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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.



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(57) 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.



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

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims priority to U.S. Application Serial No. 61/047,529, filed on April 24, 2008.

BACKGROUND

10 [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.

15 [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).

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

30 [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
5 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.

10 [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 degradation 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
15 advantageous to provide a library in which the occurrence 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,
20 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
25 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 naturally 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),
5 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
10 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
15 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
20 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,
25 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
30 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 dobbing.

[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.

5 [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
10 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 disclosure provides a cassette for displaying a wobbled heavy chain (HC) CDR3, for example, wherein the cassette comprises the cassette shown in Table 400.

15 [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
20 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
25 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
30 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).

5 [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).

10 [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.

15 [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,

20 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

25 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:

30 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 1/11 of 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 Fframework (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.

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- [0057a]** In an embodiment, there is provided 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 the diversity of the antibody family, wherein the vectors or genetic packages
- 5 comprise variegated DNA sequences that encode: (i) a heavy chain, comprising: (a) a CDR1 having the following amino acid sequence: $X_{31}YX_{33}MX_{35}$, wherein X_{31} , X_{33} and X_{35} are any amino acid except Cys or Met; (b) a CDR2 having the following amino acid sequence $X_{50}IX_{52}X_{52a}SGGX_{56}TX_{58}YADSVKG$, wherein X_{50} , X_{52} , X_{56} and X_{58} are any amino acid except Cys or Met and X_{52a} is selected from the group consisting of Gly, Ser, Pro or Tyr; and
- 10 (c) a CDR3; and (ii) a light chain.

[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

5 [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
10 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
15 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
20 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
25 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
30 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.

5 **[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
10 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
15 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
20 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
25 following equation:

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

[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,
30 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 (C1q) 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 (YEDYW₁₀₃GQGTLVTVSS (SEQ ID NO:1)) occurs more often than JH3 in native antibodies. Hence, it is preferred for the focused libraries of this invention.

5 However, JH3 (AFDIW₁₀₃GQGTMVTVSS (SEQ ID NO:2)), JH6 (YYYYYGMDVW₁₀₃GQGTTVTVSS (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 M₁₀₈. 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
10 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
15 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
20 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, Sonnhhammer EL, Buck D, Winter G. *J Mol Biol.* 1997 V270:587-97.) showed that the human immune system does not insert multiple D
25 segments and recombining 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
30 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::JH_n (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::JH_n.

[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"), RACHITTTM (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 Pe1B signal sequence) and NotI (positioned in the linker between CH1 and the anchor protein). Signal sequences other than Pe1B 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
5 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
10 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 in a yeast cell.

[00105] Other types of protein display include cell-based display (see, e.g., WO
15 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
20 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-
25 242; 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
30 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.

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CDR1, CDR2, and light-chain diversity

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 (His6 is disclosed as SEQ ID NO: 934). 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.

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.

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. E 7. The diversity of HC CDR3 is at least 221, 272, 500, 1000, 1. E 4, 1. E 5, 1. E 6, 1. E 7, 1. E 8, or 1. E 9.

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 secretes 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. E 7, 1. E 8, or 1. E 9.

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

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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.

Light Chain Diversity

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, *Sgf*I), FR2 (*Kpn*I), FR3(*Xba*I), and Fr4::Ckappa (*Bsi*WI) so that each CDR and be replaced with a varied population.

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.

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 R₂₄ASQSISSYLN₃₄ (SEQ ID NO: 935). Other VK1 loci have Q at 24. Other loci have M at 25. S₂₆ and Q₂₇ are invariant in VKI. Other VKI loci have D or G at 28. I₂₉ and L₃₃ 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 3×10^7 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. x 10 ⁵	2. x 10 ⁵	4. x 10 ⁵	8. x 10 ⁵	8. x 10 ⁵	8. x 10 ⁵	4. x 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 *	ggt, 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

5 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
10 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.

15 [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
20 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.

5 [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) 10 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 apparatus, 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.

15 [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 20 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 25 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 30 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.

- 5 [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,
 10 VK1glL8-JK5, and VK1GLO12-JK3 (amino-acid sequences shown in Table 19) into pLCSK23.

Table 19: 26 VL to be used in pLCSK23.

15	VK1O2gl-JK3		(SEQ ID NO:4)		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQSI	SYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	SYSTPFTFGP	GTKVDIK 107
20	VK1O2var1		(SEQ ID NO:5) S28D		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQDIS	SYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	SYSTPFTFGP	GTKVDIK 107
25	VK1O2var2		(SEQ ID NO:6) S91R		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQSI	SYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	RYSTPFTFGP	GTKVDIK 107
30	VK1O2var3		(SEQ ID NO:7) S91E		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQSI	SYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	EYSTPFTFGP	GTKVDIK 107
35	VK1O2var4		(SEQ ID NO:8) S31R		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQSI	RYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	SYSTPFTFGP	GTKVDIK 107
40	VK1O2var5		(SEQ ID NO:9) S31E, S93R		
	DIQMTQSPSS	LSASVGDRVT	ITCRASQSI	EYLNWYQQK	GKAPKLLIYA ASSLQSGVPS 60
	RFSGSGSGTD	FTLTISSLQP	EDFATYYCQ	SYRTPFTFGP	GTKVDIK 107
45	VK3L6gl-JK4		(SEQ ID NO:10)		
	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQK	GQAPRLLIYD ASNRATGIPA 60
	RFSGSGSGTD	FTLTISLLEP	EDFAVYYCQ	RSNWPLTFGG	GTKVEIK 107
50	VK3L6var1		(SEQ ID NO:11) S31R		
	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	RYLAWYQQK	GQAPRLLIYD ASNRATGIPA 60
	RFSGSGSGTD	FTLTISLLEP	EDFAVYYCQ	RSNWPLTFGG	GTKVEIK 107
55	VK3L6var2		(SEQ ID NO:12) S92R		

	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RRNWPLTFGG	GTKVEIK		107
	VK3L6var3 (SEQ ID NO:13) S92G						
5	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RGNWPLTFGG	GTKVEIK		107
	VK3L6var4 (SEQ ID NO:14) S92Y						
10	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RYNWPLTFGG	GTKVEIK		107
	VK3L6var5 (SEQ ID NO:15) S92E						
15	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RENWPLTFGG	GTKVEIK		107
	VK3L6var6 (SEQ ID NO:16) Y32F						
	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SFLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RSNWPLTFGG	GTKVEIK		107
20	VK3L6var7 (SEQ ID NO:17) Y32D						
	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SDLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RSNWPLTFGG	GTKVEIK		107
	VK3L6var8 (SEQ ID NO:18) N93G						
25	EIVLTQSPAT	LSLSPGERAT	LSCRASQSVS	SYLAWYQQKP	GQAPRLLIYD	ASNRATGIPA	60
	RFSGSGSGTD	FTLTISSLEP	EDFAVYYCQ	RSGWPLTFGG	GTKVEIK		107
	VK3A27gl-JK3 (SEQ ID NO:19)						
30	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
35	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
40	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						
45	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	GASSRATGIP	60
	DRFSGSGSGT	DFTLTISRLE	PEDFAVYYCQ	QYESSPFTFG	PGTKVDIK		108
	VK3A27var5 (SEQ ID NO:24) G93R						
50	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	SSYLAWYQQK	PGQAPRLLIY	GASSRATGIP	60
	DRFSGSGSGT	DFTLTISRLE	PEDFAVYYCQ	QYRSSPFTFG	PGTKVDIK		108
	VK3A27var6 (SEQ ID NO:25) S30D, G93E						
	EIVLTQSPGT	LSLSPGERAT	LSCRASQSVS	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 PEDFAVYQC QYGRSPFTFG PGTKVDIK 108

5

VK3L2g1-JK3 (SEQ ID NO:27)
 EIVMTQSPAT LSVSPGERAT LSCRASQSVS SNLAWYQQK GPAPRLLIYG ASTRATGIPA 60
 RFSGSGSGTE FTLTISSLQS EDFAVYQCQ YNNWPFTFGP GTKVDIK 107

10

VK1g1L8-JK5 (SEQ ID NO:28)
 DIQLTQSPSF LSASVGDRV ITCRASQGIS SYLAWYQQK GKAPKLLIYA ASTLQSGVPS 60
 RFSGSGSGTE FTLTISSLQP EDFATYQCQ LNSYPITFGQ GTRLEIK 107

15

VK1GLO12-JK3 (SEQ ID NO:897)
 DIQMTQSPSS LSASVGDRV TITCRASQSI SSYLNWYQQK PGKAPKLLIY AASSLQSGVP 60
 SRFSGSGSGT DFTLTISL QPEDFATYQC QSYSTPFTF GPGTKVDIKR GTVAAPSVFI 120
 FPPSDEQLKS GTASVCLL NNFYPREKV QWKVDNALQS GNSQESVTEQ DSKDSTYSL 180
 STLTLKADY EKHKVYACE VTHQGLSSPV TKSFNREGCA AAGKIPNPL LGLDST 236

20

[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.

25

[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.

30

35

[00163] Van den Brulle et al. (*Biotechniques* 45:340-3 (2008)) describe a method of synthesis of variable DNA in which type II 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 cysteines 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

64371-1229

$$j = 0.06 A + 0.06 C + 0.82 G + 0.06 T$$

$$z = 0.06 A + 0.06 C + 0.06 G + 0.82 T$$

EXEMPLIFICATION

5 [00165] The present invention is further illustrated by the following examples which should not be construed as limiting in any way.

10 [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 Ca of R₉₄ (which ends FR3) to the Ca of the W₁₀₄ (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 has suggested 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 from 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 dobbing.

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

Length 3

	...FR3-----	CDR3-	FR4-----	
V-3JH1	TAVYYCAK	FQH	WGQGT LVTVSS	(SEQ ID NO:31)
V-3JH2	TAVYYCAK	FDL	WGRGT LVTVSS	(SEQ ID NO:32)
V-3JH3	TAVYYCAK	FDI	WGQGT MVTVSS	(SEQ ID NO:33)
V-3JH4	TAVYYCAK	FDY	WGQGT LVTVSS	(SEQ ID NO:34)
V-3JH5	TAVYYCAK	FDP	WGQGT LVTVSS	(SEQ ID NO:35)
V-3JH6	TAVYYCAK	MDV	WGQGT TVTVSS	(SEQ ID NO:36)
V-3D1-1.1.1-JH1	TAVYYCAK	GTT	WGQGT LVTVSS	(SEQ ID NO:37)
V-3D1-1.1.2-JH1	TAVYYCAK	TTG	WGQGT LVTVSS	(SEQ ID NO:38)
V-3D3-3.3.3-JH2	TAVYYCAK	IFG	WGRGT LVTVSS	(SEQ ID NO:39)

Length 4

V-4JH1	TAVYYCAK	YFQH	WGQGT LVTVSS	(SEQ ID NO:40)
V-4JH2	TAVYYCAK	YFDL	WGRGT LVTVSS	(SEQ ID NO:41)
V-4JH3	TAVYYCAK	AFDI	WGQGT MVTVSS	(SEQ ID NO:42)
V-4JH4	TAVYYCAK	YFDY	WGQGT LVTVSS	(SEQ ID NO:43)
V-4JH5	TAVYYCAK	WFDP	WGQGT LVTVSS	(SEQ ID NO:44)

V-4D3-10.1a-JH2 TAVYYCAK LLWF **WGRGTLTVSS** (SEQ ID NO:46)

V-5D2-8.2a-JH2 TAVYYCAK DIVLM **WGRGTLVT**VSS (SEO ID NO:53)

```

!                                     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:5
!
!   BstEII...

```

25 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
30 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.

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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 x 10⁹ HC in phage can be expanded into a Fab library of 1.2 x 10¹¹ (1. x 10⁹ x 117). If we combined 1 x 10⁷ CDR1-2s with 10⁶ HC CDR3s, we could make a library of 5 x 10⁷ in which each CDR3 is coupled with 50 CDR1-2s. A library of 5 x 10⁷ HCs in phage could give results similar to an old-style library of 6 x 10⁹.

Table 1: Designs of very short exemplary HC CDR3s

15 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...
!
20 ! 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)
25 cag ggt act ac**G 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)
30 (ON_5) 5'-AcTggAgAcggTgAccgTAgTAcccTggcccccA-3' ! 33 bases (SEQ ID NO: 256)
(ON_5 is reverse complement of 5'-tgg ggc cag ggt act ac**G GTC ACC** gtc tcc
agt-3' (SEQ ID NO:59))
! Use ON-1 and ON-3 shown below
!-----
35 !

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...
 5
 !
 ! 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-
 !
 10
 ! G Q G T T V T V S S (SEQ ID NO:60)
 ggc cag ggt act ac**G 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-
 15 ggc cag ggt act ac-3' (SEQ ID NO:62)
 ! Use ON-1, ON-3, and ON-5
 !-----
 C3X5
 20
 ! 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.....
 25
 ! 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 ac**G GTC ACC** gtc tcc agt-3' (SEQ ID NO:64)
 ! BstEII...
 30
 (C3X5) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk nnk nnk nnk tgg-
 ggc cag ggt act ac-3' (SEQ ID NO:65)
 !-----
 aRg encodes K or R
 35

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 transferred 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
5 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 cell that are thought to have multiple states. An antibody having a very long HC CDR3 could form a protuberance that
10 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
15 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
20 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
25 built using dobbing, 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.

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

Table 4: Human JH amino-acid sequences

5		H3	

		CDR3	

		100	110
10			
	JH1	---AEYFQH WGQ GT LV TVSS	(SEQ ID NO:66)
	JH2	---YWYFDL WGR GT LV TVSS	(SEQ ID NO:67)
	JH3	-----AFDI WGQ GT MV TVSS	(SEQ ID NO:2)
	JH4	-----YFDY WGQ GT LV TVSS	(SEQ ID NO:1)
15	JH5	----NWFD PWGQ GT LV TVSS	(SEQ ID NO:68)
	JH6	YYYYYGMDV WGQ GT TV TVSS	(SEQ ID NO:3)

In each of the following designs, the amino-acid sequence begins with YYCA(K/R) (SEQ ID NO: 936) which is the end of FR3. FR4 starts with WG and is shown bold.

20 **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 (SEQ ID NO: 937), which is rich in Tyr and Ser residues, which is
25 followed by full-length JH1.

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XX::D2-2.2::XX::JH1

		1	1	2	2
FR3	1	5	0	5	0 3FR4

YYCAK DYGYCSSTSCYTYGYSYAEYFQHWGQGTLLTVSS (SEQ ID NO:898)

5 YYCAK **XXGYCSXXSCYTXXYSYAEYFQHWGQGTLLTVSS** (SEQ ID NO:69)

R GYCSSTSCYT AEYFQHWGQGTLLTVSS (JH1)

(SEQ ID NO:70) (SEQ ID NO:66)

	1	1		1	1
--	---	---	--	---	---

9	9	0	0		0	1
---	---	---	---	--	---	---

10 4 5 0 2abcdefghijklmnp3 0

Amino-acid diversity = 1.28 E 8

DNA diversity = 2.15 E 9

Stop-free = 83%

15 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

20 loop into which diversity at two points has been introduced. This CDR3 is 23 long. Using primers that include DNA up to ...YYCA (SEQ ID NO: 938) and from WGQG (SEQ ID NO: 939)..., 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

25 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

30 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** -

35 ! 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-

40 ! 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)

45 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)
 5 (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|
 10 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	GSYYYGSGSYYNVDSYYAEYFQH	<u>WGQGT</u>	LVTVSS	(SEQ ID NO:899)	
	YYCAK	XXYYYGXGSXYNXXSYAEYFQH	<u>WGQGT</u>	LVTVSS	(SEQ ID NO:80)	
20	R	YYYGSGSYYN	AEYFQH	<u>WGQGT</u>	LVTVSS (JH1)	
		(SEQ ID NO:81)		(SEQ ID NO:66)		

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

30 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
 35 ! 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
 40 |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
 45 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
 !
 50 ! 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...

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(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.

5

Design 3

1 1 2 2
1 5 0 5 0 3
YYCAK DYYYYGSGSYNSDSYSAEYFQHWGQGLVTVSS (SEQ ID NO:900)
10 YYCAK XZYZZGZGZXYNZXZYXAXZFQHWGQGLVTVSS (SEQ ID NO: 940)
R YYYGSGSYYN AEYFQHWGQGLVTVSS (JH1)
(SEQ ID NO:81) (SEQ ID NO:66)
Amino-acid diversity = 1.64 E 8
DNA diversity = 1.07 E 9
15 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)

20 and 8th residue changed to X, ZXZYX, , 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 G
(C23D310b) 5'-GCA|GtT|taT|taC|tgc|gct aRg nnk tmc tac tmc tmt ggt tmc ggc-

25 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)
30

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 YYSFSYYPYYYDSSGYYYGYSDYSYSAEYFQHWGQGLVTVSS (SEQ ID NO:901)
YYCAK YYSXSYYXZYDSZGYZYXYYSXZYZZAZZFQHWGQGLVTVSS (SEQ ID NO:87)
R YYDSSGYYY AEYFQHWGQGLVTVSS (JH1)
(SEQ ID NO:88) (SEQ ID NO:66)
40 1 1 1 1
9 9 0 0 0 1
4 5 0 2abcdefghijklmnopqrstuvmnxyab3 0
, ,

Amino-acid diversity = 1.64 E 8
45 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.

```

5  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...
10 ! 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-----
15 ! 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
20 !
   ! 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...
25 (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.

```

30

Design 5

```

1 1 1 2 2
1 5 0 5 0 3
35 YYCAK SSGYCSSTSCYTGVIYYAEYFQHWGQGLVTVSS (SEQ ID NO:902)
   YYCAK ZZGZCZZXZCZTXXYZYXZYFQHWGQGLVTVSS (SEQ ID NO:92)
   R GYCSSTSCYT AEYFQHWGQGLVTVSS (JH1)
   (SEQ ID NO:70) (SEQ ID NO:66)
Amino-acid diversity = 1.64 E 8
DNA diversity = 1.07 E 9
40 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.

```

50 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 -
 5 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
 10 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...
 15 (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)

20

Design 6

1 1 2 2 2 3 3
 1 5 0 5 0 3 5 0 5
 YYCAK SYDYYGYCSSTSCYTYYSYVSYSYSSYSYAEYFQHWGQGTTLVTVSS (SEQ ID NO:903)
 25 YYCAK ZYXZYGCZXXSCZTYZSZXZYSZYZZSYAEZFQHWGQGTTLVTVSS (SEQ ID NO:96)
 R GYCSSTSCYT D2-2.2 AEYFQHWGQGTTLVTVSS (JH1)
 (SEQ ID NO:70) (SEQ ID NO:66)

30 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%

35

C35D222JH1

40 ! 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 -
 45 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
 50 ! 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)
 55 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)

60

Design 7

5 1 1 2 2 2 3 3
 1 5 0 5 0 3 5 0 5
 YYCAK YYSYYGYCSSTSCYTYSSSVSYSSYYSSYYAEYFQHWGQGLVTVSS (SEQ ID NO:904)
 YYCAK **ZYZZYGZCZZXZCZTYZSZXZYSZYSSZYAψZJQBWGQGLVTVSS** (SEQ ID NO:100)
 R GYCSSTSCYT D2-2.2 AEYFQHWGQGLVTVSS (JH1)
 (SEQ ID NO:70) (SEQ ID NO:66)
 10 (J=FSY, B=YHND, ψ=EKQ)

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

C35D222JH1B
 20 !
 ! scabDNA 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...
 !
 25 ! 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-----
 30 ! **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**
 35 !
 ! **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

40 1 1 2 2 2 3 3
 1 5 0 5 0 3 5 0 5
 YYCAK SRSYYDYVWGSYRYTSSYSYSSYSYSSYYAEYFQHWGQGLVTVSS (SEQ ID NO:905)
 YYCAK **ZXZYZBZVWGZZRZTZSZXZYZZZYSSZAψZFQHWGQGLVTVSS** (SEQ ID NO:103)
 45 R YYDYVWGSYRYT D3-16.2 AEYFQHWGQGLVTVSS (JH1)
 (SEQ ID NO:104) (SEQ ID NO:66)
 (J=FSY, B=YHND, ψ=EKQ)

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

55

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** -

10 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

15 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
 20 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...

25 (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)

30 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
 35 YYCAK YGYSSDSYSSYYDYVWGSYRYTYSSYYAEYFQHWGQGLVTVSS (SEQ ID NO:906)
 YYCAK **ZXZZXZYZZZYBZVWGZZRZTYZSZYAψZFQHWGQGLVTVSS** (SEQ ID NO:108)
 R D3-16.2 YYDYVWGSYRYT AEYFQHWGQGLVTVSS (JH1)
 (SEQ ID NO:104) (SEQ ID NO:66)
 (J=FSY, B=YHND, ψ=EKQ)

40 Amino-acid diversity = 1.31 E 8
 DNA diversity = 5.37 E 8
 Stop-free = 91%
 Gratuitous Cys-free = 91%
 45 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 T
 ! 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 ct**G GTC ACC** gtc tcc agt-3' (SEQ ID NO:110)
 ! BstEII...

(C35D316JH1B)

30 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

35 1 5 0 5 0 4
 YYCAK GSSYYYGSGSYNSDYSAEYFQHW**GQGLTVTVSS** (SEQ ID NO:907)
 YYCAK **XZZYZZGZGXYNZXZYZAXZFQHWGQGLTVTVSS** (SEQ ID NO:112)
 40 R YYYGSGSYYN AEYFQHW**GQGLTVTVSS** (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)

50 ON_1, ON_2, ON_3, and ON_4 as above.

Design 11

1 5 0 5 0 5

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YYCAR SSRSGYCTNGVCYRSGSYWYFDLWGRGTLVTVSS (SEQ ID NO: 991)
 YYCAR ZZXZGZC32GVCZ3ZXZZ4Z12LWGRGTLVTVSS (SEQ ID NO:114)
 K GYCTNGVCYT YWYFDLWGRGTLVTVSS D2-8.2 JH2
 (SEQ ID NO:115) (SEQ ID NO:67)

5 (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 tgc 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)

10

(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)

15

(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

20

(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))

25 Design 12

			1	1	2	2	3	3
	1	5	0	5	0	5	0	5

YYCAR SSSYSGYCTNGVCYTYSYSYSSYSYWYFDLWGRGTLVTVSS (SEQ ID NO:908)
 YYCAR ZZZZZZGZC32GVCZ3ZZZZYZZYZZZ4Z12LWGRGTLVTVSS (SEQ ID NO:121)
 30 K GYCTNGVCYT YWYFDLWGRGTLVTVSS 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)

35

(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)

40

(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
 45 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 SSSYYSYSSGYCTNGVCYTYSYSYSSYWYFDLWGRGTLVTVSS (SEQ ID NO:912)
 50 YYCAR ZZZZZZZZZZGYCTNGVCYTZZZZZZZZZWZF2LWGRGTLVTVSS (SEQ ID NO:123)
 K GYCTNGVCYT YWYFDLWGRGTLVTVSS D2-8.2 JH2
 (SEQ ID NO:115) (SEQ ID NO:67)

(2=YHND)

5 (C34D282.2A) 5'-GCA|GtI|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 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
 10 NO:126) [RC])

Design 14

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

15
 1 5 0 5 0 3 5 0 5
 YYCAK YSYYSYSSYYSDYVWGSYRYTSYDSYYYAEYFQHWGQGLTVTVSS (SEQ ID NO:913)
 YYCAK ZZZZZZZZZZDZVWGSYRZTZZZZZZZZAEZFQHWGQGLTVTVSS (SEQ ID NO:127)
 R D3-16.2 YYDYVWGSYRYT AEYFQHWGQGLTVTVSS (JH1)
 20 (SEQ ID NO:104) (SEQ ID NO:66)
 (C34D316.2A) 5'-GCA|GtI|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)
 25 (C34D316.2B) 5'-Ag AgT Acc cTg gcc ccA ATg cTg gAA AKA cTc Agc gKA 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'
 30 (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.

35
 1 5 0 5 0 3 5 0 5
 YYCAK SYDYSSYSYYDYVWGSYRYTSYSGDSYAAEYFQHWGQGLTVTVSS (SEQ ID NO:914)
 YYCAK ZZZZZZZZZZDZVWGSYRZTZZZZZZZZAEZFQHWGQGLTVTVSS (SEQ ID NO:131)
 40 YYDYVWGSYRYT AEYFQHWGQGLTVTVSS
 (SEQ ID NO:104) (SEQ ID NO:66)
 (C35D316.2A) 5'-GCA|GtI|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' (SEQ ID NO:
 45 132)
 (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-
 50 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.

```

5          1      1      2  2  2      3      3
          1  5    0  5    0  3  5    0  5
YYCAK SSSYYSSYSYSGYCSGGSCYSSYYSSYYSAEYFQGWGQGLVTVSS (SEQ ID NO:915)
YYCAK ZZZZZZZZZZGZCZGGZCZSZZZZZZZZZAEZFQHWGQGLVTVSS (SEQ ID NO:135)
      R      GYCSGGSCYS 2-25.2 AEYFQHWGQGLVTVSSJH1
10          (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-
15      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.

```

20 (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 ggA gcA gKA gcc Acc gKA AcA gKA gcc gKA g-3'
25      (SEQ ID NO:140)! 93 bases

```

Design 17

```

          1      1      2  2  2      3      3
          1  5    0  5    0  3  5    0  5
30 YYCAK YSSYSYDYVWGSYRYTSSSYSSYYSAEYFQGWGQGLVTVSS (SEQ ID NO:916)
YYCAK ZZZZZZZZDZVWGZZRZTZZZZZZZZAEZFQHWGQGLVTVSS (SEQ ID NO:141)
      R      YYDYVWGSYRYT D3-16.2 AEYFQHWGQGLVTVSS (JH1)
          (SEQ ID NO:104)      (SEQ ID NO:66)

35 (C35D3162A) 5'-GCA|GtT|taT|taC|tgc|gct aRg tmt 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 ggT AKA Acg gKA AKA Acc ccA gAc-
40      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
45 YYCAK SSYYYSSSYDYVWGSYRYTSSYYSSYSAEYFQGWGQGLVTVSS (SEQ ID NO:917)
YYCAK ZZZZZZZZZZDZVWGZZRZTZZZZZZZZAEZFQHWGQGLVTVSS (SEQ ID NO:144)
      R      YYDYVWGSYRYT D3-16.2AEYFQHWGQGLVTVSS (JH1)
          (SEQ ID NO:104)      (SEQ ID NO:66)

50 (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)

5 Design 19

1 5 0 5 0 3 5 0 5
YYCAK YSGDSYSYYYDSSGYYYSSSYSSYYAEYFQGWGQGLVTVSS (SEQ ID NO:918)
10 YYCAK **ZZZZZZZZZZDSSGZZZZZZZZZZZZZZAEZFQHWGQGLVTVSS** (SEQ ID NO:147)
R YYYDSSGYYY AEYFQHWGQGLVTVSS (JH1)
(SEQ ID NO:88) (SEQ ID NO:66)
1 1 1 1
9 9 0 0 0 1
4 5 0 2abcdefghijklmnopqrstuvwxyab3 0
15 ''

Amino-acid diversity = 6.7 E 7
DNA diversity = 6.7 E 7
Stop-free = 100
20 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
25 with six Ys as Z)::ZZZZZZZZZZ::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
30 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 KIR
! |TTA|AGg|gct|gag|gaT|aCT|GCA|GtT|taT|taC|tgc|gct **aRg** -
35 !
! CDR3-----
! 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
40 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 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)
45 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	YSSYS	YYYYDSSG	YYYSSYS	SSYS	YYYAEYFQGWGQ	GLTVTVSS		(SEQ ID NO:919)
15	YYCAK	ZZZZZZ(Z)	ZZZZDSSGZZZZZZZZZZ(Z)	ZZZAEZFQHWGQGLTVTVSS					(SEQ ID NO:152)
	R		YYYDSSGYYY		AEYFQHWGQGLTVTVSS				(JH1)
			(SEQ ID NO:88)		(SEQ ID NO:66)				
		1	1			1	1		
	9	9	0	0		0	1		
20	4	5	0	3	abc	defghijklmnop	q	rstuvwx	ya4

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 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 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 Sup⁰ 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.

- 5 [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 Sup⁰ 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
- 10 the phage library.

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Table 20: Human D regions

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

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

5		RF 1 (SEQ ID NO:156)	RF 2 (SEQ ID NO:157)	RF 3 (SEQ ID NO:158)	Used in designs
	D1 1-1	GTTGT	VQLER	YNWND	
		(SEQ ID NO:159)	(SEQ ID NO:160)	(SEQ ID NO:161)	
10	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)	
15	1-26	GIVGAT	V*WELL	YSGSY	
		(SEQ ID NOS 171 & 167)	(SEQ ID NO:70)	(SEQ ID NO:168)	
20	D2 2-2	RIL**YQLLY	GYCSSTSCYT	DIVVVPAAI	1, 5, 6, 7,
		(SEQ ID NOS 169 & 392)	(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)	
25	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)	
30	D3 3-3	VLRFLWLLY	YYDFWSGYT	ITIFGVVII	
		(SEQ ID NO:179)	(SEQ ID NO:180)	(SEQ ID NOS 181 & 579)	
35	3-9	VLRYFDWLL@	YYDILTGYN	ITIF*LVII	
		(SEQ ID NO:182)	(SEQ ID NO:81)	(SEQ ID NO:183)	
	3-10	VLLWFGELL@	YYGSGSYN	ITMVRGVII	
		(SEQ ID NO:184)	(SEQ ID NO:104)	(SEQ ID NO:185)	
40	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	YYDSSGY	ITMIVVVIT	4, 19, 20
		(SEQ ID NO:189)	(SEQ ID NO:192)	(SEQ ID NO:190)	
45	D4 4-4	\$LQ@L	DYSNY	TTVT	
		(SEQ ID NO:191)	(SEQ ID NO:192)	(SEQ ID NO:193)	
50	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)	
55	4-23	\$LRW@L	DYGGNS	TTVVT	

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			(SEQ ID NO:200)	(SEQ ID NO:201)	(SEQ ID NO:202)
	D5	5-5	VDTAMV	WIQLWL	GYSYGY
5			(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	VDTAMV	WIQLWL	GYSYGY
10			(SEQ ID NO:209)	(SEQ ID NO:210)	(SEQ ID NO:211)
		5-24	VEMATl	*RWLQL	RDGYNY
			(SEQ ID NO:212)	(SEQ ID NO:213)	(SEQ ID NO:214)
15	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)
20		6-19	GYSSGWY	GIAVAG	V*QWLV
			(SEQ ID NO:221)	(SEQ ID NO:222)	(SEQ ID NO:223)
	D7	7-27	LTG	@LG	NWG
25					

Table 3: Human JH segments

JH - Amino acid sequence alignment

	H3		

	CDR3		

	100	110	
	FR4-----	Used in designs	
35	JH1	-- <u>A E Y F O H W G Q G T L V T V S S</u>	1-8, (SEQ ID NO:66)
	JH2	-- <u>Y W Y F D L W G R G T L V T V S S</u>	(SEQ ID NO:67)
	JH3	---- <u>A F D I W G Q G T M V T V S S</u>	(SEQ ID NO:2)
	JH4	---- <u>Y F D Y W G Q G T L V T V S S</u>	(SEQ ID NO:1)
	JH5	---- <u>N W F D P W G Q G T L V T V S S</u>	(SEQ ID NO:68)
40	JH6	<u>Y Y Y Y Y G M D V W G Q G T T V T V S S</u>	(SEQ ID NO:3)
	123456789		

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

```

45      !           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)
50      !           BstEII...

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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.

5	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	
10	NWN D1-1.3.2	6	
	TGT D1-1.1.3	7	
	LER D1-1.2.3	8	(SEQ ID NO: 162)
	WND D1-1.3.3	9	
	GIT D1-7.1.1	10	
15	VyL D1-7.2.1	11	*
	ITG D1-7.1.2	12	
	yLE D1-7.2.2	13	*
	LEL D1-7.2.3	14	(SEQ ID NO: 160)
	WNY D1-7.3.3	15	
20	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	*
25	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	
30	ELL D1-26.2.4	26	
	SY Y D1-26.3.4	27	
	RIL D2-2.1.1	28	(SEQ ID NO: 171)
	GYC D2-2.2.1	29	#
	DIV D2-2.3.1	30	
35	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	#
40	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	*
45	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	
50	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	
5	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	
10	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	#
15	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	
20	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	
25	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	#
30	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	#
35	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	#
40	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	
45	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	#
50	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	
55	LRF D3-3.1.2	104	
	YDF D3-3.2.2	105	
	TIF D3-3.3.2	106	

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	RFL D3-3.1.3	107	
	DFW D3-3.2.3	108	
	IFG D3-3.3.3	109	
	FLE D3-3.1.4	110	
5	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	
10	EWL D3-3.1.6	116	
	SGY D3-3.2.6	117	
	VVI D3-3.3.6	118	
	WLL D3-3.1.7	119	
	GYG D3-3.2.7	120	
15	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	
20	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	*
25	FDW D3-9.1.5	131	
	LTG D3-9.2.5	132	(SEQ ID NO: 221)
	yLV D3-9.3.5	133	*
	DWL D3-9.1.6	134	
	TGY D3-9.2.6	135	
30	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	
35	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	
40	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	
45	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	*
50	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	*
55	DYV D3-16.2.3	161	
	ITF D3-16.3.3	162	
	LRL D3-16.1.4	163	

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	YVW D3-16.2.4	164	
	TFG D3-16.3.4	165	
	RLG D3-16.1.5	166	
	VWG D3-16.2.5	167	
5	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	
10	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	
15	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	*
20	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	*
25	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	
30	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	*
35	SNY D4-4.2.3	198	
	DYG D4-17.2.1	199	
	LRw D4-17.1.2	200	* (SEQ ID NO: 197)
	YGD D4-17.2.2	201	
	RwL D4-17.1.3	202	*
40	GDY D4-17.2.3	203	
	LRW D4-23.1.2	204	(SEQ ID NO: 197)
	YGG D4-23.2.2	205	
	TVV D4-23.3.2	206	
	RWy D4-23.1.3	207	*
45	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	
50	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	
55	SYG D5-5.3.3	218	
	AMV D5-5.1.4	219	
	LWL D5-5.2.4	220	

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	YGY D5-5.3.4	221	
	VDI D5-12.1.1	222	
	WIY D5-12.2.1	223	*
	IyW D5-12.2.2	224	*
5	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	
10	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	
15	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	
20	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	
25	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	(SEQ ID NO: 214)
30	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	
35	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	
40	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	
45	yLG D7-27.2.1	265	*
	NWG D7-27.3.1	266	(SEQ ID NO: 223)

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

	GTTG D1-1.1.1	(SEQ ID NO:257)	1
50	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
55	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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	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
5	yLVI D3-9.3.5	(SEQ ID NO:571)	127
	DWLL D3-9.1.6	(SEQ ID NO:577)	128
	TGY Y 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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	VLLw D3-22.1.1	(SEQ ID NO:701)	177
	YY YD 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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	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

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	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
5	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
10	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
15	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
20	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
25	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
	QWLW	D6-19.3.3	(SEQ ID NO:890)	265
30	SGWY	D6-19.1.4	(SEQ ID NO: 941)	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
35	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
40	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
45	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
50	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
	VVVP	D2-2.3.3	(SEQ ID NO:317)	22
55	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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
40	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
45	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
50	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
55	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
5	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
10	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
15	ITMIV D3-22.3.1	(SEQ ID NO:706)	153
	LLwy 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
	LwyW D3-22.1.3	(SEQ ID NO:722)	157
20	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
25	yyWLL D3-22.1.5	(SEQ ID NO:739)	163
	SSGY D3-22.2.5	(SEQ ID NO:740)	164
	VVVIT D3-22.3.5	(SEQ ID NO:741)	165
	yWLL D3-22.1.6	(SEQ ID NO:746)	166
	SGYYY D3-22.2.6	(SEQ ID NO:747)	167
30	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
35	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
40	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
45	VDIVA D5-12.1.1	(SEQ ID NO:799)	183
	WIyWL D5-12.2.1	(SEQ ID NO:800)	184
	GYSYG 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
50	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
55	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
5	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
10	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
15	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
	yQWL V D6-19.3.2	(SEQ ID NO:886)	214
20	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
25	YSGSY 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
30	YCSSTS D2-2.2.2	(SEQ ID NO:310)	8
	IVVVP A 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
35	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
40	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
45	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
50	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
55	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
5	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
10	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
15	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
20	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
	VLRFL E D3-3.1.1	(SEQ ID NO:489)	57
	YYDFWS D3-3.2.1	(SEQ ID NO:490)	58
25	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
30	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
35	FGVVII D3-3.3.4	(SEQ ID NO:518)	68
	LEWLLY D3-3.1.5	(SEQ ID NO:525)	69
	WSGYT 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
40	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
45	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
50	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
55	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
5	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
10	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
15	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
20	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
25	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
30	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
35	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
	DSSGY Y	D3-22.2.4	(SEQ ID NO:734)	129
	IVVVIT	D3-22.3.4	(SEQ ID NO:735)	130
40	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
45	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
50	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
	VEMAT I	D5-24.1.1	(SEQ ID NO:829)	144
	yRWLQL	D5-24.2.1	(SEQ ID NO:830)	145
55	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
5	GIAVAG D6-19.2.1	(SEQ ID NO:879)	153
	VyQWL V D6-19.3.1	(SEQ ID NO:880)	154
	YSSGWY D6-19.1.2	(SEQ ID NO:887)	155

10 **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 than 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 excluded. 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 from 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.

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

Length	Parental sequence	level of doping	Comment	SEQ ID NO:
6a	yycaKAEYFQHwgggtltvss	70:10:10:10	JH1(whole)	226
6b	yycaKYDYGDYwgggtltvss	70:10:10:10	Y::D4-17(2)::FR4 of JH1	227
6c	yycaKGYSYGYwgggtltvss	70:10:10:10	D5-5(3)::FR4 of JH1	228
6d	yycaKSYFDYwgggtltvss	70:10:10:10	SY::JH4(whole)	229
8a	yycaKYAEYFQHwgggtltvss	73:9:9:9	YY:JH1(whole)	230
8b	yycaKGYSSSWYwgggtltvss	73:9:9:9	Y::D6-13(1)::FR4 of JH1	231
8c	yycaKYGDIYFDYwgggtltvss	73:9:9:9	D4-17(2)[2-5]:JH4(whole)	232
10a	yycaKYYDSSGYYYwgggtltvss	73:9:9:9	D3-22(2)::Fr4 of JH1	233
10b	yycaKGycSSTScYTWgggtltvss	73:9:9:9	D2-2(2)::Fr4 of JH1	234
10c	yycaKYYSSAEYFQHwgggtltvss	73:9:9:9	YYSS::JH1(whole) (SEQ ID NO: 942)	235
10d	yycaKGYSYGYFDYwgggtltvss	73:9:9:9	D5-5(3)::JH4(whole)	236
12a	yycaKYYDSSGYYYQHwgggtltvss	85:5:5:5	D3-22(2)::QH::Fr4 of JH1	237
12b	yycaKGycSSTScYTQHwgggtltvss	85:5:5:5	D2-2(2)::QH::Fr4 of JH1	238
12c	yycaKYDGSYSAEYFQHwgggtltvss	85:5:5:5	YDGSYS::JH1(whole) (SEQ ID NO: 943)	239
12d	yycaKYYDYVWGSYRYTWgggtltvss	85:5:5:5	D3-16(2)::Fr of JH1	240
12e	yycaKGYSYGYWYFDLwgrgtltvss	85:5:5:5	D5-5(3)::JH2(whole)	241
14a	yycaKYYDSSGYYYFQHwgggtltvss	73:9:9:9	D3-22(2)::YFQH::Fr of JH1 (SEQ ID NO: 944)	242
14b	yycaKGycSSTScYTYFQHwgggtltvss	73:9:9:9	D2-2(2)::YFQH::Fr of JH1 (SEQ ID NO: 944)	243
14c	yycaKSYGYcSSTScYTQHwgggtltvss	73:9:9:9	SY::D2-2(2)::QH::Fr of JH1	244
14d	yycaKSYRYSGYSAEYFQHwgggtltvss	73:9:9:9	SYRYSGYS::JH1(whole) (SEQ ID NO: 945)	245
14e	yycaKAYcGGDcYSNWFDpwgggtltvss	73:9:9:9	D2-21(2)::JH5(whole)	246

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15a	yycakSDGYYYDSSGYYDYwgqgtltvss	73:9:9:9	SD::D3-22.2::JH4(101ff)	930
15b	yycakGSGYCSGGSCYSFDYwgqgtltvss	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	yycakYYDSSGYYAEIFQHwgqgtltvss	73:9:9:9	D3-22(2)::JH1(whole)	247
16b	yycakGYcSSTScYTAEIFQHwgqgtltvss	73:9:9:9	D2-2(2)::JH1(whole)	248
16c	yycakSYDSYRSYGSAEIFQHwgqgtltvss	73:9:9:9	SYDSYRSYGS::JH1(whole) (SEQ ID NO: 946)	249
16d	yycakSYSYGYcSSTScYTQHwgqgtltvss	73:9:9:9	SYSY::D2-2(2)::QH::Fr JH1 (SEQ ID NO: 947)	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	yycakDGYCSGGSCYSYYYYGMDVwgqgttvt vss	73:9:9:9	D::2-15.2::JH6(-2Ys)	222
19a	yycakDGYYYDSSGYYRGYYFDYwgqgtlv tvss	73:9:9:9	D::D3-22.2::RGY::JH4(all)	223
20a	yycakYSSYYYYDSSGYYAEIFQHwgqgtl vtvss	73:9:9:9	YSSY::D3-22(2)::JH1(whole) (SEQ ID NO: 948)	251
20b	yycakSYYSGYcSSTScYTAEIFQHwgqgtlt vss	73:9:9:9	SYYS::D2-2(2)::JH1(whole) (SEQ ID NO: 949)	252
20c	yycakSGYcSSTScYTYSAEIFQHwgqgtlt 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.

```

5      !      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
      !
10     !      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)
15     !      NcoI....
      !      VH
      !
      !      FR1 (DP47/V3-23) -----
20     !      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 |
      !
25     !      -----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|
      !
30     !      -----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.
35     !
      !      -----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|
40     !      ...BstXI
      !
      !      .....CDR2.....|-----FR3-----
45     !      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-----
50     !      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----->|
55     !      82a 82b 82c 83 84 85 86 87 88 89 90 91 92 93 94

```

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!       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|
!       |AflIII |               | PstI | (2/2)
5  !
!       .....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 |
10 !
!       -----FR4----->|
!       107 108 109 110 111 112 113
!       T   M   V   T   V   S   S
1351 |act|atG|GTC|ACC|gtc|tct|agt
15 !       | BstEII | c tcg ag = XhoI.
!
! CH1
20 !
!       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
25 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
30 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
35 !
!       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.....
40 !
!       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.....
45 !
!       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.....
50 !       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....
55 !
!       .....
!       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
5  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
!
10 !   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
15 !
!   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
20 !  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
!
25 !   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
30 !  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
35 !
!   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.

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40 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

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45 !-----
!   1   AATGCTACTA CTATTAGTAG AATTGATGCC ACCTTTTCAG CTCGCGCCCC AAATGAAAAT
!   61   ATAGCTAAAC AGGTTATTGA CCATTTGCGA AATGTATCTA ATGGTCAAAC TAAATCTACT
!  121   CGTTCGCAGA ATTGGGAATC AACTGTTATA TGGAATGAAA CTTCCAGACA CCGTACTTTA
!  181   GTTGCAATTT TAAAACATGT TGAGCTACAG CATTATATTC AGCAATTAAG CTCTAAGCCA
50 !  241   TCCGCAAAAA TGACCTCTTA TCAAAAGGAG CAATTAAAGG TACTCTCTAA TCCTGACCTG
!  301   TTGGAGTTTG CTTCCGGTCT GGTTCGCTTT GAAGCTCGAA TTAAAACGCG ATATTTGAAG
!  361   TCTTTCGGGC TTCCTCTTAA TCTTTTGTAT GCAATCCGCT TTGCTTCTGA CTATAATAGT
!  421   CAGGGTAAAG ACCTGATTTT TGATTTATGG TCATTCTCGT TTTCTGAACT GTTTAAAGCA

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50860-279

	481	TTTGAGGGGG	ATTCAATGAA	TATTTATGAC	GATTCGCGCAG	TATTGGACGC	TATCCAGTCT
	541	AAACATTTTA	CTATTACCCC	CTCTGGCAAA	ACTTCTTTTG	CAAAAGCCTC	TCGCTATTTT
	601	GGTTTTTATC	GTCGTCTGGT	AAACGAGGGT	TATGATAGTG	TTGCTCTTAC	TATGCCTCGT
	661	AATTCCTTTT	GGCGTTATGT	ATCTGCATTA	GTTGAATGTG	GTATTCCTAA	ATCTCAACTG
5	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
10	1021	TGTACACCGT	TCATCTGTCC	TCTTTCAAAG	TTGGTCAGTT	CGGTTCCCTT	ATGATTGACC
	1081	GTCTGCGCCT	CGTTCCGGCT	AAGTAACATG	GAGCAGGTCTG	CGGATTTCTGA	CACAATTTAT
	1141	CAGGCGATGA	TACAAATCTC	CGTTGTACTT	TGTTTCGCGC	TTGGTATAAT	CGCTGGGGGT
	1201	CAAAGATGAG	TGTTTTAGTG	TATTCTTTTG	CCTCTTTCGT	TTAGGTTTGG	TGCCTTCGTA
	1261	GTGGCATTAC	GTATTTTACC	CGTTTAATGG	AAACTTCCTC	ATGAAAAAGT	CTTTAGTCCT
15	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	GTTCTTTTCT
20	1621	ATTCTCACTC	CGCTGAAACT	GTTGAAAGTT	GTTTAGCAAA	ATCCCATACA	GAAAATTCAT
	1681	TTACTAACGT	CTGGAAAGAC	GACAAAACCT	TAGATCGTTA	CGCTAACTAT	GAGGGCTGTC
	1741	TGTGGAATGC	TACAGGCGTT	GTAGTTTGTG	CTGGTGACGA	AACTCAGTGT	TACGGTACAT
	1801	GGGTTCTTAT	TGGGCTTGCT	ATCCCTGAAA	ATGAGGGTGG	TGGCTCTGAG	GGTGGCGGTT
	1861	CTGAGGGTGG	CGGTTCTGAG	GGTGGCGGTA	CTAAACCTCC	TGAGTACGGT	GATACACCTA
25	1921	TTCCGGGCTA	TACTTATATC	AACCCTCTCG	ACGGCACTTA	TCCGCCTGGT	ACTGAGCAAA
	1981	ACCCCGCTAA	TCCTAATCCT	TCTCTTGAGG	AGTCTCAGCC	TCTTAATACT	TTCATGTTTC
	2041	AGAATAATAG	GTTCCGAAAT	AGGCAGGGGG	CATTAAGTGT	TTATACGGGC	ACTGTTACTC
	2101	AAGGCACTGA	CCCCGTAAA	ACTTATTACC	AGTACACTCC	TGTATCATCA	AAAGCCATGT
	2161	ATGACGCTTA	CTGGAACGGT	AAATTCAGAG	ACTGCGCTTT	CCATTCTGGC	TTTAATGAGG
30	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	AACTTGATTG	TGTCGCTACT	GATTACGGTG
35	2521	CTGCTATCGA	TGGTTTCATT	GGTGACGTTT	CCGGCCTTGC	TAATGGTAAT	GGTGCTACTG
	2581	GTGATTTTGC	TGGCTCTAAT	TCCCAAATGG	CTCAAGTCGG	TGACGGTGAT	AATTCACCTT
	2641	TAATGAATAA	TTTCCGTCAA	TATTTACCTT	CCCTCCCTCA	ATCGGTGAA	TGTCGCCCTT
	2701	TTGTCTTTGG	CGCTGGTAAA	CCATATGAAT	TTTCTATTGA	TTGTGACAAA	ATAAACTTAT
	2761	TCCGTGGTGT	CTTTGCGTTT	CTTTTATATG	TTGCCACCTT	TATGTATGTA	TTTTCTACGT
40	2821	TTGCTAACAT	ACTGCGTAAT	AAGGAGTCTT	AATCATGCCA	GTTCTTTTGG	GTATTCCGTT
	2881	ATTATTGCGT	TTCCTCGGTT	TCCTTCTGGT	AACTTTGTTC	GGCTATCTGC	TTACTTTTCT
	2941	TAAAAAGGGC	TTCGGTAAGA	TAGCTATTGC	TATTTTATTG	TTTCTTGCTC	TTATTATTGG
	3001	GCTTAACTCA	ATTCTTGTGG	GTTATCTCTC	TGATATTAGC	GCTCAATTAC	CCTCTGACTT
	3061	TGTTCAAGGT	GTTTCAAGTTA	TTCTCCCGTC	TAATGCGCTT	CCCTGTTTTT	ATGTTATTCT
45	3121	CTCTGTAAAG	GCTGCTATTT	TCATTTTGTG	CGTTAAACAA	AAAATCGTTT	CTTATTTGGA
	3181	TTGGGATAAA	TAATATGGCT	GTTTATTTTG	TAAGTGGCAA	ATTAGGCTCT	GGAAAGACGC
	3241	TCGTTAGCGT	TGGTAAGATT	CAGGATAAAA	TTGTAGCTGG	GTGCAAAATA	GCAACTAATC
	3301	TTGATTTAAG	GCTTCAAAAC	CTCCCGCAAG	TCGGGAGGTT	CGCTAAAACG	CCTCGCGTTC
	3361	TTAGAATACC	GGATAAGCCT	TCTATATCTG	ATTGCTTTCG	TATTGGGCGC	GGTAATGATT
50	3421	CCTACGATGA	AAATAAAAAC	GGCTTGCTTG	TTCTCGATGA	GTGCGGTACT	TGGTTTAATA
	3481	CCCGTTCTTG	GAATGATAAG	GAAAGACAGC	CGATTATTGA	TTGGTTTCTA	CATGCTCGTA
	3541	AATTAGGATG	GGATATTATT	TTTCTTGTTT	AGGACTTATC	TATTGTTGAT	AAACAGGCGC
	3601	GTTCTGCATT	AGCTGAACAT	GTTGTTTATT	GTCGTCGTCT	GGACAGAATT	ACTTTACCTT
	3661	TTGTCGGTAC	TTTATATTCT	CTTATTACTG	GCTCGAAAAT	GCCTCTGCCT	AAATTACATG
55	3721	TTGGCGTTGT	TAAATATGGC	GATTCTCAAT	TAAGCCCTAC	TGTTGAGCGT	TGGCTTTATA
	3781	CTGGTAAGAA	TTTGTATAAC	GCATATGATA	CTAAACAGGC	TTTTTCTAGT	AATTATGATT
	3841	CCGGTGTTTA	TTCTTATTTA	ACGCCTTATT	TATCACACGG	TCGGTATTTT	AAACCATTAA

3901	ATTTAGGTCA	GAAGATGAAA	TTAACTAAAA	TATATTTGAA	AAAGTTTTCT	CGCGTTCCTT
3961	GTCTTGCGAT	TGGATTTGCA	TCAGCATTTA	CATATAGTTA	TATAACCCAA	CCTAAGCCGG
4021	AGGTAAAAA	GGTAGTCTCT	CAGACCTATG	ATTTTGATAA	ATTCCTATT	GACTCTTCTC
4081	AGCGTCTTAA	TCTAAGCTAT	CGCTATGTTT	TCAAGGATTC	TAAGGGAAAA	TTAATTAATA
5	4141	GCGACGATTT	ACAGAAGCAA	GGTTATTCAC	TCACATATAT	TGATTTATGT
	4201	TTAAAAAAGG	TAATTCAAAT	GAAATTGTTA	AATGTAATTA	ATTTTGTTTT
	4261	GTTTCATCAT	CTTCTTTTGC	TCAGGTAATT	GAAATGAATA	ATTCGCCTCT
	4321	GTAACCTGGT	ATTCAAAGCA	ATCAGGCGAA	TCCGTTATTG	TTTCTCCCGA
	4381	ACTGTTACTG	TATATTCATC	TGACGTTAAA	CCTGAAAATC	TACGCAATTT
10	4441	GTTTTACGTG	CAAATAAATT	TGATATGGTA	GGTTCTAACC	CTTCCATAAT
	4501	AATCCAAACA	ATCAGGATTA	TATTGATGAA	TTGCCATCAT	CTGATAATCA
	4561	GATAATTCCG	CTCCTTCTGG	TGGTTTCTTT	GTTCCGCAAA	ATGATAATGT
	4621	TTTAAAATTA	ATAACGTTCC	GGCAAAGGAT	TTAATACGAG	TTGTCGAATT
	4681	TCTAATACTT	CTAAATCCTC	AAATGTATTA	TCTATTGACG	GCTCTAATCT
15	4741	AGTGCTCCTA	AAGATATTTT	AGATAACCTT	CCTCAATTCC	TTTCAACTGT
	4801	ACTGACCAGA	TATTGATTGA	GGGTTTGATA	TTTGAGGTTT	AGCAAGGTGA
	4861	TTTTCATTTG	CTGCTGGCTC	TCAGCGTGGC	ACTGTTGCAG	GCGGTGTTAA
	4921	CTCACCTCTG	TTTTATCTTC	TGCTGGTGGT	TCGTTCCGTA	TTTTTAATGG
	4981	GGGCTATCAG	TTCGCGCATT	AAAGACTAAT	AGCCATTCAA	AAATATTGTC
20	5041	ATTCTTACGC	TTTCAGGTCA	GAAGGGTTCT	ATCTCTGTTG	GCCAGAATGT
	5101	ACTGGTCGTG	TGACTGGTGA	ATCTGCCAAT	GTAAATAATC	CATTTTCAGAC
	5161	CAAAATGTAG	GTATTTCCAT	GAGCGTTTTT	CCTGTTGCAA	TGGCTGGCGG
	5221	CTGGATATTA	CCAGCAAGGC	CGATAGTTTG	AGTTCTTCTA	CTCAGGCAAG
	5281	ACTAATCAAA	GAAGTATTGC	TACAACGGTT	AATTTGCGTG	ATGGACAGAC
25	5341	GGTGGCCTCA	CTGATTATAA	AAACACTTCT	CAGGATTCTG	GCGTACCGTT
	5401	ATCCCTTTAA	TCGGCCTCCT	GTTTAGCTCC	CGCTCTGATT	CTAACGAGGA
	5461	TACGTGCTCG	TCAAAGCAAC	CATAGTACGC	GCCCTGTAGC	GGCGCATTAA
	5521	TGTGGTGGTT	ACGCGCAGCG	TGACCGCTAC	ACTTGCCAGC	GCCCTAGCGC
	5581	CGCTTCTTTC	CCTTCCTTTC	TCGCCACGTT	CGCCGGCTTT	CCCCGTCAAG
30	5641	GGGGCTCCCT	TTAGGGTTCC	GATTTAGTGC	TTTACGGCAC	CTCGACCCCA
	5701	TTTGGGTGAT	GGTTCACGTA	GTGGGCCATC	GCCCTGATAG	ACGGTTTTTC
	5761	GTTGGAGTCC	ACGTTCTTTA	ATAGTGGACT	CTTGTTCCAA	ACTGGAACAA
	5821	TATCTCGGGC	TATTCTTTTG	ATTTATAAGG	GATTTTGCCG	ATTTTCGGAAC
	5881	CAGGATTTTC	GCCTGCTGGG	GCAAACCAGC	GTGGACCGCT	TGCTGCAACT
35	5941	CAGGCGGTGA	AGGGCAATCA	GCTGTTGCCC	GTCTCACTGG	TGAAAAGAAA
	6001	GATCCAAGCT	TGCAGGTGGC	ACTTTTCGGG	GAAATGTGCG	CGGAACCCCT
	6061	TTTTCTAAAT	ACATTCAAAT	ATGTATCCGC	TCATGAGACA	ATAACCCTGA
	6121	AATAATATTG	AAAAAGGAAG	AGTATGAGTA	TTCAACATTT	CCGTGTCGCC
	6181	TTTTTGCGGC	ATTTTGCCCT	CCTGTTTTTG	CTCACCCAGA	AACGCTGGTG
40	6241	ATGCTGAAGA	TCAGTTGGGC	GCACTAGTGG	GTTACATCGA	ACTGGATCTC
	6301	AGATCCTTGA	GAGTTTTTCG	CCCGAAGAAC	GTTTTCCAAT	GATGAGCACT
	6361	TGCTATGTGG	CGCGGTATTA	TCCCGTATTG	ACGCCGGGCA	AGAGCAACTC
	6421	TACACTATTC	TCAGAATGAC	TTGGTTGAGT	ACTCACCAGT	CACAGAAAAG
	6481	ATGGCATGAC	AGTAAGAGAA	TTATGCAGTG	CTGCCATAAC	CATGAGTGAT
45	6541	CCAACTTACT	TCTGACAACG	ATCGGAGGAC	CGAAGGAGCT	AACCGCTTTT
	6601	TGGGGGATCA	TGTAACCTCG	CTTGATCGTT	GGGAACCGGA	GCTGAATGAA
	6661	ACGACGAGCG	TGACACCACG	ATGCCTGTAG	CAATGGCAAC	AACGTTGCGC
	6721	CTGGCGAACT	ACTTACTCTA	GCTTCCCGGC	AACAATTAAT	AGACTGGATG
	6781	AAGTTGCAGG	ACCACTTCTG	CGCTCGGCCC	TTCCGGCTGG	CTGGTTTATT
50	6841	CTGGAGCCGG	TGAGCGTGGG	TCTCGCGGTA	TCATTGCAGC	ACTGGGGCCA
	6901	CCTCCCGTAT	CGTAGTTATC	TACACGACGG	GGAGTCAGGC	AACTATGGAT
	6961	GACAGATCGC	TGAGATAGGT	GCCTCACTGA	TTAAGCATTG	GTAAGTGTCA
	7021	ACTCATATAT	ACTTTAGATT	GATTTAAAAC	TTCATTTTAA	ATTTAAAAGG
	7081	AGATCCTTTT	TGATAATCTC	ATGACCAAAA	TCCCTTAACG	TGAGTTTTCG
55	7141	CGTAAGACCC	CCAAGCTTGT	CGACTGAATG	GCGAATGGCG	CTTTGCCTGG
	7201	CAGAAGCGGT	GCCGGAAAGC	TGGCTGGAGT	GCGATCTTCC	TGACGCTCGA

XhoI...

7261 ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCCAGGCTT TACACTTTAT GCTTCCGGCT
 7321 CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTTACA CAGGAAACAG CTATGACCAT
 7381 GATTACGCCA AGCTTTGGAG CCTTTTTTTT GGAGATTTTC AAC

5

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

	1	aatgctacta	ctattagtag	aattgatgcc	acctttttcag	ctcgcgcccc	aaatgaaaat
	61	atagctaâac	aggttattga	ccatttgcca	aatgtatcta	atgggtcaaac	taaatctact
10	121	cgttcgcaga	attgggaatc	aactgttata	tggaaatgaaa	cttccagaca	ccgtacttta
	181	gttgcatatt	taaaacatgt	tgagctacag	cattatattc	agcaattaag	ctctaagcca
	241	tccgcaaaaa	tgacctctta	tcaaaaggag	caattaaagg	tactctctaa	tcctgacctg
	301	ttggagtttg	cttccgggtc	ggttcgcttt	gaagctcgaa	ttaaaacgcg	atatttgaag
	361	tctttcgggc	ttcctcttaa	tctttttgat	gcaatccgct	ttgcttctga	ctataatagt
	421	cagggtaaag	acctgatttt	tgatttatgg	tcatctcgt	tttctgaact	gtttaaagca
15	481	tttgaggggg	attcaatgaa	tatttatgac	gattccgcag	tattggacgc	tatccagtct
	541	aaacatttta	ctattacccc	ctctggcaaa	acttcttttg	caaaagcctc	tcgctatttt
	601	ggtttttata	gtcgtctggt	aaacgaggg	tatgatagtg	ttgctcttac	tatgcctcgt
	661	aattcctttt	ggcgttatgt	atctgcatta	gttgaatgtg	gtattcctaa	atctcaactg
	721	atgaatcttt	ctacctgtaa	taatgttggt	ccgttagttc	gttttattaa	cgtagatttt
20	781	tcttcccaac	gtcctgactg	gtataatgag	ccagttctta	aaatcgcata	aggtaattca
	841	caatgattaa	agttgaaatt	aaacctctc	aagcccaatt	tactactcgt	tctgggtgtt
	901	ctcgtcaggg	caagccttat	tactgaatg	agcagctttg	ttacgttgat	ttgggtaatg
	961	aatatccggt	tcttgtcaag	attactcttg	atgaagggtc	gccagcctat	gcgcctggtc
	1021	tgtacaccgt	tcatctgtcc	tctttcaaa	ttgggtcagtt	cggttccctt	atgattgacc
25	1081	gtctgcgcct	cgttccggct	aagtaacatg	gagcaggtcg	cggatttcga	cacaatttat
	1141	caggcgatga	tacaaatctc	cgttgtactt	tgtttcgcgc	ttgggtataat	cgctgggggt
	1201	caaagatgag	tgttttagtg	tattcttttg	cctctttcgt	tttaggttgg	tgccctcgta
	1261	gtggcattac	gtattttacc	cgtttaatgg	aaacttcctc	atgaaaaagt	cttagtcct
	1321	caaagcctct	gtagccgttg	ctaccctcgt	tccgatgctg	tctttcgcgt	ctgaggggtga
30	1381	cgatcccga	aaagcggcct	ttaactccct	gcaagcctca	gcgaccgaat	atatcggtta
	1441	tgcgtgggag	atggttggtg	tcattgtcgg	cgcaactatc	ggatcaagc	tgtttaagaa
	1501	attcacctcg	aaagcaagct	gataaaccga	tacaattaaa	ggctcctttt	ggagcctttt
	1561	tttttggaga	ttttcaacgt	gaaaaaatta	ttattcgcaa	ttccttttagt	tgttcctttc
	1621	tattctcact	ccgctgaaac	tgttgaaagt	tgttttagcaa	aatcccatat	agaaaattca
35	1681	tttactaacg	tctggaaaga	cgacaaaact	ttagatcggt	acgctaacta	tgagggtcgt
	1741	ctgtggaatg	ctacaggcgt	tgtagtgtgt	actggtgacg	aaactcagtg	ttacggtaca
	1801	tgggttccta	ttgggcttgc	tatccctgaa	aatgaggggtg	gtggctctga	gggtggcggt
	1861	tctgaggggtg	gcgggttctga	gggtggcggt	actaaacctc	ctgagtacgg	tgatacacct
	1921	attccggggt	atacttatat	caaccctctc	gacggcactt	atccgcctgg	tactgagcaa
40	1981	aaccccgtta	atcctaattc	ttctcttgag	gagctctcagc	ctcttaatac	tttcatgttt
	2041	cagaataata	ggttccgaaa	taggcagggg	gcattaactg	tttatacggg	cactgttact
	2101	caaggcactg	accccgttaa	aacttattac	cagtacactc	ctgtatcatc	aaaagccatg
	2161	tatgacgctt	actggaacgg	taaattcaga	gactgcgctt	tccattctgg	ctttaatgag
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45	2281	gctggcgagg	gctctgggtg	tgggtctggt	ggcggtctctg	aggggtggtg	ctctgagggg
	2341	ggcggttctg	aggggtggcg	ctctgagggg	ggcggttccg	gtgggtggctc	tgggtccggg
	2401	gatttttgatt	atgaaaagat	ggcaaacgct	aat'aaggggg	ctatgaccga	aaatgccgat
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	2641	ttaatgaata	atctccgtca	atatttacct	tccctccctc	aatcggttga	atgtcgccct
	2701	tttgtctttg	gcgctggtaa	accatatgaa	ttttctattg	attgtgacaa	aataaactta
	2761	ttccgtgggtg	tctttgcgtt	tcttttatat	gttgccacct	ttatgtatgt	atcttctacg
	2821	tttgctaaca	tactgcgtaa	taaggagtct	taatcatgcc	agttcttttg	ggatttccgt
55	2881	tattattgag	tttccctcgg	ttccttctgg	taactttggt	cggctatctg	cttacttttc
	2941	ttaaaaaggg	cttcggtaag	atagctattg	ctatttcatt	gtttcttggc	cttattattg

	3001	ggcttaactc	aattcttgtg	ggttatctct	ctgatattag	cgctcaatta	ccctctgact
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	3121	tctctgtaaa	ggctgctatt	ttcatttttg	acgttaaaca	aaaaatcggt	tcttatttgg
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	3241	ctcggttagcg	ttggtaagat	tcaggataaa	attgtagctg	ggtgcaaaat	agcaactaat
	3301	cttgatttaa	ggcttcaaaa	cctcccgcga	gtcgggaggt	tcgctaaaac	gcctcgcggt
	3361	cttagaatac	cggataagcc	ttctatatct	gatttgcttg	ctattgggcg	cggtaatgat
	3421	tcctacgatg	aaaataaaaa	cggcttgctt	gttctcgatg	agtgcggtac	ttggtttaat
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	3541	aaattaggat	gggatattat	ttttcttgtt	caggacttat	ctattggtga	taaacaggcg
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	3721	gttggcggtg	ttaaatatgg	cgattctcaa	ttaagcccta	ctgttgagcg	ttggctttat
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	3961	tgtcttgcca	ttggatttgc	atcagcattt	acatatagtt	atataaccca	acctaagccg
	4021	gaggttaaaa	aggtagtctc	tcagacctat	gatttttgata	aattcactat	tgactcttct
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	4321	tgtaacttgg	tattcaaagc	aatcaggcga	atccgttatt	gtttctcccg	atgtaaaagg
	4381	tactgttact	gtatattcat	ctgacgttaa	acctgaaaat	ctacgcaatt	tctttatttc
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	4501	taatccaaac	aatcaggatt	atattgatga	attgccatca	tctgataatc	aggaatatga
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	4621	ttttaaaatt	aataacgttc	gggcaaagga	tttaatacga	gttgctgaat	tgtttgtaaa
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	4801	aactgaccag	atattgattg	agggtttgat	atttgagggt	cagcaagggtg	atgctttaga
	4861	tttttcatth	gctgctggct	ctcagcgtgg	cactgttgca	ggcggtgtta	atactgaccg
	4921	cctcacctct	gttttatctt	ctgctggtgg	ttcggtcggt	atthtttaattg	gcgatgtttt
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	5161	tcaaaatgta	ggtattttcca	tgagcgtttt	tctgtttgca	atggctggcg	gtaatatgtg
	5221	tctggatatt	accagcaagg	ccgatagttt	gagttcttct	actcaggcaa	gtgatgttat
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	5401	aatcccttta	atcggcctcc	tgtttagctc	ccgctctgat	tctaacgagg	aaagcacgtt
	5461	atacgtgctc	gtcaaagcaa	ccatagtagc	cgccctgtag	cggcgcattha	agcgcgcggt
	5521	gtgtggtggt	tacgcgcagc	gtgaccgcta	cacttgccag	cgccctagcg	cccgtcctt
	5581	tcgctttctt	cccttccctt	ctcgccacgt	tcgcccgtt	tcccgtcaa	gctctaaatc
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	5701	atthgggtga	tggttcacgt	agtgggccat	cgccctgata	gacggttttt	cgccctttga
	5761	cgthggagtc	cacgttcttt	aatagtggac	tcttgthcca	aactggaaca	acactcaacc
	5821	ctatctcggt	ctattctttt	gatttataag	ggattttgct	gatttcggaa	ccaccatcaa
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	5941	ccaggcggtg	aagggtcaatc	agctgttgcc	cgtctcactg	gtgaaaagaa	aaaccaccct
	6001	ggatccaagc	ttgcagggtg	cacttttcgg	ggaaatgtgc	gcggaacccc	tatttgthta
	6061	tttttctaaa	tacattcaaa	tatgtatccg	ctcatgagac	aataaccctg	ataaatgctt
	6121	caataatatt	gaaaaaggaa	gagtatgagt	attcaacatt	tccgtgtcgc	ccttattccc
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	6241	gatgctgaag	atcagttggg	cgcactagtg	ggthacatcg	aactggatct	caacagcggt
	6301	aagatccttg	agagtttttcg	ccccgaagaa	cgttttccaa	tgatgagcac	ttttaaagtt
	6361	ctgctatgtg	gcgcggtatt	atcccgtatt	gacgcggggc	aagagcaact	cggctcgccgc

5 6421 atacactatt ctcagaatga cttgggttgag tactcaccag tcacagaaaa gcatcttacg
 6481 gatggcatga cagtaagaga attatgcagt gctgccataa ccatgagtga taacactgcg
 6541 gccaaacttac ttctgacaac gatcggagga ccgaaggagc taaccgcttt tttgcacaac
 6601 atgggggatc atgtaactcg ccttgatcgt tgggaaccgg agctgaatga agccatacca
 6661 aacgacgagc gtgacaccac gatgcctgta gcaatggcaa caacggtgcg caaactatta
 6721 actggcgaac tacttactct agcttcccgg caacaattaa tagactggat ggaggcggat
 6781 aaagttgcag gaccacttct gcgctcggcc cttccggctg gctggtttat tgctgataaa
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 7021 tactcatata tacttttagat tgatttaaaa cttcattttt aatttaaaag gatctaggtg
 7081 aagatccttt ttgataatct catgaccaa atcccttaac gtgagttttc gttccactgt
 7141 acgtaagacc cccaagcttg tgcactgaat ggcgaatggc gctttgcctg gtttccggca
 7201 ccagaagcgg tgcgggaaag ctggctggag tgcgatcttc ctgacgctcg agcgcaacgc
 15 7261 aattaatgtg agttagctca ctcataggc accccaggct ttacacttta tgcttccggc
 7321 tcgtatgttg tgtggaattg tgagcggata acaatttcac acaggaaaca gctatgacca
 7381 tgattacgcc aagctttgga gccttttttt tggagatttt caacatgaaa tacctattgc
 7441 ctacggcagc cgctggattg ttattactcg cGGCCcagcc GGCCatggcc gaagttcaat
 20 7501 tgtagagtc tgggtggcgg cttgttcagc ctggtgggtc tttacgtctt tcttgcgctg
 7561 cttccggatt cactttctct tcgtacgcta tgtcttgggt tcgccaagct cctggtaaaag
 7621 gtttggagtg ggtttctgct atctctgggt ctggtggcag tacttactat gctgactccg
 7681 ttaaaggctc cttcactatc tctagagaca actctaagaa tactctctac ttgcagatga
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 8041 gcagcgtagt gaccgtgccc tccagcagct tgggcaccca gacctacatc tgcaacgtga
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 8161 cacatcatca tcaccatcac ggggcccagc acaaaaaact catctcagaa gaggatctga
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 8281 ccaacaaagg cgccatgact gagaacgctg acgagaatgc tttgcaaagc gatgccaaag
 8341 gtaagttaga cagcgtcgcg accgactatg gcgcccgcac cgacggcttt atcggcgatg
 35 8401 tcagtggttt ggccaacggc aacggagcca ccggagactt cgcaggttcg aattctcaga
 8461 tggcccagggt tggagatggg gacaacagtc cgcttatgaa caactttaga cagtaccttc
 8521 cgtctcttcc gcagagtgtc gagtgcgctc cattcgtttt cgggtgccggc aagccttacg
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 9421 accgatttag ctttatgctc tgaggcttta ttgcttaatt ttgctaattc tttgccttgc
 9481 ctgtatgatt tattggatgt t

55 Table 35: DNA sequence of pMID21: 5957 bp (SEQ ID NO:895)

1 gacgaaaggg cctcgtgata cgcctatttt tatagggttaa tgtcatgata ataatggttt

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	301	ctgaagatca	gttgggtgcc	cgagtgggtt	acatcgaaact	ggatctcaac	agcggtaaga
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	421	tatgtggcgc	ggtattatcc	cgtattgacg	ccgggcaaga	gcaactcggg	cgccgcatac
	481	actattctca	gaatgacttg	gttgagtact	caccagtcac	agaaaagcat	cttacggatg
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	601	acttacttct	gacaacgatc	ggaggaccga	aggagctaac	cgcttttttg	cacaacatgg
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	961	cccgtatcgt	agttatctac	acgacgggga	gtcaggcaac	tatggatgaa	cgaaatagac
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	1261	gctgcttgca	aacaaaaaaa	ccaccgctac	cagcgggtgt	ttgtttgccg	gatcaagagc
	1321	taccaactct	ttttccgaag	gtaactggct	tcagcagagc	gcagatacca	aatactgttc
	1381	ttctagtgtg	gccgtagtta	ggccaccact	tcaagaactc	tgtagcaccg	cctacatacc
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	2761	ttcgtggccc	agccggcctc	tgtggaagtt	caattgttag	agtctgggtg	cggctctgtt
	2821	cagcctgggtg	gttcttttacg	tctttcttgc	gctgcttccg	gagcttcaga	tctgtttgcc
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	3301	ttgtatgagg	cgggtgcaggg	agacaaatca	ccaatcccac	aggcgggttg	tctgtttgct
	3361	gggaaaccac	agcaggaggt	tgtgttggct	gcgctggaag	atacctggga	gactctttcc
	3421	aaacgctatg	gcaataatgt	gagtaactgg	aaaacaccgg	caatggcctt	aacgttccgg

3481 gcaaataatt tcttttggtgt accgcaggcc gcagcgggaag aaacgcgtca tcaggcggag
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 3661 acagttgata agcactatga agatcagctg aaaatgtacg aaaatttttg ccgtaagtgc
 5 3721 ctctggttaa cgaagcagga tgtggaggcg cataaggagt tctagagaca actctaagaa
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 4141 aggtgtataa cccccagcag ctagccatat tctctcggtc accgtctcaa gcgcctccac
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 4261 ggccctgggc tgccctggtca aggactactt cccgaaccg gtgacggtgt cgtggaactc
 15 4321 aggcgccttg accagcggcg tccacacctt cccggtgtc ctacagtcta gcggactcta
 4381 ctccctcagc agcgtagtga ccgtgccctc ttctagcttg ggcaccaga cctacatctg
 4441 caacgtgaat cacaagccca gcaacaccaa ggtggacaag aaagttgagc ccaaattctg
 4501 tgcggccgca catcatcatc accatcacgg ggccgcagaa caaaaactca tctcagaaga
 4561 ggatctgaat ggggccgcag aggctagttc tgctagtaac gcgtcttccg gtgattttga
 20 4621 ttatgaaaag atggcaaacg ctaataaggg ggctatgacc gaaaatgccg atgaaaacgc
 4681 gctacagtct gacgctaaag gcaaacttga ttctgtcgct actgattacg gtgctgctat
 4741 cgatggtttc attggtgacg tttccggcct tgctaattgt aatggtgcta ctggtgattt
 4801 tgctggctct aattcccaaa tggctcaagt cgggtgacgt gataattcac ctttaatgaa
 4861 taatttccgt caatatttac ctccctccc tcaatcggtt gaatgtcgcc cttttgtctt
 25 4921 tggcgctggt aaaccatatg aattttctat tgattgtgac aaaataaact tattccgtgg
 4981 tgtctttgcy tttcttttat atgttgccac ctttatgtat gtattttcta cgtttgctaa
 5041 catactgcgt aataaggagt cttaatgaaa cgcgtgatga gaattcactg gccgtcgttt
 5101 tacaacgtcg tgactgggaa aaccctggcg ttaccaact taatcgctt gcagcacatc
 5161 cccctttcgc cagctggcgt aatagcgaag aggcgcgac cgatcgccct tcccaacagt
 30 5221 tgcgcagcct gaatggcgaa tggcgctga tgcggtatth tctccttacg catctgtgcg
 5281 gtattttaca ccgcatacgt caaagcaacc atagtacgcg ccctgtagcg gcgcattaag
 5341 cgcggcgggt gtggtggtta cgcgcagcgt gaccgctaca ctgcccagcg ccttagcgcc
 5401 cgtccctttc gctttcttcc ctccctttct cgcacgctc gccggcttcc cccgtcaagc
 5461 tctaaatcgg gggctccctt tagggttccg atttagtgct ttacggcacc tcgaccccaa
 35 5521 aaaacttgat ttgggtgatg gttcacgtag tgggccatcg ccctgataga cggtttttcg
 5581 ccctttgacg ttggagtcca cgttctttta tagtggaactc ttgttccaaa ctggaacaac
 5641 actcaactct atctcgggct attcttttga tttataaggg attttgccga tttcggctta
 5701 ttggttaaaa aatgagctga ttttaacaaa atttaacgcg aattttaaca aaatattaac
 5761 gtttacaatt ttatggtgca gtctcagtac aatctgctct gatgccgat agttaagcca
 40 5821 gccccgacac ccgccaacac ccgtgacgc gccctgacgg gcttgtctgc tcccgcatc
 5881 cgcttacaga caagctgtga ccgtctccgg gagctgcatg tgctcagagg tttcacgcgc
 5941 atcaccgaaa cgcgcga

Table 36 pM21J containing IIIss::A27::Ckappa

45 Number of bases 5225 (SEQ ID NO:921)
 GACGAAAGGG CCTCGTGATA CGCCTATTTT TATAGGTAA TGTCATGATA ATAATGGTTT 60
 CTTAGACGTC AGGTGGCACT TTTCGGGGAA ATGTGCGCGG AACCCCTATT TGTTTATTTT 120
 TCTAAATACA TTCAAATATG TATCCGCTCA TGAGACAATA ACCCTGATAA ATGCTTCAAT 180
 50 AATATTGAAA AAGGAAGAGT ATGAGTATTC AACATTTCCG TGTCGCCCTT ATTCCCTTTT 240
 TTGCGGCATT TTGCCTTCCT GTTTTTGCTC ACCCAGAAAC GCTGGTGAAA GTAAAAGATG 300
 CTGAAGATCA GTTGGGTGCC CGAGTGGGT ACATCGAACT GGATCTCAAC AGCGGTAAGA 360
 TCCTTGAGAG TTTTCGCCCC GAAGAACGTT TTCCAATGAT GAGCACTTTT AAAGTTCTGC 420
 TATGTGGCGC GGTATTATCC CGTATTGACG CCGGGCAAGA GCAACTCGGT CGCCGCATAC 480
 55 ACTATTCTCA GAATGACTTG GTTGTGACT CACCAGTCAC AGAAAAGCAT CTTACGGATG 540
 GCATGACAGT AAGAGAATTA TGCAGTGCTG CCATAACCAT GAGTGATAAC ACTGCGGCCA 600

	ACTTACTTCT	GACAACGATC	GGAGGACCGA	AGGAGCTAAC	CGCTTTTTTG	CACAACATGG	660
	GGGATCATGT	AACTCGCCTT	GATCGTTGGG	AACCGGAGCT	GAATGAAGCC	ATACCAAACG	720
	ACGAGCGTGA	CACCACGATG	CCTGTAGCAA	TGGCAACAAC	GTTGCGCAA	CTATTAAGTG	780
	GCGAACTACT	TACTCTAGCT	TCCCGGCAAC	AATTAATAGA	CTGGATGGAG	GCGGATAAAG	840
5	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	ATTTTAAATT	TAAAAGGATC	TAGGTGAAGA	1140
10	TCCTTTTTGA	TAATCTCATG	ACCAAAATCC	CTTAACGTGA	GTTTTCGTTC	CACTGAGCGT	1200
	CAGACCCCGT	AGAAAAGATC	AAAGGATCTT	CTTGAGATCC	TTTTTTTCTG	CGCGTAATCT	1260
	GCTGCTTGCA	AACAAAAAAA	CCACCGCTAC	CAGCGGTGGT	TTGTTTGCCG	GATCAAGAGC	1320
	TACCAACTCT	TTTTCCGAAG	GTAAGTGGCT	TCAGCAGAGC	GCAGATACCA	AATACTGTTC	1380
	TTCTAGTGTA	GCCGTAGTTA	GGCCACCACT	TCAAGAACTC	TGTAGCACCG	CCTACATACC	1440
15	TCGCTCTGCT	AATCCTGTGA	CCAGTGGCTG	CTGCCAGTGG	CGATAAGTCG	TGTCTTACCG	1500
	GGTTGGACTC	AAGACGATAG	TTACCGGATA	AGGCGCAGCG	GTCGGGCTGA	ACGGGGGGTT	1560
	CGTGCAATCA	GCCCAGCTTG	GAGCGAACGA	CCTACACCGA	ACTGAGATAC	CTACAGCGTG	1620
	AGCTATGAGA	AAGCGCCACG	CTTCCCGAAG	GGAGAAAGGC	GGACAGGTAT	CCGGTAAGCG	1680
	GCAGGGTCGG	AACAGGAGAG	CGCACGAGGG	AGCTTCCAGG	GGGAAACGCC	TGGTATCTTT	1740
20	ATAGTCCTGT	CGGGTTTTCG	CACCTCTGAC	TTGAGCGTCG	ATTTTTGTGA	TGCTCGTCAG	1800
	GGGGGCGGAG	CCTATGGAAA	AACGCCAGCA	ACGCGGCCTT	TTTACGGTTC	CTGGCCTTTT	1860
	GCTGGCCTTT	TGCTCACATG	TTCTTTCTCG	CGTTATCCCC	TGATTCTGTG	GATAACCGTA	1920
	TTACCGCCTT	TGAGTGAGCT	GATACCGCTC	GCCGCAGCCG	AACGACCGAG	CGCAGCGAGT	1980
	CAGTGAGCGA	GGAAGCGGAA	GAGCGCCCAA	TACGCAAACC	GCCTCTCCCC	GCGCGTTGGC	2040
25	CGATTCATTA	ATGCAGCTGG	CACGACAGGT	TTCCCGACTG	GAAAGCGGGC	AGTGAGCGCA	2100
	ACGCAATTAA	TGTGAGTTAG	CTCACTCATT	AGGCACCCCA	GGCTTTACAC	TTTATGCTTC	2160
	CGGCTCGTAT	GTTGTGTGGA	ATTGTGAGCG	GATAACAATT	TCACACAGGA	AACAGCTATG	2220
	ACCATGATTA	CGCCAAGCTT	TGGAGCCTTT	TTTTTGAGGA	TTTTCAACAT	GAAGAACTG	2280
	CTGTCTGCTA	TCCCACTAGT	TGTCCCTTTT	TATTCTCATA	GTGAAATCGT	TCTGACCCAG	2340
30	TCCCCGGGGA	CCCTGTCTCT	GTCTCCGGGT	GAACGTGCTA	CGCTGAGCTG	TCGTGCTTCT	2400
	CAATCCGTTA	GCTCCTCTTA	TTTAGCTTGG	TATCAGCAAA	AGCCGGGTCA	AGCTCCGCGG	2460
	CTGTTGATCT	ATGGTGCCTC	TAGTCGTGCT	ACTGGCATCC	CTGATCGTTT	CTCTGGCTCT	2520
	GGCTCCGGAA	CCGATTTTAC	TCTGACCATT	TCTCGTCTCG	AGCCGGAAGA	TTTCGCTGTC	2580
	TACTATTGTC	AACAGTATGG	TTCTAGTCCG	CTGACTTTTC	GTGGCGGTAC	CAAAGTCGAA	2640
35	ATCAAGCGTG	GAAGTGTGGC	TGCACCATCT	GTCTTCATCT	TCCCGCCATC	TGATGAGCAG	2700
	TTGAAATCTG	GAAGTGCCTC	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
40	GTGACAAAGA	GCTTCAACAG	GGGAGAGTGT	TAATAAGGCG	CGCCAATTTA	ACCATCTATT	3000
	TCAAGGAACA	GTCTTAATGA	AGAAGCTCCT	CTTTGCTATC	CCGCTCGTCG	TTCCTTTTGT	3060
	GGCCCAGCCG	GCCATGGCCG	AAGTTCAATT	GTTAGAGTCT	GGTGGCGGTC	TTGTTTCAGCC	3120
	TGGTGGTTCT	TTACGTCTTT	CTTGCGCTGC	TTCCGGATTG	ACTTTCTCTC	GTTACAAGAT	3180
	GAAGTGGGTT	CGCCAAGCTC	CTGGTAAAGG	TTTGGAGTGG	GTTTCTGTGA	TCTATCCTTC	3240
45	TGGTGGCGGT	ACTGGTTATG	CTGACTCCGT	TAAAGGTGCG	TTCACTATCT	CTAGAGACAA	3300
	CTCTAAGAAT	ACTCTCTACT	TGCAGATGAA	CAGCTTAAAG	GCTGAGGACA	CTGCAGTCTA	3360
	CTATTGTGCG	AGAGTCAATT	ACTATGATAG	TAGTGGTTAC	GGTCCATATG	CTCCTGGACT	3420
	TGACTACTGG	GGCCAGGGAA	CCCTGGTCAC	CGTCTCAAGC	GCCTCCACCA	AGGGTCCGTC	3480
	GGTCTTCCCG	CTAGCACCCCT	CCTCCAAGAG	CACCTCTGGG	GGCACAGCGG	CCCTGGGCTG	3540
50	CCTGGTCAAG	GACTACTTCC	CCGAACCGGT	GACGGTGTGC	TGGAAGTCAAG	GCGCCCTGAC	3600
	CAGCGGCGTC	CACACCTTCC	CGGCTGTCTT	ACAGTCTAGC	GGACTCTACT	CCCTCAGCAG	3660
	CGTAGTGACC	GTGCCCTCTT	CTAGCTTGGG	CACCCAGACC	TACATCTGCA	ACGTGAATCA	3720
	CAAGCCCAGC	AACACCAAGG	TGGACAAGAA	AGTTGAGCCC	AAATCTTGTG	CGGCCGCACA	3780
	TCATCATCAC	CATCACGGGG	CCGCAGAACAA	AAAATCTATC	TCAGAAGAGG	ATCTGAATGG	3840
55	GGCCGCAGAG	GCTAGTTCTG	CTAGTAACGC	GTCTTCCGGT	GATTTTGATT	ATGAAAAGAT	3900
	GGCAAACGCT	AATAAGGGGG	CTATGACCGA	AAATGCCGAT	GAAAACGCGC	TACAGTCTGA	3960
	CGCTAAAGGC	AAACTTGATT	CTGTCGCTAC	TGATTACGGT	GCTGCTATCG	ATGGTTTCAT	4020

	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
5	TCTTTTATAT	GTTGCCACCT	TTATGTATGT	ATTTTCTACG	TTTGCTAACA	TACTGCGTAA	4320
	TAAGGAGTCT	TAATGAAACG	CGTGATGAGA	ATTCACGGC	CGTCGTTTTA	CAACGTCGTG	4380
	ACTGGGAAAA	CCCTGGCGTT	ACCCAACCTA	ATCGCCTTGC	AGCACATCCC	CCTTTCGCCA	4440
	GCTGGCGTAA	TAGCGAAGAG	GCCCGCACCG	ATCGCCCTTC	CCAACAGTTG	CGCAGCCTGA	4500
	ATGGCGAATG	GCGCCTGATG	CGGTATTTTC	TCCTTACGCA	TCTGTGCGGT	ATTTACACACC	4560
10	GCATACGTCA	AAGCAACCAT	AGTACGCGCC	CTGTAGCGGC	GCATTAAGCG	CGGCGGGTGT	4620
	GGTGGTTACG	GCGAGCGTGA	CCGCTACACT	TGCCAGCGCC	TTAGCGCCCG	CTCCTTTCGC	4680
	TTTCTTCCCT	TCCTTTCTCG	CCACGTTTCG	CGGCTTTCCC	CGTCAAGCTC	TAAATCGGGG	4740
	GCTCCCTTTA	GGGTTCGGAT	TTAGTGCTTT	ACGGCACCTC	GACCCCAAAA	AACTTGATTT	4800
	GGGTGATGGT	TCACGTAGTG	GGCCATCGCC	CTGATAGACG	GTTTTTCGCC	CTTTGACGTT	4860
15	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
20	AGCTGTGACC	GTCTCCGGA	GCTGCATGTG	TCAGAGGTTT	TCACCGTCAT	CACCGAAACG	5220
	CGCGA						5225

Table 40: pLCSK23 (SEQ ID NO:896)

25	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
30	301	AGAATGGCAA	AAGCTTATGC	ATTTCTTTCC	AGACTTGTTT	AACAGGCCAG	CCATTACGCT
	361	CGTCATCAAA	ATCACTCGCA	TCAACCAAAC	CGTTATTTCAT	TCGTGATTGC	GCCTGAGCGA
	421	GACGAAATAC	GCGATCGCTG	TAAAAGGAC	AATTACAAAC	AGGAATTGAA	TGCAACCGGC
	481	GCAGGAACAC	TGCCAGCGCA	TCAACAATAT	TTTCACCTGA	ATCAGGATAT	TCTTCTAATA
	541	CCTGGAATGC	TGTTTTCCCG	GGGATCGCAG	TGGTGAGTAA	CCATGCATCA	TCAGGAGTAC
35	601	GGATAAAATG	CTTGATGGTC	GGAAGAGGCA	TAAATTCGGT	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	CCCTTGTTAT	ACTGTTTATG	TAAGCAGACA
40	901	GTTTTATTGT	TCATGATGAT	ATATTTTAT	CTTGTCGAAT	GTAACATCAG	AGATTTTGAG
	961	ACACAACGTG	GCTTTCCCCC	CCCCCCCCTG	CAGGTCTCGG	GCTATTCTTG	TCAGACCAAG
	1021	TTTACTCATA	TATACTTTAG	ATTGATTTAA	AACTTCATTT	TTAATTTAAA	AGGATCTAGG
	1081	TGAAGATCCT	TTTTGATAAT	CTCATGACCA	AAATCCCTTA	ACGTGAGTTT	TCGTTCCACT
	1141	GAGCGTCAGA	CCCCGTAGAA	AAGATCAAAG	GATCTTCTTG	AGATCCTTTT	TTTCTGCGCG
45	1201	TAATCTGCTG	CTTGCAAACA	AAAAAACAC	CGTACCAGC	GGTGGTTTGT	TTGCCGGATC
	1261	AAGAGCTACC	AACTCTTTT	CCGAAGGTAA	CTGGCTTCAG	CAGAGCGCAG	ATACCAAATA
	1321	CTGTTCTTCT	AGTGTAGCCG	TAGTTAGGCC	ACCACTTCAA	GAACCTGTGA	GCACCGCCTA
	1381	CATACCTCGC	TCTGCTAATC	CTGTTACCAG	TGGCTGCTGC	CAGTGGCGAT	AAGTCGTGTC
	1441	TTACCGGGTT	GGACTCAAGA	CGATAGTTAC	CGGATAAGGC	GCAGCGGTCT	GGCTGAACGG
50	1501	GGGGTTCGTG	CATACAGCCC	AGCTTGGAGC	GAACGACCTA	CACCGAAGTG	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	TGGA AAAACG	CCAGCAACGC	GGCCTTTTAA	CGGTTCCCTG
55	1801	CCTTTTGCTG	GCCTTTTGCT	CACATGTTCT	TTCTTGCGTT	ATCCCTGAT	TCTGTGGATA
	1861	ACCGTATTAC	CGCCTTTGAG	TGAGCTGATA	CCGCTCGCCG	CAGCCGAACG	ACCGAGCGCA

5 1921 GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT CTCCCCGCGC
 1981 GTTGGCCGAT TCATTAATGC AGCTGGCAGC ACAGGTTTCC CGACTGGAAA GCGGGCAGTG
 2041 AGCGCAACGC AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA
 2101 TGCTTCCGGC TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTTAC 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
 10 2461 TTCAGTGGCA GTGGATCTGG GACAGATTTC ACTCTCACCA TCAGCAGTCT GCAACCTGAA
 2521 GATTTTGCAA CTTACTACTG TCAACAGAGT TACAGTACCC CTTTCACTTT CGGCCCTGGG
 2581 ACCAAAGTGG ATATCAAACG TGGTACcGTG GCTGCACCAT CTGTCTTCAT CTTCCCGCCA
 2641 TCTGATGAGC AGTTGAAATC TGGAAGTGGC TCTGTTGTGT GCCTGCTGAA TAACTTCTAT
 2701 CCCAGAGAGG CCAAAGTACA GTGGAAGGTG GATAACGCCC TCCAATCGGG TAACTCCCAG
 15 2761 GAGAGTGTCA CAGAGCAGGA CAGCAAGGAC AGCACCTACA GCCTCAGCAG CACCCTGACG
 2821 CTGAGCAAAG CAGACTACGA GAAACACAAA GTCTACGCCT GCGAAGTCAC CCATCAGGGC
 2881 CTGAGTTTAC CGGTGACAAA GAGCTTCAAC AGGGGAGAGT GTGCGGCCGC TGGTAAGCCT
 2941 ATCCCTAACC CTCTCCTCGG TCTCGATTCT ACGTGATAAC TTCACCGGTC AACGCGTGAT
 20 3001 GAGAATTAC 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
 25 3361 TCCGGGAGCT GCATGTGTCA GAGGTTTTCA CCGTCATCAC CGAAACGCGC GA

[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
 30 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
 35 likely than 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
 40 Cys because we do not want gratuitous 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

5 [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

10 [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.

15 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

20 [00196] Very short CDR3 can be made by dobling. 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 doobling 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,

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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.

- 5 Table 62: HC CDR3 length 4 from JH2 (V-4JH2 in Table 7) ('KYFDL' disclosed as SEQ ID NO: 953)

Position	Parental AA (source)	Allowed
94	K (VH 3-23)	K
95	Y (JH2)	Y SDRL (4:1:1:1:1)
96	F (JH2)	F SYDRL (4:1:1:1:1:1)
97	D (JH2)	D RSYL (4:1:1:1:1)
98	L (JH2)	L SYDR (4:1:1:1:1)
103	W (JH2)	W

- 10 In Table 63, there is a dobling 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.

- 15 Table 63: HC CDR3 of length four from V-4D3-10.1a in Table 8 ('KLLWF' disclosed as SEQ ID NO: 954)

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

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Example 4.4: HC CDR3 length 10 to 20

HC CDR3

Two sublibraries, both with CDR3 of length 16:

Table 52: Library 1: Diversity = 5×10^{11} , the "parental" sequence occurs at 1 in 1.5×10^6 .

5 ('KYYYDSSGYYYAEYFQHW' disclosed as SEQ ID NO: 955)

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)
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 3.0×10^{10} and the parental sequence occurs once in 3.7×10^5 . ('KGYCSSTSCYTAEYFQHW' disclosed as SEQ ID NO: 956)

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

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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

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

5 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

10 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.

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

('KDYGYCSSTSCYTYGYSYAEYFQHW' disclosed as SEQ ID NO: 957)

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

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

- 5 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
- 10 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 1.46×10^9 . The parental sequence is expected at 1 in 6.9×10^4 . Each of the singly substituted sequences is about 1/3 as likely; the doubly substituted ones are

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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.

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

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

5 ('KSGGYCSGGSCYSFDYW' disclosed as SEQ ID NO: 958)

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

Example 5: Synthetic light chain diversity

- 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
- 10 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.
- 15 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.

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

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

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.

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. *SpeI* is in the *iii* signal sequence and allows the entire LC to be inserted or removed. *XmaI*, *PpuMI*, *EcoO109I*, and *BlnI* precede CDR1. *SacII* is in FR2, separating CDR1 from CDR2. Alternatively, an *AvrII* site could be inserted at the same position. *BspEI* and *XhoI* sites are in FR3 and a *KpnI* site is in FR4.

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
20 !1QLR      GASQSVS_NYLA  DASSRAT  QQYGSSPLT
!A27      RASQSVSSSYLA  GASSRAT  QQYGSSPLT
                ** ***** *  *  *  *  *****
GASQSVS is (SEQID NO:922) NYLA (SEQ ID NO: 959)  DASSRAT is (SEQID NO:923)
QQYGSSPLY is (SEQID NO:924) QQYGSSPLT (SEQ ID NO: 966)
25 RASQSVSSSYLA is (SEQ ID NO:925)  GASSRAT is (SEQ ID NO:926)

```

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

VK3 is (SEQ ID NO: 927)
A27 is (SEQ ID NO: 960)
A11 is (SEQ ID NO: 961)
L2 is (SEQ ID NO: 962)
L16 is (SEQ ID NO: 962)
L6 is (SEQ ID NO: 963)
L20 is (SEQ ID NO: 964)
L25 is (SEQ ID NO: 965)

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

A27 and I.6 differ by G9A(FR1), Δ31a(in CDR1), G50D(CDR2), S53N(CDR2), G92S(CDR3), S93N(CDR3), S94W(CDR3) VK3 from US 7,264,963.

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CDR1

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.

10 CDR2

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 15 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.

CDR3

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, $\phi = -80$ and $\psi = -15$ 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.

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.

! IIIIsignal::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|ggt|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

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T56 7, 10A¹, 1G, 1H, 2P, 4S, 137-,
 8, 1A, 6T,

9% ERSY

Note, there are seven antibodies with an insertion of one AA.

5 CDR3 (showing "_" means that the Ab has a deletion in CDR3) ('QQYGSSPLT' disclosed as SEQ ID NO: 966)

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

10 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)

15 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, 24P², 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-,

20 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.

25

Table 71: Allowed diversity in CDR1, 2, and 3 of A27::JK4. ('RASQSVSSSYLA' disclosed as SEQ ID NO: 925; 'GASSRAT' disclosed as SEQ ID NO: 926; 'QQYGSSPLT' disclosed as SEQ ID NO: 966)

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			

¹ Seven of these come from the insertions.

² Some of these appear because of insertions.

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108(89)	Q	fixed	
109(90)	Q	fixed	
110(91)	Y	FERS	64%Y 9% other
111(92)	G	ADRSTY	52%G 8% other
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	

The parental sequence appears at 5.32×10^{-5} or 1 in 1.88×10^4 .

Sequences with a single substitution have a probability between 1.1×10^{-5} and 7.5×10^{-6} .

Sequences that have none of the parental AAs occurs at 1 in 6.7×10^{16} .

5 The allowed diversity is about 2.35×10^{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	YYDSSGYYY
4-17.2	27	DYGDY
3-3.2	25	YYDFWSGYT
6-19.1	25	GYSSGWY
7-27.1	19	LTG
5-5.3	18	GYSYGY

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6-13.1	18	GYSSSWY	215
5-12.3	13	GYSGYDY	205
6-13.2	10	GIAAAG	216
1-26.3	9	YSGSYY	284
2-15.2	9	GYCSGGSCYS	136
4-4.3	9	TTVT	190
3-10.2	8	YYYGSGSYYN	81
1-1.3	7	YNWND	262
4-4.2	7	DYSNY	754
2-2.2	6	GYCSSTSCYT	70
3-16.2	6	YYDYVWGSYRYT	104
6-6.1	6	EYSSSS	212
6-19.2	6	GIAVAG	219
3-9.1	5	VLRYFDWLL@	179
4-23.2	5	DYGGNS	198
6-6.2	5	SIAAR	213
1-7.3	4	YNWNY	270
2-2.3	4	DIVVVPAAI	168
4-23.3	4	TTVVT	199
1-7.1	3	GITGT	268
1-26.1	3	GIVGAT	282
7-27.3	3	NWG	223
3-10.1	2	VLLWFGELL@	182
3-10.2	2	ITMVRGVII	183
5-5.1	2	VDTAMV	200
5-5.2	2	WIQLWL	201
5-12.1	2	VDIVATI	203
5-24.3	2	RDGYNY	211
1-1.1	1	GTTGT	260
2-21.3	1	HIVVVTAI	175
3-3.3	1	ITIFGVVII	178
5-24.2	1	*RWLQL	210
6-6.3	1	V*QLV	214
6-19.3	1	V*QWLV	220

Table 78: D segment utilization (0.667 cutoff)

Name	Number	Sequence	SEQ ID NO:	%
None	935			0.517
7-27.1	158	LTG	221	0.087
7-27.3	98	NWG	223	0.054
5-5.3	72	GYSYGY	786	0.040
1-26.3	67	YSGSYY	166	0.037
3-22.2	46	YYDSSGYYY	187	0.025
4-17.2	38	DYGDY	195	0.021
3-3.2	37	YYDFWSGYT	177	0.020

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7-27.2	37	@LG	222	0.020
6-19.1	33	GYSSGWY	218	0.018
6-13.2	31	GIAAAG	860	0.017
6-13.1	22	GYSSSWY	215	0.012
6-6.1	18	EYSSSS	847	0.010
6-19.2	18	GIAVAG	879	0.010
4-23.2	17	DYGGNS	198	0.009
5-12.3	17	GYSGYDY	205	0.009
5-24.3	14	RDGYNY	211	0.008
2-15.2	13	GYCSGGSCYS	136	0.007
1-26.1	11	GIVGAT	164	0.006
4-4.3	11	TTVT	190	0.006
1-1.3	9	YNWND	262	0.005
2-2.2	9	GYCSSTSCYT	70	0.005
3-16.2	9	YYDYVWGSYRYT	104	0.005
2-2.3	8	DIVVVPAAI	168	0.004
3-10.2	8	YYYGSGSYYN	81	0.004
4-4.2	8	DYSNY	192	0.004
1-7.3	7	YNWNY	270	0.004
3-3.3	6	ITIFGVVII	178	0.003
6-6.2	6	SIAAR	213	0.003
3-9.1	5	VLRYFDWLL@	179	0.003
3-10.2	5	ITMVRGVII	183	0.003
6-19.3	5	V*QWL	220	0.003
1-7.1	4	GITGT	268	0.002
4-23.3	4	TTVVT	768	0.002
1-1.1	3	GTTGT	156	0.002
5-5.1	3	VDTAMV	200	0.002
5-24.2	3	*RWLQL	210	0.002
3-10.1	2	VLLWFGELL@	182	0.001
5-5.2	2	WIQLWL	201	0.001
5-12.1	2	VDIVATI	203	0.001
1-26.2	1	V*WELL	165	0.001
2-21.2	1	AYCGGDCYS	174	0.001
2-21.3	1	HIVVVTAI	175	0.001
3-3.1	1	VLRFLEWLLY	176	0.001
3-16.2	1	IMITFGGVIVI	185	0.001
6-6.3	1	V*QLV	214	0.001
6-13.3	1	V*QQLV	217	0.001

Table 78: Utilization of JH segments

JH1	17
JH2	31
JH3	452

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JH4	636
JH5	32
JH6	251

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 ('SYSY' disclosed as SEQ ID NO: 947) 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 $8^{14} = 4.3 \text{ E } 12$.

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											

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```

!   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
5  !   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
10  !           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
15  !           W  G  Q  G  T  L  V  T  V  S  S (SEQ ID NO: 968)
!           |TGg|ggt|caa|ggt|act|ttG|GTC|ACC|gtc|tct|agt (SEQ ID NO: 967)
!           | BstEII |
!
20  Table 500: Expected distribution of AA types in wobbled HC CDR3
16d

```

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

25 Amino acid disclosed as SEQ ID NO: 970
DNA sequence disclosed as SEQ ID NO: 969

```

           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
30  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
35 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

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	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
5	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
10	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
15	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
20	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
25	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   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|ggt|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

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!           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 ----->
5      !           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|ggt|ccg|TCT|AGA|ttc|tct|ggc|tct|ggt|tct|ggt|act|gat|ttt|
!           XbaI...
!
!           ----- FR3 ----->
10     !           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 ----->
15     !           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----->
20     !           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|ggt|gag|att|aag|CGT|ACG|gtg|gct|gct|ccg|tct|gtc|ttc|
!           BsiWI..
25

```

Table 900: CDR1 diversity (SEQ ID NO: 973)

												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	

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Table 1100: CDR2 diversity								
POSITION	50	51	52	53	54	55	56	Diversity
O12	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
O12	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.
O12	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.
O12	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: YYDFWSGYT), 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

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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 (SEQ ID NO: 976) goes into the Leadin pile.

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	(SEQ ID NO: 977)	
JH6	YYYYYGMDVWGQGT TVTVSS	(SEQ ID NO: 3)	
15	123456789		
B)			
13	EPIWGYYYYYGMDV	(SEQ ID NO: 978)	
JH6	YYYYYGMDVWGQGT TVTVSS	(SEQ ID NO: 3)	
20			
C)			
9	DFFTSYFDY	(SEQ ID NO: 979)	
JH4	-----YFDYWGQGT LTVTVSS	(SEQ ID NO: 1)	
25			
D)			
12	DRGVSL LGAFDI	(SEQ ID NO: 980)	
JH3	-----AFDIWGQGT MTVTVSS	(SEQ ID NO: 2)	
30			
E)			
12	DSGVVAAADFDS	(SEQ ID NO: 981)	
JH4	-----YFDYWGQGT LTVTVSS	(SEQ ID NO: 1)	
	6789		

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 (SEQ ID NO: 986) 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 (SEQ ID NO: 974). A non-negligible fraction retain the sequence YGMDV (SEQ ID NO: 982), YYGMDV (SEQ ID NO: 983), or YYYYGMDV (SEQ ID NO: 984).

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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 1/11 of 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.

10 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 (SEQ ID NO: 984). The DNA that encodes the parental sequence YYYGMDV (SEQ ID NO: 984) is synthesized with the parental amino acid at 5, 6, 7, 8, or 10 times more likely than the others.

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.

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 (SEQ ID NO: 218) 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 (SEQ ID NO: 986) is synthesized with the parental amino acid 6-x as likely as the others.

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 (SEQ ID NO: 218) 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

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allowing GSRDLY in the ratios shown. Finally is JH3 with variability in the J stump (sequence: YFDY (SEQ ID NO: 985)) which allows YGSRD with the parental amino acid 6-X as likely as the other allowed types.

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	SEQ ID NO:
JH1	1356	6.15	---AEYFQHWGQGT ~~L~~ VTVSS	66
JH2	1720	7.80	---YWYFDLWGRGT ~~L~~ VTVSS	67
JH3	5601	25.39	-----AFDIWGQGT ~~M~~ VTVSS	2
JH4	7658	34.71	-----YFDYWGQGT ~~L~~ VTVSS	1
JH5	1062	4.81	-----NWFDPWGQGT ~~L~~ VTVSS	68
JH6	4666	21.15	YYYYYGMDVWGQGT ~~T~~ VTVSS	3
Total	22063			

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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	GYCTNGVCYT
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

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Table 1030: Utilization of D segments.			SEQ ID NO:
Id	Number	Sequence	
2-21.1	0	SILWW\$LLF	173
2-21.2	24	AYCGGDCYS	174
2-21.3	6	HIVVVTAI	175
3-3.1	28	VLRFLWLLY	176
3-3.2	743	YYDFWSGYT	177
3-3.3	15	ITIFGVVII	178
3-9.1	41	VLRYFDWLL@	179
3-9.2	8	YYDILTGYN	180
3-9.3	0	ITIF*LVII	181 & 579
3-10.1	26	VLLWFGELL@	182
3-10.2	136	YYYGSGSYN	81
3-10.2	32	ITMVRGVII	183
3-16.1	0	VL\$RLGELSLY	184
3-16.2	109	YYDYVWGSYRYT	104
3-16.2	8	IMITFGGVIVI	185
3-22.1	0	VLL***WLLL	186
3-22.2	617	YYDSSGYYY	187
3-22.3	2	ITMIVVVIT	188
4-4.1	0	\$LQ@L	189
4-4.2	75	DYSNY	192
4-4.3	165	TTVT	190
4-11.1	0	\$LQ@L	191
4-11.2	0	DYSNY	192
4-11.3	0	TTVT	193
4-17.1	0	\$LR@L	194
4-17.2	392	DYGDY	195
4-17.3	0	TTVT	196
4-23.1	0	\$LRW@L	197
4-23.2	60	DYGGNS	198
4-23.3	16	TTVVT	199
5-5.1	25	VDTAMV	200
5-5.2	29	WIQLWL	201
5-5.3	292	GYSYGY	202
5-12.1	13	VDIVATI	203
5-12.2	0	WI*WLRL	204
5-12.3	200	GYSGYDY	205
5-18.1	0	VDTAMV	206
5-18.2	0	WIQLWL	207
5-18.3	0	GYSYGY	208
5-24.1	9	VEMAT I	209
5-24.2	21	*RWLQL	210
5-24.3	44	RDGYNY	211
6-6.1	87	EYSSSS	212
6-6.2	122	SIAAR	213

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Table 1030: Utilization of D segments.			SEQ ID NO:
Id	Number	Sequence	
6-6.3	1	V*QLV	214
6-13.1	399	GYSSSWY	215
6-13.2	170	GIAAAG	216
6-13.3	0	V*QQLV	217
6-19.1	441	GYSSGWY	218
6-19.2	104	GIAVAG	219
6-19.3	3	V*QWL V	220
7-27.1	257	LTG	221
7-27.2	0	@LG	222
7-27.3	64	NWG	223
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

Table 1040: JH vs Length						
Length	JH1	JH2	JH3	JH4	JH5	JH6
31	0	0	1	0	0	1
32	0	1	0	0	0	0
33	0	0	0	1	0	0
34	0	0	0	0	0	0
35	0	0	0	0	0	0
36	0	0	0	0	1	0

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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: Dipeptides 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	YSGSY	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	VLRFLWLLY	0	0	0	0	0	0	0	0	0	0	1	2	2	2	3			
3-3.2	YYDFWSGYT	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	VLRFDWLL@	0	0	0	0	0	0	0	0	1	0	0	4	5	5	5			
3-9.2	YYDILTGYN	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	YYGSGSYN	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	YYDYVWGSRYT	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	YYDSSGYYY	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			

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Table 1060a Lengths of HC CDR3s vs which D segments occur (if any) for lengths 3-17																										
Name	Sequence	SEQ ID NO:	Length																							
			3	4	5	6	7	8	9	10	11	12	13	14	15	16	17									
4-4.3	TTVT	190	0	0	0	0	2	4	11	19	23	19	25	19	10	11	9									
4-17.2	DYGDY	195	0	0	2	6	12	8	38	40	48	47	50	40	29	21	10									
4-23.2	DYGGNS	198	0	0	0	0	0	2	7	4	5	17	4	8	5	1	1									
4-23.3	TTVVT	199	0	0	0	0	0	0	2	0	1	1	2	1	2	0	0									
5-5.1	VDTAMV	200	0	0	0	0	0	0	0	1	4	8	1	3	2	0	1									
5-5.2	WQLWL	201	0	0	0	0	0	0	0	3	2	0	3	1	4	3	3									
5-5.3	GYSYGY	202	0	0	0	0	1	6	9	20	43	29	27	22	32	26	27									
5-12.1	VDIVATI	203	0	0	0	0	0	0	0	2	0	1	2	4	0	2	1									
5-12.3	GYSGYDY	205	0	0	0	0	4	10	13	15	19	15	22	27	16	15	9									
5-24.1	VEMATI	209	0	0	0	0	0	0	1	1	0	0	6	0	0	0	0									
5-24.2	*RWLQL	210	0	0	0	0	1	0	3	1	3	2	2	1	2	2	2									
5-24.3	RDGYNY	211	0	0	0	0	0	0	0	1	8	12	6	7	3	2	1									
6-6.1	EYSSSS	212	0	0	0	0	0	0	0	9	7	16	19	13	2	4	2									
6-6.2	SIAAR	213	0	0	0	1	1	0	17	8	7	13	17	6	16	16	7									
6-6.3	V*QLV	214	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0									
6-13.1	GYSSSWY	215	0	0	0	0	0	1	6	11	35	40	56	44	50	42	35									
6-13.2	GLAAAG	216	0	0	0	0	1	2	18	14	15	20	20	15	16	14	11									
6-19.1	GYSSGWY	218	0	0	0	0	1	1	4	27	57	58	48	52	45	35	30									
6-19.2	GLAVAG	219	0	0	0	0	1	1	0	7	8	20	8	13	16	8	10									
6-19.3	V*QWL	220	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0									
7-27.1	LTG	221	0	0	1	0	2	8	12	7	14	11	17	17	24	24	31									
7-27.3	NWG	223	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	SEQ ID NO:	Length														31	32								
			18	19	20	21	22	23	24	25	26	27	28	29	30											
1-1.1	GTTGT	156	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0									
1-1.2	VQLER	157	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0									
1-1.3	YNWND	158	6	5	3	4	1	1	0	0	1	0	0	0	0	0	0									
1-7.1	GITGT	159	2	2	1	0	0	0	1	0	0	0	0	0	0	0	0									

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Name	Sequence	SEQ ID NO:	Length																													
			18	19	20	21	22	23	24	25	26	27	28	29	30	31	32															
1-7.3	YNWNY	161	5	2	1	1	0	0	0	0	0	0	0	1	0	0	0															
1-26.1	GIVGAT	164	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0															
1-26.2	V*WELL	165	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0															
1-26.3	YSGSY	166	14	11	9	8	6	3	3	0	3	1	0	0	0	0	0															
2-2.2	GYCSSTSCYT	70	11	7	2	10	11	4	2	0	0	0	1	0	0	0	0															
2-2.3	DIVVPPAAI	168	2	6	3	4	3	2	1	0	0	0	0	0	0	0	0															
2-8.2	GYCTNGVCYT	115	3	0	1	1	3	1	0	0	0	0	0	0	0	0	0															
2-8.3	DIVLMVYAI	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0															
2-15.2	GYCSGGSCYS	136	20	10	7	4	8	9	3	0	0	0	0	0	0	0	0															
2-15.3	DIVVVVAAT	172	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0															
2-21.2	AYCGGDCYS	174	1	3	1	1	1	0	0	0	0	0	0	0	0	0	0															
2-21.3	HIVVVTAI	175	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0															
3-3.1	VLRFEWLLY	176	6	2	4	3	2	0	0	0	1	0	0	0	0	0	0															
3-3.2	YYDFWSGYT	177	82	97	104	67	61	32	23	7	3	4	0	0	2	1	0															
3-3.3	ITIFGVVII	178	3	2	0	0	2	0	0	0	0	0	0	0	0	0	0															
3-9.1	VLRYFDWLL@	179	7	2	2	6	1	2	1	0	0	0	0	0	0	0	0															
3-9.2	YYDILTGYN	180	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0															
3-10.1	VLLWFGELL@	182	3	4	0	0	0	0	0	0	0	0	0	0	0	0	0															
3-10.2	YYYGSGSYN	81	15	10	8	7	6	3	2	1	1	0	0	0	0	0	0															
3-10.2	ITMVRGVII	183	3	3	6	0	0	0	0	0	0	0	0	0	0	0	0															
3-16.2	YYDYVWGSYRYT	104	11	11	14	10	18	13	5	2	2	0	0	0	1	0	0															
3-16.2	IMITFGGVIVI	185	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0															
3-22.2	YYDSSGYYY	187	77	54	28	22	18	8	1	2	1	1	0	0	0	0	0															
3-22.3	ITMIVVVIT	188	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0															
4-4.2	DYSNY	192	7	2	2	1	1	0	1	0	0	0	0	0	0	0	0															
4-4.3	TTVT	190	4	2	2	1	3	0	0	0	0	0	1	0	0	0	0															
4-17.2	DYGDY	195	17	7	8	3	2	1	1	0	1	0	1	0	0	0	0															
4-23.2	DYGGNS	198	3	1	0	2	0	0	0	0	0	0	0	0	0	0	0															
4-23.3	TTVVT	199	2	2	1	1	1	0	0	0	0	0	0	0	0	0	0															
5-5.1	VDTAMV	200	0	1	1	2	1	0	0	0	0	0	0	0	0	0	0															

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Table 1060b Lengths of HC CDR3s vs which D segments occur (if any) for lengths 18-32																																		
Name	Sequence	SEQ ID NO:	Length																															
			18	19	20	21	22	23	24	25	26	27	28	29	30	31	32																	
5-5.2	WIQLWL	201	3	3	1	0	1	0	0	0	0	0	1	0	0	0	1	0																
5-5.3	GYSYGY	202	13	18	7	2	2	2	6	0	0	0	0	0	2	0	0	0																
5-12.1	VDIVATI	203	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0																
5-12.3	GYSGYDY	205	11	10	6	6	0	0	2	0	0	0	0	0	0	0	0	0																
5-24.1	VEMATII	209	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
5-24.2	*RWLQL	210	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
5-24.3	RDGYNY	211	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
6-6.1	EYSSSS	212	9	3	1	1	0	0	0	0	1	0	0	0	0	0	0	0																
6-6.2	SIAAR	213	2	3	2	6	0	0	0	0	0	0	0	0	0	0	0	0																
6-6.3	V*QLV	214	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
6-13.1	GYSSSWY	215	24	22	17	4	5	5	1	3	2	0	0	1	0	0	0	0																
6-13.2	GIAAAG	216	10	4	2	5	2	2	1	0	0	0	0	0	0	0	0	0																
6-19.1	GYSSGWY	218	23	25	13	7	7	7	3	0	2	1	0	1	0	0	0	0																
6-19.2	GIAVAG	219	6	2	2	0	0	0	0	2	0	0	0	0	0	0	0	0																
6-19.3	V*QWLIV	220	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0																
7-27.1	LTG	221	29	23	12	9	7	7	4	3	1	0	1	0	0	0	0	0																
7-27.3	NWG	223	1	0	0	0	2	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	0	1																

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Table 1082: Tally of J stumps																									
JH1	---AEYFQHWGQGLTVTVSS 6.15% (SEQ ID NO: 66)																								
	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	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	0	0	0	568	
6	0	0	0	0	0	1	1	0	0	3	0	2	1	0	1	1	0	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	---YWFYDLWGRGTLTVTVSS 7.80% (SEQ ID NO: 67)																								
	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	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	----AFDIWQGTMTVTVSS 25.4% (SEQ ID NO: 2)																								
	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	-----YFDYWQGQTLTVSS 34.7% (SEQ ID NO: 1)																				
	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	----NWFDPWGQGLTVSS 4.8% (SEQ ID NO: 68)																				
	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	YYYYGMDVWGQTTTVSS 21.1% (SEQ ID NO: 3)																				
	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

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Table 1097: HC CDR3 libraries						
Library	CDR3 Length	Lead in	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) (SEQ ID NO: 986)	WGQGTMTVTSS (JH4) (SEQ ID NO: 987)
5.002	13,14,15	6,7,8 X X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	none	none	YYYGMDV (diversity in text) (SEQ ID NO: 984)	WGQGTITVTSS (jh6) (SEQ ID NO: 988)
5.003	18,19,20	4,5,6 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YYDFWSGYT (3-3.2) (SEQ ID NO: 177)	none	YFDY (SEQ ID NO: 985)	WGQGTITVTSS (JH3) (SEQ ID NO: 989)
5.004	15,16,17	2,3,4 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYSSGWY (6-19.1) (SEQ ID NO: 218)	2X,X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	AFDI (SEQ ID NO: 986)	WGQGTMTVTSS (JH4) (SEQ ID NO: 987)
5.005	11-14	0,1,2 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYSSGWY (6-19.1) (SEQ ID NO: 218)	0,1 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YFDY (SEQ ID NO: 985)	WGQGTITVTSS (JH3) (SEQ ID NO: 989)
5.006	19-25	0,1,2,3 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	GYCSGGSCYS (2-2.2) (Cys residues constant) (SEQ ID NO: 136)	0,1,2,3 X = (1.0G, .57S, .46R, .42D, .36L, .35Y)	YYYYYGMDV (parent AA 8X others) (SEQ ID NO: 990)	WGQGTITVTSS (jh6) (SEQ ID NO: 988)

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REFERENCES**EQUIVALENTS**

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 scope of the
5 invention. Accordingly, other embodiments are within the scope of the following claims.

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 50860-279 Seq 01-DEC-10 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual
10 Property Office.

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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 the diversity of the
5 antibody family, wherein the vectors or genetic packages comprise variegated DNA
sequences that encode:
 - (i) a heavy chain, comprising:
 - (a) a CDR1 having the following amino acid sequence: $X_{31}YX_{33}MX_{35}$,
wherein X_{31} , X_{33} and X_{35} are any amino acid except Cys or Met;
 - 10 (b) a CDR2 having the following amino acid sequence
 $X_{50}IX_{52}X_{52a}SGGX_{56}TX_{58}YADSVKG$, wherein X_{50} , X_{52} , X_{56} and X_{58} are any amino acid
except Cys or Met and X_{52a} is selected from the group consisting of Gly, Ser, Pro or Tyr; and
 - (c) a CDR3;
 - and
 - 15 (ii) a light chain.
2. The library of Claim 1, 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.
3. The library of Claim 2, wherein about 50% of all the positions of the HC
20 CDR3 are Tyr, Gly, Asp, Ser, or Arg.
4. The library of Claim 2, wherein an Arg residue is present in a filler region
between V and D, between D and J, or between V and J.
5. The library of claim 1, wherein the heavy chain (HC) CDR3 consists 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,

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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; and wherein the FR3 is a 3-23 VH FR3.

- 5 6. The library of Claim 1, wherein more than about 20% of the HC CDR3 sequence consists of Tyr residues, and wherein D regions and J stumps of the HC CDR3 consist of more than about 20% of Tyr residues.
7. The library of Claim 1, wherein less than about 20% of the HC CDR3 sequence consists of Tyr residues, and wherein leadin or DJ filler positions of the HC CDR3
10 consist of less than about 20% of Tyr residues.
8. The library of Claim 1, wherein the HC CDR3 is three amino acids in length and: the first residue of HC CDR3 varies among F, S, Y, D, and R in a 3:1:1:1:1 ratio; the second residue of HC CDR3 varies among Q, E, R, S, Y, and L in a 3:1:1:1:1:1 ratio; and the third residue of HC CDR3 varies among H, D, R, S, Y, and L in a 3:1:1:1:1:1 ratio, and
15 wherein a FR3 is located at the amino terminus of the CDR3, and the last residue of the FR3 varies between K and R in a 3:1 ratio.
9. The library of Claim 1, wherein the HC CDR3 is three amino acids in length and: the first residue of HC CDR3 varies among T, Y, R, D, and L in a 5:1:1:1:1 ratio; the second residue of HC CDR3 varies among T, Y, R, D, and L in a 5:1:1:1:1 ratio; and the third
20 residue of HC CDR3 varies among G, S, Y, R, D, and L in a 5:1:1:1:1 ratio.
10. The library of Claim 1, wherein the HC CDR3 is four amino acids in length and: the first residue of HC CDR3 varies among L, S, Y, D, and R in a 4:1:1:1:1 ratio; the second residue of HC CDR3 varies among L, S, Y, D, and R in a 4:1:1:1:1 ratio; the third residue of HC CDR3 varies among W, S, Y, D, and R in a 4:1:1:1:1 ratio; and the fourth
25 residue of HC CDR3 varies among F, S, Y, D, and R in a 4:1:1:1:1 ratio, and wherein a FR3 is located at the amino terminus of the CDR3, and the last residue of the FR3 varies between K and R in a 4:1 ratio.

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11. The library of Claim 1, wherein the HC CDR3 is four amino acids in length and: the first residue of HC CDR3 varies among Y, S, D, R, and L in a 4:1:1:1:1 ratio; the second residue of HC CDR3 varies among F, S, Y, D, and R in a 4:1:1:1:1 ratio; the third residue of HC CDR3 varies among D, R, S, Y, and L in a 4:1:1:1:1 ratio; and the fourth residue of HC CDR3 varies among L, S, Y, D, and R in a 4:1:1:1:1 ratio, and wherein a FR3 is located at the amino terminus of the CDR3.
12. The library of Claim 1, wherein the HC CDR3 is sixteen amino acids in length and: the first residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the second residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the third residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the fourth residue of HC CDR3 varies among D, Y, S, R, and L in a 3:1:1:1:1 ratio; the fifth residue of HC CDR3 varies among S, Y, R, D, and L in a 3:1:1:1:1 ratio; the sixth residue of HC CDR3 varies among S, Y, R, D, and L in a 3:1:1:1:1 ratio; the seventh residue of HC CDR3 varies among G, A, S, Y, R, D, and L in a 3:1:1:1:1:1:1 ratio; the eighth residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the ninth residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the tenth residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the eleventh residue of HC CDR3 varies among A, S, Y, R, and D in a 3:1:1:1:1 ratio; the twelfth residue of HC CDR3 varies among E, R, S, Y, and L in a 3:1:1:1:1 ratio; the thirteenth residue of HC CDR3 varies among Y, S, R, D, and L in a 3:1:1:1:1 ratio; the fourteenth residue of HC CDR3 varies among F, Y, S, R, and D in a 3:1:1:1:1 ratio; the fifteenth residue of MC CDR3 varies among Q, E, R, S, and Y in a 3:1:1:1:1 ratio; and the sixteenth residue of HC CDR3 varies among H, E, R, S, Y, and L in a 3:1:1:1:1:1 ratio, and wherein a FR3 is located at the amino terminus of the CDR3, and the last residue of the FR3 varies between K and R in a 3:1 ratio.
13. The library of Claim 1, wherein the HC CDR3 is sixteen amino acids in length and: the first residue of HC CDR3 varies among G, S, V, D, R, and L in a 3:1:1:1:1:1 ratio; the second residue of HC CDR3 varies among Y, S, D, R, and L in a 3:1:1:1:1 ratio; the third residue of HC CDR3 is C; the fourth residue of HC CDR3 varies among S, Y, R, D, and L in a 3:1:1:1:1 ratio; the fifth residue of HC CDR3 varies among S, Y, R, D, and L in a 3:1:1:1:1

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ratio; the sixth residue of HC CDR3 varies among T, Y, R, D, and L in a 3:1:1:1:1 ratio; the seventh residue of HC CDR3 varies among S, Y, R, D, and L in a 3:1:1:1:1 ratio; the eighth residue of HC CDR3 is C; the ninth residue of HC CDR3 varies among Y, S, D, R, and L in a 3:1:1:1:1 ratio; the tenth residue of HC CDR3 varies among T, Y, R, D, and L in a 3:1:1:1:1 ratio; the eleventh residue of HC CDR3 varies among A, S, Y, D, R, and L in a 3:1:1:1:1:1 ratio; the twelfth residue of HC CDR3 varies among E, R, S, Y, and L in a 3:1:1:1:1 ratio; the thirteenth residue of HC CDR3 varies among Y, S, D, R, and L in a 3:1:1:1:1 ratio; the fourteenth residue of HC CDR3 varies among F, Y, S, R, D, and L in a 3:1:1:1:1:1 ratio; the fifteenth residue of HC CDR3 varies among Q, E, R, S, Y, and L in a 3:1:1:1:1:1 ratio; and the sixteenth residue of HC CDR3 varies among H, D, R, S, Y, and L in a 3:1:1:1:1:1 ratio, and wherein a FR3 is located at the amino terminus of the CDR3, and the last residue of the FR3 varies between K and R in a 3:1 ratio.

14. The library of claim 1, wherein the light chain is an A27 light chain (LC),
wherein CDR1 of the LC is diversified, wherein position 27 is about 55% Q,
15 and about 9% each of E, R, Y, S, and L; position 28 is about 46% S and about 9% each of N, T, Y, E, R, and L; position 30 is about 55% S and about 9% each of D, N, R, T, and Y; position 30a is about 46% S and about 9% each of G, N, R, T, Y, and D, and about 8% with no amino acid; position 31 is about 44% S and about 8% each of D, F, G, N, R, T, and Y; position 32 is about 44% Y and about 7% each of F, D, L, N, Q, R, S, and Y; and position 34
20 is about 70% A and about 15% each of S and Y, and

wherein CDR2 of the LC is diversified, wherein position 50 is about 55% G, and about 9% each of D, R, S, Y, and L; position 53 is about 52% S and about 8% each of N, T, S, Y, E, and R; and position 56 is about 64% T and about 9% each of E, R, S, and Y.

15. The library of Claim 1, wherein the light chain is an A27 light chain (LC),
25 wherein CDR1 of the LC is diversified, wherein position 27 is about 55% Q, and about 9% each of E, R, Y, S, and L; position 28 is about 46% S and about 9% each of N, T, Y, E, R, and L; position 30 is about 55% S and about 9% each of D, N, R, T, and Y;

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position 30a is about 46% S and about 9% each of G, N, R, T, Y, and D, and about 8% with no amino acid; position 31 is about 44% S and about 8% each of D, F, G, N, R, T, and Y; position 32 is about 44% Y and about 7% each of F, D, L, N, Q, R, S, and Y; and position 34 is about 70% A and about 15% each of S and Y, and

5 wherein CDR3 of the LC is diversified, wherein position 91 is about 64% Y, and about 9% each of F, E, R, and S; position 92 is about 52% G and about 8% each of A, D, R, S, T, and Y; position 93 is about 52% S and about 8% each of D, F, N, R, T, and Y; position 94 is about 55% S and about 9% each of W, E, R, Y, and S; position 95 is about 64% P and about 9% each of E, R, Y, and S, and about 8% with no amino acid; and position
10 96 is about 55% L and about 9% each of E, R, P, Y, and S.

16. The library of Claim 1, wherein the light chain is an A27 light chain (LC),

 wherein CDR2 of the LC is diversified, wherein position 50 is about 55% G, and about 9% each of D, R, S, Y, and L; position 53 is about 52% S and about 8% each of N, T, S, Y, E, and R; and position 56 is about 64% T and about 9% each of E, R, S, and Y, and

15 wherein CDR3 of the LC is diversified, wherein position 91 is about 64% Y, and about 9% each of F, E, R, and S; position 92 is about 52% G and about 8% each of A, D, R, S, T, and Y; position 93 is about 52% S and about 8% each of D, F, N, R, T, and Y; position 94 is about 55% S and about 9% each of W, E, R, Y, and S; position 95 is about 64% P and about 9% each of E, R, Y, and S, and about 8% with no amino acid; and
20 position 96 is about 55% L and about 9% each of E, R, P, Y, and S.

17. The library of any of Claims 1-10, wherein the light chain has diversity at one or more of LC CDR1, LC CDR2, and LC CDR3.

18. The library of Claim 17, wherein the light chain comprises a LC CDR1, a LC CDR2, or a LC CDR3 of an A27 LC,

25 wherein CDR1 of the LC is diversified, wherein position 27 is about 55% Q, and about 9% each of E, R, Y, S, and L; position 28 is about 46% S and about 9% each of N,

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T, Y, E, R, and L; position 30 is about 55% S and about 9% each of D, N, R, T, and Y; position 30a is about 46% S and about 9% each of G, N, R, T, Y, and D, and about 8% with no amino acid; position 31 is about 44% S and about 8% each of D, F, G, N, R, T, and Y; position 32 is about 44% Y and about 7% each of F, D, L, N, Q, R, S, and Y; and position 34
 5 is about 70% A and about 15% each of S and Y;

wherein CDR2 of the LC is diversified, wherein position 50 is about 55% G, and about 9% each of D, R, S, Y, and L; position 53 is about 52% S and about 8% each of N, T, S, Y, E, and R; and position 56 is about 64% T and about 9% each of E, R, S, and Y, or

wherein CDR3 of the LC is diversified, wherein position 91 is about 64% Y,
 10 and about 9% each of F, E, R, and S; position 92 is about 52% G and about 8% each of A, D, R, S, T, and Y; position 93 is about 52% S and about 8% each of D, F, N, R, T, and Y; position 94 is about 55% S and about 9% each of W, E, R, Y, and S; position 95 is about 64% P and about 9% each of E, R, Y, and S, and about 8% with no amino acid; and position 96 is about 55% L and about 9% each of E, R, P, Y, and S, or combinations thereof.

15 19. A method of preparing the library of any one of Claims 1 to 18, the method comprising digital wobbling.