

CLAIMS:

1. A method of making a mouse cell or a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

5 the mouse having a genome that

- (a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and
- (b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

- 10 (c) constructing a transgenic mouse embryonic stem cell (ES cell) comprising a transgenic antibody heavy chain locus by inserting one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma);
- 15 (d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes;
- (e) simultaneously or separately from step (c) or (d), inserting into the ES cell genome one or more ADAM6 genes; and optionally
- 20 (f) developing the ES cell into a fertile mouse or a progeny thereof whose genome is homozygous for said transgenic heavy chain locus and encodes ADAM6, wherein all or part of the endogenous heavy chain VDJ region has been deleted from both chromosomes 12 in the genome; optionally wherein said fertile mouse or progeny is male.

- 25 2. A method of making a mouse cell or a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

the mouse having a genome that

- 30 (a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and
- (b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

- (c) constructing a transgenic mouse embryonic stem cell (ES cell) comprising a transgenic antibody heavy chain locus by inserting one or more human VH gene segments, one or more

human D gene segments and one or more human JH gene segments into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma);

(d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes;

(e) developing the ES cell into a child mouse or progeny thereof whose genome comprises a said transgenic heavy chain locus;

(f) deriving a second ES cell from said mouse and inserting into the genome of said second ES cell one or more ADAM6 genes; and optionally

(g) developing the second ES cell into a fertile mouse or a progeny thereof whose genome is homozygous for said transgenic heavy chain locus and encodes ADAM6, wherein all or part of the endogenous heavy chain VDJ region has been deleted from both chromosomes 12 in the genome; optionally wherein said fertile mouse or progeny is male.

3. A method of making a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

the mouse having a genome that

(a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and

(b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

(c) constructing a transgenic mouse embryonic stem cell (ES cell) comprising a transgenic

antibody heavy chain locus by inserting one or more human VH gene segments, one or more

human D gene segments and one or more human JH gene segments into DNA of a chromosome

12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma);

(d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes;

(e) developing the ES cell into a child mouse or progeny thereof whose genome comprises a said transgenic heavy chain locus; and

(f) by breeding using said child mouse (or progeny) and a further mouse whose genome

comprises one or more ADAM6 genes, developing a fertile mouse or a progeny thereof whose genome is homozygous for said transgenic heavy chain locus and encodes ADAM6, wherein all or part of the endogenous heavy chain VDJ region has been deleted from both chromosomes 12 in the genome; optionally wherein said fertile mouse or progeny is male.

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4. Use of an ADAM6 gene in the manufacture of a mouse for producing a fertile mouse (eg, a fertile male mouse),

wherein the mouse has a genome that comprises

10 (a) a transgenic heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments inserted into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma); and

15 (b) a deletion of all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes; and

wherein one or more ADAM6 genes are inserting into the genome.

- 20 5. Use of an ADAM6 gene in the manufacture of a mouse cell for producing a cell that is capable of developing into such fertile mouse (eg, a male mouse);

wherein the mouse cell has a genome that comprises

25 (a) a transgenic heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments inserted into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma); and

30 (b) a deletion of all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes; and

wherein one or more ADAM6 genes are inserting into the genome.

- 35 6. The method or use of any preceding claim, wherein an ADAM6 gene is inserted on one or both chromosomes 12.

7. The method or use of claim 6, wherein an ADAM6 gene is inserted (i) within one or both transgenic heavy chain loci or (ii) within 20 Mb of one or both transgenic heavy chain loci.

- 40 8. The method or use of any preceding claim, wherein said human gene segments are inserted into chromosome 12 to replace all or part of the endogenous heavy chain VDJ region, so that

insertion of the human gene segments and deletion of the endogenous VDJ DNA take place simultaneously; optionally wherein the entire endogenous VDJ region is replaced.

9. The method or use of any preceding claim, wherein mouse ADAM6a and ADAM6b genes are inserted, such that the final fertile mouse can express both ADAM6a and ADAM6b proteins.

10. The method or use of any preceding claim, wherein the genome of the final fertile mouse or progeny is homozygous for each inserted ADAM6 gene; optionally wherein the genome comprises more than two copies of mouse ADAM6a and/or ADAM6b genes.

11. The method or use of any preceding claim, with the alternative that each organism is a rat instead of a mouse and each said chromosome is a chromosome 6 instead of chromosome 12; or with the alternative that each cell is a rat cell instead of a mouse cell and each said chromosome is a chromosome 6 instead of chromosome 12.

12. A mouse (optionally a male mouse) comprising a transgenic antibody heavy chain locus, the mouse having a genome that

- (i) comprises said transgenic heavy chain locus on a chromosome 12; and
- (ii) is inactivated for endogenous antibody heavy chain expression;

wherein said chromosome 12 of the genome comprises

(iii) a transgenic antibody heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments operably connected upstream of a mouse or human heavy chain constant region (optionally Cmu and/or Cgamma);

(iv) a deletion of all or part of the mouse endogenous heavy chain VDJ region of said chromosome 12 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes mouse ADAM6 genes; and

wherein the genome comprises

- (v) an insertion of one or more expressible ADAM6 genes.

13. The mouse of claim 12, wherein the genome comprises a transgenic antibody heavy chain locus on a respective copy of chromosome 12.

14. A rat (optionally a male rat) comprising a transgenic antibody heavy chain locus, the rat having a genome that

- (i) comprises said transgenic heavy chain locus on a chromosome 6; and
- (ii) is inactivated for endogenous antibody heavy chain expression;

wherein said chromosome 6 of the genome comprises

(iii) a transgenic antibody heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments operably connected upstream of a rat or human heavy chain constant region (optionally Cmu and/or Cgamma);

(iv) a deletion of all or part of the rat endogenous heavy chain VDJ region of said chromosome 6 to inactivate endogenous antibody heavy chain expression, wherein the deletion includes rat ADAM6; and

wherein the genome comprises

(v) an insertion of one or more expressible ADAM6 genes.

15. The rat of claim 13, wherein the genome comprises a transgenic antibody heavy chain locus on a respective copy of chromosome 6.

16. The mouse of claim 12 or 13 or rat of claim 14 or 15, wherein each inserted ADAM6 gene is on a (i) chromosome 12 wherein the animal is a mouse; or (ii) chromosome 6 wherein the animal is a rat.

17. The mouse or rat of any one of claims 12 to 16, wherein an inserted ADAM6 gene is inserted (i) within one of both transgenic heavy chain loci or (ii) within 20 Mb of one or both transgenic heavy chain loci.

18. The mouse or rat of any one of claims 12 to 17, wherein the human gene segments replace all or part of the endogenous VDJ region in each heavy chain locus.

19. The mouse or rat of any one of claims 12 to 18, wherein the genome comprises inserted expressible mouse ADAM6a and ADAM6b genes.

20. The mouse or rat of any one of claims 12 to 19, wherein the genome comprises an inserted expressible rat ADAM6 gene.

21. The mouse or rat of any one of claims 12 to 20, wherein the genome is homozygous for each inserted ADAM6 gene; optionally wherein the genome comprises more than two copies of ADAM6 genes selected from rat ADAM6, mouse ADAM6a and mouse ADAM6b genes.

22. The mouse or rat of any one of claims 12 to 21 wherein the genome comprises one or more transgenic light chain loci each comprising one or more human light chain V gene segments and one or more light chain J gene segments operably connected upstream of a light chain constant region (eg, an endogenous mouse or rat C kappa constant region).

23. The method, use, mouse or rat of any preceding claim, wherein the genome comprises an exogenous ADAM6.

24. The method, use, mouse or rat of any preceding claim, wherein the genome comprises ADAM6 that is inserted from a targeting vector (eg, a BAC vector).

25. The method, use, mouse or rat of any preceding claim, wherein the genome comprises ADAM6 exons is inserted by targeted or random insertion into an ES cell or zygote.

26. The method, use, mouse or rat of any preceding claim, wherein endogenous ADAM6 is deleted from the genome.

27. A non-human vertebrate (optionally a mouse or rat) or non-human vertebrate cell (optionally a mouse or rat cell) having a genome that

(i) comprises one or more transgenic antibody loci capable of expressing antibodies comprising human variable regions (optionally following antibody gene rearrangement); and

(ii) is inactivated for endogenous antibody expression;

wherein

(iii) endogenous variable region gene segments have been translocated to a chromosomal species (eg, chromosome 15) that does not contain antibody variable region gene segments in wild-type vertebrates of said non-human type, whereby endogenous antibody expression is inactivated.

28. A mouse or mouse cell having a genome that

(i) comprises one or more transgenic antibody loci capable of expressing antibodies comprising human variable regions (optionally following antibody gene rearrangement); and

(ii) is inactivated for endogenous mouse antibody expression;

wherein

(iii) a plurality of endogenous mouse variable region gene segments are absent from chromosomes 12 in the genome, but are present in germline configuration (with respect to each other) on one or more chromosomes other than chromosomes 12 (eg, the gene segments are on chromosome 15), whereby endogenous mouse antibody expression is inactivated.

29. A rat or rat cell having a genome that

(i) comprises one or more transgenic antibody loci capable of expressing antibodies comprising human variable regions (optionally following antibody gene rearrangement); and

(ii) is inactivated for endogenous rat antibody expression;

wherein

(iii) a plurality of endogenous rat variable region gene segments are absent from chromosomes 6 in the genome, but are present in germline configuration (with respect to each other) on one or more chromosomes other than chromosomes 6 (eg, the gene segments are on chromosome 15), whereby endogenous rat antibody expression is inactivated.

30. The vertebrate, mouse, rat or cell of any one of claims 27 to 29, wherein said genome comprises a transgenic antibody heavy chain locus (in heterozygous or homozygous state), the locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human DH gene segments operably connected upstream of a non-human vertebrate (eg, mouse or rat) constant region or a human constant region (optionally Cmu and/or Cgamma); and said endogenous variable region gene segments are selected from endogenous

(a) VH;

(b) D;

(c) JH;

(d) VH and D;

(e) D and JH; and

(f) VH, D and JH.

31. The vertebrate, mouse, rat or cell of any one of claims 27 to 30, wherein the genome comprises expressible endogenous ADAM6 gene(s).

32. A male vertebrate, mouse, rat or cell of any one of claims 27 to 31.

33. A method of making a non-human vertebrate cell (optionally a mouse or rat cell), the method comprising

- (i) inserting into a non-human ES cell genome one or more transgenic antibody loci comprising human variable region gene segments; and
- (ii) inactivating endogenous antibody expression by translocating endogenous variable region gene segments to a chromosomal species (eg, chromosome 15) that does not contain antibody variable region gene segments in wild-type vertebrates of said non-human type,

whereby a non-human vertebrate ES cell is produced that is capable of giving rise to a progeny cell in which endogenous antibody expression is inactivated and wherein the progeny is capable of expressing antibodies comprising human variable regions; and

- (iii) Optionally differentiating said ES cell into said progeny cell or a non-human vertebrate (eg, mouse or rat) comprising said progeny cell.

34. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises an inserted human VDJ region that includes bases 105,400,051 to 106,368,585 from human chromosome 14.

35. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome is homozygous at the Ig heavy chain, Igλ and Igκ loci.

36. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the non-human vertebrate VDJ region of the endogenous heavy chain Ig locus and the VJ region of the endogenous lambda and kappa loci have been inactivated.

37. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises

- (i) a heavy chain transgene comprising substantially the full human repertoire of IgH V, D and J regions; and
- (ii) substantially the full human repertoire of Igκ V and J regions and/or substantially the full human repertoire of Igλ V and J regions.

38. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises

- (a) Heavy chain loci, each comprising one or more human heavy chain V gene segments, one or more human heavy chain D gene segments and one or more human heavy chain JH gene segments upstream of an endogenous non-human vertebrate constant region or a human constant region (eg, Cmu and/or Cgamma);

(b) A kappa light chain locus (optionally in homozygous state) comprising one or more human kappa chain V gene segments, and one or more human kappa chain Jk gene segments upstream of an endogenous non-human vertebrate (eg, endogenous mouse or rat) kappa constant region; and optionally

5 (c) A lambda light chain locus (optionally in homozygous state) comprising one or more human lambda chain V gene segments, and one or more human lambda chain Jλ gene segments upstream of a lambda constant region; and

(d) Wherein the vertebrate is capable of producing chimaeric antibodies following rearrangement of said loci and immunisation with an antigen.

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39. The method, use, vertebrate, mouse or cell of any preceding claim, wherein the vertebrate is a mouse having a mouse genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv.

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40. The method, use, vertebrate, mouse or cell of any preceding claim, wherein the vertebrate is a mouse and the genome comprises an insertion of human light chain kappa VJ targeted into mouse chromosome 6 between coordinates 70,673,899 and 70,675,515, suitably at position 70,674,734, or an equivalent position in the lambda mouse locus on chromosome 16.

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41. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises a lambda antibody transgene comprising all or part of the human Igλ locus including at least one human Jλ region and at least one human Cλ region.

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42. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises a lambda transgene comprising a human Eλ enhancer and/or a kappa transgene comprising a human Ek enhancer.

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43. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein the genome comprises human DNA (as a source of heavy and/or light chain gene segments) from a library selected from a human RPCI-11 library, RPCI-13 library, CalTech A library, CalTech B library, CalTech C library or CalTech D library.

44. The method, use, vertebrate or cell of any preceding claim, wherein the vertebrate is a mouse, rat, rabbit, Camelid or shark.

45. The method, use, vertebrate, mouse or rat of any preceding claim, wherein the vertebrate is able to generate a diversity of at least 1×10^6 different functional chimaeric antibody sequence combinations.

46. The method, use, vertebrate, mouse, rat or cell of any preceding claim, wherein human VH which is naturally inverted or pseudogene VH is omitted from the genome.

47. A method of isolating an antibody or nucleotide sequence encoding an antibody, the method comprising

(a) immunising a vertebrate (eg, mouse or rat) as recited in preceding claim with a human target antigen such that the vertebrate produces antibodies; and

(b) isolating from the vertebrate an antibody that specifically binds to said antigen and/or isolating a nucleotide sequence encoding at least the heavy and/or the light chain variable regions of such an antibody; and

optionally subsequently joining the variable regions of said antibody to a human constant region.

48. The method of claim 47, wherein a prime and boost protocol is used for the immunisation.

49. The method of claim 47, wherein a RIMMS protocol is used for the immunisation.

50. The method of any one of claims 47 to 49, comprising after step (b) mutating the amino acid sequence of the heavy and/or the light chain variable regions of the antibody to improve affinity for binding to said antigen.

51. The method of any one of claims 47 to 50, comprising after step (b) mutating the amino acid sequence of the heavy and/or the light chain variable regions of the antibody to improve one or more biophysical characteristics of the antibody, eg, one or more of melting temperature, solution state, stability and expression (eg, in CHO or *E coli*).

52. A 4-chain antibody, a H2 antibody or an antibody fragment (eg, Fab or Fab₂) produced by the method of any one of claims 47 to 51, wherein the antibody or fragment specifically binds a human target antigen and comprises human heavy chain variable regions and a constant region; or a derivative of said antibody or fragment.
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53. An antibody produced by affinity maturing the variable regions of an antibody isolated from a vertebrate as recited in any one of claims 1 to 46, wherein the antibody specifically binds a human target antigen.
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54. An antibody produced by mutating the constant regions of an antibody isolated from a vertebrate as recited in any one of claims 1 to 46 (eg, to ablate or enhance Fc function), wherein the antibody specifically binds a human target antigen.
- 15
55. The antibody of claim 52, 53 or 54 (eg, in fully humanised form) for use as a therapeutic or prophylactic medicament.
56. Polyclonal antibodies produced in response to antigen challenge in vertebrates according to any one of claims 1 to 46.
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57. The antibodies of claim 56 (eg, in fully humanised form) for use as a therapeutic or prophylactic medicament.
- 25
58. An antibody isolated from a vertebrate as recited in any one of claims 1 to 46, or a derivative or fragment thereof, for use in medicine, wherein the antibody specifically binds a human target antigen.
59. Polyclonal antibodies isolated from vertebrates according to any one of claims 1 to 46, for use in medicine.
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60. A human variable region (eg, a domain antibody) generated from a heavy or light chain variable chain produced by the method of any one of claims 47 to 51 and which specifically binds a human target antigen.

61. A nucleotide sequence encoding an antibody, fragment, derivative or variable region according to any one of claims 52 to 60, optionally wherein the nucleotide sequence is part of a vector.

62. A pharmaceutical composition comprising

- 5 - an antibody isolated from a vertebrate as recited in any one of claims 1 to 46, or comprising a derivative of said antibody; or comprising polyclonal antibodies isolated from vertebrates of any one of claims 1 to 46 and
- a diluent, excipient or carrier;
- optionally wherein the composition is contained in an IV container (eg, and IV bag) or a
- 10 container connected to an IV syringe,

wherein the antibody(ies) specifically bind(s) a human target antigen.

63. A cell line (eg, an immortalised cell line, ES cell or iPS cell line), B-cell, hybridoma or stem cell

15 derived from a vertebrate as recited in any one of claims 1 to 46.

64. The stem cell of claim 63 wherein the cell is a BALB/c, JM8, AB2.1 or AB2.2 embryonic stem cell.

65. The cell line, cell or hybridoma of claim 63 or 64, wherein the vertebrate is a mouse BALB/c,

20 C57BL/6N, C57BL/6J, 129S5 or 129Sv strain.

CLAIMS

1. A method of making a mouse cell or a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

the mouse having a genome that

5 (a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and

(b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

10 (c) constructing a transgenic mouse embryonic stem cell comprising a transgenic antibody heavy chain locus by inserting one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region, optionally Cmu and/or Cgamma;

15 (d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes;

20 (e) simultaneously or separately from step (c) or (d), inserting into the embryonic stem cell genome one or more expressible ADAM6 genes in order to restore fertility; and optionally

25 (f) developing the embryonic stem cell into a fertile mouse or a progeny thereof whose genome is homozygous for the transgenic heavy chain locus and encodes ADAM6, in which all or part of the endogenous heavy chain VDJ region has been deleted from both chromosomes 12 in the genome; optionally in which the fertile mouse or progeny is male;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the mouse comprises a human target antigen; or

(iii) the mouse or mouse cell has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 ES line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac.

2. A method of making a mouse cell or a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

the mouse having a genome that

(a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and

(b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

(c) constructing a transgenic mouse embryonic stem cell comprising a transgenic antibody heavy chain locus by inserting one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region, optionally Cmu and/or Cgamma;

(d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes;

(e) developing the embryonic stem cell into a child mouse or progeny thereof whose genome comprises a the transgenic heavy chain locus;

(f) deriving a second embryonic stem cell from the mouse and inserting into the genome of the second embryonic stem cell one or more expressible ADAM6 genes in order to restore fertility; and optionally

(g) developing the second embryonic stem cell into a fertile mouse or a progeny thereof whose genome is homozygous for the transgenic heavy chain locus and encodes ADAM6, in which all or part of the endogenous heavy chain VDJ region

has been deleted from both chromosomes 12 in the genome; optionally in which the fertile mouse or progeny is male;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the mouse comprises a human target antigen; or

(iii) the mouse or mouse cell has a genetic background selected from 129, BALB/c, C57BL/6N, C57BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 ES line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac.

3. A method of making a fertile mouse that is homozygous for a transgenic antibody heavy chain locus,

the mouse having a genome that

(a) comprises each transgenic heavy chain locus on a respective copy of chromosome 12; and

(b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

(c) constructing a transgenic mouse embryonic stem cell comprising a transgenic antibody heavy chain locus by inserting one or more human V H gene segments, one or more human D gene segments and one or more human J H gene segments into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region, optionally C μ and/or C γ ;

(d) simultaneously or separately from step (c), deleting all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes;

(e) developing the embryonic stem cell into a child mouse or progeny thereof whose genome comprises a the transgenic heavy chain locus; and

(f) by breeding the child mouse or progeny and a further mouse whose genome comprises one or more expressible ADAM6 genes, developing a fertile mouse or a progeny thereof whose genome is homozygous for the transgenic heavy chain locus and encodes expressible ADAM6, in which all or part of the endogenous heavy chain VDJ region has been deleted from both chromosomes 12 in the genome; optionally in which the fertile mouse or progeny is male;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the mouse comprises a human target antigen; or

(iii) the mouse has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 ES line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac.

4. A method of making a fertile rodent that is homozygous for a transgenic antibody heavy chain locus,

the rodent having a genome that

(a) comprises each transgenic heavy chain locus on a respective copy of mouse chromosome 12 or equivalent chromosome for the rodent; and

(b) is inactivated for endogenous antibody heavy chain expression;

the method comprising the steps of

(c) constructing a transgenic embryonic stem cell comprising a transgenic antibody heavy chain locus by inserting one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments into DNA of the chromosome so that the human gene segments are operably connected upstream of a rodent heavy chain constant region, optionally C μ and/or C γ ;

(d) simultaneously or separately from step (c), deleting all or part of the rodent endogenous heavy chain VDJ region of the chromosome to inactivate endogenous antibody heavy chain expression, in which the deletion includes ADAM6 gene(s);

(e) simultaneously or separately from step (c) or (d), inserting into the embryonic stem cell genome one or more expressible ADAM6 genes in order to restore fertility; and

(f) developing the embryonic stem cell into a fertile rodent or a progeny thereof whose genome is homozygous for the transgenic heavy chain locus and encodes ADAM6, in which all or part of the endogenous heavy chain VDJ region has been deleted from both of the chromosomes in the genome; optionally in which the fertile rodent or progeny is male;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the rodent comprises a human target antigen; or

(iii) the rodent is a mouse that has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.

5. Use of an ADAM6 gene in the manufacture of a mouse for producing a fertile male mouse,

in which the mouse has a genome that comprises

(a) a transgenic heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments inserted into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region, optionally C μ and/or C γ ; and

(b) a deletion of all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes; and

in which one or more expressible ADAM6 genes are inserting into the genome;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the mouse comprises a human target antigen; or

(iii) the mouse has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.

6. Use of an ADAM6 gene in the manufacture of a mouse cell for producing a cell that is capable of developing into such fertile male mouse;

in which the mouse cell has a genome that comprises

(a) a transgenic heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments inserted into DNA of a chromosome 12 so that the human gene segments are operably connected upstream of a mouse or human heavy chain constant region, optionally C μ and/or C γ ; and

(b) a deletion of all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes; and in which one or more expressible ADAM6 genes are inserting into the genome;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the mouse comprises a human target antigen; or

(iii) the mouse or mouse cell has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.

7. A method or use according to any preceding claim, in which an expressible ADAM6 gene is inserted on one or both chromosomes 12 of a mouse.

8. A method or use according to any preceding claim, in which the human gene segments are inserted into mouse chromosome 12 to replace all or part of the endogenous heavy chain VDJ region, so that insertion of the human gene segments and deletion of the endogenous VDJ DNA take place simultaneously; optionally in which the entire endogenous VDJ region is replaced.

9. A method or use according to any preceding claim, in which mouse ADAM6a and ADAM6b genes or a rat ADAM6 gene are inserted, such that the final fertile mouse can express both ADAM6a and ADAM6b proteins or a rat ADAM6 protein.

10. A method or use according to any preceding claim, in which the genome of the final fertile mouse or progeny is homozygous for each inserted ADAM6 gene; optionally in which the genome comprises more than two copies of mouse ADAM6a and/or ADAM6b genes.

11. A method or use according to any preceding claim, with the alternative that each organism is a rat instead of a mouse and each chromosome is a chromosome 6 instead of chromosome 12; or with the alternative that each cell is a rat cell instead of a mouse cell and each chromosome is a chromosome 6 instead of chromosome 12; in which, option (iii) of Claims 1 to 6 does not apply.

12. A fertile mouse, optionally male, that is homozygous for a transgenic antibody heavy chain locus, the mouse having a genome that

(a) comprises a transgenic heavy chain locus on a chromosome 12; and

(b) is inactivated for endogenous antibody heavy chain expression;

in which the chromosome 12 of the genome comprises

(c) a transgenic antibody heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments operably connected upstream of a mouse or human heavy chain constant region, optionally Cmu and/or Cgamma;

(d) a deletion of all or part of the mouse endogenous heavy chain VDJ region of the chromosome 12 to inactivate endogenous antibody heavy chain expression, in which the deletion includes mouse ADAM6 genes; and

in which the genome comprises

(e) an insertion of one or more expressible ADAM6 genes;

in which

(i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

5 (ii) the mouse comprises a human target antigen; or

(iii) the mouse has a genetic background selected from 129, BALB/c, C57BL/6N, C57/BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.

10 13. A fertile rat, optionally male, that is homozygous for a transgenic antibody heavy chain locus, the rat having a genome that

(a) comprises a transgenic heavy chain locus on a chromosome 6; and

(b) is inactivated for endogenous antibody heavy chain expression;

in which the chromosome 6 of the genome comprises

15 (c) a transgenic antibody heavy chain locus comprising one or more human V H gene segments, one or more human D gene segments and one or more human J H gene segments operably connected upstream of a rat or human heavy chain constant region, optionally C μ and/or C γ ;

20 (d) a deletion of all or part of the rat endogenous heavy chain VDJ region of the chromosome 6 to inactivate endogenous antibody heavy chain expression, in which the deletion includes rat ADAM6; and

in which the genome comprises

(e) an insertion of one or more expressible ADAM6 genes;

optionally in which

25 (i) the genome comprises a lambda transgene comprising a human E λ enhancer and/or a kappa transgene comprising a human E κ enhancer; or

(ii) the rat comprises a human target antigen;

14. A mouse according to Claim 12, in which the genome comprises a transgenic antibody heavy chain locus on a respective copy of chromosome 12; or the rat according to Claim 13, in which the genome comprises a transgenic antibody heavy chain locus on a respective copy of chromosome 6.

5 15. A fertile rodent, optionally male, that is homozygous for a transgenic antibody heavy chain locus, the rodent having a genome that

(a) comprises each heavy chain locus on a respective copy of a first chromosome;
and

(b) is inactivated for endogenous antibody heavy chain expression;

10 in which each first chromosome of the genome comprises

(c) a transgenic antibody heavy chain locus comprising one or more human VH gene segments, one or more human D gene segments and one or more human JH gene segments operably connected upstream of a rodent heavy chain constant region, optionally Cmu and/or Cgamma;

15 (d) a deletion of all or part of the endogenous heavy chain VDJ region of the chromosome to inactivate endogenous antibody heavy chain expression, in which the deletion includes ADAM6; and

in which the genome comprises

(e) an insertion of one or more expressible ADAM6 genes;

20 in which

(i) the genome comprises a lambda transgene comprising a human Eλ enhancer and/or a kappa transgene comprising a human EK enhancer; or

(ii) the rodent comprises a human target antigen; or

25 (iii) the rodent is a mouse that has a genetic background selected from 129, BALB/c, C57BL/6N, C57BL/6J, JM8, AB2.1, AB2.2, 129S5 or 129Sv, in which the genetic background of the mouse is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.

16. A mouse according to Claim 12 or a rat according to Claim 13, in which each inserted ADAM6 gene is on a

(i) chromosome 12 in which the animal is a mouse; or

(ii) chromosome 6 in which the animal is a rat.

17. A method or use according to Claim 7, or a mouse, rat or rodent according to any of Claims 12 to 16, in which an ADAM6 gene is inserted

5 (i) within one or both transgenic heavy chain loci or

(ii) within 20Mb of one or both transgenic heavy chain loci.

18. A mouse, rat or rodent according to any of Claims 12 to 17, in which the human gene segments replace all or part of the endogenous VDJ region in each heavy chain locus.

19. A mouse, rat or rodent according to any of Claims 12 to 18, in which the genome
10 comprises inserted expressible mouse ADAM6a and ADAM6b genes; or in which the genome comprises an inserted expressible rat ADAM6 gene.

20. A mouse, rat or rodent according to any of Claims 12 to 19, in which the genome is homozygous for each inserted ADAM6 gene; optionally in which the genome comprises more than two copies of ADAM6 genes selected from rat ADAM6, mouse ADAM6a and mouse
15 ADAM6b genes.

21. A method, use, mouse, rat or rodent according to any preceding claim, in which the genome comprises expressible ADAM6 that is inserted from a targeting vector, for example a BAC vector.

22. A method, use, mouse, rat or rodent according to any preceding claim, in which the
20 genome comprises ADAM6 exons is inserted by targeted or random insertion into an embryonic stem cell or zygote.

23. A method, use, mouse, rat or rodent according to any preceding claim, in which the expressible ADAM6 gene(s) is a rat ADAM6 nucleotide sequence operably connected to an endogenous mouse ADAM6 promoter or a mouse ADAM6 nucleotide sequence operably
25 connected to an endogenous rat ADAM6 promoter.

24. A method, use, rodent, mouse, rat or cell according to any preceding claim, in which the genome comprises an inserted human VDJ region that includes bases 105,400,051 to 106,368,585 from human chromosome 14.

25. A method, use, rodent, mouse, rat or cell according to any preceding claim, in which the genome is homozygous at the Ig heavy chain, Ig λ and Ig κ loci.

26. A method, use, rodent, mouse, rat or cell according to any preceding claim, in which the genome comprises:

5 (a) heavy chain loci, each comprising one or more human heavy chain V gene segments, one or more human heavy chain D gene segments and one or more human heavy chain JH gene segments upstream of an endogenous rodent constant region or a human constant region, for example C μ and/or C γ ;

10 (b) a kappa light chain locus, optionally in homozygous state, comprising one or more human kappa chain V gene segments, and one or more human kappa chain J κ gene segments upstream of an endogenous rodent, for example endogenous mouse or rat, kappa constant region; and optionally

15 (c) a lambda light chain locus, optionally in homozygous state, comprising one or more human lambda chain V gene segments, and one or more human lambda chain J λ gene segments upstream of a lambda constant region; and

(d) in which the rodent, mouse or rat is capable of producing chimaeric antibodies following rearrangement of the loci and immunisation with an antigen.

27. A method, use, rodent, mouse or cell according to any preceding claim, in which the rodent is a mouse and the genome comprises an insertion of human light chain kappa VJ
20 targeted into mouse chromosome 6 between coordinates 70,673,899 and 70,675,515, suitably at position 70,674,734, or an equivalent position in the lambda mouse locus on chromosome 16.

28. A method, use, rodent, mouse, rat or cell according to any preceding claim, in which the genome comprises human DNA as a source of heavy and/or light chain gene segments from a
25 library selected from a human RPCI-11 library, RPCI-13 library, CalTech A library, CalTech B library, CalTech C library or CalTech D library.

29. A method, use, rodent or cell according to any preceding claim, in which the rodent is a mouse or rat.

30. A method, use, rodent, mouse, rat or cell according to any preceding claim, in which
30 human VH which is naturally inverted or pseudogene VH is omitted from the genome.

31. A method of isolating an antibody or nucleotide sequence encoding an antibody, the method comprising

(a) immunising a rodent, for example a mouse or rat, as recited in any preceding claim with a human target antigen such that the rodent produces antibodies; and

5 (b) isolating from the rodent an antibody that specifically binds to the antigen and/or isolating a nucleotide sequence encoding at least the heavy and/or the light chain variable regions of such an antibody; and

optionally subsequently joining the variable regions of the antibody to a human constant region.

10 32. A method according to Claim 31, in which a prime and boost protocol is used for the immunisation or in which a RIMMS protocol is used for the immunisation.

33. A method according to Claim 31 in which the antibody is formulated as a pharmaceutical composition with a diluent, excipient or carrier, and in which the composition is contained in an IV bag or a container connected to an IV syringe.

15 34. A cell line, for example an immortalised cell line, embryonic stem cell or iPS cell line, B-cell, hybridoma or stem cell derived from a rodent, mouse or rat as recited in any one of claims 1 to 30 optionally in which the stem cell is a BALB/c, JM8, AB2.1 or AB2.2 embryonic stem cell.

20 35. A cell line, cell or hybridoma according to Claim 35, in which the rodent is a mouse BALB/c, C57BL/6N, C57BL/6J, 129S5 or 129Sv strain, and in which the genetic background is not the F1 embryonic stem line F1H4 derived from 129S6/SvEvTac and C57BL/6NTac heterozygous embryos.