CLAIMS

1. A method for the production of a V_H heavy chain-only antibody in a transgenic non-human mammal comprising the steps of providing more than one heterologous V_H heavy chain locus in that mammal, wherein each V_H heavy chain locus comprises one or more V gene segments, one or more D gene segments, one or more J gene segments and a gene segment encoding a heavy chain constant region which, when expressed, does not include a C_H1 domain and expressing a V_H heavy chain-only antibody from at least one of said loci.

2. The method of claim 1, wherein each V_H heavy chain locus comprises one or multiple V gene segments.

3. The method of claim 1 or claim 2, wherein each locus comprises only one V gene segment.

4. The method of claim 3, wherein each V gene segment is different from all other V gene segments.

5. The method of claim 3, wherein each V gene segment is identical to all the other V gene segments.

6. The method of claim 5 wherein the remaining gene segments in each locus are the same as those in all the other loci.

7. The method of claim 5 wherein the remaining gene segments in each locus are different from those in all the other loci.

8. The method of claim 1 or claim 2, wherein each locus comprises multiple V gene segments.

9. The method of claim 8, wherein the V gene segments in any one locus are all derived from an organism of the same species.

10. The method of claim 8, wherein the V gene segments in any one locus are derived from organisms of different species.

11. The method of claim 9, wherein the V gene segments are of human origin.

12. The method of any one of claims 1 to 4 and 8 to 11, wherein the multiple heavy chain loci comprise any number or combination of the 39 functional human V gene segments and engineered variants thereof with improved solubility properties distributed across the multiple loci.

13. The method of any one of claims 1 to 12, wherein each different heavy chain locus is present as a single copy in the genome of the transgenic non-human mammal.

14. The method of any one of claims 1 to 13 wherein each V_H heavy chain locus comprises from one to forty D gene segments.

15. The method of any one of claims 1 to 14, wherein the D gene segments are human D gene segments.

16. The method of any one of claims 1 to 15, wherein each V_H heavy chain locus comprises from one to twenty J gene segments.

17. The method of claims 16, wherein the J gene segments are human J gene segments.

18. The method of any one of claims 1 to 17, wherein each V_H heavy chain locus contains the same D and J gene segments.

19. The method of any one of claims 1 to 17, wherein each V_H heavy chain locus contains different combinations of D and J gene segments.

20. The method of any one of claims 1 to 19, wherein each V_H heavy chain locus comprises one or more V gene segments, twenty-five functional human D gene segments and 6 human J gene segments.

21. The method of any one of claims 1 to 20, wherein each V_H heavy chain locus comprises a gene segment encoding at least one heavy chain constant region providing effector functions *in vivo* wherein the constant region when expressed does not include a C_H1 domain in the antibody.

22. The method of claim 21, wherein each locus contains only one gene segment encoding one particular constant region.

23. The method of claim 21, wherein each locus comprises more than one gene segment, each encoding a different constant region.

24. The method of any one of claims 21 to 23, wherein each locus contains the same constant region-encoding gene segment(s).

25. The method of any one of claims 21 to 23, wherein each locus has different or a different combination of constant region-encoding gene segment(s).

26. The method of any one of claims 21 to 25, wherein each gene segment encoding a heavy chain constant region comprises one or more heavy chain constant region exons of the Cδ, $C\gamma_{1-4}$, Cμ, Cε or $C\alpha_{1-2}$ classes, with the proviso that the heavy chain constant region gene segments do not express a C_H1 domain.

27. The method of any one of claims 21 to 26, wherein the heavy chain constant region is of human origin.

28. The method of any one of the preceding claims, wherein two or more heterologous V_H heavy chain loci in that mammal are present in tandem on the same chromosome.

29. The method of any one of claims 1 to 27 , wherein one or more heterologous V_H heavy chain loci in that mammal are present on different chromosomes.

30. The method of any one of claims 1 to 29, wherein the transgenic non-human mammal is a rodent.

31. The method of claim 30, wherein the rodent is a mouse.

32. A transgenic non-human mammal comprising more than one heterologous V_H heavy chain locus as defined in any one of claims 1 to 31.

33. A method for the production of heavy chain-only antibodies by immunising a transgenic non-human mammal of claim 32 with an antigen.

34. A method of producing high affinity, antigen-specific V_H heavy chain-only antibodies comprising:

immunising a transgenic non-human mammal according to claim 32 with an antigen;

generating B-cell hybridomas;

selecting cells expressing antigen-specific heavy chain-only antibody; and

isolating antigen-specific recombined affinity matured V_H heavy chain-only antibody.

CLAIMS

1. A method for the production of a V_H heavy chain-only antibody in a transgenic non-human mammal comprising the steps of:

providing more than one heterologous V_H heavy chain locus on different chromosomes in that mammal, wherein each V_H heavy chain locus comprises one or more V gene segments, one or more D gene segments, one or more J gene segments and a gene segment encoding a heavy chain constant region which, when expressed, does not include a C_H1 domain, and wherein, following antigen challenge, only one locus is recombined productively in a B cell resulting in the production of a V_H heavy chain only antibody,

and expressing said V_H heavy chain-only antibody from said productively recombined locus.

2. The method of claim 1, wherein each locus comprises only one V gene segment.

3. The method of claim 2, wherein each V gene segment is different from all other V gene segments.

4. The method of claim 2, wherein each V gene segment is identical to all the other V gene segments.

5. The method of claim 4 wherein the remaining gene segments in each locus are the same as those in all the other loci.

6. The method of claim 4 wherein the remaining gene segments in each locus are different from those in all the other loci.

7. The method of claim 1, wherein each locus comprises multiple V gene segments.

8. The method of claim 7, wherein the V gene segments in any one locus are all derived from an organism of the same species.

9. The method of claim 7, wherein the V gene segments in any one locus are derived from organisms of different species.

10. The method of claim 8, wherein the V gene segments are of human origin.

11. The method of any preceding claim, wherein the multiple heavy chain loci comprise any number or combination of the 39 functional human V gene segments and engineered variants thereof with improved solubility properties distributed across the multiple loci.

12. The method of any preceding claim, wherein each different heavy chain locus is present as a single copy in the genome of the transgenic non-human mammal.

13. The method of any preceding claim, wherein each V_H heavy chain locus comprises from one to forty D gene segments.

14. The method of any preceding claim, wherein the D gene segments are human D gene segments.

15. The method of any preceding claim, wherein each V_H heavy chain locus comprises from one to twenty J gene segments.

16. The method of any preceding claim, wherein the J gene segments are human J gene segments.

17. The method of any preceding claim, wherein each V_H heavy chain locus contains the same D and J gene segments.

18. The method of any one of claims 1-16, wherein each V_H heavy chain locus contains different combinations of D and J gene segments.

19. The method of any preceding claim, wherein each V_H heavy chain locus comprises one or more V gene segments, twenty-five functional human D gene segments and 6 human J gene segments.

20. The method of any preceding claim, wherein each V_H heavy chain locus comprises a gene segment encoding at least one heavy chain constant region providing effector functions *in vivo* wherein the constant region when expressed does not include a C_H1 domain in the antibody.

21. The method of claim 20, wherein each locus contains only one gene segment encoding one particular constant region.

22. The method of claim 20, wherein each locus comprises more than one gene segment, each encoding a different constant region.

23. The method of claim 20, wherein each locus contains the same constant region-encoding gene segment(s).

24. The method of claim 20, wherein each locus has different or a different combination of constant region-encoding gene segment(s).

25. The method of any one of claims 20-24, wherein each gene segment encoding a heavy chain constant region comprises one or more heavy chain constant region exons of the Cδ, $C\gamma_{1-4}$, Cμ, Cε or $C\alpha_{1-2}$ classes, with the proviso that the heavy chain constant region gene segments do not express a C_H1 domain.

26. The method of any one of claims 20-25, wherein the heavy chain constant region is of human origin.

27. The method of any preceding claim, wherein the transgenic non-human mammal is a rodent.

28. The method of claim 27, wherein the rodent is a mouse.

29. A transgenic non-human mammal comprising more than one heterologous V_H heavy chain locus on different chromosomes, wherein each V_H heavy chain locus comprises one or more V gene segments, one or more D gene segments, one or more J gene segments and a gene segment encoding a heavy chain constant region which, when expressed, does not include a C_H1 domain, and wherein, following antigen

challenge, only one locus is recombined productively in a B-cell resulting in the production of a V_H heavy chain only antibody.

30. A method for the production of heavy chain-only antibodies by immunising a transgenic non-human mammal of claim 29 with an antigen.

31. A method of producing high affinity, antigen-specific V_H heavy chain-only antibodies comprising:

immunising a transgenic non-human mammal according to claim 29 with an antigen;

generating B-cell hybridomas;

selecting cells expressing antigen-specific heavy chain-only antibody; and

isolating antigen-specific recombined affinity matured V_H heavy chain-only antibody.