

CLAIMS

1. A rodent comprising in its germline genome at an endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence operably linked to an endogenous heavy chain constant region gene sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of V_H3-23/X₁X₂/J_H, wherein X₁ is any amino acid, and X₂ is any amino acid, and wherein the rodent is a rat or a mouse.
2. The rodent of claim 1, wherein X₁ is Gly and X₂ is Tyr.
3. The rodent of claim 1, wherein the human J_H gene segment is selected from the group consisting of J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, and a polymorphic variant thereof.
4. The rodent of claim 1, wherein the rearranged human immunoglobulin heavy chain variable region nucleotide sequence encodes the sequence of human V_H3-23/GY/J_H4-4 (SEQ ID NO:137).
5. The rodent of claim 4, wherein the rodent is a mouse.
6. The rodent of claim 1, wherein the heavy chain constant region gene sequence is selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.
7. The rodent of claim 1, wherein substantially all endogenous functional V_H, D, and J_H gene segments are deleted from the immunoglobulin heavy chain locus of the rodent or rendered non-functional.
8. The rodent of claim 1, wherein the rodent comprises a modification that deletes or renders non-functional endogenous functional V_H, D, and J_H gene segments.
9. The rodent of claim 1, wherein an immunoglobulin heavy chain variable domain encoded

by the rearranged heavy chain variable region nucleotide sequence is not immunogenic to the non-human animal.

10. The rodent of claim 1, wherein the rodent comprises an Adam6a gene, an Adam6b gene, or both.

11. The rodent of claim 1, wherein the rodent further comprises a nucleotide sequence encoding an unrearranged human immunoglobulin light chain V gene segment (V_L) and an unrearranged human immunoglobulin light chain J gene segment (J_L), wherein the nucleotide sequence is operably linked to an immunoglobulin light chain constant region gene sequence.

12. The rodent of claim 11, wherein the light chain constant region gene sequence is selected from a rodent and a human constant region gene sequence.

13. The rodent of claim 12, wherein the rodent is selected from a mouse, a rat, and a hamster.

14. The rodent of claim 11, wherein the unrearranged human immunoglobulin light chain (V_L) gene segment and the unrearranged human immunoglobulin (J_L) gene segment are operably linked, at an endogenous rodent locus, to a rodent immunoglobulin constant region gene sequence.

15. A method of making a rodent, the method comprising:

(a) modifying a genome of a rodent to delete or render non-functional substantially all endogenous functional immunoglobulin heavy chain V_H , D, and J_H gene segments of an endogenous immunoglobulin heavy chain locus, wherein the rodent is a rat or a mouse; and

(b) placing in the genome at the endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of $V_{H3-23}/X_1X_2/J_H$ operably linked to an endogenous heavy chain constant region gene sequence,

wherein X_1 is any amino acid, and X_2 is any amino acid.

16. A modified rodent comprising in its genome:

(i) at an endogenous immunoglobulin heavy chain locus, a rearranged human heavy chain variable region nucleic acid sequence operably linked to an endogenous heavy chain constant region nucleic acid sequence, wherein the rearranged heavy chain variable region nucleic acid sequence encodes the sequence of $V_H3-23/X_1X_2/J_H$, wherein X_1 is any amino acid, and X_2 is any amino acid, wherein the rodent is a rat or a mouse; and

(ii) an immunoglobulin light chain locus comprising one or more but less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments, operably linked to a light chain constant region nucleic acid sequence.

17. The modified rodent of claim 16, wherein X_1 is Gly and X_2 is Tyr.

18. The modified rodent of claim 16, wherein the human J_H gene segment is selected from the group consisting of J_H1 , J_H2 , J_H3 , J_H4 , J_H5 , J_H6 , and a polymorphic variant thereof.

19. The modified rodent of claim 16, wherein the rearranged human immunoglobulin heavy chain variable region nucleotide sequence encodes the sequence of human $V_H3-23/GY/J_H4-4$ (SEQ ID NO:137).

20. The modified rodent of claim 16, wherein the one or more but less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments are operably linked to the light chain constant region nucleic acid sequence at an endogenous locus.

21. The modified rodent of claim 16, wherein the immunoglobulin light chain locus comprises two human V_L gene segments, $V_{\kappa1}-39$ and $V_{\kappa3}-20$.

22. The modified rodent of claim 16, wherein at least one of the human light chain V_L or J_L gene segments encode one or more histidine codons that are not encoded by a corresponding human germline light chain variable gene segment.

23. The modified rodent of claim 16, wherein at least one of the V_L gene segments comprises an addition or substitution of at least one non-histidine codon encoded by the corresponding human germline V_L segment sequence with a histidine codon.

24. The modified rodent of claim 23, wherein the added or substituted histidine codon is present in CDR3.

25. The mouse claim 5, wherein the mouse comprises an Adam6a gene, an Adam6b gene, or both.

26. The mouse of claim 5, wherein the non-human animal further comprises a nucleotide sequence encoding an unrearranged human immunoglobulin light chain V gene segment (V_L) and an unrearranged human immunoglobulin light chain J gene segment (J_L), wherein the nucleotide sequence is operably linked to an immunoglobulin light chain constant region gene sequence.

27. The rodent of claim 16, wherein the rodent is a mouse.

28. A method of producing a nucleic acid that encodes a human immunoglobulin light chain variable domain comprising:

obtaining a nucleic acid comprising a sequence encoding the human immunoglobulin light chain variable domain from a lymphocyte of a rodent, or a hybridoma produced from the lymphocyte;

wherein the rodent comprises in its germline genome

(i) at an endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence operably linked to an endogenous heavy chain constant region gene sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of $V_{H3-23}/X_1X_2/J_H$, wherein X_1 is any amino acid, and X_2 is any amino acid, and

(ii) an immunoglobulin light chain locus comprising at least one unrearranged human immunoglobulin light chain variable gene segment (V_L) and at least one unrearranged human immunoglobulin light chain J gene segment (J_L) operably linked to an immunoglobulin light chain constant region gene sequence;

wherein the lymphocyte expresses an immunoglobulin heavy chain variable domain that is (a) derived from the rearranged human immunoglobulin heavy chain variable region $V_{H3-23}/X_1X_2/J_H$ gene linked to the endogenous heavy chain constant region gene sequence and (b) cognate with the human immunoglobulin light chain variable domain.

29. The method of claim 28, further comprising immunizing the rodent with an antigen of interest and allowing the rodent to mount an immune response to the antigen before obtaining the nucleic acid.

30. The method of claim 2, wherein the lymphocyte expresses an antigen-binding protein that specifically binds the antigen of interest.

31. The method of claim 30, wherein the amplified rearranged human immunoglobulin light chain variable region gene sequence comprises at least one somatic hypermutation.

32. The method of claim 28, wherein X_1 is Gly and X_2 is Tyr.

33. The method of claim 28, wherein the human J_H gene segment is selected from the group consisting of J_{H1} , J_{H2} , J_{H3} , J_{H4} , J_{H5} , J_{H6} , and a polymorphic variant thereof.

34. The method of claim 28, wherein the rearranged human immunoglobulin heavy chain variable region gene sequence encodes the sequence of $V_{H3-23}/GY/J_{H4-4}$ (SEQ ID NO: 137).

35. The method of claim 28, wherein the heavy chain constant region gene sequence is selected from a C_{H1} , a hinge, a C_{H2} , a C_{H3} , and a combination thereof.

36. The method of claim 28, wherein substantially all endogenous functional V_H, D, and J_H gene segments are deleted from the immunoglobulin heavy chain locus of the rodent or rendered non-functional.
37. The method of claim 28, wherein the light chain constant region gene sequence is selected from a rodent and a human constant region gene sequence.
38. The method of claim 37, wherein the at least one unrearranged human immunoglobulin V_L gene segment and the at least one unrearranged human immunoglobulin J_L gene segment are operably linked, at an endogenous locus, to a rodent immunoglobulin constant region gene sequence.
39. The method of claim 28, wherein the immunoglobulin light chain locus comprises less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments.
40. The method of claim 39, wherein the less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments are operably linked to a rodent light chain constant region nucleic acid sequence at an endogenous locus.
41. The method of claim 40, wherein at least one of the human light chain V_L or J_L gene segments encode one or more histidine codons that are not encoded by a corresponding human germline light chain variable gene segment.
42. The method of claim 41, wherein the added or substituted histidine codon is present in CDR3.
43. The method of claim 40, wherein the immunoglobulin light chain locus comprises two unrearranged human V_k gene segment sequences and one or more unrearranged J_k gene segment sequences.
44. The method of claim 43, wherein the two unrearranged human V_k gene segments are human V_k1-39 and V_k3-20 gene segments each comprising one or more substitutions of a non-histidine codon with a histidine codon.

45. The method of claim 44, wherein the human Vk and Jk gene segments are capable of rearranging and encoding a human light chain variable domain comprising one or more histidines at a position selected from the group consisting of 105, 106, 107, 108, 109, 111 (according to IGMT numbering), and a combination thereof, wherein the one or more histidines are derived from the one or more substitutions.

46. The method of claim 28, wherein the lymphocyte is a B cell.

47. The method of claim 28, wherein the rodent selected from the group consisting of a hamster, a rat, and a mouse.

48. The method of claim 47, wherein the rodent is a mouse and the mouse comprises an Adam6a gene, an Adam 6b gene, or both.

49. The method of claim 28, wherein the light chain constant region gene sequence is a non-human animal constant region gene sequence, and the method further comprises cloning the nucleic acid in frame with a nucleotide comprising a sequence encoding a suitable human constant region nucleic acid sequence.

50. A nucleic acid produced according to the method of any one of claims 28-49.

51. A host cell comprising the nucleic acid of claim 50.

52. The host cell of claim 51, wherein the host cell is selected from a CHO cell, a CHO K1 cell, a DXB-11 CHO cell, a Veggie-CHO cell, a COS-7 cell, a retinal cell, a Vero cell, a CV1 cell, a kidney cell, an HEK293 cell, a 293 EBNA cell, a MSR 293 cell, a MDCK cell, a HaK cell, a BHK cell, a HeLa cell, a HepG2 cell, a WI38 cell, a MRC 5 cell, Colo205 cell, an HB 8065 cell, an HL-60 cell, a BHK21 cell, a Jurkat cell, a Daudi cell, a A431 epidermal cell, a CV-1 cell, a U937 cell, a 3T3 cell, an L cell, a C127 cell, a SP2/0 cell, a NS-0 cell, a MMT 060562 cell, a Sertoli cell, a BRL 3A cell, an HT1080 cell, a myeloma cell, and a tumor cell.

53. A method of obtaining a cell comprising a human immunoglobulin light chain variable domain:

isolating a lymphocyte from a rodent that comprises in its germline genome

(i) at an endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence operably linked to an endogenous heavy chain constant region gene sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of $V_{H3-23}/X_1X_2/J_H$, wherein X_1 is any amino acid, and X_2 is any amino acid, and

(ii) an immunoglobulin light chain locus comprising at least one unrearranged human immunoglobulin light chain variable gene segment (V_L) and at least one unrearranged human immunoglobulin light chain J gene segment (J_L) operably linked to an immunoglobulin light chain constant region gene sequence;

wherein the lymphocyte expresses an immunoglobulin heavy chain variable domain that is (a) derived from the rearranged human immunoglobulin heavy chain variable region $V_{H3-23}/X_1X_2/J_H$ gene linked to the endogenous heavy chain constant region gene sequence and (b) cognate with the human immunoglobulin light chain variable domain.

54. The method of claim 53, further comprising producing a hybridoma from the isolated lymphocyte.

55. A cell obtained by the method of any one of claims 53-54.

56. A method of making a human immunoglobulin light chain variable domain comprising culturing the cell obtained according to any one of claims 53-54 or the cell of any one of claims 51 and 55.

57. The method of claim 56, wherein the cell comprises a nucleic acid encoding a human immunoglobulin light chain variable domain that is cognate to an immunoglobulin heavy chain variable domain derived from a rearranged human immunoglobulin heavy chain variable region $V_{H3-23}/X_1X_2/J_H$ gene linked to a heavy chain constant region gene sequence.

58. The method of claim 56, wherein the cell further comprises a second nucleic acid that encodes a human immunoglobulin heavy chain variable domain that can pair with the human immunoglobulin light chain variable domain.

CLAIMS

1. A method of producing a nucleic acid that encodes a human immunoglobulin light chain variable domain comprising:

obtaining a nucleic acid comprising a sequence encoding the human immunoglobulin light chain variable domain from a lymphocyte of a rodent, or a hybridoma produced from the lymphocyte;

wherein the rodent comprises in its germline genome

(i) at an endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence operably linked to an endogenous heavy chain constant region gene sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of $V_H3-23/X_1X_2/J_H$, wherein X_1 is any amino acid, and X_2 is any amino acid, and

(ii) an immunoglobulin light chain locus comprising at least one unrearranged human immunoglobulin light chain variable gene segment (V_L) and at least one unrearranged human immunoglobulin light chain J gene segment (J_L) operably linked to an immunoglobulin light chain constant region gene sequence;

wherein the lymphocyte expresses an immunoglobulin heavy chain variable domain that is (a) derived from the rearranged human immunoglobulin heavy chain variable region $V_H3-23/X_1X_2/J_H$ gene linked to the endogenous heavy chain constant region gene sequence and (b) cognate with the human immunoglobulin light chain variable domain.

2. The method of claim 1, further comprising immunizing the rodent with an antigen of interest and allowing the rodent to mount an immune response to the antigen before obtaining the nucleic acid.

3. The method of claim 2, wherein the lymphocyte expresses an antigen-binding protein that specifically binds the antigen of interest.

4. The method of claim 3, wherein the amplified rearranged human immunoglobulin light chain variable region gene sequence comprises at least one somatic hypermutation.
5. The method of claim 1, wherein X_1 is Gly and X_2 is Tyr.
6. The method of claim 1, wherein the human J_H gene segment is selected from the group consisting of J_{H1} , J_{H2} , J_{H3} , J_{H4} , J_{H5} , J_{H6} , and a polymorphic variant thereof.
7. The method of claim 1, wherein the rearranged human immunoglobulin heavy chain variable region gene sequence encodes the sequence of $V_{H3-23}/GY/J_{H4-4}$ (SEQ ID NO: 137).
8. The method of claim 1, wherein the heavy chain constant region gene sequence is selected from a C_{H1} , a hinge, a C_{H2} , a C_{H3} , and a combination thereof.
9. The method of claim 1, wherein substantially all endogenous functional V_H , D, and J_H gene segments are deleted from the immunoglobulin heavy chain locus of the rodent or rendered non-functional.
10. The method of claim 1, wherein the light chain constant region gene sequence is selected from a rodent and a human constant region gene sequence.
11. The method of claim 10, wherein the at least one unrearranged human immunoglobulin V_L gene segment and the at least one unrearranged human immunoglobulin J_L gene segment are operably linked, at an endogenous locus, to a rodent immunoglobulin constant region gene sequence.
12. The method of claim 1, wherein the immunoglobulin light chain locus comprises less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments.
13. The method of claim 12, wherein the less than the wild type number of human immunoglobulin light chain V_L and J_L gene segments are operably linked to a rodent light chain constant region nucleic acid sequence at an endogenous locus.

14. The method of claim 13, wherein at least one of the human light chain V_L or J_L gene segments encode one or more histidine codons that are not encoded by a corresponding human germline light chain variable gene segment.
15. The method of claim 14, wherein the added or substituted histidine codon is present in CDR3.
16. The method of claim 13, wherein the immunoglobulin light chain locus comprises two unrearranged human Vk gene segment sequences and one or more unrearranged Jk gene segment sequences.
17. The method of claim 16, wherein the two unrearranged human Vk gene segments are human Vk1-39 and Vk3-20 gene segments each comprising one or more substitutions of a non-histidine codon with a histidine codon.
18. The method of claim 17, wherein the human Vk and Jk gene segments are capable of rearranging and encoding a human light chain variable domain comprising one or more histidines at a position selected from the group consisting of 105, 106, 107, 108, 109, 111 (according to IGMT numbering), and a combination thereof, wherein the one or more histidines are derived from the one or more substitutions.
19. The method of claim 1, wherein the lymphocyte is a B cell.
20. The method of claim 1, wherein the rodent selected from the group consisting of a hamster, a rat, and a mouse.
21. The method of claim 20, wherein the rodent is a mouse and the mouse comprises an Adam6a gene, an Adam 6b gene, or both.
22. The method of claim 1, wherein the light chain constant region gene sequence is a non-human animal constant region gene sequence, and the method further comprises cloning the nucleic acid in frame with a nucleotide comprising a sequence encoding a suitable human constant region nucleic acid sequence.

23. A nucleic acid produced according to the method of any one of claims 1-22.
24. A host cell comprising the nucleic acid of claim 23.
25. The host cell of claim 24, wherein the host cell is selected from a CHO cell, a CHO K1 cell, a DXB-11 CHO cell, a Veggie-CHO cell, a COS-7 cell, a retinal cell, a Vero cell, a CV1 cell, a kidney cell, an HEK293 cell, a 293 EBNA cell, a MSR 293 cell, a MDCK cell, a HaK cell, a BHK cell, a HeLa cell, a HepG2 cell, a WI38 cell, a MRC 5 cell, Colo205 cell, an HB 8065 cell, an HL-60 cell, a BHK21 cell, a Jurkat cell, a Daudi cell, a A431 epidermal cell, a CV-1 cell, a U937 cell, a 3T3 cell, an L cell, a C127 cell, a SP2/0 cell, a NS-0 cell, a MMT 060562 cell, a Sertoli cell, a BRL 3A cell, an HT1080 cell, a myeloma cell, and a tumor cell.
26. A method of obtaining a cell comprising a human immunoglobulin light chain variable domain:
- isolating a lymphocyte from a rodent that comprises in its germline genome
- (i) at an endogenous immunoglobulin heavy chain locus a rearranged human immunoglobulin heavy chain variable region nucleotide sequence operably linked to an endogenous heavy chain constant region gene sequence, wherein the rearranged heavy chain variable region nucleotide sequence encodes the sequence of $V_{H3-23}/X_1X_2/J_H$, wherein X_1 is any amino acid, and X_2 is any amino acid, and
- (ii) an immunoglobulin light chain locus comprising at least one unrearranged human immunoglobulin light chain variable gene segment (V_L) and at least one unrearranged human immunoglobulin light chain J gene segment (J_L) operably linked to an immunoglobulin light chain constant region gene sequence;
- wherein the lymphocyte expresses an immunoglobulin heavy chain variable domain that is (a) derived from the rearranged human immunoglobulin heavy chain variable region $V_{H3-23}/X_1X_2/J_H$ gene linked to the endogenous heavy chain constant region gene sequence and (b) cognate with the human immunoglobulin light chain variable domain.

27. The method of claim 26, further comprising producing a hybridoma from the isolated lymphocyte.
28. A cell obtained by the method of any one of claims 26-27.
29. A method of making a human immunoglobulin light chain variable domain comprising culturing the cell obtained according to any one of claims 26-27 or the cell of any one of claims 24 and 25.
30. The method of claim 29, wherein the cell comprises a nucleic acid encoding a human immunoglobulin light chain variable domain that is cognate to an immunoglobulin heavy chain variable domain derived from a rearranged human immunoglobulin heavy chain variable region $V_H3-23/X_1X_2/J_H$ gene linked to a heavy chain constant region gene sequence.
31. The method of claim 29, wherein the cell further comprises a second nucleic acid that encodes a human immunoglobulin heavy chain variable domain that can pair with the human immunoglobulin light chain variable domain.