Claims

WHAT IS CLAIMED IS:

1. A method of mapping mutant polypeptides formed from a template polypeptide wherein n is the number of amino acid residues in the template polypeptide, the method comprising steps of:
    a. generating n-1 separate sets of mutant polypeptides, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
    b. assaying each set of mutant polypeptides for at least one predetermined property, characteristic or activity;
    c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
    d. creating a functional map that is is used to identify (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

2. The method of claim 1 wherein X is 19 and represents the 19 naturally occurring amino acid residues not present in a predetermined position of the template polypeptide.

3. The method of claim 2 wherein said generating step comprises:
    i. subjecting a codon-containing polynucleotide encoding for said template polypeptide to polymerase-based amplification using a 64-fold degenerate oligonucleotide for each codon to be mutagenized, wherein each of said 64-fold degenerate oligonucleotides is comprised of a first homologous sequence and a

degenerate N,N,N triplet sequence, so as to generate a set of progeny polynucleotides; and

ii. subjecting said set of progeny polynucleotides to clonal amplification such that polypeptides encoded by the progeny polynucleotides are expressed to generate the mutant polypeptides.

4. A method of mapping a population of mutant antibodies formed from a template antibody having at least one complementary determining region (CDR), said at least one CDR together have a total of n amino acid residues, the method comprising:

a. generating a population comprising n sets of mutant antibodies, each set comprising mutant antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of mutant antibodies differs in the single predetermined position; and the number of different mutant antibodies generated is equivalent to n x X;

b. assaying the population for at least one predetermined property, characteristic or activity;

c. for each mutant antibody identifying any change in said property, characteristic or activity relative to the template antibody; and

d. creating a structural positional map that is used to identify (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template antibody; (b) fully mutable sites compared to the template antibody; and (c) positions and mutations which result in an up-mutant compared to the template antibody.

5. The method of claim 4, wherein the at least one complementarity determining region is six complementary determining regions (CDRs), said six CDRs together comprising n amino acid residues.

6. The method of claim 4, wherein said predetermined property, characteristic or property is at least one of binding affinity and immunogenicity.

7. A functional positional map made by the method of claim 1, or 4.

8. A method of producing a library of multi-site mutant polypeptides formed from a template polypeptide wherein n is the number of amino acid residues in the template polypeptide, the method comprising:

   a. generating n-1 separate sets of point mutation polypeptides, each set comprising point mutation polypeptides having X number of different predetermined single amino acid substitutions at a single predetermined site of the template polypeptide; wherein each set of point mutation polypeptides differs in the single predetermined site; and the number of different point mutation polypeptides generated is equivalent to [n-1] x X;
   b. screening each set of point mutation polypeptides for at least one predetermined property, characteristic or activity;
   c. identifying any change in said property, characteristic or activity relative to the template polypeptide for each point mutation polypeptide;
   d. categorizing each amino acid point mutation as one of deactivating, non-deactivating or activating;
   e. creating a functional map of such changes categorizing each site as one of non-mutable; partially mutable; or fully mutable;
   f. utilizing the functional map to select non-deactivating amino acid point mutations within partially mutable sites that are adjacent to non-mutable sites; and
   g. recombining permutations of two to twenty amino acid point mutations selected in step (f) simultaneously to generate a library of multi-site mutant polypeptides.

9. The method of claim 8 wherein the recombining step utilizes combinatorial protein synthesis.

10. The method of claim 8 wherein contact amino acid residues in the template polypeptide are categorized as non-mutable in the functional map.

11. The method of claim 8 wherein the template polypeptide is derived from a portion of an immunoglobulin molecule.

12. The method of claim 11 wherein the portion of the immunoglobulin molecule is selected from a heavy chain, light chain, variable domain, constant domain, hypervariable region, complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3).

13. The method of claim 8, further comprising:

h. screening the library of multi-site mutant polypeptides for at least one pre-determined property, characteristic or activity relative to the template polypeptide; and

i. identifying the multi-site up-mutant polypeptides as those with one or more improved properties, characteristics, or activities relative to the template polypeptide.

14. A method of selecting two or more sites in a template polypeptide for multi-site mutation, the method comprising:

a. obtaining a functional map of mutant polypeptides formed from the template polypeptide wherein n is the number of amino acid residues in the template polypeptide, said obtaining step comprising the steps of:

i. generating n-1 separate sets of mutant polypeptides, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single

predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;

ii. assaying each set for at least one predetermined property, characteristic or activity;

iii. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and

iv. creating a functional map of such changes identifying positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide;

b. utilizing the functional map to select partially mutable sites that are adjacent to non-mutable sites for multi-site mutation.

15. A method of mapping mutant polypeptides formed from a template polypeptide wherein n is the number of amino acid residues in the template polypeptide, the method comprising:

a. generating n-1 (n-2 in the case where the initial residue is methionine) separate mutant polypeptides, wherein each mutant polypeptide differs from the template polypeptide in that one amino acid is deleted at a single predetermined position;

b. assaying the population of mutant polypeptides for at least one predetermined property, characteristic or activity;

c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and

d. creating a functional map that is is used to identify those deletions which result in (a) deletions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) deletions which result in a down-mutant compared to the template polypeptide; and (c) deletions which result in an up-mutant compared to the template polypeptide.

16. A method of mapping mutant polypeptides formed from a template polypeptide wherein n is the number of amino acid residues in the template polypeptide, the method comprising:

a. generating n+[20 x (n-1)] separate mutant polypeptides, wherein each mutant polypeptide differs from the template polypeptide in that it has inserted after a specific position in the template one each of the 20 naturally occurring amino acids;
b. assaying the population for at least one predetermined property, characteristic or activity;
c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a functional map that is used to identify those insertions which result in (a) insertions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) insertions which result in a down-mutant compared to the template polypeptide; and (c) insertions which result in an up-mutant compared to the template polypeptide.

17. A method of providing an optimized protein, the method comprising:
a. selecting a template polypeptide wherein n is the number of amino acid residues in the template polypeptide;
b. generating n-1 separate sets of mutant polypeptides from the template polypeptide, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
c. assaying each mutant polypeptide for at least one predetermined property, characteristic or activity;
d. identifying any change in said property, characteristic or activity of the mutant polypeptide relative to the template polypeptide;
e. creating a functional map wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide;

f. combining two or more of each of the relevant positions and mutations identified from mutant polypeptides which result in a up-mutant compared to the template polypeptide to create a set of combinatorial polypeptides;
g. assaying each combinatorial polypeptide for said predetermined property, characteristic or activity; and
h. selecting a combinatorial polypeptide in which said property, characteristic or activity is optimized compared to up-mutant polypeptides.

18. The method of claim 1 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position in each mutant polypeptide in each set of mutant polypeptides.

19. The method of claim 4 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position of the CDR in each mutant antibody in each set of mutant antibodies.

20. The method of claim 8 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of each of the intended predetermined single amino acid substitutions at the predetermined site in each point mutation polypeptide in each set of point mutation polypeptides.

21. The method of claim 14 wherein the generating step (i) further comprises confirming the sequence of each multi-site mutant polypeptide in the library by sequencing, or some other method.

22. The method of claim 15 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid deletion at the single predetermined position.

23. The method of claim 16 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid insertion at the specific position in the template.

24. A method of evolving a template polypeptide encoding a functional protein, the method comprising:

a. generating and cloning at least 20 or more separate sets of mutant polypeptides, each set comprising mutant polypeptides having from 5 to 19 different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position;

b. confirming that each different mutant polypeptide was generated and cloned;

c. assaying each set of mutant polypeptides for at least one predetermined property, characteristic or activity;

d. performing bioinformatic analysis to identify positions of potential interest in the template polypeptide for further modification; and

e. mutating positions identified in step (d).

25. The method of claim 24, wherein in step (a), each set of mutant polypeptides comprises mutant polypeptides having from 10 to 19, from 15 to 19, or 19 different predetermined amino acid residues at a single predetermined position of the template polypeptide.

26. The method of claim 24 wherein bioinformatic analysis is generation of a functional map.

27. The method of claim 24 wherein the at least one predetermined property, characteristic or activity is modified expression.

28. The method of claim 27 wherein the modified expression is improved expression.

29. A method of evolving a template polypeptide encoding a functional protein and wherein n is the number of amino acid residues in the template polypeptide, the method comprising:

a. generating and cloning n-1 sets of mutant polypeptides, each set comprising mutant polypeptides having 19 different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the total number of different mutant polypeptides generated is equivalent to [n-1] x 19;

b. confirming that each different mutant polypeptide was generated and cloned;

c. assaying each set of mutant polypeptide for at least one predetermined property, characteristic or activity;

d. performing bioinformatic analysis to identify positions of potential interest for further modification; and

e. mutating positions identified in step (d).

30. The method of claim 29 wherein the bioinformatic analysis is generation of a functional map.

31. The method of claim 29 wherein the at least one predetermined property, characteristic or activity is modified expression.

32. The method of claim 31 wherein the modified expression is improved expression.

33. A method of evolving a polypeptide from a functional protein, the method comprising:

a. incorporating N,N,N at every codon position in a polynucleotide encoding the functional protein in order to generate a set of predetermined mutant polynucleotides containing every possible codon sequence and cloning each predetermined mutant polynucleotide;

b. confirming by sequencing, or some other method, that each predetermined mutant polynucleotide in the set was generated and cloned;

c. producing a set of predetermined mutant polypeptides from the set of predetermined mutant polynucleotides;

d. assaying each mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein; and

e. creating a functional map that is used to identify (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the functional protein; (b) fully mutable sites compared to the functional protein; and (c) positions and mutations which result in an up-mutant compared to the functional protein.

34. The method of claim 33, wherein step (b) comprises

confirming by sequencing or some other method that greater than 75%, 50%, 25%, or 1% of the predetermined mutant polynucleotides in the set were generated, cloned and expressed.

35. The method of claim 33, wherein step (b) comprises

confirming by sequencing, or some other method, that any number of the predetermined mutant polynucleotides in the set were generated, cloned and expressed.

36. The method of claim 33 wherein the at least one predetermined property, characteristic or activity is modified expression.

37. The method of claim 36 wherein the modified expression is improved expression.

38. The method of claim 33 wherein the confirming step comprises sequencing of each mutant polynucleotide.