

What is claimed is:

1.- A ~~mouse~~ method for making an antibody that binds an antigen of interest comprising:

— (a) immunizing a mouse with an antigen of interest, wherein the mouse comprises in its germline genome:

(i) two un rearranged human immunoglobulin ~~V_L~~V_K gene segments and ~~two or more~~ five un rearranged human immunoglobulin ~~J_L~~J_K gene segments operably linked to an immunoglobulin light chain constant region sequence, wherein the two un rearranged human immunoglobulin ~~V_L~~V_K gene segments are a human V_K1-39 gene segment and a human V_K3-20 gene segment, and ~~the two or more human~~ wherein the five un rearranged human J_K gene segments are a human J_K1 gene segment, a human J_K2 gene segment, a human J_K3 gene segment, a human J_K4 gene segment, and a human J_K5 gene segment; and

(ii) one or more un rearranged human V_H gene segments, one or more un rearranged human D_H gene segments, and one or more un rearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the un rearranged human heavy chain gene segments and un rearranged human kappa light chain gene segments of the mouse are capable of rearranging to generate immunoglobulin heavy chain variable region sequences and immunoglobulin kappa light chain variable region sequences, respectively, and wherein the mouse does not comprise an endogenous V_K gene segment that is capable of rearranging to form a light chain variable region sequence, and

(b) obtaining an immunoglobulin heavy chain and/or kappa light chain variable region sequence that encodes an immunoglobulin heavy chain variable domain and/or an immunoglobulin ~~J_L~~ gene segments are selected from the group consisting of a human J_K1, J_K2, J_K3, J_K4, and J_K5; and light chain variable domain, respectively, of an antibody that specifically binds the antigen of interest.

— ~~one or more human~~ (c) employing the immunoglobulin heavy chain and/or kappa light chain variable region sequence to produce an antibody that binds the antigen.

2. A method for making an antibody that binds an antigen of interest comprising the steps of:

(a) immunizing a mouse with an antigen of interest, wherein the mouse comprises in its

germline genome:

(i) two unrearranged human V_k gene segments and five unrearranged human J_k gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human V_k gene segments are a human V_k1-39 gene segment and a human V_k3-20 gene segment, and wherein the five unrearranged human J_k gene segments are a human J_k1 gene segment, a human J_k2 gene segment, a human J_k3 gene segment, a human J_k4 gene segment, and a human J_k5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human immunoglobulin- D_H gene segments, and one or more unrearranged human immunoglobulin- J_H gene segments operably linked to a non-human mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging to generate immunoglobulin heavy chain variable domains and immunoglobulin kappa light chain variable domains, respectively;

wherein the mouse generates antibodies when immunized with the antigen, wherein the antibodies each comprise a human heavy chain variable domain operably linked to a mouse heavy chain constant domain and a human light chain variable domain operably linked to a light chain constant domain; and

wherein the mouse does not comprise an endogenous V_k gene segment that is capable of rearranging to form a light chain variable region sequence; and

(b) determining a human heavy chain variable domain sequence of an antibody that specifically binds the antigen and that was generated by the genetically modified mouse and/or determining a human kappa light chain variable domain sequence of an antibody that specifically binds the antigen and that was generated by the genetically modified mouse; and

(c) employing the immunoglobulin constant region; heavy chain and/or kappa light chain variable domain sequence to produce an antibody that binds the antigen.

3. The method of claim 1, wherein employing the human heavy and/or kappa light chain variable region sequence comprises employing the human heavy chain variable region sequence of (b) operably linked to a human heavy chain constant region sequence and/or employing the human kappa light chain variable region sequence of (b) operably linked to a human light chain constant region sequence.

4. The method of claim 3, wherein employing the human heavy chain variable region sequence of (b) operably linked to a human heavy chain constant region sequence comprises expressing the human heavy chain variable region sequence of (b) and the human heavy chain constant region sequence.

5. The method of claim 3, wherein employing the human kappa light chain variable region sequence of (b) operably linked to a human light chain constant region sequence comprises expressing the human kappa light chain variable region sequence of (b) and the human light chain constant region sequence.

6. The method of claim 2, wherein employing the human heavy and/or kappa light chain variable domain sequence comprises employing the human heavy chain variable domain sequence of (b) operably linked to a human heavy chain constant domain sequence and/or employing the human kappa light chain variable domain sequence of (b) operably linked to a human light chain constant domain sequence.

7. The method of claim 2 or 6, wherein employing the human heavy chain variable domain sequence of (b) operably linked to a human heavy chain constant domain sequence comprises expressing a nucleotide sequence that encodes the human heavy chain variable domain sequence of (b) and the human heavy chain constant domain sequence.

8. The method of claim 2 or 6, wherein employing the human kappa light chain variable domain sequence of (b) operably linked to a human light chain constant domain sequence comprises expressing a nucleotide sequence that encodes the human kappa light chain variable domain sequence of (b) and the human light chain constant domain sequence.

9. The method of claim 1 or claim 2, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are operably linked to a mouse light chain constant region sequence.

10. The method of claim 9, wherein the mouse light chain constant region sequence is a mouse C κ region sequence.

11. The method of claim 9, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin light chain locus.

12. The method of claim 11, wherein the germline genome of the mouse comprises in order: the human V κ 1-39 gene segment, the human V κ 3-20 gene segment, the human J κ 1 gene segment, the human J κ 2 gene segment, the human J κ 3 gene segment, the human J κ 4 gene segment, and the human J κ 5 gene segment.

13. The method of claim 1 or claim 2, wherein the one or more unrearranged human V $_H$ gene segments, one or more unrearranged human D $_H$ gene segments, and one or more unrearranged human J $_H$ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin heavy chain locus.

14. The method of claim 13, wherein the mouse does not comprise an endogenous mouse immunoglobulin V $_H$ gene segment that is capable of rearranging to form an immunoglobulin heavy chain variable region.

15. The method of claim 1 or claim 2, wherein the mouse comprises a nonfunctional immunoglobulin λ light chain locus.

16. ~~wherein the human gene segments.~~ A method for making an antibody comprising:

(1) expressing in a single cell:

(a) a first nucleic acid sequence that encodes a first immunoglobulin heavy chain, the first nucleic acid sequence comprising a first human heavy chain variable region sequence operably linked to a human heavy chain constant region sequence, wherein the first human heavy chain variable region sequence was obtained from a B cell of a mouse, wherein the mouse has been immunized with an antigen of interest including an epitope and the mouse comprises in its germline genome:

(i) two unrearranged human V κ gene segments and five unrearranged human J κ gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human V κ gene segments are a human V κ 1-39 gene segment and a human V κ 3-20 gene segment, and wherein

the five unrearranged human J κ gene segments are a human J κ 1 gene segment, a human J κ 2 gene segment, a human J κ 3 gene segment, a human J κ 4 gene segment, and a human J κ 5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging and encoding human heavy chain variable domains and human kappa light chain variable domains of an antibody, respectively;

wherein the mouse does not comprise an endogenous V κ gene segment that is capable of rearranging to form a light chain variable region sequence; and

wherein the first human heavy chain variable region sequence encodes a first human heavy chain variable domain that, with a kappa light chain variable domain, recognizes the epitope; and

(b) a second nucleic acid sequence that encodes an immunoglobulin light chain, the second nucleic acid sequence comprising a human kappa light chain variable region sequence operably linked to a human light chain constant region sequence, wherein the human kappa light chain variable region sequence comprises: a human V κ 1-39 gene segment, a human V κ 3-20 gene segment, or a somatically hypermutated version thereof;

(2) maintaining the cell under conditions sufficient to express a fully human antibody; and

(3) isolating the antibody.

17. The method of claim 16, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are operably linked to a mouse light chain constant region sequence.

18. The method of claim 17, wherein the mouse light chain constant region sequence of the mouse is a mouse C κ region sequence.

19. The method of claim 17, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin light chain locus.

20. The method of claim 19, wherein the germline genome of the mouse comprises in order: the human V κ 1-39 gene segment, the human V κ 3-20 gene segment, the human J κ 1 gene segment, the human J κ 2 gene segment, the human J κ 3 gene segment, the human J κ 4 gene segment, and the human J κ 5 gene segment.

21. The method of claim 16, wherein the one or more unrearranged human V $_H$ gene segments, one or more unrearranged human D $_H$ gene segments, and one or more unrearranged human J $_H$ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin heavy chain locus.

22. The method of claim 21, wherein the mouse does not comprise an endogenous mouse V $_H$ gene segment that is capable of rearranging to form a heavy chain variable region.

23. The method of claim 16, wherein the mouse comprises a nonfunctional immunoglobulin λ light chain locus.

24. The method of claim 16, wherein the second nucleic acid sequence comprises a hV κ /hJ κ /mC κ junction sequence selected from SEQ ID NOs: 38-49.

25. The method of claim 16, wherein the antibody made by the method is a bispecific antibody.

26. A method for generating a human heavy and/or kappa light chain variable region sequence comprising:

(a) immunizing a genetically modified mouse with an antigen, wherein the mouse generates antibodies when immunized with the antigen, and wherein the mouse has a germline genome that comprises:

(i) two unrearranged human V κ gene segments and five unrearranged human J κ gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human V κ gene segments are a human V κ 1-39 gene segment

and a human Vk3-20 gene segment, and wherein the five unrearranged human Jk gene segments are a human Jk1 gene segment, a human Jk2 gene segment, a human Jk3 gene segment, a human Jk4 gene segment, and a human Jk5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging and encoding human heavy chain variable domains and human kappa light chain variable domains of an antibody, respectively, and wherein the mouse does not comprise an endogenous Vk gene segment that is capable of rearranging to form a light chain variable region sequence, and

(b) determining a human heavy and/or kappa light chain variable region sequence that encodes a human heavy and/or kappa light chain variable domain of an antibody that specifically binds the antigen and that was generated by the genetically modified mouse.

27. A method for generating a human heavy and/or kappa light chain variable domain sequence comprising:

(a) immunizing a genetically modified mouse with an antigen, wherein the mouse generates antibodies when immunized with the antigen, and wherein the mouse has a germline genome that comprises:

(i) two unrearranged human Vk gene segments and five unrearranged human Jk gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human Vk gene segments are a human Vk1-39 gene segment and a human Vk3-20 gene segment, and wherein the five unrearranged human Jk gene segments are a human Jk1 gene segment, a human Jk2 gene segment, a human Jk3 gene segment, a human Jk4 gene segment, and a human Jk5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging and encoding human heavy chain variable domains and human kappa light chain variable

domains of an antibody, respectively, and wherein the mouse does not comprise an endogenous V κ gene segment that is capable of rearranging to form a light chain variable region sequence, and

(b) determining a human heavy and/or kappa light chain variable domain sequence of an antibody that specifically binds the antigen and that was generated by the genetically modified mouse.

28. The method of claim 27, wherein determining a human heavy and/or kappa light chain variable domain sequence comprises determining a nucleotide sequence that encodes the human heavy and/or kappa light chain variable domain sequence.

29. The method of claim 26 or 27, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are operably linked to a mouse light chain constant region sequence.

30. The method of claim 29, wherein the mouse light chain constant region sequence is a mouse C κ region sequence.

31. The method of claim 29, wherein the two unrearranged human V κ gene segments and five unrearranged human J κ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin light chain locus.

32. The method of claim 31, wherein the germline genome of the mouse comprises in order: the human V κ 1-39 gene segment, the human V κ 3-20 gene segment, the human J κ 1 gene segment, the human J κ 2 gene segment, the human J κ 3 gene segment, the human J κ 4 gene segment, and the human J κ 5 gene segment.

33. The method of claim 26 or 27, wherein the one or more unrearranged human V $_H$ gene segments, one or more unrearranged human D $_H$ gene segments, and one or more unrearranged human J $_H$ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin heavy chain locus.

34. The method of claim 33, wherein the mouse does not comprise an endogenous mouse

immunoglobulin V_H gene segment that is capable of rearranging to form an immunoglobulin heavy chain variable region.

35. The method of claim 26 or 27, wherein the mouse comprises a nonfunctional immunoglobulin λ light chain locus.

36. A method of making a nucleotide sequence encoding fully human heavy chain and/or a fully human kappa light chain comprising the steps of:

(a) immunizing a genetically modified mouse with an antigen, wherein the mouse has a germline genome that comprises:

(i) two unrearranged human V_k gene segments and five unrearranged human J_k gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human V_k gene segments are a human V_k1-39 gene segment and a human V_k3-20 gene segment, and wherein the five unrearranged human J_k gene segments are a human J_k1 gene segment, a human J_k2 gene segment, a human J_k3 gene segment, a human J_k4 gene segment, and a human J_k5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging and encoding human heavy chain variable domains and human kappa light chain variable domains of an antibody, respectively, and wherein the mouse does not comprise an endogenous V_k gene segment that is capable of rearranging to form a light chain variable region sequence, and

(b) determining a human heavy and/or kappa light chain variable region sequence that encodes a human heavy and/or kappa light chain variable domain of an antibody, respectively, that specifically binds the antigen and that was generated by the genetically modified mouse; and

(c) operably linking the human heavy chain variable region sequence to a human heavy chain constant region sequence to form a nucleotide sequence encoding a fully human heavy chain and/or operably linking the human kappa light chain variable region sequence to a human kappa light chain constant region sequence to form a nucleotide sequence encoding a fully

human kappa light chain.

37. A method of making a fully human heavy chain and/or a fully human kappa light chain comprising the steps of:

(a) immunizing a genetically modified mouse with an antigen, wherein the mouse has a germline genome that comprises:

(i) two unrearranged human V_k gene segments and five unrearranged human J_k gene segments operably linked to a light chain constant region sequence, wherein the two unrearranged human V_k gene segments are a human V_k1-39 gene segment and a human V_k3-20 gene segment, and wherein the five unrearranged human J_k gene segments are a human J_k1 gene segment, a human J_k2 gene segment, a human J_k3 gene segment, a human J_k4 gene segment, and a human J_k5 gene segment; and

(ii) one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a mouse heavy chain constant region sequence;

wherein the unrearranged human heavy chain gene segments and unrearranged human kappa light chain gene segments of the mouse are capable of rearranging and encoding human heavy chain variable domains and human kappa light variable domains of an antibody, respectively, and wherein the mouse does not comprise an endogenous V_k gene segment that is capable of rearranging to form a light chain variable region sequence, and

(b) determining a human heavy and/or kappa light chain variable domain sequence of an antibody that specifically binds the antigen and that was generated by the genetically modified mouse; and

(c) operably linking the human heavy chain variable domain sequence to a human heavy chain constant domain sequence to form a fully human heavy chain and/or operably linking the human kappa light chain variable domain sequence to a human kappa light chain constant domain sequence to form a fully human kappa light chain.

38. The method of claim 37, wherein determining a human heavy or kappa light chain variable domain sequence comprises determining a nucleotide sequence that encodes the human heavy and/or light chain variable domain sequence, respectively.

39. The method of claim 37, wherein operably linking the human heavy and/or light chain variable domain sequence to a human heavy or light chain constant domain sequence, respectively, comprises operably linking a nucleotide sequence encoding the human heavy and/or light chain variable domain sequence to a nucleotide sequence encoding the human heavy and/or light chain constant domain sequence, respectively.

40. The method of claims 36 or 37, wherein the two unrearranged human V_κ gene segments and five unrearranged human J_κ gene segments in the germline genome of the mouse are operably linked to a mouse light chain constant region sequence.

41. The method of claim 40, wherein the mouse light chain constant region sequence is a mouse C_κ region sequence.

42. The method of claims 36 or 37, wherein the two unrearranged human V_κ gene segments and five unrearranged human J_κ gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin light chain locus.

43. The method of claim 42, wherein the germline genome of the mouse comprises in order: the human V_κ1-39 gene segment, the human V_κ3-20 gene segment, the human J_κ1 gene segment, the human J_κ2 gene segment, the human J_κ3 gene segment, the human J_κ4 gene segment, and the human J_κ5 gene segment.

44. The method of claims 36 or 37, wherein the one or more unrearranged human V_H gene segments, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments in the germline genome of the mouse are present at an endogenous immunoglobulin heavy chain locus.

45. The method of claim 44, wherein the mouse does not comprise an endogenous mouse immunoglobulin V_H gene segment that is capable of rearranging to form an immunoglobulin heavy chain variable region.

46. The method of claims 36 or 37, wherein the mouse comprises a nonfunctional immunoglobulin λ light chain locus.

47. A method of making a mouse comprising genetically modifying the germline genome of the mouse so that it includes:

(a) two unrearranged human immunoglobulin V_κ gene segments and five unrearranged human immunoglobulin J_κ gene segments operably linked to an immunoglobulin light chain constant region, wherein the two unrearranged human immunoglobulin V_κ gene segments are a human V_κ1-39 gene segment and a human V_κ3-20 gene segment, and the five unrearranged human immunoglobulin J_κ gene segments are human J_κ1, human J_κ2, human J_κ3, human J_κ4, and human J_κ5; and

(b) one or more human immunoglobulin V_H gene segments, one or more human immunoglobulin D_H gene segments, and one or more human immunoglobulin J_H gene segments operably linked to a mouse immunoglobulin constant region sequence;

wherein the human immunoglobulin kappa light chain gene segments are capable of rearranging and encoding human immunoglobulin light chain variable domains, and further wherein the mouse does not comprise an endogenous immunoglobulin V_LV_κ gene segment that is capable of rearranging to form an immunoglobulin light chain-variable region sequence.

~~2.48.~~ The ~~mouse~~method of claim ~~447~~, wherein the two unrearranged human V_Limmunoglobulin V_κ gene segments and the ~~two or more~~five unrearranged human J_Limmunoglobulin J_κ gene segments are operably linked to a mouse or rat immunoglobulin light chain constant region-sequence

~~3.~~ The ~~mouse~~ of claim 2, wherein the immunoglobulin light chain constant region sequence is a rat C_κC_κ region or a mouse C_κ region.

4.

49. The ~~mouse~~ of claim 2, wherein the ~~light chain constant region~~ is a mouse C_κ region.

~~5.~~ The ~~mouse~~method of any one of the ~~preceding~~ claims, wherein the mouse comprises five human J_L-~~gene segments~~.

~~6.~~ The mouse of claim 5, wherein the five human J_L-~~gene segments~~ are human J_κ1, J_κ2

Jk3, Jk4 and Jk5.

~~7.~~ The mouse of any one of the preceding claims 47 to 48, wherein the two unrearranged human V_Limmunoglobulin V_K gene segments and ~~two or more~~ five unrearranged human J_Limmunoglobulin J_K gene segments are present at an endogenous kappa immunoglobulin light chain locus.

~~8-50.~~ The ~~mouse~~ method of any one of the preceding claims 47 to 49, wherein the one or more human immunoglobulin V_H gene segments, one or more human V_Himmunoglobulin D_H gene segments, and one or more human D_H and ~~one or more human~~ immunoglobulin J_H gene segments are operably linked to a mouse constant region- sequence.

~~9.~~ The mouse

51. The method of any one of claims 47 to 50, wherein the human immunoglobulin heavy chain gene segments are capable of rearranging and encoding human immunoglobulin heavy chain variable domains.

52. The method of any one of the preceding claims 47 to 51, wherein the mouse comprises a functional λ immunoglobulin light chain locus.

~~10-53.~~ The ~~mouse~~ method of any one of claims ~~47 to 52~~, wherein the mouse comprises a nonfunctional λ immunoglobulin light chain locus.

~~11.~~

54. The ~~mouse~~ method of any one of the preceding claims ~~where~~ 48 to 53, wherein the two unrearranged human V_Limmunoglobulin V_K gene segments and ~~two or more~~ five unrearranged human J_Limmunoglobulin J_K gene segments operably linked to a mouse or rat light chain constant region sequence are present at ~~a~~ an endogenous mouse κ immunoglobulin light chain variable region locus comprising.

55. The method of claim 54, wherein the germline genome of the mouse comprises in order: two human V_KV_K segments which are ~~a~~ the human V_K4V_K1-39 gene segment and ~~a~~ the human

~~Vκ3~~Vκ3-20 gene segment, a human Jκ1, a human Jκ2, a human Jκ3, a human Jκ4 and a human Jκ1, Jκ2 Jκ3, Jκ4 and Jκ5- Jκ5.

~~42-56.~~ The mouse method of any one of the preceding claims where the mouse comprises the DLCJ-5J locus of Figure 9.

~~47~~

~~13.~~ The mouse of any one of the preceding claims, wherein the mouse comprises pro-B cell population in the bone marrow within the range of about 2.5×10^4 to about 1.5×10^5 cells characterized by CD19⁺, CD43⁺, c-kit⁺ expression.

~~55~~

~~14.~~ The mouse of any one of the preceding claims, wherein the mouse comprises a pre-B cell population in the bone marrow within in the range of about 1×10^6 to about 2×10^6 cells characterized by CD19⁺, CD43⁺, c-kit⁺ expression.

DLC-5J locus, wherein the DLC-5J locus

~~15.~~ The mouse of any one of the preceding claims, wherein the mouse comprises an immature B cell population in the bone marrow within the range of about 5×10^5 to about 7×10^5 cells characterized by IgM⁺, B220^{int} expression.

~~16.~~ The mouse of any one of the preceding claims, wherein the mouse comprises a mature B cell population in the bone marrow within the range of about 3×10^4 to about 1.5×10^5 cells characterized by IgM⁺, B220^{hi} expression hVκ/hJκ/mCκ junction sequence selected from SEQ ID NOs: 38-49.

~~57.~~ An isolated cell from a ~~47.~~ The mouse made by the method of any one of the preceding claims, wherein the mouse comprises a total CD19⁺ B 47 to 56.

~~58.~~ The cell population in the bone marrow within the range of about 1×10^6 to about 3×10^6 cells.

of claim 57, wherein

~~18.~~ The mouse of any one of the preceding claims, wherein the mouse comprises a CD19⁺ splenic B cell population within the range of about 2×10^6 to about 7×10^6 cells.

~~19.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a CD19⁺, IgD^{hi}, IgM^{lo} splenic B cell population within the range of about 1×10^6 to about 4×10^6 cells.

~~20.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a CD19⁺, IgD^{lo}, IgM^{hi} splenic B cell population within the range of about 9×10^5 to about 2×10^6 cells.

~~21.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a transitional T1 splenic B cell population within the range of about 2×10^6 to about 7×10^6 cells characterized by CD93⁺ B220⁺ IgM^{hi} CD23⁻ expression.

~~22.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a transitional T2 splenic B cell population within the range of about 1×10^6 to about 7×10^6 cells characterized by CD93⁺ B220⁺ IgM^{hi} CD23⁺ expression.

~~23.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a transitional T3 splenic B cell population within the range of about 1×10^6 to about 4×10^6 cells characterized by CD93⁺ B220⁺ IgM^{lo} CD23⁺ expression.

~~24.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a marginal zone splenic B cell population within the range of about 1×10^6 to about 3×10^6 cells characterized by CD93⁻ B220⁺ IgM^{hi} CD21/35^{hi} CD23⁻ expression.

~~25.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a follicular type 1 (FO-I) splenic B cell population within the range of about 3×10^6 to about 1.5×10^7 cells characterized by CD93⁻ B220⁺ CD21/35^{int} IgM^{lo} IgD^{hi} expression.

~~26.~~—The mouse of any one of the preceding claims, wherein the mouse comprises a follicular type 2 (FO-II) splenic B cell population within the range of about 1×10^6 to about 2×10^6 cells characterized by CD93⁻ B220⁺ CD21/35^{int} IgM^{int} IgD^{hi} expression.

~~27.~~—An isolated cell of the mouse of any one of the preceding claims.

~~28.~~—The cell of claim 27, wherein the cell is an embryonic stem (ES) cell.

~~29.~~ A mouse embryo that comprises the ES cell of claim 28.

~~30.~~ 59. The cell of claim 27, wherein the cell is a B cell.

3460. A hybridoma made with the B cell of claim 30.

61. A method for making an antibody that binds an antigen, ~~32.~~ Use of interest comprising immunizing a genetically modified mouse made according to any one of claims 1 to 26 to generate an antibody or to identify a nucleic acid sequence encoding a human immunoglobulin heavy chain variable region, 47 to 56 with an antigen of interest, obtaining an immunoglobulin variable region sequence from the mouse, and employing the immunoglobulin variable region sequence to produce

~~33.~~ A method for making an antibody that binds an antigen.

62. The method of interest comprising claim 61, wherein the method further comprises: immunizing mouse expressing in a single cell:

(a) a first human immunoglobulin heavy chain variable region sequence of the genetically modified mouse made according to any one of claims 1 to 26 with an antigen of interest,

obtaining an 66 which has been immunized, wherein the human immunoglobulin heavy chain variable region gene sequence from the mouse, and

employing the immunoglobulin variable region gene sequence to produce an antibody that binds the antigen.

~~34.~~ The method of claim 33, further comprising: expressing in a single cell:

—— (a) a first V_H gene sequence of the mouse according to any one of claims 1 to 26 which has been immunised, where the V_H gene sequence is fused with a human C_H gene sequence; and

—— (b) a V_L gene human immunoglobulin light chain variable region sequence, wherein the V_L gene human immunoglobulin light chain variable region sequence is fused with a human C_L gene immunoglobulin light chain

constant region sequence;
maintaining the cell under conditions sufficient to express a fully human antibody; and
isolating the antibody from the cell.

~~35-63.~~ The method according to claim ~~34~~, ~~where~~62, wherein the cell comprises a second ~~V_H-gene~~human immunoglobulin heavy chain variable region sequence of a genetically modified mouse made according to any one of claims ~~147~~ to ~~2656~~ which has been

immunized, where the V_H-gene

wherein the second human immunoglobulin heavy chain variable region
sequence is fused with a human ~~C_H-gene~~immunoglobulin heavy chain constant region
sequence, ~~where~~wherein the first ~~V_H-gene~~human immunoglobulin heavy chain variable
region sequence

encodes a ~~V_H~~ domain that recognizes a first epitope, and the second ~~V_H-gene~~ sequence
~~encodes a V_H~~human immunoglobulin heavy chain variable domain that recognizes a second
epitope, ~~wherein the first epitope and the second~~, and the second human immunoglobulin
heavy chain variable region sequence encodes a second human immunoglobulin heavy chain
variable domain that recognizes a second epitope are,

wherein the first epitope and the second epitope are not identical, and wherein the first
and the second ~~V_H~~human immunoglobulin heavy chain variable domains interact with the
~~V_L~~human immunoglobulin light chain variable domain encoded by the ~~V_L-gene~~human
immunoglobulin light chain variable region sequence.

~~36.~~ Use of a mouse according to any one of claims 1 to 26 in making a human bispecific
antibody.

~~37.~~ A method for making a human bispecific antibody, where the method comprises
immunizing a mouse according to any one of claims 1 to 26 and making the bispecific antibody
using human variable region gene sequences of B cells from the mouse.

~~38.~~ A method according to claim 37, where the method comprises:

~~(a) identifying a clonally selected lymphocyte of the mouse according to any one of~~
~~claims 1 to 26, where the mouse has been immunized and allowed to develop an immune~~

response to an antigen of interest, where the lymphocyte expresses an antibody that specifically binds the antigen of interest;

—— (b) obtaining from the lymphocyte or the antibody a nucleotide sequence that encodes a human heavy chain variable region that specifically binds the antigen of interest; and,

—— (c) employing the nucleotide sequence of (c) in making a bispecific antibody.

39. — The method of claim 38, wherein steps (a) through (c) are performed a first time for a first antigen of interest to generate a first human heavy chain variable region sequence, and steps (a) through (c) are performed a second time for a second antigen of interest to generate a second human heavy chain variable region sequence, and wherein the first human heavy chain variable region sequence is expressed fused with a first human heavy chain constant region to form a first human heavy chain, the second human heavy chain variable region sequence is expressed fused with a second human heavy chain constant region to form a second human heavy chain, wherein the first and the second human heavy chains are expressed in the presence of a single human light chain expressed from a Vk1-39 or a Vk3-20 gene segment.

40. — The method according to claim 39, where the method comprises:

(a) cloning variable heavy regions from B cells from:

— the mouse according to any one of claims 1 to 26 which has been immunised with a first immunogen; and

— the same mouse, or a different mouse which is genetically the same, which has been immunized with a second immunogen

(b) expressing in a cell the variable heavy regions of (a) with the same heavy chain constant region and the same light chain to make a bispecific antibody.

41. — The method of claim 40, wherein the first human heavy chain comprises a modification that eliminates or substantially reduces the affinity of the first human heavy chain to protein A, and the second human heavy chain retains the ability to bind protein A, where the modification that eliminates or substantially reduces affinity of the first human heavy chain to protein A is selected from a 95R (EU 435R), a 96F (EU 436F), and a combination thereof.

42. — Use of a mouse according to any one of claims 1 to 26 to select a human immunoglobulin heavy chain variable domain gene segment.

~~43. A mouse according to any one of claims 1 to 26 which has been immunized.~~

~~44.~~ 64. A method of producing an antibody comprising immunizing a genetically modified mouse made according to any one of claims ~~447~~ to ~~2656~~ with an antigen and obtaining a human immunoglobulin heavy chain variable ~~region~~domain amino acid sequence, or encoding nucleotide sequence, from a resulting antibody produced by the genetically modified mouse against the antigen, and utilizing said human immunoglobulin heavy chain variable ~~region~~domain amino acid ~~or sequence or encoding~~ nucleic acid sequence in making an antibody.

~~45.~~ 65. The method of claim ~~44~~64, wherein the method further comprises:

———(a) —identifying the human immunoglobulin heavy chain variable ~~region~~domain amino acid sequence, or encoding nucleotide sequence, from ~~at~~ two different antibodies against different epitopes of the antigen that the genetically modified mouse has been immunized with; or

———(b) —immunizing the same genetically modified mouse, or a further mouse made according to any one of claims ~~447~~ to ~~2656~~, with a different antigen, then identifying a human immunoglobulin heavy chain variable ~~region~~sequence~~domain~~ amino acid sequence, or encoding nucleotide sequence, from an antibody produced by the genetically modified mouse ~~which~~that is specific for the ~~second~~said different antigen, and generating a bispecific antibody ———~~where the method further comprises using the~~ by employing the two different human immunoglobulin heavy chain variable ~~regions to generate a bispecific antibody.~~

~~domain amino acid sequences.~~ ~~46.~~ The method of claim 44 or 45, where the method ~~further comprises also identifying the light chain variable region sequence from the antibody or two antibodies.~~ encoding nucleotide sequences.

~~47.~~ The method of claim ~~46~~, wherein the method ~~comprises also employing the~~ 66. A mouse embryonic stem (ES) cell that has been genetically modified so that it comprises in its genome:

exactly two unrearranged human immunoglobulin V κ gene segments and five unrearranged human immunoglobulin J κ gene segments operably linked to a mouse

immunoglobulin light chain constant region sequence, wherein the two unrearranged human immunoglobulin V κ gene segments are a human V κ 1-39 gene segment and a human V κ 3-20 gene segment, and wherein the five unrearranged human immunoglobulin J κ gene segments are a human J κ 1 gene segment, a human J κ 2 gene segment, a human J κ 3 gene segment, a human J κ 4 gene segment, and a human J κ 5 gene segment.

67. The genetically modified mouse ES cell of claim 66, further comprising in its genome: one or more unrearranged human immunoglobulin VH gene segments, one or more unrearranged human immunoglobulin DH gene segments, and one or more unrearranged human immunoglobulin JH gene segments operably linked to a mouse immunoglobulin heavy chain constant region sequence at the endogenous heavy chain loci.

68. The genetically modified ES cell of claim 67, wherein the genome of the ES cell does not comprise an endogenous mouse immunoglobulin VH gene segment that is capable of rearranging to form an immunoglobulin heavy chain variable region.

69. The genetically modified ES cell of any one of claims 66 to 68, wherein the two unrearranged human immunoglobulin V κ gene segments and five unrearranged human immunoglobulin J κ gene segments are present at the endogenous immunoglobulin κ light chain locus.

70. The genetically modified ES cell of claim 66 or 67, wherein the genome of the genetically modified mouse ES cell does not comprise a functional λ light chain locus.

71. The genetically modified mouse ES cell of claim 66 or 67, wherein the mouse light chain constant region is a mouse C κ constant region.

72. The genetically modified mouse ES cell of claim 71, wherein the mouse C κ constant region is an endogenous mouse C κ constant region.

73. The genetically modified mouse ES cell of claim 66 or 67, wherein the human V κ 1-39 gene segment in genome of the genetically modified mouse ES cell is a human germline V κ 1-39

gene segment and the human V κ 3-20 gene segment in the genome of the genetically modified mouse ES cell is a human germline V κ 3-20 gene segment.

74. The genetically modified mouse ES cell of claim 66 or 67, wherein the genome of the genetically modified mouse ES cell comprises in order: the human V κ 1-39 gene segment, the human V κ 3-20 gene segment, the human J κ 1 gene segment, the human J κ 2 gene segment, the human J κ 3 gene segment, the human J κ 4 gene segment, and the human J κ 5 gene segment.

75. The genetically modified mouse ES cell of any one of claims 66 to 74, wherein the genome of the genetically modified mouse ES cell comprises a DLC-5J locus, wherein the DLC-5J locus comprises a hV κ /hJ κ /mC κ junction sequence selected from SEQ ID NOs: 38-49.

76. A method of making a mouse comprising gestating a mouse embryo derived from the genetically modified ES cell of claim 66 or 67 in a surrogate mother and allowing the surrogate mother to give birth to progeny derived in whole or in part from the genetically modified ES cell.

77. A method of making a genetically modified mouse ES cell, the method comprising genetically modifying an ES cell so that it comprises in its genome exactly two unrearranged human immunoglobulin V κ gene segments and five unrearranged human immunoglobulin J κ gene segments operably linked to a mouse immunoglobulin light chain constant region sequence, wherein the two unrearranged human immunoglobulin V κ gene segments are a human V κ 1-39 gene segment and a human V κ 3-20 gene segment, and wherein the five unrearranged human immunoglobulin J κ gene segments are a human J κ 1 gene segment, a human J κ 2 gene segment, a human J κ 3 gene segment, a human J κ 4 gene segment, and a human J κ 5 gene segment.

78. The method of claim 77, wherein the genetically modified mouse ES cell further comprises in its genome one or more unrearranged human immunoglobulin V H gene segments, one or more unrearranged human immunoglobulin D H gene segments, and one or more unrearranged human immunoglobulin J H gene segments operably linked to a mouse immunoglobulin heavy chain constant region sequence at the endogenous heavy chain loci.

79. The method of claim 77 or 78, wherein the mouse light chain constant region is a mouse C κ constant region.

80. The method of claim 79, wherein the mouse light chain constant region is an endogenous mouse C κ constant region.

81. The method of claim 78, wherein the genome of the genetically modified ES cell does not comprise an endogenous mouse immunoglobulin VH gene segment that is capable of rearranging to form an immunoglobulin heavy chain variable region or regions identified in the antibody produced by the method.

82. The method of any one of claims 77 to 81, wherein the two unrearranged human immunoglobulin V κ gene segments and five unrearranged human immunoglobulin J κ gene segments are present at the endogenous immunoglobulin κ light chain locus.

83. The method of claim 77 or 78, wherein the genome of the genetically modified mouse ES cell does not comprise a functional λ light chain locus.

84. The method of claim 77 or 78, wherein the human V κ 1-39 gene segment in the genome of the genetically modified mouse ES cell is a human germline V κ 1-39 gene segment and the human V κ 3-20 gene segment in the genome of the genetically modified mouse ES cell is a human germline V κ 3-20 gene segment.

85. The method of claim 77 or 78, wherein the genome of the genetically modified mouse ES cell comprises in order: the human V κ 1-39 gene segment, the human V κ 3-20 gene segment, the human J κ 1 gene segment, the human J κ 2 gene segment, the human J κ 3 gene segment, the human J κ 4 gene segment, and the human J κ 5 gene segment.

86. The method of any one of claims 77 to 85, wherein the genetically modified mouse ES cell comprises a DLC-5J locus, wherein the DLC-5J locus comprises a hV κ /hJ κ /mC κ junction sequence selected from SEQ ID NOs: 38-49.