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Canadian Intellectual Property Office

CA 3049612 C 2023/01/10

(11)(21) 3 049 612

(12) BREVET CANADIEN
CANADIAN PATENT

(13) C

(22) Date de dépôt/Filing Date: 2009/04/24

(41) Mise à la disp. pub./Open to Public Insp.: 2009/10/29

(45) Date de délivrance/Issue Date: 2023/01/10

(62) Demande originale/Original Application: 2 968 164

(30) Priorité/Priority: 2008/04/24 (US61/047529)

(51) Cl.Int./Int.Cl. *C40B 40/02* (2006.01),
C07K 16/00 (2006.01), *C12N 15/13* (2006.01),
C12N 15/63 (2006.01), *C12Q 1/6897* (2018.01),
C40B 40/10 (2006.01), *C40B 50/06* (2006.01)

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(54) Titre : BIBLIOTHEQUES DE MATERIELS GENETIQUES COMPRENANT DE NOUVELLES CONCEPTIONS CDR1, CDR2 ET CDR3 HC ET DE NOUVELLES CONCEPTIONS CDR1, CDR2 ET CDR3 LC

(54) Title: LIBRARIES OF GENETIC PACKAGES COMPRISING NOVEL HC CDR1, CDR2, AND CDR3 AND NOVEL LC CDR1, CDR2, AND CDR3 DESIGNS

(57) Abrégé/Abstract:
Provided are compositions and methods for preparing and identifying antibodies having CDR3s that vary in sequence and in length from very short to very long which in certain embodiments may bind to a carbohydrate moiety or the active site of an enzyme. Libraries coding for antibodies with the CDR3s are also provided. The libraries can be provided by modifying a pre-existing nucleic acid library.

Abstract

Provided are compositions and methods for preparing and identifying antibodies having CDR3s that vary in sequence and in length from very short to very long which in certain embodiments may bind to a carbohydrate moiety or the active site of an enzyme. Libraries coding for antibodies with the CDR3s are also provided. The libraries can be provided by modifying a pre-existing nucleic acid library.

LIBRARIES OF GENETIC PACKAGES COMPRISING NOVEL HC CDR1, CDR2, AND CDR3 AND NOVEL LC CDR1, CDR2, AND CDR3 DESIGNS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a division of application CA 2,968,164, which is a division of application CA 2,722,409, filed April 24, 2009, and claims priority to US 61/047,529 filed April 24, 2008.

BACKGROUND

[0001] It is now common practice in the art to prepare libraries of genetic packages that individually display, display and express, or comprise a member of a diverse family of peptides, polypeptides or proteins and collectively display, display and express, or comprise at least a portion of the amino acid diversity of the family. In many common libraries, the peptides, polypeptides or proteins are related to antibodies (e.g., single chain Fv (scFv), Fv, Fab, whole antibodies or minibodies (i.e., dimers that consist of V_H linked to V_L)). Often, they comprise one or more of the CDRs and framework regions of the heavy and light chains of human antibodies.

[0002] Peptide, polypeptide or protein libraries have been produced in several ways. See, e.g., Knappik et al., *J. Mol. Biol.*, 296, pp. 57-86 (2000), which is incorporated herein by reference. One method is to capture the diversity of native donors, either naive or immunized. Another way is to generate libraries having synthetic diversity. A third method is a combination of the first two. Typically, the diversity produced by these methods is limited to sequence diversity, i.e., each member of the library has the same length but differs from the other members of the family by having different amino acids or variegation at a given position in the peptide, polypeptide or protein chain. Naturally diverse peptides, polypeptides or proteins, however, are not limited to diversity only in their amino acid sequences. For example, human antibodies are not limited to sequence diversity in their amino acids, they are also diverse in the lengths of their amino acid chains.

SUMMARY

[0003] For antibodies, heavy chain diversity in length occurs, for example, during variable region rearrangements. See e.g., Corbett et al., *J. Mol. Biol.*, 270, pp. 587-97 (1997). The joining of V genes to J genes, for example, results in the inclusion of a recognizable D segment in CDR3

in about half of the heavy chain antibody sequences, thus creating regions encoding varying lengths of amino acids. D segments are more common in antibodies having long HC CDR3s. The following also may occur during joining of antibody gene segments: (i) the end of the V gene may have zero to several bases deleted or changed; (ii) the end of the D segment may have zero to many bases removed or changed; (iii) a number of approximately random bases may be inserted between V and D or between D and J; and (iv) the 5' end of J may be edited to remove or to change several bases. These rearrangements result in antibodies that are diverse both in amino acid sequence and in length. HC CDR3s of different lengths may fold into different shapes, giving the antibodies novel shapes with which to bind antigens. The conformations depend on both the length and the sequence of the CDR3. It should be remembered that a HC CDR3 of length 8, for example, and of any sequence cannot adequately mimic the behavior of a CDR3 of length 22, for example.

[0004] Libraries that contain only amino acid sequence diversity are, thus, disadvantaged in that they do not reflect the natural diversity of the peptide, polypeptide or protein that the library is intended to mimic. Further, diversity in length may be important to the ultimate functioning of the protein, peptide or polypeptide. For example, with regard to a library comprising antibody regions, many of the peptides, polypeptides, proteins displayed, displayed and expressed, or comprised by the genetic packages of the library may not fold properly or their binding to an antigen may be disadvantaged, if diversity both in sequence and length are not represented in the library.

[0005] An additional disadvantage of such libraries of genetic packages that display, display and express, or comprise peptides, polypeptides and proteins is that they are not focused on those members that are based on natural occurring diversity and thus on members that are most likely to be functional and least likely to be immunogenic. Rather, the libraries, typically, attempt to include as much diversity or variegation as possible at every CDR position. This makes library construction time-consuming and less efficient than necessary. The large number of members that are produced by trying to capture complete diversity also makes screening more cumbersome than it needs to be. This is particularly true given that many members of the library will not be functional or will be non-specifically sticky.

[0006] In addition to the labor of constructing synthetic libraries is the question of immunogenicity. For example, there are libraries in which all CDR residues are either Tyr (Y)

or Ser (S). Although antibodies (Abs) selected from these libraries show high affinity and specificity, their very unusual composition may make them immunogenic. The present invention is directed toward making Abs that could well have come from the human immune system and so are less likely to be immunogenic. The libraries of the present invention retain as many residues from V-D-J or V-J fusions as possible. To reduce the risk of immunogenicity, it may be prudent to change each non-germline amino acid in both framework and CDRs back to germline to determine whether the change from germline is needed to retain binding affinity. Thus, a library that is biased at each varied position toward germline will reduce the likelihood of isolating Abs that have unneeded non-germline amino acids.

[0007] Abs are large proteins and are subject to various forms of degradation. One form of degradation is the deamidation of Asn and Gln residues (especially in Asn-Gly or Gln-Gly) and the isomerization of Asp residues. Another form of degration is the oxidation of methionines, tryptophan, and cysteine. Another form of degradation is the cleavage of Asp-Pro dipeptides. Another form of degradation is the formation of pyroglutamate from N-terminal Glu or Gln. It is advantageous to provide a library in which the occurance of problematic sequences is minimized.

[0008] Provided are libraries of vectors or packages that encode members of a diverse family of human antibodies comprising heavy chain (HC) CDR3s that are between about 3 amino acids in length to about 35 amino acids in length. The HC CDR3s may also, in certain embodiments, may be rich in Tyr (Y) and Ser (S) and/or comprise diversified D regions and/or comprise extended JH regions. For example, the HC CDR3s may contain greater than about 40% (e.g., between about 43% and about 80%; e.g., greater than about 40% but less than about 100%) Y and/or S residues, e.g., as provided in the examples herein. Also provided are focused libraries comprising such HC CDR3s. Also provided are designs for HC CDR1, HC CDR2, and a library of VKIII A27 with diversity in the CDRs. A library of vectors or packages that encode members of a diverse family of human antibodies comprising HC CDR3s described herein can further have diversity at one or more (e.g., at one, two or three) of HC CDR1, HC CDR2, LC CDR1, LC CDR2, and LC CDR3. For example, the library can have diversity at one or more (e.g., at one, two or three) of HC CDR1, HC CDR2, LC CDR1, LC CDR2, and LC CDR3 as described herein.

[0009] A diversified D region is a D region into which one or more amino acid changes have been introduced (e.g., as compared to the sequence of a nautrally occurring D region; for example, a stop codon can be changed to a Tyr residue).

[0010] An extended JH region is a JH region that has one or more amino acid residues present at the amino terminus of the framework sequence of the JH region (e.g., amino terminal to FR4 sequences, e.g., which commence with WGQ ...). For example, JH1 is an extended JH region. As other examples, JH2, JH3, JH4, JH5, and JH6 are extended JH regions.

[0011] Provided also are methods of making and screening the above libraries and the HC CDR3s and antibodies obtained in such screening. Compositions and kits for the practice of these methods are also described herein.

[0012] In some aspects, the disclosure features a focused library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins (e.g., a diverse family of antibodies) and collectively display, display and express, or comprise at least a portion of the diversity of the family, wherein the vectors or genetic packages comprise variegated DNA sequences that encode a heavy chain (HC) CDR3 selected from the group consisting of:

(a) a HC CDR3 that is about 3 or about 4 or about 5 amino acids in length;

(b) a HC CDR3 that is about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34 or about 35 amino acids in length (e.g., about 23 to about 35 amino acids in length); and

c) a HC CDR3 that is from about 6 to about 20 amino acids in length (e.g., about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, or about 20 amino acids in length);

wherein the HC CDR3 comprises amino acids from a D region (e.g., a diversified D region) (or fragment thereof (e.g., 3 or more amino acids of the D region, e.g., diversified D region)) or a JH region (e.g., an extended JH region).

[0013] In some embodiments, the HC CDR3 is enriched in Tyr (Y) and Ser (S) (e.g., greater than 40% of the residues of the HC CDR3 are Y and/or S).

[0014] In some embodiments, the library (e.g., the vectors or genetic packages thereof) comprises a D region or a fragment of a D region (e.g., wherein the D region is adjacent to a JH region).

[0015] In some embodiments, the library comprises a JH region, e.g., an extended JH region.

[0016] In some embodiments, the HC CDR3 comprises amino acids from a D region or a fragment of a D region (e.g., wherein the D region is adjacent to a JH region).

[0017] In some embodiments, the D region is selected from the group consisting of D2-2 (RF 2), D2-8(RF 2), D2-15(RF 2), D2-21(RF 2), D3-16(RF 2), D3-22 (RF 2), D3-3 (RF-2), D3-9 (RF 2), D3-10 (RF 2), D1-26 (RF 3), D4-11 (RF 2), D4-4 (RF 2), D5-5 (RF 3), D5-12 (RF 3), D5-18 (RF 3), D6-6 (RF1), D6-13 (RF 1), and D6-19 (RF 1).

[0018] In some embodiments, the HC CDR3 comprises amino acids from a JH region. The JH region may be an extended JH region. In some embodiments, the extended JH region is selected from the group consisting of JH1, JH2, JH3, JH4, JH5, and JH6. In some embodiments, the JH region may be enriched in Y and/or S residues, for example, it may contain greater than about 40% (e.g., between about 43% and about 80%; e.g., greater than about 40% but less than about 100%) Y and/or S residues.

[0019] In some embodiments, the D region comprises one or more cysteine (Cys) residues and in some embodiments, the one or more Cys residues are held constant (e.g., are not varied).

[0020] In some embodiments, the HC CDR3 (e.g., the DNA encoding the HC CDR3) comprises one or more filling codons between FR3 and the D region and each filling codon is individually NNK, TMY, TMT, or TMC (TMY, TMT, or TMC encode S or Y).

[0021] In some embodiments, the HC CDR3 (e.g., the DNA encoding the HC CDR3) comprises one or more filling codons between the D region and JH and each filling codon is individually NNK, TMY, TMT, or TMC.

[0022] In some embodiments, the library (e.g., the vectors or genetic packages of the library) further comprises a HC CDR1, HC CDR2, and/or a light chain and also comprises diversity in the HC CDR1, HC CDR2, or light chain comprises diversity in HC CDR1 and/or HC CDR2, and/or a light chain (e.g., kappa or lambda light chain) (respectively). For example, HC CDR3 diversity can be constructed in the background of diversity in HC CDR1, HC CDR2, and/or light chains. For example, the light-chain diversity may be encoded in the same DNA molecule as the HC diversity or the LC and HC diversities may be encoded in separate DNA molecules.

[0023] In some aspects, the disclosure features a library comprising a HC CDR3 that is 3, 4, or 5 amino acids in length, wherein the CDR3 comprises amino acids from a JH region (e.g.,

extended JH region) or from a D region (e.g., a diversified D region) (or fragment thereof (e.g., 3 or more amino acids of the D region, e.g., diversified D region)) joined to the FR4 portion of a JH region.

[0024] In some embodiments, the HC CDR3 is from a D region joined to the FR4 portion of a JH region and comprises a trimer, a tetramer, or a pentamer, wherein the trimer, tetramer, or pentamer does not comprise a cysteine residue.

[0025] In some embodiments, the HC CDR3 is from a D region joined to the FR4 portion of a JH region and comprises a trimer, a tetramer, or a pentamer, wherein the trimer, tetramer, or pentamer does not comprise a stop codon.

[0026] In some embodiments, the D region (e.g., the DNA encoding the D region) comprises a TAG codon and the TAG codon is replaced by a codon selected from the group consisting of TCG, TTG, TGG, CAG, AAG, TAT, and GAG.

[0027] In some embodiments, the D region (e.g., the DNA encoding the D region) comprises a TAA codon and the TAA codon is replaced by a codon selected from the group consisting of TCA, TTA, CAA, AAA, TAT, and GAA.

[0028] In some embodiments, the D region (e.g., the DNA encoding the D region) comprises a TGA codon and the TGA codon is replaced by a codon selected from the group consisting of TGG, TCA, TTA, AGA, and GGA.

[0029] In some embodiments, the library further comprises diversity in HC CDR1 and/or HC CDR2, and/or a light chain (e.g., kappa or lambda light chain). For example, HC CDR3 diversity can be constructed in the background of diversity in HC CDR1, HC CDR2, and/or light chains. For example, the light-chain diversity may be encoded in the same DNA molecule as the HC diversity or the LC and HC diversities may be encoded in separate DNA molecules.

[0030] In some aspects, the disclosure provides a method of diversifying a library, the method comprising mutagenizing a library described herein.

[0031] In some embodiments, the mutagenizing comprises error-prone PCR.

[0032] In some embodiments, the mutagenizing comprises wobbling.

[0033] In some embodiments, the mutagenizing comprises dobbling.

[0034] In some embodiments, the mutagenizing introduces on average about 1 to about 10 mutations (e.g., about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10 mutations; e.g., base changes) per HC CDR3.

[0035] "Wobbling" is a method of making variegated DNA so that an original sequence is favored. If the original sequence had, for example, an Ala that could be encoded with GCT the mixture (0.7 G, 0.1 A, 0.1 T, 0.1 C) can be used for the first position, (0.7 C, 0.1 A, 0.1 T, 0.1 G) at the second position, and (0.7 T, 0.1 A, 0.1 G, 0.1 C) at the third. Other ratios of "doping" can be used. This allows Ala to appear about 50% of the time while V, D, G, T, P, and S occur about 7% of the time. Other AA types occur at lower frequency.

[0036] In some aspects, the present disclosure is drawn, e.g., to keeping a (purified) HC CDR1-2 repertoire, and building synthetic HC CDR3 and LC diversity.

[0037] In some embodiments, the dislocsure provides a cassette for displaying a wobbled heavy chain (HC) CDR3, for example, wherein the cassette comprises the cassette shown in Table 400.

[0038] In some aspects, the present disclosure features a library in which Tyr levels are controlled in the HC CDR3. In some embodiments, the HC CDR3 regions contain about 15% or greater (e.g., about 16%, about 18%, about 20%, or greater) Tyr residues. In some embodiments, high levels (e.g., more than about 20%) of Tyr are inserted into the HC CDR3 of library members, e.g., at D regions and J stumps (or synthetic sequences corresponding thereto) that contain Tyr. In some embodiments, at leadin or DJ filler positions (or synthetic sequences corresponding thereto), Tyr is allowed, but at no more than 20%. In some embodiments, the HC CDR3 regions contain less than about 15% (e.g., about 14%, about 12%, about 10%, about 8%, about 6% or less)Tyr residues. In some embodiments, the HC leadin or DJ filler positions (or synthetic sequences corresponding thereto) contain less than about 15% (e.g., about 14%, about 12%, about 10%, about 8%, about 6% or less) Tyr residues.

[0039] In some aspects, the disclosure features a library of genetic packages that encode a human antibody heavy chain in which a parent amino-acid sequence comprises a VH sequence followed by zero to ten amino acids selected from the group consisting of (Y, S, D, L, R), followed by a human D-region or fragment of a D-region, followed by zero to ten amino acids

selected from the group consisting of (Y,S,R,D,L), followed by a JH segment that comprises at least W103 onward wherein the variable DNA encoding this sequence is synthesized in a way that the parental amino-acid sequence is the most likely one (e.g., by wobbling).

[0040] In some aspects, the disclosure features a library of light chains having germline framework regions and wherein the CDRs are varied such that residues remote from the combining site or having buried side groups are held constant. In some embodiments, a method of variable DNA synthesis is used so that germline sequence is the most likely one (e.g., by wobbling).

[0041] In some aspects, the disclosure features a library of diverse members encoding antigen binding variable regions as disclosed herein.

[0042] In some aspects, the disclosure features a library of diverse members encoding HC CDR3 regions as disclosed herein. In some embodiments, the library is a library of Table 1097.

[0043] In some aspects, the disclosure features a library of diverse members, each member encoding comprising a HC CDR 3, wherein

at least 1, 2, 3, 4, 5, 6, 7, or 8 positions in the HDCR3, respectively, is occupied by G, S, R, D, L, and Y in the library in the following proportions [1.0G, .57S, .46R, .42D, .36L, .35Y] and optionally,

the last 4 positions of HC CDR3 are represented as follows:

the parental amino acid is present at 3, 4, 5, 6, 7, 8, 10 times as likely as other amino-acid types, wherein the other amino-acid types comprise Y, S, D, R, G.

[0044] In some aspects, the disclosure features a library of diverse members, each member encoding comprising a HC CDR 3, wherein

at least one and preferably all of the first 1, 2, 3, 4, 5, 6, 7, or 8 positions in the HC CDR3, is occupied by G, S, R, D, L, and Y, in the library in the following proportions [1.0G, .57S, .46R, .42D, .36L, .35Y] and optionally

the last 4 positions of HCDR3 are represented as follows:

the parental amino acid is present at 3, 4, 5, 6, 7, 8, 10 times as likely as other amino-acid types, wherein the other amino-acid types comprise Y, S, D, R, G.

[0045] In some aspects, the disclosure features a library of diverse members, each member encoding a HC CDR 3, wherein

the length of HC CDR3 is 10, 11, or 12 positions;

each of the first 6, 7, or 8 positions in the HC CDR3, respectively, is occupied by G, S, R, D, L, and Y in the library in the following proportions [1.0G, .57S, .46R, .42D, .36L, .35Y];

the last 4 positions of HCDR3 are represented as follows:

the parental amino acid is present at 3, 4, 5, 6, 7, 8, 10 times as likely as other amino-acid types, wherein the other amino-acid types comprise Y, S, D, R, G.

[0046] In some embodiments, each of the last 4 HC CDR3 positions is represented in the library as 7/12 parental, plus 1/12 each of Y, S, D, R, and G.

[0047] In some embodiments, each of the last 4 HC CDR3 positions is represented in the library as A6= 7/12 A, plus 1/12 each of Y, S, D, R, and G; F7 = 7/12 F plus 1/12 each of Y, S, D, R, and G; D8 = 7/11 D plus 1/11of Y, S, R, and G; I9 = 7/12 I plus 1/12 Y, S, R, D, G.

[0048] In some embodiments, the members further encode HC CDR1, HC CDR2.

[0049] In some embodiments, the members further encode Fframwork (FR) regions 1-4.

[0050] In some embodiments, the members encode HC CDR1, HC CDR2 and FR regions 1-4.

[0051] In some embodiments, the members comprise a 3-23 HC framework.

[0052] In some embodiments, the library further comprises a LC variable region.

[0053] In some embodiments, the library comprises members encoding diverse LC variable regions.

[0054] In some embodiments, the members comprising a LC variable region comprise an A27 LC framework.

[0055] In some embodiments, the library is a display library, e.g., a phage display library.

[0056] In some embodiments, the library has at least 10^4, 10^5 10^6, 10^7, 10^8, 10^9 10^{10}, 10^{11} diverse members.

[0057] In some aspects, the disclosure features a method of selecting a library member, comprising; contacting a library described herein with a target, allowing a member to bind to said target, and recovering the member which binds the target.

[0058] These embodiments of the present invention, other embodiments, and their features and characteristics will be apparent from the description, drawings, and claims that follow.

DETAILED DESCRIPTION

[0059] Antibodies ("Ab") concentrate their diversity into those regions that are involved in determining affinity and specificity of the Ab for particular targets. These regions may be diverse in sequence or in length. Generally, they are diverse in both ways. However, within families of human antibodies the diversities, both in sequence and in length, are not truly random. Rather, some amino acid residues are preferred at certain positions of the CDRs and some CDR lengths are preferred. These preferred diversities account for the natural diversity of the antibody family.

[0060] According to this invention, and as more fully described below, libraries of vectors and genetic packages that encode members of a diverse family of human antibodies comprising heavy chain (HC) CDR3s that are between about 3 to about 35 amino acids in length may be prepared and used. The HC CDR3s may also, in certain embodiments, may be rich in Y and S and/or comprise diversified D regions. Also provided are focused libraries comprising such HC CDR3s.

[0061] When an immune cell constructs an antibody heavy chain, it connects a V segment to a D segment and that to a J segment. The D segment is optional and about 50% of human Abs have recognizable Ds. The cell may perform considerable editing at the junction sites (V-to-D, D-to-J, or V-to-J) both removing and adding bases, but not exactly randomly. The initially rearranged antibody is presented on the surface of the cell and if it binds an antigen (Ag), the cell is stimulated to perform somatic mutations to improve the affinity. There are hot spots encoded in the immunoglobulin germline genes so that certain places in the Ab gene are very likely to go through a particular set of mutations in search of a better binder to a persistent Ag. In nature, some of the mutations are in framework positions but most are in the complementarity determining regions (CDRs). Of particular interest is the CDR3 of the heavy chain (HC) because it shows not only a high degree of sequence diversity but also length diversity. Antibody (Ab) libraries have been built in which the CDRs are replaced with random DNA, and useful Abs have been obtained. However, some therapeutic Abs show a significant degree of antigenicity. It is possible that Abs that are closer to human germline would be less antigenic.

[0062] Definitions

[0063] For convenience, before further description of the present invention, certain terms employed in the specification, examples and appended claims are defined here.

[0064] The singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise.

[0065] The term "affinity" or "binding affinity" refers to the apparent association constant or K_a. The K_a is the reciprocal of the dissociation constant (K_d). A binding protein may, for example, have a binding affinity of at least 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10} and 10^{11} M^{-1} for a particular target molecule. Higher affinity binding of a binding protein to a first target relative to a second target can be indicated by a higher K_A (or a smaller numerical value K_D) for binding the first target than the K_A (or numerical value K_D) for binding the second target. In such cases, the binding protein has specificity for the first target (e.g., a protein in a first conformation or mimic thereof) relative to the second target (e.g., the same protein in a second conformation or mimic thereof; or a second protein). Differences in binding affinity (e.g., for specificity or other comparisons) can be at least 1.5, 2, 3, 4, 5, 10, 15, 20, 37.5, 50, 70, 80, 91, 100, 500, 1000, or 10^5 fold.

[0066] Binding affinity can be determined by a variety of methods including equilibrium dialysis, equilibrium binding, gel filtration, ELISA, surface plasmon resonance, or spectroscopy (e.g., using a fluorescence assay). Exemplary conditions for evaluating binding affinity are in TRIS-buffer (50mM TRIS, 150mM NaCl, 5mM $CaCl_2$ at pH7.5). These techniques can be used to measure the concentration of bound and free binding protein as a function of binding protein (or target) concentration. The concentration of bound binding protein ([Bound]) is related to the concentration of free binding protein ([Free]) and the concentration of binding sites for the binding protein on the target where (N) is the number of binding sites per target molecule by the following equation:

$$[\text{Bound}] = N \cdot [\text{Free}]/((1/K_A) + [\text{Free}]).$$

[0067] It is not always necessary to make an exact determination of K_A, though, since sometimes it is sufficient to obtain a quantitative measurement of affinity, e.g., determined using a method such as ELISA or FACS analysis, is proportional to K_A, and thus can be used for comparisons, such as determining whether a higher affinity is, e.g., 2-fold higher, to obtain a qualitative measurement of affinity, or to obtain an inference of affinity, e.g., by activity in a functional

assay, e.g., an *in vitro* or *in vivo* assay.

[0068] The term "antibody" refers to a protein that includes at least one immunoglobulin variable domain or immunoglobulin variable domain sequence. For example, an antibody can include a heavy (H) chain variable region (abbreviated herein as VH), and a light (L) chain variable region (abbreviated herein as VL). In another example, an antibody includes two heavy (H) chain variable regions and two light (L) chain variable regions. Heavy chain and light chain may also be abbreviated as HC and LC, respectively. The term "antibody" encompasses antigen-binding fragments of antibodies (e.g., single chain antibodies, Fab and sFab fragments, $F(ab')_2$, Fd fragments, Fv fragments, scFv, and domain antibodies (dAb) fragments (de Wildt et al., Eur J Immunol. 1996; **26**(3):629-39.)) as well as complete antibodies. An antibody can have the structural features of IgA, IgG, IgE, IgD, IgM (as well as subtypes thereof). Antibodies may be from any source, but primate (human and non-human primate) and primatized are preferred.

[0069] The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, termed "framework regions" ("FR"). The extent of the framework region and CDRs has been precisely defined (see, Kabat, E.A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. (1987) *J. Mol. Biol.* 196:901-917, see also www.hgmp.mrc.ac.uk). Kabat definitions are used herein. Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

[0070] The VH or VL chain of the antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. In IgGs, the heavy chain constant region includes three immunoglobulin domains, CH1, CH2 and CH3. The light chain constant region includes a CL domain. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system. The light

chains of the immunoglobulin may be of types, kappa or lambda. In one embodiment, the antibody is glycosylated. An antibody can be functional for antibody-dependent cytotoxicity and/or complement-mediated cytotoxicity.

[0071] One or more regions of an antibody can be human or effectively human. For example, one or more of the variable regions can be human or effectively human. For example, one or more of the CDRs can be human, e.g., HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3. Each of the light chain CDRs can be human. HC CDR3 can be human. One or more of the framework regions can be human, e.g., FR1, FR2, FR3, and FR4 of the HC or LC. For example, the Fc region can be human. In one embodiment, all the framework regions are human, e.g., derived from a human somatic cell, e.g., a hematopoietic cell that produces immunoglobulins or a non-hematopoietic cell. In one embodiment, the human sequences are germline sequences, e.g., encoded by a germline nucleic acid. In one embodiment, the framework (FR) residues of a selected Fab can be converted to the amino-acid type of the corresponding residue in the most similar primate germline gene, especially the human germline gene. One or more of the constant regions can be human or effectively human. For example, at least 70, 75, 80, 85, 90, 92, 95, 98, or 100% of an immunoglobulin variable domain, the constant region, the constant domains (CH1, CH2, CH3, CL), or the entire antibody can be human or effectively human.

[0072] All or part of an antibody can be encoded by an immunoglobulin gene or a segment thereof. Exemplary human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the many immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 KDa or about 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH--terminus. Full-length immunoglobulin "heavy chains" (about 50 KDa or about 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids). The length of human HC varies considerably because HC CDR3 varies from about 3 amino-acid residues to over 35 amino-acid residues.

[0073] Herein, the terms "D segment" and "D region" are used interchangeably and are identical. It is to be understood that these items have both DNA and amino-acid representations and that

which is meant is clear from the context.

[0074] A "library" or "display library" refers to a collection of nucleotide, e.g., DNA, sequences within clones; or a genetically diverse collection of polypeptides displayed on replicable display packages capable of selection or screening to provide an individual polypeptide or a mixed population of polypeptides.

[0075] The term "package" as used herein refers to a replicable genetic display package in which the particle is displaying a polypeptide at its surface. The package may be a bacteriophage which displays an antigen binding domain at its surface. This type of package has been called a phage antibody (pAb).

[0076] A "pre-determined target" refers to a target molecule whose identity is known prior to using it in any of the disclosed methods.

[0077] The term "replicable display package" as used herein refers to a biological particle which has genetic information providing the particle with the ability to replicate. The particle can display on its surface at least part of a polypeptide. The polypeptide can be encoded by genetic information native to the particle and/or artificially placed into the particle or an ancestor of it. The displayed polypeptide may be any member of a specific binding pair e.g., heavy or light chain domains based on an immunoglobulin molecule, an enzyme or a receptor etc. The particle may be, for example, a virus e.g., a bacteriophage such as fd or M13.

[0078] The term "vector" refers to a DNA molecule, capable of replication in a host organism, into which a gene is inserted to construct a recombinant DNA molecule. A "phage vector" is a vector derived by modification of a phage genome, containing an origin of replication for a bacteriophage, but not one for a plasmid. A "phagemid vector" is a vector derived by modification of a plasmid genome, containing an origin of replication for a bacteriophage as well as the plasmid origin of replication.

[0079] In discussing oligonucleotides, the notation "[RC]" indicates that the Reverse Complement of the oligonucleotide shown is the one to be used.

[0080] Human Antibody Heavy Chain CDR3s

[0081] The heavy chain ("HC") Germ-Line Gene (GLG) 3-23 (also known as VP-47) accounts for about 12% of all human Abs and is preferred as the framework in the preferred embodiment of the invention. It should, however, be understood that other well-known frameworks, such as

4-34, 3-30, 3-30.3 and 4-30.1, may also be used without departing from the principles of the focused diversities of this invention.

[0082] In addition, JH4 (YFDY**W$_{103}$GQGTLVTVSS** (SEQ ID NO:1)) occurs more often than JH3 in native antibodies. Hence, it is preferred for the focused libraries of this invention. However, JH3 (AFDI**W$_{103}$GQGTMVTVSS** (SEQ ID NO:2)), JH6 (YYYYYGMDV**W$_{103}$GQGTTVTVSS** (SEQ ID NO:3)), JH1, JH2, or JH5 could be used as well. JH2 has the advantage of having RG at 105-106 instead of QG in all the other human JHs. JH3 has the disadvantage of M$_{108}$. In a collection of 1419 Abs that were ELISA positive for at least one target, we saw 17 JH1s, 31 JH2s, 452 JH3s, 636 JH4s, 32 JH5s, and 251 JH6s. If present, the double underscored portions of the JHs are considered to be part of CDR3. In Table 3, the FR4 parts of the JHs are underscored.

[0083] The frequency at which each amino-acid appeared in the HC CDR3s of these 1419 Abs was tabulated and recorded in Table 75. Note that the most common amino acid is Tyr with Gly, Asp, Ser, and Arg following in that order. Rel. Up is the relative abundance of each type compared to Cys, the least common. Rel. Down is the abundance of each type compared to Tyr, the most common. Hence the preferred amino-acid types to substitute into HC CDR3s are Y, G, D, S, and R.

[0084] Naturally, HC CDR3s vary in length. About half of human HCs consist of the components: V::nz::D::ny::JHn where V is a V gene, nz is a series of bases that are essentially random, D is a D segment, often with heavy editing at both ends, ny is a series of bases that are essentially random, and JHn is one of the six JH segments, often with heavy editing at the 5' end. The D segments appear to provide spacer segments that allow folding of the IgG. The greatest diversity is at the junctions of V with D and of D with JH.

[0085] Corbett et al. (Corbett SJ, Tomlinson IM, Sonnhammer EL, Buck D, Winter G. **J Mol Biol**. 1997 V*270*:587-97.) showed that the human immune system does not insert multiple D segments and recombing D segments. Nevertheless, D segments have been selected to be good components of HC CDR3s and the present invention comprises HC CDR3 that contain more than one D segment.

[0086] Human D segments have some very strong biases. The tally of the 522 amino-acids in human D segments is Y 70 (13.4%), L 63 (12.1%), V 52 (10%), G 49 (9.4%), I 41 (7.9%), T 40 (7.7%), S 33 (6.3%), W 27 (5.2%), D 21 (4%), A 19 (3.6%), R 16 (3.1%), TAG 15 (2.9%), N 14

2.7%), Q 11 (2.1%), C 9 (1.7%), E 9 (1.7%), F 8 (1.5%), M 8 (1.5%), TGA 8 (1.5%), TAA 7 (1.3%), P 1 (0.2%), H 1 (0.2%), and K 0 (0%). There is one D (2-8 RF 1) that has an unpaired Cys but also a TGA stop codon, so it is little used. Thus, D segments are primarily hydrophobic. The frequencies of amino acids in human HC CDR3s are shown in Table 75. There are both similarities and differences in the frequencies. In HC CDR3s overall, Tyr is the most common and only Gly comes close (96% as common as Tyr). Asp (75% as common as Tyr), Ser (53% as common as Tyr). Leu, Val, and Ile are relatively common in the D segments if all the D segments are counted as equal. The immune system does not use the D segments with equal frequency. Table 77 shows the frequency of utilization of D segments. The D segments that are often used are very rich in Tyr, Gly, Ser, and Asp. Arg is not found in the most often used D segments nor is Arg encoded in any of the CDR portions of JH segments. Arg comes to prominence either by mutation of V, D, and J or in the filler regions between V and D, D and J, or V and J. In this sample, 50% of all the amino acids are Tyr, Gly, Asp, Ser, or Arg. In one embodiment of the present invention, substitutions of "parental" HC CDR3 sequences is limited to the set of amino acids consisting of Tyr, Gly, Ser, Asp, and Arg. In one embodiment of the present invention, Arg is made common in the filler regions between V and D, between D and J, or between V and J.

[0087] In the preferred libraries of this invention, both types of HC CDR3s are used. In HC CDR3s that have no identifiable D segment, the structure is V::nz::JHn (n=1,6) where JH is usually edited at the 5' end. In HC CDR3s that have an identifiable D segment, the structure is V::nz::D::ny::JHn.

[0088] Provided herein are HC CDR3s that are between about 3 to a about 35 amino acids in length. The HC CDR3s may also, in certain embodiments, be rich in Y and S and/or comprise diversified D regions, where a D region is present. For example, the HC CDR3s may contain between about 43% and about 80% Y and/or S residues, e.g., about 43%, about 48%, about 69%, about 63%, about 71%, about 62%, about 58%, about 68%, about 80%, about 77%, or greater than about 40%, or about 40% to less than about 100%, of the residues are Y and/or S. For example, not all of the residues in the CDR3 are Y and/or S. The HC CDR3s may, in certain embodiments, comprise an extended JH region. Exemplary HC CDR3 component designs of the preferred libraries of this invention are shown and described in Examples 1, 2, and 3.

[0089] In some embodiments, diversity (e.g., in a CDR, e.g., HC CDR3, or framework region (e.g., framework region near or adjacent to a CDR, e.g., CDR3, e.g., HC CDR3) is generated to create on average about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, or about 1 to about 10 mutations (e.g., base change), e.g., per CDR (e.g., HC CDR3) or framework region (e.g., framework region near or adjacent to a CDR, e.g., CDR3, e.g., HC CDR3). In some implementations, the mutagenesis is targeted to regions known or likely to be at the binding interface. Further, mutagenesis can be directed to framework regions near or adjacent to the CDRs. In the case of antibodies, mutagenesis can also be limited to one or a few of the CDRs, e.g., to make precise step-wise improvements. Likewise, if the identified ligands are enzymes, mutagenesis can provide antibodies that are able to bind to the active site and vicinity. The CDR or framework region (e.g., an HC CDR3 described herein) may be, in certain embodiments, subjected to error-prone PCR to generate the diversity. This approach uses a "sloppy" version of PCR, in which the polymerase has a fairly high error rate (up to 2%), to amplify the wild-type sequence, and is generally described in Pritchard, et al. (2005) *J. Theor. Biol.* 234: 497-509 and Leung et al. (1989) *Technique* 1:11-15. Other exemplary mutagenesis techniques include DNA shuffling using random cleavage (Stemmer (1994) *Nature* 389-391; termed "nucleic acid shuffling"), RACHITT™ (Coco et al. (2001) *Nature Biotech.* 19:354), site-directed mutagenesis (Zoller et al. (1987) *Nucl Acids Res* 10:6487-6504), cassette mutagenesis (Reidhaar-Olson (1991) *Methods Enzymol.* 208:564-586) and incorporation of degenerate oligonucleotides (Griffiths et al. (1994) *EMBO J.* 13:3245).

[0090] In some embodiments of the invention, D segments in which a majority of the residues are either Ser or Tyr are picked. In some embodiments, when the DNA encoding the D region is synthesized, each Ser or Tyr residue is encoded by TMT, TMC, or TMY so that the encoded amino acid is either Ser or Tyr.

[0091] In some embodiments, the HC CDR3 sequences described herein may be subjected to selection for open reading frames by fusing the sequence encoding the HC CDR3 of interest in frame to an antibiotic resistance gene, such as Kan^R gene and selecting for kanamycin resistance. Cells in which the potential CDR3 has a stop codon or a frame shift will not have the antibiotic resistance and that sequence will be eliminated.

[0092] Methods of Construction of Libraries comprising Human Antibody Heavy Chain CDR3s and Libraries comprising Human Antibody Heavy Chain CDR3s

[0093] An antibody library is a collection of proteins that include proteins that have at least one immunoglobulin variable domain sequence. For example, camelized variable domains (e.g., VH domains) can be used as a scaffold for a library of proteins that include only one immunoglobulin variable domain sequence. In another example, the proteins include two variable domains sequences, e.g., a VH and VL domain, that are able to pair. An antibody library can be prepared from a nucleic acid library (an antibody-coding library) that includes antibody-coding sequences, e.g., comprising the sequences encoding the HC CDR3s provided herein.

[0094] In cases where a display library is used, each member of the antibody-coding library can be associated with the antibody that it encodes. In the case of phage display, the antibody protein is physically associated (directly or indirectly) with a phage coat protein. A typical antibody display library member displays a polypeptide that includes a VH domain and a VL domain. The display library member can display the antibody as a Fab fragment (e.g., using two polypeptide chains) or a single chain Fv (e.g., using a single polypeptide chain). Other formats can also be used.

[0095] As in the case of the Fab and other formats, the displayed antibody can include one or more constant regions as part of a light and/or heavy chain. In one embodiment, each chain includes one constant region, e.g., as in the case of a Fab. In other embodiments, additional constant regions are included. It is also possible to add one or more constant regions to a molecule after it is identified as having useful antigen binding site. See, e.g., US 2003-0224408.

[0096] Antibody libraries can be constructed by a number of processes (see, e.g., de Haard et al. (1999) *J. Biol. Chem* 274:18218-30; Hoogenboom et al. (1998) *Immunotechnology* 4:1-20, Hoogenboom et al. (2000) *Immunol Today* 21:371-8, and Hoet et al. (2005) *Nat Biotechnol.* 23(3):344-8.

[0097] In certain embodiments for constructing libraries, the heavy chains comprising the CDR3s described herein and the kappa and lambda light chains are best constructed in separate vectors. First, a synthetic gene is designed to embody each of the synthetic variable domains. The light chains may be bounded by restriction sites for ApaLI (positioned at the very end of the signal sequence) and AscI (positioned after the stop codon). The heavy chain may be bounded

by SfiI (positioned within the PelB signal sequence) and NotI (positioned in the linker between CH1 and the anchor protein). Signal sequences other than PelB may also be used, e.g., a M13 pIII signal sequence.

[0098] The initial genes may be made with "stuffer" sequences in place of the desired CDRs. A "stuffer" is a sequence that is to be cut away and replaced by diverse DNA, but which does not allow expression of a functional antibody gene. For example, the stuffer may contain several stop codons and restriction sites that will not occur in the correct finished library vector. Stuffers are used to avoid have any one CDR sequence highly represented.

[0099] In another embodiment of the present invention, the heavy chain and the kappa or lambda light chains are constructed in a single vector or genetic packages (e.g., for display or display and expression) having appropriate restriction sites that allow cloning of these chains. The processes to construct such vectors are well known and widely used in the art. Preferably, a heavy chain and kappa light chain library and a heavy chain and lambda light chain library would be prepared separately.

[00100] Most preferably, the display is 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.

[00101] 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., pMID21 (DNA sequence shown in Table 35)) 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.

[00102] In still other embodiments, a method termed the Rapid Optimization of LIght Chains or "ROLIC", described in U.S.S.N 61/028,265 filed February 13, 2008, U.S.S.N. 61/043,938 filed April 10, 2008, and U.S.S.N. 12/371,000 filed February 13, 2009, a large population of LCs is placed in a phage vector that causes them to be displayed on phage. A small population (e.g., 3, 10, or 25) of HCs are cloned into *E. coli* so that the HCs are secreted into the periplasm, e.g., those HCs having the CDR3s described herein. The *E. coli* are then

infected with the phage vectors encoding the large population of LCs to produce the HC/LC protein pairings on the phage. The phage particles carry only a LC gene.

[00103] In another aspect, in a method termed the Economical Selection of Heavy Chains or "ESCH", also described in U.S.S.N 61/028,265 filed February 13, 2008, U.S.S.N. 61/043,938 filed April 10, 2008, and U.S.S.N. 12/371,000 filed February 13, 2009, a small population of LCs may be placed in a vector that causes them to be secreted. A new library of HCs in phage is constructed, such as those provided herein comprising the CDR3s. The LCs and HCs can then be combined by the much more efficient method of infection. Once a small set of effective HC are selected, these can be used as is, fed into ROLIC to obtain an optimal HC/LC pairing, or cloned into a Fab library of LCs for classical selection.

[00104] In another embodiment of this invention, the diversity captured by the methods of the present invention may be displayed and/or expressed using a vector suitable for expression in a eukaryotic cell, e.g., a yeast vector, e.g., for expression ina yeast cell.

[00105] Other types of protein display include cell-based display (see, e.g., WO 03/029,456); ribosome display (see, e.g., Mattheakis et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:9022 and Hanes et al. (2000) *Nat Biotechnol.* 18:1287-92); protein-nucleic acid fusions (see, e.g., U.S. Pat. No. 6,207,446); and immobilization to a non-biological tag (see, e.g., U.S. Pat. No. 5,874,214).

[00106] Antibodies isolated from the libraries of the present disclosure may be analyzed to determine the type of the LC and the closest germline gene. In a preferred embodiment, non-germline framework residues are changed back to the germline amino acid so long as binding affinity and specificity are not adversely affected to an unacceptable extent. The substitutions may be done as a group or singly. Human germline sequences are disclosed in Tomlinson, I.A. et al., 1992, *J. Mol. Biol.* 227:776-798; Cook, G. P. et al., 1995, *Immunol. Today* 16 (5): 237-242; Chothia, D. et al., 1992, *J. Mol. Bio.* 227:799-817. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I.A. et al. MRC Centre for Protein Engineering, Cambridge, UK). Antibodies are "germlined" by reverting one or more non-germline amino acids in framework regions to corresponding germline amino acids of the antibody, so long as binding properties are substantially retained. Similar methods can also be used in the constant region, e.g., in constant immunoglobulin domains.

[00107] For example, an antibody can include one, two, three, or more amino acid substitutions, e.g., in a framework, CDR, or constant region, to make it more similar to a reference germline sequence. One exemplary germlining method can include identifying one or more germline sequences that are similar (e.g., most similar in a particular database) to the sequence of the isolated antibody. Mutations (at the amino acid level) are then made in the isolated antibody, either incrementally or in combination with other mutations. For example, a nucleic acid library that includes sequences encoding some or all possible germline mutations is made. The mutated antibodies are then evaluated, e.g., to identify an antibody that has one or more additional germline residues relative to the isolated antibody and that is still useful (e.g., has a functional activity). In one embodiment, as many germline residues are introduced into an isolated antibody as possible.

[00108] In one embodiment, mutagenesis is used to substitute or insert one or more germline residues into a framework and/or constant region. For example, a germline framework and/or constant region residue can be from a germline sequence that is similar (e.g., most similar) to the non-variable region being modified. After mutagenesis, activity (e.g., binding or other functional activity) of the antibody can be evaluated to determine if the germline residue or residues are tolerated (i.e., do not abrogate activity). Similar mutagenesis can be performed in the framework regions.

[00109] Selecting a germline sequence can be performed in different ways. For example, a germline sequence can be selected if it meets a predetermined criteria for selectivity or similarity, e.g., at least a certain percentage identity, e.g., at least 75, 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 99.5% identity. The selection can be performed using at least 2, 3, 5, or 10 germline sequences. In the case of CDR1 and CDR2, identifying a similar germline sequence can include selecting one such sequence. In the case of CDR3, identifying a similar germline sequence can include selecting one such sequence, but may include using two germline sequences that separately contribute to the amino-terminal portion and the carboxy-terminal portion. In other implementations, more than one or two germline sequences are used, e.g., to form a consensus sequence.

[00110] CDR1, CDR2, and light-chain diversity

[00111] It is to be understood that the libraries of HC CDR3 are constructed in the background of diversity in HC CDR1, HC CDR2, and light chains. The light-chain diversity may be encoded in the same DNA molecule as the HC diversity or the LC and HC diversities may be encoded in separate DNA molecules. In Table 22 the fusion of a signal sequence:: VH::CH1::His6::Myc::IIIstump. CDR1 comprises residues 31-35; there is diversity at residues 31, 33, and 35. In one embodiment, residues 31, 33, and 35 can be any amino-acid type except cysteine. CDR2 comprises residues 50 through 65. There is diversity at positions 50, 52, 52a, 56, and 58. In one embodiment, residues 50, and 52 can be any of the types Ser, Gly, Val, Trp, Arg, Tyr; residue 52a can be Pro or Ser and residues 56 and 58 can be any amino-acid type except Cys. The diversity of HC CDR3 is cloned into a diversity of HC CDR1 and 2 that is at least 1. E 4, 1. E 5, 1. E 6, 1.E 7, 5. E 7, or 1. E 8.

[00112] In one embodiment, residues 31, 33, 35, 50, 52, 56, and 58 can be any amino-acid type except Cys or Met and residue 52a can be Gly, Ser, Pro, or Tyr. The diversity of HC CDR3 is cloned into a diversity of HC CDR1 and 2 that is at least 1. E 4, 1. E 5, 1. E 6, 1. E 7, 5. E 7, or 1. E 8.

[00113] In one embodiment, the diversity of the HC is cloned into a vector (phage or phagemid) that contains a diversity of light chains. This diversity is at least 25, 50, 100, 500, 1. E 3, 1. E 4, 1. E 5, 1. E 6, or 1. E7. The diversity of HC CDR3 is at least 221, 272, 500, 1000, 1. E 4, 1. E 5, 1. E 6, 1. E7, 1. E 8, or 1. E 9.

[00114] In one embodiment, the diversity of the HC is cloned into a phage vector that displays the HC on a phage protein such as III, VIII, VII, VI, or IX or a fragment of one of these sufficient to cause display and light chains are combined with the HC by infecting a cell collection wherein each cell secrets a light chain. The diversity of the light chains in the cells is at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, or 100. The diversity of HC CDR3 is at least 221, 272, 500, 1000, 1. E 4, 1. E 5, 1. E 6, 1. E7, 1. E 8, or 1. E 9.

[00115] Table 30 shows the sequence of the phage vector DY3FHC87 (SEQ ID NO:894) which carries a *bla* gene, a display cassette for heavy chains under control of a P_{lac} promoter. DY3FHC87 contains all the genes of M13 as well. Infecting F+ *E. coli* cells that harbor a diversity of light chains in a vector such as pLCSK23 (Sequence in Table 40) (SEQ ID NO:896). The vector pLCSK23 carries a Kan^R gene. Under the control of Plac promoter, there is a gene

beginning at base 2215 having a signal sequence (bases 2215-2277), a VL (in this sequence the VL encodes the sequence shown in (SEQ ID NO:897) from base 2278 to base 2598, Ckappa from base 2599 to 2922, a linker that allows an NotI site from 2923 to 2931, and a V5 tag (bases 2932-2973). There are an SfiI site at 2259-2271 and a *KpnI* site at 2602-2605 to allow easy replacement of Vkappas. (SEQ ID NO:897) is an example of the proteins that are secreted. It is to be understood that CKappa and the V5 tag are constant. All of the proteins shown in Table 19 (VK1O2gl-JK3, VK1O2var1, VK1O2var2, VK1O2var3, VK1O2var4, VK1O2var5, VK3L6gl-JK4, VK3L6var1, VK3L6var2, VK3L6var3, VK3L6var4, VK3L6var5, VK3L6var6, VK3L6var7, VK3L6var8, VK3A27gl-JK3, VK3A27var1, VK3A27var2, VK3A27var3, VK3A27var4, VK3A27var5, VK3A27var6, VK3A27var7, VK3L2gl-JK3, and VK1glL8-JK5) will have these sequences attached at the carboxy end.

[00116] Light Chain Diversity

[00117] Table 800 shows a kappa LC (light chain) that is known to pair well with 3-23 and with five CDR mutations with one HC based on 3-23, LC K1(O12)::JK1 makes a high affinity Ab to a protein target. O12 is a frequently used VKI. The gene has been designed to have useful, distinct restriction sites in the signal sequence (*Apa*LI), FR1 (*Xho*I, SgfI), FR2 (*Kpn*I), FR3(*Xba*I), and Fr4::Ckappa (*Bsi*WI) so that each CDR and be replaced with a varied population.

[00118] In human LCs, CDR3 is most important and CDR1 is next most important. CDR2 seldom makes contact with the Ag. Diversity is introduced into the CDRs as shown in Table 900 and Table 1000 (CDR1), Table 1100 and Table 1200 (CDR2), Tables 1300, 1400, and 1500 (CDR3). For Economical Selection of Heavy Chains (ESHC), a small number, for example, 50 LCs with diversity in CDR3 as in Table 1200 are picked for expression in pLCSK24 for secretion into the periplasm. More LCs can be used if several cell lines are maintained so that each cell line contains, for example, 50 or fewer LC.

[00119] Table 900 shows diversity for LC CDR1. The library can contain the O12 residue with the added diversity of the AA types shown as "allowed"; reading "allowed" as "additional allowed types" in Tables 900, 1000, 1100, 1200, 1300, 1400. O12 has R_{24}ASQSISSYLN_{34}. Other VK1 loci have Q at 24. Other loci have M at 25. S_{26} and Q_{27} are invariant in VKI. Other VKI loci have D or G at 28. I_{29} and L_{33} are invariant in VKI and the side groups are oriented

inward. Other VKI loci allow the diversity shown in Table 900 at positions 30, 31, 32, and 34. In Table 900, only seven of the eleven positions are varied and the total diversity is 576.

[00120] Table 1000 shows a higher level of diversity for LC CDR1. Here 8 of 11 positions have been varied. Those that are constant are either far from the combining site or have buried side groups.

[00121] Table 1100 shows a low level variegation for CDR2. CDR2 is far from the antigen combining site and diversity here may not be very useful. Indeed, the GL diversity is very limited. Table 1100 includes the GL diversity. Table 1200 contains a higher level of diversity, 1920 sequences allowed.

[00122] Table 1300 shows a low level of diversity for LC CDR3, 2160 sequences. Table 1400 shows a higher level which allows 105,840 sequences.

[00123] For ROLIC, about 3 x 10^7 LC are produced having the diversity shown in Tables 900, 1100, and 1300.

[00124] **Heavy Chain Diversity**

[00125] Ab HC (heavy chain) have diversity in CDR1, CDR2, and CDR3. The diversity in CDR3 is especially complex because there is both sequence and length diversity. The sequence diversity is not random. Cells making Ab genes join a V segment to a D segment to a JH segment. The D segment is optional; about half of natural human Abs have a recognizable D. There can be extensive editing at the V-D, D-J, or V-J boundaries with none to many bases added or removed. An Ab that has a germline V::D::JH could be viewed as a germline Ab.

[00126] Human D segments are shown in Table 21. Each germline (GL) D segment may appear in an Ab gene in any of the three forward reading frames. In some reading frames, some of the D segments encode stop codons. These D segments do occur rarely with the stop codon modified. Table 600 shows the frequency of each D segment as a percent of all observed D segments. Most of the examples herein that contain D segments use Ds that are fairly common (>2% of all observed Ds).

[00127] In one aspect, the present invention involves composing Ab HC genes by fusing 3-23 (or another VH, such as 4-34) to one of a) a number of amino acids picked from the set comprising (S, Y, D, R, N), b) a D region, c) a JH region, and d) the FR4 portion of a JH region. These fusions can be a GL 3-23 or a 3-23 that has synthetic diversity in CDR1 and/or CDR2.

The lengths of the HC CDR3 and be any number from about 3 to about 24. Preferably, the library would contain member with HC CDR3 of lengths 6, 8, 10, 12, 14, 16, 18, and 20. Alternatively, the lengths could be 5, 8, 11, 14, 17, and 20 or any other combination.

[00128] Table 21 shows a number of examples of designs of suitable CDR3s with lengths from 6 to 20. The codons that specify the uppercase letters in column 2 are to be synthesized with wobbling. Column 3 shows the level of doping. Table 100 shows ratios in which the various lengths of HC CDR3 could be combined to form a library that is expected to contain Abs that bind almost all protein targets.

Table 100

Length	6	8	10	12	14	16	20
Diversity	$1. \times 10^5$	$2. \times 10^5$	$4. \times 10^5$	$8. \times 10^5$	$8. \times 10^5$	$8. \times 10^5$	$4. \times 10^5$

[00129] For length 6, Table 21 four examples are given. For example, 6a has VH(3-23) joined directly to JH1 with the first six AAs wobbled, 6b has Tyr joined to D4-17 in second reading frame joined to the FR4 AAs of JH1, and 6c has D5-5(3) joined to the FR residues of JH1. Since these give different kinds of diversity, including all is preferred, but a library containing only one of these should give useful Abs.

[00130] For length 8, Table 21 shows three examples. 8a has YY fused to all of JH1 while 8b has one Y fused to D6-13(1) fused to the FR region of JH1. Lengths 10, 12, 14, 16, and 20 are also shown in Table 21. The HC CDR3 diversity could be built in a germline 3-23 or 3-23 containing synthetic diversity. Alternatively, a different VH, such as 4-34 could be used.

[00131] ROLIC is a method in which a small population of HCs are expressed in F^+ *E. coli* as soluble proteins. The population is infected with phage that carry LC::III_{stump} fusions. The phage produced obtain a HC from the periplasm of the cell that produces them. These phage can be bound to immobilized target and the binder are separated from the non-binders. The size of the population is important because when the recovered phage are propagated, the recovered phage must find the same type of cell as it came from to continue the association between LC and HC. Thus it is desirable that the number of HC be small in each cell line. Thus it may be desirable to maintain a number of cell lines with up to 10, 20, 30, or 40 different HC in each cell line. Thus we may have 1, 2, 4, 6, 8, 10, 24, 48, or 96 cell lines and we perform the same number of parallel phage productions, selections, and amplifications. After one or two rounds,

we test colonies for production of phage that bind the target by an ELISA assay. Each ELISA$^+$ colony contains a useful LC and a useful HC, but they are not on the same piece of DNA. Nevertheless, we know the start and end of each LC and each HC and can therefore use PCR on the colony to produce a Fab display or Fab secretion cassette that can be put into a display phage or phagemid or into a Fab-production plasmid.

[00132] In Efficient Selection of HCs (ESHC), we reverse the roles of LC and HC in ROLIC and have LCs in a plasmid so that they are produced as soluble proteins in the periplasm of F$^+$ *E. coli*. We produce the HC diversity in a phage vector that has no LC gene. We infect the LC-producing F$^+$ *E. coli* with the HC-carrying phage. We obtain phage that carry an HC gene and both HC and LC proteins. We select these phage for binding to the target. In many Abs, the LC is permissive and does not contribute greatly to binding affinity. Picking the best LC can greatly increase affinity, but it is usually possible to select a Fab with a very limited repertoire of LCs. Thus, we place a small set of LCs, preferable germline in the framework regions in the LC-producing F$^+$ *E. coli*. If there are, for example, 25 LC in the LC cell line, then we obtain a 25-fold reduction in the number of cell transformants that need to be made.

[00133] The libraries described have a range of HC CDR3 lengths. To favor proper folding, the HC CDR3 have either a D segment or no D segment joined to most, all, or the framework portion of a JH segment. The sequences are diversified by using wobble DNA synthesis. Although this theoretically allows any amino-acid type at any position, in practice, the actual sequences are strongly biased toward the parental sequences and AA types that are close in the genetic code table.

[00134] By using ESHC, we can sample new designs of synthetic HC CDR3 diversity. In the examples given, we use a pool of, for example, 50 LCs. A library of 5×10^8 HC should perform as well as an old-style library of 2.5×10^{10} but require far less effort.

[00135] When wobbling a sequence, picking the initial codons affects the actual mixture of AAs seen in the library. Table 300 shows which amino-acid substitutions require 1, 2, or 3 base changes from each starting parental codon. For example, if we start with gct or gcc for Ala, all three stop codons require three base changes and so are rare. If using 76:8:8:8 mixtures, Ala will appear in 57% of the cases (0.76*0.76). V, G, T, P, S will each appear in about 6% and D about 3%. E, I, L, F, Y, H, N, C, and R will be down about 10-fold. M, W, Q, K, Am, Oc, and Op will be even rarer. If we started with gca, then E would replace D in needing only one base

change, but opal and ochre stops require only two base changes, which is undesirable. The preferred codons are marked with a star (*). The choice for serine is complicate our desire to have Y substitute for S with high frequency. This brings Op and Oc into the group that differ from the parent by only two bases. This problem can be overcome by cloning the HC CDR3 repertoire before an antibiotic resistance gene such as KanR or AmpR and selecting for resistance, thus eliminating the members that contain stop codons. In addition, the library can be produced in supE *E. coli* which insert Q instead of stopping.

Table 300

Amino acid	Parental codon	1 base change	2 base changes	3 base changes
A *	gct, gcc	V, D, G, T, P, S	E, I, L, F, Y, H, N, C, R	M, W, Q, K, **Am**, **Oc**, **Op**
A	gca	V, E, G, T, P, S	D, I, L, **Oc**, Q, K, **Op**, R	M, W, H, N, C, **Am**, F, Y
A	gcg	V, E, G, T, P, S	D, M, L, **Am**, Q, K, R, W	I, F, Y, **Oc**, **Op**, H, N, C
C	tgt, tgc	Y, S, F, W, **Op**, R, G	L, H, N, D, P, T, A, V, I	**Am**, **Oc**, Q, K, E, M
D	gat, gac	E, G, A, V, N, H, Y	F, S, C, L, P, Q, K, R, **Oc**, **Am**, I, T	M, W, **Op**
E	gaa	D, G, A, V, K, Q, **Oc**	**Am**, L, I, S, P, T, R, **Op**, Y, H, N	M, F, C, W
E *	gag	D, G, A, V, K, Q, **Am**	M, L, S, P, T, Y, H, N, **Oc**, R, W	F, C, I, **Op**
F	ttt, ttc	L, I, V, S, Y, C	M, **Am**, **Op**, **Oc**, W, P, T, A, H, N, D, R, G	Q, K, E
G *	ggt, ggc	D, A, V, S, R, C	E, W, F, L, I, T, P, Y, H, N	**Am**, **Oc**, **Op**, M, Q, K
G	gga	E, A, V, R, **Oc**	D, W, L, I, S, P, T, **Op**, Q, K	**Am**, **Oc**, M, F, Y, H, N
G	ggg	E, A, V, R, W	D, **Oc**, L, M, S, P, T, **Am**, **Op**, Q, K	**Oc**, I, F, Y, H, N
H	cat, cac	Q, Y, N, D, L, P, R	F, S, C, I, T, V, A, D, G, **Am**, **Oc**	**Op**, W, M, E
I *	att, atc	M, L, F, V, T, N, S	Y, C, P, H, R, A, D, G	**Am**, **Op**, **Oc**, W, Q, K, E
I	ata	M, L, V, T, K, R	**Op**, **Oc**, S, P, Q, A, E, G, F, N	**Am**, C, D, H, W, Y
K	aaa	N, Q, **Oc**, E, P, I, R	H, Y, D, M, L, V, S, T, A, **Am**, **Op**, G	C, F, W
K *	aag	N, Q, **Am**, E, P, M, R	H, Y, D, I, L, V, S, T, A, **Oc**, G, W	C, F, **Op**

Amino acid	Parental codon	1 base change	2 base changes	3 base changes
L	tta	F, S, **Oc**, **Op**, I, V	Y, C, W, M, P, T, A, Q, K, E, R, G, **Am**	D, H, N
L	ttg	F, S, **Am**, W, M, V	Y, C, **Oc**, **Op**, P, T, A, Q, K, E, R, G, I	D, H, N
L *	ctt, ctc	F, I, V, P, H, R	M, S, Y, C, T, N, A, D, G	**Am**, **Oc**, **Op**, W, E, K, Q
L	cta	I, V, P, Q, R	F, M, S, **Oc**, **Op**, T, K, A, E, G, H	**Am**, W, D, N, C, Y
L	ctg	M, V, P, Q, R	F, I, S, **Am**, T, K, A, E, G, H, W	**Oc**, **Op**, D, N, C, Y
M	atg	L, V, T, K, R, I	F, N, S, P, A, **Am**, Q, E, W, G	**Oc**, **Op**, Y, C, H, D
N	aat, aac	K, Y, H, D, I, T, S	F, C, L, P, R, V, A, G, M, Q, E, **Am**, **Oc**	**Op**, W
P *	cct, ccc	S, T, A, L, H, R	F, Y, C, I, N, V, D, G, Q	**Am**, **Oc**, **Op**, W, M, E, K
P	cca	S, T, A, L, Q, R	**Oc**, **Op**, I, K, V, E, G, H	**Am**, W, M, D, N, C, F, Y
P	ccg	S, T, A, L, Q, R	**Am**, M, K, V, E, G, H	C, D, F, I, N, W, Y, **Oc**, **Op**
Q	caa	**Oc**, K, E, R, P, L, H	Y, **Am**, N, D, S, T, A, I, V, G, **Op**	F, C, W, M
Q *	cag	H, **Am**, K, E, R, P, L	N, D, Y, M, T, V, A, G, W, **Oc**, S	C, F, **Op**, I
R *	cgt, cgc	C, S, G, H, P, L	**Op**, W, Q, F, Y, I, T, N, V, A, D	**Am**, **Oc**, M, E, K
R	cga	G, **Op**, Q, P, L	**Oc**, S, C, W, H, I, V, T, A, E, K	**Am**, M, C, D, N, F, Y
R	cgg	G, W, Q, P, L	**Am**, **Op**, S, M, V, T, A, K, E, H, C	F, Y, I, **Oc**, D, N
R	aga	G, **Op**, S, K, T, I	C, W, N, M, L, V, P, A, **Oc**, Q, E	F, Y, H, D, **Am**
R	agg	G, W, S, K, T, M	C, **Op**, **Am**, L, I, V, A, Q, P, E, N	F, Y, H, D, **Oc**
S *	tct, tcc	F, Y, C, P, T, A	L, **Oc**, **Op**, **Am**, W, I, V, N, D, R, G, H	E, K, M, Q
S	tca	L, **Oc**, **Op**, P, T, A	F, Y, C, W, Q, R, I, K, V, E, G, **Am**	M, W, D, N, H
S	tcg	L, **Am**, W, P, T, A	F, Y, C, **Op**, **Oc**, Q, R, M, K, V, E, G	I, D, N, H
S	agt, agc	C, R, G, N, T, I	F, Y, L, P, H, V, A, D, K, W, **Op**	**Am**, **Oc**, M, E, Q
T *	act, acc	S, P, A, I, N	F, Y, C, L, H, R, M, K, V, D, G	**Am**, **Oc**, **Op**, W, E, Q

Amino acid	Parental codon	1 base change	2 base changes	3 base changes
T	aca	S, P, A, I, K, R	L, **Oc**, **Op**, Q, M, E, G, V, N	F, Y, C, **Am**, W, D, H
T	acg	S, P, A, M, K, R	I, N, L, **Am**, W, Q, V, E, G	C, F, Y, **Oc**, **Op**, D, H
V *	gtt, gtc	F, L, I, A, D, G	S, P, T, Y, H, N, E, C, R, M	**Am**, **Oc**, **Op**, W, Q, K
V	gta	L, I, A, E, G	F, M, D, S, P, T, **Oc**, **Op**, Q, R, K	**Am**, W, C, Y, H, N
V	gtg	L, M, A, E, G	F, I, D, S, P, T, **Am**, Q, R, K, W	**Oc**, **Op**, C, Y, H, N
W	tgg	C, R, G, **Am**, S, L, **Op**	P, Q, F, M, T, K, V, A, E, **Oc**, Y	D, N, H, I
Y	tat, tac	C, S, F, N, H, D, **Oc**, **Am**	L, W, Q, K, E, P, I, T, V, A, G, **Op**, R	M

Am is TAG stop, **Op** is TGA, **Oc** is TAA

[00136] **Methods of Using the Libraries**

[00137] Off-Rate Selection. Since a slow dissociation rate can be predictive of high affinity, particularly with respect to interactions between polypeptides and their targets, the methods described herein can be used to isolate ligands with a desired kinetic dissociation rate (i.e., reduced) for a binding interaction to a target.

[00138] To select for slow dissociating antibodies from a display library, the library is contacted to an immobilized target. The immobilized target is then washed with a first solution that removes non-specifically or weakly bound antibodies. Then the bound antibodies are eluted with a second solution that includes a saturating amount of free target, i.e., replicates of the target that are not attached to the particle. The free target binds to antibodies that dissociate from the target. Rebinding of the eluted antibodies is effectively prevented by the saturating amount of free target relative to the much lower concentration of immobilized target.

[00139] The second solution can have solution conditions that are substantially physiological or that are stringent (e.g., low pH, high pH, or high salt). Typically, the solution conditions of the second solution are identical to the solution conditions of the first solution. Fractions of the second solution are collected in temporal order to distinguish early from late fractions. Later fractions include antibodies that dissociate at a slower rate from the target than biomolecules in the early fractions. Further, it is also possible to recover antibodies that remain bound to the target even after extended incubation. These can either be dissociated using

chaotropic conditions or can be amplified while attached to the target. For example, phage bound to the target can be contacted to bacterial cells.

[00140] Selecting or Screening for Specificity. The display library screening methods described herein can include a selection or screening process that discards antibodies that bind to a non-target molecule. Examples of non-target molecules include, e.g., a carbohydrate molecule that differs structurally from the target molecule, e.g., a carbohydrate molecule that has a different biological property from the target molecule. In the case of a sulfated carbohydrate, a non-target may be the same carbohydrate without the sulfate or with the sulfate in a different position. In the case of a phosphopeptide, the non-target may be the same peptide without the phosphate or a different phosphopeptide.

[00141] In one implementation, a so-called "negative selection" step is used to discriminate between the target and related non-target molecule and a related, but distinct non-target molecules. The display library or a pool thereof is contacted to the non-target molecule. Members that do not bind the non-target are collected and used in subsequent selections for binding to the target molecule or even for subsequent negative selections. The negative selection step can be prior to or after selecting library members that bind to the target molecule.

[00142] In another implementation, a screening step is used. After display library members are isolated for binding to the target molecule, each isolated library member is tested for its ability to bind to a non-target molecule (e.g., a non-target listed above). For example, a high-throughput ELISA screen can be used to obtain this data. The ELISA screen can also be used to obtain quantitative data for binding of each library member to the target. The non-target and target binding data are compared (e.g., using a computer and software) to identify library members that specifically bind to the target.

[00143] In certain embodiments, the antibodies comprising the CDR3s of the invention may be able to bind carbohydrates. Methods for evaluating antibodies for carbohydrate binding include ELISA, immunohistochemistry, immunoblotting, and fluorescence-activated cell sorting. These methods can be used to identify antibodies which have a K_D of better than a threshold, e.g., better than 100 nM, 50 nM, 10 nM, 5 nM, 1 nM,500 pM, 100 pM, or 10 pM.

[00144] ELISA. Proteins encoded by a display library can also be screened for a binding property using an ELISA assay. For example, each protein is contacted to a microtitre plate whose bottom surface has been coated with the target, e.g., a limiting amount of the target. The

plate is washed with buffer to remove non-specifically bound polypeptides. Then the amount of the protein bound to the plate is determined by probing the plate with an antibody that can recognize the polypeptide, e.g., a tag or constant portion of the polypeptide. The antibody is linked to an enzyme such as alkaline phosphatase, which produces a calorimetric product when appropriate substrates are provided. The protein can be purified from cells or assayed in a display library format, e.g., as a fusion to a filamentous bacteriophage coat. Alternatively, cells (e.g., live or fixed) that express the target molecule, e.g., a target that contains a carbohydrate moiety, can be plated in a microtitre plate and used to test the affinity of the peptides/antibodies present in the display library or obtained by selection from the display library.

[00145] In another version of the ELISA assay, each polypeptide of a diversity strand library is used to coat a different well of a microtitre plate. The ELISA then proceeds using a constant target molecule to query each well.

[00146] Cell Binding Assays. Antibodies can be evaluated for their ability to interact with one or more cell types, e.g., a hematopoietic cell. Fluorescent activated cell sorting (FACS) is one exemplary method for testing an interaction between a protein and a cell. The antibody is labeled directly or indirectly with a fluorophore, before or after, binding to the cells, and then cells are counted in a FACS sorter.

[00147] Other cell types can be prepared for FACS by methods known in the art.

[00148] Homogeneous Binding Assays. The binding interaction of candidate polypeptide with a target can be analyzed using a homogenous assay, i.e., after all components of the assay are added, additional fluid manipulations are not required. For example, fluorescence resonance energy transfer (FRET) can be used as a homogenous assay (see, for example, Lakowicz et al., U.S. Pat. No. 5,631,169; Stavrianopoulos, et al., U.S. Pat. No. 4,868,103). A fluorophore label on the first molecule (e.g., the molecule identified in the fraction) is selected such that its emitted fluorescent energy can be absorbed by a fluorescent label on a second molecule (e.g., the target) if the second molecule is in proximity to the first molecule. The fluorescent label on the second molecule fluoresces when it absorbs to the transferred energy. Since the efficiency of energy transfer between the labels is related to the distance separating the molecules, the spatial relationship between the molecules can be assessed. In a situation in which binding occurs between the molecules, the fluorescent emission of the `acceptor` molecule label in the assay should be maximal. A binding event that is configured for monitoring by FRET can be

conveniently measured through standard fluorometric detection means well known in the art (e.g., using a fluorimeter). By titrating the amount of the first or second binding molecule, a binding curve can be generated to estimate the equilibrium binding constant.

[00149] Another example of a homogenous assay is Alpha Screen (Packard Bioscience, Meriden Conn.). Alpha Screen uses two labeled beads. One bead generates singlet oxygen when excited by a laser. The other bead generates a light signal when singlet oxygen diffuses from the first bead and collides with it. The signal is only generated when the two beads are in proximity. One bead can be attached to the display library member, the other to the target. Signals are measured to determine the extent of binding.

[00150] The homogenous assays can be performed while the candidate polypeptide is attached to the display library vehicle, e.g., a bacteriophage.

[00151] Surface Plasmon Resonance (SPR). The binding interaction of a molecule isolated from a display library and a target can be analyzed using SPR. SPR or Biomolecular Interaction Analysis (BIA) detects biospecific interactions in real time, without labeling any of the interactants. Changes in the mass at the binding surface (indicative of a binding event) of the BIA chip result in alterations of the refractive index of light near the surface (the optical phenomenon of surface plasmon resonance (SPR)). The changes in the refractivity generate a detectable signal, which are measured as an indication of real-time reactions between biological molecules. Methods for using SPR are described, for example, in U.S. Pat. No. 5,641,640; Raether (1988) Surface Plasmons Springer Verlag; Sjolander and Urbaniczky (1991) Anal. Chem. 63:2338-2345; Szabo et al. (1995) Curr. Opin. Struct. Biol. 5:699-705 and on-line resources provide by BIAcore International AB (Uppsala, Sweden).

[00152] Information from SPR can be used to provide an accurate and quantitative measure of the equilibrium dissociation constant (K_D), and kinetic parameters, including k_{on} and k_{off}, for the binding of a biomolecule to a target. Such data can be used to compare different biomolecules. For example, proteins encoded by nucleic acid selected from a library of diversity strands can be compared to identify individuals that have high affinity for the target or that have a slow k_{off}. This information can also be used to develop structure-activity relationships (SAR). For example, the kinetic and equilibrium binding parameters of matured versions of a parent protein can be compared to the parameters of the parent protein. Variant amino acids at given positions can be identified that correlate with particular binding parameters, e.g., high affinity

and slow k_{off}. This information can be combined with structural modeling (e.g., using homology modeling, energy minimization, or structure determination by crystallography or NMR). As a result, an understanding of the physical interaction between the protein and its target can be formulated and used to guide other design processes.

[00153] Protein Arrays. Proteins identified from the display library can be immobilized on a solid support, for example, on a bead or an array. For a protein array, each of the polypeptides is immobilized at a unique address on a support. Typically, the address is a two-dimensional address. Methods of producing polypeptide arrays are described, e.g., in De Wildt et al. (2000) Nat. Biotechnol. 18:989-994; Lueking et al. (1999) Anal. Biochem. 270:103-111; Ge (2000) Nucleic Acids Res. 28, e3, I-VII; MacBeath and Schreiber (2000) Science 289:1760-1763; WO 01/40803 and WO 99/51773A1. Polypeptides for the array can be spotted at high speed, e.g., using commercially available robotic apparati, e.g., from Genetic MicroSystems or BioRobotics. The array substrate can be, for example, nitrocellulose, plastic, glass, e.g., surface-modified glass. The array can also include a porous matrix, e.g., acrylamide, agarose, or another polymer.

[00154] Kits

[00155] Also provided are kits for use in carrying out a method according to any aspect of the invention. The kits may include the necessary vectors. One such vector will typically have an origin of replication for single stranded bacteriophage and either contain the sbp member nucleic acid or have a restriction site for its insertion in the 5' end region of the mature coding sequence of a phage capsid protein, and with a secretory leader coding sequence upstream of said site which directs a fusion of the capsid protein exogenous polypeptide to the periplasmic space.

[00156] Also provided are packages encoding the HC CDR3s as defined above and polypeptides comprising the HC CDR3s and fragments and derivatives thereof, obtainable by use of any of the above defined methods. The derivatives may comprise polypeptides fused to another molecule such as an enzyme or a Fc tail.

[00157] The kit may include a phage vector (e.g., DY3F87HC) which has a site for insertion of HC CDR3s for expression of the encoded polypeptide in free form. The kit may also include a plasmid vector for expression of soluble light chains, e.g., pLCSK23. The kit may also include a suitable cell line (e.g., TG1). The diversity of light chains encoded by pLCSK23 may be 10, 15, 20, 25, 30, or 50. The LCs in the diversity may be constructed or picked to have

certain desirable properties, such as, being germline in the framework regions and having diversity in CDR3 and/or CDR1. The germlines may be of highly utilized ones, e.g., VK1_2-O2, VK3_1-A27, VK3_5-L6, VK3_3-L2 for kappa and VL2_2a2, VL1_1c, VL1_1g, VL3_3r for lambda.

[00158] For example, one could clone genes for

[00159] VK1O2gl-JK3, VK1O2var1, VK1O2var2, VK1O2var3, VK1O2var4, VK1O2var5, VK3L6gl-JK4, VK3L6var1, VK3L6var2, VK3L6var3, VK3L6var4, VK3L6var5, VK3L6var6, VK3L6var7, VK3L6var8, VK3A27gl-JK3, VK3A27var1, VK3A27var2, VK3A27var3, VK3A27var4, VK3A27var5, VK3A27var6, VK3A27var7, VK3L2gl-JK3, VK1glL8-JK5,.and VK1GLO12-JK3 (amino-acid sequences shown in Table 19) into pLCSK23.

Table 19: 26 VL to be used in pLCSK23.

VK1O2gl-JK3 (SEQ ID NO:4)
DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYSTPFTFGP GTKVDIK 107

VK1O2var1 (SEQ ID NO:5) S28D
DIQMTQSPSS LSASVGDRVT ITCRASQDIS SYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYSTPFTFGP GTKVDIK 107

VK1O2var2 (SEQ ID NO:6) S91R
DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ RYSTPFTFGP GTKVDIK 107

VK1O2var3 (SEQ ID NO:7) S91E
DIQMTQSPSS LSASVGDRVT ITCRASQSIS SYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ EYSTPFTFGP GTKVDIK 107

VK1O2var4 (SEQ ID NO:8) S31R
DIQMTQSPSS LSASVGDRVT ITCRASQSIS RYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYSTPFTFGP GTKVDIK 107

VK1O2var5 (SEQ ID NO:9) S31E, S93R
DIQMTQSPSS LSASVGDRVT ITCRASQSIS EYLNWYQQKP GKAPKLLIYA ASSLQSGVPS 60
RFSGSGSGTD FTLTISSLQP EDFATYYCQQ SYRTPFTFGP GTKVDIK 107

VK3L6gl-JK4 (SEQ ID NO:10)
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGG GTKVEIK 107

VK3L6var1 (SEQ ID NO:11) S31R
EIVLTQSPAT LSLSPGERAT LSCRASQSVS RYLAWYQQKP GQAPRLLIYD ASNRATGIPA 60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGG GTKVEIK 107

VK3L6var2 (SEQ ID NO:12) S92R

```
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RRNWPLTFGG GTKVEIK               107

VK3L6var3                    (SEQ ID NO:13) S92G
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RGNWPLTFGG GTKVEIK               107

VK3L6var4                    (SEQ ID NO:14) S92Y
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RYNWPLTFGG GTKVEIK               107

VK3L6var5                    (SEQ ID NO:15) S92E
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RENWPLTFGG GTKVEIK               107

VK3L6var6                    (SEQ ID NO:16) Y32F
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SFLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGG GTKVEIK               107

VK3L6var7                    (SEQ ID NO:17) Y32D
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SDLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPLTFGG GTKVEIK               107

VK3L6var8                    (SEQ ID NO:18) N93G
EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA  60
RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSGWPLTFGG GTKVEIK               107

VK3A27gl-JK3                 (SEQ ID NO:19)
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPFTFG PGTKVDIK               108

VK3A27var1                   (SEQ ID NO:20) S31R
EIVLTQSPGT LSLSPGERAT LSCRASQSVS RSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPFTFG PGTKVDIK               108

VK3A27var2                   (SEQ ID NO:21) S32R
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SRYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPFTFG PGTKVDIK               108

VK3A27var3                   (SEQ ID NO:22) S32D
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SDYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGSSPFTFG PGTKVDIK               108

VK3A27var4                   (SEQ ID NO:23) G93E
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYESSPFTFG PGTKVDIK               108

VK3A27var5                   (SEQ ID NO:24) G93R
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYRSSPFTFG PGTKVDIK               108

VK3A27var6                   (SEQ ID NO:25) S30D, G93E
EIVLTQSPGT LSLSPGERAT LSCRASQSVD SSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYESSPFTFG PGTKVDIK               108
```

```
VK3A27var7                    (SEQ ID NO:26) S94R
EIVLTQSPGT LSLSPGERAT LSCRASQSVS SSYLAWYQQK PGQAPRLLIY GASSRATGIP  60
DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGRSPFTFG PGTKVDIK              108

VK3L2g1-JK3                   (SEQ ID NO:27)
EIVMTQSPAT LSVSPGERAT LSCRASQSVS SNLAWYQQKP GQAPRLLIYG ASTRATGIPA  60
RFSGSGSGTE FTLTISSLQS EDFAVYYCQQ YNNWPFTFGP GTKVDIK               107

VK1g1L8-JK5                   (SEQ ID NO:28)
DIQLTQSPSF LSASVGDRVT ITCRASQGIS SYLAWYQQKP GKAPKLLIYA ASTLQSGVPS  60
RFSGSGSGTE FTLTISSLQP EDFATYYCQQ LNSYPITFGQ GTRLEIK               107

VK1GL012-JK3                  (SEQ ID NO:897)
DIQMTQSPSS LSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP   60
SRFSGSGSGT DFTLTISSL QPEDFATYYC QQSYSTPFTF GPGTKVDIKR GTVAAPSVFI  120
FPPSDEQLKS GTASVVCLL NNFYPREAKV QWKVDNALQS GNSQESVTEQ DSKDSTYSLS  180
STLTLSKADY EKHKVYACE VTHQGLSSPV TKSFNRGECA AAGKPIPNPL LGLDST      236
```

[00160] The kits may include ancillary components required for carrying out the method, the nature of such components depending of course on the particular method employed. Useful ancillary components may comprise helper phage, PCR primers, buffers, and/or enzymes of various kinds. Buffers and enzymes are typically used to enable preparation of nucleotide sequences encoding Fv, scFv or Fab fragments derived from rearranged or unrearranged immunoglobulin genes according to the strategies described herein.

[00161] METHODS OF INTRODUCING DIVERSITY

[00162] There are many ways of generating DNA that is variable. One way is to use mixed-nucleotide synthesis (MNS). One version of MNS uses equimolar mixtures of nucleotides as shown in Table 5. For example, using NNK codons gives all twenty amino acids and one TAG stop codon. The distribution is 3(R/S/L): 2(A/G/V/T/P): 1(C/D/E/F/H/I/K/M/N/Q/W/Y) (e.g., 3 of each of Arg, Ser, and Leu, and so forth). An alternative, herein termed "wobbling", uses mixed nucleotides but not in equimolar amounts. For example, if a parental codon were TTC (encoding Phe), we could use a mixture of (0.082 T, 0.06 C, 0.06 A, and 0.06 G) in place of T and a mixture of (0.082 C, 0.06 T, 0.06 A, and 0.06 G) in place of C. This would give TTC or TTT (encoding Phe) 59% of the time and Leu 13%, S/V/I/C/Y ~5%, and other amino-acid types less often.

[00163] Van den Brulle et al. (*Biotechniques* 45:340-3 (2008)) describe a method of synthesis of variable DNA in which type IIs restriction enzymes are used to transfer

trinucleotides from an anchored hair-pin oligonucleotide (PHONs) to a so called "splinker". See also EP patents 1 181 395, EP 1 411 122, EP 1 314 783 and EP applications EP 01127864.5, EP 04001462.3, EP 08006472.8. By using mixtures of anchored PHONs and splinkers, one can build libraries in which desired amino-acid types are allowed in designer-determined ratios. Thus, one can direct that one amino-acid type is present, for example 82% of the time and 18 other amino-acid types (all non-parental amino-acid types except Cys) are present at 2% each. Herein, we will refer to such a synthesis as "dobbling" (digital wobbling). In some aspects, dobbling is preferred to wobbling, but wobbling provides useful embodiments, partly because the structure of the genetic code table causes wobbling to make mostly conservative substitutions. Dobbling does offer the possibility to exclude unwanted amino-acid types. In CDRs, unpaired cysteines are known, even in Abs approved as therapeutics, but in some embodiments, one would like to avoid them. In some embodiments, when diversifying a D region that contains a pair of cysteines, the cysteins are not allowed to vary because the disulfide-closed loop is an important structural element and because one does not want unpaired cysteines.

[00164] In addition, one can synthesize a DNA molecule that encodes a parental amino-acid sequence and subject that DNA to error-prone PCR using primers that cover the framework regions so that mutations in the framework regions are avoided.

Table 5: Standard codes for mixed nucleotides

```
N is equimolar A, C, G, T
B is equimolar C, G, T     (not A)
D is equimolar A, G, T     (not C)
H is equimolar A, C, T     (not G)
V is equimolar A, C, G     (not T)
K is equimolar G, T        (Keto)
M is equimolar A, C        (aMino)
R is equimolar A, G        (puRine)
S is equimolar C, G        (Strong)
W is equimolar A, T        (weak)
Y is equimolar C, T        (pYrimidine)
```

Table 6: Example of mixed nucleotides for wobbling

```
e = 0.82 A + 0.06 C + 0.06 G + 0.06 T
q = 0.06 A + 0.82 C + 0.06 G + 0.06 T
```

j = 0.06 A + 0.06 C + 0.82 G + 0.06 T
z = 0.06 A + 0.06 C + 0.06 G + 0.82.T

EXEMPLIFICATION

[00165] The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference.

[00166] **Prophetic Example 1: Libraries With Very Short HC CDR3s**

[00167] Very short HC CDR3s have been described in the art. Kadirvelraj et al. (2006) *Proc. Natl. Acad. Sci. USA* 103:8149-54 have described a four amino-acid HC CDR3 sequence in an antibody that binds Streptococcus Type B III Ag (GBS-Ag) but not to *Streptococcus pneumoniae* capsular Ag . GBS-Ag is sialylated at regular intervals. *S. pneumoniae* capsular Ag (SPC-Ag) is very similar but lacks the sialic acid groups. Such a short HC CDR3 creates a wide groove into which a carbohydrate could bind, and such Abs are very, very rare in existing antibody libraries. Thus, current libraries do not afford a large variety of potential binders to carbohydrates.

[00168] Ab 1B1 is the murine mAb that binds GBS-Ag; Ab 1QFU is the mAb having a known 3D structure and the closest sequence; and 1NSN is an antibody of known 3D structure having a HC CDR3 of length 4. Examination of a 3-23 HC structure gives a distance from Cα of R_{94} (which ends FR3) to the Cα of the W_{104} (which begins FR4) of ~10 Å. The CDR3 of 1B1 (NWDY (SEQ ID NO:29)) shows that the AAs need not have only small side groups or be mostly of glycine. Three amino acids (AAs) can bridge 10 Å, although PPP might not work. Indeed, we have obtained a few Fabs with CDR3s as short as 3 AAs, but they are very rare.

[00169] Although short and very short HC CDR3s have been described, no one hassuggested making an Ab library having many members (e.g., greater than about 50%, about 60%, about 70%, about 80%, about 90%, or about 95% of members) with short HC CDR3s (e.g., HC CDR3s of 3 to 5 amino acids). One approach to building an effective library is to first design amino-acid sequences that could arise from V-J or V-D-J coupling. For CDR3 length 3, 4, or 5, we start with the amino-acid sequences shown in Table 7. For example, Sequence V-3JH1 shows the C-terminal end of 3-23 FR3 (TAVYYCAK (SEQ ID NO:30)) followed by

JH1 which has been trimmed from the N-terminal end until three amino-acids before the Trp-Gly that starts FR4. V-3JH2 shows the end of FR3 followed by the trimmed JH2. The sequence following V-3JH6 are constructed by joining FR4 to a trimer taken from a human D segment followed by the FR4 region of a human JH segment. 3D3-3.3.2 would be a trimer from segment D3-3, third reading frame starting at the second amino acid. 5D5-12.2.3 is a pentamer from D5-12 in reading frame 2 starting at amino acid 3. Some of the germ-line D segments contain stop codons, yet they appear in natural antibodies when the stop codons are edited away. Here we assume that the most likely change fro TAA and TAG codons is to Tyr (Y) and that TGA stops are most likely mutated to Trp (W). Table 20 shows the amino-acid sequences of the human D segments; the types of stop codons is indicated by the use of * for TAG, @ for TAA, and $ for TGA. In Table 11 are 266 distinct trimers that can be constructed from human D segments. The TAA and TAG stops have been changed to Tyr shown as "y" (i.e., lowercase). These could also be changed to Ser, Cys, Phe, Gln, Lys, or Glu by single base changes. TAG could be changed by single base changes to Trp as well as Tyr, Gln, Lys, Glu, Ser, and Leu. Table 12 shows the 266 distinct tetramers that can be obtained by trimming human D segments. Table 13 shows the 215 pentamers that can be obtained from trimming human D segments. Table 14 shows the 155 hexamers that can be obtained by trimming human D segments. The libraries to be built have substantial diversity in HC CDR1 and HC CDR2. The sequence diversity of HC CDR3 may be less important than having a short, but acceptable sequence. The diversity of JH segments or fragments (e.g., 3 or more amino acids) of D segments provides sequences that could be built by the human immune system and so are less likely to be immunogenic.

[00170] In one embodiment, the trimers, tetramers, and pentamers that contain a Cys are eliminated.

[00171] In one embodiment, the trimers, tetramers, and pentamers that contain a Cys or the came from a D fragment containing a stop are eliminated.

[00172] The short libraries constructed using the trimers of Table 11, tetramers of Table 12, pentamers of Table 13 have substantial diversity: 266, 266, and 215 respectively. This is to be compared to the number of peptides of these lengths: 8000, 160000, and 3200000 respectively.

[00173] V-3D1-1.1.1-JH1 contains the final portion of FR3 followed by three amino acids from D1-1 (RF1), *viz.* GTT (SEQ ID NO:257). V-3D1-1.2-JH1 uses amino acids 2-4 of D1-1

(RF1) as the parental CDR3. V-3D3-3.3.3-JH2 shows the end of FR3 followed by amino acids 3-5 of D3-3 (RF 3). The invention comprises any amino-acid sequence comprising FR3::(three, four, or five stop-free AAs of a human D segment)::FR4 from a human JH. Fragments of D regions containing unpaired Cys residues are less preferred than those that are free of unpaired Cys residues. In V-5JH3, there is a Tyr shown as 'y' because JH3 has only 4 codons before the codons for Trp-Gly that define the beginning of FR4. V-5JH4 has a Ser shown as 's' for the same reason. If wobbling is used, the preferred level of purity is between 0.75 and 0.90. The invention comprises the sequences V-3JH1 through V-3JH6, V-4JH1 through V-4JH6, and V-5JH1 through V-5JH6, and libraries containing the same The invention also comprises the sequences in which the CDR region is replaced by a 3, 4, or 5 amino-acid segment from a human D region, and libraries containing the same. The invention further comprises DNA in which the parental sequence has been mutated in the CDR3 region, and libraries containing the same. A preferred embodiment is one in which the average number of base changes per CDR3 is one, two, or three. The methods of mutagenesis include error-prone PCR, wobbling, and dobbling.

Table 7: Amino-acid sequences of parental CDR3s of lengths 3, 4, 5

	...FR3-----	CDR3-	FR4--------	
Length 3				
V-3JH1	TAVYYCAK	FQH	**WGQGTLVTVSS**	(SEQ ID NO:31)
V-3JH2	TAVYYCAK	FDL	**WGRGTLVTVSS**	(SEQ ID NO:32)
V-3JH3	TAVYYCAK	FDI	**WGQGTMVTVSS**	(SEQ ID NO:33)
V-3JH4	TAVYYCAK	FDY	**WGQGTLVTVSS**	(SEQ ID NO:34)
V-3JH5	TAVYYCAK	FDP	**WGQGTLVTVSS**	(SEQ ID NO:35)
V-3JH6	TAVYYCAK	MDV	**WGQGTTVTVSS**	(SEQ ID NO:36)
V-3D1-1.1.1-JH1	TAVYYCAK	GTT	**WGQGTLVTVSS**	(SEQ ID NO:37)
V-3D1-1.1.2-JH1	TAVYYCAK	TTG	**WGQGTLVTVSS**	(SEQ ID NO:38)
V-3D3-3.3.3-JH2	TAVYYCAK	IFG	**WGRGTLVTVSS**	(SEQ ID NO:39)
Length 4				
V-4JH1	TAVYYCAK	YFQH	**WGQGTLVTVSS**	(SEQ ID NO:40)
V-4JH2	TAVYYCAK	YFDL	**WGRGTLVTVSS**	(SEQ ID NO:41)
V-4JH3	TAVYYCAK	AFDI	**WGQGTMVTVSS**	(SEQ ID NO:42)
V-4JH4	TAVYYCAK	YFDY	**WGQGTLVTVSS**	(SEQ ID NO:43)
V-4JH5	TAVYYCAK	WFDP	**WGQGTLVTVSS**	(SEQ ID NO:44)

```
V-4JH6              TAVYYCAK  GMDV WGQGTTVTVSS     (SEQ ID NO:45)
V-4D3-10.1a-JH2     TAVYYCAK  LLWF WGRGTLVTVSS     (SEQ ID NO:46)

Length 5
V-5JH1              TAVYYCAK EYFQH WGQGTLVTVSS     (SEQ ID NO:47)
V-5JH2              TAVYYCAK WYFDL WGRGTLVTVSS     (SEQ ID NO:48)
V-5JH3              TAVYYCAK yAFDI WGQGTMVTVSS     (SEQ ID NO:49)
V-5JH4              TAVYYCAK sYFDY WGQGTLVTVSS     (SEQ ID NO:50)
V-5JH5              TAVYYCAK NWFDP WGQGTLVTVSS     (SEQ ID NO:51)
V-5JH6              TAVYYCAK YGMDV WGQGTTVTVSS     (SEQ ID NO:52)
V-5D2-8.2a-JH2      TAVYYCAK DIVLM WGRGTLVTVSS     (SEQ ID NO:53)
```

Table 8: DNA encoding V-5D2-8.2a-JH2 for wobbling

```
!                                                CDR3.......
!   A   E   D   T   A   V   Y   Y   C   A   K   D   I   V   L   M
  |gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aag jez ezq jzz qzz ezj
!
!    W   G   Q   G   T   T   V   T   V   S   S    (SEQ ID NO:54)
     tgg ggc cag ggt act acG GTC ACC gtc tcc agt-3'    (SEQ ID NO:55)
!                BstEII...
```

[00174] Alternatively, one could synthesize three fragments of DNA that correspond to the region from *Xba*I to *Bst*EII and having residue 94 being K or R followed by 3, 4, or 5 NNK codons, followed by WG... of FR4. The allowed variation is $20^3 + 20^4 + 20^5 = 3{,}368{,}000$. After amplification, these DNA molecules would be mixed in the ratio 1:10:100 (so that shorter sequences are relatively oversampled) and cloned into the phagemid encoding the kappa library with HC CDR1/2 diversity. A library of 1×10^9 would give significant diversity and will allow isolation of antibodies that bind to targets that have small to medium protrusions. For example, various carbohydrates, loops of proteins that are not well ordered (such as GPCRs) may benefit from a groove in the antibody created by having a very short HC CDR3. We can also build a lambda library. The ratio of AA sequences is 1:20:400, and it may be important to sample the shorter sequences more densely. Getting a big, wide gulley in the Ab may require exactly one 3 AA CDR3, but with a 4 AA CDR3, one probably has more leeway and with 5 AAs, even more leeway. In this Example, we use the JH6 version of FR4 from the WG motif onward.

[00175] We can select from our current kappa library a collection of, for example, 25 kappa light chains that are a) germline in the framework regions, b) show suitable diversity in CDRs, and c) are of types that produce well and pair well with 3-23. These LC_s will be made in *E. coli* from a vector that carries Kan^R and no phage packaging signal. We would then build our HC library in a phage vector that has no LC. HC and LC will be crossed by infecting the LC producing cells with the HC phage. HC phage that are selected can be combined with the LC of the cell that produces $ELISA^+$ phage or the HCs can be cloned into pMID21 that have the whole LC diversity. Alternatively, the selected HC can be moved into pHCSK85 and used with ROLIC to combine with all the LCs of our collection. Lambda LCs could also be used. Thus, a library of 1×10^9 HC in phage can be expanded into a Fab library of 1.2×10^{11} ($1. \times 10^9 \times 117$). If we combined 1×10^7 CDR1-2s with 10^6 HC CDR3s, we could make a library of 5×10^7 in which each CDR3 is coupled with 50 CDR1-2s. A library of 5×10^7 HCs in phage could give results similar to an old-style library of 6×10^9.

Table 1: Designs of very short exemplary HC CDR3s

```
C3XXX
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!                                                        CDR3.......
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R any any any  W   G
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk tgg ggc-
!
!   Q   G   T   T   V   T   V   S   S     (SEQ ID NO:56)
   cag ggt act acG GTC ACC gtc tcc agt-3'     (SEQ ID NO:57)
!                BstEII...
!
(C3XXX)5'-T|GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk tgg ggc cag ggt act ac-3'
(SEQ ID NO:58)
(ON_5) 5'-AcTggAgAcggTgAccgTAgTAcccTggccccA-3' ! 33 bases    (SEQ ID NO:58)
(ON_5 is reverse complement of 5'-tgg ggc cag ggt act acG GTC ACC gtc tcc
agt-3'     (SEQ ID NO:59))
! Use ON-1 and ON-3 shown below
!-----------------------------------------------
!
```

```
C3X4
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!                                                        CDR3.....
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R any any any any  W
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk nnk tgg-
!
!   G   Q   G   T   T   V   T   V   S   S     (SEQ ID NO:60)
   ggc cag ggt act acG GTC ACC gtc tcc agt-3'     (SEQ ID NO:61)
!                  BstEII...
!
(C3X4)5'-GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk nnk tgg-
           ggc cag ggt act ac-3'     (SEQ ID NO:62)
! Use ON-1, ON-3, and ON-5
!-----------------------------------------------------------
C3X5
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!                                                        CDR3.............
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R any any any any any
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk nnk nnk-
!
!   W   G   Q   G   T   T   V   T   V   S   S     (SEQ ID NO:63)
   tgg ggc cag ggt act acG GTC ACC gtc tcc agt-3'     (SEQ ID NO:64)
!                      BstEII...
(C3X5)5'-GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk nnk nnk tgg-
           ggc cag ggt act ac-3'     (SEQ ID NO:65)
!-------------------------------------------------

aRg encodes K or R
```

Alternatively, the current HC diversity can be cloned into DY3F87HC and the CDR3 diversity described above is cloned into that diversity as XbaI - BstEII fragments. A library of, for example, 25 LC are cloned into pLCSK23 and used to create a cell line in TG1 *E. coli*. These cells are infected with the DY3F87HC phage which harbor the novel HC CDR3 (and CDR1-2) diversity. The phage obtained from this infection are selected for binding to a desired target. After two to four rounds of selection, the selected HCs are transfered to pHCSK22 and used to create a cell line which can be used with ROLIC to combine the selected HC with all the LCs in the ROLIC LC library. In this way, a library of 1. E 9 can be give Abs that normally would require construction of a library of 1. E 16 (assuming a LC diversity of 1. E 7).

[00176] Prophetic Example 2: Libraries with Very Long HC CDR3s

[00177] Sidhu *et al.* (*J Mol Biol.* 2004 **338**:299-310. and US application 20050119455A1) report high-affinity Abs selected from a library in which only Y and S were allowed in the CDRs

which were limited in length to 20 amino acids. It may be possible to generate high affinity Abs from a library that has HC CDR3s with one or more of the following forms of diversity: a) several (but not all) sites allowing Y or S, b) including 4-6 NNK codons, c) introducing D segments (with or without diversification in the D), and/or d) using error-prone PCR. We have already sampled the Ab space in which HC CDR3 is in the range ~8 to ~22 with a median length of 13. Thus, libraries in which HC CDR3 is either ~23 AAs or ~35 AAs are possible and may have advantages with certain types of targets. For example, GPCRs are integral membrane proteins with seven helical segments transversing the lipid bilayer of the call that are thought to have multiple states. An antibody having a very long HC CDR3 could form a protuberance that fits into the channel formed by the seven strands. Finding Abs that bind GPCRs has been difficult and intentionally building libraries in which all the members have very long HC CDR3s may ameliorate this problem. The lengths may be made somewhat variable, say 23, 24, or 25 in one library and 33, 34, or 35 in a second.

[00178] Below are a number of representative designs. The CDR3 have been broken up and diversity generated that lets the various parts have differing relationships depending on the value of X. A full-length JH1 has been used, and in some designs diversity allowed diversity in the CDR3 part of JH1. Other JHs could be used. In the designs, the D segments are either rich in Y or have an S-rich disulfide loop. The amino-acid sequences of human D segments are shown in Table 3. The places where the D region has either S or Y or allowed other combinations have in particular been varied. Table 4 shows the amino-acid sequences of human J regions.

[00179] Each of the libraries could be built in at least four ways: 1) DNA encoding a particular amino acid sequence is first synthesized and subjected to error-prone PCR, 2) the library can be synthesized by wobbling or with mixtures of nucleotides, 3) the library can be built using dobbling, and 4) routes (2) or (3) could be followed by error-prone PCR. As an example of route (1), in Design 12, DNA encoding SEQ ID NO:908 could be synthesized, as shown in SEQ ID NO:911. This DNA could be subjected to error-prone PCR using the primers shown in SEQ ID NO:909 and SEQ ID NO:910. Because these primers cover the framework regions, the errors will occur only in the CDR3.

[00180] A library of HCs with CDR3 with length 23 of, for example, 2 x 10^9 members and a second library with HC CDR3s of length ~35 also having 2 x 10^9 members could be built. Alternatively, the DNA could be mixed to build one library of 4 x 10^9.

Table 4: Human JH amino-acid sequences

```
          H3
         ------
          CDR3
        --------
           100       110
            |         |
JH1   ---AEYFQHWGQGTLVTVSS    (SEQ ID NO:66)
JH2   ---YWYFDLWGRGTLVTVSS    (SEQ ID NO:67)
JH3   -----AFDIWGQGTMVTVSS    (SEQ ID NO:2)
JH4   -----YFDYWGQGTLVTVSS    (SEQ ID NO:1)
JH5   ----NWFDPWGQGTLVTVSS    (SEQ ID NO:68)
JH6   YYYYYGMDVWGQGTTVTVSS    (SEQ ID NO:3)
```

[00181] In each of the following designs, the amino-acid sequence begins with YYCA(K/R) which is the end of FR3. FR4 starts with WG and is shown bold.

Design 1

SEQ ID NO:898 comprises the end of FR3 joined to two residues (DY) of types often found in the filler sequence that the immune system places between V and D. These are followed by D2-2.2, preferred because it has a disulfide loop and is rich in Ser and Tyr residues. This is followed by YGYSY, which is rich in Tyr and Ser residues, which is followed by full-length JH1.

```
XX::D2-2.2::XX::JH1
                  1    1    2  2
  FR3 1   5    0    5    0  3FR4
YYCAK DYGYCSSTSCYTYGYSYAEYFQHWGQGTLVTVSS (SEQ ID NO:898)
YYCAK XXGYCSXXSCYTXXYSYAEYFQHWGQGTLVTVSS   (SEQ ID NO:69)
    R   GYCSSTSCYT     AEYFQHWGQGTLVTVSS (JH1)
       (SEQ ID NO:70)     (SEQ ID NO:66)
           1 1               1     1
    9 9    0 0               0     1
    4 5    0 2abcdefghijklmnp3     0

Amino-acid diversity    = 1.28 E 8
DNA diversity           = 2.15 E 9
Stop-free               = 83%
Gratuitous Cys-free     = 83%
Free of stop and Cys    = 68%
```

Design 1(C23D222) has 94 being R or K, then 2 Xs, D2-2 in second reading frame with two Xs in the loop, followed by two Xs, and JH1. D2-2 2nd reading frame has a disulfide-closed loop into which diversity at two points has been introduced. This CDR3 is 23 long. Using primers that include DNA up to ...YYCA and from WGQG..., error-prone PCR on the CDR3 could be performed before amplifying out to *Xba*I and *Bst*EII for cloning into the library of kappa LC and HC CDR1/2. Thus, the AAs that are shown as fixed will be allowed to vary some. The AAs that are part of the PCR overlap region will be reinforced by the final non-error prone PCR. Error-prone PCR is not a necessary part of the design.

```
C23D222JH1
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!             XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
! CDR3-----------------------------------------------------------------
!  X   X   D2-2 RF2.........................   X   X             JH1..
!  any any  G   Y   C   S  any any  S   C   Y   T  any any  Y   S   Y   A
   nnk nnk ggt tat tgt tcc nnk nnk tct tgc tat act nnk nnk tat tcc tac gct-
!
!  CDR3---------------
!   E   Y   F   Q   H
   gaa tat ttc cag cac-
!
!   W   G   Q   G   T   L   V   T   V   S   S  (SEQ ID NO : 71)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3' (SEQ ID NO : 72)
!                        BstEII...
```

(ON_C23D222) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk nnk ggt tat tgt tcc nnk-
nnk tct tgc tat act nnk nnk tat tcc tac gct gaa tat ttc cag cac-
tgg ggc cag ggt act ct-3' ! 107 bases (SEQ ID NO : 73)
(ON_1) 5'-GCA|GtT|taT|taC|tgc|gct-3' (SEQ ID NO : 74)
(ON_2) 5'-AgAgTAcccTggccccAgAcgTccATAccgTAATAgT-3' ! 37 bases (SEQ ID NO : 75)
(ON_2 is reverse complement of 5'-ac tat tac ggt atg gac gtc tgg ggc cag ggt act ct-3') (SEQ ID NO : 76)
(ON_3) 5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|-
aac|agC|TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct-3' (SEQ ID NO : 77)
(ON_4) 5'-AcTggAgAcggTgAccAgAgTAcccTggccccA-3' ! 33 bases (SEQ ID NO : 78)
(5'-tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3' [RC] (SEQ ID NO : 79))

Design 2

```
                1    1    2  2
      1   5     0    5    0  3
YYCAK GSYYYGSGSYYNVDSYYAEYFQHWGQGTLVTVSS (SEQ ID NO:899)
YYCAK XXYYYGXGSXYNXXSYYAEYFQHWGQGTLVTVSS (SEQ ID NO:80)
    R   YYYGSGSYYN     AEYFQHWGQGTLVTVSS (JH1)
       (SEQ ID NO:81)   (SEQ ID NO:66)

Amino-acid diversity    = 1.28 E 8
DNA diversity           = 2.15 E 9
Stop-free               = 83%
Gratuitous Cys-free     = 83%
Free of stop and Cys    = 68%
```

Design 2 (C23D310) has 94 as R or K, two Xs, D3-10 (RF2) with 5th and 8th residues changed to X, 2 Xs, SYY, and JH1 . The CDR3 is 23 AA long and could be further diversified by use of error-prone PCR.

```
C23D310JH1
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!             XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3------------------------------------------------------------------
!
!  any any  Y   Y   Y   G  any  G   S  any  Y   N  any any  S   Y   Y
   nnk nnk tac tac tat ggt nnk ggc tct nnk tac aat nnk nnk tct tat tac
!
!   A   E   Y   F   Q   H
   gct gag tac ttt caa cat
!
!   JH1.....................................
!   W   G   Q   G   T   L   V   T   V   S   S    (SEQ ID NO:82)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'    (SEQ ID NO:83)
!                      BstEII...
```

(C23D310) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk nnk tac tac tat ggt nnk ggc-
tct nnk tac aat nnk nnk tct tat tac gct gag tac ttt caa cat tgg ggc cag-
ggt act ct-3' **(SEQ ID NO:84)**
ON_1, ON_2, ON_3, and ON_4 as above.

Design 3

```
                 1    1    2  2
      1   5      0    5    0  3
YYCAK DYYYYGSGSYYNSDSYSAEYFQHWGQGTLVTVSS (SEQ ID NO:900)
YYCAK XZYZZGZGZXYNZXZYZAXZFQHWGQGTLVTVSS     (SEQ ID NO:84)
    R   YYYGSGSYYN      AEYFQHWGQGTLVTVSS (JH1)
       (SEQ ID NO:81)   (SEQ ID NO:66)
Amino-acid diversity    = 1.64 E 8
DNA diversity           = 1.07 E 9
Stop-free               = 88%
Gratuitous Cys-free     = 88%
Free of stop and Cys    = 77%
```

Design 3 (C23D310B) has 94 as R or K, XZ, D3-10 (RF2) with 2nd, 3rd, 5th, and 7th as Z(Y|S) and 8th residue changed to X, ZXZYZ, , and JH1 (with the E changed to X) . Z is either Y or S. The CDR3 is 23 AA long and could be further diversified by use of error-prone PCR.

```
                A   V   Y   Y   C   A  R|K any Y|S  Y  Y|S Y|S  G  Y|S
(C23D310b) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk tmc tac tmc tmt ggt tmc ggc-

  Y|S any  Y   N  Y|S any Y|S  Y  Y|S  A  any Y|S  F   Q   H   W   G   Q
  tmt nnk tac aat tmt nnk tmc tat tmc gct nnk tmc ttt caa cat tgg ggc cag-

   G   T   L     (SEQ ID NO:85)
  ggt act ct-3'     (SEQ ID NO:86)
```

ON_1, ON_2, ON_3, and ON_4 as above.

Design 4

```
                 1    1    2  2 2    3    3
      1   5      0    5    0  3 5    0    5
YYCAK YYSFSYYPYYYDSSGYYYGYYSDYSYSYYAEYFQHWGQGTLVTVSS  (SEQ ID NO:901)
YYCAK YYSXSYYXYZYDSZGYZYXYYSXYZYZZZAZZFQHWGQGTLVTVSS  (SEQ ID NO:87)
    R          YYYDSSGYYY            AEYFQHWGQGTLVTVSS (JH1)
               (SEQ ID NO:88)           (SEQ ID NO:66)
           1 1                            1     1
    9 9    0 0                            0     1
    4 5    0 2abcdefghijklmnopqrstuvwxyab3     0
                                         ''
Amino-acid diversity    = 1.64 E 8
DNA diversity           = 1.07 E 9
Stop-free               = 88%
Gratuitous Cys-free     = 88%
Free of stop and Cys    = 77%
```

Design 4 has CDR3 of length 35. Residue 94 can be K or R, then YYS::X::SYY::X::D3-22(2nd RF with one S as X and 3 Zs)::X::YYS::X::YZZZ::JH1(with 2 Zs). Error-prone PCR could be used to add more diversity.

```
C35D322JH1
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3-------------------------------------------------------------------
!
!   Y   Y   S  any  S   Y   Y  any  Y  Y|S  Y   D   S  Y|S  G   Y  Y|S  Y
    tac tat tcc nnk tct tac tat nnk tat tmt tac gat agt tmt ggt tac tmc tat
!
   any  Y   Y   S  any  Y  Y|S  Y  Y|S Y|S Y|S  A  Y|S Y|S  F   Q   H
    nnk tac tat agc nnk tat tmc tac tmc tmt tmc gct tmt tmc ttc caa cac
!
!   W   G   Q   G   T   L   V   T   V   S   S      (SEQ ID NO:89)
    tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'   (SEQ ID NO:90)
!                       BstEII...

(c35d322B) 5'-GCA|GtT|taT|taC|tgc|gct aRg tac tat tcc nnk tct tac tat nnk-
  tat tmt tac gat agt tmt ggt tac tmc tat nnk tac tat agc nnk tat tmc tac-
  tmc tmt tmc gct tmt tmc ttc caa cac tgg ggc cag ggt act ct-3'   (SEQ ID NO:91)
ON_1, ON_2, ON_3, and ON_4 as above.
```

Design 5

```
                1    1    2  2
     1   5      0    5    0  3
YYCAK SSGYCSSTSCYTGVYYYAEYFQHWGQGTLVTVSS (SEQ ID NO:902)
YYCAK ZZGZCZZXZCZTXXYZYXZYFQHWGQGTLVTVSS   (SEQ ID NO:92)
   R   GYCSSTSCYT     AEYFQHWGQGTLVTVSS (JH1)
      (SEQ ID NO:70)   (SEQ ID NO:66)
Amino-acid diversity     = 1.64 E 8
DNA diversity            = 1.07 E 9
Stop-free                = 88%
Gratuitous Cys-free      = 88%
Free of stop and Cys     = 77%
```

Design 5(C23D222b) is like design 1 but uses many Z(Y or S) variable codons. This CDR3 is 23 long.

```
C23D222JH1b
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
```

```
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3------------------------------------------------------------------
!  Y|S Y|S  G  Y|S  C  Y|S Y|S any Y|S  C  Y|S  T  any any  Y  Y|S  Y  any
   tmc tmt ggt tmt tgc tmc tmt nnk tmt tgt tmc acc nnk nnk tat tmt tac nnk
!
!  Y|S  Y   F   Q   H
   tmt tat ttc cag cac
!
!   W   G   Q   G   T   L   V   T   V   S   S    (SEQ ID NO:93)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'    (SEQ ID NO:94)
!                       BstEII...

(C23D222JH1b) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmc tmt ggt tmt tgc tmc tmt-
 nnk tmt tgt tmc acc nnk nnk tat tmt tac nnk tmt tat ttc cag cac tgg ggc-
 cag ggt act ct-3'     (SEQ ID NO:95)
```

Design 6

```
                1    1    2  2 2    3    3
     1   5      0    5    0  3 5    0    5
YYCAK SYDYYGYCSSTSCYTYYSYVSYSSYYSYYAEYFQHWGQGTLVTVSS  (SEQ ID NO:903)
YYCAK ZYXZYGZCZZXSCZTYZSZXZYSZYZSZYAEZFQHWGQGTLVTVSS     (SEQ ID NO:96)
    R      GYCSSTSCYT D2-2.2       AEYFQHWGQGTLVTVSS (JH1)
           (SEQ ID NO:70)           (SEQ ID NO:66)
```

Amino-acid diversity	= 2.00 E 8
DNA diversity	= 5.37 E 8
Stop-free	= 91%
Gratuitous Cys-free	= 91%
Free of stop and Cys	= 83%

```
C35D222JH1
!
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!             XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3------------------------------------------------------------------
!  Y|S  Y  any Y|S  Y   G  Y|S  C  Y|S Y|S any  S   C  Y|S  T   Y  Y|S  S
   tmt tac nnk tmc tac ggc tMt tgc tmt tmc nnk tCt tgt tmc acc tat tmt tcc
!
!  Y|S any Y|S  Y   S  any  Y  Y|S  S  Y|S  Y   A   E   Y   F   Q   H
   tmt nnk tmc tat tct nnk tac tmc agt tmt tat gct gag tat ttc cag cac
!
!   W   G   Q   G   T   L   V   T   V   S   S    (SEQ ID NO:97)
  tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'     (SEQ ID NO:98)
!                       BstEII...

(C35D222JH1)5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tac nnk tmc tac ggc tat- tgc tmt tmc
nnk tmt tgt tmc acc tat tmt tcc tmt nnk tmc tat tct nnk tac-
tmc agt tmt tat gct gag tat ttc cag cac tgg ggc cag ggt act ct-3'  (SEQ ID NO:99)
```

Design 7

```
                1    1    2  2 2    3    3
     1   5      0    5    0  3 5    0    5
YYCAK YYSYYGYCSSTSCYTYSSSVSYSYYSSYYAEYFQHWGQGTLVTVSS   (SEQ ID NO:904)
YYCAK ZYZZYGZCZZXZCZTYZSZXZYSZYZSZYAψZJQBWGQGTLVTVSS (SEQ ID NO:100)
   R      GYCSSTSCYT D2-2.2        AEYFQHWGQGTLVTVSS (JH1)
            (SEQ ID NO:70)            (SEQ ID NO:66)
(J=FSY, B=YHND, ψ=EKQ)

Amino-acid diversity     = 9.44 E 8
DNA diversity            = 2.42 E 9
Stop-free                = 93%
Gratuitous Cys-free      = 93%
Free of stop and Cys     = 88%


C35D222JH1B
!
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!    L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3-------------------------------------------------------------
!  Y|S  Y  Y|S Y|S  Y   G  Y|S  C  Y|S Y|S any Y|S  C  Y|S  T   Y  Y|S  S
   tmt tac tmc tmc tac ggc tMt tgc tmt tmc nnk tmt tgt tmc acc tat tmt tcc
!
!                                                  Q       Y      N|D
!  Y|S any Y|S  Y   S  Y|S  Y  Y|S  S  Y|S  Y   A  E|K Y|S F|S  Q  H|Y
   tmt nnk tmc tat tct tmt tac tmc agt tmt tat gct Vag tmt tHc cag Nac
!
!   W   G   Q   G   T   L   V   T   V   S   S  (SEQ ID NO:101)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'   (SEQ ID NO:102)
!                      BstEII...
```

Design 8

```
                1    1    2  2 2    3    3
     1   5      0    5    0  3 5    0    5
YYCAK SRSYYDYVWGSYRYTSSYSYYSYSYSSYAEYFQHWGQGTLVTVSS  (SEQ ID NO:905)
YYCAK ZXZYZBZVWGZZRZTZSZXZYZZZYZSZAψZFQHWGQGTLVTVSS (SEQ ID NO:103)
   R    YYDYVWGSYRYT D3-16.2      AEYFQHWGQGTLVTVSS (JH1)
          (SEQ ID NO:104)            (SEQ ID NO:66)
(J=FSY,B=YHND,ψ=EKQ)

Amino-acid diversity     = 9.44 E 8
DNA diversity            = 1.61 E 9
Stop-free                = 93%
Gratuitous Cys-free      = 93%
Free of stop and Cys     = 88%
```

```
C34D316JH1A
!
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3-------------------------------------------------------------------
!                          N|D
!  Y|S any Y|S  Y  Y|S Y|H Y|S  V   W   G  Y|S Y|S  R  Y|S  T  Y|S
   tmt nnk tmc tac tmt Nat tmt gtt tgg ggt tmt tmc cgt tmt act tmt
!
!   S  Y|S any Y|S  Y  Y|S Y|S Y|S  Y  Y|S  S  Y|S
   agt tmc nnk tmt tac tmc tmt tmc tat tmc agt tmt
!
!        Q
!    A, E|K Y|S  F   Q   H
     GCT vag tmc ttc cag cat
!
!   W   G   Q   G   T   L   V   T   V   S   S   (SEQ ID NO:105)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'    (SEQ ID NO:106)
!                       BstEII...

(C34D316JH1A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt nnk tmc tac tmt Nat tmt-
 gtt tgg ggt tmt tmc cgt tmt act tmt agt tmc nnk tmt tac tmc tmt tmc tat-
 tmc agt tmt GCT vag tmc ttc cag cat tgg ggc cag ggt act ct -3'  (SEQ ID NO:107)
```

Design 9

Design 9 is like 8 except the D segment is moved to the right

```
                1    1    2  2 2    3    3
     1   5      0    5    0  3 5    0    5
YYCAK YGYSSDSYYSSYYDYVWGSYRYTYSSYYAEYFQHWGQGTLVTVSS (SEQ ID NO:906)
YYCAK ZXZZZXZYZZZYZBZVWGZZRZTYZSZYAψZFQHWGQGTLVTVSS (SEQ ID NO:108)
   R  D3-16.2   YYDYVWGSYRYT      AEYFQHWGQGTLVTVSS (JH1)
              (SEQ ID NO:104)    (SEQ ID NO:66)
(J=FSY,B=YHND,ψ=EKQ)

Amino-acid diversity     = 1.31 E 8
DNA diversity            = 5.37 E 8
Stop-free                = 91%
Gratuitous Cys-free      = 91%
Free of stop and Cys     = 83%
```

```
C34D316JH1B
!
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!             XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3-------------------------------------------------------------------
!  Y|S any Y|S Y|S Y|S any Y|S  Y  Y|S Y|S Y|S
   tmt nnk tmc tmt tmc nnk tmt tac tmc tmt tmc
!
!          N|D
!   Y  Y|S Y|H Y|S  V   W   G  Y|S Y|S  R  Y|S  I
   tac tmt Nat tmt gtt tgg ggt tmt tmc cgt tmt act
!
!   Y  Y|S  S  Y|S  Y
   tat tmc agt tmt tac
!
!        Q
!    A  E|K Y|S  F   Q   H
    GCT vag tmc ttc cag cat
!
!   W   G   Q   G   T   L   V   T   V   S   S    (SEQ ID NO:109)
   tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'    (SEQ ID NO:110)
!                        BstEII...
```

```
(C35D316JH1B)
5'-GCA|GtT|taT|taC|tgc|gct aRg tmt nnk tmc tmt tmc nnk tmt tac tmc tmt tmc
tac tmt Nat tmt gtt tgg ggt tmt tmc cgt tmt act tat tmc agt tmt tac GCT vag
tmc ttc cag cat tgg ggc cag ggt act ct-3'    (SEQ ID NO:111)
```

Design 10

```
                1    1    2   2
      1   5     0    5    0   4
YYCAK GSSYYYGSGSYYNSDYYSAEYFQHWGQGTLVTVSS  (SEQ ID NO:907)
YYCAK XZZYZZGZGZXYNZXZYZAXZFQHWGQGTLVTVSS   (SEQ ID NO:112)
    R    YYYGSGSYYN      AEYFQHWGQGTLVTVSS (JH1)
        (SEQ ID NO:81)    (SEQ ID NO:66)
```

Design 10 (C24D310B) is like Design 3, but the CDR3 is of length 24. Design 10 has 94 as R or K, XZZ, D3-10 (RF2) with 2nd, 3rd, 5th, and 7th as Z(Y|S) and 8th residue changed to X, ZXZYZ, , and JH1 (with the E changed to X) . Z is either Y or S. The CDR3 is 24 AA long and could be further diversified by use of error-prone PCR.

```
(C24D310b) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk tmc tmc tac tmc tmt ggt tmc-
  ggc tmt nnk tac aat tmt nnk tmc tat tmc gct nnk tmc ttt caa cat tgg ggc-
  cag ggt act ct-3'    (SEQ ID NO:113)
ON_1, ON_2, ON_3, and ON_4 as above.
```

Design 11

```
                1    1    2    2
      1   5     0    5    0    5
```

YYCAR SSRSGYCTNGVCYRSGSYWYFDL**WGRGTLVTVSS** (SEQ ID NO:907)
YYCAR ZZXZGZC32GVCZ3ZXZZ4Z12L**WGRGTLVTVSS** (SEQ ID NO:114)
K GYCTNGVCYT YWYFDL**WGRGTLVTVSS** D2-8.2 JH2
(SEQ ID NO:115) (SEQ ID NO:67)
(1=FYS(THT), 2=YHND(NAT), 3=ITKR(ANA), 4=LSW(TBG))

(C24D282) 5'-GCA|GtT|taT|taC|tgc|gct **aRg tmc tmt nnk tmt ggt tmc tgt ana-**
nat ggt gtc tgc tmt ana tmc nnk tmt tmt tbg tmt tht nat ctg tgg ggc-
cag ggt act ct-3'. (SEQ ID NO:116)

(C24D282.1) 5'-GCA|GtT|taT|taC|tgc|gct **aRg tmc tmt nnk tmc ggt tmc tgc ana-**
nat ggc gtc tgc tmt ana tmc nnk tmt tmt tbg tmt tht nat ctg tgg ggc-
cag ggt act ct-3' (SEQ ID NO:117)

(C24D282.1) 5'-GCA|GtT|taT|taC|tgc|gct **aRg tmc tmt nnk tmc ggt tmc tgc ana-**
nat ggc gtc tgc t-3' (SEQ ID NO:118) (needs R, M, N, K)
(C24D282.2) 5'-Ag AgT Acc cTg gcc ccA cAg ATN ADA AKA cVA AKA AKA MNN gKA TNT AKA gcA
gAc gcc ATN TNT gcA gKA Acc g-3' (SEQ ID NO:119) ! 75 bases
(5'-c ggt tmc tgc ana-
nat ggc gtc tgc tmt ana tmc nnk tmt tmt tbg tmt tht nat ctg tgg ggc-
cag ggt act ct-3' (RC) (SEQ ID NO:120) (needs N, M, K, B, H))

Design 12

1 1 2 2 3 3
1 5 0 5 0 5 0 5
YYCAR SSYYSYGYCTNGVCYTYSYSYYSYSYSYWYFDL**WGRGTLVTVSS** (SEQ ID NO:908)
YYCAR ZZZZZZGZC32GVCZ3ZZZZZYZZYZYZZ4Z12L**WGRGTLVTVSS** (SEQ ID NO:121)
K GYCTNGVCYT YWYFDL**WGRGTLVTVSS** D2-8.2 JH2
(SEQ ID NO:115) (SEQ ID NO:67)
(1=FYS, 2=YHND, 3=ITKR, 4=LSW, Z=YS)

(C33D282TP) 5'-GCA|GtT|taT|taC|tgc|gct-3' (SEQ IDNO:909)
(C33D282BP) 5'-ag agt acc ctg gcc cca-3' (SEQ ID NO:910)
(C33D282) 5'-GCA|GtT|taT|taC|tgc|gct **aRg tmt tmc tmc tmt tmc tmc** ggt-
tmt tgt ana nat ggc gtg tgc tmt ana tmc tmc tmc tmt tat **tmt tmc** tat **tmt-**
tac **tmt tmc tbg tmc tht nat** ctg tgg ggc cag ggt act ct-3'(SEQ ID NO:122)
(C33D282F) 5'-GCA|GtT|taT|taC|tgc|gct **agg tct tcc tac tat tcc tac** ggt-
tat tgt aca aat ggc gtg tgc tat aca tac tcc tac tct tat **tat tcc** tat **tct-**
tac **tct tac tgg tac ttt gat** ctg tgg ggc cag ggt act ct-3'(SEQ ID NO:911)

Design 13

Design 13 places a germ-line D segment in the middle of a sea of Zs so that one can make two pieces of DNA that overlap throughout the constant region. HC CDR3 is 34 long and diversity is $2^{23} \sim 8 \times 10^6$.

1 1 2 2 3 3
1 5 0 5 0 5 0 5
YYCAR SSSYYSYYSSGYCTNGVCYTYSSYYSSYYWYFDL**WGRGTLVTVSS** (SEQ ID NO:912)
YYCAR ZZZZZZZZZZGYCTNGVCYTZZZZZZZZZZWZF2L**WGRGTLVTVSS** (SEQ ID NO:123)
K GYCTNGVCYT YWYFDL**WGRGTLVTVSS** D2-8.2 JH2
(SEQ ID NO:115) (SEQ ID NO:67)

(2=YHND)

(C34D282.2A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tmc tmc tmt tmt tmc tmc tmt-
tmc tmc ggt tat tgt act aac ggc gtt tgc tat act-3' (SEQ ID NO:124)
(C34D282.2B) 5'-Ag AgT Acc cTg gcc ccA cAg gTN gAA AKA ccA AKA AKA AKA gKA-
gKA gKA gKA AKA AKA AgT ATA gcA AAc gcc gTT AgT AcA ATA-3' (SEQ ID NO:125)! 86
bases
(5'- tat tgt act aac ggc gtt tgc tat act tmt tmt tmc tmc tmc tmc-
tmt tmt tmt tgg tmt ttc Nac ctg tgg ggc cag ggt act ct-3' (SEQ ID
NO:126) [RC])

Design 14

Design 14 is like 9 except the D segment is mostly germline.

```
                1    1    2  2 2    3    3
      1   5     0    5    0  3 5    0    5
YYCAK YSYYSGSYYYSDYVWGSYRYTSYDSYYYAEYFQHWGQGTLVTVSS (SEQ ID NO:913)
YYCAK ZZZZZZZZZZZDYVWGSYRZTZZZZZZZAEZFQHWGQGTLVTVSS (SEQ ID NO:127)
    R  D3-16.2 YYDYVWGSYRYT       AEYFQHWGQGTLVTVSS (JH1)
               (SEQ ID NO:104)     (SEQ ID NO:66)
```

(C34D316.2A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tmc tmc tmt tmt tmc tmc tmt-
tmc tmc tmc gat tat gtc tgg ggt act tat cgt-3' (SEQ ID
NO:128)
(C34D316.2B) 5'-Ag AgT Acc cTg gcc ccA ATg cTg gAA AKA cTc Agc gKA gKA gKA-
gKA gKA gKA AKA AgT gKA Acg ATA AgT Acc ccA gAc ATA ATc-3' (SEQ ID
NO:129) ! 86 bases
(5'-gat tat gtc tgg ggt act tat cgt tmc act tmt tmc tmc tmc tmc-
tmc tmc gct gag tmt ttc cag cat tgg ggc cag ggt act ct-3'
(SEQ ID NO:130) [RC])

Design 15

Design 15 allows some diversity in the overlap, 5 two-way flip-flops. There are only 32 overlap sequences and even if there are mismatches, they will not change the allowed diversity.

```
                1    1    2  2 2    3    3
      1   5     0    5    0  3 5    0    5
YYCAK SYDYSSYSYYYDYVWGSYRYTSYSGDSYYAEYFQHWGQGTLVTVSS (SEQ ID NO:914)
YYCAK ZZZZZZZZZZZZDZVWGZZRZTZZZZZZZZZAEZFQHWGQGTLVTVSS (SEQ ID NO:131)
                YYDYVWGSYRYT         AEYFQHWGQGTLVTVSS
                  (SEQ ID NO:104)      (SEQ ID NO:66)
```

(C35D316.2A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tmc tmc tmt tmt tmc tmc tmt-
tmc tmc tmc gac tmt gtc tgg ggt tmc tmc cgt tmc acc t-3' (SEQID NO:
132)
(C35D316.2B) 5'-Ag AgT Acc cTg gcc ccA ATg cTg gAA AKA cTc Agc gKA gKA-
gKA gKA gKA gKA gKA AKA ggT gKA Acg gKA gKA Acc ccA gAc AKA gTc gKA g-3'
(SEQ ID NO:133)
(5'-c tmc gac tmt gtc tgg ggt tmc tmc cgt tmc acc tmt tmc tmc-
tmc tmc tmc tmc tmc gct gag tmt ttc cag cat tgg ggc cag ggt act ct-3' (SEQ ID
NO:134) [RC])

Design 16

Design 16 provides a CDR3 of 35. There are 4 two-way flip-flops in the overlap, thus 16 sequences.

```
                1    1    2  2 2    3    3
      1   5     0    5    0  3 5    0    5
YYCAK SSSYYSYSYSGYCSGGSCYSSYYYSSYYSAEYFQGWGQGTLVTVSS (SEQ ID NO:915)
YYCAK ZZZZZZZZZZGZCZGGZCZSZZZZZZZZZAEZFQHWGQGTLVTVSS    (SEQ ID NO:135)
    R          GYCSGGSCYS  2-25.2 AEYFQHWGQGTLVTVSSJH1
               (SEQ ID NO:136)     (SEQ ID NO:66)

(C35D225.2A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tmt tmt tmt tmt tmt tmt-
      tmc tmc ggc tmc tgt tmc ggt ggc tmc tgc tmc tcc t-3'    (SEQ ID NO:137)
(C35D225.2B) 5'-Ag AgT Acc cTg gcc ccA ATg TTg gAA AKA TTc Agc gKA gKA-
  gKA gKA gKA gKA gKA gKA gKA gKA ggA gcA gKA gcc Acc gKA AcA gKA gcc gKA g-3'
  (SEQ ID NO:138)! 96 bases
```

If we add C34D225.2A and C34D225.2B to the mixture, then we get CDR3s of lengths 33, 34, and 35.

```
(C34D225.2A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt tmt tmt tmt tmt tmt tmt-
      tmc tmc ggc tmc tgt tmc ggt ggc tmc tgc tmc tcc t-3'    (SEQ ID NO:139)
(C34D225.2B) 5'-Ag AgT Acc cTg gcc ccA ATg TTg gAA AKA TTc Agc gKA gKA-
  gKA gKA gKA gKA gKA gKA gKA gKA ggA gcA gKA gcc Acc gKA AcA gKA gcc gKA g-3'
 (SEQ ID NO:140)!  93 bases
```

Design 17

```
                1    1    2  2 2    3    3
      1   5     0    5    0  3 5    0    5
YYCAK YSSYSYYDYVWGSYRYTSSSYSYYSYYYAEYFQGWGQGTLVTVSS (SEQ ID NO:916)
YYCAK ZZZZZZZDZVWGZZRZTZZZZZZZZZZZAEZFQHWGQGTLVTVSS    (SEQ ID NO:141)
    R       YYDYVWGSYRYT D3-16.2   AEYFQHWGQGTLVTVSS (JH1)
           (SEQ ID NO:104)         (SEQ ID NO:66)

(C35D3162A) 5'- GCA|GtT|taT|taC|tgc|gct aRg tmt tmt tmt tmt tmt tmc gac-
   tmc gtc tgg ggt tmt tmc cgt tmt acc t-3'    (SEQ ID NO:142)
(C35D3162B)5'-Ag AgT Acc cTg gcc ccA gTg cTg gAA gKA cTc Agc gKA gKA gKA-
 gKA gKA gKA gKA gKA gKA gKA gKA gKA gKA ggT AKA Acg gKA AKA Acc ccA gAc-
 gKA gTc g-3'    (SEQ ID NO:143)
```

Design 18

```
                1    1    2  2 2    3    3
      1   5     0    5    0  3 5    0    5
YYCAK SSYYYSSSYYDYVWGSYRYTSSYYSYSYAEYFQGWGQGTLVTVSS (SEQ ID NO:917)
YYCAK ZZZZZZZZZZZDZVWGZZRZTZZZZZZZZZAEZFQHWGQGTLVTVSS    (SEQ ID NO:144)
    R          YYDYVWGSYRYT D3-16.2AEYFQHWGQGTLVTVSS (JH1)
               (SEQ ID NO:104)     (SEQ ID NO:66)

(C35D3162C) 5'- GCA|GtT|taT|taC|tgc|gct aRg tmt tmt tmt tmt tmt tmt tmc-
   tmc tmc tmc gac tmc gtc tgg ggt tmc tmc cgt tmc acc t-3' (SEQ ID NO:145)
82 bases
(C35D3162B) 5'-Ag AgT Acc cTg gcc ccA gTg cTg gAA gKA cTc Agc gKA gKA-
```

```
gKA gKA gKA gKA gKA gKA gKA gKA ggT gKA Acg gKA gKA Acc ccA gAc gKA-
gTc g-3'      (SEQ ID NO:146)
```

Design 19

```
                  1    1    2  2 2    3    3
      1   5       0    5    0  3 5    0    5
YYCAK YSGDSYSYYYYDSSGYYYSYYSSSYYSYYAEYFQGWGQGTLVTVSS (SEQ ID NO:918)
YYCAK ZZZZZZZZZZZZDSSGZZZZZZZZZZZZZZZZAEZFQHWGQGTLVTVSS    (SEQ ID NO:147)
    R          YYYDSSGYYY            AEYFQHWGQGTLVTVSS (JH1)
                  (SEQ ID NO:88)          (SEQ ID NO:66)
            1 1                            1     1
    9 9     0 0                            0     1
    4 5     0 2abcdefghijklmnopqrstuvwxyab3      0
                                           ''

Amino-acid diversity    = 6.7 E 7
DNA diversity           = 6.7 E 7
Stop-free               = 100
Gratuitous Cys-free     = 100
Free of stop and Cys    = 100%
```

Design 19 has CDR3 of length 35. Residue 94 can be K or R, The ZZZZZZZZZ::D3-22(2nd RF with six Ys as Z)::ZZZZZZZZZZZ::JH1(with 1 Z). Error-prone PCR could be used to add more diversity.

```
C35D322AJH1
!  scab DNA     S   R   D   N   S   K   N   T   L   Y   L   Q   M   N   S
5'-ttc|act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|aac|agC-
!              XbaI...
!
!   L   R   A   E   D   T   A   V   Y   Y   C   A  K|R
  |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aRg -
!
!  CDR3-------------------------------------------------------------------
!
!  Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S  D   S   S   G  Y|S Y|S Y|S
   tmc tmt tmc tmc tmt tmc tmt tmc tmc tmc tmc gac agc tcc ggc tmc tmc tmt
!
   Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S Y|S  A   E  Y|S  F   Q   H
   tmc tmt tmc tmc tmt tmc tmt tmc tmc tmc tmc gct gaa tmc ttc caa cac
!
!   W   G   Q   G   T   L   V   T   V   S   S     (SEQ ID NO:148)
    tgg ggc cag ggt act ctG GTC ACC gtc tcc agt-3'  (SEQ ID NO:149)
!                       BstEII...
```

(C35D322AJH1_T) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmc tmt tmc tmc tmt-
tmc tmt tmc tmc tmc tmc gac agc tcc ggc tmc tmc t-3' (SEQ ID NO:150)
(C35D322AJH1_B) 5'-cAg AgT Acc cTg gcc ccA gTg TTg gAA gKA TTc Agc gKA-
gKA gKA gKA AKA gKA AKA gKA gKA AKA gKA AKA gKA gKA gcc ggA gcT gTc-
gKA gKA g-3' (SEQ ID NO:151)

ON_1, ON_2, ON_3, and ON_4 as above.

Design 20

```
                  1    1    2  2 2      3    3
      1   5       0    5    0  3 5      0    5
YYCAK YSSYSS   YYYYDSSGYYYSSYSSYS   YYYAEYFQGWGQGTLVTVSS (SEQ ID NO:919)
YYCAK ZZZZZZ(Z)ZZZZDSSGZZZZZZZZZZ(Z)ZZZAEZFQHWGQGTLVTVSS   (SEQ ID NO:152)
    R           YYYDSSGYYY            AEYFQHWGQGTLVTVSS (JH1)
              (SEQ ID NO:88)     (SEQ ID NO:66)
          1   1                              1    1
    9 9   0   0                              0    1
    4 5   0   3abcdefghijklmnop q rstuvwxya4   0
```

```
Amino-acid diversity    = 6.7 E 7
DNA diversity           = 6.7 E 7
Stop-free               = 100
Gratuitous Cys-free     = 100
Free of stop and Cys    = 100%
```

Design 20 has CDR3s of length 33, 34, or 35. Residue 94 can be K or R, The ZZZZZZ(Z)ZZ::D3-22(2nd RF with six Ys as Z)::ZZZZZZZ(Z)ZZZ::JH1(with 1 Z). PCR combining (C35D322AJH1_T), (C34D322AJH1_T), (C35D322AJH1_B), and (C34D322AJH1_B) allows length as well as sequence diversity.

(C35D322AJH1_T) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmc tmt tmc tmc-
tmt tmc tmt tmc tmc tmc tmc gac agc tcc ggc tmc tmc t-3' (SEQ ID NO:153)
(C34D322AJH1_T) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmc tmc tmc tmt-
tmc tmt tmc tmc tmc tmc gac agc tcc ggc tmc tmc t-3' (SEQ ID NO:154)
(C35D322AJH1_B) 5'-cAg AgT Acc cTg gcc ccA gTg TTg gAA gKA TTc Agc gKA-
gKA gKA gKA AKA gKA AKA gKA gKA AKA gKA AKA gKA gKA gcc ggA gcT gTc-
gKA gKA g-3' (SEQ ID NO:920)
(C34D322AJH1_B) 5'-cAg AgT Acc cTg gcc ccA gTg TTg gAA gKA TTc Agc gKA-
gKA gKA gKA AKA gKA AKA gKA gKA AKA AKA gKA gKA gcc ggA gcT gTc-
gKA gKA g-3' (SEQ ID NO:155)

Selection against stop codons:

[00182] Because some of these libraries have NNK codons, they will have some TAG stop codons. We could remove the clones with TAG by cloning the amplified DNA into an *Xba*I-

*Bst*EII site between the signal sequence for a *bla* gene and the actual bla protein and express in Sup^0 cells. Bla^R colonies do not contain TAG stops. Alternatively, we could clone the *Xba*I-*Bst*EII fragments ahead of a kanamycin-resistance gene and select for Kan^R. We would then move the *Xba*I-*Bst*EII cassette into the phage library.

[00183] Also, because wobbling allows some stop codons, we can improve the library by removing the clones with stops by cloning the amplified DNA into an *Xba*I-*Bst*EII site between the signal sequence for a *bla* gene and the actual *bla* protein and express in Sup^0 cells. Bla^R colonies do not contain stops. Alternatively, we can clone the *Xba*I-*Bst*EII fragments ahead of a kanamycin-resistance gene and select for Kan^R. We can then move the *Xba*I-*Bst*EII cassette into the phage library.

Table 20: Human D regions

!* for TAG; @ for TAA; $ for TGA

D - Amino acid sequence alignment (RF: reading frame)

		RF 1	RF 2	RF 3	Used in designs
		(SEQ ID NO:156)	(SEQ ID NO:157)	(SEQ ID NO:158)	
D1	1-1	GTTGT	VQLER	YNWND	
		(SEQ ID NO:159)	(SEQ ID NO:160)	(SEQ ID NO:161)	
	1-7	GITGT	V*LEL	YNWNY	
		(SEQ ID NO:159)	(SEQ ID NO:162)	(SEQ ID NO:163)	
	1-20	GITGT	V*LER	YNWND	
		(SEQ ID NO:164)	(SEQ ID NO:165)	(SEQ ID NO:166)	
	1-26	GIVGAT	V*WELL	**YSGSYY**	
		(SEQ ID NO:167)	(SEQ ID NO:70)	(SEQ ID NO:168)	
D2	2-2	RIL**YQLLY	**GYCSSTSCYT**	DIVVVPAAI	1, 5, 6, 7,
		(SEQ ID NO:169)	(SEQ ID NO:115)	(SEQ ID NO:170)	
	2-8	RILY@WCMLY	**GYCTNGVCYT**	DIVLMVYAI	20, 21, 22,
		(SEQ ID NO:171)	(SEQ ID NO:136)	(SEQ ID NO:172)	
	2-15	RIL*WW*LLL	**GYCSGGSCYS**	DIVVVVAAT	25,
		(SEQ ID NO:173)	(SEQ ID NO:174)	(SEQ ID NO:175)	
	2-21	SILWW$LLF	**AYCGGDCYS**	HIVVVTAI	
		(SEQ ID NO:176)	(SEQ ID NO:177)	(SEQ ID NO:178)	
D3	3-3	VLRFLEWLLY	**YYDFWSGYYT**	ITIFGVVII	
		(SEQ ID NO:179)	(SEQ ID NO:180)	(SEQ ID NO:181)	
	3-9	VLRYFDWLL@	**YYDILTGYYN**	ITIF*LVII	
		(SEQ ID NO:182)	(SEQ ID NO:81)	(SEQ ID NO:183)	
	3-10	VLLWFGELL@	**YYYGSGSYYN**	ITMVRGVII	
		(SEQ ID NO:184)	(SEQ ID NO:104)	(SEQ ID NO:185)	
	3-16	VL$LRLGELSLY	**YYDYVWGSYRYT**	IMITFGGVIVI	8,9,14,15,17,18
		(SEQ ID NO:186)	(SEQ ID NO:187)	(SEQ ID NO:188)	
	3-22	VLL***WLLL	**YYYDSSGYYY**	ITMIVVVIT	4,19,20
		(SEQ ID NO:189)	(SEQ ID NO:88)	(SEQ ID NO:190)	
D4	4-4	$LQ@L	**DYSNY**	TTVT	
		(SEQ ID NO:191)	(SEQ ID NO:192)	(SEQ ID NO:193)	
	4-11	$LQ@L	**DYSNY**	TTVT	
		(SEQ ID NO:194)	(SEQ ID NO:195)	(SEQ ID NO:196)	
	4-17	$LR@L	DYGDY	TTVT	
		(SEQ ID NO:197)	(SEQ ID NO:198)	(SEQ ID NO:199)	
	4-23	$LRW@L	DYGGNS	TTVVT	

		(SEQ ID NO:200)	(SEQ ID NO:201)	(SEQ ID NO:202)
D5	5-5	VDTAMV	WIQLWL	**GYSYGY**
		(SEQ ID NO:203)	(SEQ ID NO:204)	(SEQ ID NO:205)
	5-12	VDIVATI	WI*WLRL	**GYSGYDY**
		(SEQ ID NO:206)	(SEQ ID NO:207)	(SEQ ID NO:208)
	5-18	VDIAMV	WIQLWL	**GYSYGY**
		(SEQ ID NO:209)	(SEQ ID NO:210)	(SEQ ID NO:211)
	5-24	VEMATI	*RWLQL	RDGYNY
		(SEQ ID NO:212)	(SEQ ID NO:213)	(SEQ ID NO:214)
D6	6-6	**EYSSSS**	SIAAR	V*QLV
		(SEQ ID NO:215)	(SEQ ID NO:216)	(SEQ ID NO:217)
	6-13	**GYSSSWY**	GIAAAG	V*QQLV
		(SEQ ID NO:218)	(SEQ ID NO:219)	(SEQ ID NO:220)
	6-19	**GYSSGWY**	GIAVAG	V*QWLV
D7	7-27	LTG	@LG	NWG

Table 3: Human JH segments

JH - Amino acid sequence alignment

```
            H3
          ------
           CDR3
         --------
            100       110
              | FR4--------     Used in designs
JH1     ---AEYFQHWGQGTLVTVSS 1-8,     (SEQ ID NO:66)
JH2     ---YWYFDLWGRGTLVTVSS          (SEQ ID NO:67)
JH3     -----AFDIWGQGTMVTVSS          (SEQ ID NO:2)
JH4     -----YFDYWGQGTLVTVSS          (SEQ ID NO:1)
JH5     ----NWFDPWGQGTLVTVSS          (SEQ ID NO:68)
JH6     YYYYYGMDVWGQGTTVTVSS          (SEQ ID NO:3)
        123456789
```

Table 10: DNA encoding V-5D2-8.2a-JH2 for wobbling

```
!                                                    CDR3.......
!   A   E   D   T   A   V   Y   Y   C   A   K   D   I   V   L   M
  |gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct aag jez ezq jzz qzz ezj
!
!    W   G   Q   G   T   T   V   T   V   S   S     (SEQ ID NO:224)
     tgg ggc cag ggt act acG GTC ACC gtc tcc agt-3'     (SEQ ID NO:225)
!                 BstEII...
```

Table 11: Trimers that can be extracted from human D segments

In Tables 11-14, the use of a lower case letter in an amino acid sequence indicates that a stop codon was changed to the residue listed as the lower case letter. For example, in the amino acid sequence "yLE", a Tyr residue was introduced in place of a stop codon.

GTT	D1-1.1.1	1	
VQL	D1-1.2.1	2	
YNW	D1-1.3.1	3	
TTG	D1-1.1.2	4	
QLE	D1-1.2.2	5	
NWN	D1-1.3.2	6	
TGT	D1-1.1.3	7	
LER	D1-1.2.3	8	
WND	D1-1.3.3	9	
GIT	D1-7.1.1	10	
VyL	D1-7.2.1	11	*
ITG	D1-7.1.2	12	
yLE	D1-7.2.2	13	*
LEL	D1-7.2.3	14	
WNY	D1-7.3.3	15	
GIV	D1-26.1.1	16	
VyW	D1-26.2.1	17	*
YSG	D1-26.3.1	18	
IVG	D1-26.1.2	19	
yWE	D1-26.2.2	20	*
SGS	D1-26.3.2	21	
VGA	D1-26.1.3	22	
WEL	D1-26.2.3	23	
GSY	D1-26.3.3	24	
GAT	D1-26.1.4	25	
ELL	D1-26.2.4	26	
SYY	D1-26.3.4	27	
RIL	D2-2.1.1	28	
GYC	D2-2.2.1	29	#
DIV	D2-2.3.1	30	
ILy	D2-2.1.2	31	*
YCS	D2-2.2.2	32	#
IVV	D2-2.3.2	33	
Lyy	D2-2.1.3	34	*
CSS	D2-2.2.3	35	#
VVV	D2-2.3.3	36	
yyY	D2-2.1.4	37	*
SST	D2-2.2.4	38	
VVP	D2-2.3.4	39	
yYQ	D2-2.1.5	40	*
STS	D2-2.2.5	41	
VPA	D2-2.3.5	42	
YQL	D2-2.1.6	43	
TSC	D2-2.2.6	44	#
PAA	D2-2.3.6	45	
QLL	D2-2.1.7	46	
SCY	D2-2.2.7	47	#
AAI	D2-2.3.7	48	
LLY	D2-2.1.8	49	

CYT	D2-2.2.8	50	#
ILY	D2-8.1.2	51	
YCT	D2-8.2.2	52	#
IVL	D2-8.3.2	53	
LYy	D2-8.1.3	54	*
CTN	D2-8.2.3	55	#
VLM	D2-8.3.3	56	
YyW	D2-8.1.4	57	*
TNG	D2-8.2.4	58	
LMV	D2-8.3.4	59	
yWC	D2-8.1.5	60	*#
NGV	D2-8.2.5	61	
MVY	D2-8.3.5	62	
WCM	D2-8.1.6	63	#
GVC	D2-8.2.6	64	#
VYA	D2-8.3.6	65	
CML	D2-8.1.7	66	#
VCY	D2-8.2.7	67	#
YAI	D2-8.3.7	68	
MLY	D2-8.1.8	69	
LyW	D2-15.1.3	70	*
CSG	D2-15.2.3	71	#
yWW	D2-15.1.4	72	*
SGG	D2-15.2.4	73	
WWy	D2-15.1.5	74	*
GGS	D2-15.2.5	75	
VVA	D2-15.3.5	76	
WyL	D2-15.1.6	77	*
GSC	D2-15.2.6	78	#
VAA	D2-15.3.6	79	
yLL	D2-15.1.7	80	*
AAT	D2-15.3.7	81	
LLL	D2-15.1.8	82	
CYS	D2-15.2.8	83	#
SIL	D2-21.1.1	84	
AYC	D2-21.2.1	85	#
HIV	D2-21.3.1	86	
ILW	D2-21.1.2	87	
YCG	D2-21.2.2	88	#
LWW	D2-21.1.3	89	
CGG	D2-21.2.3	90	#
WWw	D2-21.1.4	91	*
GGD	D2-21.2.4	92	
VVT	D2-21.3.4	93	
WwL	D2-21.1.5	94	*
GDC	D2-21.2.5	95	#
VTA	D2-21.3.5	96	
wLL	D2-21.1.6	97	*
DCY	D2-21.2.6	98	#
TAI	D2-21.3.6	99	
LLF	D2-21.1.7	100	
VLR	D3-3.1.1	101	
YYD	D3-3.2.1	102	
ITI	D3-3.3.1	103	
LRF	D3-3.1.2	104	
YDF	D3-3.2.2	105	
TIF	D3-3.3.2	106	

RFL D3-3.1.3	107	
DFW D3-3.2.3	108	
IFG D3-3.3.3	109	
FLE D3-3.1.4	110	
FWS D3-3.2.4	111	
FGV D3-3.3.4	112	
LEW D3-3.1.5	113	
WSG D3-3.2.5	114	
GVV D3-3.3.5	115	
EWL D3-3.1.6	116	
SGY D3-3.2.6	117	
VVI D3-3.3.6	118	
WLL D3-3.1.7	119	
GYY D3-3.2.7	120	
VII D3-3.3.7	121	
YYT D3-3.2.8	122	
LRY D3-9.1.2	123	
YDI D3-9.2.2	124	
RYF D3-9.1.3	125	
DIL D3-9.2.3	126	
IFy D3-9.3.3	127	*
YFD D3-9.1.4	128	
ILT D3-9.2.4	129	
FyL D3-9.3.4	130	*
FDW D3-9.1.5	131	
LTG D3-9.2.5	132	
yLV D3-9.3.5	133	*
DWL D3-9.1.6	134	
TGY D3-9.2.6	135	
LVI D3-9.3.6	136	
LLy D3-9.1.8	137	*
YYN D3-9.2.8	138	
VLL D3-10.1.1	139	
YYY D3-10.2.1	140	
ITM D3-10.3.1	141	
LLW D3-10.1.2	142	
YYG D3-10.2.2	143	
TMV D3-10.3.2	144	
LWF D3-10.1.3	145	
YGS D3-10.2.3	146	
MVR D3-10.3.3	147	
WFG D3-10.1.4	148	
GSG D3-10.2.4	149	
VRG D3-10.3.4	150	
FGE D3-10.1.5	151	
RGV D3-10.3.5	152	
GEL D3-10.1.6	153	
GVI D3-10.3.6	154	
VLw D3-16.1.1	155	*
IMI D3-16.3.1	156	
LwL D3-16.1.2	157	*
YDY D3-16.2.2	158	
MIT D3-16.3.2	159	
wLR D3-16.1.3	160	*
DYV D3-16.2.3	161	
ITF D3-16.3.3	162	
LRL D3-16.1.4	163	

YVW	D3-16.2.4	164
TFG	D3-16.3.4	165
RLG	D3-16.1.5	166
VWG	D3-16.2.5	167
FGG	D3-16.3.5	168
LGE	D3-16.1.6	169
WGS	D3-16.2.6	170
GGV	D3-16.3.6	171
ELS	D3-16.1.8	172
SYR	D3-16.2.8	173
VIV	D3-16.3.8	174
LSL	D3-16.1.9	175
YRY	D3-16.2.9	176
IVI	D3-16.3.9	177
SLY	D3-16.1.10	178
RYT	D3-16.2.10	179
LLw	D3-22.1.2	180 *
TMI	D3-22.3.2	181
Lwy	D3-22.1.3	182 *
YDS	D3-22.2.3	183
MIV	D3-22.3.3	184
wyy	D3-22.1.4	185 *
DSS	D3-22.2.4	186
yyW	D3-22.1.5	187 *
SSG	D3-22.2.5	188
yWL	D3-22.1.6	189 *
VIT	D3-22.3.7	190
wLQ	D4-4.1.1	191 *
DYS	D4-4.2.1	192
TTV	D4-4.3.1	193
LQy	D4-4.1.2	194 *
YSN	D4-4.2.2	195
TVT	D4-4.3.2	196
QyL	D4-4.1.3	197 *
SNY	D4-4.2.3	198
DYG	D4-17.2.1	199
LRw	D4-17.1.2	200 *
YGD	D4-17.2.2	201
RwL	D4-17.1.3	202 *
GDY	D4-17.2.3	203
LRW	D4-23.1.2	204
YGG	D4-23.2.2	205
TVV	D4-23.3.2	206
RWy	D4-23.1.3	207 *
GGN	D4-23.2.3	208
GNS	D4-23.2.4	209
VDT	D5-5.1.1	210
WIQ	D5-5.2.1	211
GYS	D5-5.3.1	212
DTA	D5-5.1.2	213
IQL	D5-5.2.2	214
YSY	D5-5.3.2	215
TAM	D5-5.1.3	216
QLW	D5-5.2.3	217
SYG	D5-5.3.3	218
AMV	D5-5.1.4	219
LWL	D5-5.2.4	220

YGY	D5-5.3.4	221	
VDI	D5-12.1.1	222	
WIy	D5-12.2.1	223	*
IyW	D5-12.2.2	224	*
IVA	D5-12.1.3	225	
VAT	D5-12.1.4	226	
WLR	D5-12.2.4	227	
GYD	D5-12.3.4	228	
ATI	D5-12.1.5	229	
VEM	D5-24.1.1	230	
yRW	D5-24.2.1	231	*
RDG	D5-24.3.1	232	
EMA	D5-24.1.2	233	
RWL	D5-24.2.2	234	
DGY	D5-24.3.2	235	
MAT	D5-24.1.3	236	
WLQ	D5-24.2.3	237	
GYN	D5-24.3.3	238	
LQL	D5-24.2.4	239	
YNY	D5-24.3.4	240	
EYS	D6-6.1.1	241	
SIA	D6-6.2.1	242	
VyQ	D6-6.3.1	243	*
YSS	D6-6.1.2	244	
IAA	D6-6.2.2	245	
yQL	D6-6.3.2	246	*
SSS	D6-6.1.3	247	
AAR	D6-6.2.3	248	
QLV	D6-6.3.3	249	
GIA	D6-13.2.1	250	
yQQ	D6-13.3.2	251	*
AAA	D6-13.2.3	252	
QQL	D6-13.3.3	253	
SSW	D6-13.1.4	254	
AAG	D6-13.2.4	255	
SWY	D6-13.1.5	256	
IAV	D6-19.2.2	257	
yQW	D6-19.3.2	258	*
AVA	D6-19.2.3	259	
QWL	D6-19.3.3	260	
SGW	D6-19.1.4	261	
VAG	D6-19.2.4	262	
WLV	D6-19.3.4	263	
GWY	D6-19.1.5	264	
yLG	D7-27.2.1	265	*
NWG	D7-27.3.1	266	

Table 12: Distinct tetramers that can be extracted from human D segments

GTTG	D1-1.1.1	(SEQ ID NO:257) 1
VQLE	D1-1.2.1	(SEQ ID NO:258) 2
YNWN	D1-1.3.1	(SEQ ID NO:259) 3
TTGT	D1-1.1.2	(SEQ ID NO:263) 4
QLER	D1-1.2.2	(SEQ ID NO:264) 5
NWND	D1-1.3.2	(SEQ ID NO:265) 6
GITG	D1-7.1.1	(SEQ ID NO:266) 7
VyLE	D1-7.2.1	(SEQ ID NO:267) 8

ITGT	D1-7.1.2	(SEQ ID NO:271) 9
yLEL	D1-7.2.2	(SEQ ID NO:272) 10
NWNY	D1-7.3.2	(SEQ ID NO:273) 11
yLER	D1-20.2.2	(SEQ ID NO:275) 12
GIVG	D1-26.1.1	(SEQ ID NO:276) 13
VyWE	D1-26.2.1	(SEQ ID NO:277) 14
YSGS	D1-26.3.1	(SEQ ID NO:278) 15
IVGA	D1-26.1.2	(SEQ ID NO:285) 16
yWEL	D1-26.2.2	(SEQ ID NO:286) 17
SGSY	D1-26.3.2	(SEQ ID NO:287) 18
VGAT	D1-26.1.3	(SEQ ID NO:291) 19
WELL	D1-26.2.3	(SEQ ID NO:292) 20
GSYY	D1-26.3.3	(SEQ ID NO:293) 21
RILy	D2-2.1.1	(SEQ ID NO:294) 22
GYCS	D2-2.2.1	(SEQ ID NO:295) 23
DIVV	D2-2.3.1	(SEQ ID NO:296) 24
ILyy	D2-2.1.2	(SEQ ID NO:303) 25
YCSS	D2-2.2.2	(SEQ ID NO:304) 26
IVVV	D2-2.3.2	(SEQ ID NO:305) 27
LyyY	D2-2.1.3	(SEQ ID NO:312) 28
CSST	D2-2.2.3	(SEQ ID NO:313) 29
VVVP	D2-2.3.3	(SEQ ID NO:314) 30
yyYQ	D2-2.1.4	(SEQ ID NO:321) 31
SSTS	D2-2.2.4	(SEQ ID NO:322) 32
VVPA	D2-2.3.4	(SEQ ID NO:323) 33
yYQL	D2-2.1.5	(SEQ ID NO:330) 34
STSC	D2-2.2.5	(SEQ ID NO:331) 35
VPAA	D2-2.3.5	(SEQ ID NO:332) 36
YQLL	D2-2.1.6	(SEQ ID NO:338) 37
TSCY	D2-2.2.6	(SEQ ID NO:339) 38
PAAI	D2-2.3.6	(SEQ ID NO:340) 39
QLLY	D2-2.1.7	(SEQ ID NO:343) 40
SCYT	D2-2.2.7	(SEQ ID NO:344) 41
RILY	D2-8.1.1	(SEQ ID NO:345) 42
GYCT	D2-8.2.1	(SEQ ID NO:346) 43
DIVL	D2-8.3.1	(SEQ ID NO:347) 44
ILYy	D2-8.1.2	(SEQ ID NO:354) 45
YCTN	D2-8.2.2	(SEQ ID NO:355) 46
IVLM	D2-8.3.2	(SEQ ID NO:356) 47
LYyW	D2-8.1.3	(SEQ ID NO:363) 48
CTNG	D2-8.2.3	(SEQ ID NO:364) 49
VLMV	D2-8.3.3	(SEQ ID NO:365) 50
YyWC	D2-8.1.4	(SEQ ID NO:372) 51
TNGV	D2-8.2.4	(SEQ ID NO:373) 52
LMVY	D2-8.3.4	(SEQ ID NO:374) 53
yWCM	D2-8.1.5	(SEQ ID NO:381) 54
NGVC	D2-8.2.5	(SEQ ID NO:382) 55
MVYA	D2-8.3.5	(SEQ ID NO:383) 56
WCML	D2-8.1.6	(SEQ ID NO:389) 57
GVCY	D2-8.2.6	(SEQ ID NO:390) 58
VYAI	D2-8.3.6	(SEQ ID NO:391) 59
CMLY	D2-8.1.7	(SEQ ID NO:394) 60
VCYT	D2-8.2.7	(SEQ ID NO:395) 61
ILyW	D2-15.1.2	(SEQ ID NO:401) 62
YCSG	D2-15.2.2	(SEQ ID NO:402) 63
LyWW	D2-15.1.3	(SEQ ID NO:409) 64
CSGG	D2-15.2.3	(SEQ ID NO:410) 65

VVVV	D2-15.3.3	(SEQ ID NO:411)	66
yWWy	D2-15.1.4	(SEQ ID NO:418)	67
SGGS	D2-15.2.4	(SEQ ID NO:419)	68
VVVA	D2-15.3.4	(SEQ ID NO:420)	69
WWyL	D2-15.1.5	(SEQ ID NO:427)	70
GGSC	D2-15.2.5	(SEQ ID NO:428)	71
VVAA	D2-15.3.5	(SEQ ID NO:429)	72
WyLL	D2-15.1.6	(SEQ ID NO:435)	73
GSCY	D2-15.2.6	(SEQ ID NO:436)	74
VAAT	D2-15.3.6	(SEQ ID NO:437)	75
yLLL	D2-15.1.7	(SEQ ID NO:440)	76
SCYS	D2-15.2.7	(SEQ ID NO:441)	77
SILW	D2-21.1.1	(SEQ ID NO:442)	78
AYCG	D2-21.2.1	(SEQ ID NO:443)	79
HIVV	D2-21.3.1	(SEQ ID NO:444)	80
ILWW	D2-21.1.2	(SEQ ID NO:451)	81
YCGG	D2-21.2.2	(SEQ ID NO:452)	82
LWWw	D2-21.1.3	(SEQ ID NO:459)	83
CGGD	D2-21.2.3	(SEQ ID NO:460)	84
VVVT	D2-21.3.3	(SEQ ID NO:461)	85
WWwL	D2-21.1.4	(SEQ ID NO:468)	86
GGDC	D2-21.2.4	(SEQ ID NO:469)	87
VVTA	D2-21.3.4	(SEQ ID NO:470)	88
WwLL	D2-21.1.5	(SEQ ID NO:476)	89
GDCY	D2-21.2.5	(SEQ ID NO:477)	90
VTAI	D2-21.3.5	(SEQ ID NO:478)	91
wLLF	D2-21.1.6	(SEQ ID NO:481)	92
DCYS	D2-21.2.6	(SEQ ID NO:482)	93
VLRF	D3-3.1.1	(SEQ ID NO:483)	94
YYDF	D3-3.2.1	(SEQ ID NO:484)	95
ITIF	D3-3.3.1	(SEQ ID NO:485)	96
LRFL	D3-3.1.2	(SEQ ID NO:492)	97
YDFW	D3-3.2.2	(SEQ ID NO:493)	98
TIFG	D3-3.3.2	(SEQ ID NO:494)	99
RFLE	D3-3.1.3	(SEQ ID NO:501)	100
DFWS	D3-3.2.3	(SEQ ID NO:502)	101
IFGV	D3-3.3.3	(SEQ ID NO:503)	102
FLEW	D3-3.1.4	(SEQ ID NO:510)	103
FWSG	D3-3.2.4	(SEQ ID NO:511)	104
FGVV	D3-3.3.4	(SEQ ID NO:512)	105
LEWL	D3-3.1.5	(SEQ ID NO:519)	106
WSGY	D3-3.2.5	(SEQ ID NO:520)	107
GVVI	D3-3.3.5	(SEQ ID NO:521)	108
EWLL	D3-3.1.6	(SEQ ID NO:527)	109
SGYY	D3-3.2.6	(SEQ ID NO:528)	110
VVII	D3-3.3.6	(SEQ ID NO:529)	111
WLLY	D3-3.1.7	(SEQ ID NO:532)	112
GYYT	D3-3.2.7	(SEQ ID NO:533)	113
VLRY	D3-9.1.1	(SEQ ID NO:534)	114
YYDI	D3-9.2.1	(SEQ ID NO:535)	115
LRYF	D3-9.1.2	(SEQ ID NO:542)	116
YDIL	D3-9.2.2	(SEQ ID NO:543)	117
TIFy	D3-9.3.2	(SEQ ID NO:544)	118
RYFD	D3-9.1.3	(SEQ ID NO:551)	119
DILT	D3-9.2.3	(SEQ ID NO:552)	120
IFyL	D3-9.3.3	(SEQ ID NO:553)	121
YFDW	D3-9.1.4	(SEQ ID NO:560)	122

ILTG	D3-9.2.4	(SEQ ID NO:561)	123
FyLV	D3-9.3.4	(SEQ ID NO:562)	124
FDWL	D3-9.1.5	(SEQ ID NO:569)	125
LTGY	D3-9.2.5	(SEQ ID NO:570)	126
yLVI	D3-9.3.5	(SEQ ID NO:571)	127
DWLL	D3-9.1.6	(SEQ ID NO:577)	128
TGYY	D3-9.2.6	(SEQ ID NO:578)	129
LVII	D3-9.3.6	(SEQ ID NO:579)	130
WLLy	D3-9.1.7	(SEQ ID NO:582)	131
GYYN	D3-9.2.7	(SEQ ID NO:583)	132
VLLW	D3-10.1.1	(SEQ ID NO:584)	133
YYYG	D3-10.2.1	(SEQ ID NO:585)	134
ITMV	D3-10.3.1	(SEQ ID NO:586)	135
LLWF	D3-10.1.2	(SEQ ID NO:593)	136
YYGS	D3-10.2.2	(SEQ ID NO:594)	137
TMVR	D3-10.3.2	(SEQ ID NO:595)	138
LWFG	D3-10.1.3	(SEQ ID NO:602)	139
YGSG	D3-10.2.3	(SEQ ID NO:603)	140
MVRG	D3-10.3.3	(SEQ ID NO:604)	141
WFGE	D3-10.1.4	(SEQ ID NO:611)	142
GSGS	D3-10.2.4	(SEQ ID NO:612)	143
VRGV	D3-10.3.4	(SEQ ID NO:613)	144
FGEL	D3-10.1.5	(SEQ ID NO:620)	145
RGVI	D3-10.3.5	(SEQ ID NO:621)	146
GELL	D3-10.1.6	(SEQ ID NO:626)	147
GVII	D3-10.3.6	(SEQ ID NO:627)	148
ELLy	D3-10.1.7	(SEQ ID NO:630)	149
SYYN	D3-10.2.7	(SEQ ID NO:631)	150
VLwL	D3-16.1.1	(SEQ ID NO:632)	151
YYDY	D3-16.2.1	(SEQ ID NO:633)	152
IMIT	D3-16.3.1	(SEQ ID NO:634)	153
LwLR	D3-16.1.2	(SEQ ID NO:641)	154
YDYV	D3-16.2.2	(SEQ ID NO:642)	155
MITF	D3-16.3.2	(SEQ ID NO:643)	156
wLRL	D3-16.1.3	(SEQ ID NO:650)	157
DYVW	D3-16.2.3	(SEQ ID NO:651)	158
ITFG	D3-16.3.3	(SEQ ID NO:652)	159
LRLG	D3-16.1.4	(SEQ ID NO:659)	160
YVWG	D3-16.2.4	(SEQ ID NO:660)	161
TFGG	D3-16.3.4	(SEQ ID NO:661)	162
RLGE	D3-16.1.5	(SEQ ID NO:668)	163
VWGS	D3-16.2.5	(SEQ ID NO:669)	164
FGGV	D3-16.3.5	(SEQ ID NO:670)	165
LGEL	D3-16.1.6	(SEQ ID NO:677)	166
WGSY	D3-16.2.6	(SEQ ID NO:678)	167
GGVI	D3-16.3.6	(SEQ ID NO:679)	168
GELS	D3-16.1.7	(SEQ ID NO:686)	169
GSYR	D3-16.2.7	(SEQ ID NO:687)	170
GVIV	D3-16.3.7	(SEQ ID NO:688)	171
ELSL	D3-16.1.8	(SEQ ID NO:694)	172
SYRY	D3-16.2.8	(SEQ ID NO:695)	173
VIVI	D3-16.3.8	(SEQ ID NO:696)	174
LSLY	D3-16.1.9	(SEQ ID NO:699)	175
YRYT	D3-16.2.9	(SEQ ID NO:700)	176
VLLw	D3-22.1.1	(SEQ ID NO:701)	177
YYYD	D3-22.2.1	(SEQ ID NO:702)	178
ITMI	D3-22.3.1	(SEQ ID NO:703)	179

LLwy	D3-22.1.2	(SEQ ID NO:710)	180
YYDS	D3-22.2.2	(SEQ ID NO:711)	181
TMIV	D3-22.3.2	(SEQ ID NO:712)	182
Lwyy	D3-22.1.3	(SEQ ID NO:719)	183
YDSS	D3-22.2.3	(SEQ ID NO:720)	184
MIVV	D3-22.3.3	(SEQ ID NO:721)	185
wyyW	D3-22.1.4	(SEQ ID NO:728)	186
DSSG	D3-22.2.4	(SEQ ID NO:729)	187
yyWL	D3-22.1.5	(SEQ ID NO:736)	188
SSGY	D3-22.2.5	(SEQ ID NO:737)	189
VVVI	D3-22.3.5	(SEQ ID NO:738)	190
yWLL	D3-22.1.6	(SEQ ID NO:744)	191
VVIT	D3-22.3.6	(SEQ ID NO:745)	192
WLLL	D3-22.1.7	(SEQ ID NO:748)	193
GYYY	D3-22.2.7	(SEQ ID NO:749)	194
wLQy	D4-4.1.1	(SEQ ID NO:750)	195
DYSN	D4-4.2.1	(SEQ ID NO:751)	196
TTVT	D4-4.3.1	(SEQ ID NO:752)	197
LQyL	D4-4.1.2	(SEQ ID NO:755)	198
YSNY	D4-4.2.2	(SEQ ID NO:756)	199
wLRw	D4-17.1.1	(SEQ ID NO:757)	200
DYGD	D4-17.2.1	(SEQ ID NO:758)	201
LRwL	D4-17.1.2	(SEQ ID NO:761)	202
YGDY	D4-17.2.2	(SEQ ID NO:762)	203
wLRW	D4-23.1.1	(SEQ ID NO:763)	204
DYGG	D4-23.2.1	(SEQ ID NO:764)	205
TTVV	D4-23.3.1	(SEQ ID NO:765)	206
LRWy	D4-23.1.2	(SEQ ID NO:771)	207
YGGN	D4-23.2.2	(SEQ ID NO:772)	208
TVVT	D4-23.3.2	(SEQ ID NO:773)	209
RWyL	D4-23.1.3	(SEQ ID NO:776)	210
GGNS	D4-23.2.3	(SEQ ID NO:777)	211
VDTA	D5-5.1.1	(SEQ ID NO:778)	212
WIQL	D5-5.2.1	(SEQ ID NO:779)	213
GYSY	D5-5.3.1	(SEQ ID NO:780)	214
DTAM	D5-5.1.2	(SEQ ID NO:787)	215
IQLW	D5-5.2.2	(SEQ ID NO:788)	216
YSYG	D5-5.3.2	(SEQ ID NO:789)	217
TAMV	D5-5.1.3	(SEQ ID NO:793)	218
QLWL	D5-5.2.3	(SEQ ID NO:794)	219
SYGY	D5-5.3.3	(SEQ ID NO:795)	220
VDIV	D5-12.1.1	(SEQ ID NO:796)	221
WIyW	D5-12.2.1	(SEQ ID NO:797)	222
GYSG	D5-12.3.1	(SEQ ID NO:798)	223
DIVA	D5-12.1.2	(SEQ ID NO:805)	224
IyWL	D5-12.2.2	(SEQ ID NO:806)	225
YSGY	D5-12.3.2	(SEQ ID NO:807)	226
IVAT	D5-12.1.3	(SEQ ID NO:814)	227
yWLR	D5-12.2.3	(SEQ ID NO:815)	228
SGYD	D5-12.3.3	(SEQ ID NO:816)	229
VATI	D5-12.1.4	(SEQ ID NO:820)	230
WLRL	D5-12.2.4	(SEQ ID NO:821)	231
GYDY	D5-12.3.4	(SEQ ID NO:822)	232
VEMA	D5-24.1.1	(SEQ ID NO:823)	233
yRWL	D5-24.2.1	(SEQ ID NO:824)	234
RDGY	D5-24.3.1	(SEQ ID NO:825)	235
EMAT	D5-24.1.2	(SEQ ID NO:832)	236

RWLQ	D5-24.2.2	(SEQ ID NO:833)	237
DGYN	D5-24.3.2	(SEQ ID NO:834)	238
MATI	D5-24.1.3	(SEQ ID NO:838)	239
WLQL	D5-24.2.3	(SEQ ID NO:839)	240
GYNY	D5-24.3.3	(SEQ ID NO:840)	241
EYSS	D6-6.1.1	(SEQ ID NO:841)	242
SIAA	D6-6.2.1	(SEQ ID NO:842)	243
VyQL	D6-6.3.1	(SEQ ID NO:843)	244
YSSS	D6-6.1.2	(SEQ ID NO:848)	245
IAAR	D6-6.2.2	(SEQ ID NO:849)	246
yQLV	D6-6.3.2	(SEQ ID NO:850)	247
SSSS	D6-6.1.3	(SEQ ID NO:852)	248
GYSS	D6-13.1.1	(SEQ ID NO:853)	249
GIAA	D6-13.2.1	(SEQ ID NO:854)	250
VyQQ	D6-13.3.1	(SEQ ID NO:855)	251
IAAA	D6-13.2.2	(SEQ ID NO:862)	252
yQQL	D6-13.3.2	(SEQ ID NO:863)	253
SSSW	D6-13.1.3	(SEQ ID NO:868)	254
AAAG	D6-13.2.3	(SEQ ID NO:869)	255
QQLV	D6-13.3.3	(SEQ ID NO:870)	256
SSWY	D6-13.1.4	(SEQ ID NO:872)	257
GIAV	D6-19.2.1	(SEQ ID NO:873)	258
VyQW	D6-19.3.1	(SEQ ID NO:874)	259
YSSG	D6-19.1.2	(SEQ ID NO:881)	260
IAVA	D6-19.2.2	(SEQ ID NO:882)	261
yQWL	D6-19.3.2	(SEQ ID NO:883)	262
SSGW	D6-19.1.3	(SEQ ID NO:888)	263
AVAG	D6-19.2.3	(SEQ ID NO:889)	264
QWLV	D6-19.3.3	(SEQ ID NO:890)	265
SGWY	D6-19.1.4	(SEQ ID NO:892)	266

Table 13: Pentamers that can be extracted from human D segments

GTTGT	D1-1.1.1	(SEQ ID NO:260)	1
VQLER	D1-1.2.1	(SEQ ID NO:261)	2
YNWND	D1-1.3.1	(SEQ ID NO:262)	3
GITGT	D1-7.1.1	(SEQ ID NO:268)	4
VyLEL	D1-7.2.1	(SEQ ID NO:269)	5
YNWNY	D1-7.3.1	(SEQ ID NO:270)	6
VyLER	D1-20.2.1	(SEQ ID NO:274)	7
GIVGA	D1-26.1.1	(SEQ ID NO:279)	8
VyWEL	D1-26.2.1	(SEQ ID NO:280)	9
YSGSY	D1-26.3.1	(SEQ ID NO:281)	10
IVGAT	D1-26.1.2	(SEQ ID NO:288)	11
yWELL	D1-26.2.2	(SEQ ID NO:289)	12
SGSYY	D1-26.3.2	(SEQ ID NO:290)	13
RILyy	D2-2.1.1	(SEQ ID NO:297)	14
GYCSS	D2-2.2.1	(SEQ ID NO:298)	15
DIVVV	D2-2.3.1	(SEQ ID NO:299)	16
ILyyY	D2-2.1.2	(SEQ ID NO:306)	17
YCSST	D2-2.2.2	(SEQ ID NO:307)	18
IVVVP	D2-2.3.2	(SEQ ID NO:308)	19
LyyYQ	D2-2.1.3	(SEQ ID NO:315)	20
CSSTS	D2-2.2.3	(SEQ ID NO:316)	21
VVVPA	D2-2.3.3	(SEQ ID NO:317)	22
yyYQL	D2-2.1.4	(SEQ ID NO:324)	23
SSTSC	D2-2.2.4	(SEQ ID NO:325)	24

VVPAA	D2-2.3.4	(SEQ ID NO:326)	25
yYQLL	D2-2.1.5	(SEQ ID NO:333)	26
STSCY	D2-2.2.5	(SEQ ID NO:334)	27
VPAAI	D2-2.3.5	(SEQ ID NO:335)	28
YQLLY	D2-2.1.6	(SEQ ID NO:341)	29
TSCYT	D2-2.2.6	(SEQ ID NO:342)	30
RILYy	D2-8.1.1	(SEQ ID NO:348)	31
GYCTN	D2-8.2.1	(SEQ ID NO:349)	32
DIVLM	D2-8.3.1	(SEQ ID NO:350)	33
ILYyW	D2-8.1.2	(SEQ ID NO:357)	34
YCTNG	D2-8.2.2	(SEQ ID NO:358)	35
IVLMV	D2-8.3.2	(SEQ ID NO:359)	36
LYyWC	D2-8.1.3	(SEQ ID NO:366)	37
CTNGV	D2-8.2.3	(SEQ ID NO:367)	38
VLMVY	D2-8.3.3	(SEQ ID NO:368)	39
YyWCM	D2-8.1.4	(SEQ ID NO:375)	40
TNGVC	D2-8.2.4	(SEQ ID NO:376)	41
LMVYA	D2-8.3.4	(SEQ ID NO:377)	42
yWCML	D2-8.1.5	(SEQ ID NO:384)	43
NGVCY	D2-8.2.5	(SEQ ID NO:385)	44
MVYAI	D2-8.3.5	(SEQ ID NO:386)	45
WCMLY	D2-8.1.6	(SEQ ID NO:392)	46
GVCYT	D2-8.2.6	(SEQ ID NO:393)	47
RILyW	D2-15.1.1	(SEQ ID NO:396)	48
GYCSG	D2-15.2.1	(SEQ ID NO:397)	49
ILyWW	D2-15.1.2	(SEQ ID NO:403)	50
YCSGG	D2-15.2.2	(SEQ ID NO:404)	51
IVVVV	D2-15.3.2	(SEQ ID NO:405)	52
LyWWy	D2-15.1.3	(SEQ ID NO:412)	53
CSGGS	D2-15.2.3	(SEQ ID NO:413)	54
VVVVA	D2-15.3.3	(SEQ ID NO:414)	55
yWWyL	D2-15.1.4	(SEQ ID NO:421)	56
SGGSC	D2-15.2.4	(SEQ ID NO:422)	57
VVVAA	D2-15.3.4	(SEQ ID NO:423)	58
WWyLL	D2-15.1.5	(SEQ ID NO:430)	59
GGSCY	D2-15.2.5	(SEQ ID NO:431)	60
VVAAT	D2-15.3.5	(SEQ ID NO:432)	61
WyLLL	D2-15.1.6	(SEQ ID NO:438)	62
GSCYS	D2-15.2.6	(SEQ ID NO:439)	63
SILWW	D2-21.1.1	(SEQ ID NO:445)	64
AYCGG	D2-21.2.1	(SEQ ID NO:446)	65
HIVVV	D2-21.3.1	(SEQ ID NO:447)	66
ILWWw	D2-21.1.2	(SEQ ID NO:453)	67
YCGGD	D2-21.2.2	(SEQ ID NO:454)	68
IVVVT	D2-21.3.2	(SEQ ID NO:455)	69
LWWwL	D2-21.1.3	(SEQ ID NO:462)	70
CGGDC	D2-21.2.3	(SEQ ID NO:463)	71
VVVTA	D2-21.3.3	(SEQ ID NO:464)	72
WWwLL	D2-21.1.4	(SEQ ID NO:471)	73
GGDCY	D2-21.2.4	(SEQ ID NO:472)	74
VVTAI	D2-21.3.4	(SEQ ID NO:473)	75
WwLLF	D2-21.1.5	(SEQ ID NO:479)	76
GDCYS	D2-21.2.5	(SEQ ID NO:480)	77
VLRFL	D3-3.1.1	(SEQ ID NO:486)	78
YYDFW	D3-3.2.1	(SEQ ID NO:487)	79
ITIFG	D3-3.3.1	(SEQ ID NO:488)	80
LRFLE	D3-3.1.2	(SEQ ID NO:495)	81

YDFWS	D3-3.2.2	(SEQ ID NO:496)	82
TIFGV	D3-3.3.2	(SEQ ID NO:497)	83
RFLEW	D3-3.1.3	(SEQ ID NO:504)	84
DFWSG	D3-3.2.3	(SEQ ID NO:505)	85
IFGVV	D3-3.3.3	(SEQ ID NO:506)	86
FLEWL	D3-3.1.4	(SEQ ID NO:513)	87
FWSGY	D3-3.2.4	(SEQ ID NO:514)	88
FGVVI	D3-3.3.4	(SEQ ID NO:515)	89
LEWLL	D3-3.1.5	(SEQ ID NO:522)	90
WSGYY	D3-3.2.5	(SEQ ID NO:523)	91
GVVII	D3-3.3.5	(SEQ ID NO:524)	92
EWLLY	D3-3.1.6	(SEQ ID NO:530)	93
SGYYT	D3-3.2.6	(SEQ ID NO:531)	94
VLRYF	D3-9.1.1	(SEQ ID NO:536)	95
YYDIL	D3-9.2.1	(SEQ ID NO:537)	96
ITIFy	D3-9.3.1	(SEQ ID NO:538)	97
LRYFD	D3-9.1.2	(SEQ ID NO:545)	98
YDILT	D3-9.2.2	(SEQ ID NO:546)	99
TIFyL	D3-9.3.2	(SEQ ID NO:547)	100
RYFDW	D3-9.1.3	(SEQ ID NO:554)	101
DILTG	D3-9.2.3	(SEQ ID NO:555)	102
IFyLV	D3-9.3.3	(SEQ ID NO:556)	103
YFDWL	D3-9.1.4	(SEQ ID NO:563)	104
ILTGY	D3-9.2.4	(SEQ ID NO:564)	105
FyLVI	D3-9.3.4	(SEQ ID NO:565)	106
FDWLL	D3-9.1.5	(SEQ ID NO:572)	107
LTGYY	D3-9.2.5	(SEQ ID NO:573)	108
yLVII	D3-9.3.5	(SEQ ID NO:574)	109
DWLLy	D3-9.1.6	(SEQ ID NO:580)	110
TGYYN	D3-9.2.6	(SEQ ID NO:581)	111
VLLWF	D3-10.1.1	(SEQ ID NO:587)	112
YYYGS	D3-10.2.1	(SEQ ID NO:588)	113
ITMVR	D3-10.3.1	(SEQ ID NO:589)	114
LLWFG	D3-10.1.2	(SEQ ID NO:596)	115
YYGSG	D3-10.2.2	(SEQ ID NO:597)	116
TMVRG	D3-10.3.2	(SEQ ID NO:598)	117
LWFGE	D3-10.1.3	(SEQ ID NO:605)	118
YGSGS	D3-10.2.3	(SEQ ID NO:606)	119
MVRGV	D3-10.3.3	(SEQ ID NO:607)	120
WFGEL	D3-10.1.4	(SEQ ID NO:614)	121
GSGSY	D3-10.2.4	(SEQ ID NO:615)	122
VRGVI	D3-10.3.4	(SEQ ID NO:616)	123
FGELL	D3-10.1.5	(SEQ ID NO:622)	124
RGVII	D3-10.3.5	(SEQ ID NO:623)	125
GELLy	D3-10.1.6	(SEQ ID NO:628)	126
GSYYN	D3-10.2.6	(SEQ ID NO:629)	127
VLwLR	D3-16.1.1	(SEQ ID NO:635)	128
YYDYV	D3-16.2.1	(SEQ ID NO:636)	129
IMITF	D3-16.3.1	(SEQ ID NO:637)	130
LwLRL	D3-16.1.2	(SEQ ID NO:644)	131
YDYVW	D3-16.2.2	(SEQ ID NO:645)	132
MITFG	D3-16.3.2	(SEQ ID NO:646)	133
wLRLG	D3-16.1.3	(SEQ ID NO:653)	134
DYVWG	D3-16.2.3	(SEQ ID NO:654)	135
ITFGG	D3-16.3.3	(SEQ ID NO:655)	136
LRLGE	D3-16.1.4	(SEQ ID NO:662)	137
YVWGS	D3-16.2.4	(SEQ ID NO:663)	138

TFGGV	D3-16.3.4	(SEQ ID NO:664)	139
RLGEL	D3-16.1.5	(SEQ ID NO:671)	140
VWGSY	D3-16.2.5	(SEQ ID NO:672)	141
FGGVI	D3-16.3.5	(SEQ ID NO:673)	142
LGELS	D3-16.1.6	(SEQ ID NO:680)	143
WGSYR	D3-16.2.6	(SEQ ID NO:681)	144
GGVIV	D3-16.3.6	(SEQ ID NO:682)	145
GELSL	D3-16.1.7	(SEQ ID NO:689)	146
GSYRY	D3-16.2.7	(SEQ ID NO:690)	147
GVIVI	D3-16.3.7	(SEQ ID NO:691)	148
ELSLY	D3-16.1.8	(SEQ ID NO:697)	149
SYRYT	D3-16.2.8	(SEQ ID NO:698)	150
VLLwy	D3-22.1.1	(SEQ ID NO:704)	151
YYYDS	D3-22.2.1	(SEQ ID NO:705)	152
ITMIV	D3-22.3.1	(SEQ ID NO:706)	153
LLwyy	D3-22.1.2	(SEQ ID NO:713)	154
YYDSS	D3-22.2.2	(SEQ ID NO:714)	155
TMIVV	D3-22.3.2	(SEQ ID NO:715)	156
LwyyW	D3-22.1.3	(SEQ ID NO:722)	157
YDSSG	D3-22.2.3	(SEQ ID NO:723)	158
MIVVV	D3-22.3.3	(SEQ ID NO:724)	159
wyyWL	D3-22.1.4	(SEQ ID NO:730)	160
DSSGY	D3-22.2.4	(SEQ ID NO:731)	161
IVVVI	D3-22.3.4	(SEQ ID NO:732)	162
yyWLL	D3-22.1.5	(SEQ ID NO:739)	163
SSGYY	D3-22.2.5	(SEQ ID NO:740)	164
VVVIT	D3-22.3.5	(SEQ ID NO:741)	165
yWLLL	D3-22.1.6	(SEQ ID NO:746)	166
SGYYY	D3-22.2.6	(SEQ ID NO:747)	167
wLQyL	D4-4.1.1	(SEQ ID NO:753)	168
DYSNY	D4-4.2.1	(SEQ ID NO:754)	169
wLRwL	D4-17.1.1	(SEQ ID NO:759)	170
DYGDY	D4-17.2.1	(SEQ ID NO:760)	171
wLRWy	D4-23.1.1	(SEQ ID NO:766)	172
DYGGN	D4-23.2.1	(SEQ ID NO:767)	173
TTVVT	D4-23.3.1	(SEQ ID NO:768)	174
LRWyL	D4-23.1.2	(SEQ ID NO:774)	175
YGGNS	D4-23.2.2	(SEQ ID NO:775)	176
VDTAM	D5-5.1.1	(SEQ ID NO:781)	177
WIQLW	D5-5.2.1	(SEQ ID NO:782)	178
GYSYG	D5-5.3.1	(SEQ ID NO:783)	179
DTAMV	D5-5.1.2	(SEQ ID NO:790)	180
IQLWL	D5-5.2.2	(SEQ ID NO:791)	181
YSYGY	D5-5.3.2	(SEQ ID NO:792)	182
VDIVA	D5-12.1.1	(SEQ ID NO:799)	183
WIyWL	D5-12.2.1	(SEQ ID NO:800)	184
GYSGY	D5-12.3.1	(SEQ ID NO:801)	185
DIVAT	D5-12.1.2	(SEQ ID NO:808)	186
IyWLR	D5-12.2.2	(SEQ ID NO:809)	187
YSGYD	D5-12.3.2	(SEQ ID NO:810)	188
IVATI	D5-12.1.3	(SEQ ID NO:817)	189
yWLRL	D5-12.2.3	(SEQ ID NO:818)	190
SGYDY	D5-12.3.3	(SEQ ID NO:819)	191
VEMAT	D5-24.1.1	(SEQ ID NO:826)	192
yRWLQ	D5-24.2.1	(SEQ ID NO:827)	193
RDGYN	D5-24.3.1	(SEQ ID NO:828)	194
EMATI	D5-24.1.2	(SEQ ID NO:835)	195

RWLQL	D5-24.2.2	(SEQ ID NO:836)	196
DGYNY	D5-24.3.2	(SEQ ID NO:837)	197
EYSSS	D6-6.1.1	(SEQ ID NO:844)	198
SIAAR	D6-6.2.1	(SEQ ID NO:845)	199
VyQLV	D6-6.3.1	(SEQ ID NO:846)	200
YSSSS	D6-6.1.2	(SEQ ID NO:851)	201
GYSSS	D6-13.1.1	(SEQ ID NO:856)	202
GIAAA	D6-13.2.1	(SEQ ID NO:857)	203
VyQQL	D6-13.3.1	(SEQ ID NO:858)	204
YSSSW	D6-13.1.2	(SEQ ID NO:864)	205
IAAAG	D6-13.2.2	(SEQ ID NO:865)	206
yQQLV	D6-13.3.2	(SEQ ID NO:866)	207
SSSWY	D6-13.1.3	(SEQ ID NO:871)	208
GYSSG	D6-19.1.1	(SEQ ID NO:875)	209
GIAVA	D6-19.2.1	(SEQ ID NO:876)	210
VyQWL	D6-19.3.1	(SEQ ID NO:877)	211
YSSGW	D6-19.1.2	(SEQ ID NO:884)	212
IAVAG	D6-19.2.2	(SEQ ID NO:885)	213
yQWLV	D6-19.3.2	(SEQ ID NO:886)	214
SSGWY	D6-19.1.3	(SEQ ID NO:891)	215

Table 14: All hexamers that can be extracted from human D segments

GIVGAT	D1-26.1.1	(SEQ ID NO:282)	1
VyWELL	D1-26.2.1	(SEQ ID NO:283)	2
YSGSYY	D1-26.3.1	(SEQ ID NO:284)	3
RILyyY	D2-2.1.1	(SEQ ID NO:300)	4
GYCSST	D2-2.2.1	(SEQ ID NO:301)	5
DIVVVP	D2-2.3.1	(SEQ ID NO:302)	6
ILyyYQ	D2-2.1.2	(SEQ ID NO:309)	7
YCSSTS	D2-2.2.2	(SEQ ID NO:310)	8
IVVVPA	D2-2.3.2	(SEQ ID NO:311)	9
LyyYQL	D2-2.1.3	(SEQ ID NO:318)	10
CSSTSC	D2-2.2.3	(SEQ ID NO:319)	11
VVVPAA	D2-2.3.3	(SEQ ID NO:320)	12
yyYQLL	D2-2.1.4	(SEQ ID NO:327)	13
SSTSCY	D2-2.2.4	(SEQ ID NO:328)	14
VVPAAI	D2-2.3.4	(SEQ ID NO:329)	15
yYQLLY	D2-2.1.5	(SEQ ID NO:336)	16
STSCYT	D2-2.2.5	(SEQ ID NO:337)	17
RILYyW	D2-8.1.1	(SEQ ID NO:351)	18
GYCTNG	D2-8.2.1	(SEQ ID NO:352)	19
DIVLMV	D2-8.3.1	(SEQ ID NO:353)	20
ILYyWC	D2-8.1.2	(SEQ ID NO:360)	21
YCTNGV	D2-8.2.2	(SEQ ID NO:361)	22
IVLMVY	D2-8.3.2	(SEQ ID NO:362)	23
LYyWCM	D2-8.1.3	(SEQ ID NO:369)	24
CTNGVC	D2-8.2.3	(SEQ ID NO:370)	25
VLMVYA	D2-8.3.3	(SEQ ID NO:371)	26
YyWCML	D2-8.1.4	(SEQ ID NO:378)	27
TNGVCY	D2-8.2.4	(SEQ ID NO:379)	28
LMVYAI	D2-8.3.4	(SEQ ID NO:380)	29
yWCMLY	D2-8.1.5	(SEQ ID NO:387)	30
NGVCYT	D2-8.2.5	(SEQ ID NO:388)	31
RILyWW	D2-15.1.1	(SEQ ID NO:398)	32
GYCSGG	D2-15.2.1	(SEQ ID NO:399)	33
DIVVVV	D2-15.3.1	(SEQ ID NO:400)	34

ILyWWy	D2-15.1.2	(SEQ ID NO:406)	35
YCSGGS	D2-15.2.2	(SEQ ID NO:407)	36
IVVVVA	D2-15.3.2	(SEQ ID NO:408)	37
LyWWyL	D2-15.1.3	(SEQ ID NO:415)	38
CSGGSC	D2-15.2.3	(SEQ ID NO:416)	39
VVVVAA	D2-15.3.3	(SEQ ID NO:417)	40
yWWyLL	D2-15.1.4	(SEQ ID NO:424)	41
SGGSCY	D2-15.2.4	(SEQ ID NO:425)	42
VVVAAT	D2-15.3.4	(SEQ ID NO:426)	43
WWyLLL	D2-15.1.5	(SEQ ID NO:433)	44
GGSCYS	D2-15.2.5	(SEQ ID NO:434)	45
SILWWw	D2-21.1.1	(SEQ ID NO:448)	46
AYCGGD	D2-21.2.1	(SEQ ID NO:449)	47
HIVVVT	D2-21.3.1	(SEQ ID NO:450)	48
ILWWwL	D2-21.1.2	(SEQ ID NO:456)	49
YCGGDC	D2-21.2.2	(SEQ ID NO:457)	50
IVVVTA	D2-21.3.2	(SEQ ID NO:458)	51
LWWwLL	D2-21.1.3	(SEQ ID NO:465)	52
CGGDCY	D2-21.2.3	(SEQ ID NO:466)	53
VVVTAI	D2-21.3.3	(SEQ ID NO:467)	54
WWwLLF	D2-21.1.4	(SEQ ID NO:474)	55
GGDCYS	D2-21.2.4	(SEQ ID NO:475)	56
VLRFLE	D3-3.1.1	(SEQ ID NO:489)	57
YYDFWS	D3-3.2.1	(SEQ ID NO:490)	58
ITIFGV	D3-3.3.1	(SEQ ID NO:491)	59
LRFLEW	D3-3.1.2	(SEQ ID NO:498)	60
YDFWSG	D3-3.2.2	(SEQ ID NO:499)	61
TIFGVV	D3-3.3.2	(SEQ ID NO:500)	62
RFLEWL	D3-3.1.3	(SEQ ID NO:507)	63
DFWSGY	D3-3.2.3	(SEQ ID NO:508)	64
IFGVVI	D3-3.3.3	(SEQ ID NO:509)	65
FLEWLL	D3-3.1.4	(SEQ ID NO:516)	66
FWSGYY	D3-3.2.4	(SEQ ID NO:517)	67
FGVVII	D3-3.3.4	(SEQ ID NO:518)	68
LEWLLY	D3-3.1.5	(SEQ ID NO:525)	69
WSGYYT	D3-3.2.5	(SEQ ID NO:526)	70
VLRYFD	D3-9.1.1	(SEQ ID NO:539)	71
YYDILT	D3-9.2.1	(SEQ ID NO:540)	72
ITIFyL	D3-9.3.1	(SEQ ID NO:541)	73
LRYFDW	D3-9.1.2	(SEQ ID NO:548)	74
YDILTG	D3-9.2.2	(SEQ ID NO:549)	75
TIFyLV	D3-9.3.2	(SEQ ID NO:550)	76
RYFDWL	D3-9.1.3	(SEQ ID NO:557)	77
DILTGY	D3-9.2.3	(SEQ ID NO:558)	78
IFyLVI	D3-9.3.3	(SEQ ID NO:559)	79
YFDWLL	D3-9.1.4	(SEQ ID NO:566)	80
ILTGYY	D3-9.2.4	(SEQ ID NO:567)	81
FyLVII	D3-9.3.4	(SEQ ID NO:568)	82
FDWLLy	D3-9.1.5	(SEQ ID NO:575)	83
LTGYYN	D3-9.2.5	(SEQ ID NO:576)	84
VLLWFG	D3-10.1.1	(SEQ ID NO:590)	85
YYYGSG	D3-10.2.1	(SEQ ID NO:591)	86
ITMVRG	D3-10.3.1	(SEQ ID NO:592)	87
LLWFGE	D3-10.1.2	(SEQ ID NO:599)	88
YYGSGS	D3-10.2.2	(SEQ ID NO:600)	89
TMVRGV	D3-10.3.2	(SEQ ID NO:601)	90
LWFGEL	D3-10.1.3	(SEQ ID NO:608)	91

YGSGSY	D3-10.2.3	(SEQ ID NO:609)	92
MVRGVI	D3-10.3.3	(SEQ ID NO:610)	93
WFGELL	D3-10.1.4	(SEQ ID NO:617)	94
GSGSYY	D3-10.2.4	(SEQ ID NO:618)	95
VRGVII	D3-10.3.4	(SEQ ID NO:619)	96
FGELLy	D3-10.1.5	(SEQ ID NO:624)	97
SGSYYN	D3-10.2.5	(SEQ ID NO:625)	98
VLwLRL	D3-16.1.1	(SEQ ID NO:638)	99
YYDYVW	D3-16.2.1	(SEQ ID NO:639)	100
IMITFG	D3-16.3.1	(SEQ ID NO:640)	101
LwLRLG	D3-16.1.2	(SEQ ID NO:647)	102
YDYVWG	D3-16.2.2	(SEQ ID NO:648)	103
MITFGG	D3-16.3.2	(SEQ ID NO:649)	104
wLRLGE	D3-16.1.3	(SEQ ID NO:656)	105
DYVWGS	D3-16.2.3	(SEQ ID NO:657)	106
ITFGGV	D3-16.3.3	(SEQ ID NO:658)	107
LRLGEL	D3-16.1.4	(SEQ ID NO:665)	108
YVWGSY	D3-16.2.4	(SEQ ID NO:666)	109
TFGGVI	D3-16.3.4	(SEQ ID NO:667)	110
RLGELS	D3-16.1.5	(SEQ ID NO:674)	111
VWGSYR	D3-16.2.5	(SEQ ID NO:675)	112
FGGVIV	D3-16.3.5	(SEQ ID NO:676)	113
LGELSL	D3-16.1.6	(SEQ ID NO:683)	114
WGSYRY	D3-16.2.6	(SEQ ID NO:684)	115
GGVIVI	D3-16.3.6	(SEQ ID NO:685)	116
GELSLY	D3-16.1.7	(SEQ ID NO:692)	117
GSYRYT	D3-16.2.7	(SEQ ID NO:693)	118
VLLwyy	D3-22.1.1	(SEQ ID NO:707)	119
YYYDSS	D3-22.2.1	(SEQ ID NO:708)	120
ITMIVV	D3-22.3.1	(SEQ ID NO:709)	121
LLwyyW	D3-22.1.2	(SEQ ID NO:716)	122
YYDSSG	D3-22.2.2	(SEQ ID NO:717)	123
TMIVVV	D3-22.3.2	(SEQ ID NO:718)	124
LwyyWL	D3-22.1.3	(SEQ ID NO:725)	125
YDSSGY	D3-22.2.3	(SEQ ID NO:726)	126
MIVVVI	D3-22.3.3	(SEQ ID NO:727)	127
wyyWLL	D3-22.1.4	(SEQ ID NO:733)	128
DSSGYY	D3-22.2.4	(SEQ ID NO:734)	129
IVVVIT	D3-22.3.4	(SEQ ID NO:735)	130
yyWLLL	D3-22.1.5	(SEQ ID NO:742)	131
SSGYYY	D3-22.2.5	(SEQ ID NO:743)	132
wLRWyL	D4-23.1.1	(SEQ ID NO:769)	133
DYGGNS	D4-23.2.1	(SEQ ID NO:770)	134
VDTAMV	D5-5.1.1	(SEQ ID NO:784)	135
WIQLWL	D5-5.2.1	(SEQ ID NO:785)	136
GYSYGY	D5-5.3.1	(SEQ ID NO:786)	137
VDIVAT	D5-12.1.1	(SEQ ID NO:802)	138
WIyWLR	D5-12.2.1	(SEQ ID NO:803)	139
GYSGYD	D5-12.3.1	(SEQ ID NO:804)	140
DIVATI	D5-12.1.2	(SEQ ID NO:811)	141
IyWLRL	D5-12.2.2	(SEQ ID NO:812)	142
YSGYDY	D5-12.3.2	(SEQ ID NO:813)	143
VEMATI	D5-24.1.1	(SEQ ID NO:829)	144
yRWLQL	D5-24.2.1	(SEQ ID NO:830)	145
RDGYNY	D5-24.3.1	(SEQ ID NO:831)	146
EYSSSS	D6-6.1.1	(SEQ ID NO:847)	147
GYSSSW	D6-13.1.1	(SEQ ID NO:859)	148

GIAAAG	D6-13.2.1	(SEQ ID NO:860)	149
VyQQLV	D6-13.3.1	(SEQ ID NO:861)	150
YSSSWY	D6-13.1.2	(SEQ ID NO:867)	151
GYSSGW	D6-19.1.1	(SEQ ID NO:878)	152
GIAVAG	D6-19.2.1	(SEQ ID NO:879)	153
VyQWLV	D6-19.3.1	(SEQ ID NO:880)	154
YSSGWY	D6-19.1.2	(SEQ ID NO:887)	155

Example 3: CDR3 of length 6-20.

[00184] Insertion of D segments into synthetic HC CDR3s can lead to greater stability and lower immunogenicity. Libraries are designed at the amino-acid level by joining a VH to an optional filler of some length which is joined to a D segment an optional second filler and a JH. For libraries of length six or eight, a full-length JH may follow VH and a short filler. Table 77 shows the frequency of D segments in a sampling of 1419 Abs selected from FAB-310 or FAB-410 for binding to one target or another. In the sample, 1099 Abs had no detectable D segment (i.e., less that 70% match). Where D segments are used, the D segments D1-1.3, D1-26.3, D2-2.2, D2-8.2, D2-15.2, D2-21.2, D3-16.2, **D3-22.2**, **D3-3.2**, D3-9.1, D3-9.2, D3-10.2, D3-16.2, D4-4.2, D4-4.3, D4-11.2, D4-4.2, D4-17.2, D4-23.2, **D5-5.3**, **D5-12.3**, D5-18.3, D6-6.1, D6-6.2, **D6-13.1**, D6-13.2, **D6-19.1**, D6-19.2, and D7-27.1 are preferred.

[00185] Once the parental amino-acid sequence has been designed, it can be diversified in several ways: error-prone PCR, wobbling, and dobbling. Table 14 shows a number of hexamers that can be derived from human D regions. In one embodiment, the hexamers that contain cysteine residues are exclused. In one embodiment, the fragments of D regions that contain stops are excluded. In one embodiment, any TAG codon found in the D region is replaced by a codon picked from the set comprising TCG, TTG, TGG, CAG, AAG, TAT, and GAG. In one embodiment, any TAA codon found in the D region is replaced by a codon picked form the set comprising TCA, TTA, CAA, AAA, TAT, and GAA. In one embodiment, any TGA of the D region is replaced by a codon picked from the set comprising TGG, TCA, TTA, AGA, and GGA.

[00186] Table 21 shows exemplary parental amino-acid sequences for CDR3s from 6 to 20 amino acids. These parental sequences can be combined with diversity in HC CDR1 and CDR2 to form a library. The utility is likely to improve if the CDR3 regions are diversified by, for example, wobbling, dobbling, or error-prone PCR of the CDR3s. In Table 21, sequence 6a comprises the end of VH from 3-23 fused to whole JH1. Sequence 6b contains the end of 3-23 joined to a Y joined to D4-17 (RF 2) joined to the FR4 region of JH1. Sequence 6c contains the

end of 3-23 followed by D5-5 (RF 3) followed by the FR4 part of JH1. Sequence 6d contains the end of 3-23 joined to SY joined to the whole JH4. Table 21 shows the level of doping that would be appropriate for the wobbling of the CDR3; other levels could be used as well. Other D regions or fragments of D regions could be used. Other JH sequences could be used.

Table 21: Parental amino-acid sequences for HC CDR3s of 6-20 AAs.

Length	Parental sequence	level of doping	Comment	SEQ ID NO:
6a	yycakAEYFQHwgqgtlvtvss	70:10:10:10	JH1(whole)	226
6b	yycakYDYGDYwgqgtlvtvss	70:10:10:10	Y::D4-17(2)::FR4 of JH1	227
6c	yycakGYSYGYwgqgtlvtvss	70:10:10:10	D5-5(3)::FR4 of JH1	228
6d	yycakSYYFDYwgqgtlvtvss	70:10:10:10	SY::JH4(whole)	229
8a	yycakYYAEYFQHwgqgtlvtvss	73:9:9:9	YY:JH1(whole)	230
8b	yycakYGYSSSWYwgqgtlvtvss	73:9:9:9	Y::D6-13(1)::FR4 of JH1	231
8c	yycakYGDYYFDYwgqgtlvtvss	73:9:9:9	D4-17(2)[2-5]::JH4(whole)	232
10a	yycakYYYDSSGYYYwgqgtlvtvss	73:9:9:9	D3-22(2)::Fr4 of JH1	233
10b	yycakGYcSSTScYTwgqgtlvtvss	73:9:9:9	D2-2(2)::Fr4 of JH1	234
10c	yycakYYSSAEYFQHwgqgtlvtvss	73:9:9:9	YYSS::JH1(whole)	235
10d	yycakGYSYGYYFDYwgqgtlvtvss	73:9:9:9	D5-5(3)::JH4(whole)	236
12a	yycakYYYDSSGYYYQHwgqgtlvtvss	85:5:5:5	D3-22(2)::QH::Fr4 of JH1	237
12b	yycakGYcSSTScYTQHwgqgtlvtvss	85:5:5:5	D2-2(2)::QH::Fr4 of JH1	238
12c	yycakYDGSYSAEYFQHwgqgtlvtvss	85:5:5:5	YDGSYS::JH1(whole)	239
12d	yycakYYDYVWGSYRYTwgqgtlvtvss	85:5:5:5	D3-16(2)::Fr of JH1	240
12e	yycakGYSYGYYWYFDLwgrgtlvtvss	85:5:5:5	D5-5(3)::JH2(whole)	241
14a	yycakYYYDSSGYYYYFQHwgqgtlvtvss	73:9:9:9	D3-22(2)::YFQH::Fr of JH1	242
14b	yycakGYcSSTScYTYFQHwgqgtlvtvss	73:9:9:9	D2-2(2)::YFQH::Fr of JH1	243
14c	yycakSYGYcSSTScYTQHwgqgtlvtvss	73:9:9:9	SY::D2-2(2)::QH::Fr of JH1	244
14d	yycakSYRYSGYSAEYFQHwgqgtlvtvss	73:9:9:9	SYRYSGYS::JH1(whole)	245
14e	yycakAYcGGDcYSNWFDPwgqgtlvtvss	73:9:9:9	D2-21(2)::JH5(whole)	246
15a	yycakSDGYYYDSSGYYYDYwgqgtlvtvss	73:9:9:9	SD::D3-22.2::JH4(101ff)	930
15b	yycakGSGYCSGGSCYSFDYwgqgtlvtvss	73:9:9:9	GS::D2-15.2::JH4(100ff)	931
15c	yycakGGRGYSSGWYRAFDIwgqgtmvtvss	73:9:9:9	GGR::D6-19.1::R::JH3(all)	932

16a	yycakYYYDSSGYYYAEYFQHwgqgtlvtvss	73:9:9:9	D3-22(2)::JH1(whole)	247
16b	yycakGYcSSTScYTAEYFQHwgqgtlvtvss	73:9:9:9	D2-2(2)::JH1(whole)	248
16c	yycakSYDSYRSYGSAEYFQHwgqgtlvtvss	73:9:9:9	SYDSYRSYGS::JH1(whole)	249
16d	yycakSYSYGYcSSTScYTQHwgqgtlvtvss	73:9:9:9	SYSY::D2-2(2)::QH::Fr JH1	250
17a	yycakSRPGYSSSWYYYYGMDVwgqgttvtvs s	73:9:9:9	SRP::6-13.1::JH6(-1Y)	933
18a	yycakGYCSGGSCYSYYYYGMDVwgqgttvt vss	73:9:9:9	2-15.2::JH6(-1Y)	221
18b	yycakDGYCSGGSCYSYYYGMDVwgqgttvt vss	73:9:9:9	D::2-15.2::JH6(-2Ys)	222
19a	yycakDGYYYDSSGYYYRGYYFDYwgqgtlv tvss	73:9:9:9	D::D3-22.2::RGY::JH4(all)	223
20a	yycakYSSYYYYDSSGYYYAEYFQHwgqgtl vtvss	73:9:9:9	YSSY::D3-22(2)::JH1(whole)	251
20b	yycakSYYSGYcSSTScYTAEYFQHwgqgtlvt vss	73:9:9:9	SYYS::D2-2(2)::JH1(whole)	252
20c	yycakSGYcSSTScYTYYSAEYFQHwgqgtlvt vss	73:9:9:9	S::D2-2(2)::YYS::JH1(whole)	253
20d	yycakYYYYDYVWGSYRYTSNWFDPwgqg tlvtvss	73:9:9:9	Y::D3-16(2)::S::JH5(whole)	254
20e	yycakYYYYDYVWGSYRYTSSYFDYwgqgtl vtvss	73:9:9:9	Y::D3-16(2)::SS::JH4(whole)	255

Table 22: HC display cassette

The amino-acid sequence shown in Table 22 is SEQ ID NO:892.
The DNA sequence shown in Table 22 is SEQ ID NO:893.

```
!         Signal for VH-CH1-IIIstump
!          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
   946   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
   991 gcG GCC cag ccG GCC atg gcc
!        SfiI.............
!                NgoMI...(1/2)
!                       NcoI....
!
! VH

!                                    FR1(DP47/V3-23)---------------
!                                     1   2   3   4   5   6   7   8
!                                     E   V   Q   L   L   E   S   G
  1012                               gaa|gtt|CAA|TTG|tta|gag|tct|ggt|
!                                           | MfeI  |
!
!        --------------FR1-----------------------------------------
!          9  10  11  12  13  14  15  16  17  18  19  20  21  22  23
!          G   G   L   V   Q   P   G   G   S   L   R   L   S   C   A
  1036  |ggc|ggt|ctt|gtt|cag|cct|ggt|ggt|tct|tta|cgt|ctt|tct|tgc|gct|
!
!        ----FR1-------------------->|...CDR1.............|---FR2------
!         24  25  26  27  28  29  30  31  32  33  34  35  36  37  38
!          A   S   G   F   T   F   S   S   Y   A   M   S   W   V   R
  1081  |gct|TCC|GGA|ttc|act|ttc|tct|tCG|TAC|Gct|atg|tct|tgg|gtt|cgC|
!             | BspEI |                 | BsiWI|                    |BstXI.
!
!         -------FR2---------------------------------->|...CDR2.........
!         39  40  41  42  43  44  45  46  47  48  49  50  51  52  52a
!          Q   A   P   G   K   G   L   E   W   V   S   A   I   S   G
  1126  |CAa|gct|ccT|GGt|aaa|ggt|ttg|gag|tgg|gtt|tct|gct|atc|tct|ggt|
!    ...BstXI          |
!
!       .....CDR2.....................................|---FR3---
!         53  54  55  56  57  58  59  60  61  62  63  64  65  66  67
!          S   G   G   S   T   Y   Y   A   D   S   V   K   G   R   F
  1171  |tct|ggt|ggc|agt|act|tac|tat|gct|gac|tcc|gtt|aaa|ggt|cgc|ttc|
!
!
!        --------FR3-------------------------------------------------
!         68  69  70  71  72  73  74  75  76  77  78  79  80  81  82
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
  1216  |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
!               | XbaI  |
!
!        ---FR3-------------------------------------------------------->|
!        82a 82b 82c  83  84  85  86  87  88  89  90  91  92  93  94
```

```
          N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K
  1261  |aac|agC|TTA|AGg|gct|gag|gac|aCT|GCA|Gtc|tac|tat|tgc|gct|aaa|
                |AflII |               | PstI |(2/2)

        .......CDR3.................................|----FR4--------
         95  96  97  98 98a 98b 98c  99  100 101 102 103 104 105 106
          D   Y   E   G   T   G   Y   A   F   D   I   W   G   Q   G
  1306  |gac|tat|gaa|ggt|act|ggt|tat|gct|ttc|gaC|ATA|TGg|ggt|caa|ggt|
                                                | NdeI |

        --------------FR4---------->|
         107 108 109 110 111 112 113
          T   M   V   T   V   S   S
  1351  |act|atG|GTC|ACC|gtc|tct|agt
                | BstEII |  c tcg ag = XhoI.

CH1

           A   S   T   K   G   P   S   V   F   P   L   A   P   S   S
  1372     gcc tcc acc aag ggc cca tcg gtc ttc ccG CTA GCa ccc tcc tcc
                                                 NheI....

           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
  1417     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
  1462     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
  1507     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
  1552     gga ctc tac tcc ctc agc agc gta gtg acc gtg ccc tCC Agc agc
                                                          BstXI........

           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
  1597     tTG Ggc acc cag acc tac atc tgc aac gtg aat cac aag ccc agc
  BstXI........

           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
  1642     aac acc aag gtg gac aaG AAA GTT GAG CCC AAA TCT TGT

       139 140 141  His tag.............   cMyc tag.....................
        A   A   A   H   H   H   H   H   H   G   A   A   E   Q   K   L   I
  1681 GCG GCC GCa cat cat cat cac cat cac ggg gcc gca gaa caa aaa ctc atc
       NotI......
        EagI....

       .................................
       S   E   E   D   L   N   G   A   A   E   A   S   S   A   S   N   A   S
  1732 tca gaa gag gat ctg aat ggg GCC gca gaG GCt agt tct gct agt aAC GCG Tct
```

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!                                   BglI.....(3/4)              MluI....
!
! Domain 3 (IIIstump)-----------------------------------------------------
!       S   G   D   F   D   Y   E   K   M   A   N   A   N   K   G   A
  1786 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
  1834 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
  1882 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
  1930 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
  1978 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
  2026 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
  2074 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
  2122 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
  2158 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
  2206 tct acg ttt gct aac ata ctg
!
!       R   N   K   E   S   (SEQ ID NO:892)
  2227 cgt aat aag gag tct TAA    tga aAC GCG Tga tga GAATTC (SEQ ID NO:893)
!     Intracellular anchor.           MluI....       EcoRI.
```

Table 25: The DNA sequence of DY3F85LC containing a sample germline O12 kappa light chain. The antibody sequences shown are of the form of actual antibody, but have not been identified as binding to a particular antigen.

On each line, everything after an exclamation point (!) is commentary.

The DNA of DY3F85LC is SEQ ID NO:27

```
!-------------------------------------------------------------------------
     1     AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC AAATGAAAAT
    61     ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT
   121     CGTTCGCAGA ATTGGGAATC AACTGTTATA TGGAATGAAA CTTCCAGACA CCGTACTTTA
   181     GTTGCATATT TAAAACATGT TGAGCTACAG CATTATATTC AGCAATTAAG CTCTAAGCCA
   241     TCCGCAAAAA TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
   301     TTGGAGTTTG CTTCCGGTCT GGTTCGCTTT GAAGCTCGAA TTAAAACGCG ATATTTGAAG
   361     TCTTTCGGGC TTCCTCTTAA TCTTTTTGAT GCAATCCGCT TTGCTTCTGA CTATAATAGT
   421     CAGGGTAAAG ACCTGATTTT TGATTTATGG TCATTCTCGT TTTCTGAACT GTTTAAAGCA
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 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 TATTCTTTTG 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  TTTTGGAGAT TTTCAACGTG AAAAAATTAT TATTCGCAAT TCCTTTAGTT GTTCCTTTCT
1621  ATTCTCACTC CGCTGAAACT GTTGAAAGTT GTTTAGCAAA ATCCCATACA GAAAATTCAT
1681  TTACTAACGT CTGGAAAGAC GACAAAACTT TAGATCGTTA CGCTAACTAT GAGGGCTGTC
1741  TGTGGAATGC TACAGGCGTT GTAGTTTGTA CTGGTGACGA AACTCAGTGT TACGGTACAT
1801  GGGTTCCTAT TGGGCTTGCT ATCCCTGAAA ATGAGGGTGG TGGCTCTGAG GGTGGCGGTT
1861  CTGAGGGTGG CGGTTCTGAG GGTGGCGGTA CTAAACCTCC TGAGTACGGT GATACACCTA
1921  TTCCGGGCTA TACTTATATC AACCCTCTCG ACGGCACTTA TCCGCCTGGT ACTGAGCAAA
1981  ACCCCGCTAA TCCTAATCCT TCTCTTGAGG AGTCTCAGCC TCTTAATACT TTCATGTTTC
2041  AGAATAATAG GTTCCGAAAT AGGCAGGGGG CATTAACTGT TTATACGGGC ACTGTTACTC
2101  AAGGCACTGA CCCCGTTAAA ACTTATTACC AGTACACTCC TGTATCATCA AAAGCCATGT
2161  ATGACGCTTA CTGGAACGGT AAATTCAGAG ACTGCGCTTT CCATTCTGGC TTTAATGAGG
2221  ATTTATTTGT TTGTGAATAT CAAGGCCAAT CGTCTGACCT GCCTCAACCT CCTGTCAATG
2281  CTGGCGGCGG CTCTGGTGGT GGTTCTGGTG GCGGCTCTGA GGGTGGTGGC TCTGAGGGTG
2341  GCGGTTCTGA GGGTGGCGGC TCTGAGGGAG GCGGTTCCGG TGGTGGCTCT GGTTCCGGTG
2401  ATTTTGATTA TGAAAAGATG GCAAACGCTA ATAAGGGGGC TATGACCGAA AATGCCGATG
2461  AAAACGCGCT ACAGTCTGAC GCTAAAGGCA AACTTGATTC TGTCGCTACT GATTACGGTG
2521  CTGCTATCGA TGGTTTCATT GGTGACGTTT CCGGCCTTGC TAATGGTAAT GGTGCTACTG
2581  GTGATTTTGC TGGCTCTAAT TCCCAAATGG CTCAAGTCGG TGACGGTGAT AATTCACCTT
2641  TAATGAATAA TTTCCGTCAA TATTTACCTT CCCTCCCTCA ATCGGTTGAA TGTCGCCCTT
2701  TTGTCTTTGG CGCTGGTAAA CCATATGAAT TTTCTATTGA TTGTGACAAA ATAAACTTAT
2761  TCCGTGGTGT CTTTGCGTTT CTTTTATATG TTGCCACCTT TATGTATGTA TTTTCTACGT
2821  TTGCTAACAT ACTGCGTAAT AAGGAGTCTT AATCATGCCA GTTCTTTTGG GTATTCCGTT
2881  ATTATTGCGT TTCCTCGGTT TCCTTCTGGT AACTTTGTTC GGCTATCTGC TTACTTTTCT
2941  TAAAAAGGGC TTCGGTAAGA TAGCTATTGC TATTTCATTG TTTCTTGCTC TTATTATTGG
3001  GCTTAACTCA ATTCTTGTGG GTTATCTCTC TGATATTAGC GCTCAATTAC CCTCTGACTT
3061  TGTTCAGGGT GTTCAGTTAA TTCTCCCGTC TAATGCGCTT CCCTGTTTTT ATGTTATTCT
3121  CTCTGTAAAG GCTGCTATTT TCATTTTTGA CGTTAAACAA AAAATCGTTT CTTATTTGGA
3181  TTGGGATAAA TAATATGGCT GTTTATTTTG TAACTGGCAA ATTAGGCTCT GGAAAGACGC
3241  TCGTTAGCGT TGGTAAGATT CAGGATAAAA TTGTAGCTGG GTGCAAAATA GCAACTAATC
3301  TTGATTTAAG GCTTCAAAAC CTCCCGCAAG TCGGGAGGTT CGCTAAAACG CCTCGCGTTC
3361  TTAGAATACC GGATAAGCCT TCTATATCTG ATTTGCTTGC TATTGGGCGC GGTAATGATT
3421  CCTACGATGA AAATAAAAAC GGCTTGCTTG TTCTCGATGA GTGCGGTACT TGGTTTAATA
3481  CCCGTTCTTG GAATGATAAG GAAAGACAGC CGATTATTGA TTGGTTTCTA CATGCTCGTA
3541  AATTAGGATG GGATATTATT TTTCTTGTTC AGGACTTATC TATTGTTGAT AAACAGGCGC
3601  GTTCTGCATT AGCTGAACAT GTTGTTTATT GTCGTCGTCT GGACAGAATT ACTTTACCTT
3661  TTGTCGGTAC TTTATATTCT CTTATTACTG GCTCGAAAAT GCCTCTGCCT AAATTACATG
3721  TTGGCGTTGT TAAATATGGC GATTCTCAAT TAAGCCCTAC TGTTGAGCGT TGGCTTTATA
3781  CTGGTAAGAA TTTGTATAAC GCATATGATA CTAAACAGGC TTTTTCTAGT AATTATGATT
3841  CCGGTGTTTA TTCTTATTTA ACGCCTTATT TATCACACGG TCGGTATTTC AAACCATTAA
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3901  ATTTAGGTCA GAAGATGAAA TTAACTAAAA TATATTTGAA AAAGTTTTCT CGCGTTCTTT
3961  GTCTTGCGAT TGGATTTGCA TCAGCATTTA CATATAGTTA TATAACCCAA CCTAAGCCGG
4021  AGGTTAAAAA GGTAGTCTCT CAGACCTATG ATTTTGATAA ATTCACTATT GACTCTTCTC
4081  AGCGTCTTAA TCTAAGCTAT CGCTATGTTT TCAAGGATTC TAAGGGAAAA TTAATTAATA
4141  GCGACGATTT ACAGAAGCAA GGTTATTCAC TCACATATAT TGATTTATGT ACTGTTTCCA
4201  TTAAAAAAGG TAATTCAAAT GAAATTGTTA AATGTAATTA ATTTTGTTTT CTTGATGTTT
4261  GTTTCATCAT CTTCTTTTGC TCAGGTAATT GAAATGAATA ATTCGCCTCT GCGCGATTTT
4321  GTAACTTGGT ATTCAAAGCA ATCAGGCGAA TCCGTTATTG TTTCTCCCGA TGTAAAAGGT
4381  ACTGTTACTG TATATTCATC TGACGTTAAA CCTGAAAATC TACGCAATTT CTTTATTTCT
4441  GTTTTACGTG CAAATAATTT TGATATGGTA GGTTCTAACC CTTCCATAAT TCAGAAGTAT
4501  AATCCAAACA ATCAGGATTA TATTGATGAA TTGCCATCAT CTGATAATCA GGAATATGAT
4561  GATAATTCCG CTCCTTCTGG TGGTTTCTTT GTTCCGCAAA ATGATAATGT TACTCAAACT
4621  TTTAAAATTA ATAACGTTCG GGCAAAGGAT TTAATACGAG TTGTCGAATT GTTTGTAAAG
4681  TCTAATACTT CTAAATCCTC AAATGTATTA TCTATTGACG GCTCTAATCT ATTAGTTGTT
4741  AGTGCTCCTA AAGATATTTT AGATAACCTT CCTCAATTCC TTTCAACTGT TGATTTGCCA
4801  ACTGACCAGA TATTGATTGA GGGTTTGATA TTTGAGGTTC AGCAAGGTGA TGCTTTAGAT
4861  TTTTCATTTG CTGCTGGCTC TCAGCGTGGC ACTGTTGCAG GCGGTGTTAA TACTGACCGC
4921  CTCACCTCTG TTTTATCTTC TGCTGGTGGT TCGTTCGGTA TTTTTAATGG CGATGTTTTA
4981  GGGCTATCAG TTCGCGCATT AAAGACTAAT AGCCATTCAA AAATATTGTC TGTGCCACGT
5041  ATTCTTACGC TTTCAGGTCA GAAGGGTTCT ATCTCTGTTG GCCAGAATGT CCCTTTTATT
5101  ACTGGTCGTG TGACTGGTGA ATCTGCCAAT GTAAATAATC CATTTCAGAC GATTGAGCGT
5161  CAAAATGTAG GTATTTCCAT GAGCGTTTTT CCTGTTGCAA TGGCTGGCGG TAATATTGTT
5221  CTGGATATTA CCAGCAAGGC CGATAGTTTG AGTTCTTCTA CTCAGGCAAG TGATGTTATT
5281  ACTAATCAAA GAAGTATTGC TACAACGGTT AATTTGCGTG ATGGACAGAC TCTTTTACTC
5341  GGTGGCCTCA CTGATTATAA AAACACTTCT CAGGATTCTG GCGTACCGTT CCTGTCTAAA
5401  ATCCCTTTAA TCGGCCTCCT GTTTAGCTCC CGCTCTGATT CTAACGAGGA AAGCACGTTA
5461  TACGTGCTCG TCAAAGCAAC CATAGTACGC GCCCTGTAGC GGCGCATTAA GCGCGGCGGG
5521  TGTGGTGGTT ACGCGCAGCG TGACCGCTAC ACTTGCCAGC GCCCTAGCGC CCGCTCCTTT
5581  CGCTTTCTTC CCTTCCTTTC TCGCCACGTT CGCCGGCTTT CCCCGTCAAG CTCTAAATCG
5641  GGGGCTCCCT TTAGGGTTCC GATTTAGTGC TTTACGGCAC CTCGACCCCA AAAAACTTGA
5701  TTTGGGTGAT GGTTCACGTA GTGGGCCATC GCCCTGATAG ACGGTTTTTC GCCCTTTGAC
5761  GTTGGAGTCC ACGTTCTTTA ATAGTGGACT CTTGTTCCAA ACTGGAACAA CACTCAACCC
5821  TATCTCGGGC TATTCTTTTG ATTTATAAGG GATTTTGCCG ATTTCGGAAC CACCATCAAA
5881  CAGGATTTTC GCCTGCTGGG GCAAACCAGC GTGGACCGCT TGCTGCAACT CTCTCAGGGC
5941  CAGGCGGTGA AGGGCAATCA GCTGTTGCCC GTCTCACTGG TGAAAAGAAA AACCACCCTG
6001  GATCCAAGCT TGCAGGTGGC ACTTTTCGGG GAAATGTGCG CGGAACCCCT ATTTGTTTAT
6061  TTTTCTAAAT ACATTCAAAT ATGTATCCGC TCATGAGACA ATAACCCTGA TAAATGCTTC
6121  AATAATATTG AAAAAGGAAG AGTATGAGTA TTCAACATTT CCGTGTCGCC CTTATTCCCT
6181  TTTTTGCGGC ATTTTGCCTT CCTGTTTTTG CTCACCCAGA AACGCTGGTG AAAGTAAAAG
6241  ATGCTGAAGA TCAGTTGGGC GCACTAGTGG GTTACATCGA ACTGGATCTC AACAGCGGTA
6301  AGATCCTTGA GAGTTTTCGC CCCGAAGAAC GTTTTCCAAT GATGAGCACT TTTAAAGTTC
6361  TGCTATGTGG CGCGGTATTA TCCCGTATTG ACGCCGGGCA AGAGCAACTC GGTCGCCGCA
6421  TACACTATTC TCAGAATGAC TTGGTTGAGT ACTCACCAGT CACAGAAAAG CATCTTACGG
6481  ATGGCATGAC AGTAAGAGAA TTATGCAGTG CTGCCATAAC CATGAGTGAT AACACTGCGG
6541  CCAACTTACT TCTGACAACG ATCGGAGGAC CGAAGGAGCT AACCGCTTTT TTGCACAACA
6601  TGGGGGATCA TGTAACTCGC CTTGATCGTT GGGAACCGGA GCTGAATGAA GCCATACCAA
6661  ACGACGAGCG TGACACCACG ATGCCTGTAG CAATGGCAAC AACGTTGCGC AAACTATTAA
6721  CTGGCGAACT ACTTACTCTA GCTTCCCGGC AACAATTAAT AGACTGGATG GAGGCGGATA
6781  AAGTTGCAGG ACCACTTCTG CGCTCGGCCC TTCCGGCTGG CTGGTTTATT GCTGATAAAT
6841  CTGGAGCCGG TGAGCGTGGG TCTCGCGGTA TCATTGCAGC ACTGGGGCCA GATGGTAAGC
6901  CCTCCCGTAT CGTAGTTATC TACACGACGG GGAGTCAGGC AACTATGGAT GAACGAAATA
6961  GACAGATCGC TGAGATAGGT GCCTCACTGA TTAAGCATTG GTAACTGTCA GACCAAGTTT
7021  ACTCATATAT ACTTTAGATT GATTTAAAAC TTCATTTTTA ATTTAAAAGG ATCTAGGTGA
7081  AGATCCTTTT TGATAATCTC ATGACCAAAA TCCCTTAACG TGAGTTTTCG TTCCACTGTA
7141  CGTAAGACCC CCAAGCTTGT CGACTGAATG GCGAATGGCG CTTTGCCTGG TTTCCGGCAC
7201  CAGAAGCGGT GCCGGAAAGC TGGCTGGAGT GCGATCTTCC TGACGCTCGA GCGCAACGCA
!                                                      XhoI...
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7261    ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT
7321    CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT
7381    GATTACGCCA AGCTTTGGAG CCTTTTTTTT GGAGATTTTC AAC
```

Table 30: DNA sequence of DY3FHC87 (SEQ ID NO:894)

```
   1 aatgctacta ctattagtag aattgatgcc acctttcag ctcgcgcccc aaatgaaaat
  61 atagctaaac aggttattga ccatttgcga aatgtatcta atggtcaaac taaatctact
 121 cgttcgcaga attgggaatc aactgttata tggaatgaaa cttccagaca ccgtacttta
 181 gttgcatatt taaaacatgt tgagctacag cattatattc agcaattaag ctctaagcca
 241 tccgcaaaaa tgacctctta tcaaaaggag caattaaagg tactctctaa tcctgacctg
 301 ttggagtttg cttccggtct ggttcgcttt gaagctcgaa ttaaaacgcg atatttgaag
 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 tattcttttg cctctttcgt tttaggttgg tgccttcgta
1261 gtggcattac gtatttacc cgtttaatgg aaacttcctc atgaaaaagt ctttagtcct
1321 caaagcctct gtagccgttg ctacctctcgt 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 tattctcact ccgctgaaac tgttgaaagt tgtttagcaa aatcccatac agaaaattca
1681 tttactaacg tctggaaaga cgacaaaact ttagatcgtt acgctaacta tgagggctgt
1741 ctgtggaatg ctacaggcgt tgtagtttgt actggtgacg aaactcagtg ttacggtaca
1801 tgggttccta ttgggcttgc tatccctgaa aatgagggtg gtggctctga gggtggcggt
1861 tctgagggtg gcggttctga gggtggcggt actaaacctc ctgagtacgg tgatacacct
1921 attccgggct atacttatat caacctctc gacggcactt atccgcctgg tactgagcaa
1981 aaccccgcta atcctaatcc ttctcttgag gagtctcagc ctcttaatac tttcatgttt
2041 cagaataata ggttccgaaa taggcagggg gcattaactg tttatacggg cactgttact
2101 caaggcactg accccgttaa aacttattac cagtacactc ctgtatcatc aaaagccatg
2161 tatgacgctt actggaacgg taaattcaga gactgcgctt tccattctgg ctttaatgag
2221 gatttatttg tttgtgaata tcaaggccaa tcgtctgacc tgcctcaacc tcctgtcaat
2281 gctggcggcg gctctggtgg tggttctggt ggcggctctg agggtggtgg ctctgagggt
2341 ggcggttctg agggtggcgg ctctgaggga ggcggttccg gtggtggctc tggttccggt
2401 gattttgatt atgaaaagat ggcaaacgct aaagaggggg ctatgaccga aaatgccgat
2461 gaaaacgcgc tacagtctga cgctaaaggc aaacttgatt ctgtcgctac tgattacggt
2521 gctgctatcg atggtttcat tggtgacgtt tccggccttg ctaatggtaa tggtgctact
2581 ggtgattttg ctggctctaa ttcccaaatg gctcaagtcg gtgacggtga taattcacct
2641 ttaatgaata atttccgtca tatttacct tccctccctc aatcggttga atgtcgccct
2701 tttgtctttg gcgctggtaa accatatgaa ttttctattg attgtgacaa aataaactta
2761 ttccgtggtg tctttgcgtt tcttttatat gttgccacct ttatgtatgt attttctacg
2821 tttgctaaca tactgcgtaa taaggagtct taatcatgcc agttcttttg ggtattccgt
2881 tattattgcg tttcctcggt ttccttctgg taactttgtt cggctatctg cttacttttc
2941 ttaaaaaggg cttcggtaag atagctattg ctatttcatt gtttcttgct cttattattg
```

3001 ggcttaactc aattcttgtg ggttatctct ctgatattag cgctcaatta ccctctgact
3061 ttgttcaggg tgttcagtta attctcccgt ctaatgcgct tccctgtttt tatgttattc
3121 tctctgtaaa ggctgctatt ttcatttttg acgttaaaca aaaaatcgtt tcttatttgg
3181 attgggataa ataatatggc tgtttatttt gtaactggca aattaggctc tggaaagacg
3241 ctcgttagcg ttggtaagat tcaggataaa attgtagctg ggtgcaaaat agcaactaat
3301 cttgatttaa ggcttcaaaa cctcccgcaa gtcgggaggt tcgctaaaac gcctcgcgtt
3361 cttagaatac cggataagcc ttctatatct gatttgcttg ctattgggcg cggtaatgat
3421 tcctacgatg aaaataaaaa cggcttgctt gttctcgatg agtgcggtac ttggtttaat
3481 acccgttctt ggaatgataa ggaaagacag ccgattattg attggtttct acatgctcgt
3541 aaattaggat gggatattat ttttcttgtt caggacttat ctattgttga taaacaggcg
3601 cgttctgcat tagctgaaca tgttgtttat tgtcgtcgtc tggacagaat tactttacct
3661 tttgtcggta ctttatattc tcttattact ggctcgaaaa tgcctctgcc taaattacat
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3961 tgtcttgcga ttggatttgc atcagcattt acatatagtt atataaccca acctaagccg
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5761 cgttggagtc cacgttcttt aatagtggac tcttgttcca aactggaaca acactcaacc
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6301 aagatccttg agagttttcg ccccgaagaa cgttttccaa tgatgagcac ttttaaagtt
6361 ctgctatgtg gcgcggtatt atcccgtatt gacgccgggc aagagcaact cggtcgccgc

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6541 gccaacttac ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac
6601 atgggggatc atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca
6661 aacgacgagc gtgacaccac gatgcctgta gcaatggcaa caacgttgcg caaactatta
6721 actggcgaac tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat
6781 aaagttgcag gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa
6841 tctggagccg gtgagcgtgg gtctcgcggt atcattgcag cactggggcc agatggtaag
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6961 agacagatcg ctgagatagg tgcctcactg attaagcatt ggtaactgtc agaccaagtt
7021 tactcatata tactttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg
7081 aagatccttt ttgataatct catgaccaaa atcccttaac gtgagttttc gttccactgt
7141 acgtaagacc cccaagcttg tcgactgaat ggcgaatggc gctttgcctg gtttccggca
7201 ccagaagcgg tgccggaaag ctggctggag tgcgatcttc ctgacgctcg agcgcaacgc
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7321 tcgtatgttg tgtggaattg tgagcggata acaatttcac acaggaaaca gctatgacca
7381 tgattacgcc aagctttgga gccttttttt tggagatttt caacatgaaa tacctattgc
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7981 tgaccagcgg cgtccacacc ttcccggctg tcctacagtc ctcaggactc tactccctca
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8761 tgaggccgat actgtcgtcg tcccctcaaa ctggcagatg cacggttacg atgcgcccat
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8881 gacgggttgt tactcgctca catttaatgt tgatgaaagc tggctacagg aaggccagac
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9481 ctgtatgatt tattggatgt t
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Table 35: DNA sequence of pMID21: 5957 bp (SEQ ID NO:895)

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 541 gcatgacagt aagagaatta tgcagtgctg ccataaccat gagtgataac actgcggcca
 601 acttacttct gacaacgatc ggaggaccga aggagctaac cgcttttttg cacaacatgg
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 961 cccgtatcgt agttatctac acgacgggga gtcaggcaac tatggatgaa cgaaatagac
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5881 cgcttacaga caagctgtga ccgtctccgg gagctgcatg tgtcagaggt tttcaccgtc
5941 atcaccgaaa cgcgcga
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Table 36 pM21J containing IIIss::A27::Ckappa

Number of bases 5225 (SEQ ID NO:921)

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GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTTAA TGTCATGATA ATAATGGTTT    60
CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT   120
TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT   180
AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT   240
TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG   300
CTGAAGATCA GTTGGGTGCC CGAGTGGGTT ACATCGAACT GGATCTCAAC AGCGGTAAGA   360
TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC   420
TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC   480
ACTATTCTCA GAATGACTTG GTTGAGTACT CACCAGTCAC AGAAAAGCAT CTTACGGATG   540
GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA   600
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ACTTACTTCT GACAACGATC GGAGGACCGA AGGAGCTAAC CGCTTTTTTG CACAACATGG   660
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ACGAGCGTGA CACCACGATG CCTGTAGCAA TGGCAACAAC GTTGCGCAAA CTATTAACTG   780
GCGAACTACT TACTCTAGCT TCCCGGCAAC AATTAATAGA CTGGATGGAG GCGGATAAAG   840
TTGCAGGACC ACTTCTGCGC TCGGCCCTTC CGGCTGGCTG GTTTATTGCT GATAAATCTG   900
GAGCCGGTGA GCGTGGGTCT CGCGGTATCA TTGCAGCACT GGGGCCAGAT GGTAAGCCCT   960
CCCGTATCGT AGTTATCTAC ACGACGGGGA GTCAGGCAAC TATGGATGAA CGAAATAGAC  1020
AGATCGCTGA GATAGGTGCC TCACTGATTA AGCATTGGTA ACTGTCAGAC CAAGTTTACT  1080
CATATATACT TTAGATTGAT TTAAAACTTC ATTTTTAATT TAAAAGGATC TAGGTGAAGA  1140
TCCTTTTTGA TAATCTCATG ACCAAAATCC CTTAACGTGA GTTTTCGTTC CACTGAGCGT  1200
CAGACCCCGT AGAAAAGATC AAAGGATCTT CTTGAGATCC TTTTTTTCTG CGCGTAATCT  1260
GCTGCTTGCA AACAAAAAAA CCACCGCTAC CAGCGGTGGT TTGTTTGCCG GATCAAGAGC  1320
TACCAACTCT TTTTCCGAAG GTAACTGGCT TCAGCAGAGC GCAGATACCA AATACTGTTC  1380
TTCTAGTGTA GCCGTAGTTA GGCCACCACT TCAAGAACTC TGTAGCACCG CCTACATACC  1440
TCGCTCTGCT AATCCTGTTA CCAGTGGCTG CTGCCAGTGG CGATAAGTCG TGTCTTACCG  1500
GGTTGGACTC AAGACGATAG TTACCGGATA AGGCGCAGCG GTCGGGCTGA ACGGGGGGTT  1560
CGTGCATACA GCCCAGCTTG GAGCGAACGA CCTACACCGA ACTGAGATAC CTACAGCGTG  1620
AGCTATGAGA AAGCGCCACG CTTCCCGAAG GGAGAAAGGC GGACAGGTAT CCGGTAAGCG  1680
GCAGGGTCGG AACAGGAGAG CGCACGAGGG AGCTTCCAGG GGGAAACGCC TGGTATCTTT  1740
ATAGTCCTGT CGGGTTTCGC CACCTCTGAC TTGAGCGTCG ATTTTTGTGA TGCTCGTCAG  1800
GGGGGCGGAG CCTATGGAAA AACGCCAGCA ACGCGGCCTT TTTACGGTTC CTGGCCTTTT  1860
GCTGGCCTTT TGCTCACATG TTCTTTCCTG CGTTATCCCC TGATTCTGTG GATAACCGTA  1920
TTACCGCCTT TGAGTGAGCT GATACCGCTC GCCGCAGCCG AACGACCGAG CGCAGCGAGT  1980
CAGTGAGCGA GGAAGCGGAA GAGCGCCCAA TACGCAAACC GCCTCTCCCC GCGCGTTGGC  2040
CGATTCATTA ATGCAGCTGG CACGACAGGT TTCCCGACTG GAAAGCGGGC AGTGAGCGCA  2100
ACGCAATTAA TGTGAGTTAG CTCACTCATT AGGCACCCCA GGCTTTACAC TTTATGCTTC  2160
CGGCTCGTAT GTTGTGTGGA ATTGTGAGCG GATAACAATT TCACACAGGA AACAGCTATG  2220
ACCATGATTA CGCCAAGCTT TGGAGCCTTT TTTTTGGAGA TTTTCAACAT GAAGAAACTG  2280
CTGTCTGCTA TCCCACTAGT TGTCCCTTTC TATTCTCATA GTGAAATCGT TCTGACCCAG  2340
TCCCCGGGGA CCCTGTCTCT GTCTCCGGGT GAACGTGCTA CGCTGAGCTG TCGTGCTTCT  2400
CAATCCGTTA GCTCCTCTTA TTTAGCTTGG TATCAGCAAA AGCCGGGTCA AGCTCCGCGG  2460
CTGTTGATCT ATGGTGCCTC TAGTCGTGCT ACTGGCATCC CTGATCGTTT CTCTGGCTCT  2520
GGCTCCGGAA CCGATTTCAC TCTGACCATT TCTCGTCTCG AGCCGGAAGA TTTCGCTGTC  2580
TACTATTGTC AACAGTATGG TTCTAGTCCG CTGACTTTCG GTGGCGGTAC CAAAGTCGAA  2640
ATCAAGCGTG GAACTGTGGC TGCACCATCT GTCTTCATCT TCCCGCCATC TGATGAGCAG  2700
TTGAAATCTG GAACTGCCTC TGTTGTGTGC CTGCTGAATA ACTTCTATCC CAGAGAGGCC  2760
AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA ACTCCCAGGA GAGTGTCACA  2820
GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA CCCTGACTCT GTCCAAAGCA  2880
GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC ATCAGGGCCT GAGTTCACCG  2940
GTGACAAAGA GCTTCAACAG GGGAGAGTGT TAATAAGGCG CGCCAATTTA ACCATCTATT  3000
TCAAGGAACA GTCTTAATGA AGAAGCTCCT CTTTGCTATC CCGCTCGTCG TTCCTTTTGT  3060
GGCCCAGCCG GCCATGGCCG AAGTTCAATT GTTAGAGTCT GGTGGCGGTC TTGTTCAGCC  3120
TGGTGGTTCT TTACGTCTTT CTTGCGCTGC TTCCGGATTC ACTTTCTCTC GTTACAAGAT  3180
GAAGTGGGTT CGCCAAGCTC CTGGTAAAGG TTTGGAGTGG GTTTCTGTTA TCTATCCTTC  3240
TGGTGGCGGT ACTGGTTATG CTGACTCCGT TAAAGGTCGC TTCACTATCT CTAGAGACAA  3300
CTCTAAGAAT ACTCTCTACT TGCAGATGAA CAGCTTAAGG GCTGAGGACA CTGCAGTCTA  3360
CTATTGTGCG AGAGTCAATT ACTATGATAG TAGTGGTTAC GGTCCTATAG CTCCTGGACT  3420
TGACTACTGG GGCCAGGGAA CCCTGGTCAC CGTCTCAAGC GCCTCCACCA AGGGTCCGTC  3480
GGTCTTCCCG CTAGCACCCT CCTCCAAGAG CACCTCTGGG GGCACAGCGG CCCTGGGCTG  3540
CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG TGGAACTCAG GCGCCCTGAC  3600
CAGCGGCGTC CACACCTTCC CGGCTGTCCT ACAGTCTAGC GGACTCTACT CCCTCAGCAG  3660
CGTAGTGACC GTGCCCTCTT CTAGCTTGGG CACCCAGACC TACATCTGCA ACGTGAATCA  3720
CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC AAATCTTGTG CGGCCGCACA  3780
TCATCATCAC CATCACGGGG CCGCAGAACA AAAACTCATC TCAGAAGAGG ATCTGAATGG  3840
GGCCGCAGAG GCTAGTTCTG CTAGTAACGC GTCTTCCGGT GATTTTGATT ATGAAAAGAT  3900
GGCAAACGCT AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCGC TACAGTCTGA  3960
CGCTAAAGGC AAACTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT  4020
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TGGTGACGTT TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTTG CTGGCTCTAA  4080
TTCCCAAATG GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA  4140
ATATTTACCT TCCCTCCCTC AATCGGTTGA ATGTCGCCCT TTTGTCTTTG GCGCTGGTAA  4200
ACCATATGAA TTTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG TCTTTGCGTT  4260
TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG TTTGCTAACA TACTGCGTAA  4320
TAAGGAGTCT TAATGAAACG CGTGATGAGA ATTCACTGGC CGTCGTTTTA CAACGTCGTG  4380
ACTGGGAAAA CCCTGGCGTT ACCCAACTTA ATCGCCTTGC AGCACATCCC CCTTTCGCCA  4440
GCTGGCGTAA TAGCGAAGAG GCCCGCACCG ATCGCCCTTC CCAACAGTTG CGCAGCCTGA  4500
ATGGCGAATG GCGCCTGATG CGGTATTTTC TCCTTACGCA TCTGTGCGGT ATTTCACACC  4560
GCATACGTCA AAGCAACCAT AGTACGCGCC CTGTAGCGGC GCATTAAGCG CGGCGGGTGT  4620
GGTGGTTACG CGCAGCGTGA CCGCTACACT TGCCAGCGCC TTAGCGCCCG CTCCTTTCGC  4680
TTTCTTCCCT TCCTTTCTCG CCACGTTCGC CGGCTTTCCC CGTCAAGCTC TAAATCGGGG  4740
GCTCCCTTTA GGGTTCCGAT TTAGTGCTTT ACGGCACCTC GACCCCAAAA AACTTGATTT  4800
GGGTGATGGT TCACGTAGTG GGCCATCGCC CTGATAGACG GTTTTTCGCC CTTTGACGTT  4860
GGAGTCCACG TTCTTTAATA GTGGACTCTT GTTCCAAACT GGAACAACAC TCAACTCTAT  4920
CTCGGGCTAT TCTTTTGATT TATAAGGGAT TTTGCCGATT TCGGTCTATT GGTTAAAAAA  4980
TGAGCTGATT TAACAAAAAT TTAACGCGAA TTTTAACAAA ATATTAACGT TTACAATTTT  5040
ATGGTGCAGT CTCAGTACAA TCTGCTCTGA TGCCGCATAG TTAAGCCAGC CCCGACACCC  5100
GCCAACACCC GCTGACGCGC CCTGACGGGC TTGTCTGCTC CCGGCATCCG CTTACAGACA  5160
AGCTGTGACC GTCTCCGGGA GCTGCATGTG TCAGAGGTTT TCACCGTCAT CACCGAAACG  5220
CGCGA                                                              5225
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Table 40: pLCSK23 (SEQ ID NO:896)

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   1 GACGAAAGGG CCTGCTCTGC CAGTGTTACA ACCAATTAAC CAATTCTGAT TAGAAAAACT
  61 CATCGAGCAT CAAATGAAAC TGCAATTTAT TCATATCAGG ATTATCAATA CCATATTTTT
 121 GAAAAAGCCG TTTCTGTAAT GAAGGAGAAA ACTCACCGAG GCAGTTCCAT AGGATGGCAA
 181 GATCCTGGTA TCGGTCTGCG ATTCCGACTC GTCCAACATC AATACAACCT ATTAATTTCC
 241 CCTCGTCAAA AATAAGGTTA TCAAGTGAGA AATCACCATG AGTGACGACT GAATCCGGTG
 301 AGAATGGCAA AAGCTTATGC ATTTCTTTCC AGACTTGTTC AACAGGCCAG CCATTACGCT
 361 CGTCATCAAA ATCACTCGCA TCAACCAAAC CGTTATTCAT TCGTGATTGC GCCTGAGCGA
 421 GACGAAATAC GCGATCGCTG TTAAAAGGAC AATTACAAAC AGGAATTGAA TGCAACCGGC
 481 GCAGGAACAC TGCCAGCGCA TCAACAATAT TTTCACCTGA ATCAGGATAT TCTTCTAATA
 541 CCTGGAATGC TGTTTTCCCG GGGATCGCAG TGGTGAGTAA CCATGCATCA TCAGGAGTAC
 601 GGATAAAATG CTTGATGGTC GGAAGAGGCA TAAATTCCGT CAGCCAGTTT AGTCTGACCA
 661 TCTCATCTGT AACATCATTG GCAACGCTAC CTTTGCCATG TTTCAGAAAC AACTCTGGCG
 721 CATCGGGCTT CCCATACAAT CGATAGATTG TCGCACCTGA TTGCCCGACA TTATCGCGAG
 781 CCCATTTATA CCCATATAAA TCAGCATCCA TGTTGGAATT TAATCGCGGC CTCGAGCAAG
 841 ACGTTTCCCG TTGAATATGG CTCATAACAC CCCTTGTATT ACTGTTTATG TAAGCAGACA
 901 GTTTTATTGT TCATGATGAT ATATTTTTAT CTTGTGCAAT GTAACATCAG AGATTTTGAG
 961 ACACAACGTG GCTTTCCCCC CCCCCCCCTG CAGGTCTCGG GCTATTCCTG TCAGACCAAG
1021 TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA AGGATCTAGG
1081 TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA ACGTGAGTTT TCGTTCCACT
1141 GAGCGTCAGA CCCCGTAGAA AAGATCAAAG GATCTTCTTG AGATCCTTTT TTTCTGCGCG
1201 TAATCTGCTG CTTGCAAACA AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC
1261 AAGAGCTACC AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA
1321 CTGTTCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA GCACCGCCTA
1381 CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC CAGTGGCGAT AAGTCGTGTC
1441 TTACCGGGTT GGACTCAAGA CGATAGTTAC CGGATAAGGC GCAGCGGTCG GGCTGAACGG
1501 GGGGTTCGTG CATACAGCCC AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC
1561 AGCGTGAGCT ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG
1621 TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA AACGCCTGGT
1681 ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA GCGTCGATTT TTGTGATGCT
1741 CGTCAGGGGG GCGGAGCCTA TGGAAAAACG CCAGCAACGC GGCCTTTTTA CGGTTCCTGG
1801 CCTTTTGCTG GCCTTTTGCT CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA
1861 ACCGTATTAC CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA
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1921 GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC
1981 GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC CGACTGGAAA GCGGGCAGTG
2041 AGCGCAACGC AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA
2101 TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA
2161 GCTATGACCA TGATTACGCC AAGCTTTGGA GCCTTTTTTT TGGAGATTTT CAACATGAAG
2221 AAGCTCCTCT TTGCTATCCC GCTCGTCGTT CCTTTTGTGG CCCAGCCGGC CATGGCCGAC
2281 ATCCAGATGA CCCAGTCTCC ATCCTCCCTG TCTGCATCTG TAGGAGACAG AGTCACCATC
2341 ACTTGCCGGG CAAGTCAGAG CATTAGCAGC TATTTAAATT GGTATCAGCA GAAACCAGGG
2401 AAAGCCCCTA AGCTCCTGAT CTATGCTGCA TCCAGTTTGC AAAGTGGGGT CCCATCAAGG
2461 TTCAGTGGCA GTGGATCTGG GACAGATTTC ACTCTCACCA TCAGCAGTCT GCAACCTGAA
2521 GATTTTGCAA CTTACTACTG TCAACAGAGT TACAGTACCC CTTTCACTTT CGGCCCTGGG
2581 ACCAAAGTGG ATATCAAACG TGGtACcGTG GCTGCACCAT CTGTCTTCAT CTTCCCGCCA
2641 TCTGATGAGC AGTTGAAATC TGGAACTGCC TCTGTTGTGT GCCTGCTGAA TAACTTCTAT
2701 CCCAGAGAGG CCAAAGTACA GTGGAAGGTG GATAACGCCC TCCAATCGGG TAACTCCCAG
2761 GAGAGTGTCA CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCTGACG
2821 CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC CCATCAGGGC
2881 CTGAGTTCAC CGGTGACAAA GAGCTTCAAC AGGGGAGAGT GTGCGGCCGC TGGTAAGCCT
2941 ATCCCTAACC CTCTCCTCGG TCTCGATTCT ACGTGATAAC TTCACCGGTC AACGCGTGAT
3001 GAGAATTCAC TGGCCGTCGT TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA
3061 CTTAATCGCC TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC
3121 ACCGATCGCC CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGCGCCT GATGCGGTAT
3181 TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAC GTCAAAGCAA CCATAGTCTC
3241 AGTACAATCT GCTCTGATGC CGCATAGTTA AGCCAGCCCC GACACCCGCC AACACCCGCT
3301 GACGCGCCCT GACAGGCTTG TCTGCTCCCG GCATCCGCTT ACAGACAAGC TGTGACCGTC
3361 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GA
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[00187] **Example 4: Dobbling of CDRs**

[00188] The following examples exemplify the use of dobbling in constructing synthetic libraries. The parental 3-23 heavy chain (HC) is diversified in CDR1, 2, and 3. This diversity is combined with a synthetically diversified A27 light chain (LC). The diversity will be as follows:

[00189] Example 4.1 HC CDR1

[00190] The following dobbling diversity allows 5,832 variants. See Table 50. At position 31, Ser is the germline (GL) amino-acid type. Hence we make Ser three times more likely then the other types. Since 18 types are allowed, Ser will be allowed 15% of the time and all the others are allowed at 5%. Thus, if there is no selection for the AA type at 31, we are more likely to isolate an Ab with Ser. Similarly, at 33 the GL AA type is Ala and we make Ala 3 times as likely (15%) as all the others (5%). At 35 Ser is the GL AA type and we make it three times as likely as the others. At all three positions, we have excluded Cys and Met. We exclude Cys because we do not want gratuitus disulfides or exposed unpaired cysteines that could adversely affect the solubility and reactivity of the Ab. We exclude Met because exposed methionines side groups are subject to oxidation which can alter binding properties and shelf life.

We could make the germline amino-acid type 2, 3, 4, 5, 6, 8, or 10 times more likely than the other AA types.

Table 50: Diversity for CDR1 in 3-23

Position	Parental AA	Allowed
31	S (three-times more likely as the others)	ADEFGHKLNPQRSTVWY
33	A (3-X more likely)	ADEFGHKLNPQRSTVWY
35	S (3-X more likely)	ADEFGHKLNPQRSTVWY

[00191] Throughout this disclosure, the shown "Allowed" amino acids are the amino acids that can be used at a given position. For example, in Table 50, at position 31, allowed amino acids "ADEFGHKLNPQRSTVWY" are shown. This indicates that amino acids A, D, E, F, G, H, K, L, N, P, Q, R, S, T, V, W, and Y are all allowed at position 31.

[00192] Example 4.2: HC CDR2

[00193] In CDR2, we allow diversity at positions 50, 52, 52a, 56, and 58. At 50, 52, 56, and 58 we allow all amino-acid types except Cys and Met and we make the GL AA types more likely by three fold. We could make the GL AA type 2, 3, 4, 5, 6, 8, or 10 times more likely than the other AA types.

Table 51: HC CDR2: Diversity = 419,904

Position	Parental AA	Allowed
50	A (3-X more likely)	ADEFGHKLNPQRSTVWY
52	S (3-X more likely)	ADEFGHKLNPQRSTVWY
52a	G (3-X more likely)	GPSY
56	S (3-X more likely)	ADEFGHKLNPQRSTVWY
58	Y (3-X more likely)	ADEFGHKLNPQRSTVWY

[00194] Combined CDR1 and CDR2 diversity = 2.45 E 9

[00195] Example 4.3 HC CDR3, lengths 3, 4, 5

[00196] Very short CDR3 can be made by dobbling. Table 7 shows several parental sequences for CDR3 length 3. At 94 many VH3s have Arg and we have allowed this change, but

Lys is made 3-X as likely. At 95, F is found at this position in JH1. We also allow Ser, Tyr, Asp, and Arg to allow small, large, plus charge, and minus charge. At 96, JH1 has Q. Since Q is very similar to Glu, we allow Glu as an acidic alternative plus Arg, Ser, Tyr, and Leu. At 97, His is the germline AA from JH1. We allow minus charge (D), plus charge (R), small polar (S), large hydrophobic (Y), and aliphatic (L). The parental sequence makes up 4.5% of the library, but this is combined with a large diversity in CDR1 and CDR2. The dobbling allows 360 sequences in all. The least likely sequences occur at 1 in 1792. The most likely (parental) sequence occurs about 1 in 22.

Table 60: A dobbled HC CDR3 of length 3 (V-3JH1 of Table 7)

Position	Parental amino acid (source)	Allowed
94	K (VH 3-23)	KR (3:1)
95	F (JH1)	FSYDR (3:1:1:1:1)
96	Q (JH1)	QERSYL (3:1:1:1:1:1)
97	H (JH1)	HDRSYL (3:1:1:1:1:1)
103	W (JH1)	W

[00197] Table 61 shows a dobbled HC CDR3 of length 3. Here K94 is fixed as is W103. We have made the "parental" D segment amino acid five times as likely as the other allowed AA types.

Table 61: A dobbled HC CDR3 of length 3 from a D fragment (V-3D1-1.1.2-JH1 of Table 7).

Position	Parental	Allowed
94	K (V 3-23)	K
95	T (D1-1.1.2)	TYRDL (5:1:1:1:1)
96	T (D1-1.1.2)	TYRDL (5:1:1:1:1)
97	G (D1-1.1.2)	GSYRDL (5:1:1:1:1:1)
103	W (JH1)	W

[00198] In this example (Table 62, using V-4JH2 from Table 8), 94 is fixed as Lys. At 95, JH2 has Tyr and we have allowed Ser, Asp, Arg, and Leu so that size, charge, and hydrophobicity can alter to suit the antigen. JH2 has Phe at 96 and we have allowed Ser, Tyr,

Asp, Arg, and Leu. At 97, JH2 has Asp and we have allowed Arg, Ser, Tyr, and Leu. At 98, JH2 has Leu and we have allowed Ser, Tyr, Asp, and Arg. This pattern allows 750 distinct sequences, of which the parental is the most likely (1 in 18). The least likely sequences occur at 1 in 4608 or 256 times less likely than the most likely.

Table 62: HC CDR3 length 4 from JH2 (V-4JH2 in Table 7)

Position	Parental AA (source)	Allowed
94	K (VH 3-23)	K
95	Y (JH2)	YSDRL (4:1:1:1:1)
96	F (JH2)	FSYDRL (4:1:1:1:1:1)
97	D (JH2)	DRSYL (4:1:1:1:1)
98	L (JH2)	LSYDR (4:1:1:1:1)
103	W (JH2)	W

[00199] In Table 63, there is a dobbling of V-4D3-10.1a-JH2 from Table 8. At 94, we allow Lys and Arg with Lys (the parental) four times as likely as Arg. At 95, D3-10.1a (i.e., D3-10 in the first reading frame and starting a AA 1) has Leu; we allow SYDR as well with Leu 4-X as likely as each of the other AA types. At 96, D3-10.1a has Leu again and we allow the same menu. At 97, D3-10.1a has Trp and we allow Ser, Tyr, Asp, and Arg with Trp 4-X as likely. At 98, D3-10.1a has Phe and we allow Ser, Tyr, Asp, and Arg as well.

Table 63: HC CDR3 of length four from V-4D3-10.1a in Table 8

Position	Parental AA (source)	Allowed
94	K (VH 3-23)	KR (4:1)
95	L (D3-10.1a)	LSYDR (4:1:1:1:1)
96	L (D3-10.1a)	LSYDR (4:1:1:1:1)
97	W (D3-10.1a)	WSYDR (4:1:1:1:1)
98	F (D3-10.1a)	FSYDR (4:1:1:1:1)
103	W	W

[00200] Example 4.4: HC CDR3 length 10 to 20

[00201] HC CDR3

[00202] Two sublibraries, both with CDR3 of length 16:

Table 52: Library 1: Diversity = 5 E 11, the "parental" sequence occurs at 1 in 1.5 E6.

Position	"Parental" AA (source)	Allowed
94	K (3-X more likely) (3-23)	KR (3:1)
95	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
96	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
97	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
98	D (3-X more likely) (D2-21(2))	DYSRL (3:1:1:1:1)
99	S (3-X more likely) (D2-21(2))	SYRDL (3:1:1:1:1)
100	S (3-X more likely) (D2-21(2))	SYRDL (3:1:1:1:1)
101	G (3-X more likely) (D2-21(2))	GASYRDL (3:1:1:1:1:1:1)
102	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
102a	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
102b	Y (3-X more likely) (D2-21(2))	YSRDL (3:1:1:1:1)
102c	A (3-X more likely) (JH1)	ASYRD (3:1:1:1:1)
102d	E (3-X more likely) (JH1)	ERSYL (3:1:1:1:1)
102e	Y (3-X more likely) (JH1)	YSRDL (3:1:1:1:1)
102f	F (3-X more likely) (JH1)	FYSRD (3:1:1:1:1)
102g	Q (3-X more likely) (JH1)	QERSY (3:1:1:1:1)
102h	H (3-X more likely) (JH1)	HERSYL (3:1:1:1:1:1)
103	W (JH1, fixed)	W

Table 53: Library 2: CDR3 length 16; Diversity is 3.0 E 10 and the parental sequence occurs once in 3.7 E 5.

Position	"Parental" AA (source)	Allowed
94	K (3-X more likely) (3-23)	KR (3:1)
95	G (3-X more likely) (D2-2(2))	GSYDRL (3:1:1:1:1:1)
96	Y (3-X more likely) (D2-2(2))	YSDRL (3:1:1:1:1)
97	C (fixed) (D2-2(2))	C

98	S (3-X more likely) (D2-2(2))	SYRDL (3:1:1:1:1)
99	S (3-X more likely) (D2-2(2))	SYRDL (3:1:1:1:1)
100	T (3-X more likely) (D2-2(2))	TYRDL (3:1:1:1:1)
101	S (3-X more likely) (D2-2(2))	SYRDL (3:1:1:1:1)
102	C (fixed) (D2-2(2))	C
102a	Y (3-X more likely) (D2-2(2))	YSDRL (3:1:1:1:1)
102b	T (3-X more likely) (D2-2(2))	TYRDL (3:1:1:1:1)
102c	A (3-X more likely) (JH1)	ASYDRL (3:1:1:1:1:1)
102d	E (3-X more likely) (JH1)	ERSYL (3:1:1:1:1)
102e	Y (3-X more likely) (JH1)	YSDRL (3:1:1:1:1)
102f	F (3-X more likely) (JH1)	FYSRDL (3:1:1:1:1:1)
102g	Q (3-X more likely) (JH1)	QERSYL (3:1:1:1:1:1)
102h	H (3-X more likely) (JH1)	HDRSYL (3:1:1:1:1:1)
103	W ((JH1))	W

[00203] Table 65 shows a dobbling variegation of SEQ ID NO:898. The total diversity allowed is 2.1 E 13. A synthesis that produces 1. E 8, 3. E 8, 5. E 8, 1. E 9, or 5. E 9 will sample the diversity adequately. The design of SEQ ID NO:898 was discussed above. In dobbling SEQ ID NO:898, is to allow the parental AA type at three-fold above other AA types at most positions. At positions where the parental is Tyr, then we use Tyr and Ser at equal amounts with Leu at one half that frequency. The Cys residues are fixed. Each parental AA type is allowed to go to one of Arg, Asp, Ser, Tyr, or Leu (Leu might be omitted if the parental is hydrophobic, such as Phe). The parental sequence will occur once in 1. E 8 members. The least likely sequences will occur once in 9.5 E 16. It is not important that the library actually contain the parental sequence, only that it contains many sequences that resemble the parent. Thus, a library that contains 1. E 7, 5. E7, 1.E8, 3. E8, 1. E 9, or 5. E 9, when combined with diversity in HC CDR1, HC CDR2, LC CDR1, LC CDR2, and LC CDR3 will provide a library that will contain many valuable Abs.

Table 65: Dobbling of Design 1 with SEQ ID NO:898 as parent

Position	Parental (source)	Allowed
94	K (VH 3-23)	K
95	D (No source)	DSYL (3:1:1:1)
96	Y (No source)	YSL (2:2:1)
97	G (D2-2.2)	GSYDRL (3:1:1:1:1:1)
98	Y (D2-2.2)	YSL (2:2:1)
99	C (D2-2.2)	C
100	S (D2-2.2)	SYDRL (3:1:1:1:1)
101	S (D2-2.2)	SYDRL (3:1:1:1:1)
102	T (D2-2.2)	TYDRL (3:1:1:1:1)
102a	S (D2-2.2)	SYDRL (3:1:1:1:1)
102b	C (D2-2.2)	C
102c	Y (D2-2.2)	YSL (2:2:1)
102d	T (D2-2.2)	TYDRL (3:1:1:1:1)
102e	Y (No source)	YDSL (3:1:1:1)
102f	G (No source)	GSYRD (3:1:1:1:1)
102g	Y (No source)	YSL (2:2:1)
102h	S (No source)	SYDRL (3:1:1:1)
102i	Y (No source)	YSL (2:2:1)
102j	A (JH1)	ASYDR (3:1:1:1:1)
102k	E (JH1)	ERSYL (3:1:1:1:1)
102l	Y (JH1)	YSL (2:2:1)
102m	F (JH1)	FSYDR (3:1:1:1:1)
102n	Q (JH1)	QYSDRL (3:1:1:1:1:1)
102p	H (JH1)	HSYDRL (3:1:1:1:1:1)
103	W (JH1, FR4)	W

[00204] Example 4.5 Dobbling of yycakGSGYCSGGSCYSFDYwgqgtlvtvss (SEQ ID NO:931)

[00205] Table 80 shows the dobbling of SEQ ID NO:931, an example of an HC CDR3 of length 15. Position 94 is part of FR3 and is held constant. Positions 95 and 96 have "parental" amino-acid types picked from the highly used set of (YGDRS) and are G95 and S96. The next ten positions are taken from D2-15.2 (a moderately highly used D segment containing a disulfide-closed loop). The final three positions are from the JH4 positions 100, 101, and 102 as shown in Table 3. At each position, we make the parental amino-acid type three times more likely than the other allowed types. The Cys residues are fixed. At 102e, Phe is three times more likely as are YGSRD (i.e., Phe is three times more likely as are any of amino acids Y, G, S, R, or D). The diversity allowed is 1.46 E 9. The parental sequence is expected at 1 in 6.9 E 4. Each of the singly substituted sequences is about 1/3 as likely; the doubly substituted ones are

1/9 as likely and so on. The sequences that are composed entirely of other AA types occur at only 1 in 1.1 E 11.

[00206] Each of the other sequences in Table 21 can be dobbled in the same way.

Table 80: Dobbling of yycakGSGYCSGGSCYSFDYwgqgtlvtvss (SEQ ID NO:931)

Position	Parental (source)	Allowed
94	K (VH 3-23)	K
95	G (No source)	GYSRD (3:1:1:1:1)
96	S (No source)	SGYRD (3:1:1:1:1)
97	G (D2-15.2)	GYSRD (3:1:1:1:1)
98	Y (D2-15.2)	YGSRD (3:1:1:1:1)
99	C (D2-15.2)	C
100	S (D2-15.2)	SGYRD (3:1:1:1:1)
101	G (D2-15.2)	GYSRD (3:1:1:1:1)
102	G (D2-15.2)	GYSRD (3:1:1:1:1)
102a	S (D2-15.2)	SGYRD (3:1:1:1:1)
102b	C (D2-15.2)	C
102c	Y (D2-15.2)	YGSRD (3:1:1:1:1)
102d	S (D2-15.2)	SGYRD (3:1:1:1:1)
102e	F (JH4)	FYGSRD (3:1:1:1:1:1)
102f	D (JH4)	DGSRY (3:1:1:1:1)
102g	Y (JH4)	YGSRD (3:1:1:1:1)
103	W (JH4, FR4)	W

[00207] **Example 5: Synthetic light chain diversity**

[00208] To make whole antibodies, we need to combine a library of heavy chains with a library of light chains (LC). In natural Abs, it is often observed that HC does most of the binding and many libraries have given little attention to the LC or have obtained LC diversity from human donors. To have enough diversity to give good binders to almost any target, we have designed a diversification program that exceeds what the human immune system usually provides. Nevertheless, the program is designed to yield fully functional LC that have the same kind of changes as seen in natural Abs, only a few more. Vkappa III A27 was picked as the LC.

[00209] From a library that comprises donated kappa and lambda LCs, a collection of 1266 Abs were typed. Among VKIIIs, A27 is most often seen (Table 66) and pairs well with HC 3-23.

[00210] The CDRs of A27 contain 12, 7, and 9 amino acids. Putting diversity at all of these positions might not work well: a) there might be many unstable or non-functional

members, and b) diversity at some positions might not help improve binding. We have reduced the number of variable positions from 28 to 16.

[00211] We have studied the 3D structure of 1QLR which has an A27 LC. The 1GLR structure is publicly available in the RCDB Protein Data Base. From this, the residues marked in Table 68 look useful to vary. The T56 is about 10 Å from a His in HC CDR3. Variation at 56 may be useful. G24 is only about 7 Å from an atom in HC CDR3. Germline is R24; thus, variation at 24 may be useful.

[00212] Table 69 shows a display cassette that we designed for use in pMID21. Thus, the restriction enzymes picked do not have other sites in pMID21. *Spe*I is in the *iii* signal sequence and allows the entire LC to be inserted or removed. *Xma*I, *Ppu*MI, *Eco*O109I, and *Blp*I precede CDR1. *Sac*II is in FR2, separating CDR1 from CDR2. Alternatively, an *Avr*II site could be inserted at the same position. *Bsp*EI and *Xho*I sites are in FR3 and a *Kpn*I site is in FR4.

[00213] We gathered 155 A27 sequences and analyzed what happens in the CDRs. Table 70 shows the analysis. In Table 70, we show what is found in the Abs from our library and what we would put at each position.

```
!Table 68: where to vary A27
!
!                22    3    3  5    5   89    9
!                45    0a   4  0    5   90    5
!1QLR             GASQSVS_NYLA  DASSRAT  QQYGSSPLT
!A27              RASQSVSSSYLA  GASSRAT  QQYGSSPLT
                    ** **** *  *  *  *    ******
GASQSVS is (SEQID NO:922)   DASSRAT is (SEQID NO:923)
QQYGSSPLY is (SEQID NO:924)
RASQSVSSSYLA is (SEQ ID NO:925)  GASSRAT is (SEQ ID NO:926)
```

Table 68 shows where the CDRs of A27 would be variegated.

Table 67: Compare VKIII AA seqs

VK3 is (SEQ ID NO:927)

```
       FR1.....................CDR1........FR2............CDR2...FR3.............................CDR3.....FR4.........
                                                                                                        1
            1         2         3         4         5         6         7         8         9         0
      12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
 VK3  DIVLTQSPATLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGVPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQHYTTPPTFGQGTKVEIKRT
 A27  E-------G-------------------------------------------------I-D---------------R-------------YGSS-
 A11  E---------------------G-----------------L-------D-------I-D---------------R-------------YGSS-
 L2   E--M--------V-----------------#N--------------------T----I-----------E--------QS----------YNNW-
 L16  E--M--------V-----------------#N--------------------T----I-----------E--------QS----------YNNW-
 L6   E-----------------------------#-----------------D--N----I-------------------------------RSNW-
 L20  E-------------------------G---#-----------------D--N----I--------P----------------------RSNWH
 L25  E--M-------------------------------S-----------------T----I--------------------Q----------D-NL-
```

VK3 differs from A27 by E1D, G9A, I58V, D60A, R77S.

A27 and L6 differ by G9A(FR1), Δ31a(in CDR1), G50D(CDR2), S53N(CDR2), G92S(CDR3), S93N(CDR3), S94W(CDR3)
VK3 from US 7,264,963.

[00214] CDR1

[00215] R24, A25, and S26 are too far from the combining site to help and were held constant. The side group of V29 is buried; this position was held constant as Val. At the other positions, we allowed Y or S and a charge flip-flop (RE or RD, depending on where the sample had more of E or D at the position in question) plus other types that were frequently seen. We used an Excel spread sheet to determine that this pattern of variegation would give the parental sequence at 0.8% if the "other" AAs were substituted at 5%, at 0.1% if the "other" AAs were substituted at 6.5%, and at 0.02% if "other" was at 9%. In the sample of 155, 17 have one AA deleted (including 1QLR); thus, we will arrange to have S30a deleted in ~8% of the members.

[00216] CDR2

[00217] From inspection of 1QLR, we see that CDR2 is somewhat remote from the combining site. There have even been suggestions that we keep the residues in this CDR constant. Studying the 3D structure suggests that variegation at G50, S53, and T56 could be useful. S53 is the most variable in the sample of 155, but this does not prove that these changes are useful. In 1QLR, G50 has been mutated to R50. The side group of T56 is pointed toward HC CDR3 and is about 11 Å from an atom in HC CDR3.

[00218] CDR3

[00219] Q89 and Q90 are buried and nature does not vary them often; these residues are not varied. Y91 is packed against HC CDR3 and changes here would alter the combining site and do occur. At G92, φ = -80 and ψ = -15 so putting in a non-Gly is feasible; nature does it in 47/155 cases. S93 is very often varied or deleted. S94 is highly exposed and is highly varied. P95 is exposed and varied. L96 packs against HC CDR3: changes here will affect the binding site and do occur in nature. T97 is buried and has been held constant/ the amino acid is not varied.

[00220] The parental sequence appears at 0.000246 or 1 in 4.06 E3. The allowed diversity is about 2.1 E 12. With two 8% deletions, 84.6% of the members will be full length, 7.4% will

have short CDR1 and full-length CDR3, 7.4% will have full-length CDR1 and short CDR3, and 0.6% will have both deletions.

[00221] Other germlines were not in the sample.

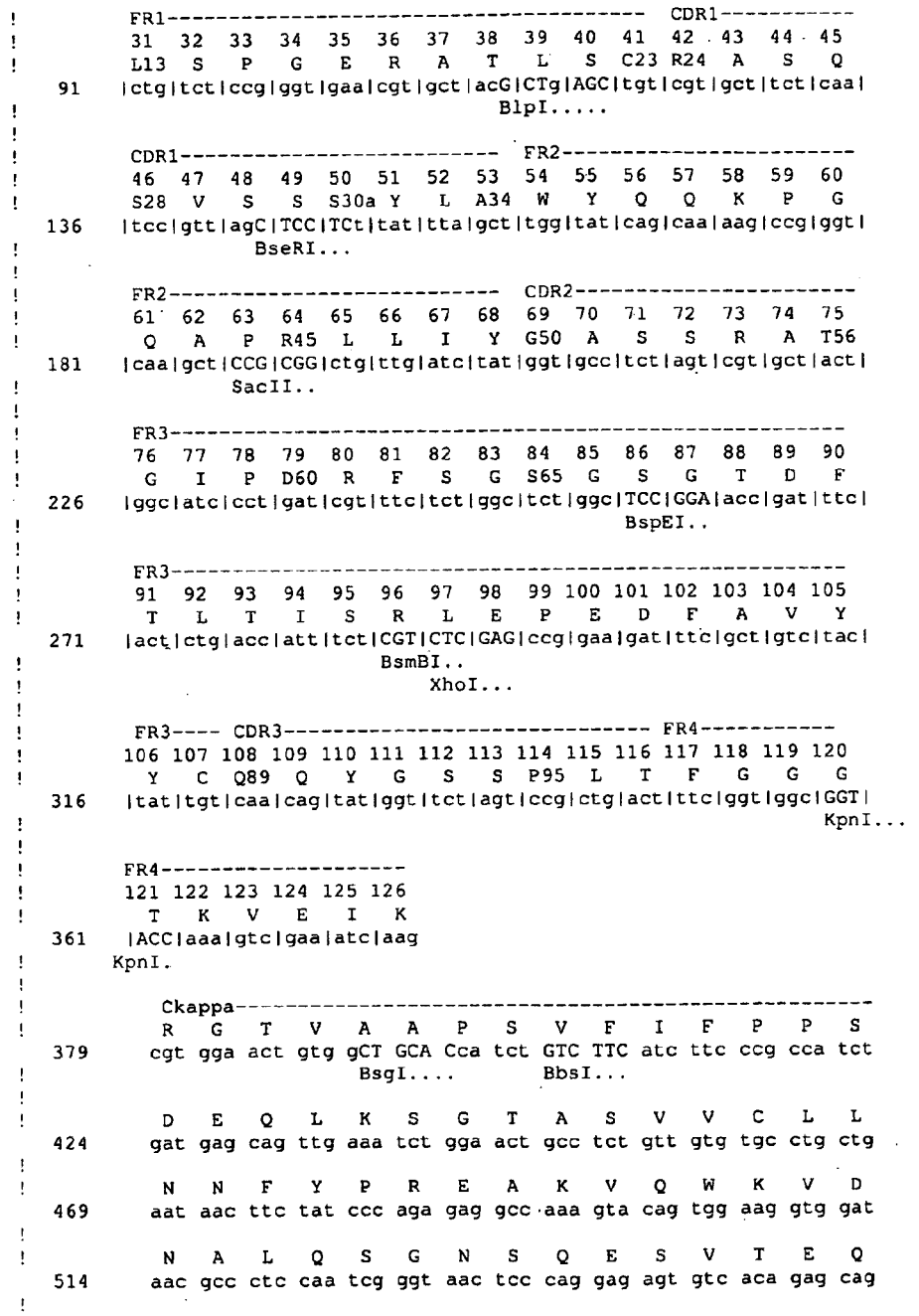
Table 66: Distribution of VLs in 1266 selected LCs.

Kappas				Lambdas			
O12	VKI	313		1a	VL1	9	
O18	VKI	1		1e	VL1	7	
A20	VKI	26		1c	VL1	55	
A30	VKI	26		1g	VL1	46	
L14	VKI	2		1b	VL1	1	118
L1	VKI	5		2c	VL2	18	
L15	VKI	1		2e	VL2	23	
L5	VKI	83		2a2	VL2	79	
L8	VKI	10		2d	VL2	1	121
L12	VKI	77	544	3r	VL3	56	
O11	VKII	4		3j	VL3	4	
A17	VKII	17		3l	VL3	31	
A19	VKII	31	52	3h	VL3	22	113
A27	VKIII	155		4a	VL4	1	1
L2	VKIII	31		5c	VL5	1	1
L6	VKIII	88		6a	VL6	8	8
L25	VKIII	16	290	10a	VL10	6	6
B3	VKIV	12	12	Number of lambdas			368
Number of kappas			898	Total Abs in sample			1266

```
!Table 69: A Display gene for A27 in pM21J.
! IIIsignal::A27::Ckappa

The amino-acid sequence of Table 69 is (SEQ ID NO:928).
The DNA sequence of Table 69 is (SEQ ID NO:929).

!        signal sequence---------------------------------------------
!          1   2   3   4   5   6   7   8   9  10  11  12  13  14  15
!          M   K   K   L   L   S   A   I   P   L   V   V   P   F   Y
     1   |atg|aaG|aaA|ctg|ctg|tct|gct|atc|ccA|CTA|GTt|gtc|cct|ttc|tat|
!                                              SpeI....
!
!       Signal-------- FR1-----------------------------------------
!         16  17  18  19  20  21  22  23  24  25  26  27  28  29  30
!          S   H   S   E1  I   V3  L   T5  Q   S7  P   G9  T   L   S12
    46   |tct|cat|agt|gaa|atc|gtt|ctg|acc|cag|tcC|CCG|GGG|aCC|Ctg|tct|
!                                                  XmaI....
!                                                        PpuMI....
!                                                        EcoO109I.(1/2)
!
```


FR1
CDR1
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
L13 S P G E R A T L S C23 R24 A S Q
91 |ctg|tct|ccg|ggt|gaa|cgt|gct|acG|CTg|AGC|tgt|cgt|gct|tct|caa|
BlpI.....
CDR1
FR2
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
S28 V S S S30a Y L A34 W Y Q Q K P G
136 |tcc|gtt|agC|TCC|TCt|tat|tta|gct|tgg|tat|cag|caa|aag|ccg|ggt|
BseRI...
FR2
CDR2
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75
Q A P R45 L L I Y G50 A S S R A T56
181 |caa|gct|CCG|CGG|ctg|ttg|atc|tat|ggt|gcc|tct|agt|cgt|gct|act|
SacII..
FR3
76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
G I P D60 R F S G S65 G S G T D F
226 |ggc|atc|cct|gat|cgt|ttc|tct|ggc|tct|ggc|TCC|GGA|acc|gat|ttc|
BspEI..
FR3
91 92 93 94 95 96 97 98 99 100 101 102 103 104 105
T L T I S R L E P E D F A V Y
271 |act|ctg|acc|att|tct|CGT|CTC|GAG|ccg|gaa|gat|ttc|gct|gtc|tac|
BsmBI..
XhoI...
FR3---- CDR3
FR4
106 107 108 109 110 111 112 113 114 115 116 117 118 119 120
Y C Q89 Q Y G S S P95 L T F G G G
316 |tat|tgt|caa|cag|tat|ggt|tct|agt|ccg|ctg|act|ttc|ggt|ggc|GGT|
KpnI...
FR4
121 122 123 124 125 126
T K V E I K
361 |ACC|aaa|gtc|gaa|atc|aag
KpnI.
Ckappa
R G T V A A P S V F I F P P S
379 cgt gga act gtg gCT GCA Cca tct GTC TTC atc ttc ccg cca tct
BsgI.... BbsI...
D E Q L K S G T A S V V C L L
424 gat gag cag ttg aaa tct gga act gcc tct gtt gtg tgc ctg ctg
N N F Y P R E A K V Q W K V D
469 aat aac ttc tat ccc aga gag gcc aaa gta cag tgg aag gtg gat
N A L Q S G N S Q E S V T E Q
514 aac gcc ctc caa tcg ggt aac tcc cag gag agt gtc aca gag cag

```
!          D   S   K   D   S   T   Y   S   L   S   S   T   L   T   L
  559      gac agc aag gac agc acc tac agc ctc agc agc acc ctg act ctg
!
!
!          S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V
  604      tcc aaa gca gac tac gag aaa cac aaa GTC TAC gcc tgc gaa gtc
!
!
!          T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R
  649      acc cat cAG GGC CTg agt tCA CCG GTG aca aag agc ttc aac agg
!                 AlwNI......       SgrAI.....
!                  EcoO109I.(2/2)    AgeI....
!
!          G   E   C   .   .
  694      gga gag tgt taa taa
!
  709                          GG CGCGCCaatt
!                              AscI.....
!                               BssHII.
```

Table 70: Tally of mutations in CDRs of A27 Abs

CDR1			
R24	1,	3G,1T,151-,	Fix
A25	2,	3T,152-,	Fix
S26	3,	1R,154-,	Fix
Q27	4,	**3E**,1H,1L,1P,**4R**,145-,	9% ERYSL
S28	5,	1A,2F,2G,1I,2L,**5N**,1P,1R,**10T**,1V,**1Y**,128-,	9% NTYERL
V29	6,	1F,19I,6L,129-,	Fix
S30	7,	2A,**2D**,8G,2H,1I,**11N**,**9R**,**6T**,**4V**,**2Y**,**108-**,	9% DNRTY
S30a	8,	1A,2F,**6G**,1H,**6N**,1P,**10R**,**6T**,**3Y**,**119-**,	9% GNRTYD
(8% delete 30a)			
S31	9,	1A,**5D**,**3F**,**4G**,1H,2I,4K,1L,**31N**,**19R**,**7T**,**7Y**,**70-**,	9% DFGNRTY
Y32	10,	5F,1K,14L,4N,4Q,2R,8S,3V,1W,**113-**,	9% FDLNQRSY
L33	11,	**16A**,1F,4I,1N,1S,8V,1Y,**123-**,	Fix
A34	12,	2G,2L,1N,1S,4V,128-,	9% SY
_	13,	2A,1G,	
_	14,	1S,	
_	15,	1S,	
_	16,	1Y,	
_	17,	1L,	
_	18,	1A,	

Note: one antibody had an insertion of six AAs in CDR1! Two other Abs had a single insertion. Seventeen Abs have a one AA deletion in CDR1.

CDR2			
G50	1,	10A,11D,1H,2R,2S,1V,7Y,**121-**,	9% DRSYL
A51	2,	7G,2I,6S,7T,2V,131-,	Fix
S52	3,	6A,3F,1G,1T,144-,	Fix
S53	4,	1A,1G,1H,5I,2K,16N,7R,16T,**106-**,	9% NTSYER
R54	5,	1A,1I,1N,1S,3T,1Y,147-,	Fix
A55	6,	2P,7R,4S,2V,140-,	Fix

T56 7, 10A[1],1G,1H,2P,4S,137-, 9% ERSY
_ 8, 1A,6T,
Note, there are seven antibodies with an insertion of one AA.

CDR3 (showing "_" means that the Ab has a deletion in CDR3)
Q89 1, 5H,1L,2M,147-, Fix
Q90 2, 1E,1F,13H,2K,2L,4R,1S,1Y,130-, Fix
Y91 3, 2A,8F,2G,2H,1L,1P,13R,4S,**122-**, 9% FERS
G92 4, 10A,3D,2H,1I,1L,2N,6R,12S,2V,3Y,**108-**,5_, 9% ADRSTY
S93 5, 1A,**2D**,**2F**,6G,2H,3I,2K,2M,**14N**,1P,1Q,**8R**,**17T**,**2Y**,**86-**,6_, 9% DFNRTY
(8% have 93 deleted)
S94 6, 3A,6F,1I,3L,3P,2R,2T,11W,**117-**,7_, 9% WERYS
P95 7, 2A,1E,1G,1K,3L,1M,7R,3S,3T,1V,**108-**,24_, 9% ERYS
L96 8, 2A,**2E**,3F,3G,1H,1I,**3K**,7L,2M,**24P**[2],6Q,**28R**,3S,3T,7V,2Y,**58-**, 9% ERPYS
T97 9, 2A,1F,2G,3I,1K,2L,3M,1N,1R,6S,3V,2Y,**128-**, Fix
_ 10, 1A,1S,34-,
_ 11, 1S,7-,
_ 12, 1A,
There is one Ab with an insertion of 3 AAs.
Five have deletions of 4 AAs, 1 has a 3 AA deletion, 1 has a 2 AA deletion, and 17 have a one AA deletion.

Table 71: Allowed diversity in CDR1, 2, and 3 of A27::JK4.

Position	parental	allowed	
CDR1			
42(24)	R	fixed	
43(25)	A	fixed	
44(26)	S	fixed	
45(27)	Q	ERYSL	55%Q 9% other
46(28)	S	NTYERL	46%S 9% other
47(29)	V	fixed	
48(30)	S	DNRTY	55%S 9% other
49(30a)	S	GNRTYD	46%S 9% other
8% have 30a deleted			
50(31)	S	DFGNRTY	44%S 8% other
51(32)	Y	FDLNQRSY	44%Y 7% other
52(33)	L	fixed	
53(34)	A	SY	70%A 15% other
CDR2			
69(50)	G	DRSYL	55%G 9% other
70(51)	A	Fixed	
71(52)	S	Fixed	
72(53)	S	NTSYER	52%S 8% other
73(54)	R	Fixed	
74(55)	A	Fixed	
75(56)	T	ERSY	64%T 9% other
CDR3			
108(89)	Q	fixed	
109(90)	Q	fixed	
110(91)	Y	FERS	64%Y 9% other
111(92)	G	ADRSTY	52%G 8% other

[1] Seven of these come from the insertions.
[2] Some of these appear because of insertions.

112(93)	S	DFNRTY	52%S 8% other
113(94)	S	WERYS	55%S 9% other
114(95)	P	ERYS	64%P 9% other
8% have P95 deleted			
115(96)	L	ERPYS	55%L 9% other
116(97)	T	fixed	

[00222] The parental sequence appears at 5.32 E -5 or 1 in 1.88 E 4.

[00223] Sequences with a single substitution have a probability between 1.1 E -5 and 7.5 E -6.

[00224] Sequences that have none of the parental AAs occurs at 1 in 6.7 E 16.

[00225] The allowed diversity is about 2.35 E 12.

Table 75: Frequencies of amino acids in HC CDR3s.

AA	Number	%	Rel up	Rel down
Y	3428	15.64	50.41	1.00
G	3244	14.80	47.71	0.95
D	2622	11.96	38.56	0.76
S	1777	8.11	26.13	0.52
R	1337	6.10	19.66	0.39
F	1328	6.06	19.53	0.39
A	1213	5.53	17.84	0.35
V	1141	5.20	16.78	0.33
L	816	3.72	12.00	0.24
I	745	3.40	10.96	0.22
P	726	3.31	10.68	0.21
T	586	2.67	8.62	0.17
W	566	2.58	8.32	0.17
M	560	2.55	8.24	0.16
N	462	2.11	6.79	0.13
E	363	1.66	5.34	0.11
K	355	1.62	5.22	0.10
H	327	1.49	4.81	0.10
Q	259	1.18	3.81	0.08
C	68	0.31	1.00	0.02
Total	21923			

Table 76: Length distribution of HC CDR3

Length	Number of Antibodies	Sum	Median
1	0		
2	0		
3	2	2	
4	21	23	
5	16	39	
6	100	139	
7	36	175	
8	78	253	
9	155	408	
10	153	561	
11	134	695	11.12
12	123	818	
13	133	951	
14	92	1043	
15	87	1130	
16	71	1201	
17	59	1260	
18	41	1301	
19	40	1341	
20	22	1363	
21	21	1384	
22	15	1399	
23	7	1406	
24	7	1413	
25	1	1414	
26	1	1415	
27	3	1418	
28	0	1418	
29	0	1418	
30	1	1419	
	1419	709.5	

Table 77: Utilization of D-segments (cut off at 0.70 match)

3-22.2	38	YYYDSSGYYY
4-17.2	27	DYGDY
3-3.2	25	YYDFWSGYYT
6-19.1	25	GYSSGWY
7-27.1	19	LTG
5-5.3	18	GYSYGY

6-13.1	18	GYSSSWY
5-12.3	13	GYSGYDY
6-13.2	10	GIAAAG
1-26.3	9	YSGSYY
2-15.2	9	GYCSGGSCYS
4-4.3	9	TTVT
3-10.2	8	YYYGSGSYYN
1-1.3	7	YNWND
4-4.2	7	DYSNY
2-2.2	6	GYCSSTSCYT
3-16.2	6	YYDYVWGSYRYT
6-6.1	6	EYSSSS
6-19.2	6	GIAVAG
3-9.1	5	VLRYFDWLL@
4-23.2	5	DYGGNS
6-6.2	5	SIAAR
1-7.3	4	YNWNY
2-2.3	4	DIVVVPAAI
4-23.3	4	TTVVT
1-7.1	3	GITGT
1-26.1	3	GIVGAT
7-27.3	3	NWG
3-10.1	2	VLLWFGELL@
3-10.2	2	ITMVRGVII
5-5.1	2	VDTAMV
5-5.2	2	WIQLWL
5-12.1	2	VDIVATI
5-24.3	2	RDGYNY
1-1.1	1	GITGT
2-21.3	1	HIVVVTAI
3-3.3	1	ITIFGVVII
5-24.2	1	*RWLQL
6-6.3	1	V*QLV
6-19.3	1	V*QWLV

Table 78: D segment utilization (0.667 cutoff)

Name	Number	Sequence	%
None	935		0.517
7-27.1	158	LTG	0.087
7-27.3	98	NWG	0.054
5-5.3	72	GYSYGY	0.040
1-26.3	67	YSGSYY	0.037
3-22.2	46	YYYDSSGYYY	0.025
4-17.2	38	DYGDY	0.021
3-3.2	37	YYDFWSGYYT	0.020

7-27.2	37	@LG	0.020
6-19.1	33	GYSSGWY	0.018
6-13.2	31	GIAAAG	0.017
6-13.1	22	GYSSSWY	0.012
6-6.1	18	EYSSSS	0.010
6-19.2	18	GIAVAG	0.010
4-23.2	17	DYGGNS	0.009
5-12.3	17	GYSGYDY	0.009
5-24.3	14	RDGYNY	0.008
2-15.2	13	GYCSGGSCYS	0.007
1-26.1	11	GIVGAT	0.006
4-4.3	11	TTVT	0.006
1-1.3	9	YNWND	0.005
2-2.2	9	GYCSSTSCYT	0.005
3-16.2	9	YYDYVWGSYRYT	0.005
2-2.3	8	DIVVVPAAI	0.004
3-10.2	8	YYYGSGSYYN	0.004
4-4.2	8	DYSNY	0.004
1-7.3	7	YNWNY	0.004
3-3.3	6	ITIFGVVII	0.003
6-6.2	6	SIAAR	0.003
3-9.1	5	VLRYFDWLL@	0.003
3-10.2	5	ITMVRGVII	0.003
6-19.3	5	V*QWLV	0.003
1-7.1	4	GITGT	0.002
4-23.3	4	TTVVT	0.002
1-1.1	3	GTTGT	0.002
5-5.1	3	VDIAMV	0.002
5-24.2	3	*RWLQL	0.002
3-10.1	2	VLLWFGELL@	0.001
5-5.2	2	WIQLWL	0.001
5-12.1	2	VDIVATI	0.001
1-26.2	1	V*WELL	0.001
2-21.2	1	AYCGGDCYS	0.001
2-21.3	1	HIVVVTAI	0.001
3-3.1	1	VLRFLEWLLY	0.001
3-16.2	1	IMITFGGVIVI	0.001
6-6.3	1	V*QLV	0.001
6-13.3	1	V*QQLV	0.001

Table 78: Utilization of JH segments

JH1	17
JH2	31
JH3	452

JH4	636
JH5	32
JH6	251

[00226] Example 6: Wobbled DNA for HC CDR3 16d

Table 400 shows a segment of DNA from an *Xba*I site in FR3 to a *Bst*EII site in FR4. The HC CDR3 consists of SYSY::D2-2(2)::QH followed by the FR4 region of JH1. The QH is found in the germline of JH1. In V-D-J joining, immune cells often edit the ends of V, D, and J. Thus the construction corresponds to what is very possible in actual immunoglobulin gene construction and maturation. By wobbling the synthesis, we obtain a large collection of genes that resemble what would come from joining 3-23 to either a D region or to a little edited JH1 followed by some mutations. In library 16d, there are two cysteines that presumably form a disulfide, these are not wobbled.

Table 500 shows the expected distribution of amino-acid types at each position in the 16d library. The wobble doping was set at 73:9:9:9. The most likely sequence is the one shown in Table 21 and should be present at a frequency of 4.8 E-5. Only 55% of the sequences are stop free and 74% are free of ochre or opel. If the library is expressed in supE cells, this is the important number. It would be valuable to remove the sequences with stop codons as discussed elsewhere herein. One can see that those positions that start as S are predicted to have S 54% of the time and Y 5.4% while those that start as Y have Y 44% of the time and S 7.2%. At each position there are 7-9 AA types that appear at >1%. There are 14 variegated positions. The sequences that will be most effectively sampled number about $8^{14} = 4.3$ E 12.

Table 400: Cassette for display of wobbled HC CDR3 16d

```
!        --------FR3-----------------------------------------------------
!         68  69  70  71  72  73  74  75  76  77  78  79  80  81  82
!          T   I   S   R   D   N   S   K   N   T   L   Y   L   Q   M
  1216  |act|atc|TCT|AGA|gac|aac|tct|aag|aat|act|ctc|tac|ttg|cag|atg|
!               | XbaI  |
!
!        ---FR3------------------------------------------------------------>|
!        82a 82b 82c  83  84  85  86  87  88  89  90  91  92  93  94
!          N   S   L   R   A   E   D   T   A   V   Y   Y   C   A   K
  1261  |aac|agC|TTA|AGg|gct|gag|gac|act|gca|gtc|tac|tat|tgc|gct|aaa|
!             . |AflII |
!
```

```
!   e = 0.73 A + 0.09 C + 0.09 G + 0.09 T
!   q = 0.09 A + 0.73 C + 0.09 G + 0.09 T
!   j = 0.09 A + 0.09 C + 0.73 G + 0.09 T
!   z = 0.09 A + 0.09 C + 0.09 G + 0.73 T
!   The values 0.73 and 0.09 are picked so that 0.73 + 3*0.09 = 1.0
!   Other ratios could be used.
!
!                                        102 102 102 102 102 102 102 102
!       95  96  97  98  99  100 101 102  a   b   c   d   e   f   g   h
!        S   Y   S   Y   G   Y   c   S   S   T   S   c   Y   T   Q   H
!       zqz zez zqz zez jjz zez TGT zqz zqz eqz zqz TGT zez eqz qej qez
!
!        ~~~~~~~~~~~~~~FR4~~~~~~~~~~~~~~~~~~~~~~~~>|
!        103 104 105 106 107 108 109 110 111 112 113
!         W   G   Q   G   T   L   V   T   V   S   S
!       |TGg|ggt|caa|ggt|act|ttG|GTC|ACC|gtc|tct|agt
!                                | BstEII |
!
```

Table 500: Expected distribution of AA types in wobbled HC CDR3 16d

"." = TGA or TAA; "b" = TAG

S	Y	S	Y	G	Y	c	S	S	T	S	c	Y	T	Q	H
zqz	zez	zqz	zez	jjz	zez	tgt	zqz	zqz	eqz	zqz	tgt	zez	eqz	qej	qez

Nominal base purity = 0.7300 others = 0.0900

	s(zqz)	y(zez)	s(zqz)	y(zez)	g(jjz)	y(zez)	C(TGT)	s(zqz)	s(zqz)	t(eqz)
1	s 5.4-01	y 4.4-01	s 5.4-01	y 4.4-01	g 5.3-01	y 4.4-01	c 1.000	s 5.4-01	s 5.4-01	t 5.3-01
2	p 6.6-02	s 7.2-02	p 6.6-02	s 7.2-02	r 7.8-02	s 7.2-02		p 6.6-02	p 6.6-02	s 1.2-01
3	a 6.6-02	f 5.4-02	a 6.6-02	f 5.4-02	a 6.6-02	f 5.4-02		a 6.6-02	a 6.6-02	a 6.6-02
4	t 6.6-02	h 5.4-02	t 6.6-02	h 5.4-02	v 6.6-02	h 5.4-02		t 6.6-02	t 6.6-02	p 6.6-02
5	f 5.4-02	n 5.4-02	f 5.4-02	n 5.4-02	s 6.2-02	n 5.4-02		f 5.4-02	f 5.4-02	i 6.0-02
6	c 5.4-02	c 5.4-02	c 5.4-02	c 5.4-02	c 5.4-02	c 5.4-02		c 5.4-02	c 5.4-02	n 5.4-02
7	y 5.4-02	d 5.4-02	y 5.4-02	d 5.4-02	d 5.4-02	d 5.4-02		y 5.4-02	y 5.4-02	r 2.0-02
8	l 2.0-02	. 5.4-02	l 2.0-02	. 5.4-02	e 1.2-02	. 5.4-02		l 2.0-02	l 2.0-02	k 1.2-02
9	. 1.2-02	b 4.8-02	. 1.2-02	b 4.8-02	l 9.6-03	b 4.8-02		. 1.2-02	. 1.2-02	l 9.6-03
10	r 9.6-03	l 2.0-02	r 9.6-03	l 2.0-02	t 8.1-03	l 2.0-02		r 9.6-03	r 9.6-03	g 8.1-03
11	g 8.1-03	k 1.2-02	g 8.1-03	k 1.2-02	p 8.1-03	k 1.2-02		g 8.1-03	g 8.1-03	v 8.1-03
12	v 8.1-03	q 1.2-02	v 8.1-03	q 1.2-02	i 7.4-03	q 1.2-02		v 8.1-03	v 8.1-03	f 6.6-03
13	i 7.4-03	e 1.2-02	i 7.4-03	e 1.2-02	. 6.6-03	e 1.2-02		i 7.4-03	i 7.4-03	c 6.6-03
14	h 6.6-03	r 9.6-03	h 6.6-03	r 9.6-03	f 6.6-03	r 9.6-03		h 6.6-03	h 6.6-03	h 6.6-03
15	n 6.6-03	t 8.1-03	n 6.6-03	t 8.1-03	h 6.6-03	t 8.1-03		n 6.6-03	n 6.6-03	d 6.6-03
16	d 6.6-03	v 8.1-03	d 6.6-03	v 8.1-03	y 6.6-03	v 8.1-03		d 6.6-03	d 6.6-03	y 6.6-03
17	w 5.9-03	a 8.1-03	w 5.9-03	a 8.1-03	n 6.6-03	a 8.1-03		w 5.9-03	w 5.9-03	m 5.9-03
18	b 5.9-03	g 8.1-03	b 5.9-03	g 8.1-03	w 5.9-03	g 8.1-03		b 5.9-03	b 5.9-03	q 1.5-03
19	q 1.5-03	p 8.1-03	q 1.5-03	p 8.1-03	q 1.5-03	p 8.1-03		q 1.5-03	q 1.5-03	e 1.5-03
20	k 1.5-03	i 7.4-03	k 1.5-03	i 7.4-03	k 1.5-03	i 7.4-03		k 1.5-03	k 1.5-03	. 1.5-03
21	e 1.5-03	w 5.9-03	e 1.5-03	w 5.9-03	m 7.3-04	w 5.9-03		e 1.5-03	e 1.5-03	w 7.3-04
22	m 7.3-04	m 7.3-04	m 7.3-04	m 7.3-04	b 7.3-04	m 7.3-04		m 7.3-04	m 7.3-04	b 7.3-04

	s(zqz)		C(TGT)	y(zez)		t(eqz)		q(qej)		h(qez)	
1	s	5.4-01	c 1.000	y	4.4-01	t	5.3-01	q	4.4-01	h	4.4-01
2	p	6.6-02		s	7.2-02	s	1.2-01	h	9.6-02	q	9.6-02
3	a	6.6-02		f	5.4-02	a	6.6-02	l	7.2-02	l	6.7-02
4	t	6.6-02		h	5.4-02	p	6.6-02	r	7.2-02	r	6.7-02
5	f	5.4-02		n	5.4-02	i	6.0-02	p	6.6-02	p	6.6-02
6	c	5.4-02		c	5.4-02	n	5.4-02	e	5.4-02	n	5.4-02
7	y	5.4-02		d	5.4-02	r	2.0-02	k	5.4-02	d	5.4-02
8	l	2.0-02		.	5.4-02	k	1.2-02	b	4.8-02	y	5.4-02
9	.	1.2-02		b	4.8-02	l	9.6-03	d	1.2-02	s	1.5-02
10	r	9.6-03		l	2.0-02	g	8.1-03	y	1.2-02	k	1.2-02
11	g	8.1-03		k	1.2-02	v	8.1-03	n	1.2-02	e	1.2-02
12	v	8.1-03		q	1.2-02	f	6.6-03	s	9.6-03	g	8.1-03
13	i	7.4-03		e	1.2-02	c	6.6-03	t	8.1-03	t	8.1-03
14	h	6.6-03		r	9.6-03	h	6.6-03	v	8.1-03	v	8.1-03
15	n	6.6-03		t	8.1-03	d	6.6-03	a	8.1-03	a	8.1-03
16	d	6.6-03		v	8.1-03	y	6.6-03	g	8.1-03	i	7.4-03
17	w	5.9-03		a	8.1-03	m	5.9-03	.	6.6-03	.	6.6-03
18	b	5.9-03		g	8.1-03	q	1.5-03	w	5.9-03	c	6.6-03
19	q	1.5-03		p	8.1-03	e	1.5-03	m	5.9-03	f	6.6-03
20	k	1.5-03		i	7.4-03	.	1.5-03	i	2.2-03	b	5.9-03
21	e	1.5-03		w	5.9-03	w	7.3-04	f	1.5-03	w	7.3-04
22	m	7.3-04		m	7.3-04	b	7.3-04	c	1.5-03	m	7.3-04

Most likely sequence has frequency = 4.8E-05
Fraction stop-free = 5.5E-01
Fraction (TAA&TGA)-free = 7.4E-01

		F%	F%	F%
D1	1-1	0.42	0.14	**2.90**
	1-7	0.42	0.28	1.24
	1-20	0.00	0.00	0.00
	1-26	0.00	0.97	1.80
D2	2-2	0.55	**4.30**	1.21
	2-8	0.00	0.67	0.41
	2-15	0.28	**4.03**	0.94
	2-21	0.00	**2.22**	0.94
D3	3-3	0.94	4.44	**3.70**
	3-9	0.67	1.82	0.00
	3-10	0.67	**5.78**	1.55
	3-16	1.08	**2.49**	0.67
	3-22	0.14	**7.87**	0.81
D4	4-4	0.28	0.69	0.28
	4-11	0.00	0.00	0.00
	4-17	0.00	**4.03**	**2.76**
	4-23	0.14	1.41	0.54
D5	5-5	1.34	0.40	**4.30**
	5-12	1.08	0.00	1.95
	5-18	0.00	0.00	0.00
	5-24	0.67	1.55	1.82
D6	6-6	1.21	1.55	0.13
	6-13	**4.84**	**2.62**	0.27
	6-19	**6.66**	1.95	0.54
D7	7-27	0.27	0.13	0.27
	Total fractional %	21.65	49.34	29.01

!Table 800: LC K1(O12)::JK1

```
!
!        ..Leader seq. ->|-------- FR1 ------------------------------>
!                                  1   2   3   4   5   6   7   8   9  10  11
!          G   V   H   S   A   Q   D   I   Q   M   T   Q   S   P   S   S   L
    1   |ggT|GTA|CAc|aGT|GCT|Cag|gat|att|cag|atg|act|caa|tct|ccC|TCG|AGt|ctg|
!           BsrGI...   ApaLI...                                       XhoI....
!
!        -------- FR1 ---------------------------------->|--- CDR1 ->
!         12  13  14  15  16  17  18  19  20  21  22  23  24  25  26
!          S   A   S   V   G   D   R   V   T   I   T   C   R   A   S
   46   |tct|gct|tct|gtc|gGC|GAT|CGC|gtt|act|att|act|tgt|cgt|gct|tcc|
!                       SgfI......
!
!        ---- CDR1 --------------------->|---- FR2 ----------------->
!         27  28  29  30  31  32  33  34  35  36  37  38  39  40  41
!          Q   S   I   S   S   Y   L   N   W   Y   Q   Q   K   P   G
   91   |cag|tcc|att|tct|agc|tat|ctg|aat|tGG|TAC|Cag|caa|aag|ccg|ggt|
!                                       KpnI....
!
!        ------ FR2 ------------------->|-- CDR2 ------------------>|
!        42  43  44  45  46  47  48  49  50  51  52  53  54  55  56
```

```
!          K   A   P   K   L   L   I   Y   A   A   S   S   L   Q   S
  136    |aag|gct|ccg|aaa|ctg|tta|atc|tat|gcc|gct|tct|agt|ctg|cag|tct|
!
!          ---------- FR3 ------------------------------------------>
!          57  58  59  60  61  62  63  64  65  66  67  68  69  70  71
!           G   V   P   S   R   F   S   G   S   G   S   G   T   D   F
  181    |ggt|gtt|ccg|TCT|AGA|ttc|tct|ggc|tct|ggt|tct|ggt|act|gat|ttt|
!                     XbaI...
!
!          ---------- FR3 ------------------------------------------>
!          72  73  74  75  76  77  78  79  80  81  82  83  84  85  86
!           T   L   T   I   S   S   L   Q   P   E   D   F   A   T   Y
  226    |act|ctg|act|att|tcc|tct|ctg|caa|ccg|gag|gac|ttt|gct|acc|tat|
!
!          - FR3->|---- CDR3 ------------------------>|--- FR4 ------>
!          87  88  89  90  91  92  93  94  95  96  97  98  99 100 101
!           Y   C   Q   Q   S   Y   S   T   P   W   T   F   G   Q   G
  271    |tac|tgc|caa|cag|tct|tat|agt|act|ccg|tgg|act|ttc|ggt|caa|ggc|
!
!          ---- FR4 ------------->|---- Ckappa---------------------->
!          102 103 104 105 106 107 108 109 110 111 112 113 114 115 116
!            T   K   V   E   I   K   R   T   V   A   A   P   S   V   F
  316    |act|aaa|gtt|gag|att|aag|CGT|ACG|gtg|gct|gct|ccg|tct|gtc|ttc|
!                                 BsiWI..
```

Table 900: CDR1 diversity												
												Diversity
Position	24	25	26	27	28	29	30	31	32	33	34	
O12	R	A	S	Q	S	I	S	S	Y	L	N	
diversity	2	2	1	1	3	1	2	2	4	1	3	576
allowed	Q	M			D		R	N	D		A	
					G				W		G	
									A			

Table 1000: Big CDR1 diversity												
												Diversity
Position	24	25	26	27	28	29	30	31	32	33	34	
O12	R	A	S	Q	S	I	S	S	Y	L	N	
diversity	3	2	4	1	5	1	4	5	5	1	6	72000
allowed	Q	M	E		D		R	N	D		A	
	E		R		G		E	E	W		G	
			Y		R		Y	R	A		D	
					Y			Y	R		R	
											Y	

Table 1100: CDR2 diversity								
POSITION	50	51	52	53	54	55	56	Diversity
012	A	A	S	S	L	Q	S	
diversity	2	1	1	3	1	2	2	24
allowed	D			N		E	T	
				T				

Table 1200: Big CDR2 diversity								
POSITION	50	51	52	53	54	55	56	Diversity
012	A	A	S	S	L	Q	S	
diversity	4	1	4	6	1	4	5	1920
allowed	D		E	N		E	T	
	R		R	T		R	Y	
	Y		Y	E		Y	R	
				R			E	
				Y				

Table 1300: CDR3 diversity											
Position	93	94	95	96	97	98	99	100	101	div.	tot.
012	Q	Q	S	Y	S	S	P	W	T		
diversity	2	2	6	3	3	5	2	1	1	2160	
allowed	L	K	Y	D	N	T	S				
			H	N	Y	L					
			F			Y					
			A			F					
			D								

Table 1400: Big CDR3 diversity											
Position	93	94	95	96	97	98	99	100	101	div.	tot.
012	Q	Q	S	Y	S	S	P	W	T		
diversity	6	1	7	7	6	5	2	6	1	105840	
allowed	L		Y	D	N	T	S	F			
	E		H	N	Y	L		Y			
	R		F	R	D	Y		H			

	Y		A	A	R	F		L			
	A		D	L	A	E		I			
			R	S		R					

[00227] Example 7: Further examples of synthetic HC CDR3s

[00228] A collection of 22,063 Fabs with distinct CDR3 which had been selected from the FAB-310 or FAB-410 library and which were ELISA positive for at least one antigen were examined. The utilization of JH chains is shown in Table 1001; the FR4 part of each JH is shown **bold**. Table 1010 shows the utilization of amino acids in the HC CDR3s. Table 1020 shows the length distribution of CDR3. The median length is 11.5.

[00229] Table 1030 shows the utilization of D segments in the CDR3s. A D segment was identified is 70% of the amino acids matched; there were 5,654 cases (25.6%). The most used Ds were 3-3.2 (743, sequence: YYDFWSGYYT), 3-22.2 (617, sequence: YYYDSSGYYY), 6-19.1 (441, sequence: GYSSGWY), 6-13.1 (399, sequence: GYSSSWY), and 4-17.2 (392, sequence: DYGDY). Of the Ds containing paired Cys residues, 2-15.2 (sequence: GYCSGGSCYS) was the most used; there were 139 examples which is 0.6% of the collection.

[00230] When V or V::D is joined to J, there is often editing of the 3' end of V or V::D and the 5' end of J. Inspection of many CDR3-FR4 sequences shows that there is often a portion of JH making up part of CDR3. Often there are mutations in the CDR3 residues corresponding to JH residues 1-9. Herein the portion of CDR3 that is thought to derive from JH is called the "J stump". The JH used in a heavy chain is determined by comparing each of the residues of the six JH chains from position 6 to 20 to fusion of the last four amino acids of CDR3 to FR4. The JH that has the fewest mismatches is selected. The CDR3 sequence is examined for a J stump by working backward in the selected JH from position 9 toward the first position of the selected JH comparing to CDR3 until the search is terminated by a) the end of JH, b) the end of CDR3, or c) two consecutive mismatches. If one of the chains ends and the last compared position is a match, then it is included in J stump. If not, it is not. Table 1070 shows several examples. The CDR is written above, the JH is below, and the J stump is underlined. In 1070 A, we start at 9, V matches V, and we continue to position 6 with matches. The search stops at 4 because of the double mismatch. GMDV goes into the J stump pile and GL goes into the "Leadin" pile. In 1070 B, the search ends with the end of JH6. The underscored residues go into the J stump pile and EPIWG goes into the Leadin pile. In 1070 E, the search terminates because of the end of

JH4, but the final residue tested (D in the CDR vs Y in JH4) is a mismatch and so the J stump is FDS and DSGVVAAAD goes into the Leadin pile.

[00231] Table 1015 shows the amino-acid distribution of CDR3s that have no D segments from which the J stump has been removed. Note that the frequency of Tyr is much lower than when the whole CDR3s were compiled. This indicates that Tyr comes into CDR3s to a large extent through incorporation of D segments and J stumps. These Tyrs are not randomly inserted, but occur in a sequence that has been selected throughout mammalian evolution. It is a feature of the present invention that high levels (more than 20%) of Tyr should be inserted into libraries through the incorporation of Ds and J stumps that contain Tyr. At leadin or DJ filler positions, Tyr is allowed, but at no more than 20%.

Table 1070: Examples of assignment of J stump

```
A)
  6          GLGMDV
JH6       YYYYYGMDVWGQGTTVTVSS
          123456789

B)
13     EPIWGYYYYGMDV
JH6         YYYYYGMDVWGQGTTVTVSS

C)
  9       DFFTSYFDY
JH4       -----YFDYWGQGTLVTVSS

D)
12    DRGVSLLGAFDI
JH3      -----AFDIWGQGTMVTVSS

E)
12    DSGVVAAADFDS
JH4      -----YFDYWGQGTLVTVSS
               6789
```

[00232] Table 1082 shows the distribution of amino-acid usage in the J stumps of each JH. Since the most common JHs are JH3, JH4, and JH6, these are the preferred JHs on which to build libraries. Table 1082 shows that most examples of JH3 retain the tetrapeptide sequence AFDI in CDR3. With JH4, a majority retain DY and a large fraction retain the sequence FDY in CDR3. With JH6, a large majority retain the sequence DV, a majority retain the sequence MDV, and a substantial fraction retain the sequence GMDV. A non-negligible fraction retain the sequence YGMDV, YYGMDV, or YYYGMDV.

[00233] Included in libraries of the present invention are libraries such as 5.001 (Table 1097). Library 5.001 contains LC and HC CDR1-2 as described elsewhere in the present application. The library contains a HC VH (such as 3-23) followed by 6, 7, or 8 amino acids allowing [GSRDLY] in proportion shown in Table 1097. In the J stump, the parental amino acid is present at 3, 4, 5, 6, 7, 8, 10 times as likely as "other" amino-acid types. The "other" amino-acid types comprise Y, S, D, R, G. Thus at A6, we allow 7/12 A, plus 1/12 each of Y, S, D, R, and G. At F7, we allow 7/12 F plus 1/12 each of Y, S, D, R, and G. At D8, we allow 7/11 D plus 1/11of Y, S, R, and G. At I9, we allow 7/12 I plus 1/12 Y, S, R, D, G. The parental amino acid could be 5, 6, 7, 8, 10 time more likely than the other amino-acid types.

[00234] Included in the libraries of the present invention is library 5.002 in Table 1097. This library comprises CDR3 of length 13, 14, and 15 and no D segment. There are 6, 7, or 8 leadin residues allowing G, S, R, D, L, or Y in the ratios 1:0.57:0.46:0.42:0.36:0.35 or reasonable approximation thereto. The CDR3 is completed with a portion of JH6: YYYGMDV. The DNA that encodes the parental sequence YYYGMDV is synthesized with the parental amino acid at 5, 6, 7, 8, or 10 times more likely than the others.

[00235] Included in the library of the present invention is library 5.003 in Table 1097. FR3 is followed by 4, 5, or 6 leadin residues allowing G, S, R, D, L, Y in the ratio 1.0:0.57:0.46:0.42:0.36:0.35. Next comes D segment 3-3.2; the DNA that encodes this region favors the parental amino acid by 5-fold and allows as other amino acids Y, G, D, R, S. There is no DJ filler and the final four amino acids come from the J stump of JH3. The DNA encoding the J stump are synthesized with the parental amino acid 5-fold more likely than the others: YSGRD.

[00236] Library 5.004 in Table 1097 is a part of the present invention. There are 2, 3, or 4 leadin residues allowing GSRDLY in the ratios shown. The DNA encoding the sequence GYSSGWY is synthesized so that the parental amino acid is 6-X as likely as the others, two DJ-filler residues are allowed with GSRDLY allowed in the ratios 1.0:0.57:0.46:0.42:0.36:0.35. The DNA to encode AFDI is synthesized with the parental amino acid 6-x as likely as the others.

[00237] Library 5.005 is part of the present invention. Library 5.005 comprises members with CDR3 lengths 11-14. After FR3, there are 0, 1, or 2 leadin residues allowing GSRDLY in the ratios shown followed by DNA that encodes the parental sequence GYSSGWY with variability that allows YGSRD such that the parental amino acid is 6-X as likely as the other

allowed types. Following the D region there is zero or one DJ filler residues allowing GSRDLY in the ratios shown. Finally is JH3 with variability in the J stump (sequence: YFDY) which allows YGSRD with the parental amino acid 6-X as likely as the other allowed types.

[00238] Library 5.006 in Table 1097 is part of the present invention. The CDR3 may be of length 19-25. There are zero to three leadin residues allowing GSRDLY in the ratios shown. Following the leadin is the D region 2-2.2. The DNA encoding 2-2.2 is synthesized so that the parental amino acid is 6-X as likely as the others (viz. YGSRD) except that the two Cys residues are fixed. Following 2-2.2 are zero to three DJ filler residues allowing GSRDLY in the ratios shown. The DNA that encodes the first nine residues of JH6 allows the parental amino acid plus YSGDR with the parental type being 6X more likely than the others.

Table 1001: Utilization of JHs

JH	Number	%	11111111112 12345678901234567890
JH1	1356	6.15	---AEYFQH**WGQGTLVTVSS**
JH2	1720	7.80	---YWYFDL**WGRGTLVTVSS**
JH3	5601	25.39	-----AFDI**WGQGTMVTVSS**
JH4	7658	34.71	-----YFDY**WGQGTLVTVSS**
JH5	1062	4.81	----NWFDP**WGQGTLVTVSS**
JH6	4666	21.15	YYYYYGMDV**WGQGTTVTVSS**
Total	22063		

Table 1010: Utilization of Amino acids in HC CDR3

AA	Number	%	Rel up	Rel dwn
Y	42863	15.47	35.87	1.00
G	37512	13.54	31.39	0.88
D	34051	12.29	28.49	0.79
S	23068	8.33	19.30	0.54
F	17813	6.43	14.91	0.42
A	15150	5.47	12.68	0.35
R	14090	5.09	11.79	0.33
V	13834	4.99	11.58	0.32
L	12351	4.46	10.34	0.29
I	10014	3.61	8.38	0.23
P	9514	3.43	7.96	0.22
W	9340	3.37	7.82	0.22
T	7544	2.72	6.31	0.18
M	6093	2.20	5.10	0.14
E	6042	2.18	5.06	0.14
N	5901	2.13	4.94	0.14
H	4403	1.59	3.68	0.10
K	3147	1.14	2.63	0.07
Q	3097	1.12	2.59	0.07
C	1195	0.43	1.00	0.03
	277022			

Table 1015: Frequency of amino acids in Leadin of CDR3s lacking D regions				
AA	Number	percent	rel up	rel dn
G	23134	18.24	46.45	1.000
S	13555	10.69	27.22	0.586
R	10562	8.33	21.21	0.457
D	9704	7.65	19.49	0.419
L	8255	6.51	16.58	0.357
Y	8099	6.39	16.26	0.350
A	7188	5.67	14.43	0.311
V	6599	5.20	13.25	0.285
P	5768	4.55	11.58	0.249
W	4804	3.79	9.65	0.208
T	4769	3.76	9.58	0.206
E	4497	3.55	9.03	0.194
N	3733	2.94	7.50	0.161
F	3616	2.85	7.26	0.156
I	3464	2.73	6.96	0.150
H	2787	2.20	5.60	0.120
K	2460	1.94	4.94	0.106
Q	2124	1.67	4.27	0.092
M	1225	0.97	2.46	0.053
C	498	0.39	1.00	0.022
	126841			

Table 1020: Lengths of HC CDR3s		
Length	Number	%
1	0	0.00
2	6	0.03
3	36	0.16
4	153	0.69
5	121	0.55
6	669	3.03
7	756	3.43
8	1066	4.83
9	2227	10.09
10	2701	12.24
11	2240	10.15
12	2071	9.39
13	2006	9.09
14	1594	7.22
15	1396	6.33
16	1254	5.68
17	1102	4.99

Table 1020: Lengths of HC CDR3s		
Length	Number	%
18	783	3.55
19	588	2.67
20	474	2.15
21	285	1.29
22	237	1.07
23	133	0.60
24	81	0.37
25	32	0.15
26	25	0.11
27	11	0.05
28	6	0.03
29	2	0.01
30	3	0.01
31	2	0.01
32	1	0.00
33	1	0.00
34	0	0.00
35	0	0.00
36	1	0.00
	22063	

Table 1030: Utilization of D segments.		
Id	Number	Sequence
1-1.1	29	GTTGT
1-1.2	6	VQLER
1-1.3	151	YNWND
1-7.1	34	GITGT
1-7.2	0	V*LEL
1-7.3	65	YNWNY
1-20.1	0	GITGT
1-20.2	0	V*LER
1-20.3	0	YNWND
1-26.1	48	GIVGAT
1-26.2	3	V*WELL
1-26.3	220	YSGSYY
2-2.1	0	RIL**YQLLY
2-2.2	102	GYCSSTSCYT
2-2.3	37	DIVVVPAAI
2-8.1	0	RILY@WCMLY
2-8.2	23	GYCINGVCYT
2-8.3	1	DIVLMVYAI
2-15.1	0	RIL*WW*LLL
2-15.2	139	GYCSGGSCYS
2-15.3	12	DIVVVVAAT
2-21.1	0	SILWW$LLF

Table 1030: Utilization of D segments.		
Id	Number	Sequence
2-21.2	24	AYCGGDCYS
2-21.3	6	HIVVVTAI
3-3.1	28	VLRFLEWLLY
3-3.2	743	YYDFWSGYYT
3-3.3	15	ITIFGVVII
3-9.1	41	VLRYFDWLL@
3-9.2	8	YYDILTGYYN
3-9.3	0	ITIF*LVII
3-10.1	26	VLLWFGELL@
3-10.2	136	YYYGSGSYYN
3-10.2	32	ITMVRGVII
3-16.1	0	VL$LRLGELSLY
3-16.2	109	YYDYVWGSYRYT
3-16.2	8	IMITFGGVIVI
3-22.1	0	VLL***WLLL
3-22.2	617	YYYDSSGYYY
3-22.3	2	ITMIVVVIT
4-4.1	0	$LQ@L
4-4.2	75	DYSNY
4-4.3	165	TTVT
4-11.1	0	$LQ@L
4-11.2	0	DYSNY
4-11.3	0	TTVT
4-17.1	0	$LR@L
4-17.2	392	DYGDY
4-17.3	0	TTVT
4-23.1	0	$LRW@L
4-23.2	60	DYGGNS
4-23.3	16	TTVVT
5-5.1	25	VDTAMV
5-5.2	29	WIQLWL
5-5.3	292	GYSYGY
5-12.1	13	VDIVATI
5-12.2	0	WI*WLRL
5-12.3	200	GYSGYDY
5-18.1	0	VDTAMV
5-18.2	0	WIQLWL
5-18.3	0	GYSYGY
5-24.1	9	VEMATI
5-24.2	21	*RWLQL
5-24.3	44	RDGYNY
6-6.1	87	EYSSSS
6-6.2	122	SIAAR
6-6.3	1	V*QLV
6-13.1	399	GYSSSWY

Table 1030: Utilization of D segments.		
Id	Number	Sequence
6-13.2	170	GIAAAG
6-13.3	0	V*QQLV
6-19.1	441	GYSSGWY
6-19.2	104	GIAVAG
6-19.3	3	V*QWLV
7-27.1	257	LTG
7-27.2	0	@LG
7-27.3	64	NWG
none	16409	

Table 1040: JH vs Length						
Length	JH1	JH2	JH3	JH4	JH5	JH6
1	0	0	0	0	0	0
2	1	4	0	1	0	0
3	20	2	3	9	0	2
4	75	3	10	45	8	12
5	47	6	10	38	8	12
6	273	14	43	280	26	33
7	88	27	194	337	30	80
8	134	43	243	503	41	102
9	121	70	**855**	886	61	234
10	116	693	623	979	68	222
11	105	81	675	**1003**	84	292
12	107	84	552	905	121	302
13	87	274	538	672	113	322
14	48	81	480	532	105	348
15	50	83	372	421	80	390
16	28	54	316	322	87	**447**
17	27	49	239	334	69	384
18	11	64	174	140	49	345
19	8	28	104	99	41	308
20	4	23	59	56	20	312
21	0	13	40	30	24	178
22	3	14	31	30	13	146
23	1	3	22	12	7	88
24	0	5	9	12	4	51
25	1	0	1	3	1	26
26	0	0	5	5	0	15
27	0	1	2	1	1	6
28	1	0	0	2	0	3
29	0	0	0	0	0	2
30	0	0	0	0	0	3
31	0	0	1	0	0	1
32	0	1	0	0	0	0
33	0	0	0	1	0	0

Table 1040: JH vs Length						
Length	JH1	JH2	JH3	JH4	JH5	JH6
34	0	0	0	0	0	0
35	0	0	0	0	0	0
36	0	0	0	0	1	0

Table 1050: Utilization of amino acids in Leadin with no D segment

AA	Number	%	Rel up	Rel dn
G	23134	18.24	46.45	1.00
S	13555	10.69	27.22	0.59
R	10562	8.33	21.21	0.46
D	9704	7.65	19.49	0.42
L	8255	6.51	16.58	0.36
Y	8099	6.39	16.26	0.35
A	7188	5.67	14.43	0.31
V	6599	5.20	13.25	0.29
P	5768	4.55	11.58	0.25
W	4804	3.79	9.65	0.21
T	4769	3.76	9.58	0.21
E	4497	3.55	9.03	0.19
N	3733	2.94	7.50	0.16
F	3616	2.85	7.26	0.16
I	3464	2.73	6.96	0.15
H	2787	2.20	5.60	0.12
K	2460	1.94	4.94	0.11
Q	2124	1.67	4.27	0.09
M	1225	0.97	2.46	0.05
C	498	0.39	1.00	0.02
	126841			

Table 1080: Dipepides in HC CDR3s, part 1							
YY	13565	FG	1073	PL	591	TV	397
FD	11637	RS	1072	TT	589	TP	390
DY	8337	SW	1014	ID	588	NA	389
SG	5979	DW	1003	DD	583	NS	388
GY	5805	LR	990	AS	570	ER	387
YG	5461	DG	989	KG	566	HG	386
DI	5448	PG	976	VD	556	VW	381
AF	4975	LL	974	VP	551	QL	378
DV	4968	AY	962	LT	540	RI	374
GG	4575	DR	923	LF	539	WN	365
SS	4491	VR	882	VL	539	YT	365
MD	4436	YM	877	FY	534	CS	360
GS	4047	AR	872	PD	533	DH	359

GM	3501	VV	869	RV	531	EA	359
YF	3438	YR	865	RF	525	WD	353
YD	3430	VA	857	AL	521	ES	350
RG	3118	RA	844	PS	510	FR	349
SY	2770	SP	820	EY	508	YC	343
GA	2611	GN	812	LW	508	PT	337
YS	2576	HY	809	PA	505	TL	326
DA	2285	SD	805	LP	500	KR	325
DS	2087	GI	804	VS	497	VF	324
WY	2079	NW	785	IR	493	MG	314
GD	2017	LS	760	YV	493	PN	313
GR	1985	LY	757	VY	478	RE	312
GL	1800	TY	749	IG	476	IV	311
DL	1777	PR	742	VT	475	KS	310
DF	1763	GE	737	TR	472	SC	310
GW	1725	SA	736	DN	471	FL	309
WS	1675	SF	728	SI	469	FF	306
AA	1671	PF	725	AD	462	CY	303
LD	1651	ND	693	LA	459	SH	302
EG	1610	ST	684	PP	451	LK	300
AG	1606	GH	683	RT	451	IT	298
RY	1558	YP	676	DT	448	LE	298
DP	1547	WL	675	RW	447	FS	296
GV	1500	SN	667	GQ	446	ED	294
RR	1498	TS	652	QG	446	RK	294
LG	1387	RD	648	TD	446	HF	292
GF	1386	YA	648	TA	437	VI	290
VG	1366	SL	644	TF	426	RH	287
GP	1339	RP	643	GK	422	MV	285
WF	1282	YL	638	YW	421	KY	284
FW	1277	IA	634	HD	420	AI	282
NY	1271	RL	627	IL	417	HS	281
PY	1209	EL	622	LV	406	YH	281
GT	1194	YN	607	IS	402	LN	278
WG	1177	AV	605	NG	398	PV	276
SR	1162	AP	600	RN	398	QY	276
TG	1142	AT	592	SV	397	WA	271

Table 1080: Dipeptides in HC CDR3s, part 2							
QH	267	KD	176	II	102	NQ	53
FQ	264	SK	176	HI	101	CF	51
LI	257	YK	176	KP	101	MP	50

Table 1080: Dipeptides in HC CDR3s, part 2							
EV	255	EF	174	MY	100	CP	49
AM	253	FN	174	RM	99	RC	47
DQ	250	HN	171	AQ	98	HE	46
HR	250	FH	165	EQ	96	VC	46
PH	248	YQ	165	QT	96	QI	45
AN	242	KN	164	LM	95	MN	44
WR	242	MA	163	HV	94	MF	43
NF	240	NN	160	IK	93	HQ	41
PI	239	KA	159	PM	93	CD	38
TN	239	SQ	157	QN	93	CL	38
TI	238	PE	156	CG	91	NC	38
PW	229	WV	154	QF	91	HM	37
IP	228	EI	153	FI	90	FM	36
QR	227	TH	153	HW	90	ME	36
EW	225	FV	152	WH	90	MK	35
YI	221	AK	151	QV	89	QM	35
FE	220	TK	151	WI	89	NM	34
IY	220	WT	151	KH	88	KM	32
EP	219	PK	150	MI	88	TC	31
NR	217	KK	148	MS	87	CR	29
DM	214	IW	145	TQ	86	CV	25
FA	212	VH	145	NV	85	HC	25
AE	210	VE	141	EM	84	WM	25
IF	210	EE	138	HK	84	AC	24
QW	208	DE	136	IN	83	FC	24
YE	208	KL	136	NH	82	CA	23
FP	201	PQ	136	NI	82	CH	21
TM	201	QP	135	HT	81	CN	21
WE	201	SM	134	WK	79	MW	21
WP	201	QD	133	KF	77	PC	19
AH	199	QS	131	VM	73	LC	17
NP	198	VQ	130	MT	71	IC	16
VN	198	QQ	129	IH	69	MM	16
HA	196	WW	129	EH	68	MH	15
LH	196	NT	128	IE	67	WC	15
AW	193	DC	118	QK	65	EC	12
HP	192	KT	118	WQ	65	CK	10
HL	191	QA	118	GC	64	CW	10
RQ	191	NK	113	KE	61	MQ	10
TW	186	KW	112	KI	61	CI	9

Table 1080: Dipeptides in HC CDR3s, part 2							
EN	185	EK	109	CT	58	CC	8
LQ	182	FT	108	FK	58	CM	8
SE	180	KV	108	IM	57	CQ	6
VK	180	MR	105	KQ	57	QC	6
ET	178	TE	104	ML	55	CE	5
DK	177	HH	103	QE	55	KC	5
NL	177	IQ	103	NE	53	MC	3

Table 1060a Lengths of HC CDR3s vs which D segments occur (if any) for lengths 3-17

Name	Sequence	Length																
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1-1.1	GTTGT	0	0	0	0	0	0	2	2	3	6	2	3	3	4	0
1-1.2	VQLER	0	0	0	0	0	0	0	0	1	0	1	0	1	2	1
1-1.3	YNWND	0	0	0	0	0	2	6	14	16	19	16	14	17	16	9
1-7.1	GITGT	0	0	0	0	0	0	0	1	2	7	6	4	4	0	4
1-7.3	YNWNY	0	0	0	0	1	0	2	5	7	8	8	6	5	9	4
1-26.1	GIVGAT	0	0	0	1	0	0	2	4	10	4	6	9	3	2	2
1-26.2	V*WELL	0	0	0	0	0	0	0	0	1	0	1	0	0	0	1
1-26.3	YSGSYY	0	0	0	0	1	0	2	10	14	24	24	27	21	26	13
2-2.2	GYCSSTSCYT	0	0	0	0	0	0	0	0	0	2	2	9	15	15	11
2-2.3	DIVVVPAAI	0	0	0	0	0	0	0	0	0	0	1	3	2	5	5
2-8.2	GYCTNGVCYT	0	0	0	0	0	0	0	0	0	1	0	2	4	3	4
2-8.3	DIVLMVYAI	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
2-15.2	GYCSGGSCYS	0	0	0	0	0	0	0	0	1	3	5	12	10	25	22
2-15.3	DIVVVVAAT	0	0	0	0	0	0	0	0	0	0	1	1	2	3	1
2-21.2	AYCGGDCYS	0	0	0	0	0	0	0	0	0	1	1	3	5	2	5
2-21.3	HIVVVTAI	0	0	0	0	0	0	0	0	0	1	2	0	2	0	1
3-3.1	VLRFLEWLLY	0	0	0	0	0	0	0	0	0	0	1	2	2	2	3
3-3.2	YYDFWSGYYT	0	0	0	0	0	0	0	1	5	8	22	38	44	72	69
3-3.3	ITIFGVVII	0	0	0	0	0	0	0	0	0	0	0	1	0	2	5
3-9.1	VLRYFDWLL@	0	0	0	0	0	0	0	0	1	0	0	4	5	5	5
3-9.2	YYDILTGYYN	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1
3-10.1	VLLWFGELL@	0	0	0	0	0	0	0	0	2	1	2	4	2	3	5
3-10.2	YYYGSGSYYN	0	0	0	0	0	0	0	2	4	7	10	13	15	18	14
3-10.2	ITMVRGVII	0	0	0	0	0	0	0	0	0	3	1	2	7	5	2
3-16.2	YYDYVWGSYRYT	0	0	0	0	0	0	0	0	0	0	0	1	7	7	7
3-16.2	IMITFGGVIVI	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
3-22.2	YYYDSSGYYY	0	0	0	0	0	0	0	0	6	30	45	56	59	108	101
3-22.3	ITMIVVVIT	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4-4.2	DYSNY	0	0	0	0	0	3	3	4	6	14	4	7	4	10	6

Table 1060a Lengths of HC CDR3s vs which D segments occur (if any) for lengths 3-17

Name	Sequence	Length														
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
4-4.3	TTVT	0	0	0	0	2	4	11	19	23	19	25	19	10	11	9
4-17.2	DYGDY	0	0	2	6	12	8	38	40	48	47	50	40	29	21	10
4-23.2	DYGGNS	0	0	0	0	0	2	7	4	5	17	4	8	5	1	1
4-23.3	TTVVT	0	0	0	0	0	0	2	0	1	1	2	1	2	0	0
5-5.1	VDTAMV	0	0	0	0	0	0	0	1	4	8	1	3	2	0	1
5-5.2	WIQLWL	0	0	0	0	0	0	0	3	2	0	3	1	4	3	3
5-5.3	GYSYGY	0	0	0	0	1	6	9	20	43	29	27	22	32	26	27
5-12.1	VDIVATI	0	0	0	0	0	0	0	2	0	1	2	4	0	2	1
5-12.3	GYSGYDY	0	0	0	0	4	10	13	15	19	15	22	27	16	15	9
5-24.1	VEMATI	0	0	0	0	0	0	1	1	0	0	6	0	0	0	0
5-24.2	*RWLQL	0	0	0	0	1	0	3	1	3	2	2	1	2	2	2
5-24.3	RDGYNY	0	0	0	0	0	0	0	1	8	12	6	7	3	2	1
6-6.1	EYSSSS	0	0	0	0	0	0	0	9	7	16	19	13	2	4	2
6-6.2	SIAAR	0	0	0	1	1	0	17	8	7	13	17	6	16	16	7
6-6.3	V*QLV	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
6-13.1	GYSSSWY	0	0	0	0	0	1	6	11	35	40	56	44	50	42	35
6-13.2	GIAAAG	0	0	0	0	1	2	18	14	15	20	20	15	16	14	11
6-19.1	GYSSGWY	0	0	0	0	1	1	4	27	57	58	48	52	45	35	30
6-19.2	GIAVAG	0	0	0	0	1	1	0	7	8	20	8	13	16	8	10
6-19.3	V*QWLV	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
7-27.1	LTG	0	0	1	0	2	8	12	7	14	11	17	17	24	24	31
7-27.3	NWG	0	0	0	1	2	11	6	5	10	6	7	5	7	1	0
none		36	153	118	660	726	1007	2063	**2463**	1851	1596	1502	1075	874	681	609

Table 1060b Lengths of HC CDR3s vs which D segments occur (if any) for lengths 18-32

Name	Sequence	Length														
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1-1.1	GTTGT	0	1	0	0	1	1	1	0	0	0	0	0	0	0	0
1-1.2	VQLER	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-1.3	YNWND	6	5	3	4	1	1	1	0	0	1	0	0	0	0	0
1-7.1	GITGT	2	2	1	0	0	0	1	0	0	0	0	0	0	0	0

Table 1060b Lengths of HC CDR3s vs which D segments occur (if any) for lengths 18-32

Name	Sequence	Length															
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1-7.3	YNWNY	5	2	1	1	0	0	0	0	0	0	0	1	0	0	0
1-26.1	GIVGAT	0	0	3	0	1	0	0	1	0	0	0	0	0	0	0
1-26.2	V*WELL	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1-26.3	YSGSYY	14	11	9	8	6	3	3	0	3	1	0	0	0	0	0
2-2.2	GYCSSTSCYT	11	7	2	10	11	4	2	0	0	0	1	0	0	0	0
2-2.3	DIVVVPAAI	2	6	3	4	3	2	1	0	0	0	0	0	0	0	0
2-8.2	GYCTNGVCYT	3	0	1	1	3	1	0	0	0	0	0	0	0	0	0
2-8.3	DIVLMVYAI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2-15.2	GYCSGGSCYS	20	10	7	4	8	9	3	0	0	0	0	0	0	0	0
2-15.3	DIVVVVAAT	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0
2-21.2	AYCGGDCYS	1	3	1	1	1	0	0	0	0	0	0	0	0	0	0
2-21.3	HIVVVTAI	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3-3.1	VLRFLEWLLY	6	2	4	3	2	0	0	0	1	0	0	0	0	0	0
3-3.2	YYDFWSGYYT	82	97	**104**	67	61	32	23	7	3	4	0	0	2	1	0
3-3.3	ITIFGVVII	3	2	0	0	2	0	0	0	0	0	0	0	0	0	0
3-9.1	VLRYFDWLL@	7	2	2	6	1	2	1	0	0	0	0	0	0	0	0
3-9.2	YYDILTGYYN	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0
3-10.1	VLLWFGELL@	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0
3-10.2	YYYGSGSYYN	15	10	8	7	6	3	2	1	1	0	0	0	0	0	0
3-10.2	ITMVRGVII	3	3	6	0	0	0	0	0	0	0	0	0	0	0	0
3-16.2	YYDYVWGSYRYT	11	11	14	10	18	13	5	2	2	0	0	0	1	0	0
3-16.2	IMITFGGVIVI	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0
3-22.2	YYYDSSGYYY	77	54	28	22	18	8	1	2	1	1	0	0	0	0	0
3-22.3	ITMIVVVIT	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
4-4.2	DYSNY	7	2	2	1	1	0	1	0	0	0	0	0	0	0	0
4-4.3	TTVT	4	2	2	1	3	0	0	0	0	0	1	0	0	0	0
4-17.2	DYGDY	17	7	8	3	2	1	1	0	1	0	1	0	0	0	0
4-23.2	DYGGNS	3	1	0	2	0	0	0	0	0	0	0	0	0	0	0
4-23.3	TTVVT	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0
5-5.1	VDIAMV	0	1	1	2	1	0	0	0	0	0	0	0	0	0	0

Table 1060b Lengths of HC CDR3s vs which D segments occur (if any) for lengths 18-32

Name	Sequence	Length															
		18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
5-5.2	WIQLWL	3	3	1	0	1	0	0	0	0	1	0	0	0	1	0
5-5.3	GYSYGY	13	18	7	2	2	6	0	0	2	0	0	0	0	0	0
5-12.1	VDIVATI	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
5-12.3	GYSGYDY	11	10	6	6	0	2	0	0	0	0	0	0	0	0	0
5-24.1	VEMATI	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
5-24.2	*RWLQL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5-24.3	RDGYNY	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
6-6.1	EYSSSS	9	3	1	1	0	0	0	1	0	0	0	0	0	0	0
6-6.2	SIAAR	2	3	2	6	0	0	0	0	0	0	0	0	0	0	0
6-6.3	V*QLV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6-13.1	GYSSSWY	24	22	17	4	5	1	3	2	0	0	1	0	0	0	0
6-13.2	GIAAAG	10	4	2	5	2	1	0	0	0	0	0	0	0	0	0
6-19.1	GYSSGWY	23	25	13	7	7	3	0	2	1	0	1	0	0	0	0
6-19.2	GIAVAG	6	2	2	0	0	0	2	0	0	0	0	0	0	0	0
6-19.3	V*QWLV	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
7-27.1	LTG	29	23	12	9	7	4	3	1	0	1	0	0	0	0	0
7-27.3	NWG	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0
none		339	217	198	85	60	36	27	13	9	2	1	1	0	0	1

Table 1082: Tally of J stumps

JH1	---AEYFQHWGQGTLVTVSS 6.15%																														
		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-								
4		41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	605									
5		1	0	1	64	0	6	0	1	0	0	0	0	0	1	2	1	1	0	0	0	568									
6		0	0	0	0	0	1	1	0	0	3	0	2	1	0	1	1	0	0	0	**211**	425									
7		1	0	1	0	**363**	3	1	0	0	8	0	0	1	0	1	6	0	3	0	6	252									
8		8	0	59	23	4	17	11	3	5	19	0	8	3	**221**	11	6	8	5	0	4	231									
9		2	1	13	2	13	3	**447**	19	2	6	3	20	1	2	0	20	3	5	4	9	71									
JH2	---YWYFDLWGRGTLVTVSS 7.80%																														
		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-								
4		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	159	1519									
5		0	0	2	0	2	11	0	0	0	1	0	1	0	0	7	1	1	1	**929**	21	701									
6		3	2	9	0	40	5	7	4	1	9	0	7	1	0	1	11	2	0	1	**1083**	492									
7		1	6	1	0	**1209**	2	6	23	0	89	30	1	2	1	1	12	0	11	1	42	240									
8		31	2	**1241**	90	4	38	30	3	1	0	1	29	3	4	2	2	3	19	0	15	160									
9		3	1	9	3	34	2	26	17	5	**1064**	36	17	30	38	33	20	8	83	1	177	71									
JH3	-----AFDIWGQGTMVTVSS 25.4%																														
		A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-								
6		**4374**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1074									
7		1	4	0	1	**4612**	2	0	8	0	56	3	2	1	1	0	29	2	15	12	74	625									
8		23	0	**4765**	51	0	28	14	0	3	0	0	15	2	1	1	2	2	6	0	4	531									
9		7	5	5	0	73	2	1	**4439**	4	64	64	43	2	1	11	54	49	113	2	18	491									

Table 1082: Tally of J stumps

JH4 -----YFDYWGQGTLVTVSS 34.7%

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1975**	5683
7	9	1	10	7	**3950**	31	6	26	0	109	4	5	24	4	5	35	7	28	16	59	3322
8	26	0	**5991**	32	5	91	19	2	1	6	1	21	7	0	8	13	3	6	2	14	1410
9	5	18	17	0	119	2	0	0	14	0	2	64	15	16	10	216	11	3	6	**6317**	823

JH5 ----NWFDPWGQGTLVTVSS 4.8%

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
5	0	0	0	0	0	0	0	0	0	0	0	274	0	0	0	0	0	0	0	0	764
6	2	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	**644**	0	390
7	1	1	0	0	**768**	2	1	1	0	13	0	0	0	0	0	1	0	2	0	4	244
8	2	0	**810**	3	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	1	218
9	3	0	3	0	3	0	3	0	0	4	0	4	**814**	1	0	14	2	0	0	0	187

JH6 YYYYYGMDVWGQGTTVTVSS 21.1%

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	-
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	690	3967
2	4	0	15	0	16	31	12	2	1	4	0	8	7	4	14	18	7	4	1	1694	2815
3	3	0	19	3	14	23	16	1	5	9	0	16	9	5	12	20	4	1	4	**2440**	2053
4	4	0	14	2	31	16	25	3	2	10	0	13	6	5	15	35	5	5	4	**2815**	1647
5	2	1	9	1	23	19	21	1	1	3	1	15	3	1	7	26	3	0	1	**3350**	1169
6	69	5	14	1	4	**3057**	8	1	0	0	0	8	7	5	4	15	3	7	0	657	792
7	3	0	1	0	4	5	0	18	2	108	**3866**	0	2	2	3	1	7	18	3	1	613
8	7	0	**4064**	5	1	17	4	1	0	2	0	11	2	0	0	3	2	3	2	6	527
9	9	0	1	0	6	1	1	19	0	7	2	1	1	0	1	4	0	**4092**	0	1	511

Table 1097: HC CDR3 libraries

Library	CDR3 Length	Leadin	D region sequence	DJ fill sequence	J stump sequence	FR4 sequence
5.001	10,11,12	6, 7, or 8 X X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	none	none	AFDI (diversity in text)	**WGQGTMVTVSS** (JH4)
5.002	13,14,15	6,7,8 X X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	none	none	YYYGMDV (diversity in text)	**WGQGTTVTVSS** (jh6)
5.003	18,19,20	4,5,6 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YYDFWSGYYT (3-3.2)	none	YFDY	**WGQGTLVTVSS** (JH3)
5.004	15,16,17	2,3,4 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYSSGWY (6-19.1)	2X,X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	AFDI	**WGQGTMVTVSS** (JH4)
5.005	11-14	0,1,2 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYSSGWY (6-19.1)	0,1 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YFDY	**WGQGTLVTVSS** (JH3)
5.006	19-25	0,1,2,3 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYCSGGSCYS (2-2.2) (Cys residues constant)	0,1,2,3 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YYYYYGMDV (parent AA 8X others)	**WGQGTTVTVSS** (jh6)

REFERENCES

[00239] The contents of all cited references including literature references, issued patents, published or non-published patent applications cited throughout this application as well as those listed below are hereby expressly incorporated by reference in their entireties. In case of conflict, the present application, including any definitions herein, will control.

[00240] U.S. Published Application 2005-0119455A1

[00241] Sidhu et al., *J Mol Biol.* 2004 **338**:299-310.

EQUIVALENTS

[00242] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are within the scope of the following claims.

CLAIMS:

1. A library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, wherein the vectors or genetic packages comprise variegated DNA sequences that encode a heavy chain (HC) CDR3, wherein 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 of the positions in the HC CDR3 of the family are varied among Tyr, Gly, Asp, Ser, and Arg residues;

wherein each of the last 4 positions in the HC CDR3 is varied among Tyr, Gly, Asp, Ser, and Arg residues; or

wherein each of the first 4 positions in the HC CDR3 is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

2. The library of claim 1, wherein about 50% of all the positions of the HC CDR3 are Tyr, Gly, Asp, Ser, or Arg.

3. The library of claim 1, wherein an Arg residue is present in a filler region between V and D, between D and J, or between V and J.

4. A library of vectors or genetic packages that display, display and express, or comprise a member of a diverse family of human antibody related peptides, polypeptides and proteins and collectively display, display and express, or comprise at least a portion of the diversity of the antibody family, wherein the vectors or genetic packages comprise variegated DNA sequences that encode a heavy chain (HC) CDR3 consisting of 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 residues, each of which independently vary among Tyr, Asn, Asp, Ser, and Arg residues, wherein a framework region (FR) 3 is located at the amino terminus of the CDR3, and a FR4 portion of a JH region is located at the carboxy terminus of the CDR3;

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wherein each of the last 4 positions in the HC CDR3 is varied among Tyr, Gly, Asp, Ser, and Arg residues; or

wherein each of the first 4 positions in the HC CDR3 is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

5. The library of claim 1 or claim 4, wherein the library is a library of genetic packages.

6. The library of claim 5, wherein the genetic packages are bacteriophages.

7. The library of claim 6, wherein the library is a phage display library.

8. The library of claim 1 or claim 4, wherein the library is a library of vectors.

9. The library of claim 8, wherein the vectors are phage vectors or phagemid vectors.

10. The library of claim 1 or claim 4, wherein the proportions of the Tyr, Gly, Asp, Ser, Arg, and Leu residues are 1.0G, 0.57S, 0.46R, 0.42D, 0.36L, and 0.35Y.

11. The library of claim 1 or claim 4, wherein the HC CDR3 are 10-15 amino acids in length, and wherein each of the first 6, 7, or 8 positions in the HC CDR3s is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

12. The library of claim 1 or claim 4, wherein the HC CDR3 are 18-20 amino acids in length, and wherein each of the first 5 or 6 positions in the HC CDR3s is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

13. The library of claim 1 or claim 4, wherein the HC CDR3 are 15-17 amino acids in length, and wherein each of the first 2, 3, or 4 positions in the HC CDR3s is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

14. The library of claim 1 or claim 4, wherein the HC CDR3 are 11-14 amino acids in length, and wherein each of the first 1 or 2 positions in the HC CDR3s is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

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15. The library of claim 1 or claim 4, wherein the HC CDR3 are 11-14 amino acids in length, and wherein each of the first 1, 2, or 3 positions in the HC CDR3s is varied among Gly, Ser, Arg, Asp, Leu, and Tyr.

16. The library of claim 4, wherein the FRs are of a VH gene selected from the group consisting of VH3-23, VH4-34, VH3-30, VH3-30.3, and VH4-30.

17. The library of claim 16, wherein the FRs are from VH 3-23.

18. The library of claim 1 or claim 4, wherein the library comprises at least 1×10^9 members.

Date Reçue/Date Received 2021-10-08