~~WE CLAIM:~~

1. A method of evolution and expression of a protein in a eukaryotic cell production host; the method comprising:
    a. selecting a template antibody;
    b. evolving the template antibody to enable production of a set of mutant antibodies in the eukaryotic cell production host;
    c. screening the mutant antibodies for at least one predetermined property, characteristic or activity;
    d. selecting an up-mutant antibody from the set of mutant antibodies based upon optimization of the at least one predetermined property, characteristic or activity compared to the template antibody; and
    e. expressing the up-mutant antibody in the same eukaryotic cell production host as in step b for any commercial scale,

    wherein the evolving step comprises one of comprehensive positional evolution (CPE) followed by confirming the mutations by a sequencing technique, comprehensive positional insertion evolution (CPI), comprehensive positional deletion evolution (CPD), comprehensive positional evolution (CPE) followed by combinatorial protein synthesis (CPS), comprehensive positional deletion evolution (CPD) followed by combinatorial protein synthesis (CPS), and comprehensive positional deletion evolution (CPD) followed by combinatorial protein synthesis (CPS), synergy evolution, flex evolution.

2. The method of claim 1, wherein step a is preceded by steps comprising:
    a1. generating an anti-antigen antibody library in the eukaryotic cell production host using eukaryotic cell surface display of antibodies; and
    a2. screening the library for the at least one predetermined property, characteristic or activity; and

    wherein the template antibody is selected from the library.

3. The method of claim 1 wherein the eukaryotic cell production host is selected from 3T3 mouse fibroblast cells; BHK21 Syrian hamster fibroblast cells; MDCK, dog epithelial cells; Hela human epithelial cells; PtK1 rat kangaroo epithelial cells; SP2/0 mouse plasma cells; and NS0 mouse mouse plasma cells; HEK 293 human embryonic kidney cells; COS monkey kidney cells; CHO, CHO-S Chinese hamster ovary cells;

R1 mouse embryonic cells; E14.1 mouse embryonic cells; H1 human embryonic cells; H9 human embryonic cells; PER C.6, human embryonic cells; S. cerevisiae yeast cells; and picchia yeast cells.

4. The method of claim 2, wherein the screening steps comprise fluorescence-activated cell sorting.

5. The method of claim 1 wherein the evolving step comprises enabling production of a set of mutant antibodies formed from the template antibody having m complementarity determining regions, wherein m is an integer of 1 to 6, each said complementarity determining region comprising n amino acid residues, the method comprising:

   a. generating m x n separate sets of antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the complementarity determining regions; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to m x n x X.

6. The method of claim 5 wherein m is 6.

7. The method of claim 1 wherein the antibody is an antibody fragment selected from a heavy chain, light chain, variable domain, constant domain, hypervariable region, complementarity determining region 1, complementarity determining region 2, and complementarity determining region 3.

8. The method of claim 1 wherein the evolving step b comprises

   h. generating n-1 separate sets of mutant polypeptides from the template antibody, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;
   and wherein the screening step c comprises

i. ~~assaying each member polypeptide for the at least one predetermined~~ property, characteristic or activity;

j. identifying any change in said property, characteristic or activity of the member polypeptide relative to the template polypeptide; and

k. creating a functional map wherein the functional map is used to identify positions and mutations in the mutant polypeptide which result in an up-mutant and/or a silent mutation compared to the template polypeptide.

9. The method of claim 8 wherein X is 19.

10. The method of claim 9 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.

11. The method of claim 8 wherein the functional map is used to identify one or more of (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.

12. The method of claim 1 wherein the predetermined property, characteristic or activity is selected from reduction of protein-protein aggregation, enhancement of protein stability, increased protein solubility, introduction of glycosylation sites, introduction of conjugation sites, reduction of immunogenicity, enhancement of protein expression, increase in antigen affinity, decrease in antigen affinity, change in binding affinity, change in immunogenicity, and enhancement of specificity.

13. The method of claim 1, wherein the eukaryotic cell production host is a mammalian cell production host.

14. The method of claim 13, wherein the mammalian cell production host is selected from CHO and HEK293.

15. The method of claim 2, wherein step a1 is preceded by a step comprising:

selecting an antigen.

16. The method of claim 2, wherein the anti-antigen antibody library is a humanized anti-antigen antibody library.

17. The method of claim 1, wherein the antibody is a full-length antibody.

18. The method of claim 1, wherein the expressing step comprises manufacturing the up-mutant antibody.

19. The method of claim 1 wherein the eukaryotic cell production host is selected from CHOK1SV and NSO host cell lines.

20. The method of claim 1 wherein the screening step comprises

creating a functional map wherein the functional map is used to identify positions and mutations in the mutant polypeptide which result in an up-mutant and/or a silent mutation compared to the template polypeptide.

WE CLAIM:

1. A method of evolution and expression of an antibody in a eukaryotic cell production host; the method comprising:
a. selecting a template antibody;
b. evolving the template antibody to enable production of a set of mutant antibodies in a eukaryotic cell production host;
c. screening the mutant antibodies for at least one predetermined property, characteristic or activity;
d. selecting an up-mutant antibody from the set of mutant antibodies based upon optimization of the at least one predetermined property, characteristic or activity as compared to a same property, characteristic or activity of the template antibody; and
e. expressing the up-mutant antibody in the same eukaryotic cell production host as in step b for any commercial scale,

wherein the evolving step comprises one of comprehensive positional evolution (CPE) followed by confirming the mutations by a sequencing technique; comprehensive positional insertion evolution (CPI); comprehensive positional deletion evolution (CPD); comprehensive positional evolution (CPE) followed by combinatorial protein synthesis (CPS); comprehensive positional deletion evolution (CPD) followed by combinatorial protein synthesis (CPS); or comprehensive positional deletion evolution (CPD) followed by combinatorial protein synthesis (CPS), synergy evolution, flex evolution.

2. The method of claim 1, wherein the selecting step (a) comprises:
a1. generating an anti-antigen antibody library in a eukaryotic cell production host with antibody cell surface display;
a2. screening the library for at least one predetermined property, characteristic or activity; and
a3. selecting a template antibody from the library.

3. The method of claim 1 wherein the eukaryotic cell production host is selected from 3T3 mouse fibroblast cells; BHK21 Syrian hamster fibroblast cells; MDCK, dog epithelial cells; Hela human epithelial cells; PtK1 rat kangaroo epithelial cells; SP2/0 mouse plasma cells; and NS0 mouse plasma cells; HEK 293 human embryonic kidney cells; COS monkey kidney cells; CHO, CHO-S Chinese hamster ovary cells; R1 mouse embryonic cells; E14.1 mouse embryonic cells; H1 human embryonic cells; H9 human embryonic cells; PER C.6, human embryonic cells; S. cerevisiae yeast cells; and picchia yeast cells.

4. The method of claim 3 wherein the eukaryotic cell production host is CHO or HEK293.

5. The method of claim 1 wherein the screening steps comprise fluorescence-activated cell sorting (FACS).

6. The method of claim 1 wherein the evolving step comprises producing a set of mutant antibodies formed from the template antibody having m complementarity determining regions (CDR), wherein m is an integer 1 to 6, each said CDR comprising n amino acid residues, the method comprising:
a. generating m x n separate sets of antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to m x n x X.

7. The method of claim 6 wherein m is 6.

8. The method of claim 1 wherein the template antibody is an antibody fragment 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).

9. The method of claim 1 wherein the evolving step (b) comprises
h. generating n-1 separate sets of mutant polypeptides from the template antibody, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;
and wherein the screening step (c) comprises:
i. assaying each member polypeptide for the at least one predetermined property, characteristic or activity;
j. identifying any change in said property, characteristic or activity of the member polypeptide relative to the template antibody;
k. creating a functional map wherein the functional map is used to identify positions and mutations in the mutant polypeptide which result in an up-mutant and/or a silent mutation compared to the template antibody.

10. The method of claim 9 wherein X is 19.

11. The method of claim 10 wherein said generating step comprises:
i. subjecting a codon-containing polynucleotide encoding for said template antibody 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 mutant polypeptides encoded by the progeny polynucleotides are expressed.

12. The method of claim 1 wherein the predetermined property, characteristic or activity is selected from reduction of antibody-protein aggregation, enhancement of antibody stability, increased antibody solubility, introduction of glycosylation sites, introduction of conjugation sites, reduction of immunogenicity, enhancement of protein expression, increase in antigen affinity, decrease in antigen affinity, change in binding affinity, change in immunogenicity, and enhancement of specificity.

13. The method of claim 2, wherein the anti-antigen antibody library is a humanized anti-antigen antibody library.

14. The method of claim 1, wherein the selecting step (d) comprises:
selecting the up-mutant antibody based upon an improved expression when compared to the template antibody; and wherein the expressing step (e) comprises manufacturing the up-mutant antibody in the same eukaryotic cell production host as in the evolving step (b).