

[0144] The mixture was incubated at 95˚ C for 5 minutes and then slowly cooled down to 50˚ C over a period of 30 minutes. Excess of oligonucleotide was washed away with 2 washes of 200 ul of NEB buffer 2. 4 U/ug of *Hinf I* was added and incubated for 1 hour at 50˚ C. Beads were mixed every 10 minutes.

[0145] After incubation the sample was purified over a Qiagen PCR purification column and was subsequently analysed on a 5% PAGE-urea gel (see FIG. 13A, cleavage was more than 70% efficient).

[0146] A schematic of the ligation of the cleaved light chains is shown in FIG. 12B. A mix of bridge/extender pairs was prepared from the Brg/Ext oligo's listed in Table 31 (total molar excess 100 fold) in 1000 U of T4 DNA Ligase (NEB) and incubated overnight at 16˚ C. After ligation of the DNA, the excess oligonucleotide was removed with a Qiagen PCR purification column and ligation was checked on a Urea-PAGE gel (see FIG. 13B; ligation was more than 95% efficient).

[0147] Multiple PCRs were performed containing 10 ng of the ligated material in an 50 ul PCR reaction using 25 pMol ON lamPlePCR and 25 pmol of an equimolar mix of Hu-CL2AscI/HuCL7AscI primer (see Example 1).

[0148] PCR was performed at 60˚ C for 15 cycles using Pfu polymerase. About 1 ug of dsDNA was recovered per PCR (see FIG. 13C) and cleaved with *ApaL1* and *AscI* for cloning the lambda light chains in pCES2.

Example 5: Capture of human heavy-chain CDR3 population.

[0149] A schematic of the cleavage and ligation of antibody light chains is shown in FIGs. 14A and 14B.

[0150] Approximately 3 ug of human heavy-chain (IgM) gene RACE material with biotin attached to 5'-end of lower strand was immobilized on 300 uL of Seradyn magnetic beads. The upper strand was removed by washing the DNA with 2 aliquots 300 uL of 0.1 M NaOH (pH 13) for 3 minutes for the first aliquot followed by 30 seconds for the second aliquot. The beads were neutralized with 300 uL of 10 mM Tris (pH 7.5) 100 mM NaCl. The REdaptors (oligonucleotides used to make single-stranded DNA locally double-stranded) shown in Table 32 were added in 30 fold molar excess in 200 uL of NEB buffer 4 (50 mM Potasium Acetate, 20 mM Tris-Acetate, 10 mM Magnesuim Acetate, 1 mM dithiothreitol pH 7.9) to the dry beads. The REadaptors were incubated with the single-stranded DNA at 80 ˚C for 5 minutes then cooled down to 55 ˚C over 30 minutes. Excess REdaptors were washed away with 2 washes of NEB buffer 4. Fifteen units of HpyCH4III (NEB) were added in NEB buffer 4 and incubated for 1 hour at 55 ˚C. The cleaved downstream DNA remaining on the beads was removed from the beads using a Qiagen Nucleotide removal column (see FIG. 15).

[0151] The Bridge/Extender pairs shown in Table 33 were added in 25 molar excess along with 1200 units of T4 DNA ligase and incubated overnight at 16 ˚C. Excess Bridge/Extender was removed with a Qiagen PCR purification column. The ligated material was amplified by PCR using primers H43.XAExtPCR2 and Hucumnest shown in Table 34 for 10 cycles with the program shown in Table 35.

[0152] The soluble PCR product was run on a gel and showed a band of approximately 500 n, as expected (see FIG. 15B). The DNA was cleaved with enzymes SfiI and *NotI*, gel purified, and ligated to similarly cleaved vector PCES1.

Example 6: Description of Phage Display Vector CJRA05, a member of the library built in vector DY3F7.

[0153] Table 36 contains an annotated DNA sequence of a member of the library, CJRA05, see FIG. 16. Table 36 is to be read as follows: on each line everything that follows an exclamation mark "!" is a comment. All occurrences of A, C, G, and T before "!" are the DNA sequence. Case is used only to show that certain bases constitute special features, such as restriction sites, ribosome binding sites, and the like, which are labeled below the DNA. CJRA05 is a derivative of phage DY3F7, obtained by cloning an *ApaLI* to *NotI* fragment into these sites in DY3F31. DY3F31 is like DY3F7 except that the light chain and heavy chain genes have been replaced by "stuffer" DNA that does not code for any antibody. DY3F7 contains an antibody that binds streptavidin, but did not come from the present library.

[0154] The phage genes start with gene ii and continue with genes x, v, vii, ix, viii, iii, vi, i, and iv. Gene iii has been slightly modified in that eight codons have been inserted between the signal sequence and the mature protein and the final amino acids of the signal sequence have been altered. This allows restriction enzyme recognition sites *EagI* and *XbaI* to be present. Following gene iv is the phage origin of replication (ori). After ori is bla which confers resistance to ampicillin (ApR). The phage genes and bla are transcribed in the same sense.

[0155] After bla, is the Fab cassette (illustrated in FIG. 17) comprising:

a) PlacZ promoter,
b) A first Ribosome Binding Site (RBS1),

c) The signal sequence form M13 iii,
d) An *ApaLI* RERS,
e) A light chain (a kappa L20::JK1 shortened by one codon at the V-J boundary in this case),
f) An *AscI* RERS,
g) A second Ribosome Binding Site (RBS2),
h) A signal sequence, preferably PelB, which contains,
i) An *SfiI* RERS,
j) A synthetic 3-23 V region with diversity in CDR1 and CDR2,
k) A captured CDR3,
l) A partially synthetic J region (FR4 after BstEII),
m) CH1,
n) A NotI RERS,
o) A His6 tag,
p) A cMyc tag,
q) An amber codon,
r) An anchor DNA that encodes the same amino-acid sequence as codons 273 to 424 of M13 iii (as shown in Table 37).
s) Two stop codons,
t) An *AvrII* RERS, and
u) A trp terminator.

[0156] The anchor (item r) encodes the same amino-acid sequence as do codons 273 to 424 of M13 iii but the DNA is approximately as different as possible from the wild-type DNA sequence. In Table 36, the III' stump runs from base 8997 to base 9455. Below the DNA, as comments, are the differences with wild-type iii for the comparable codons with "!W.T" at the ends of these lines. Note that Met and Trp have only a single codon and must be left as is. These AA types are rare. Ser codons can be changed at all three base, while Leu and Arg codons can be changed at two.

[0157] In most cases, one base change can be introduced per codon. This has three advantages: 1) recombination with the wild-type gene carried elsewhere on the phage is less likely, 2) new restriction sites can be introduced, facilitating construction; and 3) sequencing primers that bind in only one of the two regions can be designed.

[0158] The fragment of M13 III shown in CJRA05 is the preferred length for the anchor segment. Alternative longer or shorter anchor segments defined by reference to whole mature III protein may also be utilized.

[0159] The sequence of M13 III consists of the following elements: Signal Sequence::Domain 1 (D1)::Linker 1 (L1):: Domain 2 (D2)::Linker 2 (L2)::Domain 3 (D3)::Transmembrane Segment (TM):: Intracellular anchor (IC) (see Table 38).

[0160] The pIII anchor (also known as trpIII) preferably consists of D2::L2::D3::TM::IC. Another embodiment for the pIII anchor consists of D2'::L2::D3::TM::IC (where D2' comprises the last 21 residues of D2 with the first 109 residues deleted). A further embodiment of the pIII anchor consists of D2'(C>S)::L2::D3::TM::IC (where D2'(C>S) is D2' with the single C converted to S), and d) D3::TM::IC.

[0161] Table 38 shows a gene fragment comprising the *NotI* site, His6 tag, cMyc tag, an amber codon, a recombinant enterokinase cleavage site, and the whole of mature M13 III protein. The DNA used to encode this sequence is intentionally very different from the DNA of wild-type gene iii as shown by the lines denoted "W.T." containing the w.t. bases where these differ from this gene. III is divided into domains denoted "domain 1", "linker 1", "domain 2", "linker 2", "domain 3", "transmembrane segment", and "intracellular anchor".

[0162] Alternative preferred anchor segments (defined by reference to the sequence of Table 38) include:

codons 1-29 joined to codons 104-435, deleting domain 1 and retaining linker 1 to the end;
codons 1-38 joined to codons 104-435, deleting domain land retaining the rEK cleavage site plus linker 1 to the end from III;
codons 1-29 joined to codons 236-435, deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end;
codons 1-38 joined to codons 236-435, deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end and the rEK cleavage site;
codons 1-29 joined to codons 236-435 and changing codon 240 to Ser(*e.g.*, agc), deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end; and
codons 1-38 joined to codons 236-435 and changing codon 240 to Ser(*e.g.*, agc), deleting domain 1, linker 1, and most of domain 2 and retaining linker 2 to the end and the rEK cleavage site.

[0163] The constructs would most readily be made by methods similar to those of Wang and Wilkinson (Biotechniques 2001: 31(4)722-724) in which PCR is used to copy the vector except the part to be deleted and matching restriction sites are introduced or retained at either end of the part to be kept. Table 39 shows the oligonucleotides to be used in

deleting parts of the III anchor segment. The DNA shown in Table 38 has an *NheI* site before the DINDDRMA recombinant enterokinase cleavage site (rEKCS). If *NheI* is used in the deletion process with this DNA, the rEKCS site would be lost. This site could be quite useful in cleaving Fabs from the phage and might facilitate capture of very high-afffinity antibodies. One could mutagenize this sequence so that the *NheI* site would follow the rEKCS site, an Ala Ser amino-acid sequence is already present. Alternatively, one could use *SphI* for the deletions. This would involve a slight change in amino acid sequence but would be of no consequence.

Example 7 : Selection of antigen binders from an enriched library of human antibodies using phage vector DY3F31.

[0164] In this example the human antibody library used is described in de Haard et al., (Journal of Biological Chemistry, 274 (26): 18218-30 (1999). This library, consisting of a large non-immune human Fab phagemid library, was first enriched on antigen, either on streptavidin or on phenyl-oxazolone (phOx). The methods for this are well known in the art. Two preselected Fab libraries, the first one selected once on immobilized phOx-BSA (R1-ox) and the second one selected twice on streptavidin (R2-strep), were chosen for recloning.

[0165] These enriched repertoires of phage antibodies, in which only a very low percentage have binding activity to the antigen used in selection, were confirmed by screening clones in an ELISA for antigen binding. The selected Fab genes were transferred from the phagemid vector of this library to the DY3F31 vector via *ApaL1-Not1* restriction sites.

[0166] DNA from the DY3F31 phage vector was pretreated with ATP dependent DNAse to remove chromosomal DNA and then digested with *ApaL1* and *Not1*. An extra digestion with *AscI* was performed in between to prevent self-ligation of the vector. The *ApaL1*/*NotI* Fab fragment from the preselected libraries was subsequently ligated to the vector DNA and transformed into competent XL1-blue MRF' cells.

[0167] Libraries were made using vector:insert ratios of 1:2 for phOx-library and 1:3 for STREP library, and using 100 ng ligated DNA per 50 μl of electroporation-competent cells (electroporation conditions : one shock of 1700 V, 1 hour recovery of cells in rich SOC medium, plating on amplicillin-containing agar plates).

[0168] This transformation resulted in a library size of 1.6×10^6 for R1-ox in DY3F31 and 2.1×10^6 for R2-strep in DY3F31. Sixteen colonies from each library were screened for insert, and all showed the correct size insert (±1400 bp) (for both libraries).

[0169] Phage was prepared from these Fab libraries as follows. A representative sample of the library was inoculated in medium with ampicillin and glucose, and at OD 0.5, the medium exchanged for ampicillin and 1 mM IPTG. After overnight growth at 37 ˚C, phage was harvested from the supernatant by PEG-NaCl precipitation. Phage was used for selection on antigen. R1-ox was selected on phOx-BSA coated by passive adsorption onto immunotubes and R2-strep on streptavidin coated paramagnetic beads (Dynal, Norway), in procedures described in de Haard et. al. and Marks et. al., Journal of Molecular Biology, 222(3): 581-97 (1991). Phage titers and enrichments are given in Table 40.

[0170] Clones from these selected libraries, dubbed R2-ox and R3-strep respectively, were screened for binding to their antigens in ELISA. 44 clones from each selection were picked randomly and screened as phage or soluble Fab for binding in ELISA. For the libraries in DY3F31, clones were first grown in 2TY-2% glucose-50 μg/ml AMP to an OD600 of approximately 0.5, and then grown overnight in 2TY-50 μg/ml AMP +/- 1mM IPTG. Induction with IPTG may result in the production of both phage-Fab and soluble Fab. Therefore the (same) clones were also grown without IPTG. Table 41 shows the results of an ELISA screening of the resulting supernatant, either for the detection of phage particles with antigen binding (Anti-M13 HRP = anti-phage antibody), or for the detection of human Fabs, be it on phage or as soluble fragments, either with using the anti-myc antibody 9E10 which detects the myc-tag that every Fab carries at the C-terminal end of the heavy chain followed by a HRP-labeled rabbit-anti-Mouse serum (column 9E10/RAM-HRP), or with anti-light chain reagent followed by a HRP-labeled goat-anti-rabbit antiserum(anti-CK/CL Gar-HRP).

[0171] The results shows that in both cases antigen-binders are identified in the library, with as Fabs on phage or with the anti-Fab reagents (Table 41). IPTG induction yields an increase in the number of positives. Also it can be seen that for the phOx-clones, the phage ELISA yields more positives than the soluble Fab ELISA, most likely due to the avid binding of phage. Twenty four of the ELISA-positive clones were screened using PCR of the Fab-insert from the vector, followed by digestion with *BstNI.* This yielded 17 different patterns for the phOx-binding Fab's in 23 samples that were correctly analyzed, and 6 out of 24 for the streptavidin binding clones. Thus, the data from the selection and screening from this pre-enriched non-immune Fab library show that the DY3F31 vector is suitable for display and selection of Fab fragments, and provides both soluble Fab and Fab on phage for screening experiments after selection.

Example 8: Selection of Phage-antibody libraries on streptavidin magnetic beads.

[0172] The following example describes a selection in which one first depletes a sample of the library of binders to streptavidin and optionally of binders to a non-target (*i.e.*, a molecule other than the target that one does not want the selected Fab to bind). It is hypothesized that one has a molecule, termed a "competitive ligand", which binds the target

and that an antibody which binds at the same site would be especially useful.

[0173] For this procedure Streptavidin Magnetic Beads (Dynal) were blocked once with blocking solution (2% Marvel Milk, PBS (pH 7.4), 0.01% Tween-20 ("2%MPBST")) for 60 minutes at room temperature and then washed five times with 2%MPBST. 450 μL of beads were blocked for each depletion and subsequent selection set.

[0174] Per selection, 6.25 μL of biotinylated depletion target (1 mg/mL stock in PBST) was added to 0.250 mL of washed, blocked beads (from step 1). The target was allowed to bind overnight, with tumbling, at 4˚C. The next day, the beads are washed 5 times with PBST.

[0175] Per selection, 0.010 mL of biotinylated target antigen (1 mg/mL stock in PBST) was added to 0.100 mL of blocked and washed beads (from step 1). The antigen was allowed to bind overnight, with tumbling, at 4˚C. The next day, the beads were washed 5 times with PBST.

[0176] In round 1, 2 X 10^{12} up to 10^{13} plaque forming units (pfu) per selection were blocked against non-specific binding by adding to 0.500 mL of 2%MPBS (=2%MPBST without Tween) for 1 hr at RT (tumble). In later rounds, 1011 pfu per selection were blocked as done in round 1.

[0177] Each phage pool was incubated with 50 μL of depletion target beads (final wash supernatant removed just before use) on a Labquake rotator for 10 min at room temperature. After incubation, the phage supernatant was removed and incubated with another 50 μL of depletion target beads. This was repeated 3 more times using depletion target beads and twice using blocked streptavidin beads for a total of 7 rounds of depletion, so each phage pool required 350 μL of depletion beads.

[0178] A small sample of each depleted library pool was taken for titering. Each library pool was added to 0.100 mL of target beads (final wash supernatant was removed just before use) and allowed to incubate for 2 hours at room temperature (tumble).

[0179] Beads were then washed as rapidly as possible (e.g.,3 minutes total) with 5 X 0.500 mL PBST and then 2X with PBS. Phage still bound to beads after the washing were eluted once with 0.250 mL of competitive ligand (~1 μμM) in PBST for 1 hour at room temperature on a Labquake rotator. The eluate was removed, mixed with 0.500 mL Minimal A salts solution and saved. For a second selection, 0.500 mL 100 mM TEA was used for elution for 10 min at RT, then neutralized in a mix of 0.250 mL of 1 M Tris, pH 7.4 + 0.500 mL Min A salts.

[0180] After the first selection elution, the beads can be eluted again with 0.300 mL of non-biotinylated target (1 mg/mL) for 1 hr at RT on a Labquake rotator. Eluted phage are added to 0.450 mL Minimal A salts.

[0181] Three eluates (competitor from 1st selection, target from 1st selection and neutralized TEA elution from 2nd selection) were kept separate and a small aliquot taken from each for titering. 0.500 mL Minimal A salts were added to the remaining bead aliquots after competitor and target elution and after TEA elution. Take a small aliquot from each was taken for tittering.

[0182] Each elution and each set of eluted beads was mixed with 2X YT and an aliquot (e.g., 1 mL with 1. E 10/mL) of XL1-Blue MRF' E. coli cells (or other F' cell line) which had been chilled on ice after having been grown to mid-logarithmic phase, starved and concentrated (see procedure below - "Mid-Log prep of XL-1 blue MRF' cells for infection").

[0183] After approximately 30 minutes at room temperature, the phage/cell mixtures were spread onto Bio-Assay Dishes (243 X 243 X 18 mm, Nalge Nunc) containing 2XYT, 1mM IPTG agar. The plates were incubated overnight at 30˚C. The next day, each amplified phage culture was harvested from its respective plate. The plate was flooded with 35 mL TBS or LB, and cells were scraped from the plate. The resuspended cells were transferred to a centrifuge bottle. An additional 20 mL TBS or LB was used to remove any cells from the plate and pooled with the cells in the centrifuge bottle. The cells were centrifuged out, and phage in the supernatant was recovered by PEG precipitation. Over the next day, the amplified phage preps were titered.

[0184] In the first round, two selections yielded five amplified eluates. These amplified eluates were panned for 2-3 more additional rounds of selection using ~1. E 12 input phage/round. For each additional round, the depletion and target beads were prepared the night before the round was initiated.

[0185] For the elution steps in subsequent rounds, all elutions up to the elution step from which the amplified elution came from were done, and the previous elutions were treated as washes. For the bead infection amplified phage, for example, the competitive ligand and target elutions were done and then tossed as washes (see below). Then the beads were used to infect E. coli. Two pools, therefore, yielded a total of 5 final elutions at the end of the selection.

1st selection set

[0186]

A. Ligand amplified elution: elute w/ ligand for 1 hr, keep as elution

B. Target amplified elution: elute w/ ligand for 1 hr, toss as wash elute w/ target for 1 hr, keep as elution

C. Bead infect. amp. elution: elute w/ ligand for 1 hr, toss as wash elute w/ target for 1 hr, toss as wash elute w/ cell infection, keep as elution

2nd selection set

[0187]

A. TEA amplified elution; elute w/ TEA 10min, keep as elution

B. Bead infect. amp. elution; elute w/ TEA 10min, toss as wash elute w/ cell infection, keep as elution

Mid-log prep of XL1 blue MRF' cells for infection (based on Barbas et al. Phage Display manual procedure)

[0188] Culture XL1 blue MRF' in NZCYM (12.5 mg/mL tet) at 37˚C and 250 rpm overnight. Started a 500 mL culture in 2 liter flask by diluting cells 1/50 in NZCYM/tet (10 mL overnight culture added) and incubated at 37˚C at 250 rpm until OD600 of 0.45 (1.5-2 hrs) was reached. Shaking was reduced to 100 rpm for 10 min. When OD600 reached between 0.55-0.65, cells were transferred to 2 x 250 mL centrifuge bottles, centrifuged at 600 g for 15 min at 4˚C. Supernatant was poured off. Residual liquid was removed with a pipette.

[0189] The pellets were gently resuspended (not pipetting up and down) in the original volume of 1 X Minimal A salts at room temp. The resuspended cells were transferred back into 2-liter flask, shaken at 100 rpm for 45 min at 37˚C. This process was performed in order to starve the cells and restore pili. The cells were transferred to 2 x 250 mL centrifuge bottles, and centrifuged as earlier.

[0190] The cells were gently resuspended in ice cold Minimal A salts (5 mL per 500 mL original culture). The cells were put on ice for use in infections as soon as possible.

[0191] The phage eluates were brought up to 7.5 mL with 2XYT medium and 2.5 mL of cells were added. Beads were brought up to 3 mL with 2XYT and 1 mL of cells were added. Incubated at 37oC for 30 min. The cells were plated on 2XYT, 1 mM IPTG agar large NUNC plates and incubated for 18 hr at 30˚C.

Example 9: Incorporation of synthetic region in FR1/3 region.

[0192] Described below are examples for incorporating of fixed residues in antibody sequences for light chain kappa and lambda genes, and for heavy chains. The experimental conditions and oligonucleotides used for the examples below have been described in previous examples (*e.g.*, Examples 3 & 4).

[0193] The process for incorporating fixed FR1 residues in an antibody lambda sequence consists of 3 steps (see FIG. 18): (1) annealing of single-stranded DNA material encoding VL genes to a partially complementary oligonucleotide mix (indicated with Ext and Bridge), to anneal in this example to the region encoding residues 5-7 of the FR1 of the lambda genes (indicated with X..X; within the lambda genes the overlap may sometimes not be perfect); (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VL gene. In this process the first few residues of all lambda genes will be encoded by the sequences present in the oligonucleotides (Ext., Bridge or PCRpr). After the PCR, the lambda genes can be cloned using the indicated restriction site for ApaLI.

[0194] The process for incorporating fixed FR1 residues in an antibody kappa sequence (FIG. 19) consists of 3 steps : (1) annealing of single-stranded DNA material encoding VK genes to a partially complementary oligonucleotide mix (indicated with Ext and Bri), to anneal in this example to the region encoding residues 8-10 of the FR1 of the kappa genes (indicated with X..X; within the kappa genes the overlap may sometimes not be perfect); (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VK gene. In this process the first few (8) residues of all kappa genes will be encode by the sequences present in the oligonucleotides (Ext., Bridge or PCRpr.). After the PCR, the kappa genes can be cloned using the indicated restriction site for ApaLI.

[0195] The process of incorporating fixed FR3 residues in a antibody heavy chain sequence (FIG. 20) consists of 3 steps : (1) annealing of single-stranded DNA material encoding part of the VH genes (for example encoding FR3, CDR3 and FR4 regions) to a partially complementary oligonucleotide mix (indicated with Ext and Bridge), to anneal in this example to the region encoding residues 92-94 (within the FR3 region) of VH genes (indicated with X..X; within the VH genes the overlap may sometimes not be perfect); (2) ligation of this complex; (3) PCR of the ligated material with the indicated primer ('PCRpr') and for example one primer based within the VH gene (such as in the FR4 region). In this process certain residues of all VH genes will be encoded by the sequences present in the oligonucleotides used here, in particular from PCRpr (for residues 70-73), or from Ext/Bridge oligonucleotides (residues 74-91). After the PCR, the partial VH genes can be cloned using the indicated restriction site for *XbaI*.

[0196] It will be understood that the foregoing is only illustrative of the principles of this invention and that various

modifications can be made by those skilled in the art without departing from the scope of and sprit of the invention.

Table 1: Human GLG FR3 sequences

```
! VH1
!  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80
   agg gtc acc atg acc agg gac acg tcc atc agc aca gcc tac atg
!  81  82  82a 82b 82c 83  84  85  86  87  88  89  90  91  92
   gag ctg agc agg ctg aga tct gac gac acg gcc gtg tat tac tgt
!  93  94  95
   gcg aga ga ! 1-02# 1
   aga gtc acc att acc agg gac aca tcc gcg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gaa gac acg gct gtg tat tac tgt
   gcg aga ga ! 1-03# 2
   aga gtc acc atg acc agg aac acc tcc ata agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga gg ! 1-08# 3
   aga gtc acc atg acc aca gac aca tcc acg agc aca gcc tac atg
   gag ctg agg agc ctg aga tct gac gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-18# 4
   aga gtc acc atg acc gag gac aca tct aca gac aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gca aca ga ! 1-24# 5
   aga gtc acc att acc agg gac agg tct atg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac aca gcc atg tat tac tgt
   gca aga ta ! 1-45# 6
   aga gtc acc atg acc agg gac acg tcc acg agc aca gtc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-46# 7
   aga gtc acc att acc agg gac atg tcc aca agc aca gcc tac atg
   gag ctg agc agc ctg aga tcc gag gac acg gcc gtg tat tac tgt
   gcg gca ga ! 1-58# 8
   aga gtc acg att acc gcg gac gaa tcc acg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-69# 9
   aga gtc acg att acc gcg gac aaa tcc acg agc aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gcg aga ga ! 1-e# 10
   aga gtc acc ata acc gcg gac acg tct aca gac aca gcc tac atg
   gag ctg agc agc ctg aga tct gag gac acg gcc gtg tat tac tgt
   gca aca ga ! 1-f# 11
```

! VH2

agg ctc acc atc acc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cac aga c! 2-05# 12
agg ctc acc atc tcc aag gac acc tcc aaa agc cag gtg gtc ctt
acc atg acc aac atg gac cct gtg gac aca gcc aca tat tac tgt
gca cgg ata c! 2-26# 13
agg ctc acc atc tcc aag gac acc tcc aaa aac cag gtg gtc ctt
aca atg acc aac atg gac cct gtg gac aca gcc acg tat tac tgt
gca cgg ata c! 2-70# 14

! VH3

cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-07# 15
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg
caa atg aac agt ctg aga gct gag gac acg gcc ttg tat tac tgt
gca aaa gat a! 3-09#16
cga ttc acc atc tcc agg gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-11# 17
cga ttc acc atc tcc aga gaa aat gcc aag aac tcc ttg tat ctt
caa atg aac agc ctg aga gcc ggg gac acg gct gtg tat tac tgt
gca aga ga ! 3-13# 18
aga ttc acc atc tca aga gat gat tca aaa aac acg ctg tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
acc aca ga ! 3-15# 19
cga ttc acc atc tcc aga gac aac gcc aag aac tcc ctg tat ctg
caa atg aac agt ctg aga gcc gag gac acg gcc ttg tat cac tgt
gcg aga ga ! 3-20# 20
cga ttc acc atc tcc aga gac aac gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-21# 21
cgg ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gcc gta tat tac tgt
gcg aaa ga ! 3-23# 22
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3-30# 23
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3303# 24

cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aaa ga ! 3305# 25
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctg
caa atg aac agc ctg aga gcc gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-33# 26
cga ttc acc atc tcc aga gac aac agc aaa aac tcc ctg tat ctg
caa atg aac agt ctg aga act gag gac acc gcc ttg tat tac tgt
gca aaa gat a! 3-43#27
cga ttc acc atc tcc aga gac aat gcc aag aac tca ctg tat ctg
caa atg aac agc ctg aga gac gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-48# 28
aga ttc acc atc tca aga gat ggt tcc aaa agc atc gcc tat ctg
caa atg aac agc ctg aaa acc gag gac aca gcc gtg tat tac tgt
act aga ga ! 3-49# 29
cga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gcc gag gac acg gcc gtg tat tac tgt
gcg aga ga ! 3-53# 30
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg ggc agc ctg aga gct gag gac atg gct gtg tat tac tgt
gcg aga ga ! 3-64# 31
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg tat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
gcg aga ga ! 3-66# 32
aga ttc acc atc tca aga gat gat tca aag aac tca ctg tat ctg
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
gct aga ga ! 3-72# 33
agg ttc acc atc tcc aga gat gat tca aag aac acg gcg tat ctg
caa atg aac agc ctg aaa acc gag gac acg gcc gtg tat tac tgt
act aga ca ! 3-73# 34
cga ttc acc atc tcc aga gac aac gcc aag aac acg ctg tat ctg
caa atg aac agt ctg aga gcc gag gac acg gct gtg tat tac tgt
gca aga ga ! 3-74# 35
aga ttc acc atc tcc aga gac aat tcc aag aac acg ctg cat ctt
caa atg aac agc ctg aga gct gag gac acg gct gtg tat tac tgt
aag aaa ga ! 3-d# 36

! VH4

cga gtc acc ata tca gta gac aag tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-04# 37
cga gtc acc atg tca gta gac acg tcc aag aac cag ttc tcc ctg

```
aag ctg agc tct gtg acc gcc gtg gac acg gcc gtg tat tac tgt
gcg aga aa ! 4-28# 38
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4301# 39
cga gtc acc ata tca gta gac agg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gcc gtg tat tac tgt
gcc aga ga ! 4302# 40
cga gtt acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gca gac acg gcc gtg tat tac tgt
gcc aga ga ! 4304# 41
cga gtt acc ata tca gta gac acg tct aag aac cag ttc tcc ctg
aag ctg agc tct gtg act gcc gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-31# 42
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gcg gac acg gct gtg tat tac tgt
gcg aga ga ! 4-34# 43
cga gtc acc ata tcc gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gct gtg tat tac tgt
gcg aga ca ! 4-39# 44
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-59# 45
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gct gcg gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-61# 46
cga gtc acc ata tca gta gac acg tcc aag aac cag ttc tcc ctg
aag ctg agc tct gtg acc gcc gca gac acg gcc gtg tat tac tgt
gcg aga ga ! 4-b# 47
! VH5
cag gtc acc atc tca gcc gac aag tcc atc agc acc gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga ca ! 5-51# 48
cac gtc acc atc tca gct gac aag tcc atc agc act gcc tac ctg
cag tgg agc agc ctg aag gcc tcg gac acc gcc atg tat tac tgt
gcg aga  ! 5-a# 49
! VH6
cga ata acc atc aac cca gac aca tcc aag aac cag ttc tcc ctg
cag ctg aac tct gtg act ccc gag gac acg gct gtg tat tac tgt
gca aga ga ! 6-1# 50
! VH7
```

cgg ttt gtc ttc tcc ttg gac acc tct gtc agc acg gca tat ctg
cag atc tgc agc cta aag gct gag gac act gcc gtg tat tac tgt
gcg aga ga ! 74.1# 51

Table 2: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3 Typical entry:

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
BstEII Ggtnacc			2		
1: 3	48: 3				
There are 2 hits at base#			3		
MaeIII gtnac			36		
1: 4	2: 4	3: 4	4: 4	5: 4	6: 4
7: 4	8: 4	9: 4	10: 4	11: 4	37: 4
37: 58	38: 4	38: 58	39: 4	39: 58	40: 4
40: 58	91: 4	91: 58	42: 4	42: 58	93: 4
43: 58	44: 4	44: 58	45: 4	45: 58	46: 4
46: 58	47: 4	47: 58	48: 4	49: 4	50: 58
There are 24 hits at base#			**4**		
Tsp45I gtsac			33		
1: 4	2: 4	3: 4	4: 4	5: 4	6: 4
7: 4	8: 4	9: 4	10: 4	11: 4	37: 4
37: 58	38: 4	38: 58	39: 58	40: 4	40: 58
41: 58	42: 58	43: 4	43: 58	44: 4	44: 58
45: 4	45: 58	46: 4	46: 58	47: 4	97: 58
48: 4	49: 4	50: 58			
There are 21 hits at base#			4		
HphI tcacc			45		
1: 5	2: 5	3: 5	4: 5	5: 5	6: 5
7: 5	8: 5	11: 5	12: 5	12: 11	13: 5
14: 5	15: 5	16: 5	17: 5	18: 5	19: 5
20: 5	21: 5	22: 5	23: 5	24: 5	25: 5
26: 5	27: 5	28: 5	29: 5	30: 5	31: 5
32: 5	33: 5	34: 5	35: 5	36: 5	37: 5
38: 5	40: 5	43: 5	44: 5	45: 5	46: 5
47: 5	48: 5	49: 5			
There are 44 hits at base#			**5**		
NlaIII CATG			26		
1: 9	1: 42	2: 42	3: 9	3: 42	4: 9
4: 42	5: 9	5: 42	6: 42	6: 78	7: 9
7: 42	8: 21	8: 42	9: 42	10: 42	11: 42
12: 57	13: 48	13: 57	14: 57	31: 72	38: 9
48: 78	49: 78				
There are 11 hits at base# 42					
There are 1 hits at base# 48 Could cause raggedness.					
BsaJI Ccnngg			37		

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
1: 14	2: 14	5: 14	6: 14	7: 14	8: 14
8: 65	9: 14	10: 14	11: 14	12: 14	13: 14
14: 14	15: 65	17: 14	17: 65	18: 65	19: 65
20: 65	21: 65	22: 65	26: 65	29: 65	30: 65
33: 65	34: 65	35: 65	37: 65	38: 65	39: 65
40: 65	42: 65	43: 65	48: 65	49: 65	50: 65
51: 14					
There are 23 hits at base# 65					
There are 14 hits at base# 14					
AluI AGct			42		
1: 47	2: 47	3: 47	4: 47	5: 47	6: 47
7: 47	8: 47	9: 47	10: 47	11: 47	16: 63
23: 63	24: 63	25: 63	31: 63	32: 63	36: 63
37: 47	37: 52	38: 47	38: 52	39: 47	39: 52
40: 47	40: 52	41: 47	41: 52	42: 47	42: 52
43: 47	43: 52	44: 47	44: 52	45: 47	45: 52
46: 47	46: 52	47: 47	47: 52	49: 15	50: 47
There are 23 hits at base# 47					
There are 11 hits at base# 52 Only 5 bases from 47					
BlpI GCtnagc			**21**		
1: 48	**2: 48**	**3: 48**	**5: 48**	**6: 48**	**7: 48**
8: 48	**9: 48**	**10: 48**	**11: 48**	**37: 48**	**38: 48**
39: 48	**40: 48**	**41: 48**	**42: 48**	**43: 48**	**44: 48**
45: 48	**46: 48**	**47: 48**			
There are 21 hits at base# 48					
MwoI GCNNNNNngc			19		
1: 48	2: 28	19: 36	22: 36	23: 36	24: 36
25: 36	26: 36	35: 36	37: 67	39: 67	40: 67
41: 67	42: 67	43: 67	44: 67	45: 67	46: 67
47: 67					
There are 10 hits at base# 67					
There are 7 hits at base# 36					
DdeI Ctnag			71		
1: 49	1: 58	2: 49	2: 58	3: 49	3: 58
3: 65	4: 49	4: 58	5: 49	5: 58	5: 65
6: 49	6: 58	6: 65	7: 49	7: 58	7: 65
8: 49	8: 58	9: 49	9: 58	9: 65	10: 49
10: 58	10: 65	11: 49	11: 58	11: 65	15: 58
16: 58	16: 65	17: 58	18: 58	20: 58	21: 58
22: 58	23: 58	23: 65	24: 58	24: 65	25: 58
25: 65	26: 58	27: 58	27: 65	28: 58	30: 58
31: 58	31: 65	32: 58	32: 65	35: 58	36: 58
36: 65	37: 49	38: 49	39: 26	39: 49	40: 49
41: 49	42: 26	42: 49	43: 49	44: 49	45: 49
46: 49	47: 49	48: 12	49 12	51: 65	
There are 29 hits at base# 58					

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
There are 22 hits at base# 49 Only nine base from 58					
There are 16 hits at base# 65 Only seven bases from 58					
BglII Agatct			**11**		
1: 61	2: 61	3: 61	4: 61	5: 61	6: 61
7: 61	9: 61	10: 61	11: 61	51: 47	
There are 10 hits at base# 61					
BstYI Rgatcy			12		
1: 61	2: 61	3: 61	4: 61	5: 61	6: 61
7: 61	8: 61	9: 61	10: 61	11: 61	51: 47
There are 11 hits at base# 61					
Hpy188I TCNga			17		
1: 64	2: 64	3: 64	9: 64	5: 64	6: 64
7: 64	8: 64	9: 64	10: 64	11: 64	16: 57
20: 57	27: 57	35: 57	48: 67	49: 67	
There are 11 hits at base# 64					
There are 4 hits at base# 57					
There are 2 hits at base# 67 Could be ragged.					
Ms1I CAYNNnnRTG			**44**		
1: 72	**2: 72**	**3: 72**	**4: 72**	**5: 72**	**6: 72**
7: 72	**8: 72**	**9: 72**	**10: 72**	**11: 72**	**15: 72**
17: 72	**18: 72**	**19: 72**	**21: 72**	**23: 72**	**24: 72**
25: 72	**26: 72**	**28: 72**	**29: 72**	**30: 72**	**31: 72**
32: 72	**33: 72**	**34: 72**	**35: 72**	**36: 72**	**37: 72**
38: 72	**39: 72**	**40: 72**	**41: 72**	**42: 72**	**43: 72**
44: 72	**45: 72**	**46: 72**	**47: 72**	**48: 72**	**49: 72**
50: 72	**51: 72**				
There are 44 hits at base# 72					
BsiEI CGRYcg			**23**		
1: 74	**3: 74**	**4: 74**	**5: 74**	**7: 74**	**8: 74**
9: 74	**10: 74**	**11: 74**	**17: 74**	**22: 74**	**30: 74**
33: 74	**34: 74**	**37: 74**	**38: 74**	**39: 74**	**40: 74**
41: 74	**42: 74**	**45: 74**	**46: 74**	**47: 74**	
There are 23 hits at base# 74					
EaeI Yggccr			**23**		
1: 74	**3: 74**	**4: 74**	**5: 74**	**7: 74**	**8: 74**
9: 74	**10: 74**	**11: 74**	**17: 74**	**22: 74**	**30: 74**
33: 74	**34: 74**	**37: 74**	**38: 74**	**39: 74**	**40: 74**
41: 74	**42: 74**	**45: 74**	**46: 74**	**47: 74**	
There are 23 hits at base# 74					
EagI Cggccg			**23**		
1: 74	**3: 74**	**4: 74**	**5: 74**	**7: 74**	**8: 74**
9: 74	**10: 74**	**11: 74**	**17: 74**	**22: 74**	**30: 74**
33: 74	**34: 74**	**37: 74**	**38: 74**	**39: 74**	**40: 74**

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
41: 74	**42: 74**	**45: 74**	**46: 74**	**47: 74**	
There are 23 hits at base# 74					
HaeIII GGcc			27		
1: 75	3: 75	4: 75	5: 75	7: 75	8: 75
9: 75	10: 75	11: 75	16: 75	17: 75	20: 75
22: 75	30: 75	33: 75	34: 75	37: 75	38: 75
39: 75	40: 75	41: 75	42: 75	45: 75	46: 75
47: 75	48: 63	49: 63			
There are 25 hits at base# 75					
Bst4CI ACNgt 65˚C		**63 Sites There is a third isoschismer**			
1: 86	**2: 86**	**3: 86**	**4: 86**	**5: 86**	**6: 86**
7: 34	**7: 86**	**8: 86**	**9: 86**	**10: 86**	**11: 86**
12: 86	**13: 86**	**14: 86**	**15: 36**	**15: 86**	**16: 53**
16: 86	**17: 36**	**17: 86**	**18: 86**	**19: 86**	**20: 53**
20: 86	**21: 36**	**21: 86**	**22: 0**	**22: 86**	**23: 86**
24: 86	**25: 86**	**26: 86**	**27: 53**	**27: 86**	**28: 36**
28: 86	**29: 86**	**30: 86**	**31: 86**	**32: 86**	**33: 36**
33: 86	**34: 86**	**35: 53**	**35: 86**	**36: 86**	**37: 86**
38: 86	**39: 86**	**40: 86**	**41: 86**	**42: 86**	**43: 86**
44: 86	**45: 86**	**46: 86**	**47: 86**	**48: 86**	**49: 86**
50: 86	**51: 0**	**51: 86**			
There are 51 hits at base# 86 All the other sites are well away					
HpyCH4III ACNgt			**63**		
1: 86	**2: 86**	**3: 86**	**4: 86**	**5: 86**	**6: 86**
7: 34	**7: 86**	**8: 86**	**9: 86**	**10: 86**	**11: 86**
12: 86	**13: 86**	**14: 86**	**15: 36**	**15: 86**	**16: 53**
16: 86	**17: 36**	**17: 86**	**18: 86**	**19: 86**	**20: 53**
20: 86	**21: 36**	**21: 86**	**22: 0**	**22: 86**	**23: 86**
24: 86	**25: 86**	**26: 86**	**27: 53**	**27: 86**	**28: 36**
28: 86	**29: 86**	**30: 86**	**31: 86**	**32: 86**	**33: 36**
33: 86	**34: 86**	**35: 53**	**35: 86**	**36: 86**	**37: 86**
38: 86	**39: 86**	**40: 86**	**41: 86**	**42: 86**	**43: 86**
44: 86	**45: 86**	**46: 86**	**47: 86**	**48: 86**	**49: 86**
50: 86	**51: 0**	**51: 86**			
There are 51 hits at base# 86					
HinfI Gantc			**43**		
2: 2	**3: 2**	**4: 2**	**5: 2**	**6: 2**	**7: 2**
8: 2	**9: 2**	**9: 22**	**10: 2**	**11: 2**	**15: 2**
16: 2	**17: 2**	**18: 2**	**19: 2**	**19: 22**	**20: 2**
21: 2	**23: 2**	**24: 2**	**25: 2**	**26: 2**	**27: 2**
28: 2	**29: 2**	**30: 2**	**31: 2**	**32: 2**	**33: 2**
33: 22	**34: 22**	**35: 2**	**36: 2**	**37: 2**	**38: 2**
40: 2	**43: 2**	**44: 2**	**45: 2**	**46: 2**	**47: 2**
50: 60					
There are 38 hits at base# 2					
MlyI GAGTCNNNNNn			18		

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
2: 2	3: 2	4: 2	5: 2	6: 2	7: 2
8: 2	9: 2	10: 2	11: 2	37: 2	38: 2
40: 2	43: 2	44: 2	45: 2	46: 2	47: 2
There are 18 hits at base# 2					
PleI gagtc			**18**		
2: 2	**3: 2**	**4: 2**	**5: 2**	**6: 2**	**7: 2**
8: 2	**9: 2**	**10: 2**	**11: 2**	**37: 2**	**38: 2**
40: 2	**43: 2**	**44: 2**	**45: 2**	**46: 2**	**47: 2**
There are 18 hits at base# 2					
AciI Ccgc			24		
2: 26	9: 14	10: 14	11: 14	27: 74	37: 62
37: 65	38: 62	39: 65	40: 62	40: 65	41: 65
42: 65	43: 62	43: 65	44: 62	44: 65	45: 62
46: 62	47: 62	47: 65	48: 35	48: 74	49: 74
There are 8 hits at base# 62					
There are 8 hits at base# 65					
There are 3 hits at base# 14					
There are 3 hits at base# 74					
There are 1 hits at base# 26					
There are 1 hits at base# 35					
-"- Gcgg			11		
8: 91	9: 16	10: 16	11: 16	37: 67	39: 67
40: 67	42: 67	43: 67	45: 67	46: 67	
There are 7 hits at base# 67					
There are 3 hits at base# 16					
There are 1 hits at base# 91					
BsiHKAI GWGCWc			20		
2: 30	4: 30	6: 30	7: 30	9: 30	10: 30
12: 89	13: 89	14: 89	37: 51	38: 51	39: 51
40: 51	41: 51	42: 51	43: 51	44: 51	45: 51
46: 51	47: 51				
There are 11 hits at base# 51					
Bsp1286I GDGCHc			20		
2: 30	4: 30	6: 30	7: 30	9: 30	10: 30
12: 89	13: 89	14: 89	37: 51	38: 51	39: 51
40: 51	41: 51	42: 51	43: 51	44: 51	45: 51
46: 51	47: 51				
There are 11 hits at base# 51					
HgiAI GWGCWc			20		
2: 30	4: 30	6: 30	7: 30	9: 30	10: 30
12: 89	13: 89	14: 89	37: 51	38: 51	39: 51
40: 51	41: 51	42: 51	43: 51	44: 51	45: 51
46: 51	47: 51				
There are 11 hits at base# 51					

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
BsoFI GCngc			26		
2: 53	3: 53	5: 53	6: 53	7: 53	8: 53
8: 91	9: 53	10: 53	11: 53	31: 53	36: 36
37: 64	39: 64	40: 64	41: 64	42: 64	43: 64
44: 64	45: 64	46: 64	47: 64	48: 53	49: 53
50: 45	51: 53				
There are 13 hits at base# 53					
There are 10 hits at base# 64					
TseI Gcwgc			17		
2: 53	3: 53	5: 53	6: 53	7: 53	8: 53
9: 53	10: 53	11: 53	31: 53	36: 36	45: 64
46: 64	48: 53	49: 53	50: 45	51: 53	
There are 13 hits at base# 53					
MnlI gagg			**34**		
3: 67	**3: 95**	**4: 51**	**5: 16**	**5: 67**	**6: 67**
7: 67	**8: 67**	**9: 67**	**10: 67**	**11: 67**	**15: 67**
16: 67	**17: 67**	**19: 67**	**20: 67**	**21: 67**	**22: 67**
23: 67	**24: 67**	**25: 67**	**26: 67**	**27: 67**	**28: 67**
29: 67	**30: 67**	**31: 67**	**32: 67**	**33: 67**	**34: 67**
35: 67	**36: 67**	**50: 67**	**51: 67**		
There are 31 hits at base# 67					
HpyCH4V TGca			**34**		
5: 90	**6: 90**	**11: 90**	**12: 90**	**13: 90**	**14: 90**
15: 44	**16: 44**	**16: 90**	**17: 44**	**18: 90**	**19: 44**
20: 44	**21: 44**	**22: 44**	**23: 44**	**24: 44**	**25: 44**
26: 44	**27: 44**	**27: 90**	**28: 44**	**29: 44**	**33: 44**
34: 44	**35: 44**	**35: 90**	**36: 38**	**48: 44**	**49: 44**
50: 44	**50: 90**	**51: 44**	**51: 52**		
There are 21 hits at base# 44					
There are 1 hits at base# 52					
AccI GTmkac			13	5-base recognition	
7: 37	11: 24	37: 16	38: 16	39: 16	40: 16
41: 16	42: 16	43: 16	44: 16	45: 16	46: 16
47: 16					
There are 11 hits at base# 16					
SacII CCGCgg			8	6-base recognition	
9: 14	10: 14	11: 14	37: 65	39: 65	40: 65
42: 65	43: 65				
There are 5 hits at base# 65					
There are 3 hits at base# 14					
TfiI Gawtc			24		
9: 22	15: 2	16: 2	17: 2	18: 2	19: 2
19: 22	20: 2	21: 2	23: 2	24: 2	25: 2
26: 2	27: 2	28: 2	29: 2	30: 2	31: 2

(continued)

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
32: 2	33: 2	33: 22	34: 22	35: 2	36: 2
There are 20 hits at base# 2					
BsmAI Nnnnnngagac			19		
15: 11	16: 11	20: 11	21: 11	22: 11	23: 11
24: 11	25: 11	26: 11	27: 11	28: 11	28: 56
30: 11	31: 11	32: 11	35: 11	36: 11	44: 87
48: 87					
There are 16 hits at base# 11					
Bpml ctccag			19		
15: 12	16: 12	17: 12	18: 12	20: 12	21: 12
22: 12	23: 12	24: 12	25: 12	26: 12	27: 12
28: 12	30: 12	31: 12	32: 12	34: 12	35: 12
36: 12					
There are 19 hits at base# 12					
XmnI GAANNnnttc			12		
37: 30	38: 30	39: 30	40: 30	41: 30	42: 30
43: 30	44: 30	45: 30	46: 30	47: 30	50: 30
There are 12 hits at base# 30					
BsrI NCcagt			12		
37: 32	38: 32	39: 32	40: 32	41: 32	42: 32
43: 32	44: 32	45: 32	46: 32	47: 32	50: 32
There are 12 hits at base# 32					
BanII GRGCYc			11		
37: 51	38: 51	39: 51	40: 51	41: 51	42: 51
43: 51	44: 51	45: 51	46: 51	47: 51	
There are 11 hits at base# 51					
Ecl136I GAGctc			11		
37: 51	38: 51	39: 51	40: 51	41: 51	42: 51
43: 51	44: 51	45: 51	46: 51	47: 51	
There are 11 hits at base# 51					
SacI GAGCTc			11		
37: 51	38: 51	39: 51	40: 51	41: 51	42: 51
43: 51	44: 51	45: 51	46: 51	47: 51	
There are 11 hits at base# 51					

Table 3: Synthetic 3-23 FR3 of human heavy chains showning positions of possible cleavage sites

```
! Sites engineered into the synthetic gene are shown in upper case
DNA
! with the RE name between vertical bars (as in | XbaI  |).
! RERSs frequently found in GLGs are shown below the synthetic
sequence
! with the name to the right (as in gtn ac=MaeIII(24), indicating
that
! 24 of the 51 GLGs contain the site).
!
!                                                        |---FR3---
!                                                         89  90 (codon #
in
!                                                          R   F
synthetic 3-23)
                                                        |cgc|ttc|  6
!  Allowed DNA                                          |cgn|tty|
!                                                       |agr|
!                                                          ga ntc =
HinfI(38)
!                                                          ga gtc =
PleI(18)
!                                                          ga wtc =
TfiI(20)
!                                                             gtn ac =
MaeIII(24)
!                                                             gts ac =
Tsp45I(21)
!                                                              tc acc =
HphI(44)
!
!       --------FR3-----------------------------------------------
!         91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!         T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
        |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|  51
!allowed|acn|ath|tcn|cgn|gay|aay|tcn|aar|aay|acn|ttr|tay|ttr|car|atg|
!               |agy|agr|       |agy|           |ctn|   |ctn|
!               |     ga|gac = BsmAI(16)                    ag ct =
AluI(23)
!              c|tcc ag = BpmI(19)                           g ctn agc =
BlpI(21)
```

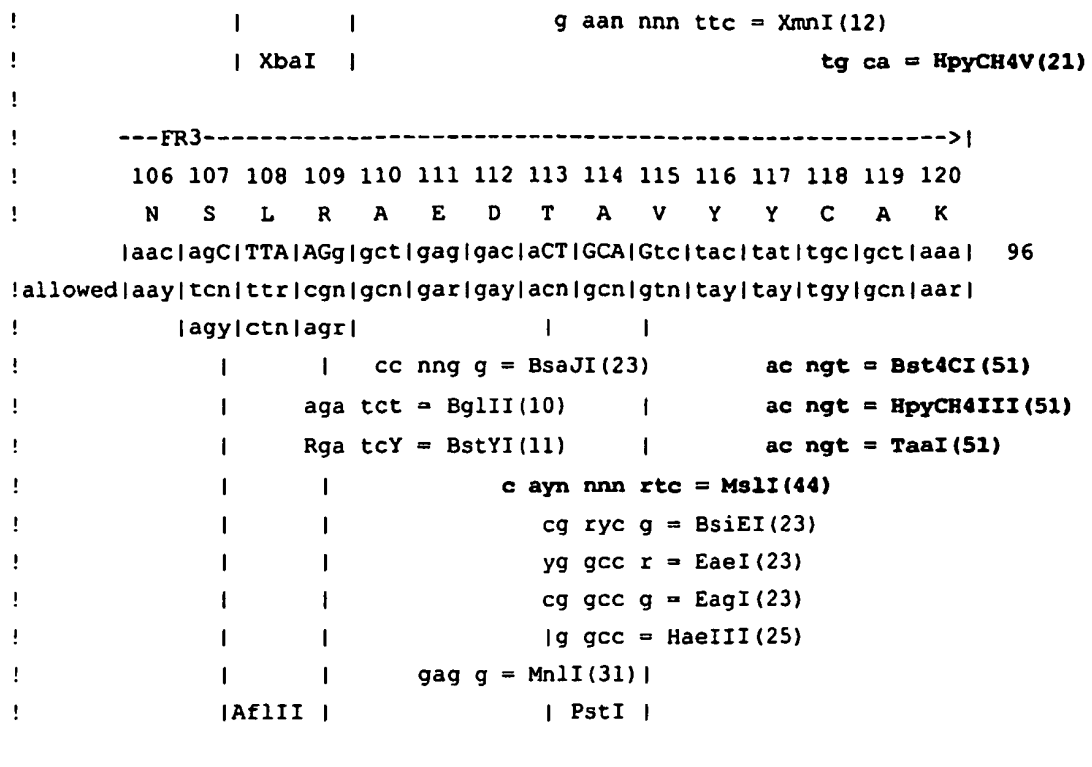

g aan nnn ttc = XmnI(12)
XbaI
tg ca = HpyCH4V(21)
---FR3----------
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
N S L R A E D T A V Y Y C A K
aac agC TTA AGg gct gag gac aCT GCA Gtc tac tat tgc gct aaa 96
allowed aay tcn ttr cgn gcn gar gay acn gcn gtn tay tay tgy gcn aar
agy ctn agr
cc nng g = BsaJI(23)
ac ngt = Bst4CI(51)
aga tct = BglII(10)
ac ngt = HpyCH4III(51)
Rga tcY = BstYI(11)
ac ngt = TaaI(51)
c ayn nnn rtc = MslI(44)
cg ryc g = BsiEI(23)
yg gcc r = EaeI(23)
cg gcc g = EagI(23)
g gcc = HaeIII(25)
gag g = MnlI(31)
AflII
PstI

Table 4: REdaptors, Extenders, and Bridges used for Cleavage and Capture of Human Heavy Chains in FR3.

A: HpyCH4V Probes of actual human HC genes

!HpyCH4V in FR3 of human HC, bases 35-56; only those with TGca site
TGca;10,
RE recognition:tgca of length 4 is expected at 10

1	6-1	**agttctccctgcagctgaactc**
2	3-11,3-07,3-21,3-72,3-48	**cactgtatctgcaaatgaacag**
3	3-09,3-43,3-20	ccctgtatctgcaaatgaacag
4	5-51	**ccgcctacctgcagtggagcag**
5	3-15,3-30,3-30.5,3-30.3,3-74,3-23,3-33	**cgctgtatctgcaaatgaacag**
6	7-4.1	cggcatatctgcagatctgcag
7	3-73	cggcgtatctgcaaatgaacag
8	5-a	ctgcctacctgcagtggagcag
9	3-49	tcgcctatctgcaaatgaacag

B: HpyCH4V REdaptors, Extenders, and Bridges

B.1 REdaptors

! Cutting HC lower strand:
! TmKeller for 100 mM NaCl, zero formamide

! Edapters for cleavage		T_m^W	T_m^K
(ON_HCFR36-1)	5'-agttctcccTGCAgctgaactc-3'	68.0	64.5
(ON_HCFR36-1A)	5'-ttctcccTGCAgctgaactc-3'	62.0	62.5
(ON_HCFR36-1B)	5'-ttctcccTGCAgctgaac-3'	56.0	59.9
(ON_HCFR33-15)	5'-cgctgtatcTGCAaatgaacag-3'	64.0	60.8
(ON_HCFR33-15A)	5'-ctgtatcTGCAaatgaacag-3'	56.0	56.3
(ON_HCFR33-15B)	5'-ctgtatcTGCAaatgaac-3'	50.0	53.1
(ON_HCFR33-11)	5'-cactgtatcTGCAaatgaacag-3'	62.0	58.9
(ON_HCFR35-51)	5'-ccgcctaccTGCAgtggagcag-3'	74.0	70.1

!

B.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

```
!                    XbaI...
!D323*  cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC
!       scab..... designed gene 3-23 gene.................
!
```

```
!    HpyCH4V
!     .. ..            AflII...
!    Ttg caG atg aac agc TtA agG . . .
!    ........................... . . .
!
```

B.3 Extender and Bridges

```
! Extender (bottom strand):
!
(ON_HCHpyEx01) 5'-cAAgTAgAgAgTATTcTTAgAgTTgTcTcTAgAcTTAgTgAAgcg-3'
! ON_HCHpyEx01 is the reverse complement of
! 5'-cgCttcacTaag tcT aga gac aaC tcT aag aaT acT ctC taC Ttg -3'
!
! Bridges (top strand, 9-base overlap):
!
(ON_HCHpyBr016-1)   5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                  aaT acT ctC taC Ttg CAgctgaac-3' {3'-term C is
blocked}
!
! 3-15 et al. + 3-11
(ON_HCHpyBr023-15)  5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                  aaT acT ctC taC Ttg CAaatgaac-3' {3'-term C is
blocked}
!

! 5-51
(ON_HCHpyBr045-51)  5'-cgCttcacTaag tcT aga gac aaC tcT aag-
                  aaT acT ctC taC Ttg CAgtggagc-3' {3'-term C is
blocked}
!
! PCR primer (top strand)
!
(ON_HCHpyPCR)       5'-cgCttcacTaag tcT aga gac-3'
!
```

C: *BlpI* Probes from human HC GLGs

```
   1          1-58,1-03,1-08,1-69,1-24,1-45,1-46,1-f,1-e
acatggaGCTGAGCagcctgag
   2                                                  1-02
acatggaGCTGAGCaggctgag
```

3 1-18
acatggagctgaggagcctgag
4 5-51,5-a
acctgcagtggagcagcctgaa
5 3-15,3-73,3-49,3-72
atctgcaaatgaacagcctgaa
6 3303,3-33,3-07,3-11,3-30,3-21,3-23,3305,3-48
atctgcaaatgaacagcctgag
7 3-20,3-74,3-09,3-43
atctgcaaatgaacagtctgag
8 74.1
atctgcagatctgcagcctaaa
9 3-66,3-13,3-53,3-d
atcttcaaatgaacagcctgag
10 3-64
atcttcaaatgggcagcctgag
11 4301,4-28,4302,4-04,4304,4-31,4-34,4-39,4-59,4-61,4-b
ccctgaaGCTGAGCtctgtgac
12 6-1
ccctgcagctgaactctgtgac
13 2-70,2-05
tccttacaatgaccaacatgga
14 2-26
tccttaccatgaccaacatgga

D: *BlpI* REdaptors, Extenders, and Bridges

D.1 REdaptors

		T_m^W	T_mK
(BlpF3HC1-58)	5'-ac atg gaG **CTG AGC** agc ctg ag-3'	70	66.4
(BlpF3HC6-1)	5'-cc ctg aag ctg agc tct gtg ac-3'	70	66.4

! BlpF3HC6-1 matches 4-30.1, not 6-1.

D.2 Segment of synthetic 3-23 gene into which captured CDR3 is to be cloned

!
BlpI
! XbaI...
... ...

```
!D323*  cgCttcacTaag TCT AGA gac aaC tcT aag aaT acT ctC taC Ttg
caG atg aac
!
!                      AflII...
!                    agC TTA AGG
```

D.3 Extender and Bridges

```
! Bridges
(BlpF3Br1) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                  taC Ttg caG Ctg a|GC agc ctg-3'
(BlpF3Br2) 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG-
                  taC Ttg caG Ctg a|gc tct gtg-3'
!                                  | lower strand is cut here
! Extender
(BlpF3Ext) 5'-TcAgcTgcAAgTAcAAAgTATTTTTAcTgTTATcTcTAgA cTgAgTgAAgcg-
3'
! BlpF3Ext is the reverse complement of:
! 5'-cgCttcacTcag tcT aga gaT aaC AGT aaA aaT acT TtG taC Ttg caG
Ctg a-3'
!
(BlpF3PCR) 5'-cgCttcacTcag tcT aga gaT aaC-3'
```

E: *HpyCH4III* Distinct GLG sequences surrounding site, bases 77-98

```
   1          102#1,118#4,146#7,169#9,1e#10,311#17,353#30,404#37,4301
ccgtgtattactgtgcgagaga
   2          103#2,307#15,321#21,3303#24,333#26,348#28,364#31,366#32
ctgtgtattactgtgcgagaga
   3                                                          108#3
ccgtgtattactgtgcgagagg
   4                                                   124#5,1f#11
ccgtgtattactgtgcaacaga
   5                                                         145#6
ccatgtattactgtgcaagata
   6                                                         158#8
ccgtgtattactgtgcggcaga
   7                                                        205#12
ccacatattactgtgcacacag
   8                                                        226#13
ccacatattactgtgcacggat
```

9	270#14
ccacgtattactgtgcacggat	
10	309#16,343#27
ccttgtattactgtgcaaaaga	
11	313#18,374#35,61#50
ctgtgtattactgtgcaagaga	
12	315#19
ccgtgtattactgtaccacaga	
13	320#20
ccttgtatcactgtgcgagaga	
14	323#22
ccgtatattactgtgcgaaaga	
15	330#23,3305#25
ctgtgtattactgtgcgaaaga	
16	349#29
ccgtgtattactgtactagaga	
17	372#33
ccgtgtattactgtgctagaga	
18	373#34
ccgtgtattactgtactagaca	
19	3d#36
ctgtgtattactgtaagaaaga	
20	428#38
ccgtgtattactgtgcgagaaa	
21	4302#40,4304#41
ccgtgtattactgtgccagaga	
22	439#44
ctgtgtattactgtgcgagaca	
23	551#48
ccatgtattactgtgcgagaca	
24	5a#49
ccatgtattactgtgcgaga	

F: *HpyCH4III* REdaptors, Extenders, and Bridges

F.1 REdaptors

```
! ONs for cleavage of HC(lower) in FR3(bases 77-97)
! For cleavage with HpyCH4III, Bst4CI, or TaaI
! cleavage is in lower chain before base 88.
!                      77 788 888 888 889 999 999 9
!                      78 901 234 567 890 123 456 7        Tm(W)
  Tm(K)
(H43.77.97.1-02#1)  5'-cc gtg tat tAC TGT gcg aga g-3'     6462.6
(H43.77.97.1-03#2)  5'-c[dU] gtg tat tAC TGT gcg aga g-3'  6260.6
(H43.77.97.108#3)   5'-cc gtg tat tAC TGT gcg aga g-3'     6462.6
(H43.77.97.323#22)  5'-cc gt[a] tat tac tgt gcg a[a]a g-3' 6058.7
(H43.77.97.330#23)  5'-c[dU] gtg tat tac tgt gcg a[a]a g-3' 6058.7
```

(H43.77.97.439#44) 5'-c[illegible] gtg tat tac tgt gcg aga [illegible]-3' 6260.6

(H43.77.97.551#48) 5'-cc [illegible]tg tat tac tgt gcg aga [illegible]-3' 6260.6

(H43.77.97.5a#49) 5'-cc [illegible]tg tat tAC TGT gcg aga [illegible]-3' 5858.3

F.2 Extender and Bridges

! XbaI and AflII sites in bridges are bunged

(H43.XABr1) 5'-ggtgtagtga-

|TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-

|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'

(H43.XABr2) 5'-ggtgtagtga-

|TCT|AGt|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-

|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'

(H43.XAExt) 5'-ATAgTAgAcT gcAgTgTccT cAgcccTTAA gcTgTTcATc TgcAAgTAgA-

gAgTATTcTT AgAgTTgTcT cTAgATcAcT AcAcc-3'

!H43.XAExt is the reverse complement of

! 5'-ggtgtagtga-

! **|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-**

! **|aac|agC|TTA|AGg|gct|gag|gac|**aCT|GCA|Gtc|tac|tat -3'

(H43.XAPCR) 5'-ggtgtagtga **|TCT|AGA|gac|aac-3'**

! XbaI and AflII sites in bridges are bunged

(H43.ABr1) 5'-ggtgtagtga-

|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aga-3'

(H43.ABr2) 5'-ggtgtagtga-

|aac|agC|TTt|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat tgt gcg aaa-3'

(H43.AExt) 5'-ATAgTAgAcTgcAgTgTccTcAgcccTTAAgcTgTTTcAcTAcAcc-3'

!(H43.AExt) is the reverse complement of 5'-ggtgtagtga-

! **|aac|agC|TTA|AGg|gct|gag|gac|**aCT|GCA|Gtc|tac|tat -3'

(H43.APCR) 5'-ggtgtagtga **|aac|agC|TTA|AGg|gct|g-3'**

Table 5: Analysis of frequency of matching REdaptors in actual V genes

A: HpyCH4V in HC at bases 35-56

		Number of mismatches.....													
Id	Nto t	0	1	2	3	4	5	6	7	8	9	10	Cut	Id	Probe
1	**510**	**5**	**11**	**274**	**9 2**	**6 1**	**2 5**	**2 2**	**1 1**	**1**	**3**	**5**	**443**	**6-1**	**agttctcccTGCAgctgaactc**
2	**192**	**54**	**42**	**32**	**2 4**	**1 5**	**2**	**3**	**1 0**	**3**	**1**	**6**	**167**	**3-11**	**cactgtatcTGCAaatgaacag**
3	58	19	7	17	6	5	1	0	1	0	2	0	54	3-09	ccctgtatcTGCAaatgaacag
4	**267**	**42**	**33**	**9**	**8**	**8**	**8 2**	**4 3**	**2 2**	**8**	**1 1**	**1**	**100**	**5-51**	**ccgcctaccTGCAgtggagcag**
5	**250**	**111**	**59**	**41**	**2 4**	**7**	**5**	**1**	**0**	**0**	**2**	**0**	**242**	**3-15**	**cgctgtatcTGCAaatgaacag**
6	7	0	2	0	1	0	0	0	0	0	4	0	3	7-4.1	cggcatatcTGCAgatctgcag
7	7	0	2	2	0	0	2	1	0	0	0	0	4	3-73	cggcgtatcTGCAaatgaacag
8	26	10	4	1	3	1	2	1	3	1	0	0	19	5-a	ctgcctaccTGCAgtggagcag
9	21	8	2	3	1	6	1	0	0	0	0	0	20	3-49	tcgcctatcTGCAaatgaacag
	1338	249	162	379	1 4 9	1 0 3	1 2 0	7 1	4 7	1 3	2 3	12	1052		
		249	411	790	9 3 9		1 1 6 2		1 2 8 0		1 3 1 6				
						1 0 4 2		1 2 3 3		1 2 9 3		1338			

Id	Probe	dotted probe
6-1	agttctcccTGCAgctgaactc	agttctccc**TGCA**gctgaactc
3-11	cactgtatcTGCAaatgaacag	cac.g.at.....aa.....ag
3-09	ccctgtatcTGCAaatgaacag	ccc.g.at.....aa.....ag

(continued)

Id	Probe	dotted probe
5-51	ccgcctaccTGCAgtggagcag	ccgc..a.....tg..g.ag
3-15	cgctgtatcTGCAaatgaacag	c.c.g.at.....aa.....ag
7-4.1	cggcatatcTGCAgatctgcag	c.gca.at.....a.ctg.ag
3-73	cggcgtatcTGCAaatgaacag	c.gcg.at.....aa.....ag
5-a	ctgcctaccTGCAgtggagcag	ctgc..a.....tg..g.ag
3-49	tcgcctatcTGCAaatgaacag	tcgc..at.....aa.....ag

Seqs with the expected RE site only.....1004
(Counts only cases with 4 or fewer mismatches)
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 48
(Counts only cases with 4 or fewer mismatches)
Seqs with no sites..... 0

B: BlpI in HC

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	Name		
1	**133**	**73**	**16**	**11**	**13**	**6**	**9**	**1**	**4**	**0**	**119**	**1-58**		**acatggaGCTGAGCagcctgag**
2	14	11	1	0	0	0	0	1	0	1	12	1-02		acatggagctgagcaggctgag
3	34	17	8	2	6	1	0	0	0	0	0	1-18		acatggagctgaggagcctgag
4	120	50	32	16	10	9	1	1	1	0	2	5-51		acctgcagtggagcagcctgaa
5	55	13	11	10	17	3	1	0	0	0	0	3-15		atctgcaaatgaacagcctgaa
6	340	186	88	41	15	6	3	0	1	0	0	3303	atctgcaaatgaacagcctgag	

(continued)

B: BlpI in HC

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	Name	
7	82	25	16	25	12	1	3	0	0	0	0	3-20	atctgcaaatgaacagtctgag
8	3	0	2	0	1	0	0	0	0	0	0	74.1	atctgcagatctgcagcctaaa
9	23	18	2	2	1	0	0	0	0	0	0	3-66	atcttcaaatgaacagcctgag
10	2	1	0	1	0	0	0	0	0	0	0	3-64	atcttcaaatgggcagcctgag
11	**486**	**249**	**78**	**81**	**38**	**21**	**10**	**4**	**4**	**1**	**467**	**4301**	**ccctgaagctgagctctgtgac**
12	16	6	3	1	0	1	1	3	1	0	1	6-1	ccctgcagctgaactctgtgac
13	28	15	8	2	2	1	0	0	0	0	0	2-70	tccttacaatgaccaacatgga
14	2	0	2	0	0	0	0	0	0	0	0	2-26	tccttaccatgaccaacatgga
											601		

Name	Full sequence	Dot mode
1-58	acatggaGCTGAGCagcctgag	acatggaGCTGAGCagcctgag
1-02	acatggagctg**a**gcaggctgag	.....g.....
1-18	acatggagctgaggagcctgag	.....g.....
5-51	acctgcagtggagcagcctgaa	..c..c..tg.....a
3-15	atctgcaaatgaacagcctgaa	.tc..c.aa...a.....a
3-30.3	atctgcaaatgaacagcctgag	.tc..c.aa...a.....
3-20	atctgcaaatgaacagtctgag	.tc..c.aa...a...t....
7-4.1	atctgcagatctgcagcctaaa	.tc..c..a.ct.....a.a
3-66	atcttcaaatgaacagcctgag	.tc.tc.aa...a.....
3-64	atcttcaaatgggcagcctgag	.tc.tc.aa..g.....

(continued)

Name	Full sequence	Dot mode
4-30.1	ccctgaagctgagctctgtgac	c.c..a.....tctg...c
6-1	ccctgcagctgaactctgtgac	c.c..c.....a.tctg...c
2-70	tccttacaatgaccaacatgga	t.c.tacaa...c..a.a..ga
2-26	tccttaccatgaccaacatgga	t.c.tacca...c..a.a..ga

Seqs with the expected RE site only..... 597 (counting sequences with 4 or fewer mismatches)
Seqs with only an unexpected site..... 2
Seqs with both expected and unexpected.... 2
Seqs with no sites..... 686
C: HpyCH4III, Bst4CI, or TaaI in HC
In scoring whether the RE site of interest is present, only ONs that have 4 or fewer mismatches are counted.
Number of sequences..... 1617

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut	acngt	acngt	acngt
1	**244**	**78**	**92**	**43**	**18**	**10**	**1**	**2**	**0**	**0**	**241**	**102#1,1**	**ccgtgtattACTGTgcgagaga**	ccgtgtattactgtgcgagaga
2	**457**	**69**	**150**	**115**	**66**	**34**	**11**	**8**	**3**	**1**	**434**	**103#2,3**	**ctgtgtattactgtgcgagaga**	.t.....
3	**173**	**52**	**45**	**36**	**22**	**14**	**3**	**0**	**0**	**1**	**169**	**108#3**	**ccgtgtattactgtgcgagagg**	.....g
4	16	0	3	2	2	1	6	0	1	1	8	124#5,1	ccgtgtattactgtgcaacaga	.....a.c...
5	4	0	0	1	0	1	1	0	1	0	2	145#6	ccatgtattactgtgcaagata	..a.....a...t.
6	15	1	0	1	0	6	4	1	1	1	8	158#8	ccgtgtattactgtgcggcaga	.....gc...
7	23	4	8	5	2	2	1	1	0	0	21	205#12	ccacatattactgtgcacacag	..aca.....acacag

(continued)

Id	Ntot	0	1	2	3	4	5	6	7	8	Nct	acngt	acngt
8	9	1	1	1	0	3	2	1	0	0	6	226#13	ccacatattactgtgcacggat	..aca.....ac.gat
9	7	1	3	1	1	0	0	1	0	0	6	270#14	ccacgtattactgtgcacggat	..ac.....ac.gat
10	23	7	3	5	5	2	1	0	0	0	22	309#16,	ccttgtattactgtgcaaaaga	..t.....a.a...
11	35	5	10	7	6	3	3	0	1	0	31	313#18,	ctgtgtattactgtgcaagaga	.t.....a.....
12	18	2	3	2	2	6	1	0	2	0	15	315#19	ccgtgtattactgtaccacaga	.....a.c.c...
13	3	1	2	0	0	0	0	0	0	0	3	320#20	ccttgtatcactgtgcgagaga	..t.....c.....
14	**117**	**29**	**23**	**28**	**22**	**8**	**4**	**2**	**1**	**0**	**110**	**323#22**	**ccgtatattactgtgcgaaaga**	....a.....a...
15	**75**	**21**	**25**	**13**	**9**	**1**	**4**	**2**	**0**	**0**	**69**	**330#23,**	**ctgtgtattactgtgcgaaaga**	.t.....a...
16	14	2	2	2	3	0	3	1	1	0	9	349#29	ccgtgtattactgtactagaga	.....a.t.....
17	2	0	0	1	0	0	1	0	0	0	1	372#33	ccgtgtattactgtgctagaga	.....t.....
18	1	0	0	1	0	0	0	0	0	0	1	373#34	ccgtgtattactgtactagaca	.....a.t...c.
19	2	0	0	0	0	0	0	0	0	2	0	3d#36	ctgtgtattactgtaagaaaga	.t.....aa..a...
20	34	4	9	9	4	5	3	0	0	0	31	428#38	ccgtgtattactgtgcgagaaa	.....a.
21	17	5	4	2	2	3	1	0	0	0	16	4302#40	ccgtgtattactgtgccagaga	.....c.....
22	**75**	**15**	**17**	**24**	**7**	**10**	**1**	**1**	**0**	**0**	**73**	**439#44**	**ctgtgtattactgtgcgagaca**	.t.....c.
23	**40**	**14**	**15**	**4**	**5**	**1**	**0**	**1**	**0**	**0**	**39**	**551#48**	**ccatgtattactgtgcgagaca**	..a.....c.

(continued)

Id	Ntot	0	1	2	3	4	5	6	7	8	Ncut		acngt		acngt
24	**213**	**26**	**56**	**60**	**42**	**20**	**7**	**2**	**0**	**0**	**204**	**5a#49**	**ccatgtattactgtgcgagaAA**	..a.................AA	
Group	337	471	363		218		130		58		23		11		6
Cumulative	337	808	1171		1389		1519		1577		1600		1611		1617
Seqs with the expected RE site only.......									1511						
Seqs with only an unexpected site.........									0						
Seqs with both expected and unexpected....									8						
Seqs with no sites.......................									0						

Table 5D:

Analysis repeated using only 8 best REdaptors

Id	Ntot	0	1	2	3	4	5	6	7	8+		
1	301	78	101	54	32	16	9	10	1	0	281	102#1 ccgtgtattactgtgcgagaga
2	493	69	155	125	73	37	14	11	3	6	459	103#2 ctgtgtattactgtgcgagaga
3	189	52	45	38	23	18	5	4	1	3	176	108#3 ccgtgtattactgtgcgagagg
4	127	29	23	28	24	10	6	5	2	0	114	323#22 ccgtatattactgtgcgaaaga
5	78	21	25	14	11	1	4	2	0	0	72	330#23 ctgtgtattactgtgcgaaaga
						6	79	15	17	25	8	11
1	2	0	0	76	439#44 ctgtgtattactgtgcgagaca							
7	43	14	15	5	5	3	0	1	0	0	42	551#48 ccatgtattactgtgcgagaca
8	307	26	63	72	51	38	24	14	13	6	250	5a#49 ccatgtattactgtgcgaga

1	102#1	ccgtgtattactgtgcgagaga	ccgtgtattactgtgcgagaga
2	103#2	ctgtgtattactgtgcgagaga	.t.....
3	108#3	ccgtgtattactgtgcgagagg	.....g
4	323#22	ccgtatattactgtgcgaaaga	....a.....a...
5	330#23	ctgtgtattactgtgcgaaaga	.t.....a...
6	439#44	ctgtgtattactgtgcgagaca	.t.....c.
7	551#48	ccatgtattactgtgcgagaca	..a.....c.
8	5a#49	ccatgtattactgtgcgagaAA ..a.....AA	

Seqs with the expected RE site only.....1463 / 1617
Seqs with only an unexpected site..... 0
Seqs with both expected and unexpected.... 7
Seqs with no sites..... 0

Table 6: Human HC GLG FR1 Sequences

VH Exon - Nucleotide sequence alignment

VH1	
1-02	CAG GTG CAG CTG GTG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG GCC TCA GTG AAG GTC
	TCC TGC AAG GCT TCT GGA TAC ACC TTC ACC
1-03	cag gtC cag ctT gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtT
	tcc tgc aag gct tct gga tac acc ttc actT
1-08	cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtc
	tcc tgc aag gct tct gga tac acc ttc acc
1-18	cag gtT cag ctg gtg cag tct ggA gct gag gtg aag aag cct ggg gcc tca gtg aag gtc
	tcc tgc aag gct tct ggT tac acc ttT acc
1-24	cag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtc
	tcc tgc aag gTt tcC gga tac acc Ctc acT
1-45	cag Atg cag ctg gtg cag tct ggg gct gag gtg aag aag Act ggg Tcc tca gtg aag gtT
	tcc tgc aag gct tcC gga tac acc ttc acc
1-46	cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg gcc tca gtg aag gtT
	tcc tgc aag gcA tct gga tac acc ttc acc
1-58	caA Atg cag ctg gtg cag tct ggg Cct gag gtg aag aag cct ggg Acc tca gtg aag gtc
	tcc tgc aag gct tct gga tTc acc ttT acT
1-69	cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag gtc
	tcc tgc aag gct tct gga GGc acc ttc aGc
1-e	cag gtg cag ctg gtg cag tct ggg gct gag gtg aag aag cct ggg Tcc tcG gtg aag gtc
	tcc tgc aag gct tct gga GGc acc ttc aGc
1-f	Gag gtC cag ctg gtA cag tct ggg gct gag gtg aag aag cct ggg gcT Aca gtg aaA Atc
	tcc tgc aag gTt tct gga tac acc ttc acc
VH2	
2-05	CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACA CAG ACC CTC ACG CTG
	ACC TGC ACC TTC TCT GGG TTC TCA CTC AGC

(continued)

VH2	
2-26	cag Gtc acc ttg aag gag tct ggt cct GTg ctg gtg aaa ccc aca Gag acc ctc acg ctg
	acc tgc acc Gtc tct ggg ttc tca ctc agc
2-70	cag Gtc acc ttg aag gag tct ggt cct Gcg ctg gtg aaa ccc aca cag acc ctc acA ctg
	acc tgc acc ttc tct ggg ttc tca ctc agc
VH3	
3-07	GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GTC CAG CCT GGG GGG TCC CTG AGA CTC
	TCC TGT GCA GCC TCT GGA TTC ACC TTT AGT
3-09	gaA gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggC Agg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttt GAt
3-11	Cag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc Aag cct ggA ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-13	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-15	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA Aag cct ggg ggg tcc ctT aga ctc
	tcc tgt gca gcc tct gga ttc acT ttC agt
3-20	gag gtg cag ctg gtg gag tct ggg gga ggT Gtg gtA cGg cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttt GAt
3-21	gag gtg cag ctg gtg gag tct ggg gga ggc Ctg gtc Aag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-23	gag gtg cag ctg Ttg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttt agC
3-30	Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-30.3	Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt

(continued)

VH3	
3-30.5	Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-33	Cag gtg cag ctg gtg gag tct ggg gga ggc Gtg gtc cag cct ggg Agg tcc ctg aga ctc
	tcc tgt gca gcG tct gga ttc acc ttC agt
3-43	gaA gtg cag ctg gtg gag tct ggg gga gTc Gtg gtA cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttt GAt
3-48	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-49	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtA cag ccA ggg Cgg tcc ctg aga ctc
	tcc tgt Aca gcT tct gga ttc acc ttt Ggt
3-53	gag gtg cag ctg gtg gag Act ggA gga ggc ttg Atc cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct ggG ttc acc GtC agt
3-64	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-66	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc GtC agt
3-72	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggA ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-73	gag gtg cag ctg gtg gag tct ggg gga ggc ttg gtc cag cct ggg ggg tcc ctg aAa ctc
	tcc tgt gca gcc tct ggG ttc acc ttC agt
3-74	gag gtg cag ctg gtg gag tcC ggg gga ggc ttA gtT cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc ttC agt
3-d	gag gtg cag ctg gtg gag tct Cgg gga gTc ttg gtA cag cct ggg ggg tcc ctg aga ctc
	tcc tgt gca gcc tct gga ttc acc GtC agt
VH4	
4-04	CAG GTG CAG CTG CAG GAG TCG GGC CCA GGA CTG GTG AAG CCT TCG GGG ACC CTG TCC CTC
	ACC TGC GCT GTC TCT GGT GGC TCC ATC AGC

(continued)

VH4	
4-28	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAC acc ctg tcc ctc
	acc tgc gct gtc tct ggt TAc tcc atc agc
4-30.1	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc atc agc
4-30.2	cag Ctg cag ctg cag gag tcC ggc Tca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc
	acc tgc gct gtc tct ggt ggc tcc atc agc
4-30.4	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc atc agc
4-31	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcA CAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc atc agc
4-34	cag gtg cag ctA cag Cag tGg ggc Gca gga ctg Ttg aag cct tcg gAg acc ctg tcc ctc
	acc tgc gct gtc tAt ggt ggG tcc Ttc agT
4-39	cag Ctg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc atc agc
4-59	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc atc agT
4-61	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc ctc
	acc tgc Act gtc tct ggt ggc tcc Gtc agc
4-b	cag gtg cag ctg cag gag tcg ggc cca gga ctg gtg aag cct tcg gAg acc ctg tcc ctc
	acc tgc gct gtc tct ggt TAc tcc atc agc
VH5	
5-51	GAG GTG CAG CTG GTG CAG TCT GGA GCA GAG GTG AAA AAG CCC GGG GAG TCT CTG AAG ATC
	TCC TGT AAG GGT TCT GGA TAC AGC TTT ACC
5-a	gaA gtg cag ctg gtg cag tct gga gca gag gtg aaa aag ccc ggg gag tct ctg aGg atc
	tcc tgt aag ggt tct gga tac agc ttt acc

(continued)

VH6 6-1	CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC TCG CAG ACC CTC TCA CTC
	ACC TGT GCC ATC TCC GGG GAC AGT GTC TCT
VH7 7-4.1	CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT GGG GCC TCA GTG AAG GTT
	TCC TGC AAG GCT TCT GGA TAC ACC TTC ACT

Table 7: RERS sites in Human HC GLG FR1s where there are at least 20 GLGs cut

BsgI GTGCAG 71 (cuts 16/14 bases to right)

1: 4	1: 13	2: 13	3: 4	3: 13	4: 13
6: 13	7: 4	7: 13	8: 13	9: 4	9: 13
10: 4	10: 13	15: 4	15: 65	16: 4	16: 65
17: 4	17: 65	18: 4	18: 65	19: 4	19: 65
20: 4	20: 65	21: 4	21: 65	22: 4	22: 65
23: 4	23: 65	24: 4	24: 65	25: 4	25: 65
26: 4	26: 65	27: 4	27: 65	28: 4	28: 65
29: 4	30: 4	30: 65	31: 4	31: 65	32: 4
32: 65	33: 4	33: 65	34: 4	39: 65	35: 4
35: 65	36: 4	36: 65	37: 4	38: 4	39: 4
41: 4	42: 4	43: 4	45: 4	46: 4	47: 4
48: 4	48: 13	49: 4	49: 13	51: 4	

There are 39 hits at base# 4

There are 21 hits at base# 65

-"- ctgcac 9

12: 63	13: 63	14: 63	39: 63	41: 63	42: 63
44: 63	45: 63	46: 63			

BbvI GCAGC 65

1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
33: 6	33: 67	39: 6	39: 67	35: 6	35: 67
36: 6	36: 67	**37**: 6	**38**: 6	**39**: 6	**40**: 6
41: 6	**42**: 6	93: 6	**44**: 6	**45**: 6	**46**: 6
47: 6	98: 6	99: 6	**50**: 12	51: 6	

There are 43 hits at base# 6 Bolded sites very near sites listed below

There are 21 hits at base# 67

-"- gctgc 13

37: 9	**38:** 9	**39:** 9	**40:** 3	**40:** 9	**41:** 9
42: 9	**44:** 3	**44:** 9	**45:** 9	**46:** 9	**47:** 9
50: 9					

There are 11 hits at base# 9

BsoFI GCnge 78

1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
33: 6	33: 67	34: 6	39: 67	35: 6	35: 67
36: 6	36: 67	37: 6	37: 9	38: 6	38: 9
39: 6	39: 9	40: 3	40: 6	40: 9	41: 6

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

41: 9	42: 6	42: 9	43: 6	44: 3	44: 6
44: 9	45: 6	45: 9	46: 6	46: 9	47: 6
47: 9	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 These often occur together.
There are 11 hits at base# 9
There are 2 hits at base# 3
There are 21 hits at base# 67

TseI Gcwgc 78

1: 6	3: 6	6: 6	7: 6	8: 6	9: 6
10: 6	15: 6	15: 67	16: 6	16: 67	17: 6
17: 67	18: 6	18: 67	19: 6	19: 67	20: 6
20: 67	21: 6	21: 67	22: 6	22: 67	23: 6
23: 67	24: 6	24: 67	25: 6	25: 67	26: 6
26: 67	27: 6	27: 67	28: 6	28: 67	29: 6
30: 6	30: 67	31: 6	31: 67	32: 6	32: 67
33: 6	33: 67	34: 6	34: 67	35: 6	35: 67
36: 6	36: 67	37: 6	37: 9	38: 6	38: 9
39: 6	39: 9	40: 3	40: 6	40: 9	41: 6
41: 9	42: 6	42: 9	43: 6	44: 3	44: 6
44: 9	45: 6	45: 9	46: 6	46: 9	47: 6
47: 9	48: 6	49: 6	50: 9	50: 12	51: 6

There are 43 hits at base# 6 Often together.
There are 11 hits at base# 9
There are 2 hits at base# 3
There are 1 hits at base# 12
There are 21 hits at base# 67

MspAlI CMGckg 48

1: 7	3: 7	4: 7	5: 7	6: 7	7: 7
8: 7	9: 7	10: 7	11: 7	15: 7	16: 7
17: 7	18: 7	19: 7	20: 7	21: 7	22: 7
23: 7	29: 7	25: 7	26: 7	27: 7	28: 7
29: 7	30: 7	31: 7	32: 7	33: 7	34: 7
35: 7	36: 7	37: 7	38: 7	39: 7	40: 1
40: 7	41: 7	42: 7	44: 1	44: 7	45: 7
46: 7	47: 7	48: 7	49: 7	50: 7	51: 7

There are 46 hits at base# 7

PvuII CAGctg 48

1: 7	3: 7	4: 7	5: 7	6: 7	7: 7
8: 7	9: 7	10: 7	11: 7	15: 7	16: 7
17: 7	18: 7	19: 7	20: 7	21: 7	22: 7
23 : 7	24: 7	25 : 7	26: 7	27: 7	28: 7
29: 7	30: 7	31: 7	32: 7	33: 7	34: 7
35: 7	36: 7	37: 7	38: 7	39: 7	40: 1
40: 7	41: 7	42: 7	44: 1	44: 7	45: 7
46: 7	47: 7	48: 7	49: 7	50: 7	51: 7

There are 46 hits at base# 7
There are 2 hits at base# 1

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

AluI AGct **54**

1: 8	2: 8	3: 8	4: 8	4: 24	5: 8
6: 8	7: 8	8: 8	9: 8	10: 8	11: 8
15: 8	16: 8	17: 8	18: 8	19: 8	20: 8
21: 8	22: 8	23: 8	24: 8	25: 8	26: 8
27: 8	28: 8	29: 8	29: 69	30: 8	31: 8
32: 8	33: 8	34: 8	35: 8	36: 8	37: 8
38: 8	39: 8	40: 2	40: 8	41: 8	42: 8
43: 8	44: 2	44: 8	45: 8	46: 8	47: 8
48: 8	48: 82	49: 8	49: 82	50: 8	51: 8

There are 48 hits at base# 8

There are 2 hits at base# 2

DdeI Ctnag 48

1: 26	1: 48	2: 26	2: 48	3: 26	3: 48
4: 26	4: 48	5: 26	5: 48	6: 26	6: 48
7: 26	7: 48	8: 26	8: 48	9: 26	10: 26
11: 26	12: 85	13: 85	14: 85	15: 52	16: 52
17: 52	18: 52	19: 52	20: 52	21: 52	22: 52
23: 52	24: 52	25: 52	26: 52	27: 52	28: 52
29: 52	30: 52	31: 52	32: 52	33: 52	35: 30
35: 52	36: 52	40: 24	49: 52	51: 26	51: 48

There are 22 hits at base# 52 52 and 48 never together.

There are 9 hits at base# 48

There are 12 hits at base# 26 26 and 24 never together.

HphI tcacc 42

1: 86	3: 86	6: 86	7: 86	8: 80	11: 86
12: 5	13: 5	14: 5	15: 80	16: 80	17: 80
18: 80	20: 80	21: 80	22: 80	23: 80	24: 80
25: 80	26: 80	27: 80	28: 80	29: 80	30: 80
31: 80	32: 80	33: 80	34: 80	35: 80	36: 80
37: 59	38: 59	39: 59	40: 59	41: 59	42: 59
43: 59	44: 59	45: 59	46: 59	47: 59	50: 59

There are 22 hits at base# 80 80 and 86 never together

There are 5 hits at base# 86

There are 12 hits at base# 59

BssKI Nccngg 50

1: 39	2: 39	3: 39	4: 39	5: 39	7: 39
8: 39	9: 39	10: 39	11: 39	15: 39	16: 39
17: 39	18: 39	19: 39	20: 39	21: 29	21: 39
22: 39	23: 39	24: 39	25: 39	26: 39	27: 39
28: 39	29: 39	30: 39	31: 39	32: 39	33: 39
34: 39	35: 19	35: 39	36: 39	37: 24	38: 24
39: 24	41: 24	42: 24	44: 24	45: 24	46: 24
47: 24	48: 39	48: 40	49: 39	49: 40	50: 24
50: 73	51: 39				

There are 35 hits at base# 39 39 and 40 together twice.

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

There are 2 hits at base# 40

BsaJI Ccnngg 47

1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
8: 40	9: 40	9: 47	10: 40	10: 47	11: 40
15: 40	18: 40	19: 40	20: 40	21: 40	22: 40
23: 40	24: 40	25: 40	26: 40	27: 40	28: 40
29: 40	30: 40	31: 40	32: 40	34: 40	35: 20
35: 40	36: 40	37: 24	38: 24	39: 24	41: 24
42: 24	44: 24	45: 24	46: 24	47: 24	48: 40
48: 41	49: 40	49: 41	50: 74	51: 40	

There are 32 hits at base# 40 40 and 41 together twice

There are 2 hits at base# 41

There are 9 hits at base# 24

There are 2 hits at base# 47

BstNI CCwgg 44

PspGI ccwgg

ScrFI(SM.HpaII) CCwgg

1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
8: 40	9: 40	10: 40	11: 40	15: 40	16: 40
17: 40	18: 40	19: 40	20: 40	21: 30	21: 40
22: 40	23: 40	24: 40	25: 40	26: 40	27: 40
28: 40	29: 40	30: 40	31: 40	32: 40	33: 40
34: 40	35: 40	36: 40	37: 25	38: 25	39: 25
41: 25	42: 25	44: 25	45: 25	46: 25	47: 25
50: 25	51: 40				

There are 33 hits at base# 40

ScrFI CCngg 50

1: 40	2: 40	3: 40	4: 40	5: 40	7: 40
8: 40	9: 40	10: 40	11: 40	15: 40	16: 40
17: 40	18: 40	19: 40	20: 40	21: 30	21: 40
22: 40	23: 40	24: 40	25: 40	26: 40	27: 40
28: 40	29: 40	30: 40	31: 40	32: 40	33: 40
34: 40	35: 20	35: 40	36: 40	37: 25	38: 25
39: 25	41: 25	42: 25	44: 25	45: 25	46: 25
47: 25	48: 40	48: 41	49: 40	49: 41	50: 25
50: 74	51: 40				

There are 35 hits at base# 40

There are 2 hits at base# 41

EcoO109I RGgnccy 34

1: 43	2: 43	3: 43	4: 43	5: 43	6: 43
7: 43	8: 43	9: 43	10: 43	15: 46	16: 46
17: 46	18: 46	19: 46	20: 46	21: 46	22: 46
23: 46	24: 46	25: 46	26: 46	27: 46	28: 46
30: 46	31: 46	32: 46	33: 46	34: 46	35: 46
36: 46	37: 46	43: 79	51: 43		

There are 22 hits at base# 46 46 and 43 never together

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

There are 11 hits at base# 43

N1aIV GGNncc 71

1: 43	2: 43	3: 43	4: 43	5: 43	6: 43
7: 43	8: 43	9: 43	9: 79	10: 43	10: 79
15: 46	15: 47	16: 47	17: 46	17: 47	18: 46
18: 47	19: 46	19: 47	20: 46	20: 47	21: 46
21: 47	22: 46	22: 47	23: 47	24: 47	25: 47
26: 47	27: 46	27: 47	28: 46	28: 47	29: 47
30: 46	30: 47	31: 46	31: 47	32: 46	32: 47
33: 46	33: 47	34: 46	34: 47	35: 46	35: 47
36: 46	36: 47	37: 21	37: 46	37: 47	37: 79
38: 21	39: 21	39: 79	40: 79	41: 21	41: 79
42: 21	42: 79	43: 79	44: 21	44: 79	45: 21
45: 79	46: 21	46: 79	47: 21	51: 43	

There are 23 hits at base# 47 46 & 47 often together

There are 17 hits at base# 46 There are 11 hits at base# 43

Sau96I Ggncc 70

1: 44	2: 3	2: 44	3: 44	4: 44	5: 3	5: 44	6: 44
7: 44	8: 22	8: 44	9: 44	10: 44	11: 3	12: 22	13: 22
14: 22	15: 33	15: 47	16: 47	17: 47	18: 47	19: 47	20: 47
21: 47	22: 47	23: 33	23: 47	24: 33	24: 47	25: 33	25: 47
26: 33	26: 47	27: 47	28: 47	29: 47	30: 47	31: 33	31: 47
32: 33	32: 47	33: 33	33: 47	34: 33	34: 47	35: 47	36: 47
37: 21	37: 22	37: 47	38: 21	38: 22	39: 21	39: 22	41: 21
41: 22	42: 21	42: 22	43: 80	44: 21	44: 22	45: 21	45: 22
46: 21	46: 22	47: 21	47: 22	50: 22	51: 44		

There are 23 hits at base# 47 These do not occur together.

There are 11 hits at base# 44

There are 14 hits at base# 22 These do occur together.

There are 9 hits at base# 21

BsmAI GTCTCNnnnn 22

1: 58	3: 58	4: 58	5: 58	8: 58	9: 58
10: 58	13: 70	36: 18	37: 70	38: 70	39: 70
40: 70	41: 70	42: 70	44: 70	45: 70	46: 70
47: 70	48: 48	49: 48	50: 85		

There are 11 hits at base# 70

-"- Nnnnnngagac 27

13: 40	15: 48	16: 48	17: 48	18: 48	20: 48
21: 48	22: 48	23: 48	24: 48	25: 48	26: 48
27: 48	28: 48	29: 48	30: 10	30: 48	31: 48
32: 48	33: 48	35: 48	36: 48	43: 40	44: 40
45: 40	46: 40	47: 40			

There are 20 hits at base# 48

AvaII Ggwcc 44

Sau96I($M.HaeIII) Ggwcc 44

2: 3	5: 3	6: 44	8: 44	9: 44	10: 44
11: 3	12: 22	13: 22	14: 22	15: 33	15: 47

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

16: 47	17: 47	18: 47	19: 47	20: 47	21: 47
22: 47	23: 33	23: 47	24: 33	24: 47	25: 33
25: 47	26: 33	26: 47	27: 47	28: 47	29: 47
30: 47	31: 33	31: 47	32: 33	32: 47	33: 33
33: 47	34: 33	34: 47	35: 47	36: 47	37: 47
43: 80	50: 22				

There are 23 hits at base# 47 44 & 47 never together

There are 4 hits at base# 44

PpuMI RGgwccy 27

6: 43	8: 43	9: 43	10: 43	15: 46	16: 46
17: 46	18: 46	19: 46	20: 46	21: 46	22: 46
23: 46	24: 46	25: 46	26: 46	27: 46	28: 46
30: 46	31: 46	32: 46	33: 46	34: 46	35: 46
36: 46	37: 46	43: 79			

There are 22 hits at base# 46 43 and 46 never occur together.

There are 4 hits at base# 43

BsmFI GGGAC 3

8: 43	37: 46	50: 77

-"- gtccc 33

15: 48	16: 48	17: 48	1: 0	1: 0	20: 48
21: 48	22: 48	23: 48	24: 48	25: 48	26: 48
27: 48	28: 48	29: 48	30: 48	31: 48	32: 48
33: 48	34: 48	35: 48	36: 48	37: 54	38: 54
39: 54	40: 54	41: 54	42: 54	43: 54	44: 54
45: 54	46: 54	47: 54			

There are 20 hits at base# 48

There are 11 hits at base# 54

HinfI Gantc 80

8: 77	12: 16	13: 16	14: 16	15: 16	15: 56
15: 77	16: 16	16: 56	16: 77	17: 16	17: 56
17: 77	18: 16	18: 56	18: 77	19: 16	19: 56
19: 77	20: 16	20: 56	20: 77	21: 16	21: 56
21: 77	22: 16	22: 56	22: 77	23: 16	23: 56
23: 77	24: 16	24: 56	24: 77	25: 16	25: 56
25: 77	26: 16	26: 56	26: 77	27: 16	27: 26
27: 56	27: 77	28: 16	28: 56	28: 77	29: 16
29: 56	29: 77	30: 56	31: 16	31: 56	31: 77
32: 16	32: 56	32: 77	33: 16	33: 56	33: 77
34: 16	35: 16	35: 56	35: 77	36: 16	36: 26
36: 56	36: 77	37: 16	38: 16	39: 16	40: 16
41: 16	42: 16	44: 16	45: 16	46: 16	47: 16
48: 46	49: 46				

There are 34 hits at base# 16

TfiI Gawtc 21

8: 77	15: 77	16: 77	17: 77	18: 77	19: 77
20: 77	21: 77	22: 77	23: 77	24: 77	25: 77

(continued)

BsgI GTGCAG 71 (cuts 16/14 bases to right)

26: 77	27: 77	28: 77	29: 77	31: 77	32: 77
33: 77	35: 77	36: 77			

There are 21 hits at base# 77

MlyI GAGTC 38

12: 16	13: 16	14: 16	15: 16	16: 16	17: 16
18: 16	19: 16	20: 16	21: 16	22: 16	23: 16
24: 16	25: 16	26: 16	27: 16	27: 26	28: 16
29: 16	31: 16	32: 16	33: 16	34: 16	35: 16
36: 16	36: 26	37: 16	38: 16	39: 16	40: 16
41: 16	42: 16	44: 16	45: 16	46: 16	47: 16
48: 46	49: 46				

There are 34 hits at base# 16

-"- GACTC 21

15: 56	16: 56	17: 56	18: 56	19: 56	20: 56
21: 56	22: 56	23: 56	24: 56	25: 56	26: 56
27: 56	28: 56	29: 56	30: 56	31: 56	32: 56
33: 56	35: 56	36: 56			

There are 21 hits at base# 56

PleI gagtc 38

12: 16	13: 16	14: 16	15: 16	16: 16	17: 16
18: 16	19: 16	20: 16	21: 16	22: 16	23: 16
24: 16	25: 16	26: 16	27: 16	27: 26	28: 16
29: 16	31: 16	32: 16	33: 16	34: 16	35: 16
36: 16	36: 26	37: 16	38: 16	39: 16	40: 16
41: 16	42: 16	44: 16	45: 16	46: 16	47: 16
48: 46	49: 46				

There are 34 hits at base# 16

-"- gactc 21

15: 56	16: 56	17: 56	18: 56	19: 56	20: 56
21: 56	22: 56	23: 56	24: 56	25: 56	26: 56
27: 56	28: 56	29: 56	30: 56	31: 56	32: 56
33: 56	35: 56	36: 56			

There are 21 hits at base# 56

AlwNI CAGNNNctg 26

15: 68	16: 68	17: 68	18: 68	19: 68	20: 68
21: 68	22: 68	23: 68	24: 68	25: 68	26: 68
27: 68	28: 68	29: 68	30: 68	31: 68	32: 68
33: 68	34: 68	35: 68	36: 68	39: 46	40: 46
41: 46	42: 46				

There are 22 hits at basel 68

Table 8: Kappa FR1 GLGs

! 1	2	3	4	5	6	7	8	9	10	11	12		
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
! 13	14	15	16	17	18	19	20	21	22	23			
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		O12
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		O2
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		O18
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		O8
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		A20
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		A30
AAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	GCC	ATG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L14
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L1
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L15
GCC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		L4
GCC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		L18
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	TCC	GTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L5
GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCT	TCT	GTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L19
GAC	ATC	CAG	TTG	ACC	CAG	TCT	CCA	TCC	TTC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		L8
GCC	ATC	CGG	ATG	ACC	CAG	TCT	CCA	TTC	TCC	CTG	TCT		
GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGC	!		L23
GCC	ATC	CGG	ATG	ACC	CAG	TCT	CCA	TCC	TCA	TTC	TCT		
GCA	TCT	ACA	GGA	GAC	AGA	GTC	ACC	ATC	ACT	TGT	!		L9

GTC ATC TGG ATG ACC CAG TCT CCA TCC TTA CTC TCT
GCA TCT ACA GGA GAC AGA GTC ACC ATC AGT TGT ! L24

GCC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L11

GAC ATC CAG ATG ACC CAG TCT CCT TCC ACC CTG TCT
GCA TCT GTA GGA GAC AGA GTC ACC ATC ACT TGC ! L12

GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O11

GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! O1

GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A17

GAT GTT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A1

GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A18

GAT ATT GTG ATG ACC CAG ACT CCA CTC TCT CTG TCC
GTC ACC CCT GGA CAG CCG GCC TCC ATC TCC TGC ! A2

GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A19

GAT ATT GTG ATG ACT CAG TCT CCA CTC TCC CTG CCC
GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGC ! A3

GAT ATT GTG ATG ACC CAG ACT CCA CTC TCC TCA CCT
GTC ACC CTT GGA CAG CCG GCC TCC ATC TCC TGC ! A23

GAA ATT GTG TTG ACG CAG TCT CCA GGC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A27

GAA ATT GTG TTG ACG CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! A11

GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L2

GAA ATA GTG ATG ACG CAG TCT CCA GCC ACC CTG TCT
GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L16

GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC ! L6

GAA ATT GTG TTG ACA CAG TCT CCA GCC ACC CTG TCT

```
TTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC  !     L20
GAA ATT GTA ATG ACA CAG TCT CCA GCC ACC CTG TCT
TTG TCT CCA GGG GAA. AGA GCC ACC CTC TCC TGC  !     L25
GAC ATC GTG ATG ACC CAG TCT CCA GAC TCC CTG GCT
GTG TCT CTG GGC GAG AGG GCC ACC ATC AAC TGC  !     B3
GAA ACG ACA CTC ACG CAG TCT CCA GCA TTC ATG TCA
GCG ACT CCA GGA GAC AAA GTC AAC ATC TCC TGC  !     B2
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC  !     A26
GAA ATT GTG CTG ACT CAG TCT CCA GAC TTT CAG TCT
GTG ACT CCA AAG GAG AAA GTC ACC ATC ACC TGC  !     A10
GAT GTT GTG ATG ACA CAG TCT CCA GCT TTC CTC TCT
GTG ACT CCA GGG GAG AAA GTC ACC ATC ACC TGC  !     A14
```

Table 9 RERS sites found in Human Kappa FR1 GLGs

	MslI	FokI --> <-- ->	PflFI	BsrI	BsmAI	MnlI	HpyCH 4V
VKI							
O12 1-69	3	3 23	12 49	15	18 47	26	36
O2 101.169	103	103 123	112 149	115	118 147	126	136
O18 201-269	203	203 223	212 249	215	218 247	226	236
O8 301-369	303	303 323	312 349	315	318 347	326	336
A20 401-469	403	403 423	412 449	415	418 447	426	436
A30 501-569	503	503 523	512 549	515	518 547	526	536
L14 601-669	603	603	612 649	615	618 647	-	636
L1 701-769	703	703 723	712 749	715	718 747	726	736
L15 801-869	803	803 823	812 849	815	818 847	826	836
L4 901-969	-	903 923	912 949	906 915	918 947	926	936
L18 1001-1069	-	1003	101 1049	1006 1015	1018 1047	1026	1036
L5 1101-1169	1103	-	1112 1149	1115	1118 1147	-	1136
L19 1201-1269	1203	1203	1212 1249	1215	1218 1247	-	1236
L8 1301-1369	-	1303 1323	1312 1349	1306 1315	1318 1347	-	1336
L23 1401-1469	1403	14031408	1412 1449	1415	1418 1447	-	1436
L9 1501-1569	1503	1503 1508 1523	1512 1549	1515	1518 1547	1526	1536
L24 1601-1669	1603	1608 1623	1612 1649	1615	1618 1647	-	1636
L11 1701-1769	1703	1703 1723	1712 1749	1715	1718 1747	1726	1736
L12 1801-1869	1803	1803	1812 1849	1815	1818 1847	-	1836
VKII							
O11 1901-1969	-	-	-	-	-	1956	-
O1 2001-2069	-	-	-	-		2056	-

(continued)

VKII							
A17 2101-2169	-	-	2112	-	2118	2156	-
A1 2201-2269	-	-	2212	-	2218	2256	-
A18 2301-2369	-	-	-	-	-	2356	-
A2 2401-2469	-	-	-	-	-	2456	-
A19 2501-2569	-	-	2512	-	2518	2556	-
A3 2601-2669	-	-	2612	-	2618	2656	-
A23 2701-2769	-	-	-	-	-	2729 2756	-
VKIII							
A27 2801-2869	-	-	2812	-	2818 2839	2860	-
A11 2901-2969	-	-	2912	-	2918 2939	2960	-
L2 3001-3069	-	-	3012	-	3018 3039	3060	-
L16 3101-3169	-	-	3112	-	3118 3139	3160	-
L6 3201-3269	-	-	3212	-	3218 3239	3260	-
L20 3301-3369	-	-	3312	-	3318 3339	3360	-
L25 3401-3469	-	-	3412	-	3418 3439	3460	-
VKIV							
B3 3501-3569	3503	-	3512	3515	3518 3539	3551<	-
VKV							
82 3601-3669	-	-	3649	-	3618 3647		-
VKVI							
A26 3701-3769	-	-	3712	-	3718		-
A10 3801-3869	-	-	3812	-	3818		-

(continued)

VKVI							
A14 3901-3969	-	-	3912	-	3918	3930>	-
VKI							
O12 1-69	37	41	53	53	55	56	-
O2 101-169	137	141	153	153	155	156	-
O18 201-269	237	241	253	253	255	256	-
O8 301-369	337	341	353	353	355	356	-
A20 401-469	437	441	453	453	455	456	-
A30 501-569	537	541	553	553	555	556	-
L14 601-669	637	641	653	653	655	656	-
L1 701-769	737	741	753	753	755	756	-
L15 801-869	837	841	853	853	855	856	-
L4 901-969	937	941	953	953	955	956	-
L18 1001-1069	1037	1041	1053	1053	1055	1056	-
L5 1101-1169	1137	1141	1153	1153	1155	1156	-
L19 1201-1269	1237	1241	1253	1253	1255	1256	-
L8 1301-1369	1337	1341	1353	1353	1355	1356	-
L23 1401-1469	1437	1441	1453	1453	1455	1456	1406
L9 1501-1569	1537	1541	1553	1553	1555	1556	1506
	SfaNI	SfcI	HinfI	MlyI --> --> <--	MaeIII Tsp45I same sites	HphI xx38 xx56 xx62	HpaII MspI xx06 xx52
L24 1601-1669	1637	1641	1653	1653	1655	1656	
L11 1701-1769	1737	1741	1753	1753	1755	1756	
L12 1801-1869	1837	1841	1853	1853	1855	1856	

(continued)

VKII							
O11 1901-1969	-	-	1918	1918	1937	1938	1952
O1 2001-2069	-	-	2018	2018	2037	2038	2052
A17 2101-2169	-	-	2112	2112	2137	2138	2152
A1 2201-2269	-	-	2212	2212	2237	2238	2252
A18 2301-2369	-	-	2318	2318	2337	2338	2352
A2 2401-2469	-	-	2418	2418	2437	2438	2452
A19 2501-2569	-	-	2512	2512	2537	2538	2552
A3 2601-2669	-	-	2612	2612	2637	2638	2652
A23 2701-2769	-	-	2718	2718	2737	2731* 2738*	-
VKIII							
A27 2801-2869	-	-	-	-			-
A11 2901-2969	-	-	-	-			-
L2 3001-3069	-	-	-	-			-
L16 3101-3169	-	-	-	-			-
L6 3201-3269	-	-	-	-			-
L20 3301-3369	-	-	-	-			-
L25 3401-3469	-	-	-	-			-
VKIV							
B3 3501-3569	-	-	3525	3525			-
VKV							
B2 3601-3669	-	-	3639	3639			-
VKVI							
A26 3701-3769	-	-	3712 3739	3712 3739	3737 3755	3756 3762	-
A10 3801-3869	-	-	3812 3839	3812 3839	3837 3855	3856 3862	-
A14 3901-3969	-	-	3939	3939	3937 3955	3956 3962	-

(continued)

	BsaJI xx29 xx42 xx43	BsskI (NstNI) xx22 xx30 xx43	BpmI xx20 xx41 xx44 --> --> <--	BsrFI Cac8I NaeI NgoMIV	HaeIII	Tsp509I
VKI						
O12 1-69	-	-	-	-	-	-
O2 101-169	-	-	-	-	-	-
O18 201-269	-	-	-	-	-	-
O8 301-369	-	-	-	-	-	-
A20 401-469	-	-	-	-	-	-
A30 501-569	-	-	-	-	-	-
L14 601-669	-	-	-	-	-	-
L1 701-769	-	-	-	-	-	-
L15 801-869	-	-	-	-	-	-
L4 901-969	-	-	-	-	-	-
L18 1001-1069	-	-	-	-	-	-
L5 1101-1169	-	-	-	-	-	-
L19 1201-1269	-	-	-	-	-	-
L8 1301-1369	-	-	-	-	-	-
L23 1401-1469	-	-	-	-	-	-
L9 1501-1569	-	-	-	-	-	-
L24 1601-1669	-	-	-	-	-	-
L11 1701-1769	-	-	-	-	-	-
L12 1801-1869	-	-	-	-	-	-
VKII						
O11 1901-1969	1942	1943	1944	1951	1954	-
O1 2001-2069	2042	2043	2044	2051	2054	-
A17 2101-2169	2142	-	-	2151	2154	-
A1 2201-2269	2242	-	-	2251	2254	-

(continued)

VKII						
A18 2301-2369	2342	2343	-	2351	2354	-
A2 2401-2469	2442	2443	-	2451	2454	-
A19 2501-2569	2542	2543	2544	2551	2554	-
A3 2601-2669	2642	2643	2644	2651	2654	-
A23 2701-2769	2742	-	-	2751	2754	-
VKIII						
A27 2801-2869	2843	2822 2843	2820 2841	-	-	2803
A11 2901-2969	2943	2943	2920 2941	-	-	2903
L2 3001-3069	3043	3043	3041	-	-	-
L16 3101-3169	3143	3143	3120 3141	-	-	-
L6 3201-3269	3243	3243	3220 3241	-	-	3203
L20 3301-3369	3343	3343	3320 3341	-	-	3303
L25 3401-3469	3443	3443	3420 3441	-	-	3403
VKIV						
B3 3501-3569	3529	3530	3520	-	3554	
VKV						
B2 3601-3669		3643	3620 3641	-	-	
VKVI						
A26 3701-3769		-	3720	-	-	3703
A10 3801-3869		-	3820	-	-	3803
A14 3901-3969	3943	3943	3920 3941	-	-	-

Table 10 Lambda FR1 GLG sequences

! VL1

CAG TCT GTG CTG ACT CAG CCA CCC TCG GTG TCT GAA
GCC CCC AGG CAG AGG GTC ACC ATC TCC TGT ! 1a

cag tct gtg ctg acG cag ccG ccc tcA gtg tct gGG
gcc ccA Ggg cag agg gtc acc atc tcc tgC ! 1e

cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1c

cag tct gtg ctg act cag cca ccc tcA gCg tct gGG
Acc ccc Ggg cag agg gtc acc atc tcT tgt ! 1g

cag tct gtg Ttg acG cag ccG ccc tcA gtg tct gCG
gcc ccA GgA cag aAg gtc acc atc tcc tgC ! 1b

! VL2

CAG TCT GCC CTG ACT CAG CCT CCC TCC GCG TCC GGG
TCT CCT GGA CAG TCA GTC ACC ATC TCC TGC ! 2c

cag tct gcc ctg act cag cct cGc tcA gTg tcc ggg
tct cct gga cag tca gtc acc atc tcc tgc! 2e

cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
tct cct gga cag tcG Atc acc atc tcc tgc ! 2a2

cag tct gcc ctg act cag cct ccc tcc gTg tcc ggg
tct cct gga cag tca gtc acc atc tcc tgc ! 2d

cag tct gcc ctg act cag cct Gcc tcc gTg tcT ggg
tct cct gga cag tcG Atc acc atc tcc tgc ! 2b2

! VL3

TCC TAT GAG CTG ACT CAG CCA CCC TCA GTG TCC GTG
TCC CCA GGA CAG ACA GCC AGC ATC ACC TGC! 3r

tcc tat gag ctg act cag cca cTc tca gtg tcA gtg
Gcc cTG gga cag acG gcc agG atT acc tgT ! 3j

tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
tcc cca gga caA acG gcc agG atc acc tgc! 3p

tcc tat gag ctg acA cag cca ccc tcG gtg tcA gtg
tcc cTa gga cag aTG gcc agG atc acc tgc ! 3a

tcT tCt gag ctg act cag GAC ccT GcT gtg tcT gtg
Gcc TTG gga cag aca gTc agG atc acA tgc ! 3l

(continued)

tcc tat gTg ctg act cag cca ccc tca gtg tcA gtg
Gcc cca gga Aag acG gcc agG atT acc tgT ! 3h
tcc tat gag ctg acA cag cTa ccc tcG gtg tcA gtg
tcc cca gga cag aca gcc agG atc acc tgc ! 3e
tcc tat gag ctg aTG cag cca ccc tcG gtg tcA gtg
tcc cca gga cag acG gcc agG atc acc tgc ! 3m
tcc tat gag ctg acA cag cca Tcc tca gtg tcA gtg
tcT ccG gga cag aca gcc agG atc acc tgc ! V2-19

VL4

CTG CCT GTG CTG ACT CAG CCC CCG TCT GCA TCT GCC
TTG CTG GGA GCC TCG ATC AAG CTC ACC TGC ! 4c
cAg cct gtg ctg act caA TcA TcC tct gcC tct gcT
tCC ctg gga Tcc tcg Gtc aag ctc acc tgc ! 4a
cAg cTt gtg ctg act caA TcG ccC tct gcC tct gcc
tCC ctg gga gcc tcg Gtc aag ctc acc tgc ! 4b

! VL5

CAG CCT GTG CTG ACT CAG CCA CCT TCC TCC TCC GCA
TCT CCT GGA GAA TCC GCC AGA CTC ACC TGC ! 5e
cag Gct gtg ctg act cag ccG Gct tcc CTc tcT gca
tct cct gga gCa tcA gcc agT ctc acc tgc ! 5c
cag cct gtg ctg act cag cca Tct tcc CAT tcT gca
tct Tct gga gCa tcA gTc aga ctc acc tgc ! 5b

! VL6

AAT TTT ATG CTG ACT CAG CCC CAC TCT GTG TCG GAG
TCT CCG GGG AAG ACG GTA ACC ATC TCC TGC ! 6a

! VL7

CAG ACT GTG GTG ACT CAG GAG CCC TCA CTG ACT GTG
TCC CCA GGA GGG ACA GTC ACT CTC ACC TGT ! 7a
cag Gct gtg gtg act cag gag ccc tca ctg act gtg
tcc cca gga ggg aca gtc act ctc acc tgt ! 7b

(continued)

! VL8

CAG ACT GTG GTG ACC CAG GAG CCA TCG TTC TCA GTG
TCC CCT GGA GGG ACA GTC ACA CTC ACT TGT ! 8a

! VL9

CAG CCT GTG CTG ACT CAG CCA CCT TCT GCA TCA GCC
TCC CTG GGA GCC TCG GTC ACA CTC ACC TGC ! 9a

! VL10

CAG GCA GGG CTG ACT CAG CCA CCC TCG GTG TCC AAG
GGC TTG AGA CAG ACC GCC ACA CTC ACC TGC ! 10a

Table 11 RERSs found in human lambda FR1 GLGs

! There are 31 lambda GLGs

MlyI NnnnnnGACTC 25

1: 6	3: 6	4: 6	6: 6	7: 6	8: 6
9: 6	10: 6	11: 6	12: 6	15: 6	16: 6
20: 6	21: 6	22: 6	23: 6	23: 50	24: 6
25: 6	25: 50	26: 6	27: 6	28: 6	30: 6
31: 6					

There are 23 hits at base# 6

-"- GAGTCNNNNNn 1

26: 34

MwoI GCNNNNNngc 20

1: 9	2: 9	3: 9	4: 9	11: 9	11: 56
12: 9	13: 9	14: 9	16: 9	17: 9	18: 9
19: 9	20: 9	23: 9	24: 9	25: 9	26: 9
30: 9	31: 9				

There are 19 hits at base# 9

HinfI Gantc 27

1: 12	3: 12	4: 12	6: 12	7: 12	8: 12
9: 12	10: 12	11: 12	12: 12	15: 12	16: 12
20: 12	21: 12	22: 12	23: 12	23: 46	23: 56
24: 12	25: 12	25: 56	26: 12	26: 34	27: 12
28: 12	30: 12	31: 12			

There are 23 hits at base# 12

PleI gactc 25

1: 12	3: 12	4: 12	6: 12	7: 12	8: 12
9: 12	10: 12	11: 12	12: 12	15: 12	16: 12
20: 12	21: 12	22: 12	23: 12	23: 56	24: 12
25: 12	25: 56	26: 12	27: 12	28: 12	30: 12
31: 12					

There are 23 hits at base# 12

-"- gagtc 1

26: 34

DdeI Ctnag 32

1: 14	2: 24	3: 14	3: 24	4: 14	4: 24

(continued)

5: 24	6: 14	7: 14	7: 24	8: 14	9: 14
10: 14	11: 14	11: 24	12: 14	12: 24	15: 5
15: 14	16: 14	16: 24	19: 24	20: 14	23: 14
24: 14	25: 14	26: 14	27: 14	28: 14	29: 30
30: 14	31: 14				

There are 21 hits at base# 14

BsaJI Ccnngg 38

1: 23	1: 40	2: 39	2: 40	3: 39	3: 40
4: 39	9: 40	5: 39	11: 39	12: 38	12: 39
13: 23	13: 39	14: 23	14: 39	15: 38	16: 39
17: 23	17: 39	18: 23	18: 39	21: 38	21: 39
21: 47	22: 38	22: 39	22: 47	26: 40	27: 39
28: 39	29: 14	29: 39	30: 38	30: 39	30: 47
31: 23	31: 32				

There are 17 hits at base# 39

There are 5 hits at base# 38

There are 5 hits at base# 40 Makes cleavage ragged.

Mn1I cctc 35

1: 23	2: 23	3: 23	4: 23	5: 23	6: 19
6: 23	7: 19	8: 23	9: 19	9: 23	10: 23
11: 23	13: 23	14: 23	16: 23	17: 23	18: 23
19: 23	20: 47	21: 23	21: 29	21: 47	22: 23
22: 29	22: 35	22: 47	23: 26	23: 29	24: 27
27: 23	28: 23	30: 35	30: 47	31: 23	

There are 21 hits at base# 23

There are 3 hits at base# 19

There are 3 hits at base# 29

There are 1 hits at base# 26

There are 1 hits at base# 27 These could make cleavage ragged.

-"- gagg 7

1: 48	2: 48	3: 48	4: 48	27: 44	28: 44
29: 44					

BssKI Nccngg 39

1: 40	2: 39	3: 39	3: 40	4: 39	9: 40
5: 39	6: 31	6: 39	7: 31	7: 39	8: 39
9: 31	9: 39	10: 39	11: 39	12: 38	12: 52
13: 39	13: 52	14: 52	16: 39	16: 52	17: 39
17: 52	18: 39	18: 52	19: 39	19: 52	21: 38
22: 38	23: 39	24: 39	26: 39	27: 39	28: 39
29: 14	29: 39	30: 38			

There are 21 hits at base# 39

There are 4 hits at base# 38

There are 3 hits at base# 31

There are 3 hits at base# 40 Ragged

BstNI CCwgg 30

1: 41	2: 40	5: 40	6: 40	7: 40	8: 40
9: 40	10: 40	11: 40	12: 39	12: 53	13: 40
13: 53	14: 53	16: 40	16: 53	17: 40	17: 53

(continued)

18: 40	18: 53	19: 53	21: 39	22: 39	23: 40
24: 40	27: 40	28: 40	29: 15	29: 40	30: 39

There are 17 hits at base# 40
There are 7 hits at base# 53
There are 4 hits at base# 39
There are 1 hits at base# 41 Ragged

PspGI ccwgg 30

1: 41	2: 40	5: 40	6: 40	7: 40	8: 40
9: 40	10: 40	11: 40	12: 39	12: 53	13: 40
13: 53	14: 53	16: 40	16: 53	17: 40	17: 53
18: 40	18: 53	19: 53	21: 39	22: 39	23: 40
24: 40	27: 40	28: 40	29: 15	29: 40	30: 39

There are 17 hits at base# 40
There are 7 hits at base# 53
There are 4 hits at base# 39
There are 1 hits at base# 41

ScrFI CCngg 39

1: 41	2: 40	3: 40	3: 41	4: 40	4: 41
5: 40	6: 32	6: 40	7: 32	7: 40	8: 40
9: 32	9: 40	10: 40	11: 40	12: 39	12: 53
13: 40	13: 53	14: 53	16: 40	16: 53	17: 40
17: 53	18: 40	18: 53	19: 40	19: 53	21: 39
22: 39	23: 40	29: 40	26: 40	27: 40	28: 40
29: 15	29: 40	30: 39			

There are 21 hits at base# 40
There are 4 hits at base# 39
There are 3 hits at base# 41

MaeIII gtnac 16

1: 52	2: 52	3: 52	4: 52	5: 52	6: 52
7: 52	9: 52	26: 52	27: 10	27: 52	28: 10
28: 52	29: 10	29: 52	30: 52		

There are 13 hits at base# 52

Tsp45I gtsac 15

1: 52	2: 52	3: 52	4: 52	5: 52	6: 52
7: 52	9: 52	27: 10	27: 52	28: 10	28: 52
29: 10	29: 52	30: 52			

There are 12 hits at base# 52

HphI tcacc 26

1: 53	2: 53	3: 53	4: 53	5: 53	6: 53
7: 53	8: 53	9: 53	10: 53	11: 59	13: 59
14: 59	17: 59	18: 59	19: 59	20: 59	21: 59
22: 59	23: 59	24: 59	25: 59	27: 59	28: 59
30: 59	31: 59				

There are 16 hits at base# 59
There are 10 hits at base# 53

(continued)

BspMI ACCTGCNNNNn			14		
11: 61	13: 61	14: 61	17: 61	18: 61	19: 61
20: 61	21: 61	22: 61	23: 61	24: 61	25: 61
30: 61	31: 61				

There are 14 hits at base# 61 Goes into CDR1

Table 12: Matches to URE FR3 adapters in 79 human HC.

A. List of Heavy-chains genes sampled

AF008566	AF103367	HSA235674	HSU94417	S83240
AF035043	AF103368	HSA235673	HSU94418	SABVH369
AF103026	AF103369	HSA240559	HSU96389	SADEIGVH
af103033	AF103370	HSCB201	HSU96391	SAH2IGVH
AF103061	af103371	HSIGGVHC	HSU96392	SDA3IGVH
Af103072	AF103372	HSU44791	HSU96395	SIGVHTTD
af103078	AF158381	HSU44793	HSZ93849	SUK4IGVH
AF103099	E05213	HSU82771	HSZ93850	
AF103102	E05886	HSU82949	HSZ93851	
AF103103	E05887	HSU82950	HSZ93853	
AF103174	HSA235661	HSU82952	HSZ93855	
AF103186	HSA235664	HSU82961	HSZ93857	
af103187	HSA235660	HSU86522	HSZ93860	
AF103195	HSA235659	HSU86523	HSZ93863	
af103277	HSA235678	HSU92452	MCOMFRAA	
af103286	HSA235677	HSU94412	MCOMFRVA	
AF103309	HSA235676	HSU94415	582745	
af103343	HSA235675	HSU94416	S82764	

Table 12B. Testing all distinct GLGs from bases 89.1 to 93.2 of the heavy variable domain

Id	Nb	0	1	2	3	4		SEQ ID NO:
1	38	15	11	10	0	2	Seq1 gtgtattactgtgc	25
2	19	7	6	4	2	0	Seq2 gtAtattactgtgc	26
3	1	0	0	1	0	0	Seq3 gtgtattactgtAA	27
4	7	1	5	1	0	0	Seq4 gtgtattactgtAc	28
5	0	0	0	0	0	0	Seq5 Ttgtattactgtgc	29
6	0	0	0	0	0	0	Seq6 TtgtatCactgtgc	30
7	3	1	0	1	1	0	Seq7 ACAtattactgtgc	31
8	2	0	2	0	0	0	Seq8 ACgtattactgtgc	32
9	9	2	2	4	1	0	Seq9 ATqtattactqtqc	33
Group		26	26	21	4	2		
Cumulative		26	52	73	77	79		

Table 12C Most important URE recognition seqs in FR3 Heavy

1	VHSzyl	GTGtattactgtgc	(ON_SHC103)	(SEQ ID NO:25)
2	VHSzy2	GTAtattactgtgc	(ON_SHC323)	(SEQ ID NO:26)
3	VHSzy4	GTGtattactgtac	(ON_SHC349)	(SEQ ID NO:28)
4	VHSzy9	ATGtattactgtgc	(ON_SHC5a)	(SEQ ID NO:33)

Table 12D, testing 79 human HC V genes with four probes

Number of sequences									79
Number of bases									29143
Number of mismatches									
Id	Best	0	1	2	3	4	5		
1	39	15	11	10	1	2	0	Seq1 gtgtattactgtgc	(SEQ ID NO:25)
2	22	7	6	5	3	0	1	Seq2 gtAtattactgtgc	(SEQ ID NO:26)
3	7	1	5	1	0	0	0	Seq4 gtgtattactgtAc	(SEQ ID NO:28)
4	11	2	4	4	1	0	0	Seq9 ATgtattactgtgc	(SEQ ID NO:33)
Group	25	26	20	5	2				
Cumulative	25	51	71	76	78				

One sequence has five mismatches with sequences 2, 4, and 9; it is scored as best for 2.
Id is the number of the adapter.
Best is the number of sequence for which the identified adapter was the best available.
The rest of the table shows how well the sequences match the adapters. For example, there are 10 sequences that match VHSzy1(Id=1 with 2 mismatches and are worse for all other adapters. In this sample, 90% come within 2 bases of one of the four adapters.

Table 13

The following list of enzymes was taken from http://rebase.neb.com/cqi-bin/asymmlist.
I have removed the enzymes that a) cut within the recognition, b) cut on both sides of the recognition, or c) have fewer than 2 bases between recognition and closest cut site.
REBASE Enzymes 04/13/2001
Type II restriction enzymes with asymmetric recognition sequences:

Enzymes	Recognition Sequence	Isoschizomers	Suppliers
AarI	CACCTGCNNNN^NNNN_	-	y
AceIII	CAGCTCNNNNNNN^NNNN_	-	-
Bbr7I	GAAGACNNNNNNN^NNNN_	-	-
BbvI	GCAGCNNNNNNNN^NNNN_		y
BbvII	GAAGACNN^NNNN_		
Bce83I	CTTGAGNNNNNNNNNNNNNN_NN^_	-	-
BceAI	ACGGCNNNNNNNNNNNN^NN_	-	y
BcefI	ACGGCNNNNNNNNNNNN^N_	-	-
BciVI	GTATCCNNNNN_N^	BfuI	y
BfiI	ACTGGGNNNN_N^	BmrI	y
BinI	GGATCNNNN^N_		
BscAI	GCATCNNNN^NN_	-	-
BseRI	GAGGAGNNNNNNNN_NN^	-	y
BsmFI	GGGACNNNNNNNNNN^NNNN_	BspLU11III	y
BspMI	ACCTGCNNNN^NNNN_	Acc36I	y
EciI	GGCGGANNNNNNNNN_NN^	-	y
Eco57I	CTGAAGNNNNNNNNNNNNNNN_NN^	BspKT5I	y
FauI	CCCGCNNNN^NN_	BstFZ438I	y
FokI	GGATGNNNNNNNNN^NNNN_	BstPZ418I	y
GsuI	NN^ CTGGAGNNNNNNNNNNNNNN_ NN^	-	y
HgaI	GACGCNNNNN^NNNNN_	-	y
HphI	GGTGANNNNNNN N^	AsuHPI	y
MboII	GAAGANNNNNNN_N^	-	y

(continued)

Enzymes	Recognition Sequence	Isoschizomers	Suppliers
MlyI	GAGTCNNNNN^	SchI	y
MmeI	TCCRACNNNNNNNNNNNNNNNNN_NN^	-	-
MnlI	CCTCNNNNNN_N^	-	y
PleI	GAGTCNNNN^N_	PpsI	y
RleAI	CCCACANNNNNNNNN_NNN^	-	-
SfaNI	GCATCNNNNN^NNNN_	BspST5I	y
SspD5I	GGTGANNNNNNNN^	-	-
Sth132I	CCCGNNNN^NNNN_	-	-
StsI	GGATGNNNNNNNNNN^NNNN_	-	-
TaqII	GACCGANNNNNNNNN_NN^, CACCCANNNNNNNNN_NN^	-	-
TthIIIII	CAARCANNNNNNNNN_NN^	-	-
UbaPI	CGAACG	-	-

The notation is ^ means cut the upper strand and _ means cut the lower strand. If the upper and lower strand are cut at the same place, then only ^ appears.

Table 14

(FOKlact) 5'-**cAcATccgTg** TTgTT **cAcggATgTg**-3' a revoir pour les emies DNA

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAgAgAgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'

! note that VHEx881 is the reverse complement of the ON below

! **[RC]** 5'-cgCttcacTaag-

! Scab.....

! Synthetic 3-23 as in Table 206

! |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|XbaI...

! |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3' Af1II...

(VHBA881) 5'-cgCttcacTaag|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg||aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'

(VHBB881) 5'-cgCttcacTaag|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg||aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'

(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'

Table 15: Use of *Fok*I as "Universal Restriction Enzyme"

*Fok*I - for dsDNA, I represents sites of cleavage

sites of cleavage 5'-cacGGATGtq--nnnnnnn|nnnnnnn-3'(SEQ ID NO:15)

```
3'-gtgCCTACac--nnnnnnnnnnn|nnn-5'(SEQ ID NO:16)
   RECOG
   NITion of FokI
```

Case I

5'-...gtg|tatt-actgtgc..Substrate....-3' (SEQ ID NO:17)

```
3'-cac-ataa|tgacacg¬
                   gtGTAGGcac\
              5'- caCATCCgtg/(SEQ ID NO:18)
```

Case II

5'-...gtgtatt|agac-tgc..Substrate....-3'(SEQ ID NO:19)

```
          ⌐cacataa-tctg|acg-5'
  /gtgCCTACac
  \cacGGATGtg-3'(SEQ ID NO:20)
```

Case III (Case I rotated 180 degrees)

```
/gtgCCTACac-5'
\cacGGATGtg¬
          gtgtctt|acag-tcc-3' Adapter (SEQ ID NO:21)
```

3'-...cacagaa-tgtc|agg..substrate....-5'(SEQ ID NO:22)

Case IV (Case II rotated 180 degrees)

```
              3'- gtGTAGGcac\  (SEQ ID NO:23)
                ⌐caCATCCgtg/
5'-gag|tctc-actgagc
```

Substrate 3'-...ctc-agag|tgactcg...-5'(SEQ ID NO:24)

Improved *Fok*I adapters

*Fok*I - for dsDNA, I represents sites of cleavage

Case I

Stem 11, loop 5, stem 11, recognition 17

```
5'-...catgtg|tatt-actgtgc..Substrate....-3'
   3'-gtacac-ataa|tgacacg¬          ⌐T¬
                          gtGTAGGcacG  T
                    5'- caCATCCgtgc  C
                                    ⌊TT⌋
```

Case II

Stem 10, loop 5, stem 10, recognition 18

```
          5'-...gtgtatt|agac-tgctgcc..Substrate....-3'
 ⌐T¬        ⌐cacataa-tctg|acgacgg-5'
 T  gtgCCTACac
 C  cacGGATGtg-3'
 ⌊TT⌋
```

Case III (Case I rotated 180 degrees)

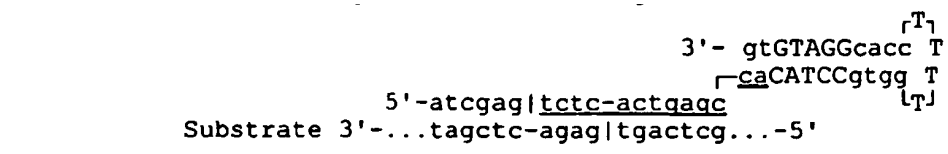
Stem 11, loop 5, stem 11, recognition 20

```
⌐ T¬
T  TgtgCCTACac-5'
G  AcacGGATGtg¬
⌊TT⌋
          gtgtctt|acag-tccattctg-3' Adapter
   3'-...cacagaa-tgtc|aggtaagac..substrate....-5'
```

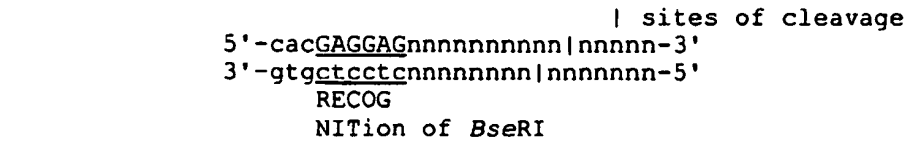
Case IV (Case II rotated 180 degrees)

Stem 11, loop 4, stem 11, recognition 17

(continued)



BseRI



Stem 11, loop 5, stem 11, recognition 19

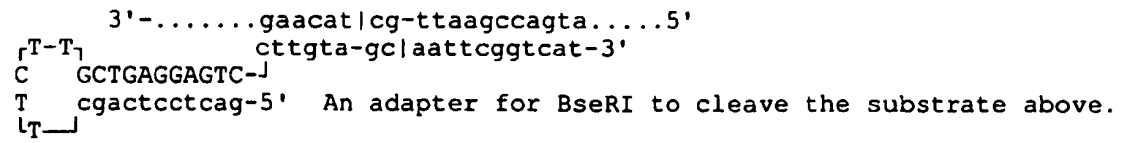


Table 16 Human heavy chains bases 88.1 to 94.2

Number of sequences..... 840

Id	Ntot	Number of Mismatchers..... 0	1	2	3	4	5	6	7	Name	Probe Sequence.....	Dot form.....
1	364	152	97	76	26	7	4	2	0	VHS881-1.1	gctgtgtattactgtgcgag	gctgtgtattactgtgcgag
2	265	150	60	33	13	5	4	0	0	VHS881-1.2	gccgtgtattactgtgcgag	..c..................
3	96	14	34	16	10	5	7	9	1	VHS881-2.1	gccgtatattactgtgcgag	..c..a..............
4	20	0	3	4	9	2	2	0	0	VHS881-4.1	gccgtgtattactgtacgag	..c............a....
5	95	25	36	18	11	2	2	0	1	VHS881-9.1	gccatgtattactgtgcgag	..ca................
	840	341	230	147	69	21	19	11	2			
		341	571	718	787	808	827	838	840			

```
                 88 89 90 91 92 93 94 95 Codon number as in Table 195
                 Recognition.....        Stem..... Loop. Stem.....
(VHS881-1.1) 5'-gctgtgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-1.2) 5'-gccgtgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-2.1) 5'-gccgtatat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-4.1) 5'-gccgtgtat|tact-gtacgag  cAcATccgTg TTgTT cAcggATgTg-3'
(VHS881-9.1) 5'-gccatgtat|tact-gtgcgag  cAcATccgTg TTgTT cAcggATgTg-3'
                                 | site of substrate cleavage
```

(FOKIact) 5'-cAcATccgTg TTgTT cAcggATgTg-3'

(VHEx881) 5'-AATAgTAgAc TgcAgTgTcc TcAgcccTTA AgcTgTTcAT cTgcAAgTAg-
AgAgTATTcT TAgAgTTgTc TcTAgAcTTA gTgAAgcg-3'
! note that VHEx881 is the reverse complement of the ON below
! **[RC]** 5'-cgCttcacTaag-
! Scab.....
! Synthetic 3-23 as in Table 206
! |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
! XbaI...
! |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|t-3'

```
                    !          AflII...
(VHBA881)                5'-cgCttcacTaag-
                            |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                            |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt gcg ag-3'
(VHBB881)                5'-cgCttcacTaag-
                            |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
                            |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgt Acg ag-3'
(VH881PCR) 5'-cgCttcacTaag|TCT|AGA|gac|aac -3'
```

Table 17: Kappa, bases 12-30

ID	Ntot	0	1	2	3	4	5	6	Name	Sequence.....	Dot Form.....
1	84	40	21	20	1	2	0	0	SK12O12	gacccagtctccatcctcc	gacccagtctccatcctcc
2	32	19	3	6	2	1	0	1	SK12A17	gactcagtctccactctcc	...t.....ct....
3	26	17	8	1	0	0	0	0	SK12A27	gacgcagtctccaggcacc	...g.....gg.a..
4	40	21	18	1	0	0	0	0	SK12A11	gacgcagtctccagccacc	...g.....g..a..
	182	97	50	28	3	3	0	1			
		97	147	175	178	181	181	182			

URE adapters:

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-O12) 5'-cAcATccgTg TTgTT cAcggATgTg ggAggATggAgAcTgggTc-3'

[RC] 5'-gacccagtctccatcctcc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A17) 5'-cAcATccgTg TTgTT cAcggATgTg ggAgAgTggAgAcTgAgTc-3'

[RC] 5'-gactcagtctccactctcc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A27) 5'-cAcATccgTg TTgTT cAcggATgTg ggTgccTggAgAcTgcgTc-3'

[RC] 5'-gacgcagtctccaggcacc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

FokI. FokI.

Stem..... Loop. Stem..... Recognition.....

(SzKB1230-A11) 5'-cAcATccgTg TTgTT cAcggATgTg ggTggcTggAgAcTgcgTc-3'

[RC] 5'-gacgcagtctccagccacc **cAcATccgTg** AAcAA **cAcggATgTg**-3'

Recognition..... Stem..... loop. Stem.....

```
!                          FokI.        FokI.
```

What happens in the upper strand:

```
(SzKB1230-O12*)              5'-gac cca gtc|tcc a-tc ctc c-3'
!                             | Site of cleavage in substrate
!
(SzKB1230-A17*)              5'-gac tca gtc|tcc a-ct ctc c-3'
!
(SzKB1230-A27*)              5'-gac gca gtc|tcc a-gg cac c-3'
!
(SzKB1230-A11*)              5'-gac gca gtc|tcc a-gc cac c-3'

(kapextURE)     5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg-3' !sense strand
                   Scab.............ApaLI.

(kapextUREPCR) 5'-ccTctactctTgTcAcAgTg-3'
                   Scab.............

(kaBR01UR)    5'-ggAggATggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-tc ctc c-3' ON above is R.C. of this one
(kaBR02UR)    5'-ggAgAgTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-ct ctc c-3' ON above is R.C. of this one
(kaBR03UR)    5'-ggTgccTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gg cac c-3' ON above is R.C. of this one
(kaBR04UR)    5'-ggTggcTggA cTggATgTcT TgTgcAcTgT gAcAAgAgTA gAgg-3'
!        [RC] 5'-ccTctactctTgTcAcAgTgcAcAA gAc ATc cAg tcc a-gc cac c-3' ON above is R.C. of this one
                   Scab.............ApaLI.
```

```
Table 18 Lambda URE adapters  bases 13.3 to 19.3
!
! Number of sequences.......... 128
!
!        Number of mismatches.............
! Id Ntot  0  1  2  3  4  5  6  7  8  Name       Sequence.......... Dot form..........
! 1   58  45  7  1  0  0  0  2  2  1 · VL133-2a2 gtctcctggacagtcgatc gtctcctggacagtcgatc
! 2   16  10  1  0  1  0  1  1  0  2  VL133-3l  ggccttgggacagacagtc .g.cttg......a.ag..
! 3   17   6  0  0  0  4  1  1  5  0  VL133-2c  gtctcctggacagtcagtc ...............ag..
· ! 4   37   3  0 10  4  4  3  7  4  2  VL133-1c  ggccccagggcagagggtc .g.c..a..g...ag.g..
!    128  64  8 11  5  8  5 11 11  5
!         64 72 83 88 96 101 112 123 128
!
!              Stem...... loop. Stem...... Recognition........
(VL133-2a2)     5'-cAcATccgTg TTgTT cAcggATgTg gATcgAcTgTccAggAgAc-3'
!         [RC] 5'-gtctcctggacagtcgatc cAcATccgTg AAcAA cAcggATgTg-3'
!              Recognition........ Stem...... Loop. Stem......
!
!              Stem...... loop. Stem...... Recognition........
(VL133-3l)     5'-cAcATccgTg TTgTT cAcggATgTg gAcTgTcTgTcccAAggcc-3'
!         [RC] 5'-ggccttgggacagacagtc cAcATccgTg AAcAA cAcggATgTg-3'
!              Recognition........ Stem...... Loop. Stem......
!
!              Stem...... loop. Stem...... Recognition........
(VL133-2c)     5'-cAcATccgTg TTgTT cAcggATgTg gAcTgAcTgTccAggAgAc-3'
          [RC] 5'-gtctcctggacagtcagtc cAcATccgTg AAcAA cAcggATgTg-3'
!              Recognition........ Stem...... Loop. Stem......
!
!              Stem...... loop. Stem...... Recognition........
(VL133-1c)     5'-cAcATccgTg TTgTT cAcggATgTg gAcccTcTgcccTggggcc-3'
          [RC] 5'-ggccccagggcagagggtc cAcATccgTg AAcAA cAcggATgTg-3'
```

What happens in the top strand:

```
!                    | site of cleavage in the upper strand
(VL133-2a2*)   5'-g tct cct g|ga cag tcg atc
!
(VL133-3l*)    5'-g gcc ttg g|ga cag aca gtc
!
(VL133-2c*)    5'-g tct cct g|ga cag tca gtc
!
(VL133-1c*)    5'-g gcc cca g|gg cag agg gtc
!
! The following Extenders and Bridges all encode the AA sequence of 2a2 for codons 1-15
!                        1
(ON_LamEx133)  5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
        AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
        tcC ccG g ! 2a2
!                        1
(ON_LamB1-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
        AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
        tcC ccG g ga cag tcg at-3' ! 2a2  N.B. the actual seq is the
!                                  reverse complement of the
!                                  one shown.
!
(ON_LamB2-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
        AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
        tcC ccG g ga cag aca gt-3' ! 3l  N.B. the actual seq is the
!                                  reverse complement of the
!                                  one shown.
!
!
(ON_LamB3-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
!
!       2  3  4  5  6  7  8  9  10 11 12
        AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-
!
!       13 14 15
        tcC ccG g ga cag tca gt -3'! 2c  N.B. the actual seq is the
!                                  reverse complement of the
!                                  one shown.
!(ON_LamB4-133) [RC] 5'-ccTcTgAcTgAgT gcA cAg -
```

```
!
!            2   3   4   5   6   7   8   9   10  11  12
            AGt gcT TtA acC caA ccG gcT AGT gtT AGC ggT-s
!
!            13  14  15
            tcC ccG g gg cag agg gt-3' ! 1c  N.B. the actual seq is the
!                                              reverse complement of the
!                                              one shown.
!
(ON_Lam133PCR)  5'-ccTcTgAcTgAgT gcA cAg AGt gc-3'
```

Table 19: Cleavage of 75 human light chains.

Enzyme	Recognition*	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
Af1II	Cttaag	0	0	HC FR3
AgeI	Accggt	0	0	
AscI	GGcgcgcc	0	0	After LC
BglII	Agatct	0	0	
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
BstBI	TTcgaa	0	0	
DraIII	CACNNNgtg	0	0	
EagI	Cggccg	0	0	
FseI	GGCCGGcc	0	0	
FspI	TGCgca	0	0	
HpaI	GTTaac	0	0	
MfeI	Caattg	0	0	HC FR1
MluI	Acgcgt	0	0	
NcoI	Ccatgg	0	0	Heavy chain signal
NheI	Gctagc	0	0	HC/anchor linker
NotI	GCggccgc	0	0	In linker after HC
NruI	TCGcga	0	0	
PacI	TTAATtaa	0	0	
PmeI	GTTTaaac	0	0	
PmlI	CACgtg	0	0	
PvuI	CGATcg	0	0	
SacII	CCGCgg	0	0	
SalI	Gtcgac	0	0	
SfiI	GGCCNNNNnggcc	0	0	Heavy Chain signal
SgfI	GCGATcgc	0	0	
SnaBI	TACgta	0	0	
StuI	AGGcct	0	0	
XbaI	Tctaga	0	0	HC FR3
AatII	GACGTc	1	1	
AclI	AAcgtt	1	1	
AseI	ATtaat	1	1	
BsmI	GAATGCN	1	1	
BspEI	Tccgga	1	1	HC FR1
BstXI	CCANNNNNTGG	1	1	HC FR2
DrdI	GACNNNNnngtc	1	1	

(continued)

Enzyme	Recognition*	Nch	Ns	Planned location of site
HindIII	Aagctt	1	1	
PciI	Acatgt	1	1	
SapI	gaagagc	1	1	
ScaI	AGTact	1	1	
SexAI	Accwggt	1	1	
SpeI	Actagt	1	1	
TliI	Ctcgag	1	1	
XhoI	Ctcgag	1	1	
BcgI	cgannnnnntgc	2	2	
BlpI	GCtnagc	2	2	
BssSI	Ctcgtg	2	2	
BstAPI	GCANNNNntgc	2	2	
EspI	GCtnagc	2	2	
KasI	Ggcgcc	2	2	
PflMI	CCANNNNntgg	2	2	
XmnI	GAANNnnttc	2	2	
ApaLI	Gtgcac	3	3	LC signal seq
NaeI	GCCggc	3	3	
NgoMI	Gccggc	3	3	
PvuII	CAGctg	3	3	
RsrII	CGgwccg	3	3	
BsrBI	GAGcgg	4	4	
BsrDI	GCAATGNNn	4	4	
BstZ17I	GTAtac	4	4	
EcoRI	Gaattc	4	4	
SphI	GCATGc	4	4	
SspI	AATatt	4	4	
AccI	GTmkac	5	5	
BclI	Tgatca	5	5	
BsmBI	Nnnnnngagacg	5	5	
BsrGI	Tgtaca	5	5	
OraI	TTTaaa	6	6	
NdeI	CAtatg	6	6	HC FR4
SwaI	ATTTaaat	6	6	
BamHI	Ggatcc	7	7	
SacI	GAGCTc	7	7	
BciVI	GTATCCNNNNNN	8	8	
BsaBI	GATNNnnatc	8	8	
NsiI	ATGCAt	8	8	
Bsp120I	Gggccc	9	9	CH1
ApaI	GGGCCc	9	9	CH1
PspOOM1	Gggccc	9	9	
BspHI	Tcatga	9	11	
EcoRV	GATatc	9	9	
AhdI	GACNNNnngtc	11	11	
BbsI	GAAGAC	11	14	
PsiI	TTAtaa	12	12	
BsaI	GGTCTCNnnnn	13	15	
XmaI	Cccggg	13	14	
AvaI	Cycgrg	14	16	

(continued)

Enzyme	Recognition*	Nch	Ns	Planned location of site
BglI	GCCNNNNnggc	14	17	
AlwNI	CAGNNNctg	16	16	
BspMI	ACCTGC	17	19	
XcmI	CCANNNNNnnnntgg	17	26	
BstEII	Ggtnacc	19	22	HC FR4
Sse8387I	CCTGCAgg	20	20	
AvrII	Cctagg	22	22	
HincII	GTYrac	22	22	
BsgI	GTGCAG	27	29	
MscI	TGGcca	30	34	
BseRI	NNnnnnnnnnctcctc	32	35	
Bsu36I	CCtnagg	35	37	
PstI	CTGCAg	35	40	
EciI	nnnnnnnnntccgcc	38	40	
PpuMI	RGgwccy	41	50	
StyI	Ccwwgg	44	73	
EcoO109I	RGgnccy	46	70	
Acc65I	Ggtacc	50	51	
KpnI	GGTACc	50	51	
BpmI	ctccag	53	82	
AvaII	Ggwcc	71	124	

* cleavage occurs in the top strand after the last upper-case base. For REs that cut palindromic sequences, the lower strand is cut at the symmetrical site.

Table 20: Cleavage of 79 human heavy chains

Enzvme	Recognition	Nch	Ns	Planned location of site
AfeI	AGCgct	0	0	
AflII	**Cttaag**	**0**	**0**	**HC FR3**
AscI	**GGcgcgcc**	**0**	**0**	**After LC**
BsiWI	Cgtacg	0	0	
BspDI	ATcgat	0	0	
BssHII	Gcgcgc	0	0	
FseI	GGCCGGcc	0	0	
HpaI	GTTaac	0	0	
NheI	**Gctagc**	**0**	**0**	**HC Linker**
NotI	**Gcggccgc**	**0**	**0**	**In linker, HC/anchor**
NruI	TCGcga	0	0	
NsiI	ATGCAt	0	0	
PacI	TTAATtaa	0	0	
PciI	Acatgt	0	0	
PmeI	GTTTaaac	0	0	
PvuI	CGATcg	0	0	
RsrII	CGgwccg	0	0	
SapI	gaagagc	0	0	
SfiI	**GGCCIJNNNnggcc**	**0**	**0**	**HC signal seq**
SgfI	GCGATcgc	0	0	
SwaI	ATTTaaat	0	0	
AclI	AAcgtt	1	1	
AgeI	Accggt	1	1	

(continued)

Enzvme	Recognition	Nch	Ns	Planned location of site
AseI	ATtaat	1	1	
AvrII	Cctagg	1	1	
BsmI	GAATGCN	1	1	
BsrBI	GAGcgg	1	1	
BsrDI	GCAATGNNn	1	1	
DraI	TTTaaa	1	1	
FspI	TGCgca	1	1	
HindIII	Aagctt	1	1	
MfeI	**Caattg**	**1**	**1**	**HC FR1**
NaeI	GCCggc	1	1	
NgoMI	Gccggc	1	1	
SpeI	Actagt	1	1	
Acc65I	Ggtacc	2	2	
BstBI	TTcgaa	2	2	
KpnI	GGTACc	2	2	
MluI	Acgcgt	2	2	
NcoI	**Ccatgg**	**2**	**2**	**In HC signal seq**
NdeI	**CAtatg**	**2**	**2**	**HC FR4**
PmlI	CACgtg	2	2	
XcmI	CCANNNNNnnnntgg	2	2	
BcgI	cgannnnnntgc	3	3	
BclI	Tgatca	3	3	
BglI	GCCNNNNnggc	3	3	
BsaBI	GATNNnnatc	3	3	
BsrGI	Tgtaca	3	3	
SnaBI	TACgta	3	3	
Sse8387I	CCTGCAgg	3	3	
ApaLI	**Gtgcac**	**4**	**4**	**LC Signal/FR1**
BspHI	Tcatga	4	4	
BssSI	Ctcgtg	4	4	
PsiI	TTAtaa	4	5	
SphI	GCATGc	4	4	
AhdI	GACNNNnngtc	5	5	
BspEI	**Tccgga**	**5**	**5**	**HC FR1**
MscI	TGGcca	5	5	
SacI	GAGCTc	5	5	
ScaI	AGTact	5	5	
SexAI	Accwggt	5	6	
SspI	AATatt	5	5	
TliI	Ctcgag	5	5	
XhoI	Ctcgag	5	5	
BbsI	GAAGAC	7	8	
BstAPI	GCANNNNntgc	7	8	
BstZ17I	GTAtac	7	7	
EcoRV	GATatc	7	7	
EcoRI	Gaattc	8	8	
BlpI	GCtnagc	9	9	
Bsu36I	CCtnagg	9	9	
DraIII	CACNNNgtg	9	9	
EspI	GCtnagc	9	9	

(continued)

Enzvme	Recognition	Nch	Ns	Planned location of site
StuI	AGGcct	9	13	
XbaI	**Tctaga**	**9**	**9**	**HC FR3**
Bsp120I	**Gggccc**	**10**	**11**	**CH1**
ApaI	**GGGCCc**	**10**	**11**	**CH1**
PspOOMI	Gggccc	10	11	
BciVI	GTATCCNNNNNN	11	11	
SalI	Gtcgac	11	12	
DrdI	GACNNNNnngtc	12	12	
KasI	Ggcgcc	12	12	
XmaI	Cccggg	12	14	
BglII	Agatct	14	14	
HincII	GTYrac	16	18	
BamHI	Ggatcc	17	17	
PflMI	CCANNNNntgg	17	18	
BsmBI	Nnnnnngagacg	18	21	
BstXI	**CCANNNNNntgg**	**18**	**19**	**HC FR2**
XmnI	GAANNnnttc	18	18	
SacII	CCGCgg	19	19	
PstI	CTGCAg	20	24	
PvuII	CAGctg	20	22	
AvaI	Cycgrg	21	24	
EagI	Cggccg	21	22	
AatII	GACGTc	22	22	
BspMI	ACCTGC	27	33	
AccI	GTmkac	30	43	
StyI	Ccwwgg	36	49	
AlwNI	CAGNNNctg	38	44	
BsaI	GGTCTCNnnnn	38	44	
PpuMI	RGgwccy	43	46	
BsgI	GTGCAG	44	54	
BseRI	NNnnnnnnnnctcctc	48	60	
EciI	nnnnnnnnntccgcc	52	57	
BstEII	**Ggtnacc**	**54**	**61**	**HC Fr4, 47/79 have one**
EcoO109I	RGgnccy	54	86	
BpmI	ctccag	60	121	
AvaII	Ggwcc	71	140	

Table 21: MALIA3, annotated

```
! MALIA3  9532 bases
!-----------------------------------------------------------------------
     1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc
!    gene ii continued
    49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta
    97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act
   145 gtt aca tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta
   193 aaa cat gtt gag cta cag cac cag att cag caa tta agc tct aag cca
   241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct
   289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct
   337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt
   385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac
   433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca
   481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac
!         RBS?......      Start gene x, ii continues
   529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct
   577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac
   625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg
   673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg
   721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt att
   769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt
   817 ctt aaa atc gca TAA
!                    End X & II
   832 ggtaattca ca
!
!       M1               E5                  Q10                 T15
   843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt
!      Start gene V
!
!      S17         S20                 P25                 E30
   891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt
!
!              V35                 E40                 V45
   939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act
!
!          D50                 A55                 L60
   987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat
!                                                   BsrGI...
!      L65                 V70                 S75                 R80
  1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt
!
!                      P85     K87 end of V
  1083 ctg cgc ctc gtt ccg gct aag TAA C
!
  1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
!      Start gene VII
!
  1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
!
!                        VII and IX overlap.
!                        ..... S2  V3  L4  V5                 S10
  1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttc gcc tct ttc gtt
!                          End VII
!                        |start IX
!      L13     W15                 G20                 T25             E29
  1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa
```

```
!
  1293 act tcc tc
!
!       .... stop of IX, IX and VIII overlap by four bases
  1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc ctc
!      Start signal sequence of viii.
!
  1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa gcg
!                                 mature VIII --->
  1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg
  1445 tgg gcg atg gtt gtt gtc att
  1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
  1499 aaa ttc acc tcg aaa gca ! 1515
!       ...........  -35  ..
!
  1517       agc tga taaaccgat acaattaaag gctccttttg
!                     ..... -10   ...
!
  1552 gagccttttt ttttGGAGAt ttt ! S.D. underlined
!
!           <------ III signal sequence ----------------------------->
!            M   K   K   L   L   F   A   I   P   L   V
  1575 caac GTG aaa aaa tta tta ttc gca att cct tta gtt ! 1611
!
!        V   P   F   Y   S   H   S   A   Q
  1612 gtt cct ttc tat tct cac aGT gcA Cag tCT
!                               ApaLI...
!
  1642      GTC GTG ACG CAG CCG CCC TCA GTG TCT GGG GCC CCA GGG CAG
            AGG GTC ACC ATC TCC TGC ACT GGG AGC AGC TCC AAC ATC GGG GCA
!             BstEII...
  1729      GGT TAT GAT GTA CAC TGG TAC CAG CAG CTT CCA GGA ACA GCC CCC AAA
  1777      CTC CTC ATC TAT GGT AAC AGC AAT CGG CCC TCA GGG GTC CCT GAC CGA
  1825      TTC TCT GGC TCC AAG TCT GGC ACC TCA GCC TCC CTG GCC ATC ACT
  1870      GGG CTC CAG GCT GAG GAT GAG GCT GAT TAT
  1900      TAC TGC CAG TCC TAT GAC AGC AGC CTG AGT
  1930      GGC CTT TAT GTC TTC GGA ACT GGG ACC AAG GTC ACC GTC
!                                                 BstEII...
  1969      CTA GGT CAG CCC AAG GCC AAC CCC ACT GTC ACT
  2002      CTG TTC CCG CCC TCC TCT GAG GAG CTC CAA GCC AAC AAG GCC ACA CTA
  2050      GTG TGT CTG ATC AGT GAC TTC TAC CCG GGA GCT GTG ACA GTG GCC TGG
  2098      AAG GCA GAT AGC AGC CCC GTC AAG GCG GGA GTG GAG ACC ACC ACA CCC
  2146      TCC AAA CAA AGC AAC AAC AAG TAC GCG GCC AGC AGC TAT CTG AGC CTG
  2194      ACG CCT GAG CAG TGG AAG TCC CAC AGA AGC TAC AGC TGC CAG GTC ACG
  2242      CAT GAA GGG AGC ACC GTG GAG AAG ACA GTG GCC CCT ACA GAA TGT TCA
  2290      TAA TAA ACCG CCTCCACCGG GCGCGCCAAT TCTATTTCAA GGAGACAGTC ATA
!                               AscI.....
!
!           PelB signal---------------------------------------------->
!            M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
  2343      ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC
!
!           16  17  18  19  20      21  22
!           A   A   Q   P   A       M   A
  2388     gcG GCC cag ccG GCC       atg gcc
!            SfiI.............
!                    NgoMI...(1/2)
!                           NcoI.........
```

```
!
!                                        FR1(DP47/V3-23)---------------
!                                        23  24  25  26  27  28  29  30
!                                         E   V   Q   L   L   E   S   G
5     2409                               gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
!                                              | MfeI  |
!
!       --------------FR1-----------------------------------------------
!        31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!         G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
10    2433  |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
!
!       ----FR1---------------->|...CDR1.............|---FR2------
!        46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!         A   S   G   F   T   F   S   S   Y   A   M   S   W   V   R
15    2478  |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|
!           | BspEI |                 | BsiWI|                      |BstXI.
!
!        -------FR2-------------------------------->|...CDR2.........
!        61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!         Q   A   P   G   K   G   L   E   W   V   S   A   I   S   G
20    2523  |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|
!     ...BstXI          |
!
!       .....CDR2.........................................|---FR3---
!        76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!         S   G   G   S   T   Y   Y   A   D   S   V   K   G   R   F
25    2568  |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
!
!
!       --------FR3-------------------------------------------------
!         91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
30    2613  |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
!               | XbaI  |
!
!       ---FR3-------------------------------------------------------->|
!        106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!         N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K
35    2658  |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|
!             |AflII |                 | PstI |
!
!       .......CDR3.................|----FR4------------------------
!        121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!         D   Y   E   G   T   G   Y   A   F   D   I   W   G   Q   G
40    2703  |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
!                                              | NdeI |(1/4)
!
!       --------------FR4---------->|
!        136 137 138 139 140 141 142
!         T   M   V   T   V   S   S
45    2748  |act|atG|GTC|ACC|gtc|tct|agt
!               | BstEII |
!  From BstEII onwards, pV323 is same as pCES1, except as noted.
!  BstEII sites may occur in light chains; not likely to be unique in final
50!  vector.
```

```
                          143 144 145 146 147 148 149 150 151 152
                           A   S   T   K   G   P   S   V   F   P
 2769                      gcc tcc acc aaG GGC CCa tcg GTC TTC ccc
                                       Bsp120I.      BbsI...(2/2)
                                       ApaI....

      153 154 155 156 157 158 159 160 161 162 163 164 165 166 167
       L   A   P   S   S   K   S   T   S   G   G   T   A   A   L
 2799 ctg gca ccC TCC TCc aag agc acc tct ggg ggc aca gcg gcc ctg
              BseRI...(2/2)

        168 169 170 171 172 173 174 175 176 177 178 179 180 181 182
         G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S
 2844    ggc tgc ctg GTC AAG GAC TAC TTC CCc gaA CCG GTg acg gtg tcg
                                           AgeI....

        183 184 185 186 187 188 189 190 191 192 193 194 195 196 197
         W   N   S   G   A   L   T   S   G   V   H   T   F   P   A
 2889    tgg aac tca GGC GCC ctg acc agc ggc gtc cac acc ttc ccg gct
                 KasI...(1/4)

        198 199 200 201 202 203 204 205 206 207 208 209 210 211 212
         V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T
 2934    gtc cta cag tCt agc GGa ctc tac tcc ctc agc agc gta gtg acc
                 (Bsu36I...)(knocked out)

        213 214 215 216 217 218 219 220 221 222 223 224 225 226 227
         V   P   S   S   S   L   G   T   Q   T   Y   I   C   N   V
 2979    gtg ccC tCt tct agc tTG Ggc acc cag acc tac atc tgc aac gtg
                (BstXI.....)N.B. destruction of BstXI & BpmI sites.

        228 229 230 231 232 233 234 235 236 237 238 239 240 241 242
         N   H   K   P   S   N   T   K   V   D   K   K   V   E   P
 3024    aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag ccc

        243 244 245
         K   S   C   A   A   A   H   H   H   H   H   H   S   A
 3069    aaa tct tgt GCG GCC GCt cat cac cac cat cat cac tct gct
                 NotI......

         E   Q   K   L   I   S   E   E   D   L   N   G   A   A
 3111    gaa caa aaa ctc atc tca gaa gag gat ctg aat ggt gcc gca

         D   I   N   D   D   R   M     A   S   G   A
 3153    GAT ATC aac gat gat cgt atg   gct AGC  ggc gcc
         rEK cleavage site.........   NheI...  KasI...
         EcoRV..

 Domain 1 ------------------------------------------------------------
           A   E   T   V   E   S   C   L   A
 3183     gct gaa act gtt gaa agt tgt tta gca


       K   P   H   T   E   I   S   F
 3210 aaa ccc cat aca gaa aat tca ttt

       T   N   V   W   K   D   D   K   T
 3234 aCT AAC GTC TGG AAA GAC GAC AAA Act
```

```
!
!       L   D   R   Y   A   N   Y   E   G   C   L   W   N   A   T   G   V
  3261 tta gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc gtt
!                                                       BsmI____
!
!       V   V   C   T   G   D   E   T   Q   C   Y   G   T   W   V   P   I
  3312 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att
!
!       G   L   A   I   P   E   N
  3363 ggg ctt gct atc cct gaa aat
!
! L1 linker ------------------------------------
!       E   G   G   G   S   E   G   G   G   S
  3384 gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!       E   G   G   G   S   E   G   G   G   T
  3414 gag ggt ggc ggt tct gag ggt ggc ggt act
!
!  Domain 2 ------------------------------------
  3444 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac
  3495 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct
  3546 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat
!                    BseRI__
  3597 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act
  3645 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct
  3693 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA
!                                                          AlwNI
  3741 GAC TGc gct ttc cat tct ggc ttt aat gaa gat cca ttc gtt tgt gaa
!       AlwNI
  3789 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
!
  3834 ggc ggc ggc tct
!  start L2 ------------------------------------------------------------
  3846 ggt ggt ggt tct
  3858 ggt ggc ggc tct
  3870 gag ggt ggt ggc tct gag ggt ggc ggt tct
  3900 gag ggt ggc ggc tct gag gga ggc ggt tcc
  3930 ggt ggt ggc tct ggt    ! end L2
!
! Domain 3 ------------------------------------------------------------
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  3945 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  3993 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  4041 aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  4089 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  4137 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  4185 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa
```

```
!
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  4233 tcg gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa
!
!       F   S   I   D   C   D   K   I   N   L   F   R
  4281 ttt tct att gat tgt gac aaa ata aac tta ttc cgt
!                                                   End Domain 3
!
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V  F140
  4317 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt
!      start transmembrane segment
!
!       S   T   F   A   N   I   L
  4365 tct acg ttt gct aac ata ctg
!
!       R   N   K   E   S
  4386 cgt aat aag gag tct TAA ! stop of iii
!     Intracellular anchor.
!
!           M1  P2  V   L  L5   G   I   P   L  L10  L   R   F   L  G15
  4404  tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt
!          Start VI
!
  4451 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag
  4499 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att
  4547 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct
  4595 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct
  4643 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att
  4691 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat
!
!                 M1  A2  V3      F5                  L10         G13
  4739 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga
!       end VI   Start gene I
!
!       14  15  16  17  18  19  20  21  22  23  24  25  26  27  28
!       K   T   L   V   S   V   G   K   I   Q   D   K   I   V   A
  4785 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct
!
!       29  30  31  32  33  34  35  36  37  38  39  40  41  42  43
!       G   C   K   I   A   T   N   L   D   L   R   L   Q   N   L
  4830 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc
!
!       44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
!       P   Q   V   G   R   F   A   K   T   P   R   V   L   R   I
  4875 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata
!
!       59  60  61  62  63  64  65  66  67  68  69  70  71  72  73
!       P   D   K   P   S   I   S   D   L   L   A   I   G   R   G
  4920 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt
!
!       74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
!       N   D   S   Y   D   E   N   K   N   G   L   L   V   L   D
  4965 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat
!
!       89  90  91  92  93  94  95  96  97  98  99 100 101 102 103
!       E   C   G   T   W   F   N   T   R   S   W   N   D   K   E
  5010 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa
!
```

```
!       104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
!        R   Q   P   I   I   D   W   F   L   H   A   R   K   L   G
  5055 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga
!
!       119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
!        W   D   I   I   F   L   V   Q   D   L   S   I   V   D   K
  5100 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa
!
!       134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
!        Q   A   R   S   A   L   A   E   H   V   V   Y   C   R   R
  5145 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt
!
!       149 150 151 152 153 154 155 156 157 158 159 160 161 162 163
!        L   D   R   I   T   L   P   F   V   G   T   L   Y   S   L
  5190 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt
!
!       164 165 166 167 168 169 170 171 172 173 174 175 176 177 178
!        I   T   G   S   K   M   P   L   P   K   L   H   V   G   V
  5235 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt
!
!       179 180 181 182 183 184 185 186 187 188 189 190 191 192 193
!        V   K   Y   G   D   S   Q   L   S   P   T   V   E   R   W
  5280 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg
!
!       194 195 196 197 198 199 200 201 202 203 204 205 206 207 208
!        L   Y   T   G   K   N   L   Y   N   A   Y   D   T   K   Q
  5325 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag
!
!       209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
!        A   F   S   S   N   Y   D   S   G   V   Y   S   Y   L   T
  5370 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg
!
!       224 225 226 227 228 229 230 231 232 233 234 235 236 237 238
!        P   Y   L   S   H   G   R   Y   F   K   P   L   N   L   G
  5415 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt
!
!       239 240 241 242 243 244 245 246 247 248 249 250 251 252 253
!        Q   K   M   K   L   T   K   I   Y   L   K   K   F   S   R
  5460 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc
!
!       254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
!        V   L   C   L   A   I   G   F   A   S   A   F   T   Y   S
  5505 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt
!
!       269 270 271 272 273 274 275 276 277 278 279 280 281 282 283
!        Y   I   T   Q   P   K   P   E   V   K   K   V   V   S   Q
  5550 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag
!
!       284 285 286 287 288 289 290 291 292 293 294 295 296 297 298
!        T   Y   D   F   D   K   F   T   I   D   S   S   Q   R   L
  5595 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt
!
!       299 300 301 302 303 304 305 306 307 308 309 310 311 312 313
!        N   L   S   Y   R   Y   V   F   K   D   S   K   G   K   L
  5640 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA
!                                                               PacI
!
```

```
!      314 315 316 317 318 319 320 321 322 323 324 325 326 327 328
!       I   N   S   D   D   L   Q   K   Q   G   Y   S   L   T   Y

  5685 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat
!     PacI
!
!      329 330 331 332 333 334 335 336 337 338 339 340 341 342 343
!      i  I   D   L   C   T   V   S   I   K   K   G   N   S   N   E
!     iv                                                        M1  K
  5730   att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa
!                                                              Start IV
!
!         344 345 346 347 348 349
!     i    I   V   K   C   N   .End of I
!     iv    L3  L   N5  V   I7  N    F  V10
  5775    att gtt aaa tgt aat TAA T TTT GTT
!  IV continued.....
  5800 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg
  5848 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa tca
  5896 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta
  5944 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct
  5992 gtt tta cgt gct aat aat ttt gat atg gtt ggt tca att cct tcc ata
  6040 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca
  6088 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt
  6136 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat
  6184 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag
  6232 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct aat
  6280 cta tta gtt gtt TCT gca cct aaa gat att tta gat aac ctt cct caa
!                     ApaLI removed
  6328 ttc ctt tct act gtt gat ttg cca act gac cag ata ttg att gag ggt
  6376 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct
  6424 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc
  6472 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt aat
  6520 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat
  6568 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag
  6616 ggt tct atc tct gtT GGC CAg aat gtc cct ttt att act ggt cgt gtg
!                       MscI____
  6664 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag cgt
  6712 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc
  6760 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct
  6808 tct act cag gca agt gat gtt att act aat caa aga agt att gct aca
  6856 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act
  6904 gat tat aaa aac act tct caa gat tct ggc gta ccg ttc ctg tct aaa
  6952 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcc aac gag
  7000 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc ctg
  7048 TAG cggcgcatt
!      End IV
  7060 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc
  7120 gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcGCCGGCt ttccccgtca
!                                                    NgoMI_
  7180 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc
  7240 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat agacggtttt
!                                 DraIII____
  7300 tcgccctttG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc aaactggaac
!              DrdI__________
  7360 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga
  7420 accaccatca aacaggattt tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa
  7480 ctctctcagg gccaggcggt gaagggcaat CAGCTGttgc cCGTCTCact ggtgaaaaga
!                                    PvuII.      BsmBI.
  7540 aaaaccaccc tGGATCC  AAGCTT
!                BamHI   HindIII (½)
```

```
!                  Insert carrying bla gene
  7563    gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
  7600 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct
!                                          BciVI
  7660 gataaatgct tcaataatat tgaaaaAGGA AGAgt
!                                RBS.?...
!      Start bla gene
  7695 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt
  7746 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct
  7797 gaa gat cag ttg ggC gCA CGA Gtg ggt tac atc gaa ctg gat ctc aac agc
!                         BssSI...
!                      ApaLI removed
  7848 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc
  7899 act ttt aaa gtt ctg cta tgt cat aca cta tta tcc cgt att gac gcc ggg
  7950 caa gaG CAA CTC GGT CGc cgg gcg cgg tat tct cag aat gac ttg gtt gAG
!            BcgI_____________                                      ScaI
  8001 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
!      ScaI_
  8052 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt
  8103 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac aac atg
!               PvuI____
  8154 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc
  8205 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg cca aca acg
  8256 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa
!       FspI....
!
  8307 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg
  8358 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
!      BglI__________
  8409 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt
!       BsaI____
  8460 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat
!                            AhdI___________
  8511 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt
!                                                          stop
  8560 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa
  8620 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt
  8680 cgttccactg tacgtaagac cccc
  8704 AAGCTT   GTCGAC tgaa tggcgaatgg cgctttgcct
!      HindIII  SalI..
!      (2/2)    HincII
  8740 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
!
  8790 CCTGAGG
!      Bsu36I_
  8797       ccgat actgtcgtcg tcccctcaaa ctggcagatg
  8832 cacggttacg atgcgcccat ctacaccaac gtaacctatc ccattacggt caatccgccg
  8892 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc
  8952 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg ttaaaaaatg
  9012 agctgattta acaaaaattt aacgcgaatt ttaacaaaat attaacgttt acaATTTAAA
!                                                            SwaI...
  9072 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc GGGGTAcat
!                                                          RBS?
  9131 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc
!      Start gene II
  9182 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata
!                                                  BglII...
  9233 gct acc ctc tcc ggc atg aat tta tca gct aga acg gtt gaa tat cat att
```

```
   9284 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct
   9335 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt
   9386 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat
   9437 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt
   9488 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt ! 9532
! gene II continues
```

Table 21B: Sequence of MALIA3, condensed

```
LOCUS       MALIA3      9532              CIRCULAR
ORIGIN

1          AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC AAATGAAAAT
61         ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT
121        CGTTCGCAGA ATTGGGAATC AACTGTTACA TGGAATGAAA CTTCCAGACA CCGTACTTTA
181        GTTGCATATT TAAAACATGT TGAGCTACAG CACCAGATTC AGCAATTAAG CTCTAAGCCA
241        TCCGCAAAAA TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
361        TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT
421        CAGGGTAAAG ACCTGATTTT TGATTTATGG TCATTCTCGT TTTCTGAACT GTTTAAAGCA
481        TTTGAGGGGG ATTCAATGAA TATTTATGAC GATTCCGCAG TATTGGACGC TATCCAGTCT
541        AAACATTTTA CTATTACCCC CTCTGGCAAA ACTTCTTTTG CAAAAGCCTC TCGCTATTTT
601        GGTTTTTATC GTCGTCTGGT AAACGAGGGT TATGATAGTG TTGCTCTTAC TATGCCTCGT
661        AATTCCTTTT GGCGTTATGT ATCTGCATTA GTTGAATGTG GTATTCCTAA ATCTCAACTG
721        ATGAATCTTT CTACCTGTAA TAATGTTGTT CCGTTAGTTC GTTTTATTAA CGTAGATTTT
781        TCTTCCCAAC GTCCTGACTG GTATAATGAG CCAGTTCTTA AAATCGCATA AGGTAATTCA
841        CAATGATTAA AGTTGAAATT AAACCATCTC AAGCCCAATT TACTACTCGT TCTGGTGTTT
901        CTCGTCAGGG CAAGCCTTAT TCACTGAATG AGCAGCTTTG TTACGTTGAT TTGGGTAATG
961        AATATCCGGT TCTTGTCAAG ATTACTCTTG ATGAAGGTCA GCCAGCCTAT GCGCCTGGTC
1021       TGTACACCGT TCATCTGTCC TCTTTCAAAG TTGGTCAGTT CGGTTCCCTT ATGATTGACC
1081       GTCTGCGCCT CGTTCCGGCT AAGTAACATG GAGCAGGTCG CGGATTTCGA CACAATTTAT
1141       CAGGCGATGA TACAAATCTC CGTTGTACTT TGTTTCGCGC TTGGTATAAT CGCTGGGGGT
1201       CAAAGATGAG TGTTTTAGTG TATTCTTTCG CCTCTTTCGT TTTAGGTTGG TGCCTTCGTA
1261       GTGGCATTAC GTATTTTACC CGTTTAATGG AAACTTCCTC ATGAAAAAGT CTTTAGTCCT
1321       CAAAGCCTCT GTAGCCGTTG CTACCCTCGT TCCGATGCTG TCTTTCGCTG CTGAGGGTGA
1381       CGATCCCGCA AAAGCGGCCT TTAACTCCCT GCAAGCCTCA GCGACCGAAT ATATCGGTTA
1441       TGCGTGGGCG ATGGTTGTTG TCATTGTCGG CGCAACTATC GGTATCAAGC TGTTTAAGAA
1501       ATTCACCTCG AAAGCAAGCT GATAAACCGA TACAATTAAA GGCTCCTTTT GGAGCCTTTT
1561       TTTTTGGAGA TTTTCAACGT GAAAAAATTA TTATTCGCAA TTCCTTTAGT TGTTCCTTTC
1621       TATTCTCACA GTGCACAGTC TGTCGTGACG CAGCCGCCCT CAGTGTCTGG GGCCCCAGGG
1681       CAGAGGGTCA CCATCTCCTG CACTGGGAGC AGCTCCAACA TCGGGGCAGG TTATGATGTA
1741       CACTGGTACC AGCAGCTTCC AGGAACAGCC CCCAAACTCC TCATCTATGG TAACAGCAAT
1801       CGGCCCTCAG GGGTCCCTGA CCGATTCTCT GGCTCCAAGT CTGGCACCTC AGCCTCCCTG
1861       GCCATCACTG GGCTCCAGGC TGAGGATGAG GCTGATTATT ACTGCCAGTC CTATGACAGC
1921       AGCCTGAGTG GCCTTTATGT CTTCGGAACT GGGACCAAGG TCACCGTCCT AGGTCAGCCC
1981       AAGGCCAACC CCACTGTCAC TCTGTTCCCG CCCTCCTCTG AGGAGCTCCA AGCCAACAAG
2041       GCCACACTAG TGTGTCTGAT CAGTGACTTC TACCCGGGAG CTGTGACAGT GGCCTGGAAG
2101       GCAGATAGCA GCCCCGTCAA GGCGGGAGTG GAGACCACCA CACCCTCCAA ACAAAGCAAC
2161       AACAAGTACG CGGCCAGCAG CTATCTGAGC CTGACGCCTG AGCAGTGGAA GTCCCACAGA
2221       AGCTACAGCT GCCAGGTCAC GCATGAAGGG AGCACCGTGG AGAAGACAGT GGCCCCTACA
2281       GAATGTTCAT AATAAACCGC CTCCACCGGG CGCGCCAATT CTATTTCAAG GAGACAGTCA
2341       TAATGAAATA CCTATTGCCT ACGGCAGCCG CTGGATTGTT ATTACTCGCG GCCCAGCCGG
2401       CCATGGCCGA AGTTCAATTG TTAGAGTCTG GTGGCGGTCT TGTTCAGCCT GGTGGTTCTT
2461       TACGTCTTTC TTGCGCTGCT TCCGGATTCA CTTTCTCTTC GTACGCTATG TCTTGGGTTC
2521       GCCAAGCTCC TGGTAAAGGT TTGGAGTGGG TTTCTGCTAT CTCTGGTTCT GGTGGCAGTA
2581       CTTACTATGC TGACTCCGTT AAAGGTCGCT TCACTATCTC TAGAGACAAC TCTAAGAATA
2641       CTCTCTACTT GCAGATGAAC AGCTTAAGGG CTGAGGACAC TGCAGTCTAC TATTGCGCTA
2701       AAGACTATGA AGGTACTGGT TATGCTTTCG ACATATGGGG TCAAGGTACT ATGGTCACCG
2761       TCTCTAGTGC CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGCACCCTCC TCCAAGAGCA
2821       CCTCTGGGGG CACAGCGGCC CTGGGCTGCC TGGTCAAGGA CTACTTCCCC GAACCGGTGA
2881       CGGTGTCGTG GAACTCAGGC GCCCTGACCA GCGGCGTCCA CACCTTCCCG GCTGTCCTAC
2941       AGTCTAGCGG ACTCTACTCC CTCAGCAGCG TAGTGACCGT GCCCTCTTCT AGCTTGGGCA
3001       CCCAGACCTA CATCTGCAAC GTGAATCACA AGCCCAGCAA CACCAAGGTG GACAAGAAAG
3061       TTGAGCCCAA ATCTTGTGCG GCCGCTCATC ACCACCATCA TCACTCTGCT GAACAAAAAC
3121       TCATCTCAGA AGAGGATCTG AATGGTGCCG CAGATATCAA CGATGATCGT ATGGCTGGCG
3181       CCGCTGAAAC TGTTGAAAGT TGTTTAGCAA AACCCCATAC AGAAAATTCA TTTACTAACG
3241       TCTGGAAAGA CGACAAAACT TTAGATCGTT ACGCTAACTA TGAGGGTTGT CTGTGGAATG
```

```
3301  CTACAGGCGT TGTAGTTTGT ACTGGTGACG AAACTCAGTG TTACGGTACA TGGGTTCCTA
3361  TTGGGCTTGC TATCCCTGAA AATGAGGGTG GTGGCTCTGA GGGTGGCGGT TCTGAGGGTG
3421  GCGGTTCTGA GGGTGGCGGT ACTAAACCTC CTGAGTACGG TGATACACCT ATTCCGGGCT
3481  ATACTTATAT CAACCCTCTC GACGGCACTT ATCCGCCTGG TACTGAGCAA AACCCCGCTA
3541  ATCCTAATCC TTCTCTTGAG GAGTCTCAGC CTCTTAATAC TTTCATGTTT CAGAATAATA
3601  GGTTCCGAAA TAGGCAGGGG GCATTAACTG TTTATACGGG CACTGTTACT CAAGGCACTG
3661  ACCCCGTTAA AACTTATTAC CAGTACACTC CTGTATCATC AAAAGCCATG TATGACGCTT
3721  ACTGGAACGG TAAATTCAGA GACTGCGCTT TCCATTCTGG CTTTAATGAA GATCCATTCG
3781  TTTGTGAATA TCAAGGCCAA TCGTCTGACC TGCCTCAACC TCCTGTCAAT GCTGGCGGCG
3841  GCTCTGGTGG TGGTTCTGGT GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT GGCGGTTCTG
3901  AGGGTGGCGG CTCTGAGGGA GGCGGTTCCG GTGGTGGCTC TGGTTCCGGT GATTTTGATT
3961  ATGAAAAGAT GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC
4021  TACAGTCTGA CGCTAAAGGC AAACTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG
4081  ATGGTTTCAT TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTTG
4141  CTGGCTCTAA TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA
4201  ATTTCCGTCA ATATTTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTA
4261  GCGCTGGTAA ACCATATGAA TTTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG
4321  TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG TTTGCTAACA
4381  TACTGCGTAA TAAGGAGTCT TAATCATGCC AGTTCTTTTG GGTATTCCGT TATTATTGCG
4441  TTTCCTCGGT TTCCTTCTGG TAACTTTGTT CGGCTATCTG CTTACTTTTC TTAAAAAGGG
4501  CTTCGGTAAG ATAGCTATTG CTATTTCATT GTTTCTTGCT CTTATTATTG GGCTTAACTC
4561  AATTCTTGTG GGTTATCTCT CTGATATTAG CGCTCAATTA CCCTCTGACT TTGTTCAGGG
4621  TGTTCAGTTA ATTCTCCCGT CTAATGCGCT TCCCTGTTTT TATGTTATTC TCTCTGTAAA
4681  GGCTGCTATT TTCATTTTTG ACGTTAAACA AAAAATCGTT TCTTATTTGG ATTGGGATAA
4741  ATAATATGGC TGTTTATTTT GTAACTGGCA AATTAGGCTC TGGAAAGACG CTCGTTAGCG
4801  TTGGTAAGAT TCAGGATAAA ATTGTAGCTG GGTGCAAAAT AGCAACTAAT CTTGATTTAA
4861  GGCTTCAAAA CCTCCCGCAA GTCGGGAGGT TCGCTAAAAC GCCTCGCGTT CTTAGAATAC
4921  CGGATAAGCC TTCTATATCT GATTTGCTTG CTATTGGGCG CGGTAATGAT TCCTACGATG
4981  AAAATAAAAA CGGCTTGCTT GTTCTCGATG AGTGCGGTAC TTGGTTTAAT ACCCGTTCTT
5041  GGAATGATAA GGAAAGACAG CCGATTATTG ATTGGTTTCT ACATGCTCGT AAATTAGGAT
5101  GGGATATTAT TTTTCTTGTT CAGGACTTAT CTATTGTTGA TAAACAGGCG CGTTCTGCAT
5161  TAGCTGAACA TGTTGTTTAT TGTCGTCGTC TGGACAGAAT TACTTTACCT TTTGTCGGTA
5221  CTTTATATTC TCTTATTACT GGCTCGAAAA TGCCTCTGCC TAAATTACAT GTTGGCGTTG
5281  TTAAATATGG CGATTCTCAA TTAAGCCCTA CTGTTGAGCG TTGGCTTTAT ACTGGTAAGA
5341  ATTTGTATAA CGCATATGAT ACTAAACAGG CTTTTTCTAG TAATTATGAT TCCGGTGTTT
5401  ATTCTTATTT AACGCCTTAT TTATCACACG GTCGGTATTT CAAACCATTA AATTTAGGTC
5461  AGAAGATGAA ATTAACTAAA ATATATTTGA AAAAGTTTTC TCGCGTTCTT TGTCTTGCGA
5521  TTGGATTTGC ATCAGCATTT ACATATAGTT ATATAACCCA ACCTAAGCCG GAGGTTAAAA
5581  AGGTAGTCTC TCAGACCTAT GATTTTGATA AATTCACTAT TGACTCTTCT CAGCGTCTTA
5641  ATCTAAGCTA TCGCTATGTT TTCAAGGATT CTAAGGGAAA ATTAATTAAT AGCGACGATT
5701  TACAGAAGCA AGGTTATTCA CTCACATATA TTGATTTATG TACTGTTTCC ATTAAAAAAG
5761  GTAATTCAAA TGAAATTGTT AAATGTAATT AATTTTGTTT TCTTGATGTT TGTTTCATCA
5821  TCTTCTTTTG CTCAGGTAAT TGAAATGAAT AATTCGCCTC TGCGCGATTT TGTAACTTGG
5881  TATTCAAAGC AATCAGGCGA ATCCGTTATT GTTTCTCCCG ATGTAAAAGG TACTGTTACT
5941  GTATATTCAT CTGACGTTAA ACCTGAAAAT CTACGCAATT TCTTTATTTC TGTTTTACGT
6001  GCTAATAATT TTGATATGGT TGGTTCAATT CCTTCCATAA TTCAGAAGTA TAATCCAAAC
6061  AATCAGGATT ATATTGATGA ATTGCCATCA TCTGATAATC AGGAATATGA TGATAATTCC
6121  GCTCCTTCTG GTGGTTTCTT TGTTCCGCAA AATGATAATG TTACTCAAAC TTTTAAAATT
6181  AATAACGTTC GGGCAAAGGA TTTAATACGA GTTGTCGAAT TGTTTGTAAA GTCTAATACT
6241  TCTAAATCCT CAAATGTATT ATCTATTGAC GGCTCTAATC TATTAGTTGT TTCTGCACCT
6301  AAAGATATTT TAGATAACCT TCCTCAATTC CTTTCTACTG TTGATTTGCC AACTGACCAG
6361  ATATTGATTG AGGGTTTGAT ATTTGAGGTT CAGCAAGGTG ATGCTTTAGA TTTTTCATTT
6421  GCTGCTGGCT CTCAGCGTGG CACTGTTGCA GGCGGTGTTA ATACTGACCG CCTCACCTCT
6481  GTTTTATCTT CTGCTGGTGG TTCGTTCGGT ATTTTTAATG GCGATGTTTT AGGGCTATCA
6541  GTTCGCGCAT TAAAGACTAA TAGCCATTCA AAAATATTGT CTGTGCCACG TATTCTTACG
6601  CTTTCAGGTC AGAAGGGTTC TATCTCTGTT GGCCAGAATG TCCCTTTTAT TACTGGTCGT
6661  GTGACTGGTG AATCTGCCAA TGTAAATAAT CCATTTCAGA CGATTGAGCG TCAAAATGTA
6721  GGTATTTCCA TGAGCGTTTT TCCTGTTGCA ATGGCTGGCG GTAATATTGT TCTGGATATT
```

```
6781  ACCAGCAAGG CCGATAGTTT GAGTTCTTCT ACTCAGGCAA GTGATGTTAT TACTAATCAA
6841  AGAAGTATTG CTACAACGGT TAATTTGCGT GATGGACAGA CTCTTTTACT CGGTGGCCTC
6901  ACTGATTATA AAAACACTTC TCAAGATTCT GGCGTACCGT TCCTGTCTAA AATCCCTTTA
6961  ATCGGCCTCC TGTTTAGCTC CCGCTCTGAT TCCAACGAGG AAAGCACGTT ATACGTGCTC
7021  GTCAAAGCAA CCATAGTACG CGCCCTGTAG CGGCGCATTA AGCGCGGCGG GTGTGGTGGT
7081  TACGCGCAGC GTGACCGCTA CACTTGCCAG CGCCCTAGCG CCCGCTCCTT TCGCTTTCTT
7141  CCCTTCCTTT CTCGCCACGT TCGCCGGCTT TCCCCGTCAA GCTCTAAATC GGGGGCTCCC
7201  TTTAGGGTTC CGATTTAGTG CTTTACGGCA CCTCGACCCC AAAAAACTTG ATTTGGGTGA
7261  TGGTTCACGT AGTGGGCCAT CGCCCTGATA GACGGTTTTT CGCCCTTTGA CGTTGGAGTC
7321  CACGTTCTTT AATAGTGGAC TCTTGTTCCA AACTGGAACA ACACTCAACC CTATCTCGGG
7381  CTATTCTTTT GATTTATAAG GGATTTTGCC GATTTCGGAA CCACCATCAA ACAGGATTTT
7441  CGCCTGCTGG GGCAAACCAG CGTGGACCGC TTGCTGCAAC TCTCTCAGGG CCAGGCGGTG
7501  AAGGGCAATC AGCTGTTGCC CGTCTCACTG GTGAAAAGAA AAACCACCCT GGATCCAAGC
7561  TTGCAGGTGG CACTTTTCGG GGAAATGTGC GCGGAACCCC TATTTGTTTA TTTTTCTAAA
7621  TACATTCAAA TATGTATCCG CTCATGAGAC AATAACCCTG ATAAATGCTT CAATAATATT
7681  GAAAAAGGAA GAGTATGAGT ATTCAACATT TCCGTGTCGC CCTTATTCCC TTTTTTGCGG
7741  CATTTTGCCT TCCTGTTTTT GCTCACCCAG AAACGCTGGT GAAAGTAAAA GATGCTGAAG
7801  ATCAGTTGGG CGCACGAGTG GGTTACATCG AACTGGATCT CAACAGCGGT AAGATCCTTG
7861  AGAGTTTTCG CCCCGAAGAA CGTTTTCCAA TGATGAGCAC TTTTAAAGTT CTGCTATGTC
7921  ATACACTATT ATCCCGTATT GACGCCGGGC AAGAGCAACT CGGTCGCCGG GCGCGGTATT
7981  CTCAGAATGA CTTGGTTGAG TACTCACCAG TCACAGAAAA GCATCTTACG GATGGCATGA
8041  CAGTAAGAGA ATTATGCAGT GCTGCCATAA CCATGAGTGA TAACACTGCG GCCAACTTAC
8101  TTCTGACAAC GATCGGAGGA CCGAAGGAGC TAACCGCTTT TTTGCACAAC ATGGGGGATC
8161  ATGTAACTCG CCTTGATCGT TGGGAACCGG AGCTGAATGA AGCCATACCA AACGACGAGC
8221  GTGACACCAC GATGCCTGTA GCAATGCCAA CAACGTTGCG CAAACTATTA ACTGGCGAAC
8281  TACTTACTCT AGCTTCCCGG CAACAATTAA TAGACTGGAT GGAGGCGGAT AAAGTTGCAG
8341  GACCACTTCT GCGCTCGGCC CTTCCGGCTG GCTGGTTTAT TGCTGATAAA TCTGGAGCCG
8401  GTGAGCGTGG GTCTCGCGGT ATCATTGCAG CACTGGGGCC AGATGGTAAG CCCTCCCGTA
8461  TCGTAGTTAT CTACACGACG GGGAGTCAGG CAACTATGGA TGAACGAAAT AGACAGATCG
8521  CTGAGATAGG TGCCTCACTG ATTAAGCATT GGTAACTGTC AGACCAAGTT TACTCATATA
8581  TACTTTAGAT TGATTTAAAA CTTCATTTTT AATTTAAAAG GATCTAGGTG AAGATCCTTT
8641  TTGATAATCT CATGACCAAA ATCCCTTAAC GTGAGTTTTC GTTCCACTGT ACGTAAGACC
8701  CCCAAGCTTG TCGACTGAAT GGCGAATGGC GCTTTGCCTG GTTTCCGGCA CCAGAAGCGG
8761  TGCCGGAAAG CTGGCTGGAG TGCGATCTTC CTGAGGCCGA TACTGTCGTC GTCCCCTCAA
8821  ACTGGCAGAT GCACGGTTAC GATGCGCCCA TCTACACCAA CGTAACCTAT CCCATTACGG
8881  TCAATCCGCC GTTTGTTCCC ACGGAGAATC CGACGGGTTG TTACTCGCTC ACATTTAATG
8941  TTGATGAAAG CTGGCTACAG GAAGGCCAGA CGCGAATTAT TTTTGATGGC GTTCCTATTG
9001  GTTAAAAAAT GAGCTGATTT AACAAAAATT TAACGCGAAT TTTAACAAAA TATTAACGTT
9061  TACAATTTAA ATATTTGCTT ATACAATCTT CCTGTTTTTG GGGCTTTTCT GATTATCAAC
9121  CGGGGTACAT ATGATTGACA TGCTAGTTTT ACGATTACCG TTCATCGATT CTCTTGTTTG
9181  CTCCAGACTC TCAGGCAATG ACCTGATAGC CTTTGTAGAT CTCTCAAAAA TAGCTACCCT
9241  CTCCGGCATG AATTTATCAG CTAGAACGGT TGAATATCAT ATTGATGGTG ATTTGACTGT
9301  CTCCGGCCTT TCTCACCCTT TTGAATCTTT ACCTACACAT TACTCAGGCA TTGCATTTAA
9361  AATATATGAG GGTTCTAAAA ATTTTTATCC TTGCGTTGAA ATAAAGGCTT CTCCCGCAAA
9421  AGTATTACAG GGTCATAATG TTTTTGGTAC AACCGATTTA GCTTTATGCT CTGAGGCTTT
9481  ATTGCTTAAT TTTGCTAATT CTTTGCCTTG CCTGTATGAT TTATTGGATG TT
```

Table 22: Primers used in RACE amplification:

Heavy chain	
HuCμ-FOR (1st PCR)	5'-TGG AAG AGG CAC GTT CTT TTC TTT-3'
HuCμ-Nested (2nd PCR)	5' CTT TTC TTT GTT GCC GTT GGG GTG-3'
Kappa light chain	
HuCkFor (1st PCR)	5'-ACA CTC TCC CCT GTT GAA GCT CTT-3'
HuCkForAscI(2nd PCR)	**5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA ACA CTC TCC CCT GTT GAA GCT CTT-3'**
Lambda light chain	
HuClambdaFor (1st PCR)	
HuCL2-FOR	5'-TGA ACA TTC TGT AGG GGC CAC TG-3'
HuCL7-FOR	5'-AGA GCA TTC TGC AGG GGC CAC TG-3'
HuClambdaForAscI (2nd PCR)	
HuCL2-FOR-ASC	**5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA TGA ACA TTC TGT AGG GGC CAC TG-3'**
HuCL7-FOR-ASC	**5'-ACC GCC TCC ACC GGG CGC GCC TTA TTA AGA GCA TTC TGC AGG GGC CAC TG-3'**
GeneRAcer 5' Primers provided with the kit (Invitrogen)	
5'A 1st PCR	5'CGACTGGAGCACGAGGACACTGA 3'
5'NA 2nd pCR	5'GGACACTGACATGGACTGAAGGAGTA-3'

Table 23: ONs used in Capture of kappa light chains using CJ method and *BsmA*I

All ONs are written 5' to 3'.

REdapters (6)		
ON_20SK15012		gggAggATggAgAcTgggTc
ON_20SK15L12		gggAAgATggAgAcTgggTc
ON_20SK15A17		gggAgAgTggAgAcTgAgTc
ON_20SK15A27		gggTgccTggAgAcTgcgTc
ON_20SK15A11		gggTggcTggAgAcTgcgTc
ON_20SK15B3	gggAgTcTggAgAcTgggTc	
Bridges (6)		
kapbri1012	gggAggATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
kapbri1L12	gggAAgATggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
kapbrilA17	gggAgAgTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
kapbri1A27	gggTgccTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
kapbri1A11	gggTggcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
kapbrilB3	gggAgTcTggAgAcTgggTcATcTggATgTcTTgTgcAcTgTgAcAgAgg	
Extender (5' biotinylated)		
kapextl bio	ccTcTgTcAcAgTgcAcAAgAcATccAgATgAcccAgTcTcc	
Primers		
kaPCRtl	ccTcTgTcAcAgTgeAcAAgAc	
kapfor		5'-aca ctc tcc cct gtt gaa gct ctt-3'

Table 24: PCR program for amplification of kappa DNA

95˚C	5 minutes
95˚C	15 seconds
65˚C	30 seconds
72˚C	1 minute
72˚C	7 minutes
4˚C	hold

Reagents (100 ul reaction):

Template	50 ng
10x turbo PCR buffer	1x
turbo Pfu	4U
dNTPs	200 μM each
kaPCRtl	300 nM
kapfor	300 nM

Table 25: h3401-h2 captured Via CJ with BsmAI

```
! 1  2  3  4  5  6  7  8  9  10 11 12 13 14 15
! S  A  Q  D  I  Q  M  T  Q  S  P  A  T  L  S
 aGT GCA Caa gac atc cag atg acc cag tct cca gcc acc ctg tct
! ApaLI...                    a gcc acc ! L25,L6,L20,L2,L16,A11
! Extender.....................Bridge...

! 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
! V  S  P  G  E  R  A  T  L  S  C  R  A  S  Q
 gtg tct cca ggg gaa agg gcc acc ctc tcc tgc agg gcc agt cag

! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
! S  V  S  N  N  L  A  W  Y  Q  Q  K  P  G  Q
 agt gtt agt aac aac tta gcc tgg tac cag cag aaa cct ggc cag

! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
! V  P  R  L  L  I  Y  G  A  S  T  R  A  T  D
 gtt ccc agg ctc ctc atc tat ggt gca tcc acc agg gcc act gat

! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
! I  P  A  R  F  S  G  S  G  S  G  T  D  F  T
 atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gac ttc act

! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
! L  T  I  S  R  L  E  P  E  D  F  A  V  Y  Y
 ctc acc atc agc aga ctg gag cct gaa gat ttt gca gtg tat tac

! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
! C  Q  R  Y  G  S  S  P  G  W  T  F  G  Q  G
 tgt cag cgg tat ggt agc tca ccg ggg tgg acg ttc ggc caa ggg

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
! T  K  V  E  I  K  R  T  V  A  A  P  S  V  F
 acc aag gtg gaa atc aaa cga act gtg gct gca cca tct gtc ttc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
! I  F  P  P  S  D  E  Q  L  K  S  G  T  A  S
 atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
! V  V  C  L  L  N  N  F  Y  P  R  E  A  K  V
 gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
! Q  W  K  V  D  N  A  L  Q  S  G  N  S  Q  E
 cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
! S  V  T  E  Q  D  S  K  D  S  T  Y  S  L  S
 agt gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc
```

```
! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
! S  T  L  T  L  S  K  A  D  Y  E  K  H  K  V
  agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
! Y  A  C  E  V  T  H  Q  G  L  S  S  P  V  T
  tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct gtc aca

! 211 212 213 214 215 216 217 218 219 220 221 222 223
! K  S  F  N  K  G  E  C  K  G  E  F  A
  aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc.....
```

Table 26: h3401-d8 KAPPA captured with CJ and *Bsm*AI

```
! 1  2  3  4  5  6  7  8  9  10 11 12 13 14 15
! S  A  Q  D  I  Q  M  T  Q  S  P  A  T  L  S
 aGT GCA Caa gac atc cag atg acc cag tct cct gcc acc ctg tct
! ApaLI...Extender.....a gcc acc ! L25,L6,L20,L2,L16,A11
!                        A GCC ACC CTG TCT ! L2

! 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
! V  S  P  G  E  R  A  T  L  S  C  R  A  S  Q
 gtg tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag
! GTG TCT CCA GGG GAA AGA GCC ACC CTC TCC TGC !    L2

! 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
! N  L  L  S  N  L  A  W  Y  Q  Q  K  P  G  Q
 aat ctt ctc agc aac tta gcc tgg tac cag cag aaa cct ggc cag

! 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
! A  P  R  L  L  I  Y  G  A  S  T  G  A  I  G
 gct ccc agg ctc ctc atc tat ggt gct tcc acc ggg gcc att ggt

! 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
! I  P  A  R  F  S  G  S  G  S  G  T  E  F  T
 atc cca gcc agg ttc agt ggc agt ggg tct ggg aca gag ttc act

! 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
! L  T  I  S  S  L  Q  S  E  D  F  A  V  Y  F
 ctc acc atc agc agc ctg cag tct gaa gat ttt gca gtg tat ttc

! 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
! C  Q  Q  Y  G  T  S  P  P  T  F  G  G  G  T
 tgt cag cag tat ggt acc tca ccg ccc act ttc ggc gga ggg acc

! 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
! K  V  E  I  K  R  T  V  A  A  P  S  V  F  I
 aag gtg gag atc aaa cga act gtg gct gca cca tct gtc ttc atc

! 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
! F  P  P  S  D  E  Q  L  K  S  G  T  A  S  V
 ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt

! 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
! V  C  P  L  N  N  F  Y  P  R  E  A  K  V  Q
 gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag

! 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
! W  K  V  D  N  A  L  Q  S  G  N  S  Q  E  S
 tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt

! 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
! V  T  E  Q  D  N  K  D  S  T  Y  S  L  S  S
 gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc
```

```
! 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
! T  L  T  L  S  K  V  D  Y  E  K  H  E  V  Y
  acc ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac

! 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
! A  C  E  V  T  H  Q  G  L  S  S  P  V  T  K
  gcc tgc gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag

! 211 212 213 214 215 216 217 218 219 220 221 222 223
! S  F  N  R  G  E  C  K  K  E  F  V
  agc ttc aac agg gga gag tgt aag aaa gaa ttc gtt t
```

Table 27: V3-23 VH framework with variegated codons shown

```
!
!                        17 18 19 20 21 22
!                         A  Q  P  A  M  A
5           5'-ctg tct gaa cG GCC cag ccG GCC atg gcc    29
            3'-gac aga ctt gc cgg gtc ggc cgg tac cgg
!             Scab.....SfiI.....
!                          NgoMI...
!                              NcoI....
!
10          !                        FR1(DP47/V3-23)----------
            !                        23 24 25 26 27 28 29 30
            !                         E  V  Q  L  L  E  S  G
                                     gaa|gtt|CAA|TTG|tta|gag|tct|ggt|   53
            !                        ctt|caa|gtt|aac|aat|ctc|aga|cca|
15          !                            | MfeI |
            !
            !    ----------FR1-------------------------------------
            !     31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
            !      G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A
                |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|   98
20          !   |ccg|cca|gaa|caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|
            !
            !    Sites to be varied--->     ***   ***   ***
            !    ---FR1---------->|...CDR1.....|---FR2------
            !     46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
            !      A  S  G  F  T  F  S  S  Y  A  M  S  W  V  R
25              |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|   143
            !   |cga|agg|cct|aag|tga|aag|aga|agc|atg|cga|tac|aga|cc|caa|gcg|
            !     | BspEI |          | BsiWI|              |BstXI.
            !
            !                Sites to be varies---> ***   *** ***
            !    -------FR2---------------------->|...CDR2.....
30          !     61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
            !      Q  A  P  G  K  G  L  E  W  V  S  A  I  S  G
                |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|   188
            !   |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|cga|tag|aga|cca|
            ! ...BstXI       |
35          !
            !          ***   ***
            !    .....CDR2.....|---FR3---
            !     76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
            !      S  G  G  S  T  Y  Y  A  D  S  V  K  G  R  F
                |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|   233
40          !   |aga|cca|ccg|tca|tga|atg|ata|cga|ctg|agg|caa|ttt|cca|gcg|aag|
            !
            !    --------FR3-------------------------------------------
            !     91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
            !      T  I  S  R  D  N  S  K  N  T  L  Y  L  Q  M
                |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|   278
45          !   |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|
            !        | XbaI |
            !
            !    ---FR3------------------------------------------------>|
            !     106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
50          !      N  S  L  R  A  E  D  T  A  V  Y  Y  C  A  K
                |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|   323
            !   |ttg|tcg|aat|tcc|cga|ctc|ctg|tga|cgt|cag|atg|ata|acg|cga|ttt|
            !
55
```

```
!          |AflII|          |PstI|
!
!     .....CDR3.....|----FR4----------
!      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!       D  Y  E  G  T  G  Y  A  F  D  I  W  G  Q  G
      |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|   368
!     |ctg|ata|ctt|cca|tga|cca|ata|cga|aag|ctg|tat|acc|cca|gtt|cca|
!                                 |NdeI|
!
!     ----------FR4--------->|
!      136 137 138 139 140 141 142
!       T  M  V  T  V  S  S
      |act|atG|GTC|ACC|gtc|tct|agt-     389
!     |tga|tac|cag|tgg|cag|aga|tca-
!         |BstEII|
!
!                 143 144 145 146 147 148 149 150 151 152
!                  A  S  T  K  G  P  S  V  F  P
                  gcc tcc acc aaG GGC CCa tcg GTC TTC ccc-3'   419
!                 cgg agg tgg ttc ccg ggt agc cag aag ggg-5'
!                         Bsp120I.    BbsI...(2/2)
!                         ApaI....

(SFPRMET) 5'-ctg tct gaa cG GCC cag ccG-3'
(TOPFR1A) 5'-ctg tct gaa cG GCC cag ccG GCC atg gcc-
          gaa|gtt|CAA|TTG|tta|gag|tct|ggt|-
         |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta-3'
(BOTFR1B)          3'-caa|gtc|gga|cca|cca|aga|aat|gca|gaa|aga|acg|cga|-
         |cga|agg|cct|aag|tga|aag-5' ! bottom strand
(BOTFR2) 3'-acc|caa|gcg|-
         |gtt|cga|gga|cca|ttt|cca|aac|ctc|acc|caa|aga|-5' ! bottom strand
(BOTFR3) 3'- a|cga|ctg|agg|caa|ttt|cca|gcg|aag|-
         |tga|tag|aga|tct|ctg|ttg|aga|ttc|tta|tga|gag|atg|aac|gtc|tac|-
       |ttg|tcg|aat|tcc|cga|ctc|ctg|tga-5'
(F06)      5'-gC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|-
      |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|c-3'
(BOTFR4) 3'-cga|aag|ctg|tat|acc|cca|gtt|cca|-
         |tga|tac|cag|tgg|cag|aga|tca-
            cgg agg tgg ttc ccg ggt agc cag aag ggg-5' ! bottom strand
(BOTPRCPRIM)         3'-gg ttc ccg ggt agc cag aag ggg-5'
!
! CDR1 diversity
!
(ON-vgC1)  5'-|gct|TCC|GGA|ttc|act|ttc|tct|<1>|TAC|<1>|atg|<1>|
!                          CDR1.....6859
           |tgg|gtt|cgC|CAa|gct|ccT|GG-3'
!
!<1> stands for an equimolar mix of {ADEFGHIKLMNPQRSTVWY}; no C
!                    (this is not a sequence)
!
! CDR2 diversity
!
(ON-vgC2)  5'-ggt|ttg|gag|tgg|gtt|tct|<2>|atc|<2>|<3>|-
!                        CDR2.....
            |tct|ggt|ggc|<1>|act|<1>|tat|gct|gac|tcc|gtt|aaa|gg-3'
!           CDR2.....
! <1> is an equimolar mixture of {ADEFGHIKLMNPQRSTVWY}; no C
! <2> is an equimolar mixture of {YRWVGS}; no ACDEFHIKLMNPQT



  ! <3> is an equimolar mixture of {PS}; no ACDEFGHIKLMNQRTVWY
```

Table 28: Snuffer used in VH

1 TCCGGAGCTT CAGATCTGTT TGCCTTTTTG TGGGGTGGTG CAGATCGCGT TACGGAGATC
61 GACCGACTGC TTGAGCAAAA GCCACGCTTA ACTGCTGATC AGGCATGGGA TGTTATTCGC
121 CAAACCAGTC GTCAGGATCT TAACCTGAGG CTTTTTTTAC CTACTCTGCA AGCAGCGACA
181 TCTGGTTTTGA CACAGAGCGA TCCGCGTCGT CAGTTGGTAG AAACATTAAC ACGTTGGGAT
241 GGCATCAATT TGCTTAATGA TGATGGTAAA ACCTGGCAGC AGCCAGGCTC TGCCATCCTG
301 AACGTTTGGC TGACCAGTAT GTTGAAGCGT ACCGTAGTGG CTGCCGTACC TATGCCATTT
361 GATAAGTGGT ACAGCGCCAG TGGCTACGAA ACAACCCAGG ACGGCCCAAC TGGTTCGCTG
421 AATATAAGTG TTGGAGCAAA AATTTTGTAT GAGGCGGTGC AGGGAGACAA ATCACCAATC
481 CCACAGGCGG TTGATCTGTT TGCTGGGAAA CCACAGCAGG AGGTTGTGTT GGCTGCGCTG
541 GAAGATACCT GGGAGACTCT TTCCAAACGC TATGGCAATA ATGTGAGTAA CTGGAAAACA
601 CCTGCAATGG CCTTAACGTT CCGGGCAAAT AATTTCTTTG GTGTACCGCA GGCCGCAGCG
661 GAAGAAACGC GTCATCAGGC GGAGTATCAA AACCGTGGAA CAGAAAACGA TATGATTGTT
721 TTCTCACCAA CGACAAGCGA TCGTCCTGTG CTTGCCTGGG ATGTGGTCGC ACCCGGTCAG
781 AGTGGGTTTA TTGCTCCCGA TGGAACAGTT GATAAGCACT ATGAAGATCA GCTGAAAATG
841 TACGAAAATT TTGGCCGTAA GTCGCTCTGG TTAACGAAGC AGGATGTGGA GGCGCATAAG
901 GAGTCGTCTA GA

Table 29: DNA sequence of pCES5

```
! pCES5  6680  bases = pCes4 with stuffers in CDR1-2 and CDR3 2000.12.13
!
! Ngene =  6680
! Useful REs (cut MAnoLI fewer than 3 times) 2000.06.05
!
! Non-cutters
!Acc65I Ggtacc      AfeI AGCgct       AvrII Cctagg
!BsaBI GATNNnnatc   BsiWI Cgtacg      BsmFI Nnnnnnnnnnnnnnngtccc
!BsrGI Tgtaca      BstAPI GCANNNNntgc  BstBI TTcgaa
!BstZ17I GTAtac    BtrI CACgtg       Ecl136I GAGctc
!EcoRV GATatc      FseI GGCCGGcc     KpnI GGTACc
!MscI TGGcca       NruI TCGcga       NsiI ATGCAt
!PacI TTAATtaa     PmeI GTTTaaac     PmlI CACgtg
!PpuMI RGgwccy     PshAI GACNNnngtc  SacI GAGCTc
!SacII CCGCgg      SbfI CCTGCAgg     SexAI Accwggt
!SgfI GCGATcgc     SnaBI TACgta      SpeI Actagt
!SphI GCATGc       Sse8387I CCTGCAgg  StuI AGGcct
!SwaI ATTTaaat     XmaI Cccggg
!
! cutters
! Enzymes that cut more than   3 times.
!AlwNI CAGNNNctg          5
!BsgI ctgcac            4
!BsrFI Rccggy            5
!EarI CTCTTCNnnn         4
!FauI nNNNNNNGCGGG       10
!
! Enzymes that cut from 1 to   3 times.
!
!EcoO109I RGgnccy        3    7  2636  4208
!BssSI Ctcgtg           1   12
!-"-  Cacgag            1  1703
!BspHI Tcatga           3   43  148  1156
!AatII GACGTc           1   65
```

!BciVI GTATCCNNNNNN 2 140 1667
!Eco57I CTGAAG 1 301
!-"- cttcag 2 1349
!AvaI Cycgrg 3 319 2347 6137
!BsiHKAI GWGCWc 3 401 2321 4245
!HgiAI GWGCWc 3 401 2321 4245
!BcgI gcannnnnntcg 1 461
!ScaI AGTact 1 505
!PvuI CGATcg 3 616 3598 5926
!FspI TGCgca 2 763 5946
!BglI GCCNNNNnggc 3 864 2771 5952
!BpmI CTGGAG 1 898
!-"- ctccag 1 4413
!BsaI GGTCTCNnnnn 1 916
!AhdI GACNNNnngtc 1 983
!EamI105I GACNNNnngtc 1 983
!DrdI GACNNNNnngtc 3 1768 6197 6579
!SapI gaagagc 1 1998
!PvuII CAGctg 3 2054 3689 5896
!PflMI CCANNNNntgg 3 2233 3943 3991
!HindIII Aagctt 1 2235
!ApaLI Gtgcac 1 2321
!BspMI Nnnnnnnnngcaggt 1 2328
!-"- ACCTGCNNNNn 2 3460
!PstI CTGCAg 1 2335
!AccI GTmkac 2 2341 2611
!HincII GTYrac 2 2341 3730
!SalI Gtcgac 1 2341
!TliI Ctcgag 1 2347
!XhoI Ctcgag 1 2347
!BbsI gtcttc 2 2383 4219
!BlpI GCtnagc 1 2580
!EspI GCtnagc 1 2580
!SgrAI CRccggyg 1 2648
!AgeI Accggt 2 2649 4302
!AscI GGcgcgcc 1 2689
!BssHII Gcgcgc 1 2690

!SfiI GGCCNNNNnggcc 1 2770
!NaeI GCCggc 2 2776 6349
!NgoMIV Gccggc 2 2776 6349
!BtgI Ccrygg 3 2781 3553 5712
!DsaI Ccrygg 3 2781 3553 5712
!NcoI Ccatgg 1 2781
!StyI Ccwwgg 3 2781 4205 4472
!MfeI Caattg 1 2795
!BspEI Tccgga 1 2861
!BglII Agatct 1 2872
!BclI Tgatca 1 2956
!Bsu36I CCtnagg 3 3004 4143 4373
!XcmI CCANNNNNnnnntgg 1 3215
!MluI Acgcgt 1 3527
!HpaI GTTaac 1 3730
!XbaI Tctaga 1 3767
!
!AflII Cttaag 1 3811
!BsmI NGcattc 1 3821
!-"- GAATGCN 1 4695
!RsrII CGgwccg 1 3827
!NheI Gctagc 1 4166
!BstEII Ggtnacc 1 4182
!BsmBI CGTCTCNnnnn 2 4188 6625
!-"- Nnnnnngagacg 1 6673
!ApaI GGGCCc 1 4209
!BanII GRGCYc 3 4209 4492 6319
!Bsp120I Gggccc 1 4209
!PspOMI Gggccc 1 4209
!BseRI NNnnnnnnnnctcctc 1 4226
!-"- GAGGAGNNNNNNNNNN 1 4957
!EcoNI CCTNNnnnagg 1 4278
!PflFI GACNnngtc 1 4308
!Tth111I GACNnngtc 1 4308
!KasI Ggcgcc 2 4327 5967
!BstXI CCANNNNNntgg 1 4415
!NotI GCggccgc 1 4507

```
!EagI Cggccg              1   4508
!BamHI Ggatcc             1   5169
!BspDI ATcgat             1   5476
!NdeI CAtatg              1   5672
!EcoRI Gaattc             1   5806
!PsiI TTAtaa              1   6118
!DraIII CACNNNgtg         1   6243
!BsaAI YACgtr             1   6246
!-------------------------------------------------------------------------
   1   gacgaaaggg cCTCGTGata cgcctatttt tataggttaa tgtcatgata ataatggttt
!              BssSI.(1/2)
  61   cttaGACGTC aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt
!        AatII.
 121   tctaaatаca ttcaaatatG TATCCgctca tgagacaata accctgataa atgcttcaat
!                  BciVI..(1 of 2)
 181   aatattgaaa aaggaagagt
! Base #  201 to 1061 = ApR gene from pUC119 with some RE sites removed
!
!       1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
!      fM  S  I  Q  H  F  R  V  A  L  I  P  F  F  A
 201   atg agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg
!
!      16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
!       A  F  C  L  P  V  F  A  H  P  E  T  L  V  K
 246   gca ttt tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa
!
!      31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!       V  K  D  A  E  D  Q  L  G  A  R  V  G  Y  I
 291   gta aaa gat gct gaa gat cag ttg ggt gcc cga gtg ggt tac atc
!
!      46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
!       E  L  D  L  N  S  G  K  I  L  E  S  F  R  P
 336   gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt cgc ccc
!
!      61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
!       E  E  R  F  P  M  M  S  T  F  K  V  L  L  C
 381   gaa gaa cgt ttt cca atg atg agc act ttt aaa gtt ctg cta tgt
```

```
!
!      76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
!      G  A  V  L  S  R  I  D  A  G  Q  E  Q  L  G
  426  ggc gcg gta tta tcc cgt att gac gcc ggg caa gaG CAa ctc ggT
!                                  BcgI.....
!
!      91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
!      R  R  I  H  Y  S  Q  N  D  L  V  E  Y  S  P
  471  CGc cgc ata cac tat tct cag aat gac ttg gtt gAG TAC Tca cca
!..BcgI.....                        ScaI....
!
!      106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!      V  T  E  K  H  L  T  D  G  M  T  V  R  E  L
  516  gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta
!
!      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!      C  S  A  A  I  T  M  S  D  N  T  A  A  N  L
  561  tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta
!
!      136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!      L  L  T  T  I  G  G  P  K  E  L  T  A  F  L
  606  ctt ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg
!             PvuI.... (1/2)
!
!      151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!      H  N  M  G  D  H  V  T  R  L  D  R  W  E  P
  651  cac aac atg ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg
!
!      166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!      E  L  N  E  A  I  P  N  D  E  R  D  T  T  M
  696  gag ctg aat gaa gcc ata cca aac gac gag cgt gac acc acg atg
!
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!      P  V  A  M  A  T  T  L  R  K  L  L  T  G  E
  741  cct gta GCA ATG gca aca acg tTG CGC Aaa cta tta act ggc gaa
!          BsrDI..(1/2)      FspI.... (1/2)
!
```

```
!       196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!       L  L  T  L  A  S  R  Q  Q  L  I  D  W  M  E
  786   cta ctt act cta gct tcc cgg caa caa tta ata gac tgg atg gag
!
!       211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!       A  D  K  V  A  G  P  L  L  R  S  A  L  P  A
  831   gcg gat aaa gtt gca gga cca ctt ctg cgc tcg gcc ctt ccg gct
!
!       226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!       G  W  F  I  A  D  K  S  G  A  G  E  R  G  S
  876   ggc tgg ttt att gct gat aaa tCT GGA Gcc ggt gag cgt gGG TCT
!                           BpmI....(1/2)         BsaI....
!
!       241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!       R  G  I  I  A  A  L  G  P  D  G  K  P  S  R
  921   Cgc ggt atC ATT GCa gca ctg ggg cca gat ggt aag ccc tcc cgt
! BsaI.....       BsrDI...(2/2)
!
!       256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!       I  V  V  I  Y  T  T  G  S  Q  A  T  M  D  E
  966   atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa
!                     AhdI.....
!
!       271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!       R  N  R  Q  I  A  E  I  G  A  S  L  I  K  H
 1011   cga aat aga cag atc gct gag ata ggt gcc tca ctg att aag cat
!
!       286 287
!       W  .
 1056   tgg taa
 1062                                   ctgtcagac caagtttact
 1081   catatatact ttagattgat ttaaaacttc atttttaatt taaaaggatc taggtgaaga
 1141   tccttttga taatctcatg accaaaatcc cttaacgtga gttttcgttc cactgagcgt
 1201   cagaccccgt agaaaagatc aaaggatctt cttgagatcc ttttttctg cgcgtaatct
 1261   gctgcttgca aacaaaaaaa ccaccgctac cagcggtggt ttgtttgccg gatcaagagc
 1321   taccaactct ttttccgaag gtaactggct tcagcagagc gcagatacca aatactgtcc
 1381   ttctagtgta gccgtagtta ggccaccact tcaagaactc tgtagcaccg cctacatacc
```

```
 1441   tcgctctgct aatcctgtta ccagtggctg ctgccagtgg cgataagtcg tgtcttaccg
 1501   ggttggactc aagacgatag ttaccggata aggcgcagcg gtcgggctga acgggggggtt
 1561   cgtgcataca gcccagcttg gagcgaacga cctacaccga actgagatac ctacagcgtg
 1621   agcattgaga aagcgccacg cttcccgaag ggagaaaggc ggacagGTAT CCggtaagcg
!                                          BciVI.. (2 of 2)
 1681   gcagggtcgg aacaggagag cgCACGAGgg agcttccagg gggaaacgcc tggtatcttt
!                    BssSI.(2/2)
 1741   atagtcctgt cgggtttcgc cacctctgac ttgagcgtcg atttttgtga tgctcgtcag
 1801   gggggcggag cctatggaaa aacgccagca acgcggcctt tttacggttc ctggccttttt
 1861   gctggccttt tgctcACATG Ttctttcctg cgttatcccc tgattctgtg gataaccgta
!              PciI...
 1921   ttaccgcctt tgagtgagct gataccgctc gccgcagccg aacgaccgag cgcagcgagt
 1981   cagtgagcga ggaagcgGAA GAGCgcccaa tacgcaaacc gcctctcccc gcgcgttggc
!                SapI....
 2041   cgattcatta atgCAGCTGg cacgacaggt ttcccgactg gaaagcgggc agtgagcgca
!                 PvuII.(1/3)
 2101   acgcaattTAA TGTgagttag ctcactcatt aggcacccca ggcTTTACAc tttatgcttc
!                ..-35..      Plac              ..-10.
 2161   cggctcgtat gttgtgtgga attgtgagcg gataacaatt tcacaCAGGA AACAGCTATG
!                                          M13Rev_seq_primer
 2221   ACcatgatta cgCCAAGCTT TGGagccttt tttttggaga ttttcaac
!             PflMI.....
!              Hind3.
! signal::linker::CLight
!
!       1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
!      fM  K  K  L  L  F  A  I  P  L  V  V  P  F  Y
 2269   gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
!
!                Linker..... End of FR4
!       16 17 18 19   20 21 22 23 24 25 26 27 28 29 30
!       S  H  S  A    Q  V  Q  L  Q  V  D  L  E  I  K
 2314   tct cac aGT GCA   Cag gtc caa CTG CAG GTC GAC CTC GAG atc aaa
!          ApaLI.....       PstI...     XhoI...
!                         BspMI...
!                            SalI...
!                            AccI...(1/2)
```

```
!                                HincII.(1/2)
!
! Vlight domains could be cloned in as ApaLI-XhoI fragments.
! VL-CL(kappa) segments can be cloned in as ApaLI-AscI fragments. <--------
!
!       Ckappa-----------------------------------------------------
!       31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!       R  G  T  V  A  A  P  S  V  F  I  F  P  P  S
 2359    cgt gga act gtg gct gca cca tct GTC TTC atc ttc ccg cca tct
!                                BbsI...(1/2)
!
!       46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
!       D  E  Q  L  K  S  G  T  A  S  V  V  C  L  L
 2404    gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg
!
!       61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
!       N  N  F  Y  P  R  E  A  K  V  Q  W  K  V  D
 2449    aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat
!
!       76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
!       N  A  L  Q  S  G  N  S  Q  E  S  V  T  E  Q
 2494    aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag
!
!       91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
!       D  S  K  D  S  T  Y  S  L  S  S  T  L  T  L
 2539    gac agc aag gac agc acc tac agc ctc agc agc acc ctg acG CTG
!                                                 EspI...
!
!      106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!       S  K  A  D  Y  E  K  H  K  V  Y  A  C  E  V
 2584    AGC aaa gca gac tac gag aaa cac aaa GTC TAC gcc tgc gaa gtc
! ...EspI....                      AccI...(2/2)
!
!      121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!       T  H  Q  G  L  S  S  P  V  T  K  S  F  N  R
 2629    acc cat cag ggc ctg agt tcA CCG GTg aca aag agc ttc aac agg
!                                AgeI....(1/2)
```

```
!
!       136 137 138 139 140
!       G  E  C  .  .
 2674    gga gag tgt taa taa  GG CGCGCCaatt
!                          AscI.....
!                           BssHII.
!
 2701    ctatttcaag gagacagtca ta
!
! PelB::3-23(stuffed)::CH1::III fusion gene
!
!       1  2  3  4  5  6  7  8  9 10 11 12 13 14 15
!      M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L
 2723   atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc
!
!----------------------------------------
!
!     16 17 18 19 20 21 22
!     A  A  Q  P  A  M  A
 2768 gcG GCC cag ccG GCC atg gcc
!      SfiI.....
!           NgoMIV..(1/2)
!               NcoI....
!
!                          FR1(DP47/V3-23)----------
!                          23 24 25 26 27 28 29 30
!                           E  V  Q  L  L  E  S  G
 2789                        gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
!                              |MfeI |
!
!     ----------FR1-----------------------------------------
!     31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
!      G  G  L  V  Q  P  G  G  S  L  R  L  S  C  A
 2813  |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
!
```

```
!     ----FR1-----
!      46 47 48
!       A  S  G
 2858 |gct|TCC|GGA|
!       | BspEI |
!
!      Stuffer for CDR1, FR2, and CDR2---------------------------------->
!      There are no stop codons in this stuffer.
 2867                                  gcttcAGATC Tgtttgcctt
!                                       BglII..
 2887   tttgtggggt ggtgcagatc gcgttacgga gatcgaccga ctgcttgagc aaaagccacg
 2947   cttaactgcT GATCAggcat gggatgttat tcgccaaacc agtcgtcagg atcttaacct
!          BclI...
 3007   gaggcttttt ttacctactc tgcaagcagc gacatctggt ttgacacaga gcgatccgcg
 3067   tcgtcagttg gtagaaacat taacacgttg ggatggcatc aatttgctta atgatgatgg
 3127   taaaacctgg cagcagccag gctctgccat cctgaacgtt tggctgacca gtatgttgaa
 3187   gcgtaccgta gtggctgccg tacctatgCC Atttgataag TGGtacagcg ccagtggcta
!                      XcmI.....
 3247   cgaaacaacc caggacggcc caactggttc gctgaatata agtgttggag caaaaatttt
 3307   gtatgaggcg gtgcagggag acaaatcacc aatcccacag gcggttgatc tgtttgctgg
 3367   gaaaccacag caggaggttg tgttggctgc gctggaagat acctgggaga ctctttccaa
 3427   acgctatggc aataatgtga gtaactggaa aacacctgca atggccttaa cgttccgggc
 3487   aaataatttc tttggtgtac cgcaggccgc agcggaagaa ACGCGTcatc aggcggagta
!                                  MluI..
 3547   tcaaaaccgt ggaacagaaa acgatatgat tgttttctca ccaacgacaa gcgatcgtcc
 3607   tgtgcttgcc tgggatgtgg tcgcacccgg tcagagtggg tttattgctc ccgatggaac
 3667   agttgataag cactatgaag atcagctgaa aatgtacgaa aattttggcc gtaagtcgct
!                     PvuII.
 3727   ctgGTTAACg aagcaggatg tggaggcgca taaggagtcg
!         HpaI..
!         HincII(2/2)
!
!     --------FR3-----------------------------------------------------
!           4  5  6  7  8  9  10 11 12 13 14 15 16
!           93 94 95 96 97 98 99 100 101 102 103 104 105
!           S  R  D  N  S  K  N  T  L  Y  L  Q  M
 3767       |TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
```

```
!          | XbaI |
!
!    ---FR3---------------------------------------------------->|
!      17 18 19 20
!     106 107 108 109
!     N  S  L  s  l  s  i  r  s  g
 3806 |aac|agC|TTA|AG t ctg agc att CGG TCC G
!         |AflII |            RsrII..
!
!         q  h  s  p  t  .
 3834    gg caa cat tct cca aac tga   ccagacga cacaaacggc
 3872    ttacgctaaa tcccgcgcat gggatggtaa agaggtggcg tctttgctgg cctggactca
 3932    tcagatgaag gccaaaaatt ggcaggagtg gacacagcag gcagcgaaac aagcactgac
 3992    catcaactgg tactatgctg atgtaaacgg caatattggt tatgttcata ctggtgctta
 4052    tccagatcgt caatcaggcc atgatccgcg attacccgtt cctggtacgg gaaaatggga
 4112    ctggaaaggg ctattgcctt ttgaaatgaa ccctaaggtg tataaccccc ag
 4164        aa GCTAGC ctgcggcttc
!            NheI..
!
 4182    G|GTC|ACC|                          gtc tca agc
!      | BstEII |
!

!       136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
!       A  S  T  K  G  P  S  V  F  P  L  A  P  S  S
 4198    gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc
!
!       151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!       K  S  T  S  G  G  T  A  A  L  G  C  L  V  K
 4243    aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag
!
!       166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!       D  Y  F  P  E  P  V  T  V  S  W  N  S  G  A
 4288    gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc
!
```

```
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!      L  T  S  G  V  H  T  F  P  A  V  L  Q  S  S
 4333   ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tcc tca
!
!      196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!      G  L  Y  S  L  S  S  V  V  T  V  P  S  S  S
 4378   gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc
!
!      211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!      L  G  T  Q  T  Y  I  C  N  V  N  H  K  P  S
 4423   ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc
!
!      226 227 228 229 230 231 232 233 234 235 236 237 238
!      N  T  K  V  D  K  K  V  E  P  K  S  C
 4468   aac acc aag gtg gac aaG AAA GTT GAG CCC AAA TCT TGT
!                    ON-TQHCforw.....
!
!                    Poly His linker
!             139 140 141 142 143 144 145 146 147 148 149 150
!              A   A   A   H   H   H   H   H   H   G   A   A
 4507           GCG GCC GCa cat cat cat cac cat cac ggg gcc gca
!             NotI.....
!             EagI....
!
!     151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
!     E  Q  K  L  I  S  E  E  D  L  N  G  A  A  .
 4543  gaa caa aaa ctc atc tca gaa gag gat ctg aat ggg gcc gca tag
!
!     Mature III---------------------------------------------->...
!     166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
!     T  V  E  S  C  L  A  K  P  H  T  E  N  S  F
 4588  act gtt gaa agt tgt tta gca aaa cct cat aca gaa aat tca ttt
!
!     181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!     T  N  V  W  K  D  D  K  T  L  D  R  Y  A  N
 4633  act aac gtc tgg aaa gac gac aaa act tta gat cgt tac gct aac
!
```

```
!     196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!     Y  E  G  C  L  W  N  A  T  G  V  V  V  C  T
 4678  tat gag ggc tgt ctg tgG AAT GCt aca ggc gtt gtg gtt tgt act
!                  BsmI....
!
!     211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!     G  D  E  T  Q  C  Y  G  T  W  V  P  I  G  L
 4723  ggt gac gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt
!
!     226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!     A  I  P  E  N  E  G  G  G  S  E  G  G  G  S
 4768  gct atc cct gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!     241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!     E  G  G  G  S  E  G  G  G  T  K  P  P  E  Y
 4813  gag ggt ggc ggt tct gag ggt ggc ggt act aaa cct cct gag tac
!
!     256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!     G  D  T  P  I  P  G  Y  T  Y  I  N  P  L  D
 4858  ggt gat aca cct att ccg ggc tat act tat atc aac cct ctc gac
!
!     271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!     G  T  Y  P  P  G  T  E  Q  N  P  A  N  P  N
 4903  ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct aat
!
!     286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!     P  S  L  E  E  S  Q  P  L  N  T  F  M  F  Q
 4948  cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag
!           BseRI..(2/2)
!
!     301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!     N  N  R  F  R  N  R  Q  G  A  L  T  V  Y  T
 4993  aat aat agg ttc cga aat agg cag ggt gca tta act gtt tat acg
!
!     316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!     G  T  V  T  Q  G  T  D  P  V  K  T  Y  Y  Q
 5038  ggc act gtt act caa ggc act gac ccc gtt aaa act tat tac cag
```

```
!
!     331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!      Y  T  P  V  S  S  K  A  M  Y  D  A  Y  W  N
 5083  tac act cct gta tca tca aaa gcc atg tat gac gct tac tgg aac
!
!     346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!      G  K  F  R  D  C  A  F  H  S  G  F  N  E  D
 5128  ggt aaa ttc aga gac tgc gct ttc cat tct ggc ttt aat gaG GAT
!                                          BamHI..
!
!     361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!      P  F  V  C  E  Y  Q  G  Q  S  S  D  L  P  Q
 5173  CCa ttc gtt tgt gaa tat caa ggc caa tcg tct gAC CTG Cct caa
! BamHI...                                 BspMI...(2/2)
!
!     376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!      P  P  V  N  A  G  G  G  S  G  G  G  S  G  G
 5218  cct cct gtc aat gct ggc ggc ggc tct ggt ggt ggt tct ggt ggc
!
!     391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!      G  S  E  G  G  G  S  E  G  G  G  S  E  G  G
 5263  ggc tct gag ggt ggc ggc tct gag ggt ggc ggt tct gag ggt ggc
!
!     406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!      G  S  E  G  G  G  S  G  G  G  S  G  S  G  D
 5308  ggc tct gag ggt ggc ggt tcc ggt ggc ggc tcc ggt tcc ggt gat
!
!     421 422 423 424 425 426 427 428 429 430 431 432 433 434 435
!      F  D  Y  E  K  M  A  N  A  N  K  G  A  M  T
 5353  ttt gat tat gaa aaa atg gca aac gct aat aag ggg gct atg acc
!
!     436 437 438 439 440 441 442 443 444 445 446 447 448 449 450
!      E  N  A  D  E  N  A  L  Q  S  D  A  K  G  K
 5398  gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc aaa
!
```

```
!     451 452 453 454 455 456 457 458 459 460 461 462 463 464 465
!     L  D  S  V  A  T  D  Y  G  A  A  I  D  G  F
 5443  ctt gat tct gtc gct act gat tac ggt gct gct ATC GAT ggt ttc
!                                    BspDI..
!
!     466 467 468 469 470 471 472 473 474 475 476 477 478 479 480
!     I  G  D  V  S  G  L  A  N  G  N  G  A  T  G
 5488  att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt
!
!     481 482 483 484 485 486 487 488 489 490 491 492 493 494 495
!     D  F  A  G  S  N  S  Q  M  A  Q  V  G  D  G
 5533  gat ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt
!
!     496 497 498 499 500 501 502 503 504 505 506 507 508 509 510
!     D  N  S  P  L  M  N  N  F  R  Q  Y  L  P  S
 5578  gat aat tca cct tta atg aat aat ttc cgt caa tat tta cct tct
!
!     511 512 513 514 515 516 517 518 519 520 521 522 523 524 525
!     L  P  Q  S  V  E  C  R  P  Y  V  F  G  A  G
 5623  ttg cct cag tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct ggt
!
!     526 527 528 529 530 531 532 533 534 535 536 537 538 539 540
!     K  P  Y  E  F  S  I  D  C  D  K  I  N  L  F
 5668  aaa cCA TAT Gaa ttt tct att gat tgt gac aaa ata aac tta ttc
!        NdeI....
!
!     541 542 543 544 545 546 547 548 549 550 551 552 553 554 555
!     R  G  V  F  A  F  L  L  Y  V  A  T  F  M  Y
 5713  cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat
!
!     556 557 558 559 560 561 562 563 564 565 566 567 568 569 570
!     V  F  S  T  F  A  N  I  L  R  N  K  E  S  .
 5758  gta ttt tcg acg ttt gct aac ata ctg cgt aat aag gag tct taa
!

!    571
!     .
 5803  taa GAATTC
!       EcoRI.
 5812    actggccgt cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc
 5871    gccttgcagc acatcccccc tttcgccagct ggcgtaatag cgaagaggcc cgcacCGATC
!                                                    PvuI..
 5931    Gcccttccca acagtTGCGC Agcctgaatg gcgaatGGCG CCtgatgcgg tattttctcc
! ...PvuI... (3/3)       FspI... (2/2)       KasI...(2/2)
 5991    ttacgcatct gtgcggtatt tcacaccgca tataaattgt aaacgttaat attttgttaa
 6051    aattcgcgtt aaattttgt taaatcagct catttttaa ccaataggcc gaaatcggca
 6111    aaatcccTTA TAAatcaaaa gaatagcccg agatagggtt gagtgttgtt ccagtttgga
!          PsiI...
 6171    acaagagtcc actattaaag aacgtggact ccaacgtcaa agggcgaaaa accgtctatc
 6231    agggcgatgg ccCACtacGT Gaaccatcac ccaaatcaag ttttttgggg tcgaggtgcc
!              DraIII....
 6291 gtaaagcact aaatcggaac cctaaaggga gcccccgatt tagagcttga cggggaaaGC
!                                                  NgoMIV..
 6351    CGGCgaacgt ggcgagaaag gaagggaaga aagcgaaagg agcgggcgct agggcgctgg
!   ..NgoMIV.(2/2)
 6411    caagtgtagc ggtcacgctg cgcgtaacca ccacacccgc cgcgcttaat gcgccgctac
 6471    agggcgcgta ctatggttgc tttgacgggt gcagtctcag tacaatctgc tctgatgccg
 6531    catagttaag ccagccccga cacccgccaa cacccgctga cgcgccctga cgggcttgtc
 6591    tgctcccggc atccgcttac agacaagctg tgaccgtctc cgggagctgc atgtgtcaga
 6651    ggttttcacc gtcatcaccg aaacgcgcga
```

Table 30: Oligonucleotides used to clone CDR1/2 diversity

All sequences are 5' to 3'.

1) ON_CD1Bsp, 30 bases

A c c T c A c T g g c T T c c g g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T T c A c T T T c T c T
19 20 21 22 23 24 25 26 27 28 29 30

2) ON_Br12, 42 bases

A g A A A c c c A c T c c A A A c c
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

T T T A c c A g g A g c T T g g c g
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

A A c c c A
37 38 39 40 41 42

3) ON_CD2Xba. 51 bases

g g A A g g c A g T g A T c T A g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

g A T A g T g A A g c g A c c T T T
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

A A c g g A g T c A g c A T A
37 38 39 40 41 42 43 44 45 46 47 48 49 50 51

4) ON_BotXba, 23 bases

g g A A g g c A g T g A T c T A g A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

g A T A g
19 20 21 22 23

Table 31: Bridge/Extender Oligonucleotides

ON_Lam1aB7(rc)	.....GTGCTGACTCAGCCACCCTC.	20
ON_Lam2aB7(rc)	.....GCCCTGACTCAGCCTGCCTC.	20
ON_Lam31B7(rc)	.....GAGCTGACTCAGG.ACCCTGC	20
ON_Lam3rB7(rc)	.....GAGCTGACTCAGCCACCCTC.	20
ON_LamHf1cBrg(rc)	CCTCGACAGCGAAGTGCACAGAGCGTCTTGACTCAGCC.....	38
ON_LamHf1cExt	CCTCGACAGCGAAGTGCACAGAGCGTCTTG.....	30
ON_LamHf2b2Brg (rc)	CCTCGACAGCGAAGTGCACAGAGCGCTTTGACTCAGCC.....	38
ON_LamHf2b2Ext	CCTCGACAGCGAAGTGCACAGAGCGCTTTG.....	30
ON_LamHf2dBrg(rc)	CCTCGACAGCTAAGTGCACAGAGCGCTTTGACTCAGCC.....	38
ON_LamHf2dExt	CCTCGACAGCGAAGTGCACAGAGCGCTTTG.....	30
ON_LamHf31Brg(rc)	CCTCGACAGCGAAGTGCACAGAGCGAATTGACTCAGCC.....	38
ON_LamHf31Ext	CCTCGACAGCGAAGTGCACAGAGCGAATTG.....	30
ON_LamHf3rBrg(rc)	CCTCGACAGCGAAGTGCACAGTACGAATTGACTCAGCC.....	38
ON_LamHf3rExt	CCTCGACAGCGAAGTGCACAGTACGAATTG.....	30
ON_lamPlePCR Consensus	CCTCGACAGCGAAGTGCACAG.....	21

Table 32: Oligonucleotides used to make SSDNA locally double-stranded

Adapters (8)	
H43HF3.1?02#1	5'-cc gtg tat tac tgt gcg aga g-3'
H43.77.97.1-03#2	5'-ct gtg tat tac tgt gcg aga g-3'
H43.77.97.323#22	5'-cc gta tat tac tgt gcg aaa g-3'
H43.77.97.330#23	5'-ct gtg tat tac tgt gcg aaa g-3'
H43.77.97.439#44	5'-ct gtg tat tac tgt gcg aga gcg-3'
H43.77.97.551#48	5'-cc atg tat tac tgt gcg aga gcg-3'

Table 33: Bridge/extender pairs

Bridges (2)
H43.XABrl
5'ggtgtagtgaTCTAGtgacaactctaagaatactctctacttgcagatgaacagCTTtAGgg ctgaggacaCTGCAGtctactattgtgcgaga-3'
H43.XABr2
5'ggtgtagtgaTCTAGtgacaactctaagaatactctctacttgcagatgaacagCTTtAGgg ctgaggacaCTGCAGtctactattgtgcgaaa-3'
Extender
H43.XAExt
5'ATAgTAgAcTgcAgTgTccTcAgcccTTAAgcTgTTcATcTgcAAgTAgAgAgTATTcTTAg AgTTgTcTcTAgATcAcTAcAcc-3'

Table 34: PCR primers

Primers	
H43.XAPCR2	gactgggTgTAgTgATcTAg
Hucmnest	cttttctttgttgccgttggggtg

Table 35: PCR program for amplification of heavy chain CDR3 DNA

95 degrees C	5 minutes	
95 degrees C	20 seconds	
60 degrees C	30 seconds	repeat 20x
72 degrees C	1 minute	
72 degrees C	7 minutes	
4 degrees C	hold	

Reagents (100 ul reaction):

Template	5ul ligation mix
10x PCR buffer	1x
Taq	5U
dNTPs	200 uM each
MgCl2	2mM
H43.XAPCR2-biotin	400 nM
Hucmnest	200 nM

```
! Table 36: Annotated sequence of CJR DY3F7(CJR-A05)  10251 bases
!
! Non-cutters
!
!BclI Tgatca          BsiWI Cgtacg         BssSI Cacgag
!BstZ17I GTAtac       BtrI CACgtg          EcoRV GATatc
!FseI GGCCGGcc        HpaI GTTaac          MluI Acgcgt
!PmeI GTTTaaac        PmlI CACgtg          PpuMI RGgwccy
!RsrII CGgwccg        SapI GCTCTTC         SexAI Accwggt
!SgfI GCGATcgc        SgrAI CRccggyg       SphI GCATGc
!StuI AGGcct          XmaI Cccggg
!
! cutters
!
! Enzymes that cut from 1 to 4 times and other features
!
!End of genes II and X                829
!Start gene V                         843
!BsrGI Tgtaca                  1     1021
!BspMI Nnnnnnnnngcaggt          3     1104  5997  9183
!-"-   ACCTGCNNNNn              1     2281
!End of gene V                        1106
!Start gene VII                       1108
!BsaBI GATNNnnatc               2     1149  3967
!Start gene IX                        1208
!End gene VII                         1211
!SnaBI TACgta                  2     1268  7133
!BspHI Tcatga                  3     1299  6085  7093
!Start gene VIII                      1301
!End gene IX                          1304
!End gene VIII                        1522
!Start gene III                       1578
!EagI Cggccg                   2     1630  8905
!XbaI Tctaga                   2     1643  8436
!KasI Ggcgcc                   4     1650  8724  9039  9120
!BsmI GAATGCN                  2     1769  9065
!BseRI GAGGAGNNNNNNNNNN        2     2031  8516
!-"-   NNnnnnnnnnctcctc        2     7603  8623
!AlwNI CAGNNNctg               3     2210  8072  8182
!BspDI ATcgat                  2     2520  9883
!NdeI CAtatg                   3     2716  3796  9847
!End gene III                         2846
!Start gene VI                        2848
!AfeI AGCgct                   1     3032
!End gene VI                          3187
!Start gene I                         3189
!EarI CTCTTCNnnn               2     4067  9274
!-"-  Nnnnngaagag              2     6126  8953
!PacI TTAATtaa                 1     4125
!Start gene IV                        4213
!End gene I                           4235
!BsmFI Nnnnnnnnnnnnnnngtccc    2     5068  9515
!MscI TGGcca                   3     5073  7597  9160
!PsiI TTAtaa                   2     5349  5837
!End gene IV                          5493
!Start ori                            5494
!NgoMIV Gccggc                 3     5606  8213  9315
!BanII GRGCYc                  4     5636  8080  8606  8889
!DraIII CACNNNgtg              1     5709
!DrdI GACNNNNnngtc             1     5752
!AvaI Cycgrg                   2     5818  7240
```

!PvuII CAGctg	1	5953		
!BsmBI CGTCTCNnnnn	3	5964	8585	9271
!End ori region		5993		
!BamHI Ggatcc	1	5994		
!HindIII Aagctt	3	6000	7147	7384
!BciVI GTATCCNNNNNN	1	6077		
!Start bla		6138		
!Eco57I CTGAAG	2	6238	7716	
!SpeI Actagt	1	6257		
!BcgI gcannnnnntcg	1	6398		
!ScaI AGTact	1	6442		
!PvuI CGATcg	1	6553		
!FspI TGCgca	1	6700		
!BglI GCCNNNNnggc	3	6801	8208	8976
!BsaI GGTCTCNnnnn	1	6853		
!AhdI GACNNNnngtc	1	6920		
!Eam1105I GACNNNnngtc	1	6920		
!End bla		6998		
!AccI GTmkac	2	7153	8048	
!HincII GTYrac	1	7153		
!SalI Gtcgac	1	7153		
!XhoI Ctcgag	1	7240		
!Start PlacZ region		7246		
!End PlacZ region		7381		
!PflMI CCANNNNntgg	1	7382		
!RBS1		7405		
!start M13-iii signal seq for LC		7418		
!ApaLI Gtgcac	1	7470		
!end M13-iii signal seq		7471		
!Start light chain kappa L20:JK1		7472		
!PflFI GACNnngtc	3	7489	8705	9099
!SbfI CCTGCAgg	1	7542		
!PstI CTGCAg	1	7543		
!KpnI GGTACc	1	7581		
!XcmI CCANNNNNnnnntgg	2	7585	9215	
!NsiI ATGCAt	2	7626	9503	
!BsgI ctgcac	1	7809		
!BbsI gtcttc	2	7820	8616	
!BlpI GCtnagc	1	8017		
!EspI GCtnagc	1	8017		
!EcoO109I RGgnccy	2	8073	8605	
!Ecl136I GAGctc	1	8080		
!SacI GAGCTc	1	8080		
!End light chain		8122		
!AscI GGcgcgcc	1	8126		
!BssHII Gcgcgc	1	8127		
!RBS2		8147		
!SfiI GGCCNNNNnggcc	1	8207		
!NcoI Ccatgg	1	8218		
!Start 3-23, FR1		8226		
!MfeI Caattg	1	8232		
!BspEI Tccgga	1	8298		
!Start CDR1		8316		
!Statt FR2		8331		
!BstXI CCANNNNNntgg	2	8339	8812	
!EcoNI CCTNNnnnnagg	2	8346	8675	
!Start FR3		8373		
!XbaI Tctaga	2	8436	1643	
!AflII Cttaag	1	8480		
!Start CDR3		8520		
!AatII GACGTc	1	8556		

```
!Start FR4                          8562
!PshAI GACNNnngtc              2    8573  9231
!BstEII Ggtnacc                1    8579
!Start CH1                          8595
!ApaI GGGCCc                   1    8606
!Bsp120I Gggccc                1    8606
!PspOMI Gggccc                 1    8606
!AgeI Accggt                   1    8699
!Bsu36I CCtnagg                2    8770  9509
!End of CH1                         8903
!NotI GCggccgc                 1    8904
!Start His6 tag                     8913
!Start cMyc tag                     8931
!Amber codon                        8982
!NheI Gctagc                   1    8985
!Start M13 III Domain 3             8997
!NruI TCGcga                   1    9106
!BstBI TTcgaa                  1    9197
!EcoRI Gaattc                  1    9200
!XcmI CCANNNNNnnnntgg          1    9215
!BstAPI GCANNNNntgc            1    9337
!SacII CCGCgg                  1    9365
!End IIIstump anchor                9455
!AvrII Cctagg                  1    9462
!trp terminator                     9470
!SwaI ATTTaaat                 1    9784
!Start gene II                      9850
!BglII Agatct                  1    9936
!-------------------------------------------------------------------------
     1 aat gct act act att agt aga att gat gcc acc ttt tca gct cgc gcc
!    gene ii continued
    49 cca aat gaa aat ata gct aaa cag gtt att gac cat ttg cga aat gta
    97 tct aat ggt caa act aaa tct act cgt tcg cag aat tgg gaa tca act
   145 gtt aTa tgg aat gaa act tcc aga cac cgt act tta gtt gca tat tta
   193 aaa cat gtt gag cta cag caT TaT att cag caa tta agc tct aag cca
   241 tcc gca aaa atg acc tct tat caa aag gag caa tta aag gta ctc tct
   289 aat cct gac ctg ttg gag ttt gct tcc ggt ctg gtt cgc ttt gaa gct
   337 cga att aaa acg cga tat ttg aag tct ttc ggg ctt cct ctt aat ctt
   385 ttt gat gca atc cgc ttt gct tct gac tat aat agt cag ggt aaa gac
   433 ctg att ttt gat tta tgg tca ttc tcg ttt tct gaa ctg ttt aaa gca
   481 ttt gag ggg gat tca ATG aat att tat gac gat tcc gca gta ttg gac
!                        Start gene x, ii continues
   529 gct atc cag tct aaa cat ttt act att acc ccc tct ggc aaa act tct
   577 ttt gca aaa gcc tct cgc tat ttt ggt ttt tat cgt cgt ctg gta aac
   625 gag ggt tat gat agt gtt gct ctt act atg cct cgt aat tcc ttt tgg
   673 cgt tat gta tct gca tta gtt gaa tgt ggt att cct aaa tct caa ctg
   721 atg aat ctt tct acc tgt aat aat gtt gtt ccg tta gtt cgt ttt att
   769 aac gta gat ttt tct tcc caa cgt cct gac tgg tat aat gag cca gtt
   817 ctt aaa atc gca TAA
!                    End X & II
   832 ggtaattca ca
!
!       M1              E5                  Q10                 T15
   843 ATG att aaa gtt gaa att aaa cca tct caa gcc caa ttt act act cgt
!      Start gene V
!
!      S17         S20                 P25                 E30
   891 tct ggt gtt tct cgt cag ggc aag cct tat tca ctg aat gag cag ctt
!
!              V35                 E40                 V45
   939 tgt tac gtt gat ttg ggt aat gaa tat ccg gtt ctt gtc aag att act
```

```
!
!          D50                  A55                  L60
!   987 ctt gat gaa ggt cag cca gcc tat gcg cct ggt cTG TAC Acc gtt cat
!                                                    BsrGI...
!       L65                  V70                  S75                  R80
!  1035 ctg tcc tct ttc aaa gtt ggt cag ttc ggt tcc ctt atg att gac cgt
!
!                       P85     K87 end of V
!  1083 ctg cgc ctc gtt ccg gct aag TAA C
!
!  1108 ATG gag cag gtc gcg gat ttc gac aca att tat cag gcg atg
!       Start gene VII
!
!  1150 ata caa atc tcc gtt gta ctt tgt ttc gcg ctt ggt ata atc
!
!                         VII and IX overlap.
!                         ..... S2  V3  L4  V5                 S10
!  1192 gct ggg ggt caa agA TGA gt gtt tta gtg tat tct ttT gcc tct ttc gtt
!                            End VII
!                         |start IX
!       L13     W15                  G20                  T25             E29
!  1242 tta ggt tgg tgc ctt cgt agt ggc att acg tat ttt acc cgt tta atg gaa
!
!  1293 act tcc tc
!
!        .... stop of IX, IX and VIII overlap by four bases
!  1301 ATG aaa aag tct tta gtc ctc aaa gcc tct gta gcc gtt gct acc ctc
!       Start signal sequence of viii.
!
!  1349 gtt ccg atg ctg tct ttc gct gct gag ggt gac gat ccc gca aaa gcg
!                                  mature VIII --->
!  1397 gcc ttt aac tcc ctg caa gcc tca gcg acc gaa tat atc ggt tat gcg
!  1445 tgg gcg atg gtt gtt gtc att
!  1466 gtc ggc gca act atc ggt atc aag ctg ttt aag
!
! bases 1499-1539 are probable promoter for iii
!  1499 aaa ttc acc tcg aaa gca ! 1515
!       ..........  -35  ..
!
!  1517      agc tga taaaccgat acaattaaag gctccttttg
!                       ..... -10   ...
!
!  1552 gagccttttt ttt GGAGAt ttt ! S.D. uppercase, there may be 9 Ts
!
!           <------ III signal sequence ----------------------------->
!            M   K   K   L   L   F   A   I   P   L   V   V   P   F
!  1574 caac GTG aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc ! 1620
!
!        Y   S   G   A   A   E   S   H   L   D   G   A
!  1620 tat tct ggc gCG GCC Gaa tca caT CTA GAc ggc gcc
!                  EagI....         XbaI....
!
! Domain 1 ------------------------------------------------------------
!           A   E   T   V   E   S   C   L   A
!  1656     gct gaa act gtt gaa agt tgt tta gca
!
!        K   S   H   T   E   I   S   F   T   N   V   W   K   D   D   K   T
!  1683 aaA Tcc cat aca gaa aat tca ttt aCT AAC GTC TGG AAA GAC GAC AAA ACt
!
!        L   D   R   Y   A   N   Y   E   G   S   L   W   N   A   T   G   V
!  1734 tta gat cgt tac gct aac tat gag ggC tgt ctg tgG AAT GCt aca ggc gtt
```

```
!                                                        BsmI....
!
!       V   V   C   T   G   D   E   T   Q   C   Y   G   T   W   V   P   I
  1785 gta gtt tgt act ggt GAC GAA ACT CAG TGT TAC GGT ACA TGG GTT cct att
!
!       G   L   A   I   P   E   N
  1836 ggg ctt gct atc cct gaa aat
!
! L1 linker ------------------------------------
!       E   G   G   G   S   E   G   G   G   S
  1857 gag ggt ggt ggc tct gag ggt ggc ggt tct
!
!       E   G   G   G   S   E   G   G   G   T
  1887 gag ggt ggc ggt tct gag ggt ggc ggt act
!
!  Domain 2 -----------------------------------
  1917 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac
  1968 cct ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct
  2019 aat cct tct ctt GAG GAG tct cag cct ctt aat act ttc atg ttt cag aat
!                     BseRI..
  2070 aat agg ttc cga aat agg cag ggg gca tta act gtt tat acg ggc act
  2118 gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act cct
  2166 gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttC AGA
!                                                          AlwNI
  2214 GAC TGc gct ttc cat tct ggc ttt aat gaG gat TTa ttT gtt tgt gaa
!       AlwNI
  2262 tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct
!
  2307 ggc ggc ggc tct
!  start L2 ------------------------------------------------------------
  2319 ggt ggt ggt tct
  2331 ggt ggc ggc tct
  2343 gag ggt ggt ggc tct gag gga ggc ggt tcc
  2373 ggt ggt ggc tct ggt    ! end L2
!
! Many published sequences of M13-derived phage have a longer linker
! than shown here by repeats of the EGGGS motif two more times.
!
! Domain 3 ------------------------------------------------------------
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  2388 tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct
!
!       M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
  2436 atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc
!
!       K   L   D   S   V   A   T   D   Y   G   A   A   M   D   G   F
  2484 aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc
!
!       I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  2532 att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat
!
!       F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  2580 ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat
!
!       S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  2628 tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa
!
!       S   V   E   C   R   P   F   V   F   G   A   G   K   P   Y   E
  2676 tcg gtt gaa tgt cgc cct ttt gtc ttt Ggc gct ggt aaa cca tat gaa
!
!       F   S   I   D   C   D   K   I   N   L   F   R
```

```
2724 ttt tct att gat tgt gac aaa ata aac tta ttc cgt
                                                End Domain 3

      G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V  F140
2760 ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt
     start transmembrane segment

      S   T   F   A   N   I   L
2808 tct acg ttt gct aac ata ctg

      R   N   K   E   S
2829 cgt aat aag gag tct TAA ! stop of iii
    Intracellular anchor.

          M1  P2  V   L  L5   G   I   P   L  L10  L   R   F   L  G15
2847  tc ATG cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt
         Start VI

2894 ttc ctt ctg gta act ttg ttc ggc tat ctg ctt act ttt ctt aaa aag
2942 ggc ttc ggt aag ata gct att gct att tca ttg ttt ctt gct ctt att
2990 att ggg ctt aac tca att ctt gtg ggt tat ctc tct gat att agc gct
3038 caa tta ccc tct gac ttt gtt cag ggt gtt cag tta att ctc ccg tct
3086 aat gcg ctt ccc tgt ttt tat gtt att ctc tct gta aag gct gct att
3134 ttc att ttt gac gtt aaa caa aaa atc gtt tct tat ttg gat tgg gat

               M1  A2  V3      F5                 L10         G13
3182 aaa TAA t ATG gct gtt tat ttt gta act ggc aaa tta ggc tct gga
      end VI   Start gene I

      K   T   L   V   S   V   G   K   I   Q   D   K   I   V   A
3228 aag acg ctc gtt agc gtt ggt aag att cag gat aaa att gta gct

      G   C   K   I   A   T   N   L   D   L   R   L   Q   N   L
3273 ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa aac ctc

      P   Q   V   G   R   F   A   K   T   P   R   V   L   R   I
3318 ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga ata

      P   D   K   P   S   I   S   D   L   L   A   I   G   R   G
3363 ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt

      N   D   S   Y   D   E   N   K   N   G   L   L   V   L   D
3408 aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat

      E   C   G   T   W   F   N   T   R   S   W   N   D   K   E
3453 gag tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa

      R   Q   P   I   I   D   W   F   L   H   A   R   K   L   G
3498 aga cag ccg att att gat tgg ttt cta cat gct cgt aaa tta gga

      W   D   I   I   F   L   V   Q   D   L   S   I   V   D   K
3543 tgg gat att att ttt ctt gtt cag gac tta tct att gtt gat aaa

      Q   A   R   S   A   L   A   E   H   V   V   Y   C   R   R
3588 cag gcg cgt tct gca tta gct gaa cat gtt gtt tat tgt cgt cgt

      L   D   R   I   T   L   P   F   V   G   T   L   Y   S   L
3633 ctg gac aga att act tta cct ttt gtc ggt act tta tat tct ctt

      I   T   G   S   K   M   P   L   P   K   L   H   V   G   V
3678 att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc gtt
```

```
!
!       V   K   Y   G   D   S   Q   L   S   P   T   V   E   R   W
  3723 gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg
!
!       L   Y   T   G   K   N   L   Y   N   A   Y   D   T   K   Q
  3768 ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag
!
!       A   F   S   S   N   Y   D   S   G   V   Y   S   Y   L   T
  3813 gct ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg
!
!       P   Y   L   S   H   G   R   Y   F   K   P   L   N   L   G
  3858 cct tat tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt
!
!       Q   K   M   K   L   T   K   I   Y   L   K   K   F   S   R
  3903 cag aag atg aaa tta act aaa ata tat ttg aaa aag ttt tct cgc
!
!       V   L   C   L   A   I   G   F   A   S   A   F   T   Y   S
  3948 gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt aca tat agt
!
!       Y   I   T   Q   P   K   P   E   V   K   K   V   V   S   Q
  3993 tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc tct cag
!
!       T   Y   D   F   D   K   F   T   I   D   S   S   Q   R   L
  4038 acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt ctt
!
!       N   L   S   Y   R   Y   V   F   K   D   S   K   G   K   L
  4083 aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa TTA
!                                                             PacI
!
!       I   N   S   D   D   L   Q   K   Q   G   Y   S   L   T   Y
  4128 ATT AAt agc gac gat tta cag aag caa ggt tat tca ctc aca tat
!     PacI
!
!      i  I   D   L   C   T   V   S   I   K   K   G   N   S   N   E
!     iv                                                        M1  K
  4173   att gat tta tgt act gtt tcc att aaa aaa ggt aat tca aAT Gaa
!                                                            Start IV
!
!     i     I   V   K   C   N   .End of I
!     iv     L3  L   N5  V   I7  N    F  V10
  4218     att gtt aaa tgt aat TAA T TTT GTT
!  IV continued.....
  4243 ttc ttg atg ttt gtt tca tca tct tct ttt gct cag gta att gaa atg
  4291 aat aat tcg cct ctg cgc gat ttt gta act tgg tat tca aag caa tca
  4339 ggc gaa tcc gtt att gtt tct ccc gat gta aaa ggt act gtt act gta
  4387 tat tca tct gac gtt aaa cct gaa aat cta cgc aat ttc ttt att tct
  4435 gtt tta cgt gcA aat aat ttt gat atg gtA ggt tcT aAC cct tcc atT
  4483 att cag aag tat aat cca aac aat cag gat tat att gat gaa ttg cca
  4531 tca tct gat aat cag gaa tat gat gat aat tcc gct cct tct ggt ggt
  4579 ttc ttt gtt ccg caa aat gat aat gtt act caa act ttt aaa att aat
  4627 aac gtt cgg gca aag gat tta ata cga gtt gtc gaa ttg ttt gta aag
  4675 tct aat act tct aaa tcc tca aat gta tta tct att gac ggc tct aat
  4723 cta tta gtt gtt agt gcT cct aaa gat att tta gat aac ctt cct caa
  4771 ttc ctt tcA act gtt gat ttg cca act gac cag ata ttg att gag ggt
  4819 ttg ata ttt gag gtt cag caa ggt gat gct tta gat ttt tca ttt gct
  4867 gct ggc tct cag cgt ggc act gtt gca ggc ggt gtt aat act gac cgc
  4915 ctc acc tct gtt tta tct tct gct ggt ggt tcg ttc ggt att ttt aat
  4963 ggc gat gtt tta ggg cta tca gtt cgc gca tta aag act aat agc cat
  5011 tca aaa ata ttg tct gtg cca cgt att ctt acg ctt tca ggt cag aag
  5059 ggt tct atc tct gtT GGC CAg aat gtc cct ttt att act ggt cgt gtg
!                        MscI....
```

```
  5107 act ggt gaa tct gcc aat gta aat aat cca ttt cag acg att gag cgt
  5155 caa aat gta ggt att tcc atg agc gtt ttt cct gtt gca atg gct ggc
  5203 ggt aat att gtt ctg gat att acc agc aag gcc gat agt ttg agt tct
  5251 tct act cag gca agt gat gtt att act aat caa aga agt att gct aca
  5299 acg gtt aat ttg cgt gat gga cag act ctt tta ctc ggt ggc ctc act
  5347 gat tat aaa aac act tct caG gat tct ggc gta ccg ttc ctg tct aaa
  5395 atc cct tta atc ggc ctc ctg ttt agc tcc cgc tct gat tcT aac gag
  5443 gaa agc acg tta tac gtg ctc gtc aaa gca acc ata gta cgc gcc ctg
  5491 TAG cggcgcatt
!      End IV
  5503 aagcgcggcg ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc
  5563 gcccgctcct ttcgctttct tcccttcctt tctcgccacg ttcGCCGGCt ttccccgtca
!                                                    NgoMI.
  5623 agctctaaat cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc
  5683 caaaaaactt gatttgggtg atggttCACG TAGTGggcca tcgccctgat agacggtttt
!                                  DraIII....
  5743 tcgccctttG ACGTTGGAGT Ccacgttctt taatagtgga ctcttgttcc aaactggaac
!              DrdI..........
  5803 aacactcaac cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga
  5863 accaccatca aacaggattt tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa
  5923 ctctctcagg gccaggcggt gaagggcaat CAGCTGttgc cCGTCTCact ggtgaaaaga
!                                     PvuII.      BsmBI.
  5983 aaaaccaccc tGGATCC  AAGCTT
!                BamHI   HindIII (1/2)
!                Insert carrying bla gene
  6006    gcaggtg gcacttttcg gggaaatgtg cgcggaaccc
  6043 ctatttgttt atttttctaa atacattcaa atatGTATCC gctcatgaga caataaccct
!                                        BciVI
  6103 gataaatgct tcaataatat tgaaaaAGGA AGAgt
!                                  RBS.?...
!      Start bla gene
  6138 ATG agt att caa cat ttc cgt gtc gcc ctt att ccc ttt ttt gcg gca ttt
  6189 tgc ctt cct gtt ttt gct cac cca gaa acg ctg gtg aaa gta aaa gat gct
  6240 gaa gat cag ttg ggC gcA CTA GTg ggt tac atc gaa ctg gat ctc aac agc
!                         SpeI....
!                      ApaLI & BssSI Removed
  6291 ggt aag atc ctt gag agt ttt cgc ccc gaa gaa cgt ttt cca atg atg agc
  6342 act ttt aaa gtt ctg cta tgt GGC GcG Gta tta tcc cgt att gac gcc ggg
  6393 caa gaG CAA CTC GGT CGc cgC ATA cAC tat tct cag aat gac ttg gtt gAG
!           BcgI.............                                        ScaI
  6444 TAC Tca cca gtc aca gaa aag cat ctt acg gat ggc atg aca gta aga gaa
!      ScaI.
  6495 tta tgc agt gct gcc ata acc atg agt gat aac act gcg gcc aac tta ctt
  6546 ctg aca aCG ATC Gga gga ccg aag gag cta acc gct ttt ttg cac aac atg
!              PvuI....
  6597 ggg gat cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc
  6648 ata cca aac gac gag cgt gac acc acg atg cct gta gca atg Gca aca acg
  6699 tTG CGC Aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa caa
!       FspI....
!
  6750 tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg cgc tcg
  6801 GCC ctt ccG GCt ggc tgg ttt att gct gat aaa tct gga gcc ggt gag cgt
!      BglI..........
  6852 gGG TCT Cgc ggt atc att gca gca ctg ggg cca gat ggt aag ccc tcc cgt
!       BsaI....
  6903 atc gta gtt atc tac acG ACg ggg aGT Cag gca act atg gat gaa cga aat
!                           AhdI...........
  6954 aga cag atc gct gag ata ggt gcc tca ctg att aag cat tgg TAA ctgt
!                                                       stop
  7003 cagaccaagt ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa
  7063 ggatctaggt gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt
```

```
  7123 cgttccactg tacgtaagac cccc
  7147 AAGCTT   GTCGAC tgaa tggcgaatgg cgctttgcct
!      HindIII  SalI..
!      (2/2)    HincII
  7183 ggtttccggc accagaagcg gtgccggaaa gctggctgga gtgcgatctt
!
! Start of Fab-display cassette, the Fab DSR-A05, selected for
! binding to a protein antigen.
!
  7233 CCTGAcG CTCGAG
!      xBsu36I XhoI..
!
! PlacZ promoter is in the following block
!
  7246                              cgcaacgc aattaatgtg agttagctca
  7274    ctcattaggc accccaggct ttacacttta tgcttccggc tcgtatgttg
  7324    tgtggaattg tgagcggata acaatttcac acaggaaaca gctatgacca
  7374    tgattacgCC AagcttTGGa gccttttttt tggagatttt caac
!               PflMI.......
!                  Hind3. (there are 3)
! Gene iii signal sequence:
!         1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!         M   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
  7418    gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
!
!         16  17  18          Start light chain (L20:JK1)
!          S   H   S   A   Q   D   I   Q   M   T   Q   S   P   A
  7463    tct cac aGT GCA Caa gac atc cag atg acc cag tct cca gcc
!                 ApaLI...
!                 Sequence supplied by extender.....
!
!               T   L   S   L
  7505          acc ctg tct ttg
!
!          S   P   G   E   R   A   T   L   S   C   R   A   S   Q   G
  7517    tct cca ggg gaa aga gcc acc ctc tcc tgc agg gcc agt cag Ggt
!
!          V   S   S   Y   L   A   W   Y   Q   Q   K   P   G   Q   A
  7562    gtt agc agc tac tta gcc tgg tac cag cag aaa cct ggc cag gct
!
!          P   R   L   L   I   Y   D   A   S   S   R   A   T   G   I
  7607    ccc agg ctc ctc atc tat gAt gca tcc aAc agg gcc act ggc atc
!
!          P   A   R   F   S   G   S   G   P   G   T   D   F   T   L
  7652    cca gCc agg ttc agt ggc agt ggg Cct ggg aca gac ttc act ctc
!
!          T   I   S   S   L   E   P   E   D   F   A   V   Y   Y   C
  7697    acc atc agc agC ctA gag cct gaa gat ttt gca gtT tat tac tgt
!
!          Q   Q   R   S   W   H   P   W   T   F   G   Q   G   T   R
  7742    cag cag CGt aAc tgg cat ccg tgg ACG TTC GGC CAA GGG ACC AAG
!
!          V   E   I   K   R   T   V   A   A   P   S   V   F   I   F
  7787    gtg gaa atc aaa cga act gtg gCT GCA Cca tct gtc ttc atc ttc
!                                      BsgI....
!
!          P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V
  7832    ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt gtg
!
!          C   L   L   N   N   F   Y   P   R   E   A   K   V   Q   W
  7877    tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg
```

```
!
!          K   V   D   N   A   L   Q   S   G   N   S   Q   E   S   V
  7922     aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc
!
!          T   E   R   D   S   K   D   S   T   Y   S   L   S   S   T
  7967     aca gag cgg gac agc aag gac agc acc tac agc ctc agc agc acc
!
!          L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A
  8012     ctg acG CTG AGC aaa gca gac tac gag aaa cac aaa gtc tac gcc
!               EspI.....
!
!          C   E   V   T   H   Q   G   L   S   S   P   V   T   K   S
  8057     tgc gaa gtc acc cat cag ggc ctG AGC TCg ccc gtc aca aag agc
!                                        SacI....
!
!          F   N   R   G   E   C   .   .
  8102     ttc aac agg gga gag tgt taa taa
!
  8126         GGCGCG CCaattctat ttcaaGGAGA cagtcata
!              AscI.....              RBS2.
!
!          PelB signal sequence------(22 codons)----->
!           1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!          M   K   Y   L   L   P   T   A   A   A   G   L   L   L   L
  8160     atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta tta ctc
!
!          ...PelB signal------------> Start VH, FR1----------------->
!           16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!           A   A   Q   P   A   M   A   E   V   Q   L   L   E   S   G
  8205     gcG GCC cag ccG GCC atg gcc gaa gtt CAA TTG tta gag tct ggt
!            SfiI.............                MfeI...
!                          NcoI....
!
!          31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
!          G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
  8250     ggc ggt ctt gtt cag cct ggt ggt tct tta cgt ctt tct tgc gct
!
!          ...FR1-------------------> CDR1--------------> FR2-------->
!           46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
!           A   S   G   F   T   F   S   T   Y   E   M   R   W   V   R
  8295     gct TCC GGA ttc act ttc tct act tac gag atg cgt tgg gtt cgC
!              BspEI..                                              BstXI...
!
!          FR2---------------------------------------> CDR2 ---------->
!          61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
!          Q   A   P   G   K   G   L   E   W   V   S   Y   I   A   P
  8340     CAa gct ccT GGt aaa ggt ttg gag tgg gtt tct tat atc gct cct
!  BstXI.............
!
!        ...CDR2-------------------------------------------> FR3---->
!          76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
!          S   G   G   D   T   A   Y   A   D   S   V   K   G   R   F
  8385     tct ggt ggc gat act gct tat gct gac tcc gtt aaa ggt cgc ttc
!
!          91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
  8430     act atc TCT AGA gac aac tct aag aat act ctc tac ttg cag atg
!                 XbaI...
!                 Supplied by extender-------------------------------
!
!          ----------------------------------------FR3------------->
```

```
!          106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
!           N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   R
  8475     aac agC TTA AGg gct gag gac act gca gtc tac tat tgt gcg agg
!             AflII...
!          from extender---------------------------------->
!
!          CDR3------------------------------------------------------> FR4-->
!          121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
!           R   L   D   G   Y   I   S   Y   Y   Y   G   M   D   V   W
  8520     agg ctc gat ggc tat att tcc tac tac tac ggt atg GAC GTC tgg
!                                                        AatII..
!
!          136 137 138 139 140 141 142 143 144 145
!           G   Q   G   T   T   V   T   V . S   S
  8565     ggc caa ggg acc acG GTC ACC gtc tca agc
!                        BstEII...
!
!           CH1 of IgG1---------->
!            A   S   T   K   G   P   S   V   F   P   L   A   P   S   S
  8595      gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc
!
!            K   S   T   S   G   G   T   A   A   L   G   C   L   V   K
  8640      aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag
!
!            D   Y   F   P   E   P   V   T   V   S   W   N   S   G   A
  8685      gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc
!
!            L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S
  8730      ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tCC TCA
!                                                              Bsu36I....
!
!            G   L   Y   S   L   S   S   V   V   T   V   P   S   S   S
  8775      GGa ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc
!  Bsu36I....
!
!            L   G   T   Q   T   Y   I   C   N   V   N   H   K   P   S
  8820      ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc
!
!            N   T   K   V   D   K   K   V   E   P   K   S   C   A   A
  8865      aac acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt GCG GCC
!                                                          NotI......
!
!            A   H   H   H   H   H   H   G   A   A   E   Q   K   L   I
  8910      GCa cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc atc
!  ..NotI....  H6 tag.................. Myc-Tag.........................
!
!            S   E   E   D   L   N   G   A   A   q   A   S   S   A
  8955      tca gaa gag gat ctg aat ggg gcc gca tag GCT AGC tct gct
!           Myc-Tag.....................          ... NheI...
!                                              Amber
!
! III'stump
!
! Domain 3 of III ------------------------------------------------------
!
!        S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  8997 agt ggc gac ttc gac tac gag aaa atg gct aat gcc aac aaa GGC GCC
!      tcc   t   t   t   t   t   a   g       a   c   t   t   g   g   t !W.T.
!                                                          KasI...(2/4)
!
!        M   T   E   N   A   D   E   N   A   L   Q   S   D   A   K   G
```

```
  9045 atG ACT GAG AAC GCT GAC GAG aat gct ttg caa agc gat gcc aag ggt
!            c   a   t   c   t   a   c   g c a   g tct   c   t   a   c !W.T.
!
!        K   L   D   S   V   A   T   D   Y   G   A   A   I   D   G   F
  9093 aag tta gac agc gTC GCG Acc gac tat GGC GCC gcc ATC GAc ggc ttt
!        a c t   t tct       t   t   t   c   t   t   t       t   t   c !W.T.
!                      NruI....          KasI...(3/4)
!
!        I   G   D   V   S   G   L   A   N   G   N   G   A   T   G   D
  9141 atc ggc gat gtc agt ggt tTG GCC Aac ggc aac gga gcc acc gga gac
!        t   t   c   t tcc   c c t   t   t   t   t   t   t   t   t   t !W.T.
!                          MscI....(3/3)
!
!        F   A   G   S   N   S   Q   M   A   Q   V   G   D   G   D   N
  9189 ttc GCA GGT tcG AAT TCt cag atg gcC CAG GTT GGA GAT GGg gac aac
!        t   t   c   t       c   a     · t   a   c   t   c   t   t   t !W.T.
!          BspMI.. (2/2)               XcmI.............
!                  EcoRI...
!
!        S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
  9237 agt ccg ctt atg aac aac ttt aga cag tac ctt ccg tct ctt ccg cag
!       tca   t t a       t   t   c c t   a   t t a   t   c   c   t   a !W.T.
!
!        S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
  9285 agt gtc gag tgc cgt cca ttc gtt ttc tct gcc ggc aag cct tac gag
!       tcg   t   a   t   c   t   t   c   t agc   t   t   a   a   t   a !W.T.
!
!        F   S   I   D   C   D   K   I   N   L   F   R
  9333 ttc aGC Atc gac TGC gat aag atc aat ctt ttC CGC
!        t tct   t   t   t   c   a   a   c t a   c   t  !W.T.
!          BstAPI........                       SacII...
!                                                 End Domain 3
!
!        G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V   F
  9369 GGc gtt ttc gct ttc ttg cta tac gtc gct act ttc atg tac gtt ttc
!        t   c   t   g   t c t t a   t   t   c   c   t       t   a   t !W.T.
!       start transmembrane segment
!
!        S   T   F   A   N   I   L     R   N   K   E   S
  9417 aGC ACT TTC GCC AAT ATT TTA   Cgc aac aaa gaa agc
!       tct   g   t   t   c   a c g     t   t   g   g tct !W.T.
!                                   Intracellular anchor.
!
!                .   .
  9453          tag tga tct CCT AGG
!                          AvrII..
!
  9468 aag ccc gcc taa tga gcg ggc ttt ttt ttt ct  ggt
!        | Trp terminator                     |
!
! End Fab cassette
!
  9503   ATGCAT CCTGAGG  ccgat actgtcgtcg tcccctcaaa ctggcagatg
!        NsiI.. Bsu36I.(3/3)
  9551 cacggttacg atgcgcccat ctacaccaac gtgacctatc ccattacggt caatccgccg
  9611 tttgttccca cggagaatcc gacgggttgt tactcgctca catttaatgt tgatgaaagc
  9671 tggctacagg aaggccagac gcgaattatt tttgatggcg ttcctattgg ttaaaaaatg
  9731 agctgattta acaaaaattt aaTgcgaatt ttaacaaaat attaacgttt acaATTTAAA
!                                                              SwaI...
  9791 Tatttgctta tacaatcttc ctgtttttgg ggcttttctg attatcaacc GGGGTAcat
  9850 ATG att gac atg cta gtt tta cga tta ccg ttc atc gat tct ctt gtt tgc
```

```
!      Start gene II
  9901 tcc aga ctc tca ggc aat gac ctg ata gcc ttt gtA GAT CTc tca aaa ata
!                                                    BglII...
  9952 gct acc ctc tcc ggc atT aat tta tca gct aga acg gtt gaa tat cat att
 10003 gat ggt gat ttg act gtc tcc ggc ctt tct cac cct ttt gaa tct tta cct
 10054 aca cat tac tca ggc att gca ttt aaa ata tat gag ggt tct aaa aat ttt
 10105 tat cct tgc gtt gaa ata aag gct tct ccc gca aaa gta tta cag ggt cat
 10156 aat gtt ttt ggt aca acc gat tta gct tta tgc tct gag gct tta ttg ctt
 10207 aat ttt gct aat tct ttg cct tgc ctg tat gat tta ttg gat gtt !
! gene II continues
!------------------------ End of Table --------------------------------
```

Table 37: DNA seq of w.t. M13 gene iii

```
       1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
      fM   K   K   L   L   F   A   I   P   L   V   V   P   F   Y
1579  gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat
      Signal sequence.....................................

      16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
      S   H   S   A   E   T   V   E   S   C   L   A   K   P   H
1624 tct cac tcc gct gaa act gtt gaa agt tgt tta gca aaa ccc cat
Signal sequence> Domain 1-------------------------------------

      31  32  33  34  35  36  37  38  39  40  41  42  43  44  45
      T   E   N   S   F   T   N   V   W   K   D   D   K   T   L
1669 aca gaa aat tca ttt act aac gtc tgg aaa gac gac aaa act tta
     Domain 1-----------------------------------------------

      46  47  48  49  50  51  52  53  54  55  56  57  58  59  60
      D   R   Y   A   N   Y   E   G   C   L   W   N   A   T   G
1714 gat cgt tac gct aac tat gag ggt tgt ctg tgG AAT GCt aca ggc
                                            BsmI....
     Domain 1-----------------------------------------------

      61  62  63  64  65  66  67  68  69  70  71  72  73  74  75
      V   V   V   C   T   G   D   E   T   Q   C   Y   G   T   W
1759 gtt gta gtt tgt act ggt gac gaa act cag tgt tac ggt aca tgg
     Domain 1-----------------------------------------------

      76  77  78  79  80  81  82  83  84  85  86  87  88  89  90
      V   P   I   G   L   A   I   P   E   N   E   G   G   G   S
1804 gtt cct att ggg ctt gct atc cct gaa aat gag ggt ggt ggc tct
     Domain 1----------------------------> Linker 1-----------

      91  92  93  94  95  96  97  98  99 100 101 102 103 104 105
      E   G   G   G   S   E   G   G   G   S   E   G   G   G   T
1849 gag ggt ggc ggt tct gag ggt ggc ggt tct gag ggt ggc ggt act
     Linker 1----------------------------------------------->

     106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
      K   P   P   E   Y   G   D   T   P   I   P   G   Y   T   Y
1894 aaa cct cct gag tac ggt gat aca cct att ccg ggc tat act tat
     Domain 2-----------------------------------------------

     121 122 123 124 125 126 127 128 129 130 131 132 133 134 135
      I   N   P   L   D   G   T   Y   P   P   G   T   E   Q   N
1939 atc aac cct ctc gac ggc act taT CCG CCt ggt act gag caa aac
                                   EciI....
     Domain 2-----------------------------------------------

     136 137 138 139 140 141 142 143 144 145 146 147 148 149 150
      P   A   N   P   N   P   S   L   E   E   S   Q   P   L   N
1984 ccc gct aat cct aat cct tct ctt GAG GAG tct cag cct ctt aat
                                     BseRI..
     Domain 2-----------------------------------------------

     151 152 153 154 155 156 157 158 159 160 161 162 163 164 165
      T   F   M   F   Q   N   N   R   F   R   N   R   Q   G   A
2029 act ttc atg ttt cag aat aat agg ttc cga aat agg cag ggg gca
     Domain 2-----------------------------------------------

     166 167 168 169 170 171 172 173 174 175 176 177 178 179 180
```

```
!       L   T   V   Y   T   G   T   V   T   Q   G   T   D   P   V
  2074 tta act gtt tat acg ggc act gtt act caa ggc act gac ccc gtt
!      Domain 2-------------------------------------------------
!
!      181 182 183 184 185 186 187 188 189 190 191 192 193 194 195
!       K   T   Y   Y   Q   Y   T   P   V   S   S   K   A   M   Y
  2119 aaa act tat tac cag tac act cct gta tca tca aaa gcc atg tat
!      Domain 2-------------------------------------------------
!
!      196 197 198 199 200 201 202 203 204 205 206 207 208 209 210
!       D   A   Y   W   N   G   K   F   R   D   C   A   F   H   S
  2164 gac gct tac tgg aac ggt aaa ttC AGa gaC TGc gct ttc cat tct
!                                    AlwNI.......
!      Domain 2-------------------------------------------------
!
!      211 212 213 214 215 216 217 218 219 220 221 222 223 224 225
!       G   F   N   E   D   P   F   V   C   E   Y   Q   G   Q   S
  2209 ggc ttt aat gaG GAT CCa ttc gtt tgt gaa tat caa ggc caa tcg
!                  BamHI...
!      Domain 2-------------------------------------------------
!
!      226 227 228 229 230 231 232 233 234 235 236 237 238 239 240
!       S   D   L   P   Q   P   P   V   N   A   G   G   G   S   G
  2254 tct gac ctg cct caa cct cct gtc aat gct ggc ggc ggc tct ggt
!      Domain 2------------------------------> Linker 2-----------
!
!      241 242 243 244 245 246 247 248 249 250 251 252 253 254 255
!       G   G   S   G   G   G   S   E   G   G   G   S   E   G   G
  2299 ggt ggt tct ggt ggc ggc tct gag ggt ggt ggc tct gag ggt ggc
!      Linker 2-------------------------------------------------
!
!      256 257 258 259 260 261 262 263 264 265 266 267 268 269 270
!       G   S   E   G   G   G   S   E   G   G   G   S   G   G   G
  2344 ggt tct gag ggt ggc ggc tct gag gga ggc ggt tcc ggt ggt ggc
!      Linker 2-------------------------------------------------
!
!      271 272 273 274 275 276 277 278 279 280 281 282 283 284 285
!       S   G   S   G   D   F   D   Y   E   K   M   A   N   A   N
  2389 tct ggt tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat
!Linker 2>     Domain 3-------------------------------------------
!
!      286 287 288 289 290 291 292 293 294 295 296 297 298 299 300
!       K   G   A   M   T   E   N   A   D   E   N   A   L   Q   S
  2434 aag ggg gct atg acc gaa aat gcc gat gaa aac gcg cta cag tct
!      Domain 3-------------------------------------------------
!
!      301 302 303 304 305 306 307 308 309 310 311 312 313 314 315
!       D   A   K   G   K   L   D   S   V   A   T   D   Y   G   A
  2479 gac gct aaa ggc aaa ctt gat tct gtc gct act gat tac ggt gct
!      Domain 3-------------------------------------------------
!
!      316 317 318 319 320 321 322 323 324 325 326 327 328 329 330
!       A   I   D   G   F   I   G   D   V   S   G   L   A   N   G
  2524 gct atc gat ggt ttc att ggt gac gtt tcc ggc ctt gct aat ggt
!      Domain 3-------------------------------------------------
!
!      331 332 333 334 335 336 337 338 339 340 341 342 343 344 345
!       N   G   A   T   G   D   F   A   G   S   N   S   Q   M   A
  2569 aat ggt gct act ggt gat ttt gct ggc tct aat tcc caa atg gct
!      Domain 3-------------------------------------------------
!
```

```
!      346 347 348 349 350 351 352 353 354 355 356 357 358 359 360
!       Q   V   G   D   G   D   N   S   P   L   M   N   N   F   R
  2614 caa gtc ggt gac ggt gat aat tca cct tta atg aat aat ttc cgt
!      Domain 3-----------------------------------------------
!
!      361 362 363 364 365 366 367 368 369 370 371 372 373 374 375
!       Q   Y   L   P   S   L   P   Q   S   V   E   C   R   P   F
  2659 caa tat tta cct tcc ctc cct caa tcg gtt gaa tgt cgc cct ttt
!      Domain 3-----------------------------------------------
!
!      376 377 378 379 380 381 382 383 384 385 386 387 388 389 390
!       V   F   S   A   G   K   P   Y   E   F   S   I   D   C   D
  2704 gtc ttt agc gct ggt aaa cca tat gaa ttt tct att gat tgt gac
!      Domain 3-----------------------------------------------
!
!      391 392 393 394 395 396 397 398 399 400 401 402 403 404 405
!       K   I   N   L   F   R   G   V   F   A   F   L   L   Y   V
  2749 aaa ata aac tta ttc cgt ggt gtc ttt gcg ttt ctt tta tat gtt
!      Domain 3-------------> Transmembrane segment-------------
!
!      406 407 408 409 410 411 412 413 414 415 416 417 418 419 420
!       A   T   F   M   Y   V   F   S   T   F   A   N   I   L   R
  2794 gcc acc ttt atg tat gta ttt tct acg ttt gct aac ata ctg cgt
!      Transmembrane segment---------------------------------> ICA--
!
!      421 422 423 424 425
!       N   K   E   S   .
  2839 aat aag gag tct taa ! 2853
!      ICA----------->           ICA = intracellular anchor
!
!------------------ End of Table ----------------------------------------
```

Table 38: Whole mature III anchor M13-III derived anchor with recoded DNA

```
!
!           1   2   3
!           A   A   A
     1     GCG gcc gca
!          NotI......
!
!           4   5   6   7   8   9  10  11  12  13  14  15  16  17
!           H   H   H   H   H   H   G   A   A   E   Q   K   L   I
    10     cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc atc
!
!          18  19  20  21  22  23  24  25  26  27  28  29
!           S   E   E   D   L   N   G   A   A   .   A   S
    52     tca gaa gag gat ctg aat ggg gcc gca Tag GCT AGC
!                                              NheI...
!
!         30  31  32  33  34  35  36    37  38  39
!          D   I   N   D   D   R   M     A   S   T
    88    GAT ATC aac gat gat cgt atg   gct tct act
! (ON_G37bot) [RC] 5'-c aac gat gat cgt atg gcG CAt Gct gcc gag aca g-3'
!         EcoRV..
!         Enterokinase cleavage site.
!
!  Start mature III (recoded)  Domain 1 ---->
!               40  41  42  43
!                A   E   T   V
   118         |gcC|gaG|acA|gtC|
!                 t   a   t   t ! W.T.
!
!         44  45  46  47  48  49  50  51  52  53  54  55  56  57  58
!          E   S   C   L   A   K   P   H   T   E   N   S   F   T   N
   130   |gaa|TCC|tgC|CTG|GCC|AaG|ccT|caC|acT|gaG|aat|AGT|ttC|aCA|Aat|
!            agt   t t a   a   a   c   t   a   a     tca   t   t   c ! W.T.
!                  MscI....
!
!        59  60  61  62  63  64  65  66  67  68  69  70  71  72  73
!         V   W   K   D   D   K   T   L   D   R   Y   A   N   Y   E
   175 |gtg|TGG|aaG|gaT|gaT|aaG|acC|CtT|gAT|CGA|TaT|gcC|aaT|taC|gaA|
!         c       a   c   c   a   t t a       t   c   t   c   t   g ! W.T.
!                                  BspDI...
!
!        74  75  76  77  78  79  80  81  82  83  84  85  86  87  88
!         G   C   L   W   N   A   T   G   V   V   V   C   T   G   D
   220 |ggC|tgC|TtA|tgg|aat|gcC|ACC|GGC|GtC|gtT|gtC|TGC|ACG|ggC|gaT|
!         t   t c g           t   a       t   a   t   t   t   t   c ! W.T.
!                           SgrAI......         BsgI....
!
!        89  90  91  92  93  94  95  96  97  98  99 100 101 102 103
!         E   T   Q   C   Y   G   T   W   V   P   I   G   L   A   I
   265 |gaG|acA|caA|tgC|taT|ggC|ACG|TGg|gtG|ccG|atA|gGC|TTA|GCC|atA|
!         a   t   g   t   c   t   a       t   t   t   g c t   t   c ! W.T.
!                           PmlI....              BlpI.....
!
!    Domain 1-----> Linker 1---------------->
!        104 105 106 107 108 109 110 111 112 113 114 115 116 117 118
!         P   E   N   E   G   G   G   S   E   G   G   G   S   E   G
   310 |ccG|gaG|aaC|gaA|ggC|ggC|ggT|AGC|gaA|ggC|ggT|ggC|AGC|gaA|ggC|
!         t   a   t   g   t   t   c tct   g   t   c   t tct   g   t ! W.T.
!
!        Linker 1---------------------> Domain 2--------------->
```

```
!       119 120 121 122 123 124 125 126 127 128 129 130 131 132 133
!        G   G   S   E   G   G   G   T   K   P   P   E   Y   G   D
  355 |ggT|GGA|TCC|gaA|ggA|ggT|ggA|acC|aaG|ccG|ccG|gaA|taT|ggC|gaC|
!         c   t   t   g   t   c   t   t   a   t   t   g   c   t   t ! W.T.
!           BamHI..(2/2)
!
!       134 135 136 137 138 139 140 141 142 143 144 145 146 147 148
!        T   P   I   P   G   Y   T   Y   I   N   P   L   D   G   T
  400 |acT|ccG|atA|CCT|GGT|taC|acC|taC|atT|aaT|ccG|TtA|gaT|ggA|acC|
!         a   t   t   g   c   t   t   t   c   c   t c c   c   c   t ! W.T.
!                 SexAI....
!
!       149 150 151 152 153 154 155 156 157 158 159 160 161 162 163
!        Y   P   P   G   T   E   Q   N   P   A   N   P   N   P   S
  445 |taC|ccT|ccG|ggC|acC|gaA|caG|aaT|ccT|gcC|aaC|ccG|aaC|ccA|AGC|
!         T   G   t   t   t   g   a   c   c   t   t   t   t tct ! W.T.
!                                                       HindIII...
!
!       164 165 166 167 168 169 170 171 172 173 174 175 176 177 178
!        L   E   E   S   Q   P   L   N   T   F   M   F   Q   N   N
  490 |TTA|gaA|gaA|AGC|caA|ccG|TtA|aaC|acC|ttT|atg|ttC|caA|aaC|aaC|
!       c t   G   G tct   g   t c t   t   t   c       t   g   t   t ! W.T.
! HindIII.
!
!       179 180 181 182 183 184 185 186 187 188 189 190 191 192 193
!        R   F   R   N   R   Q   G   A   L   T   V   Y   T   G   T
  535 |CgT|ttT|AgG|aaC|CgT|caA|gGT|GCT|CtT|acC|gTG|TAC|AcT|ggA|acC|
!       a g   c c a   t a g   g   g   a t a   t   t   t   g   c   t ! W.T.
!                             HgiAI...        BsrGI...
!
!       194 195 196 197 198 199 200 201 202 203 204 205 206 207 208
!        V   T   Q   G   T   D   P   V   K   T   Y   Y   Q   Y   T
  580 |gtC|acC|caG|GGT|ACC|gaT|ccT|gtC|aaG|acC|taC|taT|caA|taT|acC|
!         t   t   a   c   t   c   c   t   a   t   t   c   g   c   t ! W.T.
!                   KpnI...
!
!       209 210 211 212 213 214 215 216 217 218 219 220 221 222 223
!        P   V   S   S   K   A   M   Y   D   A   Y   W   N   G   K
  625 |ccG|gtC|TCG|AGt|aaG|gcT|atg|taC|gaT|gcC|taT|tgg|aaT|ggC|aaG|
!         t   a   a tca   a   c       t   c   t   c       c   t   a ! W.T.
!        BsaI....
!            XhoI....
!
!       224 225 226 227 228 229 230 231 232 233 234 235 236 237 238
!        F   R   D   C   A   F   H   S   G   F   N   E   D   P   F
  670 |ttT|CgT|gaT|tgT|gcC|ttT|caC|AGC|ggT|ttC|aaC|gaa|gac|CCt|ttT|
!         C A a   C   c   t   c   t tct   c   t   t   G   T   a   c ! W.T.
!
!       239 240 241 242 243 244 245 246 247 248 249 250 251 252 253
!        V   C   E   Y   Q   G   Q   S   S   D   L   P   Q   P   P
  715 |gtC|tgC|gaG|taC|caG|ggT|caG|AGT|AGC|gaT|TtA|ccG|caG|ccA|CCG|
!         t   t   a   t   a   c   a tcg tct   c c g   t   a   t   t ! W.T.
! DrdI.....                                               AgeI.....
!
!  Domain 2--------->  Linker 2---------------------->
!       254 255 256 257 258 259 260 261 262 263 264 265 266 267 268
!        V   N   A   G   G   G   S   G   G   G   S   G   G   G   S
  760  |GTT|AAC|gcG|ggT|ggT|ggT|AGC|ggC|ggA|ggC|AGC|ggC|ggT|ggT|AGC|
!         c   t   t   c   c   c tct   t   t   t tct   t   c   c tct ! W.T.
! AgeI.....
!        HpaI...
```

```
!        HincII.
!
!      Linker 2-------------------------------------------> Domain 3-->
!      269 270 271 272 273 274 275 276 277 278 279 280 281 282 283
!       E   G   G   G   S   E   G   G   G   S   G   G   G   S   G
  805 |gaA|ggC|ggA|ggT|AGC|gaA|ggA|ggT|ggC|AGC|ggA|ggC|ggT|AGC|ggC|
!        g   t   t   c tct   g   t   c   t tct   g   t   c tct   t  ! W.T.
!
!      ------------Domain 3------------------>
!      284 285 286 287 288 289 290 291 292 293 294 295 296 297 298
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G
  850 |AGT|ggC|gac|ttc|gac|tac|gag|aaa|atg|gct|aat|gcc|aac|aaa|GGC|
!      tcc   t   t   t   t   t   a   g       a   c   t   t   g   g ! W.T.
!                                                          KasI....
!
!      299 300 301 302 303 304 305 306 307 308 309 310 311 312 313
!       A   M   T   E   N   A   D   E   N   A   L   Q   S   D   A
  895 |GCC|atg|act|gag|aac|gct|gac|gaG|AAT|GCA|ctg|caa|agt|gat|gCC|
!        t       c   a   t   c   t   a   c   g   a   g tct   c   t ! W.T.
!  KasI....                          BsmI....                  StyI...
!
!      314 315 316 317 318 319 320 321 322 323 324 325 326 327 328
!       K   G   K   L   D   S   V   A   T   D   Y   G   A   A   I
  940 |AAG|GGt|aag|tta|gac|agc|gTC|GCc|Aca|gac|tat|ggT|GCt|gcc|atc|
!        a   c   a c t   t tct       t   t   t   c           t     ! W.T.
!  StyI......          PflFI......
!
!      329 330 331 332 333 334 335 336 337 338 339 340 341 342 343
!       D   G   F   I   G   D   V   S   G   L   A   N   G   N   G
  985 |gac|ggc|ttt|atc|ggc|gat|gtc|agt|ggt|ctg|gct|aac|ggc|aac|gga|
!        t   t   c   t   t   c   t tcc   c c t       t   t   t   t ! W.T.
!
!      344 345 346 347 348 349 350 351 352 353
!       A   T   G   D   F   A   G   S   N   S
 1030 |gcc|acc|gga|gac|ttc|GCA|GGT|tcG|AAT|TCt|
!        t   t   t   t   t   t   c   t       c ! W.T.
!                              BstBI...
!                                  EcoRI...
!                        BspMI..
!
!      354 355 356 357 358 359 360 361 362 363
!       Q   M   A   Q   V   G   D   G   D   N
 1060  cag atg gcC CAG GTT GGA GAT GGg gac aac
!        a       t   a   c   t   c   t   t   t ! W.T.
!                XcmI...............
!
!     364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379
!      S   P   L   M   N   N   F   R   Q   Y   L   P   S   L   P   Q
 1090 agt ccg ctt atg aac aac ttt aga cag tac ctt ccg tct ctt ccg cag
!      tca   t t a       t   t   c c t   a   t t a   t   c   c   t   a ! W.T.
!
!      380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395
!       S   V   E   C   R   P   F   V   F   S   A   G   K   P   Y   E
 1138 agt gtc gag tgc cgt cca ttc gtt ttc tct gcc ggc aag cct tac gag
!      tcg   t   a   t   c   t   t   c   t agc   t   t   a   a   t   a ! W.T.
!
!      Domain 3------------------------------------>
!      396 397 398 399 400 401 402 403 404 405 406 407
!       F   S   I   D   C   D   K   I   N   L   F   R
 1186 ttc aGC Atc gac TGC gat aag atc aat ctt ttC CGC
!       t tct   t   t   t   c   a   a   c t a       t
```

```
!          BstAPI.....                          SacII...
!
!      transmembrane segment------------>
!      408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423
!       G   V   F   A   F   L   L   Y   V   A   T   F   M   Y   V   F
  1222 GGc gtt ttc gct ttc ttg cta tac gtc gct act ttc atg tac gtt ttc
!       t   c   t   g   t c t t a   t   t   c   c   t       t   a   t ! W.T.
!
!      424 425 426 427 428 429 430   431 432 433 434 435
!       S   T   F   A   N   I   L     R   N   K   E   S
  1270 aGC ACT TTC GCC AAT ATT TTA   Cgc aac aaa gaa agc
!      tct   g   t   t   c   a c g     t   t   g   g tct ! W.T.
!                                   Intracellular anchor.
!
!              .   .
  1306         tag tga tct CCT AGG
!                        AvrII..
!
  1321 aag ccc gcc taa tga gcg ggc ttt ttt ttt ct  ggt
!       | Trp terminator                     |
!
! End Fab cassette
!--------------------------- End of Table ------------------------
```

Table 39: ONs to make deletions in III

```
! ONs for use with NheI
!
N
(ON_G29bot)                          5'-c gTT gAT ATc gcT Agc cTA Tgc-3'   !
22
! this is the reverse complement of 5'-gca tag gct agc gat atc aac g-3'
!                                                NheI... scab.........
(ON_G104top) 5'-g|ata|ggc|tta|gcT|aGC|ccg|gag|aac|gaa|gg-3'               !
30
!               Scab.........NheI... 104 105 106 107 108
(ON_G236top) 5'-c|ttt|cac|agc|ggt|ttc|GCT|AGC|gac|cct|ttt|gtc|tgc-3'      !
37
!                                    NheI... 236 237 238 239 240
(ON_G236tCS) 5'-c|ttt|cac|agc|ggt|ttc|GCT|AGC|gac|cct|ttt|gtc|Agc-
!                                    NheI... 236 237 238 239 240
               gag|tac|cag|ggt|c-3'                                          !
50

! ONs for use with SphI G CAT Gc
(ON_X37bot)       5'-gAc TgT cTc ggc Agc ATg cgc cAT Acg ATc ATc gTT g-3'  !
37
!                       N   D   D   R   M   A   H   A
!(ON_X37bot)=[RC] 5'-c aac gat gat cgt atg gcG CAt Gct gcc gag aca gtc-3'
!                                              SphI....Scab.....
(ON_X104top) 5'-g|gtG ccg|ata|ggc|ttG|CAT|GCa|ccg|gag|aac|gaa|gg-3'       !
36
!               Scab.............SphI....  104 105 106 107 108
(ON_X236top) 5'-c|ttt|cac|agc|ggt|ttG|CaT|gCa|gac|cct|ttt|gtc|tgc-3'      !
37
!                                    SphI....  236 237 238 239 240
(ON_X236tCS) 5'-c|ttt|cac|agc|ggt|ttG|CaT|gCa|gac|cct|ttt|gtc|Agc-
!                                    NheI... 236 237 238 239 240
               gag|tac|cag|ggt|c-3'                                          !
50
```

Table 40: Phage titers and enrichments of a selections with a DY3F31-based human Fab library

	Input (total cfu)	Output (total cfu)	Output/input ratio
R1-ox selected on phOx-BSA	$4,5 \times 10^{12}$	$3,4 \times 10^{5}$	$7,5 \times 10^{-8}$
R2-Strep selected on Strep-beads	$9,2 \times 10^{12}$	3×10^{8}	$3,3 \times 10^{-5}$

Table 41: Frequency of ELISA positives in DY3F31-based Fab libraries

	Anti-M13 HRP	9E10/RAMHRP	Anti-CK/CL Gar-HRP
R2-ox (with IPTG induction) R2-ox (without IPTG)	18/44 13/44	10/44 ND	10/44 ND
R3-strep (with IPTG)	39/44	38/44	36/44
R3-strep (without IPTG)	33/44	ND	ND

SEQUENCE LISTING

[0197]

<110> LADNER, ROBERT C.
COHEN, EDWARD H.
NASTRI, HORACIO G.
ROOKEY, KRISTIN L.
HOET, RENE
HOOGENBOOM, HENDRICUS R. J. M.

<120> NOVEL METHODS OF CONSTRUCTING LIBRARIES COMPRISING DISPLAYED AND/OR EXPRESSED MEMBERS OF A DIVERSE FAMILY OF PEPTIDES, POLYPEPTIDES OR PROTEINS AND THE NOVEL LIBRARIES

<130> DYAX/002 CIP2

<140> 10/045,674
<141> 2001-10-25

<150> 06/198,069
<151> 2000-04-17

<150> 09/837,306
<151> 2001-04-17

<160> 635

<170> PatentIn Ver. 2.1

<210> 1
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 1
catgtgtatt actgtgc 17

<210> 2
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 2
cacatccgtg cttcttgcac ggatgtggca cagtaataca catg 44

<210> 3
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 3
gtgtattaga ctgctgcc 18

<210> 4
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 4
ggcagcagtc taatacacca catccgtgtt cttcacggat gtg 43

<210> 5
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 5
cacatccgtg tttgttacac ggatgtggtg tcttacagtc cattctg 47

<210> 6
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 6
cagaatggac tgtaagacac 20

<210> 7
<211> 43
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 7
atcgagtctc actgagccac atccgtggtt ttccacggat gtg 43

<210> 8
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 8
gctcagtgag actcgat 17

<210> 9
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (10)..(24)
<223> A, T, C, G, other or unknown

<400> 9
cacgaggagn nnnnnnnnn nnnn 24

<210> 10
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 10
atgaccgaat tgctacaag 19

<210> 11
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 11
gactcctcag cttcttgctg aggagtcctt gtagcaattc ggtcat 46

<210> 12

<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: 6 His tag

<400> 12

His His His His His His
1 5

<210> 13
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6)..(10)
<223> A, T, C, G, other or unknown

<400> 13
gtctcnnnnn 10

<210> 14
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(6)
<223> A, T, C, G, other or unknown

<400> 14
nnnnnngaga c 11

<210> 15
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (11)..(24)
<223> A, T, C, G, other or unknown

<400> 15
cacggatgtg nnnnnnnnnn nnnn 24

<210> 16
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(14)
<223> A, T, C, G, other or unknown

<400> 16
nnnnnnnnnn nnnncacatc cgtg 24

<210> 17
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 17
gtgtattact gtgc 14

<210> 18
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 18
cacatccgtg cacggatgtg gcacagtaat acac 34

<210> 19
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 19
gtgtattaga ctgc 14

<210> 20
<211> 34
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 20
gcagtctaat acaccacatc cgtgcacgga tgtg 34

<210> 21
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 21
cacatccgtg cacggatgtg gtgtcttaca gtcc 34

<210> 22
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 22
ggactgtaag acac 14

<210> 23
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 23
gagtctcact gagccacatc cgtgcacgga tgtg 34

<210> 24
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 24
gctcagtgag actc 14

<210> 25
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 25

gtgtattact gtgc 14

<210> 26
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 26
gtatattact gtgc 14

<210> 27
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 27
gtgtattact gtaa 14

<210> 28
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 28
gtgtattact gtac 14

<210> 29
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 29
ttgtattact gtgc 14

<210> 30
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 30
ttgtatcact gtgc 14

<210> 31

<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 31
acatattact gtgc 14

<210> 32
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 32
acgtattact gtgc 14

<210> 33
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 33
atgtattact gtgc 14

<210> 34
<211> 101
<212> DNA
<213> Homo sapiens

<400> 34

```
agggtcacca tgaccaggga cacgtccatc agcacagcct acatgabcga gctgagcagg 60
ctgagatctg acgacacggc cgtgtattac tgtgcgagag a                     101
```

<210> 35
<211> 98
<212> DNA
<213> Homo sapiens

<400> 35

```
agagtcacca ttaccaggga cacatccgcg agcacagcct acatggagct gagcagcctg 60
agatctgaag acacggctgt gtattactgt gcgagaga                         98
```

<210> 36
<211> 98
<212> DNA
<213> Homo sapiens

<400> 36

agagtcacca tgaccaggaa cacctccata agcacagcct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcgagagg 98

<210> 37
<211> 98
<212> DNA
<213> Homo sapiens

<400> 37

agagtcacca tgaccacaga cacatccacg agcacagcct acatggagct gaggagcctg 60
agatctgacg acacggccgt gtattactgt gcgagaga 98

<210> 38
<211> 98
<212> DNA
<213> Homo sapiens

<400> 38

agagtcacca tgaccgagga cacatctaca gacacagcct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcaacaga 98

<210> 39
<211> 98
<212> DNA
<213> Homo sapiens

<400> 39

agagtcacca ttaccaggga caggtctatg agcacagcct acatggagct gagcagcctg 60
agatctgagg acacagccat gtattactgt gcaagata 98

<210> 40
<211> 98
<212> DNA
<213> Homo sapiens

<400> 40

agagtcacca tgaccaggga cacgtccacg agcacagtct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcgagaga 98

<210> 41
<211> 98
<212> DNA
<213> Homo sapiens

<400> 41

agagtcacca ttaccaggga catgtccaca agcacagcct acatggagct gagcagcctg 60
agatccgagg acacggccgt gtattactgt gcggcaga 98

<210> 42
<211> 98
<212> DNA
<213> Homo sapiens

<400> 42

agagtcacga ttaccgcgga cgaatccacg agcacagcct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcgagaga 98

<210> 43
<211> 98
<212> DNA
<213> Homo sapiens

<400> 43

agagtcacga ttaccgcgga caaatccacg agcacagcct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcgagaga 98

<210> 44
<211> 98
<212> DNA
<213> Homo sapiens

<400> 44

agagtcacca taaccgcgga cacgtctaca gacacagcct acatggagct gagcagcctg 60
agatctgagg acacggccgt gtattactgt gcaacaga 98

<210> 45
<211> 100
<212> DNA
<213> Homo sapiens

<400> 45

aggctcacca tcaccaagga cacctccaaa aaccaggtgg tccttacaat gaccaacatg 60
gaccctgtgg acacagccac atattactgt gcacacagac 100

<210> 46
<211> 100
<212> DNA
<213> Homo sapiens

<400> 46

aggctcacca tctccaagga cacctccaaa agccaggtgg tccttaccat gaccaacatg 60
gaccctgtgg acacagccac atattactgt gcacggatac 100

<210> 47
<211> 100
<212> DNA
<213> Homo sapiens

<400> 47

aggctcacca tctccaagga cacctccaaa aaccaggtgg tccttacaat gaccaacatg 60
gaccctgtgg acacagccac gtattactgt gcacggatac 100

<210> 48
<211> 98
<212> DNA
<213> Homo sapiens

<400> 48

cgattcacca tctccagaga caacgccaag aactcactgt atctgcaaat gaacagcctg 60
agagccgagg acacggctgt gtattactgt gcgagaga 98

<210> 49
<211> 100
<212> DNA
<213> Homo sapiens

<400> 49

cgattcacca tctccagaga caacgccaag aactccctgt atctgcaaat gaacagtctg 60
agagctgagg acacggcctt gtattactgt gcaaaagata 100

<210> 50
<211> 98
<212> DNA
<213> Homo sapiens

<400> 50

cgattcacca tctccaggga caacgccaag aactcactgt atctgcaaat gaacagcctg 60
agagccgagg acacggccgt gtattactgt gcgagaga 98

<210> 51
<211> 98
<212> DNA
<213> Homo sapiens

<400> 51

cgattcacca tctccagaga aaatgccaag aactccttgt atcttcaaat gaacagcctg 60
agagccgggg acacggctgt gtattactgt gcaagaga 98

<210> 52
<211> 98
<212> DNA

<213> Homo sapiens

<400> 52

agattcacca tctcaagaga tgattcaaaa aacacgctgt atctgcaaat gaacagcctg 60
aaaaccgagg acacagccgt gtattactgt accacaga 98

<210> 53
<211> 98
<212> DNA
<213> Homo sapiens

<400> 53

cgattcacca tctccagaga caacgccaag aactccctgt atctgcaaat gaacagtctg 60
agagccgagg acacggcctt gtatcactgt gcgagaga 98

<210> 54
<211> 98
<212> DNA
<213> Homo sapiens

<400> 54

cgattcacca tctccagaga caacgccaag aactcactgt atctgcaaat gaacagcctg 60
agagccgagg acacggctgt gtattactgt gcgagaga 98

<210> 55
<211> 98
<212> DNA
<213> Homo sapiens

<400> 55

cggttcacca tctccagaga caattccaag aacacgctgt atctgcaaat gaacagcctg 60
agagccgagg acacggccgt atattactgt gcgaaaga 98

<210> 56
<211> 98
<212> DNA
<213> Homo sapiens

<400> 56

cgattcacca tctccagaga caattccaag aacacgctgt atctgcaaat gaacagcctg 60
agagctgagg acacggctgt gtattactgt gcgaaaga 98

<210> 57
<211> 98
<212> DNA
<213> Homo sapiens

<400> 57

cgattcacca tctccagaga caattccaag aacacgctgt atctgcaaat gaacagcctg 60
agagctgagg acacggctgt gtattactgt gcgagaga 98

<210> 58
<211> 98
<212> DNA
<213> Homo sapiens

<400> 58

cgattcacca tctccagaga caattccaag aacacgctgt atctgcaaat gaacagcctg 60
agagctgagg acacggctgt gtattactgt gcgaaaga 98

<210> 59
<211> 98
<212> DNA
<213> Homo sapiens

<400> 59

cgattcacca tctccagaga caattccaag aacacgctgt atctgcaaat gaacagcctg 60
agagccgagg acacggctgt gtattactgt gcgagaga 98

<210> 60
<211> 100
<212> DNA
<213> Homo sapiens

<400> 60

cgattcacca tctccagaga caacagcaaa aactccctgt atctgcaaat gaacagtctg 60
agaactgagg acaccgcctt gtattactgt gcaaaagata 100

<210> 61
<211> 98
<212> DNA
<213> Homo sapiens

<400> 61

cgattcacca tctccagaga caatgccaag aactcactgt atctgcaaat gaacagcctg 60
agagacgagg acacggctgt gtattactgt gcgagaga 98

<210> 62
<211> 98
<212> DNA
<213> Homo sapiens

<400> 62

agattcacca tctcaagaga tggttccaaa agcatcgcct atctgcaaat gaacagcctg 60
aaaaccgagg acacagccgt gtattactgt actagaga 98

<210> 63
<211> 98
<212> DNA
<213> Homo sapiens

<400> 63

cgattcacca tctccagaga caattccaag aacacgctgt atcttcaaat gaacagcctg 60
agagccgagg acacggccgt gtattactgt gcgagaga 98

<210> 64
<211> 98
<212> DNA
<213> Homo sapiens

<400> 64

agattcacca tctccagaga caattccaag aacacgctgt atcttcaaat gggcagcctg 60
agagctgagg acatggctgt gtattactgt gcgagaga 98

<210> 65
<211> 98
<212> DNA
<213> Homo sapiens

<400> 65

agattcacca tctccagaga caattccaag aacacgctgt atcttcaaat gaacagcctg 60
agagctgagg acacggctgt gtattactgt gcgagaga 98

<210> 66
<211> 98
<212> DNA
<213> Homo sapiens

<400> 66

agattcacca tctcaagaga tgattcaaag aactcactgt atctgcaaat gaacagcctg 60
aaaaccgagg acacggccgt gtattactgt gctagaga 98

<210> 67
<211> 98
<212> DNA
<213> Homo sapiens

<400> 67

aggttcacca tctccagaga tgattcaaag aacacggcgt atctgcaaat gaacagcctg 60
aaaaccgagg acacggccgt gtattactgt actagaca 98

<210> 68
<211> 98
<212> DNA

<213> Homo sapiens

<400> 68

cgattcacca tctccagaga caacgccaag aacacgctgt atctgcaaat gaacagtctg 60
agagccgagg acacggctgt gtattactgt gcaagaga 98

<210> 69
<211> 98
<212> DNA
<213> Homo sapiens

<400> 69

agattcacca tctccagaga caattccaag aacacgctgc atcttcaaat gaacagcctg 60
agagctgagg acacggctgt gtattactgt aagaaaga 98

<210> 70
<211> 98
<212> DNA
<213> Homo sapiens

<400> 70

cgagtcacca tatcagtaga caagtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgcgg acacggccgt gtattactgt gcgagaga 98

<210> 71
<211> 98
<212> DNA
<213> Homo sapiens

<400> 71

cgagtcacca tgtcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgtgg acacggccgt gtattactgt gcgagaaa 98

<210> 72
<211> 98
<212> DNA
<213> Homo sapiens

<400> 72

cgagttacca tatcagtaga cacgtctaag aaccagttct ccctgaagct gagctctgtg 60
actgccgcgg acacggccgt gtattactgt gcgagaga 98

<210> 73
<211> 98
<212> DNA
<213> Homo sapiens

<400> 73

cgagtcacca tatcagtaga caggtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgcgg acacggccgt gtattactgt gccagaga 98

<210> 74
<211> 98
<212> DNA
<213> Homo sapiens

<400> 74

cgagttacca tatcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
actgccgcag acacggccgt gtattactgt gccagaga 98

<210> 75
<211> 98
<212> DNA
<213> Homo sapiens

<400> 75

cgagttacca tatcagtaga cacgtctaag aaccagttct ccctgaagct gagctctgtg 60
actgccgcgg acacggccgt gtattactgt gcgagaga 98

<210> 76
<211> 98
<212> DNA
<213> Homo sapiens

<400> 76

cgagtcacca tatcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgcgg acacggctgt gtattactgt gcgagaga 98

<210> 77
<211> 98
<212> DNA
<213> Homo sapiens

<400> 77

cgagtcacca tatccgtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgcag acacggctgt gtattactgt gcgagaca 98

<210> 78
<211> 98
<212> DNA
<213> Homo sapiens

<400> 78

cgagtcacca tatcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgctgcgg acacggccgt gtattactgt gcgagaga 98

<210> 79
<211> 98
<212> DNA
<213> Homo sapiens

<400> 79

cgagtcacca tatcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgctgcgg acacggccgt gtattactgt gcgagaga 98

<210> 80
<211> 98
<212> DNA
<213> Homo sapiens

<400> 80

cgagtcacca tatcagtaga cacgtccaag aaccagttct ccctgaagct gagctctgtg 60
accgccgcag acacggccgt gtattactgt gcgagaga 98

<210> 81
<211> 98
<212> DNA
<213> Homo sapiens

<400> 81

caggtcacca tctcagccga caagtccatc agcaccgcct acctgcagtg gagcagcctg 60
aaggcctcgg acaccgccat gtattactgt gcgagaca 98

<210> 82
<211> 96
<212> DNA
<213> Homo sapiens

<400> 82

cacgtcacca tctcagctga caagtccatc agcactgcct acctgcagtg gagcagcctg 60
aaggcctcgg acaccgccat gtattactgt gcgaga 96

<210> 83
<211> 98
<212> DNA
<213> Homo sapiens

<400> 83

cgaataacca tcaacccaga cacatccaag aaccagttct ccctgcagct gaactctgtg 60
actcccgagg acacggctgt gtattactgt gcaagaga 98

<210> 84
<211> 98
<212> DNA

<213> Homo sapiens

<400> 84

cggtttgtct tctccttgga cacctctgtc agcacggcat atctgcagat ctgcagccta 60
aaggctgagg acactgccgt gtattactgt gcgagaga 98

<210> 85
<211> 11
<212> DNA
<213> Artificial Sequence

<220> <223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (3)..(9)
<223> A, T, C, G, other or unknown

<400> 85
gcnnnnnnng c 11

<210> 86
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 86
caynnnnrtg 10

<210> 87
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6)..(11)
<223> A, T, C, G, other or unknown

<400> 87
gagtcnnnnn n 11

<210> 88
<211> 11
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(6)
<223> A, T, C, G, other or unknown

<400> 88
nnnnnngaga c 11

<210> 89
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 89
gaannnnttc 10

<210> 90
<211> 90
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic 3-23 FR3 nucleotide sequence

<220>
<221> CDS
<222> (1)..(90)

<220>
<221> modified_base
<222> (3)
<223> A, T, C or G

<220>
<221> modified_base
<222> (9)
<223> A, T, C or G

<220>
<221> modified_base
<222> (12)
<223> A, T, C or G

<220>
<221> modified_base

<222> (21)
<223> A, T, C or G

<220>
<221> modified_base
<222> (30)
<223> A, T, C or G

<220>
<221> modified_base
<222> (36)
<223> A, T, C or G

<220>
<221> modified_base
<222> (51)
<223> A, T, C or G

<220>
<221> modified_base
<222> (57)
<223> A, T, C or G

<220>
<221> modified_base
<222> (60)
<223> A, T, C or G

<220>
<221> modified_base
<222> (69)
<223> A, T, C or G

<220>
<221> modified_base
<222> (72)
<223> A, T, C or G

<220>
<221> modified_base
<222> (75)
<223> A, T, C or G

<220>
<221> modified_base
<222> (78)
<223> A, T, C or G

<220>
<221> modified base
<222> (87)
<223> A, T, C or G

<400> 90

```
acn ath wsn mgn gay aay wsn aar aay acn ytn tay ttn car atg aay   48
Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn
  1               5                  10                  15

wsn ttr mgn gcn gar gay acn gcn gtn tay tay tgy gcn aar           90
Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
            20                  25                  30
```

<210> 91
<211> 30
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic 3-23 FR3 protein sequence

<400> 91

```
Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn
  1               5                  10                  15

Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys
            20                  25                  30
```

<210> 92
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 92
agttctccct gcagctgaac tc 22

<210> 93
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 93
cactgtatct gcaaatgaac ag 22

<210> 94
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 94
ccctgtatct gcaaatgaac ag 22

<210> 95

<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 95
ccgcctacct gcagtggagc ag 22

<210> 96
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 96
cgctgtatct gcaaatgaac ag 22

<210> 97
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 97
cggcatatct gcagatctgc ag 22

<210> 98
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 98
cggcgtatct gcaaatgaac ag 22

<210> 99
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 99
ctgcctacct gcagtggagc ag 22

<210> 100
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 100
tcgcctatct gcaaatgaac ag 22

<210> 101
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 101

cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agg 63

<210> 102
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 102
caagtagaga gtattcttag agttgtctct agacttagtg aagcg 45

<210> 103
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 103
cgcttcacta agtctagaga caactctaag aatactctct acttgcagct gaac 54

<210> 104
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 104
cgcttcacta agtctagaga caactctaag aatactctct acttgcaaat gaac 54

<210> 105
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 105
cgcttcacta agtctagaga caactctaag aatactctct acttgcagtg gagc 54

<210> 106
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 106
cgcttcacta agtctagaga c 21

<210> 107
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 107
acatggagct gagcagcctg ag 22

<210> 108
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 108
acatggagct gagcaggctg ag 22

<210> 109
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 109
acatggagct gaggagcctg ag 22

<210> 110
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 110
acctgcagtg gagcagcctg aa 22

<210> 111
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 111
atctgcaaat gaacagcctg aa 22

<210> 112
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 112
atctgcaaat gaacagcctg ag 22

<210> 113
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 113
atctgcaaat gaacagtctg ag 22

<210> 114
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 114
atctgcagat ctgcagccta aa 22

<210> 115
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 115
atcttcaaat gaacagcctg ag 22

<210> 116
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 116
atcttcaaat gggcagcctg ag 22

<210> 117
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 117
ccctgaagct gagctctgtg ac 22

<210> 118
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 118
ccctgcagct gaactctgtg ac 22

<210> 119
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 119
tccttacaat gaccaacatg ga 22

<210> 120
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 120
tccttaccat gaccaacatg ga 22

<210> 121
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 121
acatggagct gagcagcctg ag 22

<210> 122
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 122
ccctgaagct gagctctgtg ac 22

<210> 123
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 123
cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaac 54

<210> 124
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 124 cgcttcactc agtctagaga taacagtaaa aatactttgt acttgcagct gagcagcctg 60

<210> 125
<211> 60
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 125
cgcttcactc agtctagaga taacagtaaa aatactttgt acttgcagct gagctctgtg 60

<210> 126
<211> 52
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 126
tcagctgcaa gtacaaagta tttttactgt tatctctaga ctgagtgaag cg 52

<210> 127
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 127
cgcttcactc agtctagaga taac 24

<210> 128
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 128
ccgtgtatta ctgtgcgaga ga 22

<210> 129
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 129
ctgtgtatta ctgtgcgaga ga 22

<210> 130
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 130
ccgtgtatta ctgtgcgaga gg 22

<210> 131
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 131
ccgtgtatta ctgtgcaaca ga 22

<210> 132
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 132
ccatgtatta ctgtgcaaga ta 22

<210> 133
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 133
ccgtgtatta ctgtgcggca ga 22

<210> 134
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 134
ccacatatta ctgtgcacac ag 22

<210> 135
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 135
ccacatatta ctgtgcacgg at 22

<210> 136
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 136
ccacgtatta ctgtgcacgg at 22

<210> 137
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 137
ccttgtatta ctgtgcaaaa ga 22

<210> 138
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 138
ctgtgtatta ctgtgcaaga ga 22

<210> 139
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 139
ccgtgtatta ctgtaccaca ga 22

<210> 140
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 140
ccttgtatca ctgtgcgaga ga 22

<210> 141
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 141
ccgtatatta ctgtgcgaaa ga 22

<210> 142
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 142
ctgtgtatta ctgtgcgaaa ga 22

<210> 143
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 143
ccgtgtatta ctgtactaga ga 22

<210> 144
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 144
ccgtgtatta ctgtgctaga ga 22

<210> 145
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 145
ccgtgtatta ctgtactaga ca 22

<210> 146
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 146
ctgtgtatta ctgtaagaaa ga 22

<210> 147
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 147

ccgtgtatta ctgtgcgaga aa 22

<210> 148
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 148
ccgtgtatta ctgtgccaga ga 22

<210> 149
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 149
ctgtgtatta ctgtgcgaga ca 22

<210> 150
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 150
ccatgtatta ctgtgcgaga ca 22

<210> 151
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 151
ccatgtatta ctgtgcgaga 20

<210> 152
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 152
ccgtgtatta ctgtgcgaga g 21

<210> 153

<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 153
ctgtgtatta ctgtgcgaga g 21

<210> 154
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 154
ccgtgtatta ctgtgcgaga g 21

<210> 155
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 155
ccgtatatta ctgtgcgaaa g 21

<210> 156
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 156
ctgtgtatta ctgtgcgaaa g 21

<210> 157
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 157
ctgtgtatta ctgtgcgaga c 21

<210> 158
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 158
ccatgtatta ctgtgcgaga c 21

<210> 159
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 159
ccatgtatta ctgtgcgaga 20

<210> 160
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 160

```
ggtgtagtga tctagtgaca actctaagaa tactctctac ttgcagatga acagctttag 60
ggctgaggac actgcagtct actattgtgc gaga                              94
```

<210> 161
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 161

```
ggtgtagtga tctagtgaca actctaagaa tactctctac ttgcagatga acagctttag 60
ggctgaggac actgcagtct actattgtgc gaaa                              94
```

<210> 162
<211> 85
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 162

```
atagtagact gcagtgtcct cagcccttaa gctgttcatc tgcaagtaga gagtattctt 60
agagttgtct ctagatcact acacc                                        85
```

<210> 163
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 163
ggtgtagtga tctagagaca ac 22

<210> 164
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 164
ggtgtagtga aacagcttta gggctgagga cactgcagtc tactattgtg cgaga 55

<210> 165
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 165
ggtgtagtga aacagcttta gggctgagga cactgcagtc tactattgtg cgaaa 55

<210> 166
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 166
atagtagact gcagtgtcct cagcccttaa gctgtttcac tacacc 46

<210> 167
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 167
ggtgtagtga aacagcttaa gggctgagga cactgcagtc tactat 46

<210> 168
<211> 26
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 168
ggtgtagtga aacagcttaa gggctg 26

<210> 169
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 169
agttctccct gcagctgaac tc 22

<210> 170
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 170
cactgtatct gcaaatgaac ag 22

<210> 171
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 171
ccctgtatct gcaaatgaac ag 22

<210> 172
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 172
ccgcctacct gcagtggagc ag 22

<210> 173
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic probe

<400> 173
cgctgtatct gcaaatgaac ag 22

<210> 174
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 174
cggcatatct gcagatctgc ag 22

<210> 175
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 175
cggcgtatct gcaaatgaac ag 22

<210> 176
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 176
ctgcctacct gcagtggagc ag 22

<210> 177
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 177
tcgcctatct gcaaatgaac ag 22

<210> 178
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 178
acatggagct gagcagcctg ag 22

<210> 179
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 179
acatggagct gagcaggctg ag 22

<210> 180
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 180
acatggagct gaggagcctg ag 22

<210> 181
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 181
acctgcagtg gagcagcctg aa 22

<210> 182
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 182
atctgcaaat gaacagcctg aa 22

<210> 183
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 183
atctgcaaat gaacagcctg ag 22

<210> 184
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 184
atctgcaaat gaacagtctg ag 22

<210> 185
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 185
atctgcagat ctgcagccta aa 22

<210> 186
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 186
atcttcaaat gaacagcctg ag 22

<210> 187
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 187
atcttcaaat gggcagcctg ag 22

<210> 188
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 188
ccctgaagct gagctctgtg ac 22

<210> 189
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 189
ccctgcagct gaactctgtg ac 22

<210> 190
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 190
tccttacaat gaccaacatg ga 22

<210> 191
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 191
tccttaccat gaccaacatg ga 22

<210> 192
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 192
ccgtgtatta ctgtgcgaga ga 22

<210> 193
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 193
ctgtgtatta ctgtgcgaga ga 22

<210> 194
<211> 22
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 194
ccgtgtatta ctgtgcgaga gg 22

<210> 195
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 195
ccgtgtatta ctgtgcaaca ga 22

<210> 196
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 196
ccatgtatta ctgtgcaaga ta 22

<210> 197
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 197
ccgtgtatta ctgtgcggca ga 22

<210> 198
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 198
ccacatatta ctgtgcacac ag 22

<210> 199
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 199

ccacatatta ctgtgcacgg at 22

<210> 200
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 200
ccacgtatta ctgtgcacgg at 22

<210> 201
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 201
ccttgtatta ctgtgcaaaa ga 22

<210> 202
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 202
ctgtgtatta ctgtgcaaga ga 22

<210> 203
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 203
ccgtgtatta ctgtaccaca ga 22

<210> 204
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 204
ccttgtatca ctgtgcgaga ga 22

<210> 205

<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 205
ccgtatatta ctgtgcgaaa ga 22

<210> 206
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 206
ctgtgtatta ctgtgcgaaa ga 22

<210> 207
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 207
ccgtgtatta ctgtactaga ga 22

<210> 208
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 208
ccgtgtatta ctgtgctaga ga 22

<210> 209
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 209
ccgtgtatta ctgtactaga ca 22

<210> 210
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 210
ctgtgtatta ctgtaagaaa ga 22

<210> 211
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 211
ccgtgtatta ctgtgcgaga aa 22

<210> 212
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 212
ccgtgtatta ctgtgccaga ga 22

<210> 213
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 213
ctgtgtatta ctgtgcgaga ca 22

<210> 214
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 214
ccatgtatta ctgtgcgaga ca 22

<210> 215
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 215
ccatgtatta ctgtgcgaga aa 22

<210> 216
<211> 90
<212> DNA
<213> Homo sapiens

<400> 216

caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggata caccttcacc 90

<210> 217
<211> 90
<212> DNA
<213> Homo sapiens

<400> 217

caggtccagc ttgtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtt 60
tcctgcaagg cttctggata caccttcact 90

<210> 218
<211> 90
<212> DNA
<213> Homo sapiens

<400> 218

caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggata caccttcacc 90

<210> 219
<211> 90
<212> DNA
<213> Homo sapiens

<400> 219

caggttcagc tggtgcagtc tggagctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg cttctggtta caccttacc 90

<210> 220
<211> 90
<212> DNA
<213> Homo sapiens

<400> 220

caggtccagc tggtacagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact 90

<210> 221
<211> 90
<212> DNA
<213> Homo sapiens

<400> 221

cagatgcagc tggtgcagtc tggggctgag gtgaagaaga ctgggtcctc agtgaaggtt 60
tcctgcaagg cttccggata caccttcacc 90

<210> 222
<211> 90
<212> DNA
<213> Homo sapiens

<400> 222

caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtt 60
tcctgcaagg catctggata caccttcacc 90

<210> 223
<211> 90
<212> DNA
<213> Homo sapiens

<400> 223

caaatgcagc tggtgcagtc tgggcctgag gtgaagaagc ctgggacctc agtgaaggtc 60
tcctgcaagg cttctggatt cacctttact 90

<210> 224
<211> 90
<212> DNA
<213> Homo sapiens

<400> 224

caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc 90

<210> 225
<211> 90
<212> DNA
<213> Homo sapiens

<400> 225

caggtgcagc tggtgcagtc tggggctgag gtgaagaagc ctgggtcctc ggtgaaggtc 60
tcctgcaagg cttctggagg caccttcagc 90

<210> 226
<211> 90
<212> DNA

<213> Homo sapiens

<400> 226

gaggtccagc tggtacagtc tggggctgag gtgaagaagc ctggggctac agtgaaaatc 60
tcctgcaagg tttctggata caccttcacc 90

<210> 227
<211> 90
<212> DNA
<213> Homo sapiens

<400> 227

cagatcacct tgaaggagtc tggtcctacg ctggtgaaac ccacacagac cctcacgctg 60
acctgcacct tctctgggtt ctcactcagc 90

<210> 228
<211> 90
<212> DNA
<213> Homo sapiens

<400> 228

caggtcacct tgaaggagtc tggtcctgtg ctggtgaaac ccacagagac cctcacgctg 60
acctgcaccg tctctgggtt ctcactcagc 90

<210> 229
<211> 90
<212> DNA
<213> Homo sapiens

<400> 229

caggtcacct tgaaggagtc tggtcctgcg ctggtgaaac ccacacagac cctcacactg 60
acctgcacct tctctgggtt ctcactcagc 90

<210> 230
<211> 90
<212> DNA
<213> Homo sapiens

<400> 230

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt cacctttagt 90

<210> 231
<211> 90
<212> DNA
<213> Homo sapiens

<400> 231

gaagtgcagc tggtggagtc tgggggaggc ttggtacagc ctggcaggtc cctgagactc 60
tcctgtgcag cctctggatt caccttttgat 90

<210> 232
<211> 90
<212> DNA
<213> Homo sapiens

<400> 232

caggtgcagc tggtggagtc tgggggaggc ttggtcaagc ctggagggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 233
<211> 90
<212> DNA
<213> Homo sapiens

<400> 233

gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 234
<211> 90
<212> DNA
<213> Homo sapiens

<400> 234

gaggtgcagc tggtggagtc tgggggaggc ttggtaaagc ctgggggGtc ccttagactc 60
tcctgtgcag cctctggatt cactttcagt 90

<210> 235
<211> 90
<212> DNA
<213> Homo sapiens

<400> 235

gaggtgcagc tggtggagtc tgggggaggt gtggtacggc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt caccttTgat 90

<210> 236
<211> 90
<212> DNA
<213> Homo sapiens

<400> 236

gaggtgcagc tggtggagtc tgggggaggc ctggtcaagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 237
<211> 90
<212> DNA
<213> Homo sapiens

<400> 237

gaggtgcagc tgttggagtc tgggggaggc ttggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt cacctttagc 90

<210> 238
<211> 90
<212> DNA
<213> Homo sapiens

<400> 238

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 239
<211> 90
<212> DNA
<213> Homo sapiens

<400> 239

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 240
<211> 90
<212> DNA
<213> Homo sapiens

<400> 240

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 241
<211> 90
<212> DNA
<213> Homo sapiens

<400> 241

caggtgcagc tggtggagtc tgggggaggc gtggtccagc ctgggaggtc cctgagactc 60
tcctgtgcag cgtctggatt caccttcagt 90

<210> 242
<211> 90
<212> DNA

<213> Homo sapiens

<400> 242

gaagtgcagc tggtggagtc tgggggagtc gtggtacagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt cacctttgat 90

<210> 243
<211> 90
<212> DNA
<213> Homo sapiens

<400> 243

gaggtgcagc tggtggagtc tgggggaggc ttggtacagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 244
<211> 90
<212> DNA
<213> Homo sapiens

<400> 244

gaggtgcagc tggtggagtc tgggggaggc ttggtacagc cagggcggtc cctgagactc 60
tcctgtacag cttctggatt cacctttggt 90

<210> 245
<211> 90
<212> DNA
<213> Homo sapiens

<400> 245

gaggtgcagc tggtggagac tggaggaggc ttgatccagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctgggtt caccgtcagt 90

<210> 246
<211> 90
<212> DNA
<213> Homo sapiens

<400> 246

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggGtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 247
<211> 90
<212> DNA
<213> Homo sapiens

<400> 247

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt caccgtcagt 90

<210> 248
<211> 90
<212> DNA
<213> Homo sapiens

<400> 248

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctggagggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 249
<211> 90
<212> DNA
<213> Homo sapiens

<400> 249

gaggtgcagc tggtggagtc tgggggaggc ttggtccagc ctgggggtc cctgaaactc 60
tcctgtgcag cctctgggtt caccttcagt 90

<210> 250
<211> 90
<212> DNA
<213> Homo sapiens

<400> 250

gaggtgcagc tggtggagtc cgggggaggc ttagttcagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt caccttcagt 90

<210> 251
<211> 90
<212> DNA
<213> Homo sapiens

<400> 251

gaggtgcagc tggtggagtc tcggggagtc ttggtacagc ctgggggtc cctgagactc 60
tcctgtgcag cctctggatt caccgtcagt 90

<210> 252
<211> 90
<212> DNA
<213> Homo sapiens

<400> 252

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcgggac cctgtccctc 60
acctgcgctg tctctggtgg ctccatcagc 90

<210> 253
<211> 90
<212> DNA
<213> Homo sapiens

<400> 253

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggacac cctgtccctc 60
acctgcgctg tctctggtta ctccatcagc 90

<210> 254
<211> 90
<212> DNA
<213> Homo sapiens

<400> 254

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagc 90

<210> 255
<211> 90
<212> DNA
<213> Homo sapiens

<400> 255

cagctgcagc tgcaggagtc cggctcagga ctggtgaagc cttcacagac cctgtccctc 60
acctgcgctg tctctggtgg ctccatcagc 90

<210> 256
<211> 90
<212> DNA
<213> Homo sapiens

<400> 256

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagc 90

<210> 257
<211> 90
<212> DNA
<213> Homo sapiens

<400> 257

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagc 90

<210> 258
<211> 90
<212> DNA

<213> Homo sapiens

<400> 258

caggtgcagc tacagcagtg gggcgcagga ctgttgaagc cttcggagac cctgtccctc 60
acctgcgctg tctatggtgg gtccttcagt 90

<210> 259
<211> 90
<212> DNA
<213> Homo sapiens

<400> 259

cagctgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagc 90

<210> 260
<211> 90
<212> DNA
<213> Homo sapiens

<400> 260

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc 60
acctgcactg tctctggtgg ctccatcagt 90

<210> 261
<211> 90
<212> DNA
<213> Homo sapiens

<400> 261

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc 60
acctgcactg tctctggtgg ctccgtcagc 90

<210> 262
<211> 90
<212> DNA
<213> Homo sapiens

<400> 262

caggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcggagac cctgtccctc 60
acctgcgctg tctctggtta ctccatcagc 90

<210> 263
<211> 90
<212> DNA
<213> Homo sapiens

<400> 263

gaggtgcagc tggtgcagtc tggagcagag gtgaaaaagc ccggggagtc tctgaagatc 60
tcctgtaagg gttctggata cagctttacc 90

<210> 264
<211> 90
<212> DNA
<213> Homo sapiens

<400> 264

gaagtgcagc tggtgcagtc tggagcagag gtgaaaaagc ccggggagtc tctgaggatc 60
tcctgtaagg gttctggata cagctttacc 90

<210> 265
<211> 90
<212> DNA
<213> Homo sapiens

<400> 265

caggtacagc tgcagcagtc aggtccagga ctggtgaagc cctcgcagac cctctcactc 60
acctgtgcca tctccgggga cagtgtctct 90

<210> 266
<211> 90
<212> DNA
<213> Homo sapiens

<400> 266

caggtgcagc tggtgcaatc tgggtctgag ttgaagaagc ctggggcctc agtgaaggtt 60
tcctgcaagg cttctggata caccttcact 90

<210> 267
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 267
ccgtgtatta ctgtgcgaga ga 22

<210> 268
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 268
ctgtgtatta ctgtgcgaga ga 22

<210> 269
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 269
ccgtgtatta ctgtgcgaga gg 22

<210> 270
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 270
ccgtatatta ctgtgcgaaa ga 22

<210> 271
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 271
ctgtgtatta ctgtgcgaaa ga 22

<210> 272
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 272
ctgtgtatta ctgtgcgaga ca 22

<210> 273
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 273
ccatgtatta ctgtgcgaga ca 22

<210> 274
<211> 22
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 274
ccatgtatta ctgtgcgaga aa 22

<210> 275
<211> 69
<212> DNA
<213> Homo sapiens

<400> 275

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 276
<211> 69
<212> DNA
<213> Homo sapiens

<400> 276

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 277
<211> 69
<212> DNA
<213> Homo sapiens

<400> 277

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 278
<211> 69
<212> DNA
<213> Homo sapiens

<400> 278

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 279
<211> 69
<212> DNA
<213> Homo sapiens

<400> 279

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 280
<211> 69
<212> DNA
<213> Homo sapiens

<400> 280

gacatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 281
<211> 69
<212> DNA
<213> Homo sapiens

<400> 281

aacatccaga tgacccagtc tccatctgcc atgtctgcat ctgtaggaga cagagtcacc 60
atcacttgt 69

<210> 282
<211> 69
<212> DNA
<213> Homo sapiens

<400> 282

gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgt 69

<210> 283
<211> 69
<212> DNA
<213> Homo sapiens

<400> 283

gacatccaga tgacccagtc tccatcctca ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgt 69

<210> 284
<211> 69
<212> DNA
<213> Homo sapiens

<400> 284

gccatccagt tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 285
<211> 69
<212> DNA
<213> Homo sapiens

<400> 285

gccatccagt tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 286
<211> 69
<212> DNA
<213> Homo sapiens

<400> 286

gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgt 69

<210> 287
<211> 69
<212> DNA
<213> Homo sapiens

<400> 287

gacatccaga tgacccagtc tccatcttct gtgtctgcat ctgtaggaga cagagtcacc 60
atcacttgt 69

<210> 288
<211> 69
<212> DNA
<213> Homo sapiens

<400> 288

gacatccagt tgacccagtc tccatccttc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 289
<211> 69
<212> DNA
<213> Homo sapiens

<400> 289

gccatccgga tgacccagtc tccattctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 290
<211> 69
<212> DNA

<213> Homo sapiens

<400> 290

gccatccgga tgacccagtc tccatcctca ttctctgcat ctacaggaga cagagtcacc 60
atcacttgt 69

<210> 291
<211> 69
<212> DNA
<213> Homo sapiens

<400> 291

gtcatctgga tgacccagtc tccatcctta ctctctgcat ctacaggaga cagagtcacc 60
atcagttgt 69

<210> 292
<211> 69
<212> DNA
<213> Homo sapiens

<400> 292

gccatccaga tgacccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 293
<211> 69
<212> DNA
<213> Homo sapiens

<400> 293

gacatccaga tgacccagtc tccttccacc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgc 69

<210> 294
<211> 69
<212> DNA
<213> Homo sapiens

<400> 294

gatattgtga tgacccagac tccactctcc ctgcccgtca cccctggaga gccggcctcc 60
atctcctgc 69

<210> 295
<211> 69
<212> DNA
<213> Homo sapiens

<400> 295

gatattgtga tgacccagac tccactctcc ctgcccgtca cccctggaga gccggcctcc 60
atctcctgc 69

<210> 296
<211> 69
<212> DNA
<213> Homo sapiens

<400> 296

gatgttgtga tgactcagtc tccactctcc ctgcccgtca cccttggaca gccggcctcc 60
atctcctgc 69

<210> 297
<211> 69
<212> DNA
<213> Homo sapiens

<400> 297

gatgttgtga tgactcagtc tccactctcc ctgcccgtca cccttggaca gccggcctcc 60
atctcctgc 69

<210> 298
<211> 69
<212> DNA
<213> Homo sapiens

<400> 298

gatattgtga tgacccagac tccactctct ctgtccgtca cccctggaca gccggcctcc 60
atctcctgc 69

<210> 299
<211> 69
<212> DNA
<213> Homo sapiens

<400> 299

gatattgtga tgacccagac tccactctct ctgtccgtca cccctggaca gccggcctcc 60
atctcctgc 69

<210> 300
<211> 69
<212> DNA
<213> Homo sapiens

<400> 300

gatattgtga tgactcagtc tccactctcc ctgcccgtca cccctggaga gccggcctcc 60
atctcctgc 69

<210> 301
<211> 69
<212> DNA
<213> Homo sapiens

<400> 301

gatattgtga tgactcagtc tccactctcc ctgcccgtca cccctggaga gccggcctcc 60
atctcctgc 69

<210> 302
<211> 69
<212> DNA
<213> Homo sapiens

<400> 302

gatattgtga tgacccagac tccactctcc tcacctgtca cccttggaca gccggcctcc 60
atctcctgc 69

<210> 303
<211> 69
<212> DNA
<213> Homo sapiens

<400> 303

gaaattgtgt tgacgcagtc tccaggcacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 304
<211> 69
<212> DNA
<213> Homo sapiens

<400> 304

gaaattgtgt tgacgcagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 305
<211> 69
<212> DNA
<213> Homo sapiens

<400> 305

gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 306
<211> 69
<212> DNA

<213> Homo sapiens

<400> 306

gaaatagtga tgacgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 307
<211> 69
<212> DNA
<213> Homo sapiens

<400> 307

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 308
<211> 69
<212> DNA
<213> Homo sapiens

<400> 308

gaaattgtgt tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 309
<211> 69
<212> DNA
<213> Homo sapiens

<400> 309

gaaattgtaa tgacacagtc tccagccacc ctgtctttgt ctccagggga aagagccacc 60
ctctcctgc 69

<210> 310
<211> 69
<212> DNA
<213> Homo sapiens

<400> 310

gacatcgtga tgacccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgc 69

<210> 311
<211> 69
<212> DNA
<213> Homo sapiens

<400> 311

gaaacgacac tcacgcagtc tccagcattc atgtcagcga ctccaggaga caaagtcaac 60
atctcctgc 69

<210> 312
<211> 69
<212> DNA
<213> Homo sapiens

<400> 312

gaaattgtgc tgactcagtc tccagacttt cagtctgtga ctccaaagga gaaagtcacc 60
atcacctgc 69

<210> 313
<211> 69
<212> DNA
<213> Homo sapiens

<400> 313

gaaattgtgc tgactcagtc tccagacttt cagtctgtga ctccaaagga gaaagtcacc 60
atcacctgc 69

<210> 314
<211> 69
<212> DNA
<213> Homo sapiens

<400> 314

gatgttgtga tgacacagtc tccagctttc ctctctgtga ctccagggga gaaagtcacc 60
atcacctgc 69

<210> 315
<211> 66
<212> DNA
<213> Homo sapiens

<400> 315

cagtctgtgc tgactcagcc accctcggtg tctgaagccc ccaggcagag ggtcaccatc 60
tcctgt 66

<210> 316
<211> 66
<212> DNA
<213> Homo sapiens

<400> 316

cagtctgtgc tgacgcagcc gccctcagtg tctggggccc cagggcagag ggtcaccatc 60
tcctgc 66

<210> 317
<211> 66
<212> DNA
<213> Homo sapiens

<400> 317

cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60
tcttgt 66

<210> 318
<211> 66
<212> DNA
<213> Homo sapiens

<400> 318

cagtctgtgc tgactcagcc accctcagcg tctgggaccc ccgggcagag ggtcaccatc 60
tcttgt 66

<210> 319
<211> 66
<212> DNA
<213> Homo sapiens

<400> 319

cagtctgtgt tgacgcagcc gccctcagtg tctgcggccc caggacagaa ggtcaccatc 60
tcctgc 66

<210> 320
<211> 66
<212> DNA
<213> Homo sapiens

<400> 320

cagtctgccc tgactcagcc tccctccgcg tccgggtctc ctggacagtc agtcaccatc 60
tcctgc 66

<210> 321
<211> 66
<212> DNA
<213> Homo sapiens

<400> 321

cagtctgccc tgactcagcc tcgctcagtg tccgggtctc ctggacagtc agtcaccatc 60
tcctgc 66

<210> 322
<211> 66
<212> DNA
<213> Homo sapiens

<400> 322

cagtctgccc tgactcagcc tgcctccgtg tctgggtctc ctggacagtc gatcaccatc 60
tcctgc 66

<210> 323
<211> 66
<212> DNA
<213> Homo sapiens

<400> 323

cagtctgccc tgactcagcc tccctccgtg tccgggtctc ctggacagtc agtcaccatc 60
tcctgc 66

<210> 324
<211> 66
<212> DNA
<213> Homo sapiens

<400> 324

cagtctgccc tgactcagcc tgcctccgtg tctgggtctc ctggacagtc gatcaccatc 60
tcctgc 66

<210> 325
<211> 66
<212> DNA
<213> Homo sapiens

<400> 325

tcctatgagc tgactcagcc accctcagtg tccgtgtccc caggacagac agccagcatc 60
acctgc 66

<210> 326
<211> 66
<212> DNA
<213> Homo sapiens

<400> 326

tcctatgagc tgactcagcc actctcagtg tcagtggccc tgggacagac ggccaggatt 60
acctgt 66

<210> 327
<211> 66
<212> DNA

<213> Homo sapiens

<400> 327

tcctatgagc tgacacagcc accctcggtg tcagtgtccc caggacaaac ggccaggatc 60
acctgc 66

<210> 328
<211> 66
<212> DNA
<213> Homo sapiens

<400> 328

tcctatgagc tgacacagcc accctcggtg tcagtgtccc taggacagat ggccaggatc 60
acctgc 66

<210> 329
<211> 66
<212> DNA
<213> Homo sapiens

<400> 329

tcttctgagc tgactcagga ccctgctgtg tctgtggcct tgggacagac agtcaggatc 60
acatgc 66

<210> 330
<211> 66
<212> DNA
<213> Homo sapiens

<400> 330

tcctatgtgc tgactcagcc accctcagtg tcagtggccc caggaaagac ggccaggatt 60
acctgt 66

<210> 331
<211> 66
<212> DNA
<213> Homo sapiens

<400> 331

tcctatgagc tgacacagct accctcggtg tcagtgtccc caggacagac agccaggatc 60
acctgc 66

<210> 332
<211> 66
<212> DNA
<213> Homo sapiens

<400> 332

tcctatgagc tgatgcagcc accctcggtg tcagtgtccc caggacagac ggccaggatc 60
acctgc 66

<210> 333
<211> 66
<212> DNA
<213> Homo sapiens

<400> 333

tcctatgagc tgacacagcc atcctcagtg tcagtgtctc cgggacagac agccaggatc 60
acctgc 66

<210> 334
<211> 66
<212> DNA
<213> Homo sapiens

<400> 334

ctgcctgtgc tgactcagcc cccgtctgca tctgccttgc tgggagcctc gatcaagctc 60
acctgc 66

<210> 335
<211> 66
<212> DNA
<213> Homo sapiens

<400> 335

cagcctgtgc tgactcaatc atcctctgcc tctgcttccc tgggatcctc ggtcaagctc 60
acctgc 66

<210> 336
<211> 66
<212> DNA
<213> Homo sapiens

<400> 336

cagcttgtgc tgactcaatc gccctctgcc tctgcctccc tgggagcctc ggtcaagctc 60
acctgc 66

<210> 337
<211> 66
<212> DNA
<213> Homo sapiens

<400> 337

cagcctgtgc tgactcagcc accttcctcc tccgcatctc ctggagaatc cgccagactc 60
acctgc 66

<210> 338
<211> 66
<212> DNA
<213> Homo sapiens

<400> 338

caggctgtgc tgactcagcc ggcttccctc tctgcatctc ctggagcatc agccagtctc 60
acctgc 66

<210> 339
<211> 66
<212> DNA
<213> Homo sapiens

<400> 339

cagcctgtgc tgactcagcc atcttcccat tctgcatctt ctggagcatc agtcagactc 60
acctgc 66

<210> 340
<211> 66
<212> DNA
<213> Homo sapiens

<400> 340

aattttatgc tgactcagcc ccactctgtg tcggagtctc cggggaagac ggtaaccatc 60
tcctgc 66

<210> 341
<211> 66
<212> DNA
<213> Homo sapiens

<400> 341

cagactgtgg tgactcagga gccctcactg actgtgtccc caggagggac agtcactctc 60
acctgt 66

<210> 342
<211> 66
<212> DNA
<213> Homo sapiens

<400> 342

caggctgtgg tgactcagga gccctcactg actgtgtccc caggagggac agtcactctc 60
acctgt 66

<210> 343
<211> 66
<212> DNA

<213> Homo sapiens

<400> 343

cagactgtgg tgacccagga gccatcgttc tcagtgtccc ctggagggac agtcacactc 60
acttgt 66

<210> 344
<211> 66
<212> DNA
<213> Homo sapiens

<400> 344

cagcctgtgc tgactcagcc accttctgca tcagcctccc tgggagcctc ggtcacactc 60
acctgc . 66

<210> 345
<211> 66
<212> DNA
<213> Homo sapiens

<400> 345

caggcagggc tgactcagcc accctcggtg tccaagggct tgagacagac cgccacactc 60
acctgc 66

<210> 346
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1) .. (6)
<223> A, T, C, G, other or unknown

<400> 346
nnnnnngact c 11

<210> 347
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (11)
<223> A, T, C, G, other or unknown

<400> 347
gagtcnnnnn n 11

<210> 348
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (3) .. (9)
<223> A, T, C, G, other or unknown

<400> 348
gcnnnnnnng c 11

<210> 349
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (11)
<223> A, T, C, G, other or unknown

<400> 349
acctgcnnnn n 11

<210> 350
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 350
cacatccgtg ttgttcacgg atgtg 25

<210> 351
<211> 88
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 351

aatagtagac tgcagtgtcc tcagccctta agctgttcat ctgcaagtag agagtattct 60
tagagttgtc tctagactta gtgaagcg 88

<210> 352
<211> 88
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 352

cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agggctgagg acactgcagt ctactatt 88

<210> 353
<211> 95
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 353

cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agggctgagg acactgcagt ctactattgt gcgag 95

<210> 354
<211> 95
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 354

cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agggctgagg acactgcagt ctactattgt acgag 95

<210> 355
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 355
cgcttcacta agtctagaga caac 24

<210> 356

<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (8) .. (15)
<223> A, T, C, G, other or unknown

<400> 356 cacctgcnnn nnnnn 15

<210> 357
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 357
cagctcnnnn nnnnnnn 17

<210> 358
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 358
gaagacnnnn nnnnnnn 17

<210> 359
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (17)
<223> A, T, C, G, other or unknown

<400> 359
gcagcnnnnn nnnnnnn 17

<210> 360
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (12)
<223> A, T, C, G, other or unknown

<400> 360
gaagacnnnn nn 12

<210> 361
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (22)
<223> A, T, C, G, other or unknown

<400> 361
cttgagnnnn nnnnnnnnnn nn 22

<210> 362
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (19)
<223> A, T, C, G, other or unknown

<400> 362
acggcnnnnn nnnnnnnnn 19

<210> 363
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (18)
<223> A, T, C, G, other or unknown

<400> 363
acggcnnnnn nnnnnnnn 18

<210> 364
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (12)
<223> A, T, C, G, other or unknown

<400> 364
gtatccnnnn nn 12

<210> 365
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (11)
<223> A, T, C, G, other or unknown

<400> 365
actgggnnnn n 11

<210> 366
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (10)
<223> A, T, C, G, other or unknown

<400> 366
ggatcnnnnn 10

<210> 367
<211> 11

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (11)
<223> A, T, C, G, other or unknown

<400> 367
gcatcnnnnn n 11

<210> 368
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (16)
<223> A, T, C, G, other or unknown

<400> 368
gaggagnnnn nnnnnn 16

<210> 369
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide
<220>
<221> modified_base
<222> (6) .. (19)
<223> A, T, C, G, other or unknown

<400> 369
gggacnnnnn nnnnnnnnn 19

<210> 370
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (14)
<223> A, T, C, G, other or unknown

<400> 370
acctgcnnnn nnnn 14

<210> 371
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 371
ggcggannnn nnnnnnn 17

<210> 372
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (22)
<223> A, T, C, G, other or unknown

<400> 372
ctgaagnnnn nnnnnnnnnn nn 22

<210> 373
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (11)
<223> A, T, C, G, other or unknown

<400> 373
cccgcnnnnn n 11

<210> 374
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (18)
<223> A, T, C, G, other or unknown

<400> 374
ggatgnnnnn nnnnnnnn 18

<210> 375
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (22)
<223> A, T, C, G, other or unknown

<400> 375
ctggagnnnn nnnnnnnnnn nn 22

<210> 376
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6)..(15)
<223> A, T, C, G, other or unknown

<400> 376
gacgcnnnnn nnnnn 15

<210> 377
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (13)
<223> A, T, C, G, other or unknown

<400> 377
ggtgannnnn nnn 13

<210> 378
<211> 13

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (13)
<223> A, T, C, G, other or unknown

<400> 378
gaagannnnn nnn 13

<210> 379
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (10)
<223> A, T, C, G, other or unknown

<400> 379
gagtcnnnnn 10

<210> 380
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (26)
<223> A, T, C, G, other or unknown

<400> 380
tccracnnnn nnnnnnnnnn nnnnnn 26

<210> 381
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5) .. (11)
<223> A, T, C, G, other or unknown

<400> 381
cctcnnnnnn n 11

<210> 382
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (10)
<223> A, T, C, G, other or unknown

<400> 382
gagtcnnnnn 10

<210> 383
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (18)
<223> A, T, C, G, other or unknown

<400> 383
cccacannnn nnnnnnnn 18

<210> 384
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (14)
<223> A, T, C, G, other or unknown

<400> 384
gcatcnnnnn nnnn 14

<210> 385
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (13)
<223> A, T, C, G, other or unknown

<400> 385
ggtgannnnn nnn 13

<210> 386
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5) .. (12)
<223> A, T, C, G, other or unknown

<400> 386
cccgnnnnnn nn 12

<210> 387
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (6) .. (19)
<223> A, T, C, G, other or unknown

<400> 387
ggatgnnnnn nnnnnnnnn 19

<210> 388
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 388
gaccgannnn nnnnnnn 17

<210> 389
<211> 17

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 389
cacccannnn nnnnnnn 17

<210> 390
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (7) .. (17)
<223> A, T, C, G, other or unknown

<400> 390
caarcannnn nnnnnnn 17

<210> 391
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 391
gctgtgtatt actgtgcgag 20

<210> 392
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 392
gccgtgtatt actgtgcgag 20

<210> 393
<211> 20
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic probe

<400> 393
gccgtatatt actgtgcgag 20

<210> 394
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 394
gccgtgtatt actgtacgag 20

<210> 395
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic probe

<400> 395
gccatgtatt actgtgcgag 20

<210> 396
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 396
cacatccgtg ttgttcacgg atgtg 25

<210> 397
<211> 88
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 397

```
aatagtagac tgcagtgtcc tcagccctta agctgttcat ctgcaagtag agagtattct 60
tagagttgtc tctagactta gtgaagcg                                    88
```

<210> 398
<211> 95
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 398

cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agggctgagg acactgcagt ctactattgt gcgag 95

<210> 399
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 399
cgcttcacta agtctagaga caac 24

<210> 400
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 400
cacatccgtg ttgttcacgg atgtgggagg atggagactg ggtc 44

<210> 401
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 401
cacatccgtg ttgttcacgg atgtgggaga gtggagactg agtc 44

<210> 402
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 402
cacatccgtg ttgttcacgg atgtgggtgc ctggagactg cgtc 44

<210> 403
<211> 44
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 403
cacatccgtg ttgttcacgg atgtgggtgg ctggagactg cgtc 44

<210> 404
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 404
cctctactct tgtcacagtg cacaagacat ccag 34

<210> 405
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 405
cctctactct tgtcacagtg 20

<210> 406
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 406
ggaggatgga ctggatgtct tgtgcactgt gacaagagta gagg 44

<210> 407
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 407
ggagagtgga ctggatgtct tgtgcactgt gacaagagta gagg 44

<210> 408
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 408

ggtgcctgga ctggatgtct tgtgcactgt gacaagagta gagg 44

<210> 409
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 409
ggtggctgga ctggatgtct tgtgcactgt gacaagagta gagg 44

<210> 410
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 410
cacatccgtg ttgttcacgg atgtggatcg actgtccagg agac 44

<210> 411
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 411
cacatccgtg ttgttcacgg atgtggactg tctgtcccaa ggcc 44

<210> 412
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 412
cacatccgtg ttgttcacgg atgtggactg actgtccagg agac 44

<210> 413
<211> 44
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 413
cacatccgtg ttgttcacgg atgtggaccc tctgccctgg ggcc 44

<210> 414

<211> 59
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 414
cctctgactg agtgcacaga gtgctttaac ccaaccggct agtgttagcg gttccccgg 59

<210> 415
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 415

cctctgactg agtgcacaga gtgctttaac ccaaccggct agtgttagcg gttccccggg 60
acagtcgat 69

<210> 416
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 416

cctctgactg agtgcacaga gtgctttaac ccaaccggct agtgttagcg gttccccggg 60
acagacagt 69

<210> 417
<211> 69
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 417

cctctgactg agtgcacaga gtgctttaac ccaaccggct agtgttagcg gttccccggg 60
acagtcagt 69

<210> 418
<211> 70
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 418

cctctgactg agtgcacaga gtgctttaac ccaaccggct agtgttagcg gtstccccgg 60
ggcagagggt 70

<210> 419
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 419
cctctgactg agtgcacaga gtgc 24

<210> 420
<211> 13
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5)..(9)
<223> A, T, C, G, other or unknown

<400> 420
ggccnnnnng gcc 13

<210> 421
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (12)
<223> A, T, C, G, other or unknown

<400> 421
ccannnnnnn nntgg 15

<210> 422
<211> 12
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (9)
<223> A, T, C, G, other or unknown

<400> 422
cgannnnnnt gc 12

<210> 423
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (8)
<223> A, T, C, G, other or unknown

<400> 423
gccnnnnngg c 11

<210> 424
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (7)
<223> A, T, C, G, other or unknown

<400> 424
gatnnnnatc 10

<210> 425
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (8)
<223> A, T, C, G, other or unknown

<400> 425
gacnnnnngt c 11

<210> 426
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (8)
<223> A, T, C, G, other or unknown

<400> 426
gcannnnntg c 11

<210> 427
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (12)
<223> A, T, C, G, other or unknown

<400> 427
gtatccnnnn nn 12

<210> 428
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (9)
<223> A, T, C, G, other or unknown

<400> 428
gacnnnnnng tc 12

<210> 429
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (4) .. (8)
<223> A, T, C, G, other or unknown

<400> 429
ccannnnntg g 11

<210> 430
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1) .. (6)
<223> A, T, C, G, other or unknown

<400> 430
nnnnnngaga cg 12

<210> 431
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (9)
<223> A, T, C, G, other or unknown

<400> 431
ccannnnnnt gg 12

<210> 432
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (7)
<223> A, T, C, G, other or unknown

<400> 432
gaannnnttc 10

<210> 433
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (11)
<223> A, T, C, G, other or unknown

<400> 433
ggtctcnnnn n 11

<210> 434
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1) .. (10)
<223> A, T, C, G, other or unknown

<400> 434
nnnnnnnnnn ctcctc 16

<210> 435
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1) .. (9)
<223> A, T, C, G, other or unknown

<400> 435
nnnnnnnnnt ccgcc 15

<210> 436
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5)..(9)
<223> A, T, C, G, other or unknown

<400> 436
ggccnnnnng gcc 13

<210> 437
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 437
ccannnnnnt gg 12

<210> 438
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 438
gacnnnnnng tc 12

<210> 439
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 439
cgannnnnnt gc 12

<210> 440
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 440
gcannnnntg c 11

<210> 441
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 441
ccannnnntg g 11

<210> 442
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide
<220>
<221> modified_base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 442
gaannnnttc 10

<210> 443
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(6)
<223> A, T, C, G, other or unknown

<400> 443
nnnnnngaga cg 12

<210> 444
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(12)
<223> A, T, C, G, other or unknown

<400> 444
gtatccnnnn nn 12

<210> 445
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, C, G, other or unknown

<400> 445
gacnnnnngt c 11

<210> 446
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 446
ggtctcnnnn n 11

<210> 447
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 447
gccnnnnngg c 11

<210> 448
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(12)
<223> A, T, C, G, other or unknown

<400> 448
ccannnnnnn nntgg 15

<210> 449
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (1)..(10)
<223> A, T, C, G, other or unknown

<400> 449
nnnnnnnnnn ctcctc 16

<210> 450
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(9)
<223> A, T, C, G, other or unknown

<400> 450
nnnnnnnnnt ccgcc 15

<210> 451
<211> 9532
<212> DNA
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3 nucleotide sequence

<220>
<221> CDS

<222> (1579)..(1638)

<220>
<221> CDS
<222> (2343)..(3443)

<220>
<221> CDS
<222> (3945)..(4400)

<220>
<221> CDS
<222> (4406)..(4450)

<220>
<221> CDS
<222> (4746)..(5789)

<400> 451

```
aatgctacta ctattagtag aattgatgcc acctttcag ctcgcgcccc aaatgaaaat 60

atagctaaac aggttattga ccatttgcga aatgtatcta atggtcaaac taaatctact 120

cgttcgcaga attgggaatc aactgttaca tggaatgaaa cttccagaca ccgtacttta 180

gttgcatatt taaaacatgt tgagctacag caccagattc agcaattaag ctctaagcca 240

tccgcaaaaa tgacctctta tcaaaaggag caattaaagg tactctctaa tcctgacctg 300

ttggagtttg cttccggtct ggttcgcttt gaagctcgaa ttaaaacgcg atatttgaag 360

tctttcgggc ttcctcttaa tctttttgat gcaatccgct ttgcttctga ctataatagt 420

cagggtaaag acctgatttt tgatttatgg tcattctcgt tttctgaact gtttaaagca 480

tttgaggggg attcaatgaa tatttatgac gattccgcag tattggacgc tatccagtct 540

aaacatttta ctattacccc ctctggcaaa acttcttttg caaaagcctc tcgctatttt 600

ggtttttatc gtcgtctggt aaacgagggt tatgatagtg ttgctcttac tatgcctcgt 660

aattccttttt ggcgttatgt atctgcatta gttgaatgtg gtattcctaa atctcaactg 720

atgaatcttt ctacctgtaa taatgttgtt ccgttagttc gttttattaa cgtagatttt 780

tcttcccaac gtcctgactg gtataatgag ccagttctta aaatcgcata aggtaattca 840

caatgattaa agttgaaatt aaaccatctc aagcccaatt tactactcgt tctggtgttt 900

ctcgtcaggg caagccttat tcactgaatg agcagctttg ttacgttgat ttgggtaatg 960

aatatccggt tcttgtcaag attactcttg atgaaggtca gccagcctat gcgcctggtc 1020

tgtacaccgt tcatctgtcc tctttcaaag ttggtcagtt cggttccctt atgattgacc 1080

gtctgcgcct cgttccggct aagtaacatg gagcaggtcg cggatttcga cacaatttat 1140

caggcgatga tacaaatctc cgttgtactt tgtttcgcgc ttggtataat cgctgggggt 1200

caaagatgag tgttttagtg tattctttcg cctctttcgt tttaggttgg tgccttcgta 1260

gtggcattac gtattttacc cgtttaatgg aaacttcctc atgaaaaagt ctttagtcct 1320

caaagcctct gtagccgttg ctaccctcgt tccgatgctg tctttcgctg ctgagggtga 1380

cgatcccgca aaagcggcct ttaactccct gcaagcctca gcgaccgaat atatcggtta 1440

tgcgtgggcg atggttgttg tcattgtcgg cgcaactatc ggtatcaagc tgtttaagaa 1500

attcacctcg aaagcaagct gataaaccga tacaattaaa ggctcctttt ggagcctttt 1560

tttttggaga ttttcaac gtg aaa aaa tta tta ttc gca att cct tta gtt   1611
                  Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val
                    1               5                  10

gtt cct ttc tat tct cac agt gca cag tctgtcgtga cgcagccgcc         1658
Val Pro Phe Tyr Ser His Ser Ala Gln
```

```
                15                  20

ctcagtgtct ggggccccag ggcagagggt caccatctcc tgcactggga gcagctccaa 1718

catcggggca ggttatgatg tacactggta ccagcagctt ccaggaacag cccccaaact 1778

cctcatctat ggtaacagca atcggccctc aggggtccct gaccgattct ctggctccaa 1838

gtctggcacc tcagcctccc tggccatcac tgggctccag gctgaggatg aggctgatta 1898

ttactgccag tcctatgaca gcagcctgag tggcctttat gtcttcggaa ctgggaccaa 1958

ggtcaccgtc ctaggtcagc ccaaggccaa ccccactgtc actctgttcc cgccctcctc 2018

tgaggagctc caagccaaca aggccacact agtgtgtctg atcagtgact tctacccggg 2078

agctgtgaca gtggcctgga aggcagatag cagccccgtc aaggcgggag tggagaccac 2138

cacaccctcc aaacaaagca acaacaagta cgcggccagc agctatctga gcctgacgcc 2198

tgagcagtgg aagtcccaca gaagctacag ctgccaggtc acgcatgaag ggagcaccgt 2258

ggagaagaca gtggccccta cagaatgttc ataataaacc gcctccaccg ggcgcgccaa 2318

ttctatttca aggagacagt cata atg aaa tac cta ttg cct acg gca gcc    2369
                          Met Lys Tyr Leu Leu Pro Thr Ala Ala
                                                  25

gct gga ttg tta tta ctc gcg gcc cag ccg gcc atg gcc gaa gtt caa   2417
Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met Ala Glu Val Gln
 30                  35                  40                  45

ttg tta gag tct ggt ggc ggt ctt gtt cag cct ggt ggt tct tta cgt   2465
Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
                50                  55                  60

ctt tct tgc gct gct tcc gga ttc act ttc tct tcg tac gct atg tct   2513
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser
            65                  70                  75

tgg gtt cgc caa gct cct ggt aaa ggt ttg gag tgg gtt tct gct atc   2561
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile
        80                  85                  90

tct ggt tct ggt ggc agt act tac tat gct gac tcc gtt aaa ggt cgc   2609
Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg
    95                  100                 105

ttc act atc tct aga gac aac tct aag aat act ctc tac ttg cag atg   2657
Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
110                 115                 120                 125

aac agc tta agg gct gag gac act gca gtc tac tat tgc gct aaa gac   2705
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp
                130                 135                 140

tat gaa ggt act ggt tat gct ttc gac ata tgg ggt caa ggt act atg   2753
Tyr Glu Gly Thr Gly Tyr Ala Phe Asp Ile Trp Gly Gln Gly Thr Met
```

```
                145                 150                 155

gtc acc gtc tct agt gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg   2801
Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu
        160                 165                 170

gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc   2849
Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys
    175                 180                 185

ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca   2897
Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser
190                 195                 200                 205

ggc gcc ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tct   2945
Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser
                210                 215                 220

agc gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tct tct agc   2993
Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser
            225                 230                 235

ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac   3041
Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn
        240                 245                 250

acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gcg gcc gct cat   3089
Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Ala Ala Ala His
    255                 260                 265

cac cac cat cat cac tct gct gaa caa aaa ctc atc tca gaa gag gat   3137
His His His His His Ser Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp
270                 275                 280                 285

ctg aat ggt gcc gca gat atc aac gat gat cgt atg gct ggc gcc gct   3185
Leu Asn Gly Ala Ala Asp Ile Asn Asp Asp Arg Met Ala Gly Ala Ala
                290                 295                 300

gaa act gtt gaa agt tgt tta gca aaa ccc cat aca gaa aat tca ttt   3233
Glu Thr Val Glu Ser Cys Leu Ala Lys Pro His Thr Glu Asn Ser Phe
            305                 310                 315

act aac gtc tgg aaa gac gac aaa act tta gat cgt tac gct aac tat   3281
Thr Asn Val Trp Lys Asp Asp Lys Thr Leu Asp Arg Tyr Ala Asn Tyr
        320                 325                 330

gag ggt tgt ctg tgg aat gct aca ggc gtt gta gtt tgt act ggt gac   3329
Glu Gly Cys Leu Trp Asn Ala Thr Gly Val Val Val Cys Thr Gly Asp
    335                 340                 345

gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt gct atc cct   3377
Glu Thr Gln Cys Tyr Gly Thr Trp Val Pro Ile Gly Leu Ala Ile Pro
350                 355                 360                 365

gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct gag ggt ggc ggt   3425
Glu Asn Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
                370                 375                 380
```

```
tct gag ggt ggc ggt act aaacctcctg agtacggtga tacacctatt          3473
Ser Glu Gly Gly Gly Thr
            385

ccgggctata cttatatcaa ccctctcgac ggcacttatc cgcctggtac tgagcaaaac 3533

cccgctaatc ctaatccttc tcttgaggag tctcagcctc ttaatacttt catgtttcag 3593

aataataggt tccgaaatag gcaggggggca ttaactgttt atacgggcac tgttactcaa 3653

ggcactgacc ccgttaaaac ttattaccag tacactcctg tatcatcaaa agccatgtat 3713

gacgcttact ggaacggtaa attcagagac tgcgctttcc attctggctt taatgaagat 3773

ccattcgttt gtgaatatca aggccaatcg tctgacctgc ctcaacctcc tgtcaatgct 3833

ggcggcggct ctggtggtgg ttctggtggc ggctctgagg gtggtggctc tgagggtggc 3893

ggttctgagg gtggcggctc tgagggaggc ggttccggtg gtggctctgg t tcc ggt  3950
                                                        Ser Gly

gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct atg acc   3998
Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr
390                 395                 400                 405

gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc aaa ctt   4046
Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu
                410                 415                 420

gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc att ggt   4094
Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly
            425                 430                 435

gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat ttt gct   4142
Asp Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala
        440                 445                 450

ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat tca cct   4190
Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro
    455                 460                 465

tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa tcg gtt   4238
Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val
470                 475                 480                 485

gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa ttt tct   4286
Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser
                490                 495                 500

att gat tgt gac aaa ata aac tta ttc cgt ggt gtc ttt gcg ttt ctt   4334
Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu
            505                 510                 515

tta tat gtt gcc acc ttt atg tat gta ttt tct acg ttt gct aac ata   4382
Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile
        520                 525                 530

ctg cgt aat aag gag tct taatc atg cca gtt ctt ttg ggt att ccg tta 4432
```

```
Leu Arg Asn Lys Glu Ser       Met Pro Val Leu Leu Gly Ile Pro Leu
    535                       540                 545

tta ttg cgt ttc ctc ggt ttccttctgg taactttgtt cggctatctg          4480
Leu Leu Arg Phe Leu Gly
    550

cttacttttc ttaaaaaggg cttcggtaag atagctattg ctatttcatt gtttcttgct 4540

cttattattg ggcttaactc aattcttgtg ggttatctct ctgatattag cgctcaatta 4600

ccctctgact ttgttcaggg tgttcagtta attctcccgt ctaatgcgct tccctgtttt 4660

tatgttattc tctctgtaaa ggctgctatt ttcatttttg acgttaaaca aaaaatcgtt 4720

tcttatttgg attgggataa ataat atg gct gtt tat ttt gta act ggc aaa   4772
                           Met Ala Val Tyr Phe Val Thr Gly Lys
                           555                 560

tta ggc tct gga aag acg ctc gtt agc gtt ggt aag att cag gat aaa   4820
Leu Gly Ser Gly Lys Thr Leu Val Ser Val Gly Lys Ile Gln Asp Lys
    565                 570                 575

att gta gct ggg tgc aaa ata gca act aat ctt gat tta agg ctt caa   4868
Ile Val Ala Gly Cys Lys Ile Ala Thr Asn Leu Asp Leu Arg Leu Gln
580                 585                 590                 595

aac ctc ccg caa gtc ggg agg ttc gct aaa acg cct cgc gtt ctt aga   4916
Asn Leu Pro Gln Val Gly Arg Phe Ala Lys Thr Pro Arg Val Leu Arg
                600                 605                 610

ata ccg gat aag cct tct ata tct gat ttg ctt gct att ggg cgc ggt   4964
Ile Pro Asp Lys Pro Ser Ile Ser Asp Leu Leu Ala Ile Gly Arg Gly
            615                 620                 625

aat gat tcc tac gat gaa aat aaa aac ggc ttg ctt gtt ctc gat gag   5012
Asn Asp Ser Tyr Asp Glu Asn Lys Asn Gly Leu Leu Val Leu Asp Glu
        630                 635                 640

tgc ggt act tgg ttt aat acc cgt tct tgg aat gat aag gaa aga cag   5060
Cys Gly Thr Trp Phe Asn Thr Arg Ser Trp Asn Asp Lys Glu Arg Gln
    645                 650                 655

ccg att att gat tgg ttt cta cat gct cgt aaa tta gga tgg gat att   5108
Pro Ile Ile Asp Trp Phe Leu His Ala Arg Lys Leu Gly Trp Asp Ile
660                 665                 670                 675

att ttt ctt gtt cag gac tta tct att gtt gat aaa cag gcg cgt tct   5156
Ile Phe Leu Val Gln Asp Leu Ser Ile Val Asp Lys Gln Ala Arg Ser
                680                 685                 690

gca tta gct gaa cat gtt gtt tat tgt cgt cgt ctg gac aga att act   5204
Ala Leu Ala Glu His Val Val Tyr Cys Arg Arg Leu Asp Arg Ile Thr
            695                 700                 705

tta cct ttt gtc ggt act tta tat tct ctt att act ggc tcg aaa atg   5252
Leu Pro Phe Val Gly Thr Leu Tyr Ser Leu Ile Thr Gly Ser Lys Met
        710                 715                 720
```

```
cct ctg cct aaa tta cat gtt ggc gtt gtt aaa tat ggc gat tct caa   5300
Pro Leu Pro Lys Leu His Val Gly Val Val Lys Tyr Gly Asp Ser Gln
    725                 730                 735

tta agc cct act gtt gag cgt tgg ctt tat act ggt aag aat ttg tat   5348
Leu Ser Pro Thr Val Glu Arg Trp Leu Tyr Thr Gly Lys Asn Leu Tyr
740                 745                 750                 755

aac gca tat gat act aaa cag gct ttt tct agt aat tat gat tcc ggt   5396
Asn Ala Tyr Asp Thr Lys Gln Ala Phe Ser Ser Asn Tyr Asp Ser Gly
                760                 765                 770

gtt tat tct tat tta acg cct tat tta tca cac ggt cgg tat ttc aaa   5444
Val Tyr Ser Tyr Leu Thr Pro Tyr Leu Ser His Gly Arg Tyr Phe Lys
            775                 780                 785

cca tta aat tta ggt cag aag atg aaa tta act aaa ata tat ttg aaa   5492
Pro Leu Asn Leu Gly Gln Lys Met Lys Leu Thr Lys Ile Tyr Leu Lys
        790                 795                 800

aag ttt tct cgc gtt ctt tgt ctt gcg att gga ttt gca tca gca ttt   5540
Lys Phe Ser Arg Val Leu Cys Leu Ala Ile Gly Phe Ala Ser Ala Phe
    805                 810                 815

aca tat agt tat ata acc caa cct aag ccg gag gtt aaa aag gta gtc   5588
Thr Tyr Ser Tyr Ile Thr Gln Pro Lys Pro Glu Val Lys Lys Val Val
820                 825                 830                 835

tct cag acc tat gat ttt gat aaa ttc act att gac tct tct cag cgt   5636
Ser Gln Thr Tyr Asp Phe Asp Lys Phe Thr Ile Asp Ser Ser Gln Arg
                840                 845                 850

ctt aat cta agc tat cgc tat gtt ttc aag gat tct aag gga aaa tta   5684
Leu Asn Leu Ser Tyr Arg Tyr Val Phe Lys Asp Ser Lys Gly Lys Leu
            855                 860                 865

att aat agc gac gat tta cag aag caa ggt tat tca ctc aca tat att   5732
Ile Asn Ser Asp Asp Leu Gln Lys Gln Gly Tyr Ser Leu Thr Tyr Ile
        870                 875                 880

gat tta tgt act gtt tcc att aaa aaa ggt aat tca aat gaa att gtt   5780
Asp Leu Cys Thr Val Ser Ile Lys Lys Gly Asn Ser Asn Glu Ile Val
    885                 890                 895

aaa tgt aat taattttgtt ttcttgatgt ttgtttcatc atcttctttt           5829
Lys Cys Asn
900

gctcaggtaa ttgaaatgaa taattcgcct ctgcgcgatt ttgtaacttg gtattcaaag 5889

caatcaggcg aatccgttat tgtttctccc gatgtaaaag gtactgttac tgtatattca 5949

tctgacgtta aacctgaaaa tctacgcaat ttctttattt ctgttttacg tgctaataat 6009

tttgatatgg ttggttcaat tccttccata attcagaagt ataatccaaa caatcaggat 6069

tatattgatg aattgccatc atctgataat caggaatatg atgataattc cgctccttct 6129
```

ggtggtttct ttgttccgca aaatgataat gttactcaaa cttttaaaat taataacgtt 6189

cgggcaaagg atttaatacg agttgtcgaa ttgtttgtaa agtctaatac ttctaaatcc 6249

tcaaatgtat tatctattga cggctctaat ctattagttg tttctgcacc taaagatatt 6309

ttagataacc ttcctcaatt ccttttctact gttgatttgc caactgacca gatattgatt 6369

gagggtttga tatttgaggt tcagcaaggt gatgctttag atttttcatt tgctgctggc 6429

tctcagcgtg gcactgttgc aggcggtgtt aatactgacc gcctcacctc tgttttatct 6489

tctgctggtg gttcgttcgg tatttttaat ggcgatgttt tagggctatc agttcgcgca 6549

ttaaagacta atagccattc aaaaatattg tctgtgccac gtattcttac gctttcaggt 6609

cagaagggtt ctatctctgt tggccagaat gtccctttta ttactggtcg tgtgactggt 6669

gaatctgcca atgtaaataa tccatttcag acgattgagc gtcaaaatgt aggtatttcc 6729

atgagcgttt ttcctgttgc aatggctggc ggtaatattg ttctggatat taccagcaag 6789

gccgatagtt tgagttcttc tactcaggca agtgatgtta ttactaatca aagaagtatt 6849

gctacaacgg ttaatttgcg tgatggacag actcttttac tcggtggcct cactgattat 6909

aaaaacactt ctcaagattc tggcgtaccg ttcctgtcta aaatcccttt aatcggcctc 6969

ctgtttagct cccgctctga ttccaacgag gaaagcacgt tatacgtgct cgtcaaagca 7029

accatagtac gcgccctgta gcggcgcatt aagcgcggcg ggtgtggtgg ttacgcgcag 7089

cgtgaccgct acacttgcca gcgccctagc gcccgctcct ttcgctttct tcccttcctt 7149

tctcgccacg ttcgccggct ttccccgtca agctctaaat cgggggctcc ctttagggtt 7209

ccgatttagt gctttacggc acctcgaccc caaaaaactt gatttgggtg atggttcacg 7269

tagtgggcca tcgccctgat agacggtttt tcgcccttg acgttggagt ccacgttctt 7329

taatagtgga ctcttgttcc aaactggaac aacactcaac cctatctcgg gctattcttt 7389

tgatttataa gggattttgc cgatttcgga accaccatca aacaggattt tcgcctgctg 7449

gggcaaacca gcgtggaccg cttgctgcaa ctctctcagg gccaggcggt gaagggcaat 7509

cagctgttgc ccgtctcact ggtgaaaaga aaaaccaccc tggatccaag cttgcaggtg 7569

gcacttttcg gggaaatgtg cgcggaaccc ctatttgttt atttttctaa atacattcaa 7629

atatgtatcc gctcatgaga caataaccct gataaatgct tcaataatat tgaaaaagga 7689

agagtatgag tattcaacat ttccgtgtcg cccttattcc ctttttgcg gcattttgcc 7749

ttcctgtttt tgctcaccca gaaacgctgg tgaaagtaaa agatgctgaa gatcagttgg 7809

gcgcacgagt gggttacatc gaactggatc tcaacagcgg taagatcctt gagagttttc 7869

gccccgaaga acgttttcca atgatgagca cttttaaagt tctgctatgt catacactat 7929

tatcccgtat tgacgccggg caagagcaac tcggtcgccg ggcgcggtat tctcagaatg 7989

acttggttga gtactcacca gtcacagaaa agcatcttac ggatggcatg acagtaagag 8049

aattatgcag tgctgccata accatgagtg ataacactgc ggccaactta cttctgacaa 8109

cgatcggagg accgaaggag ctaaccgctt ttttgcacaa catgggggat catgtaactc 8169

gccttgatcg ttgggaaccg gagctgaatg aagccatacc aaacgacgag cgtgacacca 8229

cgatgcctgt agcaatgcca acaacgttgc gcaaactatt aactggcgaa ctacttactc 8289

tagcttcccg gcaacaatta atagactgga tggaggcgga taaagttgca ggaccacttc 8349

tgcgctcggc ccttccggct ggctggttta ttgctgataa atctggagcc ggtgagcgtg 8409

ggtctcgcgg tatcattgca gcactggggc cagatggtaa gccctcccgt atcgtagtta 8469

tctacacgac ggggagtcag gcaactatgg atgaacgaaa tagacagatc gctgagatag 8529

gtgcctcact gattaagcat tggtaactgt cagaccaagt ttactcatat atactttaga 8589

ttgatttaaa acttcatttt taatttaaaa ggatctaggt gaagatcctt tttgataatc 8649

tcatgaccaa aatcccttaa cgtgagtttt cgttccactg tacgtaagac ccccaagctt 8709

gtcgactgaa tggcgaatgg cgctttgcct ggtttccggc accagaagcg gtgccggaaa 8769

gctggctgga gtgcgatctt cctgaggccg atactgtcgt cgtcccctca aactggcaga 8829

tgcacggtta cgatgcgccc atctacacca acgtaaccta tcccattacg gtcaatccgc 8889

cgtttgttcc cacggagaat ccgacgggtt gttactcgct cacatttaat gttgatgaaa 8949

gctggctaca ggaaggccag acgcgaatta tttttgatgg cgttcctatt ggttaaaaaa 9009

tgagctgatt taacaaaaat ttaacgcgaa ttttaacaaa atattaacgt ttacaattta 9069

aatatttgct tatacaatct tcctgttttt ggggcttttc tgattatcaa ccggggtaca 9129

tatgattgac atgctagttt tacgattacc gttcatcgat tctcttgttt gctccagact 9189

ctcaggcaat gacctgatag cctttgtaga tctctcaaaa atagctaccc tctccggcat 9249

gaatttatca gctagaacgg ttgaatatca tattgatggt gatttgactg tctccggcct 9309

ttctcaccct tttgaatctt tacctacaca ttactcaggc attgcattta aaatatatga 9369

gggttctaaa aatttttatc cttgcgttga ataaaggct tctcccgcaa aagtattaca 9429

gggtcataat gtttttggta caaccgattt agctttatgc tctgaggctt tattgcttaa 9489

ttttgctaat tctttgcctt gcctgtatga tttattggat gtt 9532

<210> 452
<211> 20
<212> PRT
<213> Unknown Organism

<220>

<223> Description of Unknown Organism: MALIA3 peptide sequence

<400> 452

```
Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
 1               5                  10                  15

His Ser Ala Gln
            20
```

<210> 453
<211> 367
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3 protein sequence

<400> 453

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
 1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly
            20                  25                  30

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
        35                  40                  45

Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly
    50                  55                  60

Lys Gly Leu Glu Trp Val Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr
65                  70                  75                  80

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
                85                  90                  95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Lys Asp Tyr Glu Gly Thr Gly Tyr Ala
        115                 120                 125

Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser
    130                 135                 140

Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
145                 150                 155                 160

Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
```

```
                    165                 170                 175

Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
            180                 185                 190

His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
        195                 200                 205

Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
    210                 215                 220

Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
225                 230                 235                 240

Glu Pro Lys Ser Cys Ala Ala Ala His His His His His His Ser Ala
                245                 250                 255

Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala Asp Ile
            260                 265                 270

Asn Asp Asp Arg Met Ala Gly Ala Ala Glu Thr Val Glu Ser Cys Leu
        275                 280                 285

Ala Lys Pro His Thr Glu Asn Ser Phe Thr Asn Val Trp Lys Asp Asp
    290                 295                 300

Lys Thr Leu Asp Arg Tyr Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala
305                 310                 315                 320

Thr Gly Val Val Val Cys Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr
                325                 330                 335

Trp Val Pro Ile Gly Leu Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser
            340                 345                 350

Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Thr
        355                 360                 365
```

<210> 454
<211> 152
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3 protein sequence

<400> 454

```
Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala
  1               5                  10                  15

Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly
             20                  25                  30

Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe
         35                  40                  45
```

```
Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp
 50                  55                  60

Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn
65                  70                  75                  80

Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln
                85                  90                  95

Ser Val Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu
            100                 105                 110

Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala
        115                 120                 125

Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala
    130                 135                 140

Asn Ile Leu Arg Asn Lys Glu Ser
145                 150
```

<210> 455
<211> 15
<212> PRT
<213> Unknown Organism
<220>
<223> Description of Unknown Organism: MALIA3 peptide sequence

<400> 455

```
Met Pro Val Leu Leu Gly Ile Pro Leu Leu Leu Arg Phe Leu Gly
 1               5                  10                  15
```

<210> 456
<211> 34B
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3 protein sequence

<400> 456

```
Met Ala Val Tyr Phe Val Thr Gly Lys Leu Gly Ser Gly Lys Thr Leu
 1               5                  10                  15

Val Ser Val Gly Lys Ile Gln Asp Lys Ile Val Ala Gly Cys Lys Ile
            20                  25                  30

Ala Thr Asn Leu Asp Leu Arg Leu Gln Asn Leu Pro Gln Val Gly Arg
        35                  40                  45

Phe Ala Lys Thr Pro Arg Val Leu Arg Ile Pro Asp Lys Pro Ser Ile
    50                  55                  60
```

```
Ser Asp Leu Leu Ala Ile Gly Arg Gly Asn Asp Ser Tyr Asp Glu Asn
 65                  70                  75                  80

Lys Asn Gly Leu Leu Val Leu Asp Glu Cys Gly Thr Trp Phe Asn Thr
                 85                  90                  95

Arg Ser Trp Asn Asp Lys Glu Arg Gln Pro Ile Ile Asp Trp Phe Leu
            100                 105                 110

His Ala Arg Lys Leu Gly Trp Asp Ile Ile Phe Leu Val Gln Asp Leu
        115                 120                 125

Ser Ile Val Asp Lys Gln Ala Arg Ser Ala Leu Ala Glu His Val Val
    130                 135                 140

Tyr Cys Arg Arg Leu Asp Arg Ile Thr Leu Pro Phe Val Gly Thr Leu
145                 150                 155                 160

Tyr Ser Leu Ile Thr Gly Ser Lys Met Pro Leu Pro Lys Leu His Val
                165                 170                 175

Gly Val Val Lys Tyr Gly Asp Ser Gln Leu Ser Pro Thr Val Glu Arg
            180                 185                 190

Trp Leu Tyr Thr Gly Lys Asn Leu Tyr Asn Ala Tyr Asp Thr Lys Gln
        195                 200                 205

Ala Phe Ser Ser Asn Tyr Asp Ser Gly Val Tyr Ser Tyr Leu Thr Pro
    210                 215                 220

Tyr Leu Ser His Gly Arg Tyr Phe Lys Pro Leu Asn Leu Gly Gln Lys
225                 230                 235                 240

Met Lys Leu Thr Lys Ile Tyr Leu Lys Lys Phe Ser Arg Val Leu Cys
                245                 250                 255

Leu Ala Ile Gly Phe Ala Ser Ala Phe Thr Tyr Ser Tyr Ile Thr Gln
            260                 265                 270

Pro Lys Pro Glu Val Lys Lys Val Val Ser Gln Thr Tyr Asp Phe Asp
        275                 280                 285

Lys Phe Thr Ile Asp Ser Ser Gln Arg Leu Asn Leu Ser Tyr Arg Tyr
    290                 295                 300

Val Phe Lys Asp Ser Lys Gly Lys Leu Ile Asn Ser Asp Asp Leu Gln
305                 310                 315                 320

Lys Gln Gly Tyr Ser Leu Thr Tyr Ile Asp Leu Cys Thr Val Ser Ile
                325                 330                 335

Lys Lys Gly Asn Ser Asn Glu Ile Val Lys Cys Asn
            340                 345
```

<210> 457
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 457
tggaagaggc acgttctttt cttt 24

<210> 458
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 458
cttttctttg ttgccgttgg ggtg 24

<210> 459
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 459
acactctccc ctgttgaagc tctt 24

<210> 460
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 460
accgcctcca ccgggcgcgc cttattaaca ctctcccctg ttgaagctct t 51

<210> 461
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 461
tgaacattct gtaggggcca ctg 23

<210> 462
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 462
agagcattct gcaggggcca ctg 23

<210> 463
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 463
accgcctcca ccgggcgcgc cttattatga acattctgta ggggccactg 50

<210> 464
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 464
accgcctcca ccgggcgcgc cttattaaga gcattctgca ggggccactg 50

<210> 465
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 465
cgactggagc acgaggacac tga 23

<210> 466
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 466
ggacactgac atggactgaa ggagta 26

<210> 467
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 467
gggaggatgg agactgggtc 20

<210> 468
<211> 20
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 468
gggaagatgg agactgggtc 20

<210> 469
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 469
gggagagtgg agactgagtc 20

<210> 470
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 470
gggtgcctgg agactgcgtc 20

<210> 471
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 471
gggtggctgg agactgcgtc 20

<210> 472
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 472
gggaggatgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 473
<211> 50
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 473
gggaagatgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 474
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 474
gggagagtgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 475
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 475
gggtgcctgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 476
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 476
gggtggctgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 477
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 477
gggagtctgg agactgggtc atctggatgt cttgtgcact gtgacagagg 50

<210> 478
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 478

cctctgtcac agtgcacaag acatccagat gacccagtct cc 42

<210> 479
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 479
cctctgtcac agtgcacaag ac 22

<210> 480
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 480
acactctccc ctgttgaagc tctt 24

<210> 481
<211> 668
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(668)

<400> 481

```
agt gca caa gac atc cag atg acc cag tct cca gcc acc ctg tct gtg   48
Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val
  1               5                  10                  15

tct cca ggg gaa agg gcc acc ctc tcc tgc agg gcc agt cag agt gtt   96
Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val
             20                  25                  30

agt aac aac tta gcc tgg tac cag cag aaa cct ggc cag gtt ccc agg   144
Ser Asn Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Arg
         35                  40                  45

ctc ctc atc tat ggt gca tcc acc agg gcc act gat atc cca gcc agg   192
Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg
     50                  55                  60

ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga   240
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
 65                  70                  75                  80

ctg gag cct gaa gat ttt gca gtg tat tac tgt cag cgg tat ggt agc   288
Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser
                 85                  90                  95

tca ccg ggg tgg acg ttc ggc caa ggg acc aag gtg gaa atc aaa cga   336
Ser Pro Gly Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105                 110

act gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag   384
Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        115                 120                 125

ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat   432
Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    130                 135                 140

ccc aga gag gcc aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg   480
Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145                 150                 155                 160

ggt aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc   528


Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                165                 170                 175

tac agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa   576
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            180                 185                 190

cac aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg cct   624
His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        195                 200                 205

gtc aca aag agc ttc aac aaa gga gag tgt aag ggc gaa ttc gc        668
Val Thr Lys Ser Phe Asn Lys Gly Glu Cys Lys Gly Glu Phe Ala
    210                 215                 220
```

<210> 482
<211> 223
<212> PRT
<213> Homo sapiens

<400> 482

```
Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val
  1               5                  10                  15

Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val
            20                  25                  30

Ser Asn Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Val Pro Arg
         35                  40                  45

Leu Leu Ile Tyr Gly Ala Ser Thr Arg Ala Thr Asp Ile Pro Ala Arg
     50                  55                  60

Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg
 65                  70                  75                  80

Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Arg Tyr Gly Ser
                85                  90                  95

Ser Pro Gly Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
            100                 105                 110

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        115                 120                 125

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    130                 135                 140

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
145                 150                 155                 160

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                165                 170                 175

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            180                 185                 190

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        195                 200                 205

Val Thr Lys Ser Phe Asn Lys Gly Glu Cys Lys Gly Glu Phe Ala
    210                 215                 220
```

<210> 483
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 483
agccaccctg tct 13

<210> 484
<211> 700
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(699)

<400> 484

```
agt gca caa gac atc cag atg acc cag tct cct gcc acc ctg tct gtg   48
Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val
  1               5                  10                  15

tct cca ggt gaa aga gcc acc ctc tcc tgc agg gcc agt cag gtg tct   96
Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Val Ser
             20                  25                  30

cca ggg gaa aga gcc acc ctc tcc tgc aat ctt ctc agc aac tta gcc   144
Pro Gly Glu Arg Ala Thr Leu Ser Cys Asn Leu Leu Ser Asn Leu Ala
         35                  40                  45

tgg tac cag cag aaa cct ggc cag gct ccc agg ctc ctc atc tat ggt   192
Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly
     50                  55                  60

gct tcc acc ggg gcc att ggt atc cca gcc agg ttc agt ggc agt ggg   240
Ala Ser Thr Gly Ala Ile Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
 65                  70                  75                  80

tct ggg aca gag ttc act ctc acc atc agc agc ctg cag tct gaa gat   288
Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu Asp
                 85                  90                  95

ttt gca gtg tat ttc tgt cag cag tat ggt acc tca ccg ccc act ttc   336
Phe Ala Val Tyr Phe Cys Gln Gln Tyr Gly Thr Ser Pro Pro Thr Phe
            100                 105                 110
```

```
ggc gga ggg acc aag gtg gag atc aaa cga act gtg gct gca cca tct   384
Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser
        115                 120                 125

gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc   432
Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
    130                 135                 140

tct gtt gtg tgc ccg ctg aat aac ttc tat ccc aga gag gcc aaa gta   480
Ser Val Val Cys Pro Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
145                 150                 155                 160

cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt   528
Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
                165                 170                 175

gtc aca gag cag gac aac aag gac agc acc tac agc ctc agc agc acc   576
Val Thr Glu Gln Asp Asn Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
            180                 185                 190

ctg acg ctg agc aaa gta gac tac gag aaa cac gaa gtc tac gcc tgc   624
Leu Thr Leu Ser Lys Val Asp Tyr Glu Lys His Glu Val Tyr Ala Cys
        195                 200                 205

gaa gtc acc cat cag ggc ctt agc tcg ccc gtc acg aag agc ttc aac   672
Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
    210                 215                 220

agg gga gag tgt aag aaa gaa ttc gtt t                            700
Arg Gly Glu Cys Lys Lys Glu Phe Val
225                 230
```

<210> 485
<211> 233
<212> PRT
<213> Homo sapiens

<400> 485

```
Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Val
  1               5                  10                  15

Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Val Ser
             20                  25                  30

Pro Gly Glu Arg Ala Thr Leu Ser Cys Asn Leu Leu Ser Asn Leu Ala
         35                  40                  45

Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly
     50                  55                  60

Ala Ser Thr Gly Ala Ile Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly
 65                  70                  75                  80

Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Ser Glu Asp
                 85                  90                  95

Phe Ala Val Tyr Phe Cys Gln Gln Tyr Gly Thr Ser Pro Pro Thr Phe
```

```
                100                 105                 110

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser
        115                 120                 125

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
    130                 135                 140

Ser Val Val Cys Pro Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
145                 150                 155                 160

Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
                165                 170                 175

Val Thr Glu Gln Asp Asn Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
            180                 185                 190

Leu Thr Leu Ser Lys Val Asp Tyr Glu Lys His Glu Val Tyr Ala Cys
        195                 200                 205

Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
    210                 215                 220

Arg Gly Glu Cys Lys Lys Glu Phe Val
225                 230
```

<210> 486
<211> 419
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic 3-23 VH nucleotide sequence

<220>
<221> CDS
<222> (12)..(419)

<400> 486

```
ctgtctgaac g gcc cag ccg gcc atg gcc gaa gtt caa ttg tta gag tct  50
            Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser
              1               5                  10

ggt ggc ggt ctt gtt cag cct ggt ggt tct tta cgt ctt tct tgc gct   98
Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
     15                  20                  25

gct tcc gga ttc act ttc tct tcg tac gct atg tct tgg gtt cgc caa   146
Ala Ser Gly Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln
 30                  35                  40                  45

gct cct ggt aaa ggt ttg gag tgg gtt tct gct atc tct ggt tct ggt   194
Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ala Ile Ser Gly Ser Gly
                50                  55                  60
```

```
ggc agt act tac tat gct gac tcc gtt aaa ggt cgc ttc act atc tct   242
Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
            65                  70                  75

aga gac aac tct aag aat act ctc tac ttg cag atg aac agc tta agg   290
Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
        80                  85                  90

gct gag gac act gca gtc tac tat tgc gct aaa gac tat gaa ggt act   338
Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asp Tyr Glu Gly Thr
    95                  100                 105

ggt tat gct ttc gac ata tgg ggt caa ggt act atg gtc acc gtc tct   386
Gly Tyr Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser
110                 115                 120                 125

agt gcc tcc acc aag ggc cca tcg gtc ttc ccc                      419
Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
            130                 135
```

<210> 487
<211> 136
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic 3-23 VH protein sequence

<400> 487

```
Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly
  1               5                  10                  15

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
            20                  25                  30

Phe Thr Phe Ser Ser Tyr Ala Met Ser Trp Val Arg Gln Ala Pro Gly
        35                  40                  45

Lys Gly Leu Glu Trp Val Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr
    50                  55                  60

Tyr Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
65                  70                  75                  80

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
                85                  90                  95

Thr Ala Val Tyr Tyr Cys Ala Lys Asp Tyr Glu Gly Thr Gly Tyr Ala
            100                 105                 110

Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser
        115                 120                 125

Thr Lys Gly Pro Ser Val Phe Pro
    130                 135
```

<210> 488
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 488
ctgtctgaac ggcccagccg 20

<210> 489
<211> 83
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 489

```
ctgtctgaac ggcccagccg gccatggccg aagttcaatt gttagagtct ggtggcggtc 60
ttgttcagcc tggtggttct tta                                         83
```

<210> 490
<211> 54
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 490
gaaagtgaat ccggaagcag cgcaagaaag acgtaaagaa ccaccaggct gaac 54

<210> 491
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 491
agaaacccac tccaaacctt taccaggagc ttggcgaacc ca 42

<210> 492
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 492

```
agtgtcctca gcccttaagc tgttcatctg caagtagaga gtattcttag agttgtctct 60
agagatagtg aagcgacctt taacggagtc agca                             94
```

<210> 493
<211> 81

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 493

gcttaagggc tgaggacact gcagtctact attgcgctaa agactatgaa ggtactggtt 60
atgctttcga catatggggt c 81

<210> 494
<211> 72
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 494

ggggaagacc gatgggccct tggtggaggc actagagacg gtgaccatag taccttgacc 60
tatgtcgaaa gc 72

<210> 495
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 495
ggggaagacc gatgggccct tgg 23

<210> 496
<211> 56
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (22)..(24)
<223> A, T, C, G, other or unknown

<220>
<221> modified_base
<222> (28)..(30)
<223> A, T, C, G, other or unknown

<220>
<221> modified_base
<222> (34)..(36)

<223> A, T, C, G, other or unknown

<220>
<223> nnn codes for any amino acid but Cys

<400> 496
gcttccggat tcactttctc tnnntacnnn atgnnntggg ttcgccaagc tcctgg 56

<210> 497
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (19)..(21)
<223> A, T, C or G

<220>
<221> modified_base
<222> (25)..(30)
<223> A, T, C or G

<220>
<221> modified_base
<222> (40)..(42)
<223> A, T, C or G

<220>
<221> modified_base
<222> (46)..(48)
<223> A, T, C or G

<400> 497

ggtttggagt gggtttctnn natcnnnnnn tctggtggcn nnactnnnta tgctgactcc 60
gttaaagg 68

<210> 498
<211> 912
<212> DNA
<213> Escherichia coli

<400> 498

```
tccggagctt cagatctgtt tgccttttg tggggtggtg cagatcgcgt tacggagatc 60
gaccgactgc ttgagcaaaa gccacgctta actgctgatc aggcatggga tgttattcgc 120
caaaccagtc gtcaggatct taacctgagg ctttttttac ctactctgca agcagcgaca 180
tctggtttga cacagagcga tccgcgtcgt cagttggtag aaacattaac acgttgggat 240
ggcatcaatt tgcttaatga tgatggtaaa acctggcagc agccaggctc tgccatcctg 300
aacgtttggc tgaccagtat gttgaagcgt accgtagtgg ctgccgtacc tatgccattt 360
gataagtggt acagcgccag tggctacgaa acaacccagg acggcccaac tggttcgctg 420
aatataagtg ttggagcaaa aattttgtat gaggcggtgc agggagacaa atcaccaatc 480
ccacaggcgg ttgatctgtt tgctgggaaa ccacagcagg aggttgtgtt ggctgcgctg 540
gaagatacct gggagactct ttccaaacgc tatggcaata atgtgagtaa ctggaaaaca 600
cctgcaatgg ccttaacgtt ccgggcaaat aatttctttg gtgtaccgca ggccgcagcg 660
gaagaaacgc gtcatcaggc ggagtatcaa aaccgtggaa cagaaaacga tatgattgtt 720
ttctcaccaa cgacaagcga tcgtcctgtg cttgcctggg atgtggtcgc acccggtcag 780
agtgggttta ttgctcccga tggaacagtt gataagcact atgaagatca gctgaaaatg 840
tacgaaaatt ttggccgtaa gtcgctctgg ttaacgaagc aggatgtgga ggcgcataag 900
gagtcgtcta ga                                                      912
```

<210> 499
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220> .
<221> modified_base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 499
gatnnnnatc 10

<210> 500
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(15)
<223> A, T, C, G, other or unknown

<400> 500
nnnnnnnnnn nnnnngtccc 20

<210> 501
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 501
gcannnnntg c 11

<210> 502
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (9)..(7)
<223> A, T, C, G, other or unknown

<400> 502
gacnnnngtc 10

<210> 503
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(7)
<223> A, T, C, G, other or unknown

<400> 503
nnnnnnngcg gg 12

<210> 504
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(12)
<223> A, T, C, G, other or unknown

<400> 504
gtatccnnnn nn 12

<210> 505
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 505
gcannnnnnt cg 12

<210> 506
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 506
gccnnnnngg c 11

<210> 507
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 507
ggtctcnnnn n 11

<210> 508
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(11)
<223> A, T, C, G, other or unknown

<400> 508
gacnnnnngt c 11

<210> 509
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 509
gacnnnnngt c 11

<210> 510
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 510
gacnnnnnng tc 12

<210> 511
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 511 ccannnnntg g 11

<210> 512
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (1)..(9)
<223> A, T, C, G, other or unknown

<400> 512
nnnnnnnnng caggt 15

<210> 513
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 513
acctgcnnnn n 11

<210> 514
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5)..(9)
<223> A, T, C, G, other or unknown

<400> 514
ggccnnnnng gcc 13

<210> 515
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(12)
<223> A, T, C, G, other or unknown

<400> 515
ccannnnnnn nntgg 15

<210> 516
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (11)
<223> A, T, C, G, other or unknown

<400> 516
cgtctcnnnn n 11

<210> 517
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(6)
<223> A, T, C, G, other or unknown

<400> 517
nnnnnngaga cg 12

<210> 518
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(10)
<223> A, T, C, G, other or unknown

<400> 518
nnnnnnnnnn ctcctc 16

<210> 519
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(16)
<223> A, T, C, G, other or unknown

<400> 519
gaggagnnnn nnnnnn 16

<210> 520
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 520
cctnnnnnag g 11

<210> 521
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 521
ccannnnnnt gg 12

<210> 522
<211> 6680
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 nucleotide sequence

<220>
<221> CDS
<222> (201)..(1058)

<220>
<221> CDS
<222> (2269)..(2682)

<220>
<221> CDS
<222> (2723)..(2866)

<220>
<221> CDS
<222> (3767)..(3850)

<220>
<221> CDS

<222> (4198)..(5799)

<400> 522

```
gacgaaaggg cctcgtgata cgcctatttt ataggttaa tgtcatgata ataatggttt 60

cttagacgtc aggtggcact tttcggggaa atgtgcgcgg aacccctatt tgtttatttt 120

tctaaataca ttcaaatatg tatccgctca tgagacaata accctgataa atgcttcaat 180

aatattgaaa aaggaagagt atg agt att caa cat ttc cgt gtc gcc ctt att 233
                    Met Ser Ile Gln His Phe Arg Val Ala Leu Ile
                      1               5                  10

ccc ttt ttt gcg gca ttt tgc ctt cct gtt ttt gct cac cca gaa acg   281
Pro Phe Phe Ala Ala Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr
            15                  20                  25

ctg gtg aaa gta aaa gat gct gaa gat cag ttg ggt gcc cga gtg ggt   329
Leu Val Lys Val Lys Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly
        30                  35                  40

tac atc gaa ctg gat ctc aac agc ggt aag atc ctt gag agt ttt cgc   377
Tyr Ile Glu Leu Asp Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg
    45                  50                  55

ccc gaa gaa cgt ttt cca atg atg agc act ttt aaa gtt ctg cta tgt   425
Pro Glu Glu Arg Phe Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys
60                  65                  70                  75

ggc gcg gta tta tcc cgt att gac gcc ggg caa gag caa ctc ggt cgc   473
Gly Ala Val Leu Ser Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg
                80                  85                  90

cgc ata cac tat tct cag aat gac ttg gtt gag tac tca cca gtc aca   521
Arg Ile His Tyr Ser Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr
            95                  100                 105

gaa aag cat ctt acg gat ggc atg aca gta aga gaa tta tgc agt gct   569
Glu Lys His Leu Thr Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala
        110                 115                 120

gcc ata acc atg agt gat aac act gcg gcc aac tta ctt ctg aca acg   617
Ala Ile Thr Met Ser Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr
    125                 130                 135

atc gga gga ccg aag gag cta acc gct ttt ttg cac aac atg ggg gat   665
Ile Gly Gly Pro Lys Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp
140                 145                 150                 155

cat gta act cgc ctt gat cgt tgg gaa ccg gag ctg aat gaa gcc ata   713
His Val Thr Arg Leu Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile
                160                 165                 170

cca aac gac gag cgt gac acc acg atg cct gta gca atg gca aca acg   761
Pro Asn Asp Glu Arg Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr
            175                 180                 185

ttg cgc aaa cta tta act ggc gaa cta ctt act cta gct tcc cgg caa   809
```

```
Leu Arg Lys Leu Leu Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln
        190                 195                 200

caa tta ata gac tgg atg gag gcg gat aaa gtt gca gga cca ctt ctg   857
Gln Leu Ile Asp Trp Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu
    205                 210                 215

cgc tcg gcc ctt ccg gct ggc tgg ttt att gct gat aaa tct gga gcc   905
Arg Ser Ala Leu Pro Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala
220                 225                 230                 235

ggt gag cgt ggg tct cgc ggt atc att gca gca ctg ggg cca gat ggt   953
Gly Glu Arg Gly Ser Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly
                240                 245                 250

aag ccc tcc cgt atc gta gtt atc tac acg acg ggg agt cag gca act   1001
Lys Pro Ser Arg Ile Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr
            255                 260                 265

atg gat gaa cga aat aga cag atc gct gag ata ggt gcc tca ctg att   1049
Met Asp Glu Arg Asn Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile
        270                 275                 280

aag cat tgg taactgtcag accaagttta ctcatatata ctttagattg           1098
Lys His Trp
    285

atttaaaact tcatttttaa tttaaaagga tctaggtgaa gatccttttt gataatctca 1158

tgaccaaaat cccttaacgt gagttttcgt tccactgagc gtcagacccc gtagaaaaga 1218

tcaaaggatc ttcttgagat cctttttttc tgcgcgtaat ctgctgcttg caaacaaaaa 1278

aaccaccgct accagcggtg gtttgtttgc cggatcaaga gctaccaact ctttttccga 1338

aggtaactgg cttcagcaga gcgcagatac caaatactgt ccttctagtg tagccgtagt 1398

taggccacca cttcaagaac tctgtagcac cgcctacata cctcgctctg ctaatcctgt 1458

taccagtggc tgctgccagt ggcgataagt cgtgtcttac cgggttggac tcaagacgat 1518

agttaccgga taaggcgcag cggtcgggct gaacgggggg ttcgtgcata cagcccagct 1578

tggagcgaac gacctacacc gaactgagat acctacagcg tgagcattga gaaagcgcca 1638

cgcttcccga agggagaaag gcggacaggt atccggtaag cggcagggtc ggaacaggag 1698

agcgcacgag ggagcttcca gggggaaacg cctggtatct ttatagtcct gtcgggtttc 1758

gccacctctg acttgagcgt cgatttttgt gatgctcgtc aggggggcgg agcctatgga 1818

aaaacgccag caacgcggcc tttttacggt tcctggcctt ttgctggcct tttgctcaca 1878

tgttctttcc tgcgttatcc cctgattctg tggataaccg tattaccgcc tttgagtgag 1938

ctgataccgc tcgccgcagc cgaacgaccg agcgcagcga gtcagtgagc gaggaagcgg 1998

aagagcgccc aatacgcaaa ccgcctctcc ccgcgcgttg gccgattcat taatgcagct 2058
```

```
ggcacgacag gtttcccgac tggaaagcgg gcagtgagcg caacgcaatt aatgtgagtt 2118

agctcactca ttaggcaccc caggctttac actttatgct tccggctcgt atgttgtgtg 2178

gaattgtgag cggataacaa tttcacacag gaaacagcta tgaccatgat tacgccaagc 2238

tttggagcct ttttttgga gattttcaac gtg aaa aaa tta tta ttc gca att  2292
                                Met Lys Lys Leu Leu Phe Ala Ile
                                                290

cct tta gtt gtt cct ttc tat tct cac agt gca cag gtc caa ctg cag   2340
Pro Leu Val Val Pro Phe Tyr Ser His Ser Ala Gln Val Gln Leu Gln
295                 300                 305                 310

gtc gac ctc gag atc aaa cgt gga act gtg gct gca cca tct gtc ttc   2388
Val Asp Leu Glu Ile Lys Arg Gly Thr Val Ala Ala Pro Ser Val Phe
                315                 320                 325

atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc tct gtt   2436
Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
            330                 335                 340

gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta cag tgg   2484
Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
        345                 350                 355

aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca   2532
Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr
    360                 365                 370

gag cag gac agc aag gac agc acc tac agc ctc agc agc acc ctg acg   2580
Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
375                 380                 385                 390

ctg agc aaa gca gac tac gag aaa cac aaa gtc tac gcc tgc gaa gtc   2628
Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
                395                 400                 405

acc cat cag ggc ctg agt tca ccg gtg aca aag agc ttc aac agg gga   2676
Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
            410                 415                 420

gag tgt taataaggcg cgccaattct atttcaagga gacagtcata atg aaa tac   2731
Glu Cys                                             Met Lys Tyr
                                                    425

cta ttg cct acg gca gcc gct gga ttg tta tta ctc gcg gcc cag ccg   2779
Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro
        430                 435                 440

gcc atg gcc gaa gtt caa ttg tta gag tct ggt ggc ggt ctt gtt cag   2827
Ala Met Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
    445                 450                 455

cct ggt ggt tct tta cgt ctt tct tgc gct gct tcc gga gcttcagatc    2876
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
460                 465                 470
```

```
tgtttgcctt tttgtggggt ggtgcagatc gcgttacgga gatcgaccga ctgcttgagc 2936

aaaagccacg cttaactgct gatcaggcat gggatgttat tcgccaaacc agtcgtcagg 2996

atcttaacct gaggcttttt ttacctactc tgcaagcagc gacatctggt ttgacacaga 3056

gcgatccgcg tcgtcagttg gtagaaacat taacacgttg ggatggcatc aatttgctta 3116

atgatgatgg taaaacctgg cagcagccag gctctgccat cctgaacgtt tggctgacca 3176

gtatgttgaa gcgtaccgta gtggctgccg tacctatgcc atttgataag tggtacagcg 3236

ccagtggcta cgaaacaacc caggacggcc caactggttc gctgaatata agtgttggag 3296

caaaaatttt gtatgaggcg gtgcagggag acaaatcacc aatcccacag gcggttgatc 3356

tgtttgctgg gaaaccacag caggaggttg tgttggctgc gctggaagat acctgggaga 3416

ctctttccaa acgctatggc aataatgtga gtaactggaa aacacctgca atggccttaa 3476

cgttccgggc aaataatttc tttggtgtac cgcaggccgc agcggaagaa acgcgtcatc 3536

aggcggagta tcaaaaccgt ggaacagaaa acgatatgat tgttttctca ccaacgacaa 3596

gcgatcgtcc tgtgcttgcc tgggatgtgg tcgcacccgg tcagagtggg tttattgctc 3656

ccgatggaac agttgataag cactatgaag atcagctgaa aatgtacgaa aattttggcc 3716

gtaagtcgct ctggttaacg aagcaggatg tggaggcgca taaggagtcg tct aga     3772
                                                      Ser Arg

gac aac tct aag aat act ctc tac ttg cag atg aac agc tta agt ctg    3820
Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Ser Leu
475                 480                 485                 490

agc att cgg tcc ggg caa cat tct cca aac tgaccagacg acacaaacgg     3870
Ser Ile Arg Ser Gly Gln His Ser Pro Asn
                495                 500

cttacgctaa atcccgcgca tgggatggta aagaggtggc gtctttgctg gcctggactc 3930

atcagatgaa ggccaaaaat tggcaggagt ggacacagca ggcagcgaaa caagcactga 3990

ccatcaactg gtactatgct gatgtaaacg gcaatattgg ttatgttcat actggtgctt 4050

atccagatcg tcaatcaggc catgatccgc gattacccgt tcctggtacg ggaaaatggg 4110

actggaaagg gctattgcct tttgaaatga accctaaggt gtataacccc cagaagctag 4170

cctgcggctt cggtcaccgt ctcaagc gcc tcc acc aag ggc cca tcg gtc ttc 4224
                              Ala Ser Thr Lys Gly Pro Ser Val Phe
                                                  505

ccc ctg gca ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg    4272
Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
510                 515                 520                 525
```

```
ggc tgc ctg gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg   4320
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                530                 535                 540

aac tca ggc gcc ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta   4368
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            545                 550                 555

cag tcc tca gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc   4416
Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        560                 565                 570

agc agc ttg ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc   4464
Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
    575                 580                 585

agc aac acc aag gtg gac aag aaa gtt gag ccc aaa tct tgt gcg gcc   4512
Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Ala Ala
590                 595                 600                 605

gca cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca   4560
Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser
                610                 615                 620

gaa gag gat ctg aat ggg gcc gca tag act gtt gaa agt tgt tta gca   4608
Glu Glu Asp Leu Asn Gly Ala Ala     Thr Val Glu Ser Cys Leu Ala
            625                 630                 635

aaa cct cat aca gaa aat tca ttt act aac gtc tgg aaa gac gac aaa   4656
Lys Pro His Thr Glu Asn Ser Phe Thr Asn Val Trp Lys Asp Asp Lys
            640                 645                 650

act tta gat cgt tac gct aac tat gag ggc tgt ctg tgg aat gct aca   4704
Thr Leu Asp Arg Tyr Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala Thr
        655                 660                 665

ggc gtt gtg gtt tgt act ggt gac gaa act cag tgt tac ggt aca tgg   4752
Gly Val Val Val Cys Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr Trp
    670                 675                 680

gtt cct att ggg ctt gct atc cct gaa aat gag ggt ggt ggc tct gag   4800
Val Pro Ile Gly Leu Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser Glu
685                 690                 695                 700

ggt ggc ggt tct gag ggt ggc ggt tct gag ggt ggc ggt act aaa cct   4848
Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Thr Lys Pro
                705                 710                 715

cct gag tac ggt gat aca cct att ccg ggc tat act tat atc aac cct   4896
Pro Glu Tyr Gly Asp Thr Pro Ile Pro Gly Tyr Thr Tyr Ile Asn Pro
            720                 725                 730

ctc gac ggc act tat ccg cct ggt act gag caa aac ccc gct aat cct   4944
Leu Asp Gly Thr Tyr Pro Pro Gly Thr Glu Gln Asn Pro Ala Asn Pro
        735                 740                 745

aat cct tct ctt gag gag tct cag cct ctt aat act ttc atg ttt cag   4992
Asn Pro Ser Leu Glu Glu Ser Gln Pro Leu Asn Thr Phe Met Phe Gln
```

```
    750                 755                 760

aat aat agg ttc cga aat agg cag ggt gca tta act gtt tat acg ggc   5040
Asn Asn Arg Phe Arg Asn Arg Gln Gly Ala Leu Thr Val Tyr Thr Gly
765                 770                 775                 780

act gtt act caa ggc act gac ccc gtt aaa act tat tac cag tac act   5088
Thr Val Thr Gln Gly Thr Asp Pro Val Lys Thr Tyr Tyr Gln Tyr Thr
                785                 790                 795

cct gta tca tca aaa gcc atg tat gac gct tac tgg aac ggt aaa ttc   5136
Pro Val Ser Ser Lys Ala Met Tyr Asp Ala Tyr Trp Asn Gly Lys Phe
            800                 805                 810

aga gac tgc gct ttc cat tct ggc ttt aat gag gat cca ttc gtt tgt   5184
Arg Asp Cys Ala Phe His Ser Gly Phe Asn Glu Asp Pro Phe Val Cys
        815                 820                 825

gaa tat caa ggc caa tcg tct gac ctg cct caa cct cct gtc aat gct   5232
Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro Pro Val Asn Ala
    830                 835                 840

ggc ggc ggc tct ggt ggt ggt tct ggt ggc ggc tct gag ggt ggc ggc   5280
Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly
845                 850                 855                 860

tct gag ggt ggc ggt tct gag ggt ggc ggc tct gag ggt ggc ggt tcc   5328
Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser
                865                 870                 875

ggt ggc ggc tcc ggt tcc ggt gat ttt gat tat gaa aaa atg gca aac   5376
Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn
            880                 885                 890

gct aat aag ggg gct atg acc gaa aat gcc gat gaa aac gcg cta cag   5424
Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln
        895                 900                 905

tct gac gct aaa ggc aaa ctt gat tct gtc gct act gat tac ggt gct   5472
Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala
    910                 915                 920

gct atc gat ggt ttc att ggt gac gtt tcc ggc ctt gct aat ggt aat   5520
Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn
925                 930                 935                 940

ggt gct act ggt gat ttt gct ggc tct aat tcc caa atg gct caa gtc   5568
Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val
                945                 950                 955

ggt gac ggt gat aat tca cct tta atg aat aat ttc cgt caa tat tta   5616
Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu
            960                 965                 970

cct tct ttg cct cag tcg gtt gaa tgt cgc cct tat gtc ttt ggc gct   5664
Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Tyr Val Phe Gly Ala
        975                 980                 985
```

```
ggt aaa cca tat gaa ttt tct att gat tgt gac aaa ata aac tta ttc   5712
Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe
    990                 995                1000

cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat gta   5760
Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val
1005              1010                1015                1020

ttt tcg acg ttt gct aac ata ctg cgt aat aag gag tct taataagaat    5809
Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys Glu Ser
               1025                1030

tcactggccg tcgttttaca acgtcgtgac tgggaaaacc ctggcgttac ccaacttaat 5869

cgccttgcag cacatccccc tttcgccagc tggcgtaata gcgaagaggc ccgcaccgat 5929

cgcccttccc aacagttgcg cagcctgaat ggcgaatggc gcctgatgcg gtattttctc 5989

cttacgcatc tgtgcggtat ttcacaccgc atataaattg taaacgttaa tattttgtta 6049

aaattcgcgt taaatttttg ttaaatcagc tcatttttta accaataggc cgaaatcggc 6109

aaaatccctt ataaatcaaa agaatagccc gagatagggt tgagtgttgt tccagtttgg 6169

aacaagagtc cactattaaa gaacgtggac tccaacgtca aagggcgaaa aaccgtctat 6229

cagggcgatg gcccactacg tgaaccatca cccaaatcaa gtttttgggg gtcgaggtgc 6289

cgtaaagcac taaatcggaa ccctaaaggg agcccccgat ttagagcttg acggggaaag 6349

ccggcgaacg tggcgagaaa ggaagggaag aaagcgaaag gagcgggcgc tagggcgctg 6409

gcaagtgtag cggtcacgct gcgcgtaacc accacacccg ccgcgcttaa tgcgccgcta 6469

cagggcgcgt actatggttg ctttgacggg tgcagtctca gtacaatctg ctctgatgcc 6529

gcatagttaa gccagccccg acacccgcca acacccgctg acgcgccctg acgggcttgt 6589

ctgctcccgg catccgctta cagacaagct gtgaccgtct ccgggagctg catgtgtcag 6649

aggttttcac cgtcatcacc gaaacgcgcg a                                 6680
```

<210> 523
<211> 286
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 protein sequence

<400> 523

```
Met Ser Ile Gln His Phe Arg Val Ala Leu Ile Pro Phe Phe Ala Ala
  1               5                  10                  15

Phe Cys Leu Pro Val Phe Ala His Pro Glu Thr Leu Val Lys Val Lys
             20                  25                  30
```

```
Asp Ala Glu Asp Gln Leu Gly Ala Arg Val Gly Tyr Ile Glu Leu Asp
         35                  40                  45

Leu Asn Ser Gly Lys Ile Leu Glu Ser Phe Arg Pro Glu Glu Arg Phe
     50                  55                  60

Pro Met Met Ser Thr Phe Lys Val Leu Leu Cys Gly Ala Val Leu Ser
 65                  70                  75                  80

Arg Ile Asp Ala Gly Gln Glu Gln Leu Gly Arg Arg Ile His Tyr Ser
                 85                  90                  95

Gln Asn Asp Leu Val Glu Tyr Ser Pro Val Thr Glu Lys His Leu Thr
            100                 105                 110

Asp Gly Met Thr Val Arg Glu Leu Cys Ser Ala Ala Ile Thr Met Ser
        115                 120                 125

Asp Asn Thr Ala Ala Asn Leu Leu Leu Thr Thr Ile Gly Gly Pro Lys
    130                 135                 140

Glu Leu Thr Ala Phe Leu His Asn Met Gly Asp His Val Thr Arg Leu
145                 150                 155                 160

Asp Arg Trp Glu Pro Glu Leu Asn Glu Ala Ile Pro Asn Asp Glu Arg
                165                 170                 175

Asp Thr Thr Met Pro Val Ala Met Ala Thr Thr Leu Arg Lys Leu Leu
            180                 185                 190

Thr Gly Glu Leu Leu Thr Leu Ala Ser Arg Gln Gln Leu Ile Asp Trp
        195                 200                 205

Met Glu Ala Asp Lys Val Ala Gly Pro Leu Leu Arg Ser Ala Leu Pro
    210                 215                 220

Ala Gly Trp Phe Ile Ala Asp Lys Ser Gly Ala Gly Glu Arg Gly Ser
225                 230                 235                 240

Arg Gly Ile Ile Ala Ala Leu Gly Pro Asp Gly Lys Pro Ser Arg Ile
                245                 250                 255

Val Val Ile Tyr Thr Thr Gly Ser Gln Ala Thr Met Asp Glu Arg Asn
            260                 265                 270

Arg Gln Ile Ala Glu Ile Gly Ala Ser Leu Ile Lys His Trp
        275                 280                 285
```

<210> 524
<211> 138
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 protein sequence

<400> 524

```
Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
 1               5                  10                  15

His Ser Ala Gln Val Gln Leu Gln Val Asp Leu Glu Ile Lys Arg Gly
            20                  25                  30

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
        35                  40                  45

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
    50                  55                  60

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
65                  70                  75                  80

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
                85                  90                  95

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
            100                 105                 110

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
        115                 120                 125

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
    130                 135
```

<210> 525
<211> 48
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 protein sequence

<400> 525

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
 1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly
            20                  25                  30

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
        35                  40                  45
```

<210> 526
<211> 28
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 protein sequence

<400> 526

```
Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu
  1               5                  10                  15

Ser Leu Ser Ile Arg Ser Gly Gln His Ser Pro Asn
            20                  25
```

<210> 527
<211> 533
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Vector pCES5 protein sequence

<400> 527

```
Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
  1               5                  10                  15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
            20                  25                  30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
        35                  40                  45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
    50                  55                  60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65                  70                  75                  80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
                85                  90                  95

Lys Val Glu Pro Lys Ser Cys Ala Ala Ala His His His His His His
            100                 105                 110

Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly Ala
        115                 120                 125

Ala Thr Val Glu Ser Cys Leu Ala Lys Pro His Thr Glu Asn Ser Phe
    130                 135                 140

Thr Asn Val Trp Lys Asp Asp Lys Thr Leu Asp Arg Tyr Ala Asn Tyr
145                 150                 155                 160

Glu Gly Cys Leu Trp Asn Ala Thr Gly Val Val Val Cys Thr Gly Asp
                165                 170                 175

Glu Thr Gln Cys Tyr Gly Thr Trp Val Pro Ile Gly Leu Ala Ile Pro
            180                 185                 190

Glu Asn Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
        195                 200                 205
```

```
Ser Glu Gly Gly Gly Thr Lys Pro Pro Glu Tyr Gly Asp Thr Pro Ile
    210                 215                 220

Pro Gly Tyr Thr Tyr Ile Asn Pro Leu Asp Gly Thr Tyr Pro Pro Gly
225                 230                 235                 240

Thr Glu Gln Asn Pro Ala Asn Pro Asn Pro Ser Leu Glu Glu Ser Gln
                245                 250                 255

Pro Leu Asn Thr Phe Met Phe Gln Asn Asn Arg Phe Arg Asn Arg Gln
            260                 265                 270

Gly Ala Leu Thr Val Tyr Thr Gly Thr Val Thr Gln Gly Thr Asp Pro
        275                 280                 285

Val Lys Thr Tyr Tyr Gln Tyr Thr Pro Val Ser Ser Lys Ala Met Tyr
    290                 295                 300

Asp Ala Tyr Trp Asn Gly Lys Phe Arg Asp Cys Ala Phe His Ser Gly
305                 310                 315                 320

Phe Asn Glu Asp Pro Phe Val Cys Glu Tyr Gln Gly Gln Ser Ser Asp
                325                 330                 335

Leu Pro Gln Pro Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser
            340                 345                 350

Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly
        355                 360                 365

Gly Gly Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp
    370                 375                 380

Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu
385                 390                 395                 400

Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp
                405                 410                 415

Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp
            420                 425                 430

Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly
        435                 440                 445

Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu
    450                 455                 460

Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu
465                 470                 475                 480

Cys Arg Pro Tyr Val Phe Gly Ala Gly Lys Pro Tyr Glu Phe Ser Ile
                485                 490                 495

Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu
            500                 505                 510

Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu
        515                 520                 525

Arg Asn Lys Glu Ser
    530
```

<210> 528
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 528
acctcactgg cttccggatt cactttctct 30

<210> 529
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 529
agaaacccac tccaaacctt taccaggagc ttggcgaacc ca 42

<210> 530
<211> 51
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 530
ggaaggcagt gatctagaga tagtgaagcg acctttaacg gagtcagcat a 51

<210> 531
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 531
ggaaggcagt gatctagaga tag 23

<210> 532
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 532
gtgctgactc agccaccctc 20

<210> 533
<211> 20
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 533
gccctgactc agcctgcctc 20

<210> 534
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 534
gagctgactc aggaccctgc 20

<210> 535
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 535
gagctgactc agccaccctc 20

<210> 536
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 536
cctcgacagc gaagtgcaca gagcgtcttg actcagcc 38

<210> 537
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 537
cctcgacagc gaagtgcaca gagcgtcttg 30

<210> 538
<211> 38
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 538
cctcgacagc gaagtgcaca gagcgctttg actcagcc 38

<210> 539
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 539
cctcgacagc gaagtgcaca gagcgctttg 30

<210> 540
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 540
cctcgacagc taagtgcaca gagcgctttg actcagcc 38

<210> 541
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 541
cctcgacagc gaagtgcaca gagcgctttg 30

<210> 542
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 542
cctcgacagc gaagtgcaca gagcgaattg actcagcc 38

<210> 543
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 543

cctcgacagc gaagtgcaca gagcgaattg 30

<210> 544
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 544
cctcgacagc gaagtgcaca gtacgaattg actcagcc 38

<210> 545
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 545
cctcgacagc gaagtgcaca gtacgaattg 30

<210> 546
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 546
cctcgacagc gaagtgcaca g 21

<210> 547
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 547
ccgtgtatta ctgtgcgaga g 21

<210> 548
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 548
ctgtgtatta ctgtgcgaga g 21

<210> 549

<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 549
ccgtatatta ctgtgcgaaa g 21

<210> 550
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 550
ctgtgtatta ctgtgcgaaa g 21

<210> 551
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 551
ctgtgtatta ctgtgcgaga c 21

<210> 552
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 552
ccatgtatta ctgtgcgaga c 21

<210> 553
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 553

ggtgtagtga tctagtgaca actctaagaa tactctctac ttgcagatga acagctttag 60
ggctgaggac actgcagtct actattgtgc gaga 94

<210> 554

<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 554

ggtgtagtga tctagtgaca actctaagaa tactctctac ttgcagatga acagctttag 60
ggctgaggac actgcagtct actattgtgc gaaa 94

<210> 555
<211> 85
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 555

atagtagact gcagtgtcct cagcccttaa gctgttcatc tgcaagtaga gagtattctt 60
agagttgtct ctagatcact acacc 85

<210> 556
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 556
gactgggtgt agtgatctag 20

<210> 557
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 557
cttttctttg ttgccgttgg ggtg 24

<210> 558
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>

<221> modified_base
<222> (1)..(9)
<223> A, T, C, G, other or unknown

<400> 558
nnnnnnnnng caggt 15

<210> 559
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 559
acctgcnnnn n 11

<210> 560
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 560
gatnnnnatc 10

<210> 561
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(16)
<223> A, T, C, G, other or unknown

<400> 561
gaggagnnnn nnnnnn 16

<210> 562
<211> 16
<212> DNA

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(10)
<223> A, T, C, G, other or unknown

<400> 562
nnnnnnnnnn ctcctc 16

<210> 563
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(10)
<223> A, T, C, G, other or unknown

<400> 563
ctcttcnnnn 10

<210> 564
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(5)
<223> A, T, C, G, other or unknown

<400> 564
nnnnngaaga g 11

<210> 565
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (1)..(15)
<223> A, T, C, G, other or unknown

<400> 565
nnnnnnnnnn nnnnngtccc 20

<210> 566
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 566
gacnnnnnng tc 12

<210> 567
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 567
cgtctcnnnn n 11

<210> 568
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7) .. (12)
<223> A, T, C, G, other or unknown

<400> 568
gtatccnnnn nn 12

<210> 569
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 569
gcannnnnnt cg 12

<210> 570
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (9)..(8)
<223> A, T, C, G, other or unknown

<400> 570
gccnnnnngg c 11

<210> 571
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(11)
<223> A, T, C, G, other or unknown

<400> 571
ggtctcnnnn n 11

<210> 572
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 572
gacnnnnngt c 11

<210> 573
<211> 11

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 573
gacnnnnngt c 11

<210> 574
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 574
ccannnnntg g 11

<210> 575
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(12)
<223> A, T, C, G, other or unknown

<400> 575
ccannnnnnn nntgg 15

<210> 576
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (5)..(9)
<223> A, T, C, G, other or unknown

<400> 576
ggccnnnnng gcc 13

<210> 577
<211> 12
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(9)
<223> A, T, C, G, other or unknown

<400> 577
ccannnnnnt gg 12

<210> 578
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 578
cctnnnnnag g 11

<210> 579
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(7)
<223> A, T, C, G, other or unknown

<400> 579
gacnnnngtc 10

<210> 580
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4) .. (12)
<223> A, T, C, G, other or unknown

<400> 580
ccannnnnnn nntgg 15

<210> 581
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (4)..(8)
<223> A, T, C, G, other or unknown

<400> 581
gcannnnntg c 11

<210> 582
<211> 10251
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 nucleotide sequence

<220>
<221> CDS
<222> (1578)..(1916)

<220>
<221> CDS
<222> (2388)..(2843)

<220>
<221> CDS
<222> (2849)..(2893)

<220>
<221> CDS
<222> (3189)..(4232)

<220>
<221> CDS
<222> (7418)..(8119)

<220>
<221> CDS
<222> (8160)..(9452)

<400> 582

```
aatgctacta ctattagtag aattgatgcc acctttcag ctcgcgcccc aaatgaaaat 60

atagctaaac aggttattga ccatttgcga aatgtatcta atggtcaaac taaatctact 120

cgttcgcaga attgggaatc aactgttata tggaatgaaa cttccagaca ccgtacttta 180


gttgcatatt taaaacatgt tgagctacag cattatattc agcaattaag ctctaagcca 240

tccgcaaaaa tgacctctta tcaaaaggag caattaaagg tactctctaa tcctgacctg 300

ttggagtttg cttccggtct ggttcgcttt gaagctcgaa ttaaaacgcg atatttgaag 360

tctttcgggc ttcctcttaa tctttttgat gcaatccgct ttgcttctga ctataatagt 420

cagggtaaag acctgatttt tgatttatgg tcattctcgt tttctgaact gtttaaagca 480

tttgaggggg attcaatgaa tatttatgac gattccgcag tattggacgc tatccagtct 540

aaacatttta ctattacccc ctctggcaaa acttcttttg caaaagcctc tcgctatttt 600

ggtttttatc gtcgtctggt aaacgagggt tatgatagtg ttgctcttac tatgcctcgt 660

aattcctttt ggcgttatgt atctgcatta gttgaatgtg gtattcctaa atctcaactg 720

atgaatcttt ctacctgtaa taatgttgtt ccgttagttc gttttattaa cgtagatttt 780

tcttcccaac gtcctgactg gtataatgag ccagttctta aaatcgcata aggtaattca 840

caatgattaa agttgaaatt aaaccatctc aagcccaatt tactactcgt tctggtgttt 900

ctcgtcaggg caagccttat tcactgaatg agcagctttg ttacgttgat ttgggtaatg 960

aatatccggt tcttgtcaag attactcttg atgaaggtca gccagcctat gcgcctggtc 1020

tgtacaccgt tcatctgtcc tctttcaaag ttggtcagtt cggttccctt atgattgacc 1080

gtctgcgcct cgttccggct aagtaacatg gagcaggtcg cggatttcga cacaatttat 1140

caggcgatga tacaaatctc cgttgtactt tgtttcgcgc ttggtataat cgctgggggt 1200

caaagatgag tgttttagtg tattcttttg cctctttcgt tttaggttgg tgccttcgta 1260

gtggcattac gtattttacc cgtttaatgg aaacttcctc atgaaaaagt ctttagtcct 1320

caaagcctct gtagccgttg ctaccctcgt tccgatgctg tctttcgctg ctgagggtga 1380

cgatcccgca aaagcggcct ttaactccct gcaagcctca gcgaccgaat atatcggtta 1440

tgcgtgggcg atggttgttg tcattgtcgg cgcaactatc ggtatcaagc tgtttaagaa 1500

attcacctcg aaagcaagct gataaaccga tacaattaaa ggctcctttt ggagcctttt 1560

ttttggagat tttcaac gtg aaa aaa tta tta ttc gca att cct tta gtt    1610
                  Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val
                    1               5                  10

gtt cct ttc tat tct ggc gcg gcc gaa tca cat cta gac ggc gcc gct   1658
Val Pro Phe Tyr Ser Gly Ala Ala Glu Ser His Leu Asp Gly Ala Ala
            15                  20                  25

gaa act gtt gaa agt tgt tta gca aaa tcc cat aca gaa aat tca ttt   1706
Glu Thr Val Glu Ser Cys Leu Ala Lys Ser His Thr Glu Asn Ser Phe
        30                  35                  40
```

```
act aac gtc tgg aaa gac gac aaa act tta gat cgt tac gct aac tat   1754
Thr Asn Val Trp Lys Asp Asp Lys Thr Leu Asp Arg Tyr Ala Asn Tyr
     45                  50                  55

gag ggc tgt ctg tgg aat gct aca ggc gtt gta gtt tgt act ggt gac   1802
Glu Gly Cys Leu Trp Asn Ala Thr Gly Val Val Val Cys Thr Gly Asp
 60                  65                  70                  75

gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt gct atc cct   1850
Glu Thr Gln Cys Tyr Gly Thr Trp Val Pro Ile Gly Leu Ala Ile Pro
                 80                  85                  90

gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct gag ggt ggc ggt   1898
Glu Asn Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
             95                 100                 105

tct gag ggt ggc ggt act aaacctcctg agtacggtga tacacctatt         1946
Ser Glu Gly Gly Gly Thr
        110

ccgggctata cttatatcaa ccctctcgac ggcacttatc cgcctggtac tgagcaaaac 2006

cccgctaatc ctaatccttc tcttgaggag tctcagcctc ttaatacttt catgtttcag 2066

aataataggt tccgaaatag gcaggggggca ttaactgttt atacgggcac tgttactcaa 2126

ggcactgacc ccgttaaaac ttattaccag tacactcctg tatcatcaaa agccatgtat 2186

gacgcttact ggaacggtaa attcagagac tgcgctttcc attctggctt taatgaggat 2246

ttatttgttt gtgaatatca aggccaatcg tctgacctgc ctcaacctcc tgtcaatgct 2306

ggcggcggct ctggtggtgg ttctggtggc ggctctgagg gtggtggctc tgagggaggc 2366

ggttccggtg gtggctctgg t tcc ggt gat ttt gat tat gaa aag atg gca   2417
                       Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala
                           115                 120

aac gct aat aag ggg gct atg acc gaa aat gcc gat gaa aac gcg cta   2465
Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn Ala Leu
    125                 130                 135

cag tct gac gct aaa ggc aaa ctt gat tct gtc gct act gat tac ggt   2513
Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly
140                 145                 150                 155

gct gct atc gat ggt ttc att ggt gac gtt tcc ggc ctt gct aat ggt   2561
Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu Ala Asn Gly
                160                 165                 170

aat ggt gct act ggt gat ttt gct ggc tct aat tcc caa atg gct caa   2609
Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln Met Ala Gln
            175                 180                 185

gtc ggt gac ggt gat aat tca cct tta atg aat aat ttc cgt caa tat   2657
Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr
        190                 195                 200
```

```
tta cct tcc ctc cct caa tcg gtt gaa tgt cgc cct ttt gtc ttt ggc   2705
Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Phe Val Phe Gly
    205                 210                 215

gct ggt aaa cca tat gaa ttt tct att gat tgt gac aaa ata aac tta   2753
Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu
220                 225                 230                 235

ttc cgt ggt gtc ttt gcg ttt ctt tta tat gtt gcc acc ttt atg tat   2801
Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr
                240                 245                 250

gta ttt tct acg ttt gct aac ata ctg cgt aat aag gag tct taatc atg 2851
Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys Glu Ser       Met
            255                 260                 265

cca gtt ctt ttg ggt att ccg tta tta ttg cgt ttc ctc ggt           2893
Pro Val Leu Leu Gly Ile Pro Leu Leu Leu Arg Phe Leu Gly
            270                 275                 280

ttccttctgg taactttgtt cggctatctg cttacttttc ttaaaaaggg cttcggtaag 2953

atagctattg ctatttcatt gtttcttgct cttattattg ggcttaactc aattcttgtg 3013

ggttatctct ctgatattag cgctcaatta ccctctgact ttgttcaggg tgttcagtta 3073

attctcccgt ctaatgcgct tccctgtttt tatgttattc tctctgtaaa ggctgctatt 3133

ttcatttttg acgttaaaca aaaaatcgtt tcttatttgg attgggataa ataat atg  3191
                                                              Met

gct gtt tat ttt gta act ggc aaa tta ggc tct gga aag acg ctc gtt   3239
Ala Val Tyr Phe Val Thr Gly Lys Leu Gly Ser Gly Lys Thr Leu Val
            285                 290                 295

agc gtt ggt aag att cag gat aaa att gta gct ggg tgc aaa ata gca   3287
Ser Val Gly Lys Ile Gln Asp Lys Ile Val Ala Gly Cys Lys Ile Ala
        300                 305                 310

act aat ctt gat tta agg ctt caa aac ctc ccg caa gtc ggg agg ttc   3335
Thr Asn Leu Asp Leu Arg Leu Gln Asn Leu Pro Gln Val Gly Arg Phe
    315                 320                 325

gct aaa acg cct cgc gtt ctt aga ata ccg gat aag cct tct ata tct   3383
Ala Lys Thr Pro Arg Val Leu Arg Ile Pro Asp Lys Pro Ser Ile Ser
330                 335                 340                 345

gat ttg ctt gct att ggg cgc ggt aat gat tcc tac gat gaa aat aaa   3431
Asp Leu Leu Ala Ile Gly Arg Gly Asn Asp Ser Tyr Asp Glu Asn Lys
                350                 355                 360

aac ggc ttg ctt gtt ctc gat gag tgc ggt act tgg ttt aat acc cgt   3479
Asn Gly Leu Leu Val Leu Asp Glu Cys Gly Thr Trp Phe Asn Thr Arg
            365                 370                 375

tct tgg aat gat aag gaa aga cag ccg att att gat tgg ttt cta cat   3527
Ser Trp Asn Asp Lys Glu Arg Gln Pro Ile Ile Asp Trp Phe Leu His
        380                 385                 390
```

```
gct cgt aaa tta gga tgg gat att att ttt ctt gtt cag gac tta tct   3575
Ala Arg Lys Leu Gly Trp Asp Ile Ile Phe Leu Val Gln Asp Leu Ser
    395                 400                 405

att gtt gat aaa cag gcg cgt tct gca tta gct gaa cat gtt gtt tat   3623
Ile Val Asp Lys Gln Ala Arg Ser Ala Leu Ala Glu His Val Val Tyr
410                 415                 420                 425

tgt cgt cgt ctg gac aga att act tta cct ttt gtc ggt act tta tat   3671
Cys Arg Arg Leu Asp Arg Ile Thr Leu Pro Phe Val Gly Thr Leu Tyr
                430                 435                 440

tct ctt att act ggc tcg aaa atg cct ctg cct aaa tta cat gtt ggc   3719
Ser Leu Ile Thr Gly Ser Lys Met Pro Leu Pro Lys Leu His Val Gly
            445                 450                 455

gtt gtt aaa tat ggc gat tct caa tta agc cct act gtt gag cgt tgg   3767
Val Val Lys Tyr Gly Asp Ser Gln Leu Ser Pro Thr Val Glu Arg Trp
        460                 465                 470

ctt tat act ggt aag aat ttg tat aac gca tat gat act aaa cag gct   3815
Leu Tyr Thr Gly Lys Asn Leu Tyr Asn Ala Tyr Asp Thr Lys Gln Ala
    475                 480                 485

ttt tct agt aat tat gat tcc ggt gtt tat tct tat tta acg cct tat   3863
Phe Ser Ser Asn Tyr Asp Ser Gly Val Tyr Ser Tyr Leu Thr Pro Tyr
490                 495                 500                 505

tta tca cac ggt cgg tat ttc aaa cca tta aat tta ggt cag aag atg   3911
Leu Ser His Gly Arg Tyr Phe Lys Pro Leu Asn Leu Gly Gln Lys Met
                510                 515                 520

aaa tta act aaa ata tat ttg aaa aag ttt tct cgc gtt ctt tgt ctt   3959
Lys Leu Thr Lys Ile Tyr Leu Lys Lys Phe Ser Arg Val Leu Cys Leu
            525                 530                 535

gcg att gga ttt gca tca gca ttt aca tat agt tat ata acc caa cct   4007
Ala Ile Gly Phe Ala Ser Ala Phe Thr Tyr Ser Tyr Ile Thr Gln Pro
        540                 545                 550

aag ccg gag gtt aaa aag gta gtc tct cag acc tat gat ttt gat aaa   4055
Lys Pro Glu Val Lys Lys Val Val Ser Gln Thr Tyr Asp Phe Asp Lys
    555                 560                 565

ttc act att gac tct tct cag cgt ctt aat cta agc tat cgc tat gtt   4103
Phe Thr Ile Asp Ser Ser Gln Arg Leu Asn Leu Ser Tyr Arg Tyr Val
570                 575                 580                 585

ttc aag gat tct aag gga aaa tta att aat agc gac gat tta cag aag   4151
Phe Lys Asp Ser Lys Gly Lys Leu Ile Asn Ser Asp Asp Leu Gln Lys
                590                 595                 600

caa ggt tat tca ctc aca tat att gat tta tgt act gtt tcc att aaa   4199
Gln Gly Tyr Ser Leu Thr Tyr Ile Asp Leu Cys Thr Val Ser Ile Lys
            605                 610                 615

aaa ggt aat tca aat gaa att gtt aaa tgt aat taattttgtt ttcttgatgt 4252
```

Lys Gly Asn Ser Asn Glu Ile Val Lys Cys Asn
620 625

ttgtttcatc atcttctttt gctcaggtaa ttgaaatgaa taattcgcct ctgcgcgatt 4312

ttgtaacttg gtattcaaag caatcaggcg aatccgttat tgtttctccc gatgtaaaag 4372

gtactgttac tgtatattca tctgacgtta aacctgaaaa tctacgcaat ttctttattt 4432

ctgttttacg tgcaaataat tttgatatgg taggttctaa cccttccatt attcagaagt 4492

ataatccaaa caatcaggat tatattgatg aattgccatc atctgataat caggaatatg 4552

atgataattc cgctccttct ggtggtttct ttgttccgca aaatgataat gttactcaaa 4612

cttttaaaat taataacgtt cgggcaaagg atttaatacg agttgtcgaa ttgtttgtaa 4672

agtctaatac ttctaaatcc tcaaatgtat tatctattga cggctctaat ctattagttg 4732

ttagtgctcc taaagatatt ttagataacc ttcctcaatt cctttcaact gttgatttgc 4792

caactgacca gatattgatt gagggtttga tatttgaggt tcagcaaggt gatgctttag 4852

atttttcatt tgctgctggc tctcagcgtg gcactgttgc aggcggtgtt aatactgacc 4912

gcctcacctc tgttttatct tctgctggtg gttcgttcgg tatttttaat ggcgatgttt 4972

tagggctatc agttcgcgca ttaaagacta atagccattc aaaaatattg tctgtgccac 5032

gtattcttac gctttcaggt cagaagggtt ctatctctgt tggccagaat gtcccttta 5092

ttactggtcg tgtgactggt gaatctgcca atgtaaataa tccatttcag acgattgagc 5152

gtcaaaatgt aggtatttcc atgagcgttt ttcctgttgc aatggctggc ggtaatattg 5212

ttctggatat taccagcaag gccgatagtt tgagttcttc tactcaggca agtgatgtta 5272

ttactaatca aagaagtatt gctacaacgg ttaatttgcg tgatggacag actcttttac 5332

tcggtggcct cactgattat aaaaacactt ctcaggattc tggcgtaccg ttcctgtcta 5392

aaatcccttt aatcggcctc ctgtttagct cccgctctga ttctaacgag gaaagcacgt 5452

tatacgtgct cgtcaaagca accatagtac gcgccctgta gcggcgcatt aagcgcggcg 5512

ggtgtggtgg ttacgcgcag cgtgaccgct acacttgcca gcgccctagc gcccgctcct 5572

ttcgctttct tcccttcctt tctcgccacg ttcgccggct ttccccgtca agctctaaat 5632

cgggggctcc ctttagggtt ccgatttagt gctttacggc acctcgaccc caaaaaactt 5692

gatttgggtg atggttcacg tagtgggcca tcgccctgat agacggtttt tcgccctttg 5752

acgttggagt ccacgttctt taatagtgga ctcttgttcc aaactggaac aacactcaac 5812

cctatctcgg gctattcttt tgatttataa gggattttgc cgatttcgga accaccatca 5872

aacaggattt tcgcctgctg gggcaaacca gcgtggaccg cttgctgcaa ctctctcagg 5932

```
gccaggcggt gaagggcaat cagctgttgc ccgtctcact ggtgaaaaga aaaaccaccc 5992

tggatccaag cttgcaggtg gcacttttcg gggaaatgtg cgcggaaccc ctatttgttt 6052

atttttctaa atacattcaa atatgtatcc gctcatgaga caataaccct gataaatgct 6112

tcaataatat tgaaaaagga agagtatgag tattcaacat ttccgtgtcg cccttattcc 6172

ctttttgcg gcattttgcc ttcctgtttt tgctcaccca gaaacgctgg tgaaagtaaa 6232

agatgctgaa gatcagttgg gcgcactagt gggttacatc gaactggatc tcaacagcgg 6292

taagatcctt gagagttttc gccccgaaga acgttttcca atgatgagca cttttaaagt 6352

tctgctatgt ggcgcggtat tatcccgtat tgacgccggg caagagcaac tcggtcgccg 6412

catacactat tctcagaatg acttggttga gtactcacca gtcacagaaa agcatcttac 6472

ggatggcatg acagtaagag aattatgcag tgctgccata accatgagtg ataacactgc 6532

ggccaactta cttctgacaa cgatcggagg accgaaggag ctaaccgctt ttttgcacaa 6592

catgggggat catgtaactc gccttgatcg ttgggaaccg gagctgaatg aagccatacc 6652

aaacgacgag cgtgacacca cgatgcctgt agcaatggca acaacgttgc gcaaactatt 6712

aactggcgaa ctacttactc tagcttcccg gcaacaatta atagactgga tggaggcgga 6772

taaagttgca ggaccacttc tgcgctcggc ccttccggct ggctggttta ttgctgataa 6832

atctggagcc ggtgagcgtg ggtctcgcgg tatcattgca gcactggggc cagatggtaa 6892

gccctcccgt atcgtagtta tctacacgac ggggagtcag gcaactatgg atgaacgaaa 6952

tagacagatc gctgagatag gtgcctcact gattaagcat tggtaactgt cagaccaagt 7012

ttactcatat atactttaga ttgatttaaa acttcatttt taatttaaaa ggatctaggt 7072

gaagatcctt tttgataatc tcatgaccaa aatcccttaa cgtgagtttt cgttccactg 7132

tacgtaagac ccccaagctt gtcgactgaa tggcgaatgg cgctttgcct ggtttccggc 7192

accagaagcg gtgccggaaa gctggctgga gtgcgatctt cctgacgctc gagcgcaacg 7252

caattaatgt gagttagctc actcattagg caccccaggc tttacacttt atgcttccgg 7312

ctcgtatgtt gtgtggaatt gtgagcggat aacaatttca cacaggaaac agctatgacc 7372

atgattacgc caagctttgg agccttttt ttggagattt tcaac gtg aaa aaa tta 7429
                                                Met Lys Lys Leu
                                                    630

tta ttc gca att cct tta gtt gtt cct ttc tat tct cac agt gca caa   7477
Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser His Ser Ala Gln
       635                 640                 645

gac atc cag atg acc cag tct cca gcc acc ctg tct ttg tct cca ggg   7525
```

```
Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
    650                 655                 660

gaa aga gcc acc ctc tcc tgc agg gcc agt cag ggt gtt agc agc tac  7573
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly Val Ser Ser Tyr
665                 670                 675                 680

tta gcc tgg tac cag cag aaa cct ggc cag gct ccc agg ctc ctc atc  7621
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
                685                 690                 695

tat gat gca tcc aac agg gcc act ggc atc cca gcc agg ttc agt ggc  7669
Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly
            700                 705                 710

agt ggg cct ggg aca gac ttc act ctc acc atc agc agc cta gag cct  7717
Ser Gly Pro Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
        715                 720                 725

gaa gat ttt gca gtt tat tac tgt cag cag cgt aac tgg cat ccg tgg  7765
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Asn Trp His Pro Trp
    730                 735                 740

acg ttc ggc caa ggg acc aag gtg gaa atc aaa cga act gtg gct gca  7813
Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
745                 750                 755                 760

cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct gga  7861
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
                765                 770                 775

act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc  7909
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
            780                 785                 790

aaa gta cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag  7957
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
        795                 800                 805

gag agt gtc aca gag cgg gac agc aag gac agc acc tac agc ctc agc  8005
Glu Ser Val Thr Glu Arg Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
    810                 815                 820

agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc tac  8053
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
825                 830                 835                 840

gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca aag agc  8101
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
                845                 850                 855

ttc aac agg gga gag tgt taataaggcg cgccaattct atttcaagga         8149
Phe Asn Arg Gly Glu Cys
            860

gacagtcata atg aaa tac cta ttg cct acg gca gcc gct gga ttg tta    8198
          Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu
                  865                 870                 875
```

```
tta ctc gcg gcc cag ccg gcc atg gcc gaa gtt caa ttg tta gag tct   8246
Leu Leu Ala Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser
                880                 885                 890

ggt ggc ggt ctt gtt cag cct ggt ggt tct tta cgt ctt tct tgc gct   8294
Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
            895                 900                 905

gct tcc gga ttc act ttc tct act tac gag atg cgt tgg gtt cgc caa   8342
Ala Ser Gly Phe Thr Phe Ser Thr Tyr Glu Met Arg Trp Val Arg Gln
        910                 915                 920

gct cct ggt aaa ggt ttg gag tgg gtt tct tat atc gct cct tct ggt   8390
Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Tyr Ile Ala Pro Ser Gly
    925                 930                 935

ggc gat act gct tat gct gac tcc gtt aaa ggt cgc ttc act atc tct   8438
Gly Asp Thr Ala Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
940                 945                 950                 955

aga gac aac tct aag aat act ctc tac ttg cag atg aac agc tta agg   8486
Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
                960                 965                 970

gct gag gac act gca gtc tac tat tgt gcg agg agg ctc gat ggc tat   8534
Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Arg Leu Asp Gly Tyr
            975                 980                 985

att tcc tac tac tac ggt atg gac gtc tgg ggc caa ggg acc acg gtc   8582
Ile Ser Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val
        990                 995                1000

acc gtc tca agc gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca   8630
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
   1005                1010                1015

ccc tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg   8678
Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
1020                1025                1030                1035

gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc   8726
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
               1040                1045                1050

gcc ctg acc agc ggc gtc cac acc ttc ccg gct gtc cta cag tcc tca   8774
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
           1055                1060                1065

gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tcc agc agc ttg   8822
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
       1070                1075                1080

ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc   8870
Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
   1085                1090                1095

aag gtg gac aag aaa gtt gag ccc aaa tct tgt gcg gcc gca cat cat   8918
```

```
Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Ala Ala Ala His His
1100                1105                1110                1115

cat cac cat cac ggg gcc gca gaa caa aaa ctc atc tca gaa gag gat   8966
His His His His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp
                1120                1125                1130

ctg aat ggg gcc gca tag gct agc tct gct wsy ggy gay tty gay tay   9014
Leu Asn Gly Ala Ala Gln Ala Ser Ser Ala Ser Gly Asp Phe Asp Tyr
            1135                1140                1145

gar aar atg gct aaw gcy aay aar ggs gcy atg acy gar aay gcy gay   9062
Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp
        1150                1155                1160

gar aay gck ytr car wsy gay gcy aar ggy aar ytw gay wsy gtc gck   9110
Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala
    1165                1170                1175

acy gay tay ggy gcy gcc atc gay ggy tty aty ggy gay gtc wsy ggy   9158
Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly
1180                1185                1190                1195

ytk gcy aay ggy aay ggy gcy acy ggw gay tty gcw ggy tck aat tcy   9206
Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser
                1200                1205                1210

car atg gcy car gty ggw gay ggk gay aay wsw cck ytw atg aay aay   9254
Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn
            1215                1220                1225

tty mgw car tay ytw cck tcy cty cck car wsk gty gar tgy cgy ccw   9302
Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro
        1230                1235                1240

tty gty tty wsy gcy ggy aar ccw tay gar tty wsy aty gay tgy gay   9350
Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp
    1245                1250                1255

aar atm aay ytw tty cgy ggy gty tty gck tty ytk yta tay gty gcy   9398
Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala
1260                1265                1270                1275

acy tty atg tay gtw tty wsy ack tty gcy aay atw ytr cgy aay aar   9446
Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys
                1280                1285                1290

gar wsy tagtgatctc ctaggaagcc cgcctaatga gcgggctttt tttttctggt   9502
Glu Ser

atgcatcctg aggccgatac tgtcgtcgtc ccctcaaact ggcagatgca cggttacgat 9562

gcgcccatct acaccaacgt gacctatccc attacggtca atccgccgtt tgttcccacg 9622

gagaatccga cgggttgtta ctcgctcaca tttaatgttg atgaaagctg gctacaggaa 9682

ggccagacgc gaattatttt tgatggcgtt cctattggtt aaaaaatgag ctgatttaac 9742
```

aaaaatttaa tgcgaatttt aacaaaatat taacgtttac aatttaaata tttgcttata 9802

caatcttcct gtttttgggg cttttctgat tatcaaccgg ggtacatatg attgacatgc 9862

tagttttacg attaccgttc atcgattctc ttgtttgctc cagactctca ggcaatgacc 9922

tgatagcctt tgtagatctc tcaaaaatag ctaccctctc cggcattaat ttatcagcta 9982

gaacggttga atatcatatt gatggtgatt tgactgtctc cggcctttct caccctttg 10042

aatctttacc tacacattac tcaggcattg catttaaaat atatgagggt tctaaaaatt 10102

tttatccttg cgttgaaata aaggcttctc ccgcaaaagt attacagggt cataatgttt 10162

ttggtacaac cgatttagct ttatgctctg aggctttatt gcttaatttt gctaattctt 10222

tgccttgcct gtatgattta ttggatgtt 10251

<210> 583
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 protein sequence

<400> 583

Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
1 5 10 15

Gly Ala Ala Glu Ser His Leu Asp Gly Ala Ala Glu Thr Val Glu Ser
20 25 30

Cys Leu Ala Lys Ser His Thr Glu Asn Ser Phe Thr Asn Val Trp Lys
35 40 45

Asp Asp Lys Thr Leu Asp Arg Tyr Ala Asn Tyr Glu Gly Cys Leu Trp
50 55 60

Asn Ala Thr Gly Val Val Val Cys Thr Gly Asp Glu Thr Gln Cys Tyr
65 70 75 80

Gly Thr Trp Val Pro Ile Gly Leu Ala Ile Pro Glu Asn Glu Gly Gly
85 90 95

Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
100 105 110

Thr

<210> 584
<211> 152
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 protein sequence

<400> 584

```
Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala
 1               5                  10                  15

Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly
            20                  25                  30

Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe
        35                  40                  45

Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp
    50                  55                  60

Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn
65                  70                  75                  80

Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln
                85                  90                  95

Ser Val Glu Cys Arg Pro Phe Val Phe Gly Ala Gly Lys Pro Tyr Glu
            100                 105                 110

Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala
        115                 120                 125

Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala
    130                 135                 140

Asn Ile Leu Arg Asn Lys Glu Ser
145                 150
```

<210> 585
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 peptide sequence

<400> 585

```
Met Pro Val Leu Leu Gly Ile Pro Leu Leu Leu Arg Phe Leu Gly
 1               5                  10                  15
```

<210> 586
<211> 348
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 protein sequence

<400> 586

```
Met Ala Val Tyr Phe Val Thr Gly Lys Leu Gly Ser Gly Lys Thr Leu
 1               5                  10                  15

Val Ser Val Gly Lys Ile Gln Asp Lys Ile Val Ala Gly Cys Lys Ile
            20                  25                  30

Ala Thr Asn Leu Asp Leu Arg Leu Gln Asn Leu Pro Gln Val Gly Arg
        35                  40                  45

Phe Ala Lys Thr Pro Arg Val Leu Arg Ile Pro Asp Lys Pro Ser Ile
    50                  55                  60

Ser Asp Leu Leu Ala Ile Gly Arg Gly Asn Asp Ser Tyr Asp Glu Asn
65                  70                  75                  80

Lys Asn Gly Leu Leu Val Leu Asp Glu Cys Gly Thr Trp Phe Asn Thr
                85                  90                  95

Arg Ser Trp Asn Asp Lys Glu Arg Gln Pro Ile Ile Asp Trp Phe Leu
            100                 105                 110

His Ala Arg Lys Leu Gly Trp Asp Ile Ile Phe Leu Val Gln Asp Leu
        115                 120                 125

Ser Ile Val Asp Lys Gln Ala Arg Ser Ala Leu Ala Glu His Val Val
    130                 135                 140

Tyr Cys Arg Arg Leu Asp Arg Ile Thr Leu Pro Phe Val Gly Thr Leu
145                 150                 155                 160

Tyr Ser Leu Ile Thr Gly Ser Lys Met Pro Leu Pro Lys Leu His Val
                165                 170                 175

Gly Val Val Lys Tyr Gly Asp Ser Gln Leu Ser Pro Thr Val Glu Arg
            180                 185                 190

Trp Leu Tyr Thr Gly Lys Asn Leu Tyr Asn Ala Tyr Asp Thr Lys Gln
        195                 200                 205

Ala Phe Ser Ser Asn Tyr Asp Ser Gly Val Tyr Ser Tyr Leu Thr Pro
    210                 215                 220

Tyr Leu Ser His Gly Arg Tyr Phe Lys Pro Leu Asn Leu Gly Gln Lys
225                 230                 235                 240

Met Lys Leu Thr Lys Ile Tyr Leu Lys Lys Phe Ser Arg Val Leu Cys
                245                 250                 255

Leu Ala Ile Gly Phe Ala Ser Ala Phe Thr Tyr Ser Tyr Ile Thr Gln
            260                 265                 270

Pro Lys Pro Glu Val Lys Lys Val Val Ser Gln Thr Tyr Asp Phe Asp
        275                 280                 285

Lys Phe Thr Ile Asp Ser Ser Gln Arg Leu Asn Leu Ser Tyr Arg Tyr
```

```
    290                 295                 300

Val Phe Lys Asp Ser Lys Gly Lys Leu Ile Asn Ser Asp Asp Leu Gln
305                 310                 315                 320

Lys Gln Gly Tyr Ser Leu Thr Tyr Ile Asp Leu Cys Thr Val Ser Ile
                325                 330                 335

Lys Lys Gly Asn Ser Asn Glu Ile Val Lys Cys Asn
            340                 345
```

<210> 587
<211> 234
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 protein sequence

<400> 587

```
Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
  1               5                  10                  15

His Ser Ala Gln Asp Ile Gln Met Thr Gln Ser Pro Ala Thr Leu Ser
             20                  25                  30

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Gly
         35                  40                  45

Val Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro
     50                  55                  60

Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala
 65                  70                  75                  80

Arg Phe Ser Gly Ser Gly Pro Gly Thr Asp Phe Thr Leu Thr Ile Ser
                 85                  90                  95

Ser Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Arg Asn
            100                 105                 110

Trp His Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
        115                 120                 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
    130                 135                 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145                 150                 155                 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
                165                 170                 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Arg Asp Ser Lys Asp Ser Thr
            180                 185                 190
```

```
Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
        195                 200                 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
    210                 215                 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230
```

<210> 588
<211> 431
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05 protein sequence

<400> 588

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Glu Val Gln Leu Leu Glu Ser Gly Gly Gly
             20                  25                  30

Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
         35                  40                  45

Phe Thr Phe Ser Thr Tyr Glu Met Arg Trp Val Arg Gln Ala Pro Gly
     50                  55                  60

Lys Gly Leu Glu Trp Val Ser Tyr Ile Ala Pro Ser Gly Gly Asp Thr
 65                  70                  75                  80

Ala Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
                 85                  90                  95

Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
            100                 105                 110

Thr Ala Val Tyr Tyr Cys Ala Arg Arg Leu Asp Gly Tyr Ile Ser Tyr
        115                 120                 125

Tyr Tyr Gly Met Asp Val Trp Gly Gln Gly Thr Thr Val Thr Val Ser
    130                 135                 140

Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
145                 150                 155                 160

Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
                165                 170                 175

Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
            180                 185                 190

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
        195                 200                 205
```

```
Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
    210                 215                 220

Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
225                 230                 235                 240

Lys Lys Val Glu Pro Lys Ser Cys Ala Ala Ala His His His His His
                245                 250                 255

His Gly Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Gly
            260                 265                 270

Ala Ala Gln Ala Ser Ser Ala Ser Gly Asp Phe Asp Tyr Glu Lys Met
        275                 280                 285

Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu Asn Ala
    290                 295                 300

Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr Asp Tyr
305                 310                 315                 320

Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu Ala Asn
                325                 330                 335

Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln Met Ala
            340                 345                 350

Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn Phe Arg Gln
        355                 360                 365

Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro Phe Val Phe
    370                 375                 380

Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp Lys Ile Asn
385                 390                 395                 400

Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala Thr Phe Met
                405                 410                 415

Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys Glu Ser
            420                 425                 430
```

<210> 589
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative peptide

<400> 589

```
Glu Gly Gly Gly Ser
  1               5
```

<210> 590
<211> 1275
<212> DNA
<213> Unknown Organism

<220>

<221> CDS
<222> (1) .. (1272)

<220>
<223> Description of Unknown Organism: M13 nucleotide sequence

<400> 590

```
gtg aaa aaa tta tta ttc gca att cct tta gtt gtt cct ttc tat tct   48
Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
  1               5                  10                  15

cac tcc gct gaa act gtt gaa agt tgt tta gca aaa ccc cat aca gaa   96
His Ser Ala Glu Thr Val Glu Ser Cys Leu Ala Lys Pro His Thr Glu
             20                  25                  30

aat tca ttt act aac gtc tgg aaa gac gac aaa act tta gat cgt tac   144
Asn Ser Phe Thr Asn Val Trp Lys Asp Asp Lys Thr Leu Asp Arg Tyr
         35                  40                  45

gct aac tat gag ggt tgt ctg tgg aat gct aca ggc gtt gta gtt tgt   192
Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala Thr Gly Val Val Val Cys
     50                  55                  60

act ggt gac gaa act cag tgt tac ggt aca tgg gtt cct att ggg ctt   240
Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr Trp Val Pro Ile Gly Leu
 65                  70                  75                  80

gct atc cct gaa aat gag ggt ggt ggc tct gag ggt ggc ggt tct gag   288
Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu
                 85                  90                  95

ggt ggc ggt tct gag ggt ggc ggt act aaa cct cct gag tac ggt gat   336
Gly Gly Gly Ser Glu Gly Gly Gly Thr Lys Pro Pro Glu Tyr Gly Asp
            100                 105                 110

aca cct att ccg ggc tat act tat atc aac cct ctc gac ggc act tat   384
Thr Pro Ile Pro Gly Tyr Thr Tyr Ile Asn Pro Leu Asp Gly Thr Tyr
        115                 120                 125

ccg cct ggt act gag caa aac ccc gct aat cct aat cct tct ctt gag   432
Pro Pro Gly Thr Glu Gln Asn Pro Ala Asn Pro Asn Pro Ser Leu Glu
    130                 135                 140

gag tct cag cct ctt aat act ttc atg ttt cag aat aat agg ttc cga   480
Glu Ser Gln Pro Leu Asn Thr Phe Met Phe Gln Asn Asn Arg Phe Arg
145                 150                 155                 160

aat agg cag ggg gca tta act gtt tat acg ggc act gtt act caa ggc   528
Asn Arg Gln Gly Ala Leu Thr Val Tyr Thr Gly Thr Val Thr Gln Gly
                165                 170                 175
```

```
act gac ccc gtt aaa act tat tac cag tac act cct gta tca tca aaa   576
Thr Asp Pro Val Lys Thr Tyr Tyr Gln Tyr Thr Pro Val Ser Ser Lys
            180                 185                 190

gcc atg tat gac gct tac tgg aac ggt aaa ttc aga gac tgc gct ttc   624
Ala Met Tyr Asp Ala Tyr Trp Asn Gly Lys Phe Arg Asp Cys Ala Phe
        195                 200                 205

cat tct ggc ttt aat gag gat cca ttc gtt tgt gaa tat caa ggc caa   672
His Ser Gly Phe Asn Glu Asp Pro Phe Val Cys Glu Tyr Gln Gly Gln
    210                 215                 220

tcg tct gac ctg cct caa cct cct gtc aat gct ggc ggc ggc tct ggt   720
Ser Ser Asp Leu Pro Gln Pro Pro Val Asn Ala Gly Gly Gly Ser Gly
225                 230                 235                 240

ggt ggt tct ggt ggc ggc tct gag ggt ggt ggc tct gag ggt ggc ggt   768
Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
                245                 250                 255

tct gag ggt ggc ggc tct gag gga ggc ggt tcc ggt ggt ggc tct ggt   816
Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly
            260                 265                 270

tcc ggt gat ttt gat tat gaa aag atg gca aac gct aat aag ggg gct   864
Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala
        275                 280                 285

atg acc gaa aat gcc gat gaa aac gcg cta cag tct gac gct aaa ggc   912
Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly
    290                 295                 300

aaa ctt gat tct gtc gct act gat tac ggt gct gct atc gat ggt ttc   960
Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe
305                 310                 315                 320

att ggt gac gtt tcc ggc ctt gct aat ggt aat ggt gct act ggt gat   1008
Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp
                325                 330                 335

ttt gct ggc tct aat tcc caa atg gct caa gtc ggt gac ggt gat aat   1056
Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn
            340                 345                 350

tca cct tta atg aat aat ttc cgt caa tat tta cct tcc ctc cct caa   1104
Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln
        355                 360                 365

tcg gtt gaa tgt cgc cct ttt gtc ttt agc gct ggt aaa cca tat gaa   1152
Ser Val Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu
    370                 375                 380

ttt tct att gat tgt gac aaa ata aac tta ttc cgt ggt gtc ttt gcg   1200
Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala
385                 390                 395                 400

ttt ctt tta tat gtt gcc acc ttt atg tat gta ttt tct acg ttt gct   1248
Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala
```

405 410 415

aac ata ctg cgt aat aag gag tct taa 1275
Asn Ile Leu Arg Asn Lys Glu Ser
420

<210> 591
<211> 424
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: M13 protein sequence

<400> 591

Met Lys Lys Leu Leu Phe Ala Ile Pro Leu Val Val Pro Phe Tyr Ser
1 5 10 15

His Ser Ala Glu Thr Val Glu Ser Cys Leu Ala Lys Pro His Thr Glu
20 25 30

Asn Ser Phe Thr Asn Val Trp Lys Asp Asp Lys Thr Leu Asp Arg Tyr
35 40 45

Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala Thr Gly Val Val Val Cys
50 55 60

Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr Trp Val Pro Ile Gly Leu
65 70 75 80

Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu
85 90 95

Gly Gly Gly Ser Glu Gly Gly Gly Thr Lys Pro Pro Glu Tyr Gly Asp
100 105 110

Thr Pro Ile Pro Gly Tyr Thr Tyr Ile Asn Pro Leu Asp Gly Thr Tyr
115 120 125

Pro Pro Gly Thr Glu Gln Asn Pro Ala Asn Pro Asn Pro Ser Leu Glu
130 135 140

Glu Ser Gln Pro Leu Asn Thr Phe Met Phe Gln Asn Asn Arg Phe Arg
145 150 155 160

Asn Arg Gln Gly Ala Leu Thr Val Tyr Thr Gly Thr Val Thr Gln Gly
165 170 175

Thr Asp Pro Val Lys Thr Tyr Tyr Gln Tyr Thr Pro Val Ser Ser Lys
180 185 190

Ala Met Tyr Asp Ala Tyr Trp Asn Gly Lys Phe Arg Asp Cys Ala Phe
195 200 205

His Ser Gly Phe Asn Glu Asp Pro Phe Val Cys Glu Tyr Gln Gly Gln
210 215 220

```
Ser Ser Asp Leu Pro Gln Pro Pro Val Asn Ala Gly Gly Gly Ser Gly
225                 230                 235                 240

Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
                245                 250                 255

Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly
            260                 265                 270

Ser Gly Asp Phe Asp Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala
        275                 280                 285

Met Thr Glu Asn Ala Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly
    290                 295                 300

Lys Leu Asp Ser Val Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe
305                 310                 315                 320

Ile Gly Asp Val Ser Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp
                325                 330                 335

Phe Ala Gly Ser Asn Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn
            340                 345                 350

Ser Pro Leu Met Asn Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln
        355                 360                 365

Ser Val Glu Cys Arg Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu
    370                 375                 380

Phe Ser Ile Asp Cys Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala
385                 390                 395                 400

Phe Leu Leu Tyr Val Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala
                405                 410                 415

Asn Ile Leu Arg Asn Lys Glu Ser
            420
```

<210> 592
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 592
caacgatgat cgtatggcgc atgctgccga gacag 35

<210> 593
<211> 1355
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: M13-III nucleotide sequence

<220>
<221> CDS
<222> (1) .. (1305)

<400> 593

```
gcg gcc gca cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc   48
Ala Ala Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu
  1               5                  10                  15

atc tca gaa gag gat ctg aat ggg gcc gca tag gct agc gat atc aac   96
Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala     Ala Ser Asp Ile Asn
             20                  25                  30

gat gat cgt atg gct tct act gcy gar acw gty gaa wsy tgy ytr gcm   144
Asp Asp Arg Met Ala Ser Thr Ala Glu Thr Val Glu Ser Cys Leu Ala
             35                  40                  45

aar ccy cay acw gar aat wsw tty acw aay gts tgg aar gay gay aar   192
Lys Pro His Thr Glu Asn Ser Phe Thr Asn Val Trp Lys Asp Asp Lys
         50                  55                  60

acy ytw gat cgw tay gcy aay tay gar ggy tgy ytr tgg aat gcy acm   240
Thr Leu Asp Arg Tyr Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala Thr
     65                  70                  75

ggc gty gtw gty tgy ack ggy gay gar acw car tgy tay ggy acr tgg   288
Gly Val Val Val Cys Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr Trp
 80                  85                  90                  95

gtk cck atw ggs ytw gcy atm cck gar aay gar ggy ggy ggy wsy gar   336
Val Pro Ile Gly Leu Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser Glu
                100                 105                 110

ggy ggy ggy wsy gar ggy ggy ggw tcy gar ggw ggy ggw acy aar cck   384
Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Thr Lys Pro
            115                 120                 125

cck gar tay ggy gay acw cck atw cck ggy tay acy tay aty aay cck   432
Pro Glu Tyr Gly Asp Thr Pro Ile Pro Gly Tyr Thr Tyr Ile Asn Pro
        130                 135                 140

ytm gay ggm acy tay cck cck ggy acy gar car aay ccy gcy aay cck   480
Leu Asp Gly Thr Tyr Pro Pro Gly Thr Glu Gln Asn Pro Ala Asn Pro
    145                 150                 155

aay ccw wsy ytw gar gar wsy car cck ytw aay acy tty atg tty car   528
Asn Pro Ser Leu Glu Glu Ser Gln Pro Leu Asn Thr Phe Met Phe Gln
160                 165                 170                 175

aay aay mgk tty mgr aay mgk car ggk gcw ytw acy gtk tay ack ggm   576
Asn Asn Arg Phe Arg Asn Arg Gln Gly Ala Leu Thr Val Tyr Thr Gly
                180                 185                 190
```

```
acy gty acy car ggy acy gay ccy gty aar acy tay tay car tay acy   624
Thr Val Thr Gln Gly Thr Asp Pro Val Lys Thr Tyr Tyr Gln Tyr Thr
            195                 200                 205

cck gtm tcr wsw aar gcy atg tay gay gcy tay tgg aay ggy aar tty   672
Pro Val Ser Ser Lys Ala Met Tyr Asp Ala Tyr Trp Asn Gly Lys Phe
        210                 215                 220

mgw gay tgy gcy tty cay wsy ggy tty aay gar gay ccw tty gty tgy   720
Arg Asp Cys Ala Phe His Ser Gly Phe Asn Glu Asp Pro Phe Val Cys
    225                 230                 235

gar tay car ggy car wsk wsy gay ytr cck car ccw cck gty aay gck   768
Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro Pro Val Asn Ala
240                 245                 250                 255

ggy ggy ggy wsy ggy ggw ggy wsy ggy ggy ggy wsy gar ggy ggw ggy   816
Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly
                260                 265                 270

wsy gar ggw ggy ggy wsy ggr ggy ggy wsy ggy wsy ggy gay tty gay   864
Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp
            275                 280                 285

tay gar aar atg gcw aay gcy aay aar ggs gcy atg acy gar aay gcy   912
Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala
        290                 295                 300

gay gar aay gcr ctr car wst gay gcy aar ggy aar ytw gay wsy gtc   960
Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val
    305                 310                 315

gcy acw gay tay ggt gct gcy atc gay ggy tty aty ggy gay gty wsy  1008
Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser
320                 325                 330                 335

ggy ctk gct aay ggy aay ggw gcy acy ggw gay tty gcw ggy tck aat  1056
Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn
                340                 345                 350

tcy car atg gcy car gty ggw gay ggk gay aay wsw cck ytw atg aay  1104
Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn
            355                 360                 365

aay tty mgw car tay ytw cck tcy cty cck car wsk gty gar tgy cgy  1152
Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg
        370                 375                 380

ccw tty gty tty wsy gcy ggy aar ccw tay gar tty wsy aty gay tgy  1200
Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys
    385                 390                 395

gay aar atm aay ytw ttc cgy ggy gty tty gck tty ytk yta tay gty  1248
Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val
400                 405                 410                 415

gcy acy tty atg tay gtw tty wsy ack tty gcy aay atw ytr cgy aay  1296
Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn
```

420 425 430

aar gar wsy tagtgatctc ctaggaagcc cgcctaatga gcgggctttt 1345
Lys Glu Ser

tttttctggt 1355

<210> 594
<211> 434
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: M13-III protein sequence

<400> 594

```
Ala Ala Ala His His His His His His Gly Ala Ala Glu Gln Lys Leu
 1               5                  10                  15

Ile Ser Glu Glu Asp Leu Asn Gly Ala Ala Ala Ser Asp Ile Asn Asp
            20                  25                  30

Asp Arg Met Ala Ser Thr Ala Glu Thr Val Glu Ser Cys Leu Ala Lys
        35                  40                  45

Pro His Thr Glu Asn Ser Phe Thr Asn Val Trp Lys Asp Asp Lys Thr
    50                  55                  60

Leu Asp Arg Tyr Ala Asn Tyr Glu Gly Cys Leu Trp Asn Ala Thr Gly
65                  70                  75                  80

Val Val Val Cys Thr Gly Asp Glu Thr Gln Cys Tyr Gly Thr Trp Val
                85                  90                  95

Pro Ile Gly Leu Ala Ile Pro Glu Asn Glu Gly Gly Gly Ser Glu Gly
            100                 105                 110

Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Thr Lys Pro Pro
        115                 120                 125

Glu Tyr Gly Asp Thr Pro Ile Pro Gly Tyr Thr Tyr Ile Asn Pro Leu
    130                 135                 140

Asp Gly Thr Tyr Pro Pro Gly Thr Glu Gln Asn Pro Ala Asn Pro Asn
145                 150                 155                 160

Pro Ser Leu Glu Glu Ser Gln Pro Leu Asn Thr Phe Met Phe Gln Asn
                165                 170                 175

Asn Arg Phe Arg Asn Arg Gln Gly Ala Leu Thr Val Tyr Thr Gly Thr
            180                 185                 190

Val Thr Gln Gly Thr Asp Pro Val Lys Thr Tyr Tyr Gln Tyr Thr Pro
        195                 200                 205
```

```
Val Ser Ser Lys Ala Met Tyr Asp Ala Tyr Trp Asn Gly Lys Phe Arg
    210                 215                 220

Asp Cys Ala Phe His Ser Gly Phe Asn Glu Asp Pro Phe Val Cys Glu
225                 230                 235                 240

Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro Pro Val Asn Ala Gly
                245                 250                 255

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly Ser
            260                 265                 270

Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr
        275                 280                 285

Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp
    290                 295                 300

Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala
305                 310                 315                 320

Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly
                325                 330                 335

Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser
            340                 345                 350

Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn
        355                 360                 365

Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro
    370                 375                 380

Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp
385                 390                 395                 400

Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala
                405                 410                 415

Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys
            420                 425                 430

Glu Ser
```

<210> 595
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 595
cgttgatatc gctagcctat gc 22

<210> 596
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 596
gataggctta gctagcccgg agaacgaagg 30

<210> 597
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 597
ctttcacagc ggtttcgcta gcgacccttt tgtctgc 37

<210> 598
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 598
ctttcacagc ggtttcgcta gcgacccttt tgtcagcgag taccagggtc 50

<210> 599
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 599
gactgtctcg gcagcatgcg ccatacgatc atcgttg 37

<210> 600
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> CDS
<222> (2)..(25)

<400> 600

c aac gat gat cgt atg gcg cat gct gccgagacag tc 37
Asn Asp Asp Arg Met Ala His Ala
1 5

<210> 601
<211> 8
<212> PRT

<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 601

Asn Asp Asp Arg Met Ala His Ala
1 5

<210> 602
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 602
ctttcacagc ggtttgcatg cagacccttt tgtctgc 37

<210> 603
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 603
ctttcacagc ggtttgcatg cagacccttt tgtcagcgag taccagggtc 50

<210> 604
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative peptide

<400> 604

Tyr Ala Asp Ser Val Lys Gly
1 5

<210> 605
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 605
cctcgacagc gaagtgcaca g 21

<210> 606
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 606
ggctgagtca agacgctctg tgcacttcgc tgtcgagg 38

<210> 607
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative peptide <400> 607

Gln Ser Ala Leu Thr Gln Pro
1 5

<210> 608
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 608
cctctgtcac agtgcacaag ac 22

<210> 609
<211> 42
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 609
cctctgtcac agtgcacaag acatccagat gacccagtct cc 42

<210> 610
<211> 50
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 610
gggaggatgg agactgggtc gtctggatgt cttgtgcact gtgacagagg 50

<210> 611
<211> 11

<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative peptide

<400> 611

Gln Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
1 5 10

<210> 612
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 612
gactgggtgt agtgatctag 20

<210> 613
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 613
ggtgtagtga tcttctagtg acaactct 28

<210> 614
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 614

Val Ser Ser Arg Asp Asn
1 5

<210> 615
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> CDS

<222> (1)..(15)

<400> 615

```
tac tat tgt gcg aaa                                                    15
Tyr Tyr Cys Ala Lys
 1               5
```

<210> 616
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic peptide

<400> 616

```
Tyr Tyr Cys Ala Lys
 1               5
```

<210> 617
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 617
ggtgccgata ggcttgcatg caccggagaa cgaagg 36

<210> 618
<211> 95
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 618

```
cgcttcacta agtctagaga caactctaag aatactctct acttgcagat gaacagctta 60
agggctgagg acactgcagt ctactattgt acgag                             95
```

<210> 619
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base

<222> (4) .. (7)
<223> A, T, C, G, other or unknown

<400> 619
gatnnnnatc 10

<210> 620
<211> 10
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3-derived peptide

<400> 620

```
Met Lys Leu Leu Asn Val Ile Asn Phe Val
 1               5                  10
```

<210> 621
<211> 29
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05-derived peptide

<400> 621

```
Met Ser Val Leu Val Tyr Ser Phe Ala Ser Phe Val Leu Gly Trp Cys
 1               5                  10                  15

Leu Arg Ser Gly Ile Thr Tyr Phe Thr Arg Leu Met Glu
            20                  25
```

<210> 622
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Illustrative nucleotide sequence

<400> 622
ttttttttt ttttt 15

<210> 623
<211> 87
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3-derived peptide

<400> 623

```
Met Ile Lys Val Glu Ile Lys Pro Ser Gln Ala Gln Phe Thr Thr Arg
 1               5                  10                  15

Ser Gly Val Ser Arg Gln Gly Lys Pro Tyr Ser Leu Asn Glu Gln Leu
            20                  25                  30

Cys Tyr Val Asp Leu Gly Asn Glu Tyr Pro Val Leu Val Lys Ile Thr
        35                  40                  45

Leu Asp Glu Gly Gln Pro Ala Tyr Ala Pro Gly Leu Tyr Thr Val His
    50                  55                  60

Leu Ser Ser Phe Lys Val Gly Gln Phe Gly Ser Leu Met Ile Asp Arg
65                  70                  75                  80

Leu Arg Leu Val Pro Ala Lys
                85
```

<210> 624
<211> 29
<212> PRT
<213> Unknown Organism

<220>
<223> Description of Unknown Organism: MALIA3-derived peptide

<400> 624

```
Met Ser Val Leu Val Tyr Ser Phe Ala Ser Phe Val Leu Gly Trp Cys
 1               5                  10                  15

Leu Arg Ser Gly Ile Thr Tyr Phe Thr Arg Leu Met Glu
            20                  25
```

<210> 625
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<220>
<221> modified_base
<222> (7)..(10)
<223> A, T, C, G, other or unknown

<400> 625
ctcttcnnnn 10

<210> 626
<211> 87
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05-derived peptide

<400> 626

Met Ile Lys Val Glu Ile Lys Pro Ser Gln Ala Gln Phe Thr Thr Arg
1 5 10 15

Ser Gly Val Ser Arg Gln Gly Lys Pro Tyr Ser Leu Asn Glu Gln Leu
20 25 30

Cys Tyr Val Asp Leu Gly Asn Glu Tyr Pro Val Leu Val Lys Ile Thr
35 40 45

Leu Asp Glu Gly Gln Pro Ala Tyr Ala Pro Gly Leu Tyr Thr Val His
50 55 60

Leu Ser Ser Phe Lys Val Gly Gln Phe Gly Ser Leu Met Ile Asp Arg

65 70 75 80

Leu Arg Leu Val Pro Ala Lys
85

<210> 627
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: CJRA05-derived peptide

<400> 627

Met Lys Leu Leu Asn Val Ile Asn Phe Val
1 5 10

<210> 628
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 628
gacccagtct ccatcctcc 19

<210> 629
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 629
gactcagtct ccactctcc 19

<210> 630
<211> 19

<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 630
gacgcagtct ccaggcacc 19

<210> 631
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 631
gacgcagtct ccagccacc 19

<210> 632
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 632
gtctcctgga cagtcgatc 19

<210> 633
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 633
ggccttggga cagacagtc 19

<210> 634
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 634
gtctcctgga cagtcagtc 19

<210> 635
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic oligonucleotide

<400> 635
ggccccaggg cagagggtc 19

Claims

1. A method for producing a population or library of immunoglobin genes that comprises the steps of:

   (i) introducing synthetic diversity into at least one of VH CDR1 or VH CDR2 of those genes; and
   (ii) combining the diversity from step (i) with VH CDR3 diversity captured from B cells.

2. The method according to claim 1, wherein synthetic diversity is introduced into both VH CDR1 and VH CDR2.

3. The method according to claim 2, wherein the synthetic diversity comprises:

   (a) a VH CDR1 having an amino acid sequence according to the formula -X1-Y-X2-M-X3-, wherein X1, X2, and X3 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; and
   (b) a VH CDR2, having an amino acid sequence according to the formula X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G-, wherein X4 and X5 are independently selected from the group consisting of Y, R, W, V, G, and S, X6 is selected from the group consisting of P and S, and X7 and X8 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y.

4. A library comprising a collection of genetic packages that display a member of a diverse family of peptides, polypeptides or proteins and that collectively display at least a portion of the family, the displayed peptides, polypeptides or proteins being encoded by DNA sequences comprising sequences encoding

   (a) a VH CDR1 having an amino acid sequence according to the formula -X1-Y-X2-M-X3-, wherein X1, X2, and X3 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y;
   (b) a VH CDR2 having an amino acid sequence according to the formula X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G-, wherein X4 and X5 are independently selected from the group consisting of Y, R, W, V, G, and S, X6 is selected from the group consisting of P and S, and X7 and X8 are independently selected from the group consisting of A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y; and
   (c) a sequence encoding a VH CDR3, wherein said VH CDR3 is a VH CDR3 captured from the VH CDR3 region of an immunoglobin gene from a B cell.

5. The library according to claim 4, wherein said DNA sequences further comprise a sequence encoding an immunoglobin light chain.

6. The library according to claim 5, wherein said sequence encoding an immunoglobin light chain is captured from a B cell.

7. The library according to claim 6, wherein said B cell is isolated from a blood sample from an autoimmune patient.

8. The library according to claim 7, wherein the autoimmune patient is diagnosed with a disorder selected from the group consisting of systemic lupus erythematosus, systemic sclerosis, rheumatoid arthritis, antiphospholipid syndrome and vasculitis.

9. The library according to any of claims 4 or 5 to 8, wherein said DNA sequences further comprise sequences encoding VH 3-23 framework regions.

10. The library according to any of claims 4 or 5 to 8, wherein said genetic packages are M13 phages.

11. The library according to claim 10, wherein said DNA sequences are in a phage vector.

12. The library according to claim 10 or 11, wherein said phage comprises a wild-type gene iii and a truncated gene iii for display of peptides, polypeptides or proteins.

13. The library according to any of claims 4 or 5 to 8, wherein said DNA sequences are in a phagemid vector.

14. The library according to any of claims 10 to 13, wherein said displayed peptides, polypeptides, or proteins are displayed through a short linker to the final portion of M13 gene III.

Patentansprüche

1. Verfahren zur Herstellung einer Population oder Bibliothek von Immunglobulingenen, welches die Schritte umfasst:

   (i) Einführung einer synthethischen Diversität in mindestens eine der VH-CDR1 oder VH-CDR2 dieser Gene; und
   (ii) Kombination der Diversität aus Schritt (i) mit VH-CDR3-Diversität, welche von B-Zellen gewonnen wurde.

2. Verfahren gemäß Anspruch 1, wobei synthethische Diversität sowohl in VH-CDR1 als auch in VH-CDR2 eingeführt wird.

3. Verfahren gemäß Anspruch 2, wobei die synthetische Diversität umfasst

   (a) eine VH-CDR1, welche eine Aminosäuresequenz gemäß der Formel -X1-Y-X2-M-X3- hat, wobei X1, X2 und X3 unabhängig ausgewählt sind aus der Gruppe bestehend aus A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W und Y; und
   (b) eine VH-CDR2, welche eine Aminosäuresequenz gemäß der Formel X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G- hat, wobei X4 und X5 unabhängig ausgewählt sind aus der Gruppe bestehend aus Y, R, W, V, G und S, X6 ausgewählt ist aus der Gruppe bestehend aus P und S, und X7 sowie X8 unabhängig ausgewählt sind aus der Gruppe bestehend aus A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W und Y.

4. Bibliothek, umfassend eine Auswahl von genetischen Paketen, die ein Mitglied einer unterschiedlichen Familie von Peptiden, Polypeptiden oder Proteinen darstellen und die kollektiv mindestens einen Teil der Familie darstellen, wobei die gezeigten Peptide, Polypeptide oder Proteine durch DNA-Sequenzen codiert werden, die Sequenzen umfassen, die

   (a) eine VH-CDR1 codieren, die eine Aminosäuresequenz gemäß der Formel -X1-X2-M-X3- hat, wobei X1, X2 und X3 unabhängig ausgewählt sind aus der Gruppe bestehend aus A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W und Y;
   (b) eine VH-CDR2 codieren, die eine Aminosäuresequenz gemäß der Formel X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G- hat, wobei X4 und X5 unabhängig ausgewählt sind aus der Gruppe bestehend aus Y, R, W, V, G und S, X6 ausgewählt ist aus der Gruppe bestehend aus P und S, und X7 sowie X8 unabhängig ausgewählt sind aus der Gruppe bestehend aus A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W und Y; und
   (c) eine Sequenz codieren, die VH-CDR3 codiert, wobei die VH-CDR3 eine VH-CDR3 ist, die aus der VH-CDR3-Region eines Immunglobulingens von einer B-Zelle gewonnen wurde.

5. Bibliothek gemäß Anspruch 4, wobei die DNA-Sequenzen ferner eine Sequenz umfassen, die eine leichte Kette eines Immunglobulins codiert.

6. Bibliothek gemäß Anspruch 5, wobei die Sequenz, die eine leichte Kette eines Immunglobulins codiert, aus einer B-Zelle gewonnen wurde.

7. Bibliothek gemäß Anspruch 6, wobei die B-Zelle aus einer Blutprobe von einem Autoimmunpatienten isoliert wurde.

8. Bibliothek gemäß Anspruch 7, wobei bei dem Autoimmunpatienten eine Krankheit ausgewählt aus der Gruppe bestehend aus systemischem Lupus erythematodes, systemischer Sklerose, rheumatoider Arthritis, Antiphospholipidsyndrom und Vaskulitis diagnostiziert wurde.

9. Bibliothek gemäß einem der Ansprüche 4 oder 5 bis 8, wobei die DNA-Sequenzen ferner Sequenzen umfassen, die VH 3-23-Gerüstregionen codieren.

10. Bibliothek gemäß einem der Ansprüche 4 oder 5 bis 8, wobei die genetischen Pakete M13-Phagen sind.

11. Bibliothek gemäß Anspruch 10, wobei die DNA-Sequenzen in einem Phagenvektor sind.

12. Bibliothek gemäß Anspruch 10 oder 11, wobei der Phage ein Wildtyp-Gen iii und ein verkürztes Gen iii zur Darstellung von Peptiden, Polypeptiden oder Proteinen umfasst.

13. Bibliothek gemäß einem der Ansprüche 4 oder 5 bis 8, wobei die DNA-Sequenzen in einem Phagemid-Vektor sind.

14. Bibliothek gemäß einem der Ansprüche 10 bis 13, wobei die gezeigten Peptide, Polypeptide, oder Proteine durch einen kurzen Linker zu dem finalen Teil des M13-Gens III gezeigt sind.

Revendications

1. Procédé de production d'une population ou d'une banque de gènes d'immunoglobulines qui comprend les étapes consistant à :

(i) introduire une diversité synthétique dans au moins une des VH-CDR1 ou VH-CDR2 de ces gènes; et
(ii) combiner la diversité introduite à l'étape (i) à la diversité de VH-CDR3 capturée à partir de cellules B.

2. Procédé selon la revendication 1, dans lequel la diversité synthétique est introduite à la fois dans VH-CDR1 et VH-CDR2.

3. Procédé selon la revendication 2, dans lequel la diversité synthétique comprend :

(a) une VH-CDR1 ayant une séquence d'acides aminés répondant à la formule - X1-Y-X2-M-X3-, dans laquelle X1, X2, et X3 sont indépendamment choisis dans le groupe constitué par A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, et Y; et
(b) une VH-CDR2 ayant une séquence d'acides aminés répondant à la formule X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G-, dans laquelle X4 et X5 sont indépendamment choisis dans le groupe constitué par Y, R, W, V, G et S, X6 est choisi dans le groupe constitué par P et S, et X7 et X8 sont indépendamment choisis dans le groupe constitué par A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, et Y.

4. Banque comprenant une collection d'ensembles génétiques qui présentent un membre d'une famille diverse de peptides, polypeptides ou protéines et qui présentent collectivement au moins une partie de la famille, les peptides, polypeptides ou protéines présentés étant codés par des séquences ADN comprenant des séquences codant pour

(a) une VH-CDR1 ayant une séquence d'acides aminés répondant à la formule - X1-Y-X2-M-X3-, dans laquelle X1, X2, et X3 sont indépendamment choisis dans le groupe constitué par A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, et Y;
(b) une VH-CDR2 ayant une séquence d'acides aminés répondant à la formule X4-I-X5-X6-S-G-G-X7-T-X8-Y-A-D-S-V-K-G-, dans laquelle X4 et X5 sont indépendamment choisis dans le groupe constitué par Y, R, W, V, G et S, X6 est choisi dans le groupe constitué par P et S, et X7 et X8 sont indépendamment choisis dans le groupe constitué par A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, et Y; et
(c) une séquence codant pour une VH-CDR3, dans laquelle ladite VH-CDR3 est une VH-CDR3 capturée à partir de la région VH-CDR3 d'un gène d'immunoglobuline provenant d'une cellule B.

5. Banque selon la revendication 4, dans laquelle lesdites séquences ADN comprennent en outre une séquence codant pour une chaîne légère d'immunoglobuline.

6. Banque selon la revendication 5, dans laquelle ladite séquence codant pour une chaîne légère d'immunoglobuline est capturée à partir d'une cellule B.

7. Banque selon la revendication 6, dans laquelle ladite cellule B est isolée à partir d'un échantillon de sang prélevé sur un patient auto-immun.

8. Banque selon la revendication 7, dans laquelle le patient auto-immun est diagnostiqué comme étant atteint d'un

trouble choisi dans le groupe constitué par le lupus érythémateux systémique, la sclérose systémique, la polyarthrite rhumatoïde, le syndrome des antiphospholipides et la vasculite.

9. Banque selon l'une quelconque des revendications 4 ou 5 à 8, dans laquelle lesdites séquences ADN comprennent en outre des séquences codant pour des régions charpentes de VH 3-23.

10. Banque selon l'une quelconque des revendications 4 ou 5 à 8, dans laquelle lesdits ensembles génétiques sont les phages M 13.

11. Banque selon la revendication 10, dans laquelle lesdites séquences ADN sont dans un vecteur phagique.

12. Banque selon les revendications 10 ou 11, dans laquelle ledit phage comprend un gène de type sauvage iii et un gène iii tronqué pour la présentation des peptides, des polypeptides ou des protéines.

13. Banque selon l'une quelconque des revendications 4 ou 5 à 8, dans laquelle lesdites séquences ADN sont dans un vecteur phagemide.

14. Banque selon l'une quelconque des revendications 10 à 13, dans laquelle lesdits peptides, polypeptides, ou protéines présentés sont présentés par l'intermédiaire d'un lieur de petite taille attaché à la partie finale du gène III de M13.

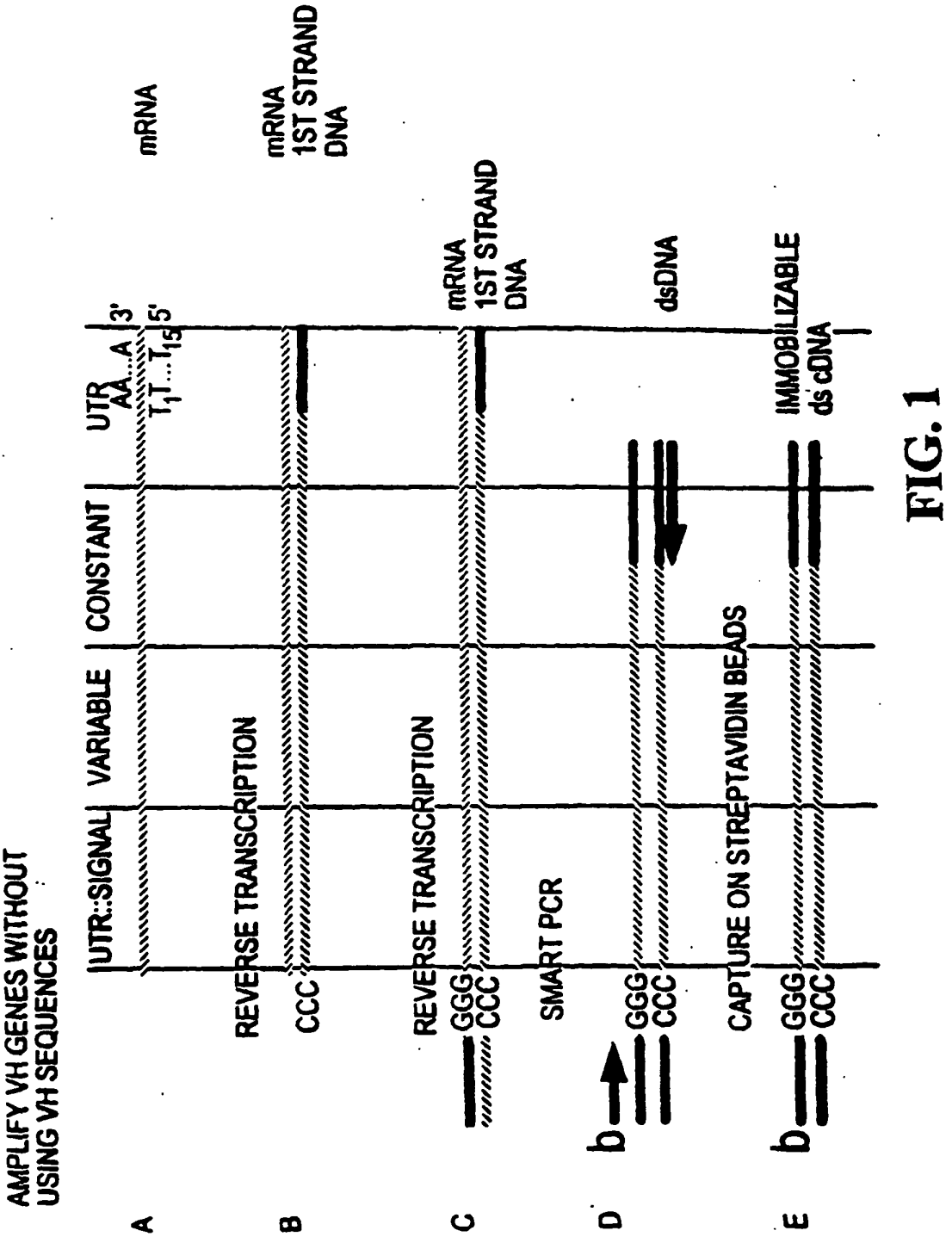

AMPLIFY VH GENES WITHOUT USING VH SEQUENCES
UTR::SIGNAL
VARIABLE
CONSTANT
UTR
AA...A 3'
T1T...T15 5'
mRNA
A
REVERSE TRANSCRIPTION
mRNA
1ST STRAND DNA
B
CCC
REVERSE TRANSCRIPTION
mRNA
1ST STRAND DNA
C
GGG
CCC
SMART PCR
dsDNA
D
b
GGG
CCC
CAPTURE ON STREPTAVIDIN BEADS
IMMOBILIZABLE ds cDNA
E
b
GGG
CCC

FIG. 1

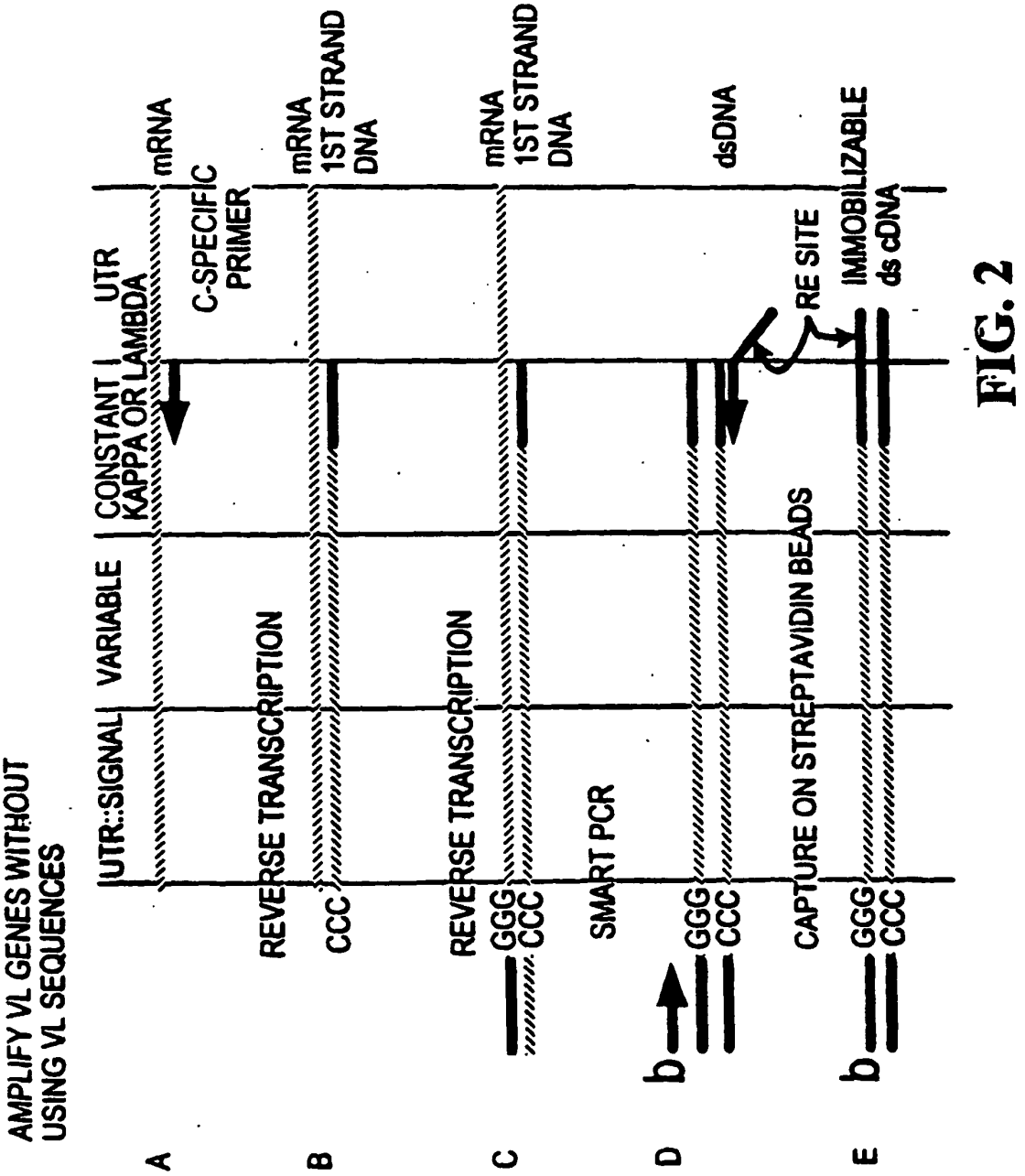

AMPLIFY VL GENES WITHOUT USING VL SEQUENCES
A
B
C
D
E
UTR::SIGNAL
VARIABLE
CONSTANT KAPPA OR LAMBDA
UTR
C-SPECIFIC PRIMER
mRNA
REVERSE TRANSCRIPTION
CCC
mRNA 1ST STRAND DNA
REVERSE TRANSCRIPTION
GGG
CCC
mRNA 1ST STRAND DNA
SMART PCR
b
GGG
CCC
dsDNA
CAPTURE ON STREPTAVIDIN BEADS
RE SITE
IMMOBILIZABLE ds cDNA
b
GGG
CCC

FIG. 2

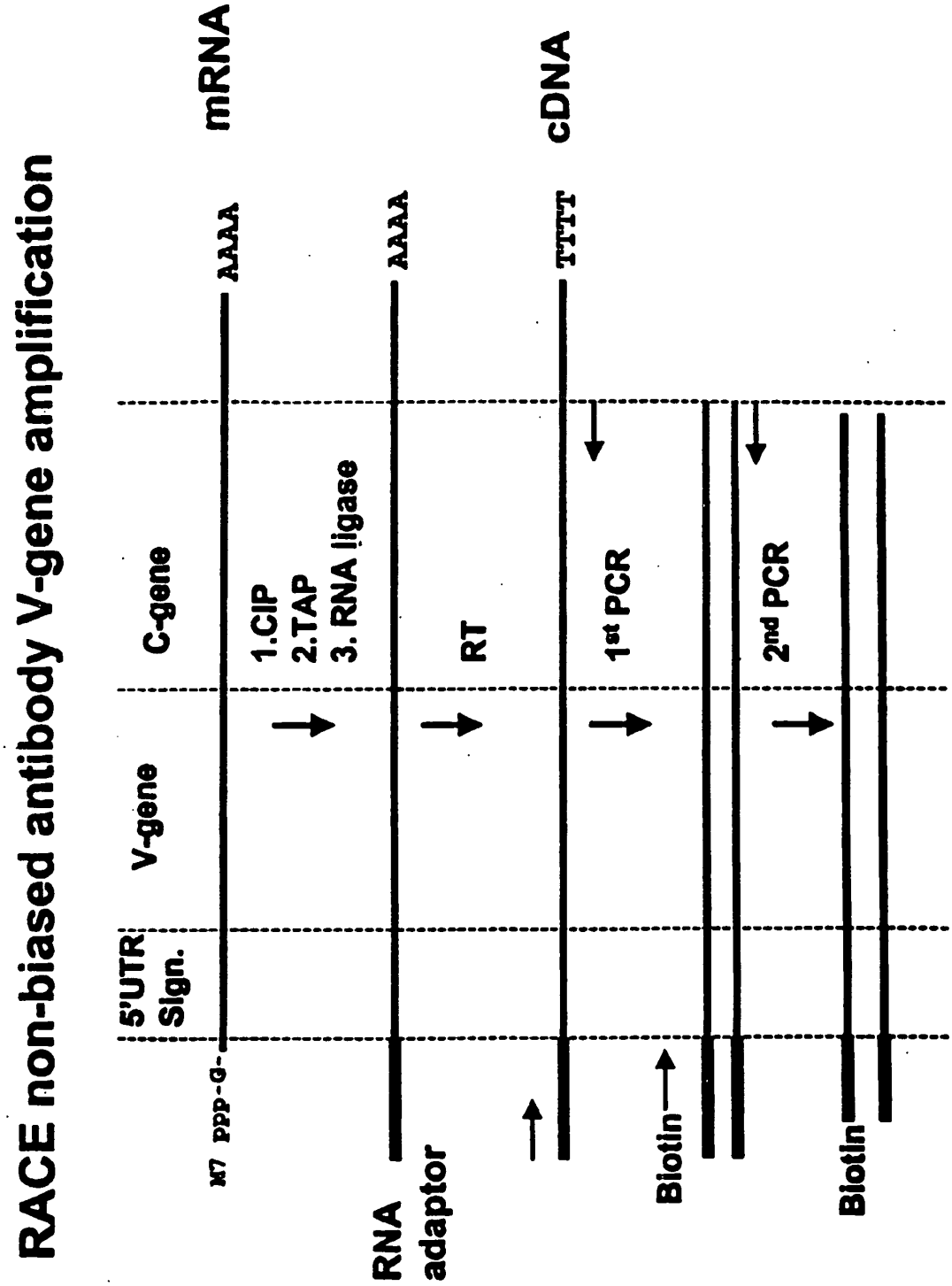
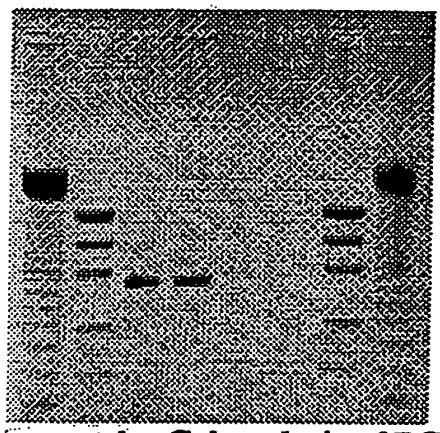

RACE non-biased antibody V-gene amplification
mRNA
cDNA
AAAA
AAAA
TTTT
5'UTR Sign.
V-gene
C-gene
1.CIP
2.TAP
3. RNA ligase
RT
1st PCR
2nd PCR
M7 ppp-G-
RNA adaptor
Biotin
Biotin

FIG. 3


1st PCR light chains
1 2 3 4 M1-2
Kappa
Lambda
1st PCR heavy chains
1, 2, 3 and 4 are patient samples

FIG. 4

1 2 3 4 5 6 7 8

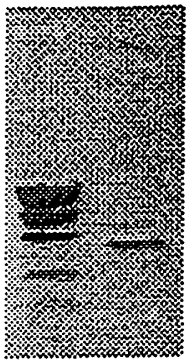


Gel analysis of PCR product from extender-kappa amplification
Approx. 75ng/5µl → 15ng/µl

1 - 100bp
2 - LDM
3 - 50ng template
4 - 10ng template
5 - ssDNA unligated
6 - negative control
7 - LDM
8 - 100bp

FIG. 5

1 2

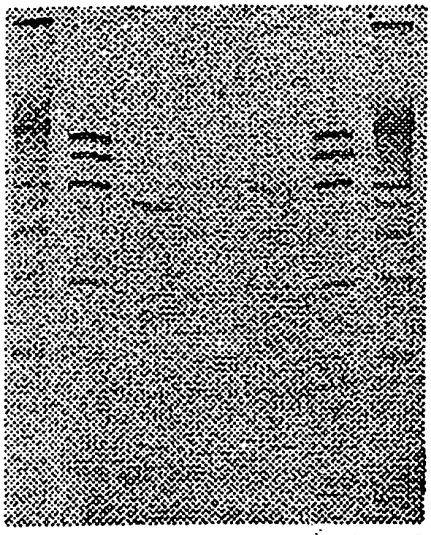


Gel purified PCR product from extender-kappa amplification
Concentration : ± 35ng/µl

1 - LDM
2 - 1µl purif.

FIG. 6

1 2 3 4 5 6 7

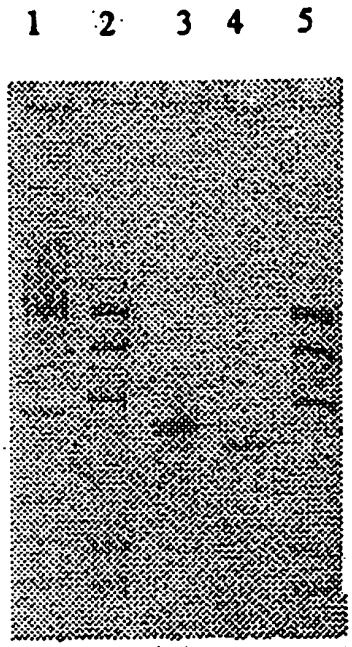


Gel-analysis of digested κ-ssDNA

1µl digested ssDNA ≈ 8ng ssDNA
Total volume of 50µl = 400ng ssDNA
→ 400ng ssDNA available for ligation of the bridge-extenders

1 - 100bp
2 - LDM
3 - 1µl ssDNA pure
4 - 4µl beads after dig.
5 - 8µl beads after dig.
6 - LDM
7 - 100bp

FIG. 7



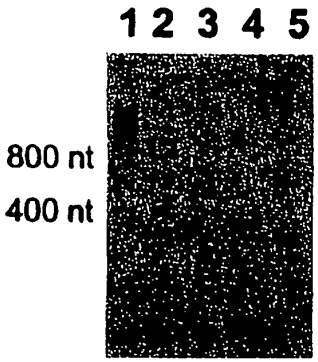
Gel analysis of extender – cleaved kappa ligation
20ng/5µl eluted material → 4ng/µl

1- 100bp
2 - LDM
3 - Ligationmix, 4µl
4 - Unligated ssDNA
5 - LDM

FIG. 8

Cleavage and ligation Kappa light chains

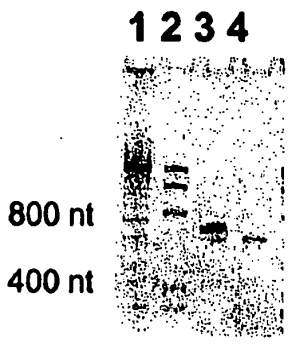
A) BsmA1 cleavage



1. 100 bp marker
2. LDM marker
3. Sup. ssDNA after dig.
4. beads after dig. (uncleaved material)
5. DNA before cleavage

80% cleavage

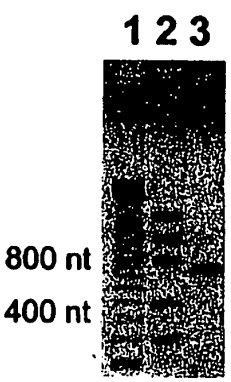
B) Bridge Ligation



1. 100 bp marker
2. LDM marker
3. Ligationmix
4. Unligated ssDNA

90% ligation

C) PCR



1. 100 bp marker
2. LDM marker
3. 50 ng template (13 cycles)

FIG. 9

VH-CDR1
1 Y 1 M 1

VH-CDR2
2 I 2 3 S G G 1 T 1 YADSVKG

FIG. 10

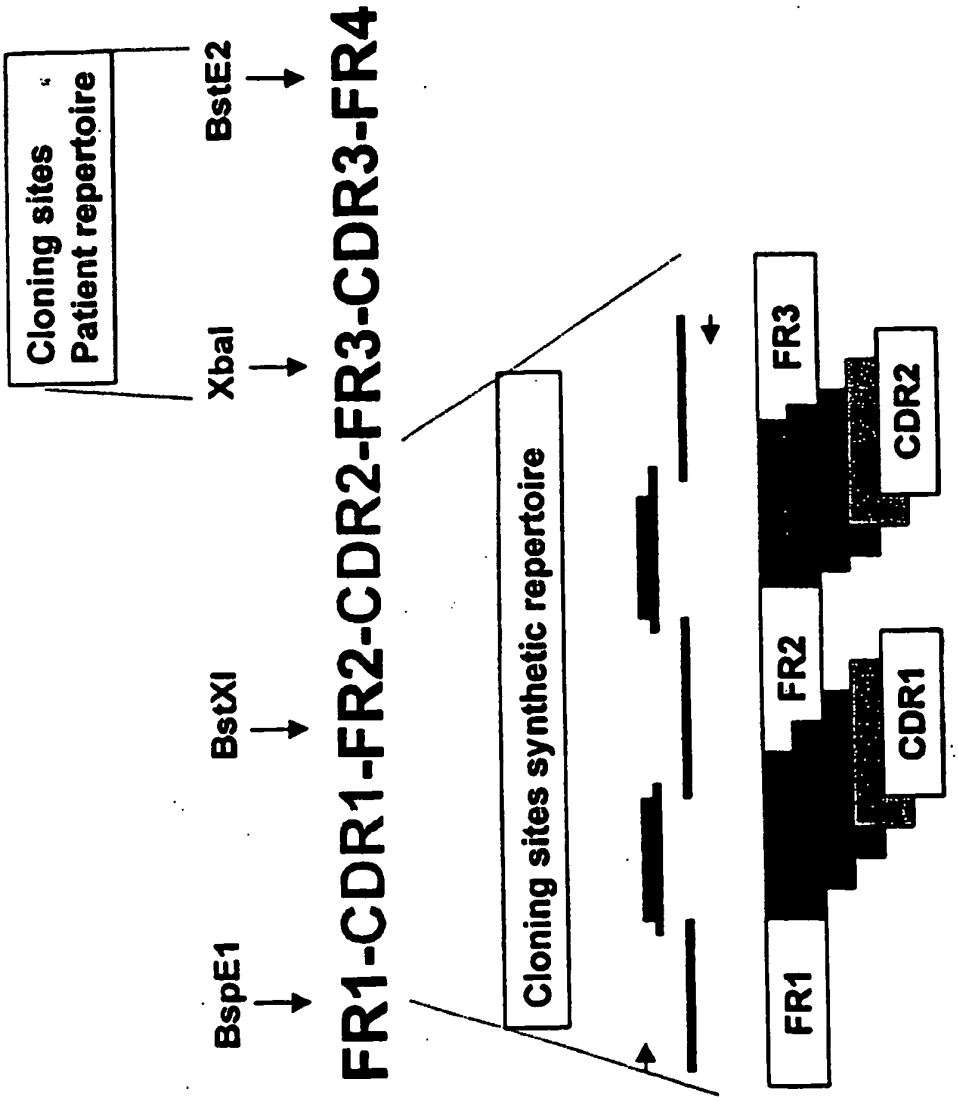

Cloning sites
Patient repertoire
BspE1
BstXI
XbaI
BstE2
FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4
Cloning sites synthetic repertoire
FR1
CDR1
FR2
CDR2
FR3

FIG. 11

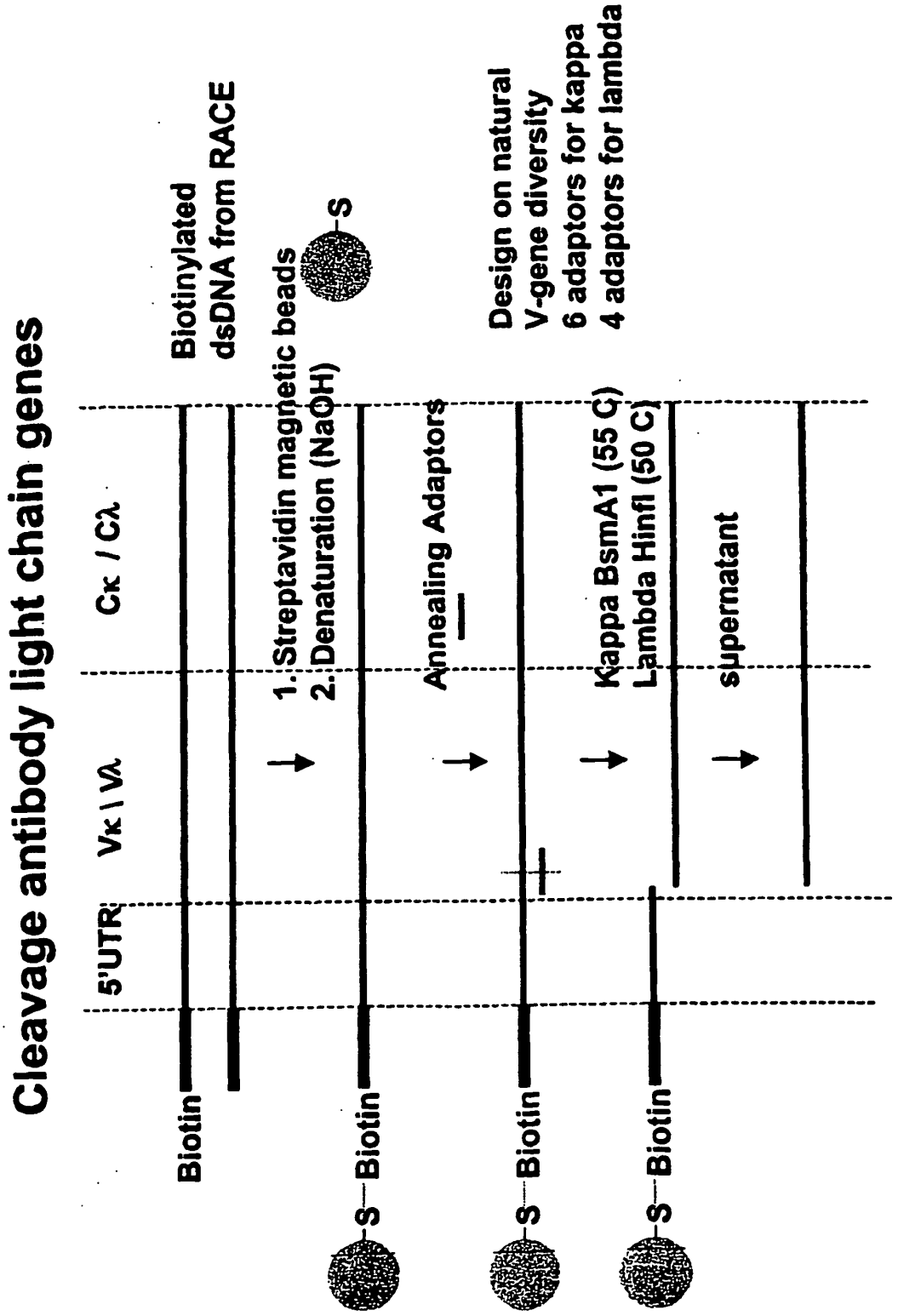

Cleavage antibody light chain genes
5'UTR
Vκ \ Vλ
Cκ / Cλ
Biotinylated dsDNA from RACE
1. Streptavidin magnetic beads
2. Denaturation (NaOH)
Annealing Adaptors
Design on natural V-gene diversity
6 adaptors for kappa
4 adaptors for lambda
Kappa BsmA1 (55 C)
Lambda HinfI (50 C)
supernatant
Biotin
S
Biotin
S
Biotin
S
Biotin
S

FIG. 12A

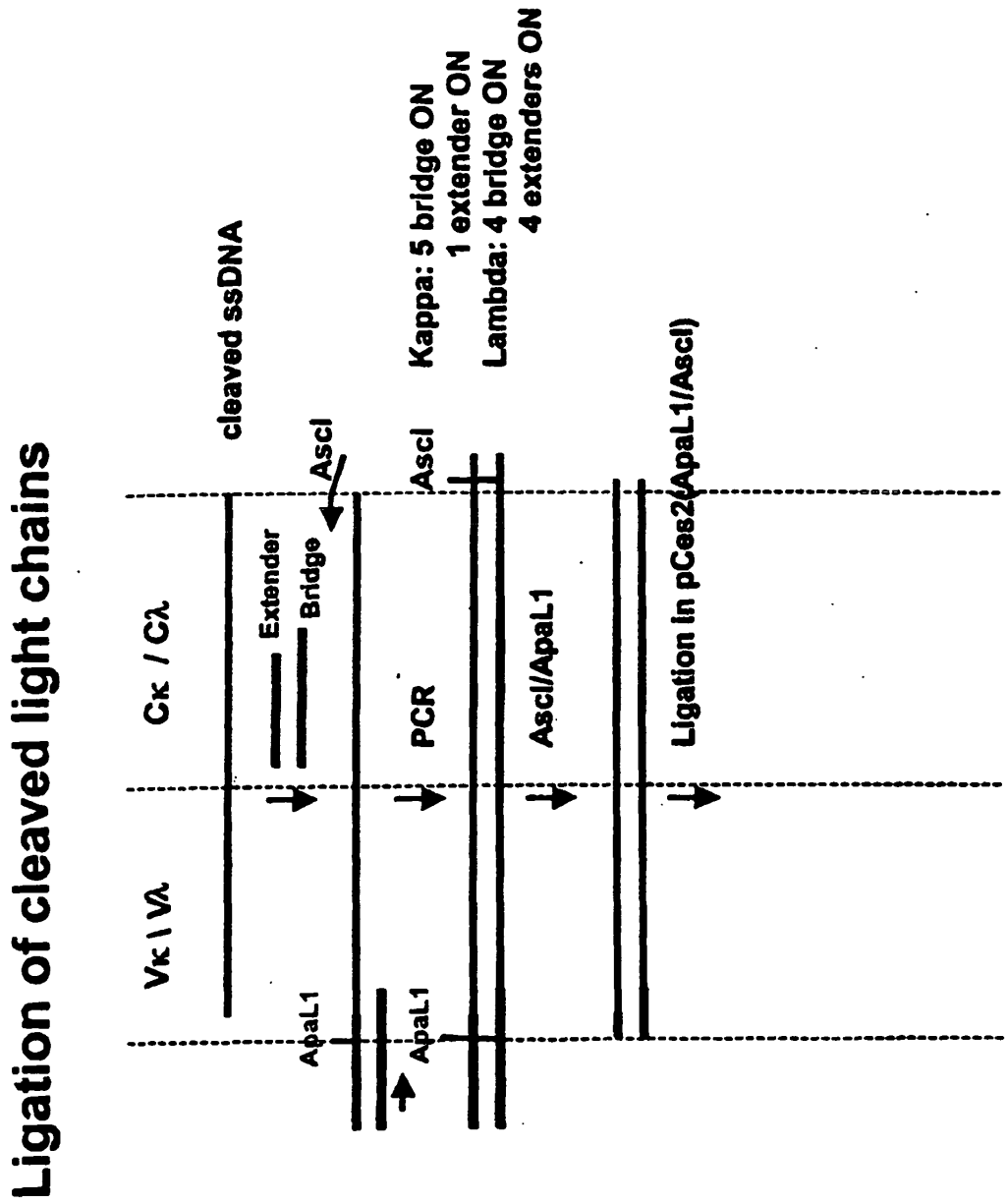
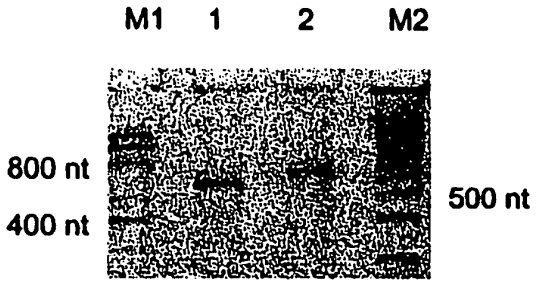

Ligation of cleaved light chains
Vκ \ Vλ
Cκ / Cλ
cleaved ssDNA
Extender
Bridge
AscI
ApaL1
ApaL1
PCR
AscI
Kappa: 5 bridge ON
1 extender ON
Lambda: 4 bridge ON
4 extenders ON
AscI/ApaL1
Ligation in pCes2(ApaL1/AscI)

FIG. 12B

Figure 3: Cleavage and ligation lambda light chains

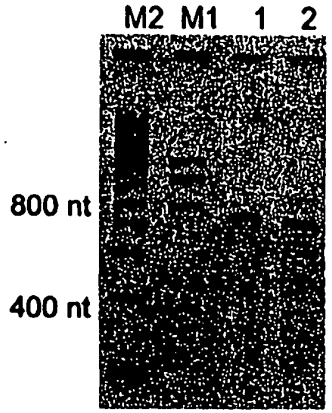
A) HinfI cleavage



M1 = LDM MW marker
1 = sup. after Hinf-cleavage (10ng ssDNA)
2 = beads after Hinf-cleavage (10ng ssDNA (not cleaved)
M2 = 100bp marker , bottom to top: 300nt, 400nt, 500nt, 600nt, 800nt

70% cleavage

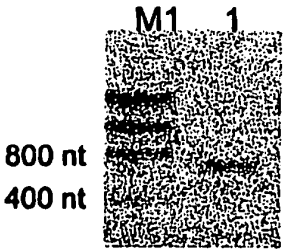
B) Bridge ligation



1=Ligated DNA
2= Unligated DNA

95% ligation

C) PCR



1= 1ul purified PCR product / 50 ul (15 cycles)

FIG. 13

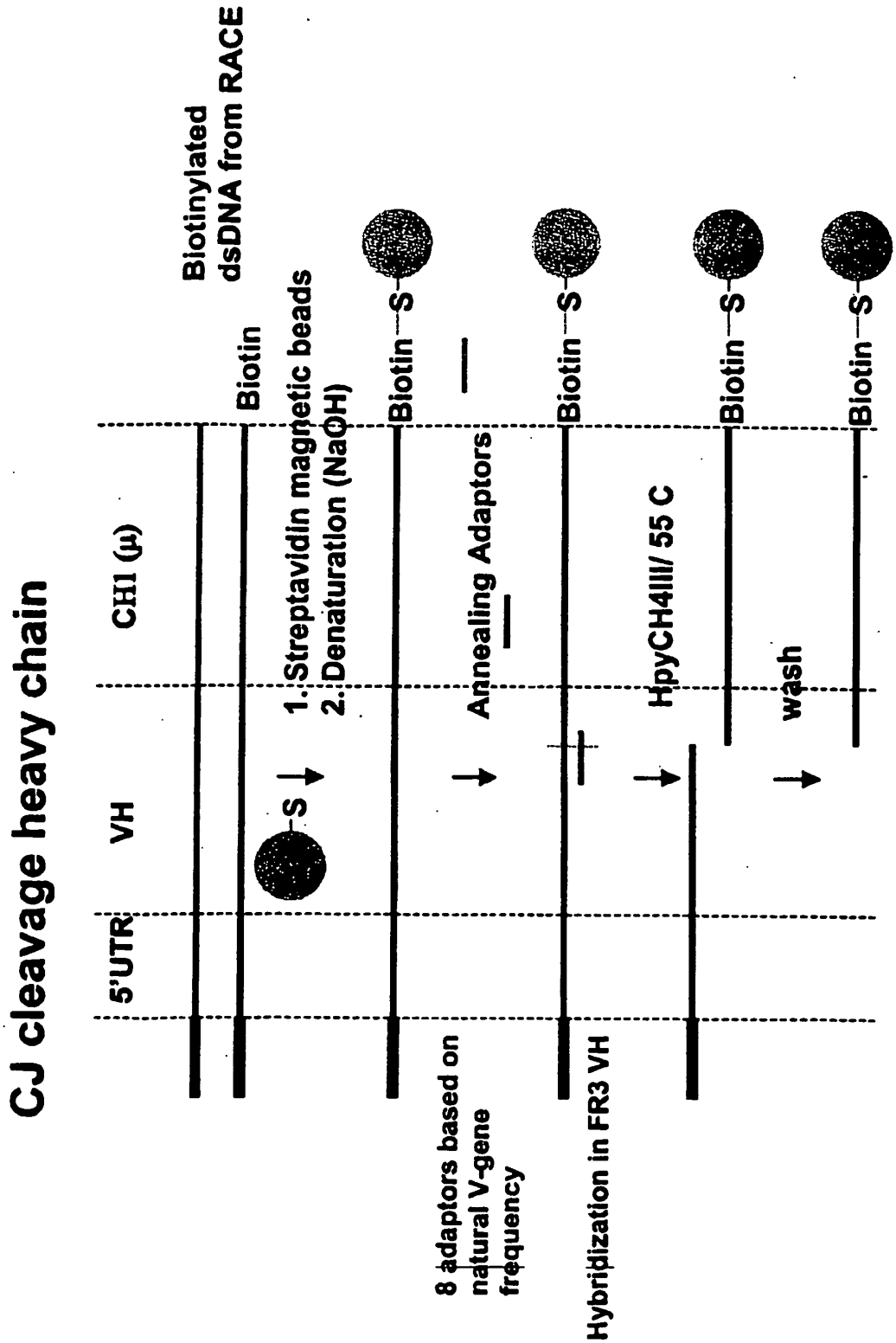

CJ cleavage heavy chain
5'UTR
VH
CH1 (μ)
Biotinylated dsDNA from RACE
Biotin
1. Streptavidin magnetic beads
2. Denaturation (NaOH)
Biotin—S
Annealing Adaptors
8 adaptors based on natural V-gene frequency
Hybridization in FR3 VH
HpyCH4III/ 55 C
wash

FIG.14A

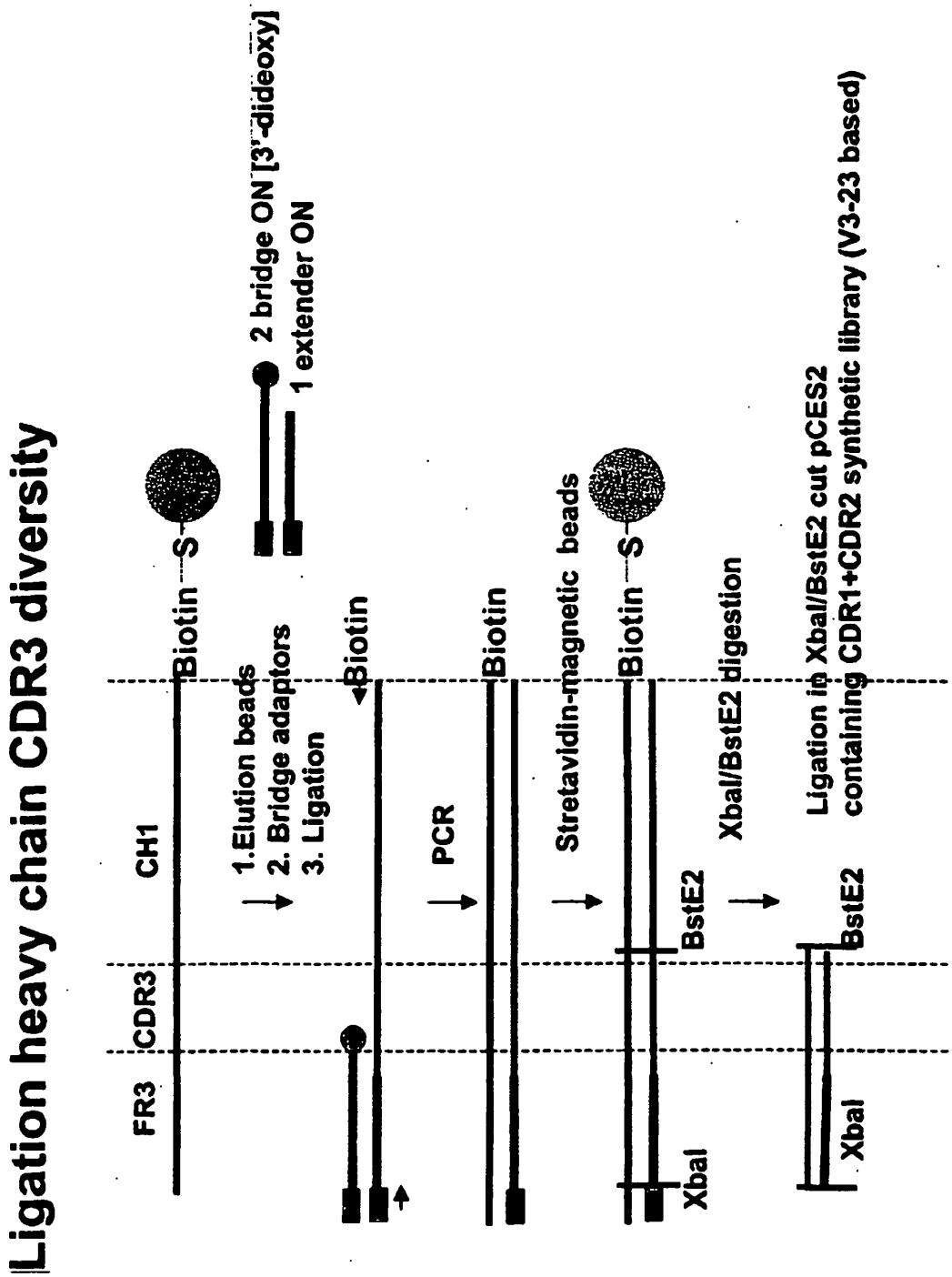
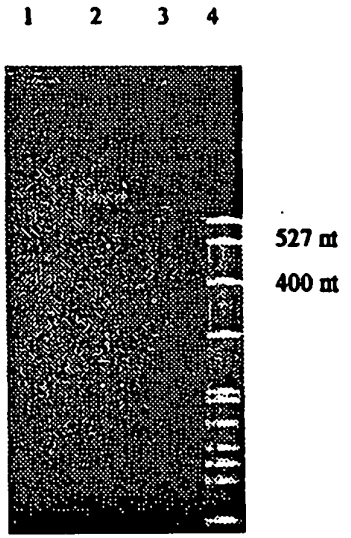

Ligation heavy chain CDR3 diversity
FR3
CDR3
CH1
Biotin
S
1.Elution beads
2. Bridge adaptors
3. Ligation
2 bridge ON [3'-dideoxy]
1 extender ON
Biotin
PCR
Biotin
Stretavidin-magnetic beads
Biotin
S
BstE2
XbaI/BstE2 digestion
Ligation in XbaI/BstE2 cut pCES2
containing CDR1+CDR2 synthetic library (V3-23 based)
BstE2
XbaI

FIG. 14B

Cleavage and ligation Heavy Chain

A) HpyCH4III cleavage



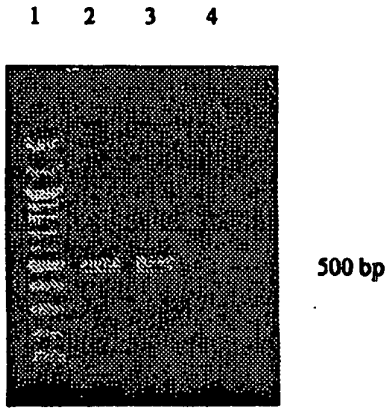
1 = Cleaved DNA eluted from PN column

2 = Beads after HpyCH4III digestion

3 = Supernatant after cleavage

4 = MspI digest of pBR322

B) PCR



1 = NEB 100bp ladder

2 = 5ul/100ul PCR product 20 cycles; sample A

3 = 5ul/100ul PCR product 20 cycles; sample B

4 = no template

FIG. 15

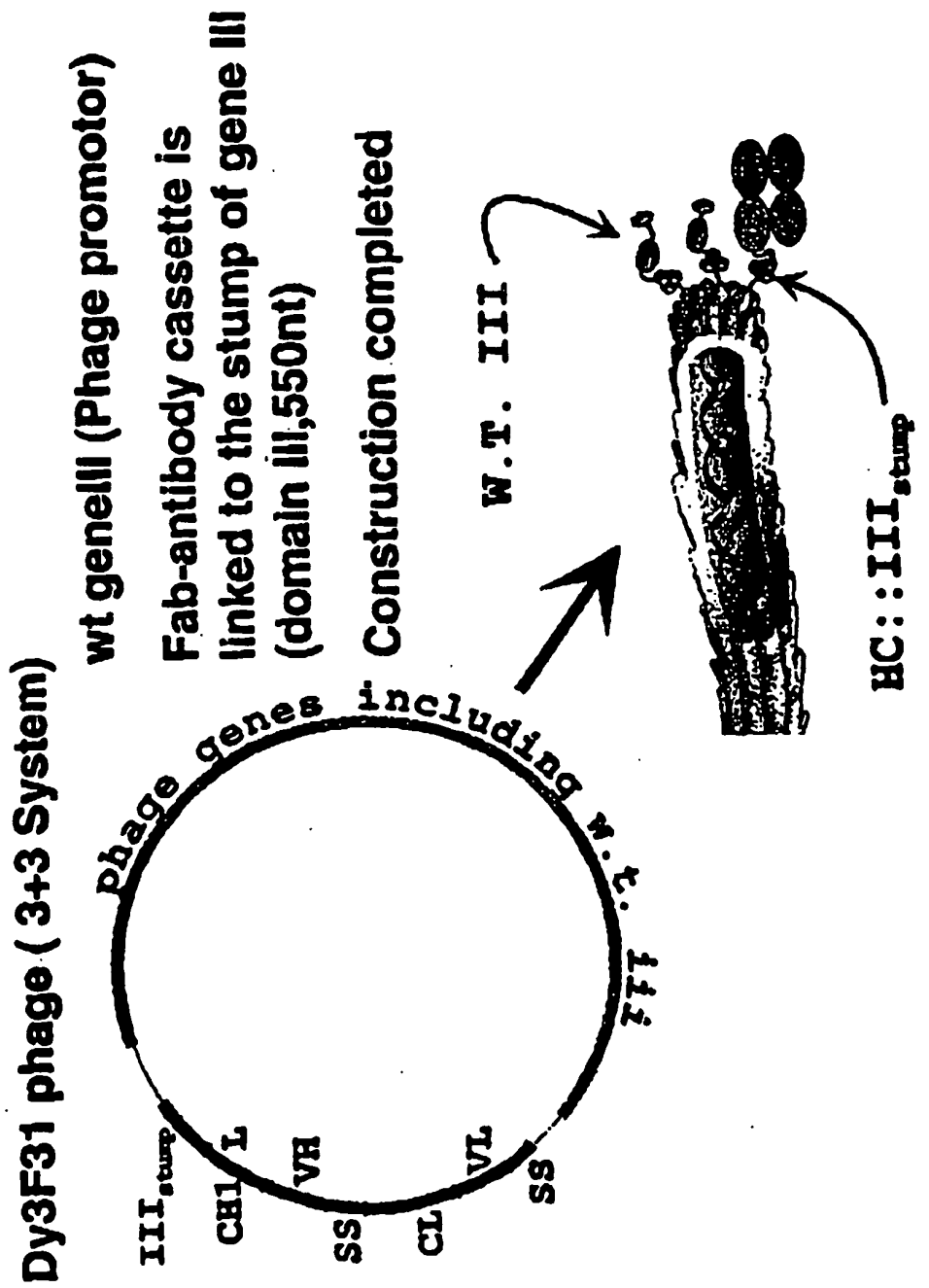
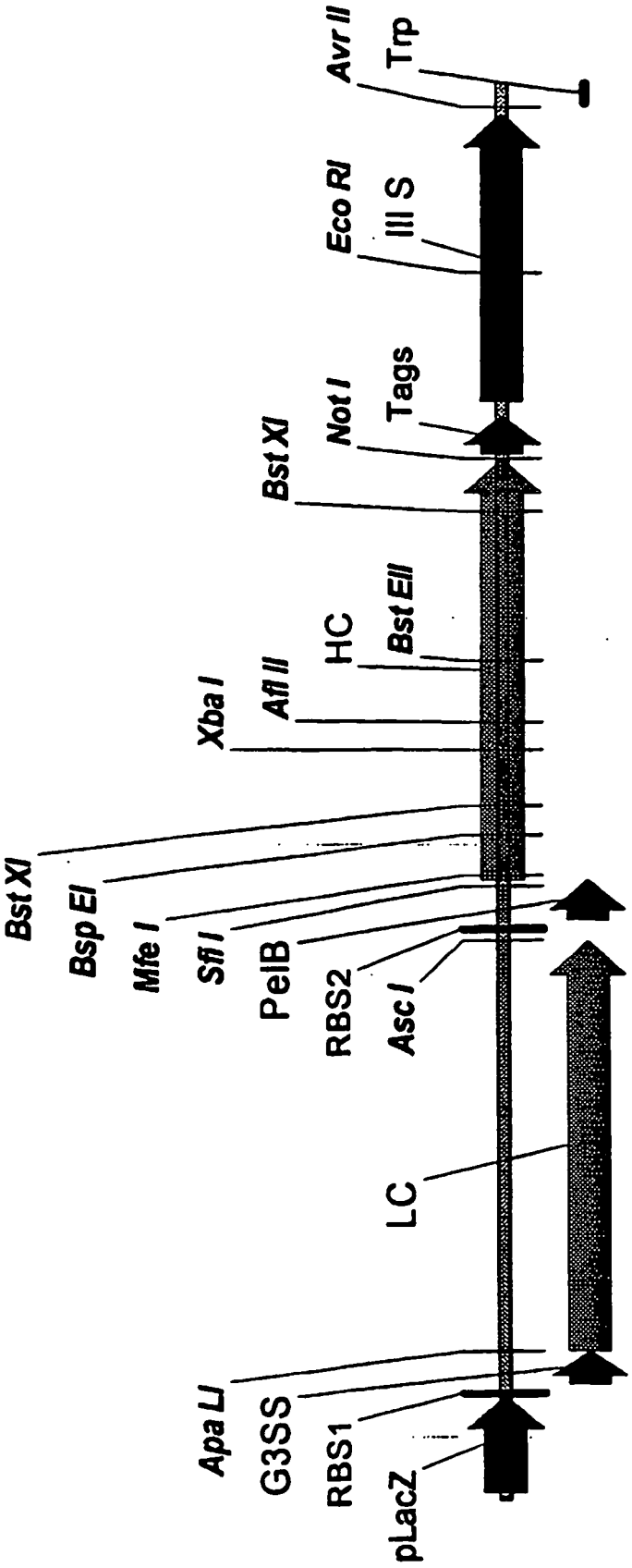

Dy3F31 phage (3+3 System)
wt geneIII (Phage promotor)
Fab-antibody cassette is
linked to the stump of gene III
(domain III,550nt)
Construction completed
Phage genes including w.t. III
III,stump
CH1
L
VH
SS
CL
VL
SS
W.T. III
HC::III,stump

FIG. 16



Fab Cassette
2263 bp

FIG. 17

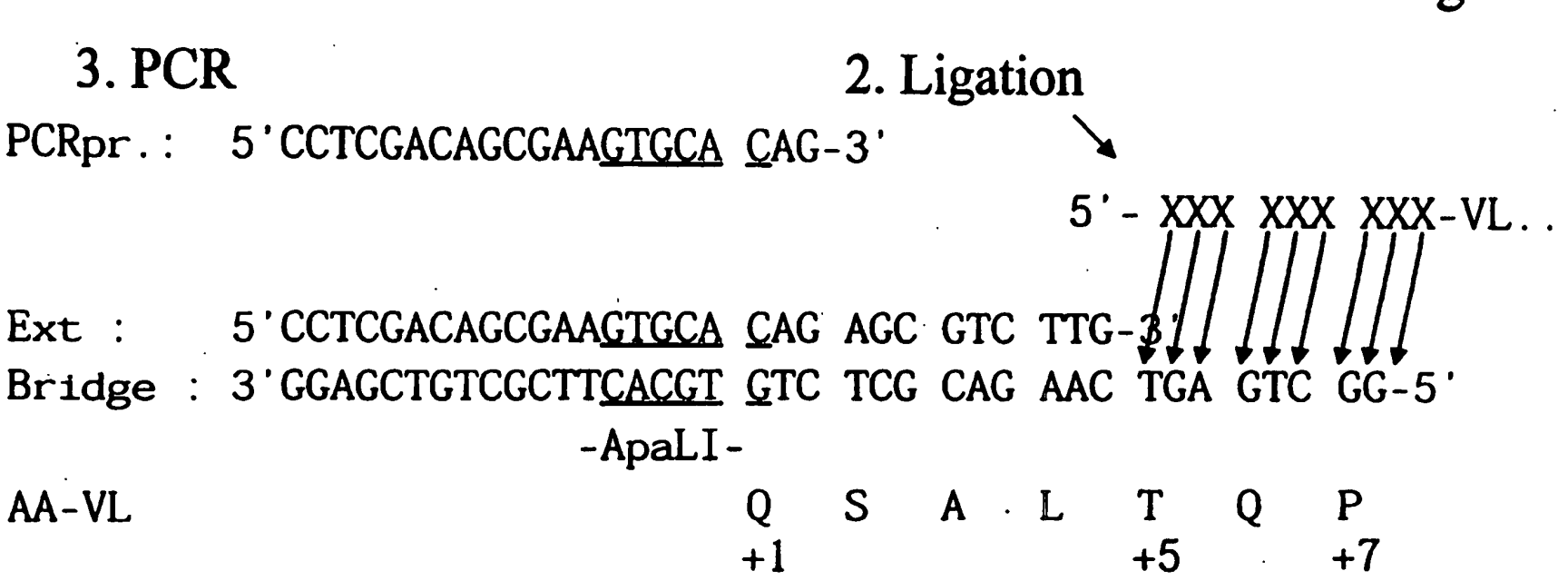


FIG. 18

EP 1 578 903 B1

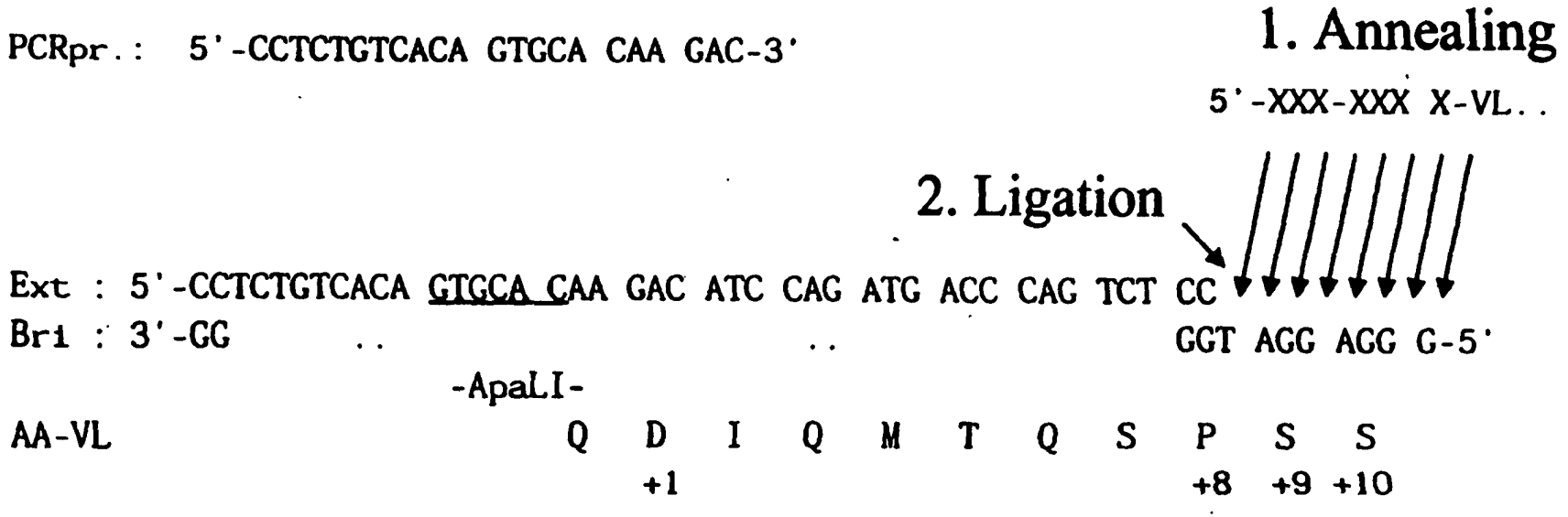

3. PCR
PCRpr.: 5'-CCTCTGTCACA GTGCA CAA GAC-3'
1. Annealing
5'-XXX-XXX X-VL..
2. Ligation
Ext : 5'-CCTCTGTCACA GTGCA CAA GAC ATC CAG ATG ACC CAG TCT CC
Bri : 3'-GG .. .. GGT AGG ACG G-5'
-ApaLI-
AA-VL Q D I Q M T Q S P S S
+1 +8 +9 +10

FIG. 19

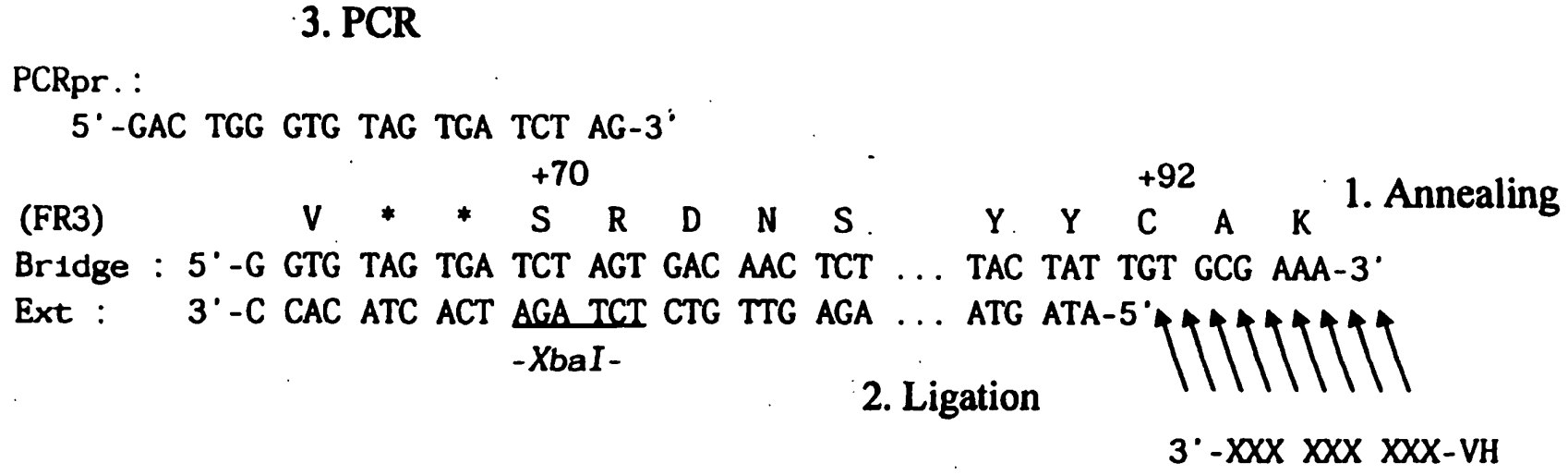

3. PCR
PCRpr. :
5'-GAC TGG GTG TAG TGA TCT AG-3'
+70
+92
1. Annealing
(FR3) V * * S R D N S Y Y C A K
Bridge : 5'-G GTG TAG TGA TCT AGT GAC AAC TCT ... TAC TAT TGT GCG AAA-3'
Ext : 3'-C CAC ATC ACT AGA TCT CTG TTG AGA ... ATG ATA-5'
-XbaI-
2. Ligation
3'-XXX XXX XXX-VH

FIG. 20

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- US 60198069 B **[0001]**
- US 09837306 B **[0001] [0197]**
- US 0112454 W **[0001]**
- US 10000516 B **[0001]**
- US 10045674 B **[0001] [0197]**
- EP 368684 B1 **[0010]**
- US 5869644 A **[0024]**
- US 06198069 B **[0197]**

Non-patent literature cited in the description

- **HJ de Haard et al.** *Journal of Biological Chemistry,* 1999, vol. 279 (26), 18218-30 **[0028]**
- **Frohman, M.A. ; Dush, M.K. ; Martin, G.R.** *Proc. Natl. Acad. Sci. USA,* 1988, 8998-9002 **[0037]**
- **Wang ; Wilkinson.** *Biotechniques,* 2001, vol. 31 (4), 722-724 **[0163]**
- **de Haard et al.** *Journal of Biological Chemistry,* 1999, vol. 274 (26), 18218-30 **[0164]**
- **de Haard ; Marks.** *Journal of Molecular Biology,* 1991, vol. 222 (3), 581-97 **[0169]**

专利名称(译)	构建文库的新方法包括多肽，多肽或蛋白质家族的展示和/或表达成员以及新文库		
公开(公告)号	EP1578903A4	公开(公告)日	2007-05-23
申请号	EP2002762148	申请日	2002-04-17
[标]申请(专利权)人(译)	拉德纳ROBERT CHARLES COHEN爱德华^ h NASTRI HORACIO摹 ROOKEY KRISTIN大号 HOET RENE HOOGENBOOM HENDRICUS - [R缩略词		
申请(专利权)人(译)	拉德纳，ROBERT CHARLES COHEN，EDWARD H. NASTRI奥拉西奥·G. ROOKEY克莉丝汀L. HOET，RENE HOOGENBOOM，HENDRICUS R.J.M.		
当前申请(专利权)人(译)	拉德纳，ROBERT CHARLES COHEN，EDWARD H. NASTRI奥拉西奥·G. ROOKEY克莉丝汀L. HOET，RENE HOOGENBOOM，HENDRICUS R.J.M.		
[标]发明人	LADNER ROBERT CHARLES COHEN EDWARD H NASTRI HORACIO G ROOKEY KRISTIN L HOET RENE HOOGENBOOM HENDRICUS R J M		
发明人	LADNER, ROBERT CHARLES COHEN, EDWARD H. NASTRI, HORACIO G. ROOKEY, KRISTIN L. HOET, RENE HOOGENBOOM, HENDRICUS R.J.M.		
IPC分类号	C12N15/00 C07K1/04 C07K16/00 C07K17/00 C12N1/00 C12N15/09 C12N15/10 C12N15/11 C12N15/13 C12N15/63 C12N15/66 C12N15/70 C12N15/74 C12P21/04 C12P21/06 C12Q1/44 C12Q1/68 C12Q1/70 C12Q3/00 C40B40/02 G01N33/53		
CPC分类号	C07K16/005 C07K2317/51 C07K2317/515 C07K2317/55 C07K2317/622 C12N15/10 C12N15/1037 C12N15/1093 C12N15/66 C40B40/02 C40B40/08		
代理机构(译)	法思博事务所		
优先权	09/837306 2001-04-17 US		
其他公开文献	EP1578903A2 EP1578903B2 EP1578903B1 EP1578903B8		

外部链接 Espacenet

摘要(译)

含有遗传包装的集合的文库，其显示多肽家族的肽，多肽或蛋白质的成员并且共同显示该家族的至少一部分，所展示的肽，多肽或蛋白质由包含编码序列的序列的DNA序列编码。描述了重链CDR区。

Table 2: Enzymes that either cut 15 or more human GLGs or have 5+-base recognition in FR3 Typical entry:

REname Recognition GLGid#:base#	GLGid#:base#		#sites GLGid#:base#.....		
BstEII Ggtnacc			2		
1: 3	48: 3				
There are 2 hits at base#			3		
MaeIII gtnac			36		
1: 4	2: 4	3: 4	4: 4	5: 4	6: 4
7: 4	8: 4	9: 4	10: 4	11: 4	37: 4
37: 58	38: 4	38: 58	39: 4	39: 58	40: 4
40: 58	91: 4	91: 58	42: 4	42: 58	93: 4
43: 58	44: 4	44: 58	45: 4	45: 58	46: 4
46: 58	47: 4	47: 58	48: 4	49: 4	50: 58
There are 24 hits at base#			**4**		
Tsp45I gtsac			33		
1: 4	2: 4	3: 4	4: 4	5: 4	6: 4
7: 4	8: 4	9: 4	10: 4	11: 4	37: 4
37: 58	38: 4	38: 58	39: 58	40: 4	40: 58
41: 58	42: 58	43: 4	43: 58	44: 4	44: 58
45: 4	45: 58	46: 4	46: 58	47: 4	97: 58
48: 4	49: 4	50: 58			
There are 21 hits at base#			4		
HphI tcacc			45		
1: 5	2: 5	3: 5	4: 5	5: 5	6: 5
7: 5	8: 5	11: 5	12: 5	12: 11	13: 5
14: 5	15: 5	16: 5	17: 5	18: 5	19: 5
20: 5	21: 5	22: 5	23: 5	24: 5	25: 5
26: 5	27: 5	28: 5	29: 5	30: 5	31: 5
32: 5	33: 5	34: 5	35: 5	36: 5	37: 5
38: 5	40: 5	43: 5	44: 5	45: 5	46: 5
47: 5	48: 5	49: 5			
There are 44 hits at base#			**5**		
NlaIII CATG			26		
1: 9	1: 42	2: 42	3: 9	3: 42	4: 9
4: 42	5: 9	5: 42	6: 42	6: 78	7: 9
7: 42	8: 21	8: 42	9: 42	10: 42	11: 42
12: 57	13: 48	13: 57	14: 57	31: 72	38: 9
48: 78	49: 78				
There are 11 hits at base# 42					
There are 1 hits at base# 48 Could cause raggedness.					
BsaJI Ccnngg			37		