

We claim:

1. A mouse having a restricted immunoglobulin heavy chain locus characterized by the presence of a single human V_H gene segment, one or more human D_H gene segments, and one or more human J_H gene segments, wherein the single human V_H gene segment is a polymorphic V_H gene segment.
2. The mouse of claim 1, wherein the mouse comprises a deletion of all or substantially all endogenous V_H, D_H, and J_H gene segments.
3. The mouse of claim 1, wherein the single human V_H gene segment is selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, and V_H3-23.
4. The mouse of claim 3, wherein the single human V_H gene segment is V_H1-69.
5. The mouse of claim 3, wherein the single human V_H gene segment is V_H1-2.
6. The mouse of claim 1, wherein the single human V_H gene segment is operably linked to a human or non-human immunoglobulin constant region gene.
7. The mouse of claim 6, wherein the immunoglobulin constant region gene is a mouse, rat, or human constant region gene.
8. The mouse of claim 1, further comprising a one or more human immunoglobulin V_L gene segments operably linked to one or more human J_L gene segments.
9. The mouse of claim 8, wherein the one or more human V_L gene segments and/or the one or more human J_L gene segments are selected from human κ and human λ gene segments.
10. The mouse of claim 8, wherein the one or more human immunoglobulin V_L gene segments and one or more human J_L gene segments are operably linked to a non-human light chain constant gene.

11. The mouse of claim 10, wherein the non-human light chain constant gene is selected from a mouse or rat κ or λ constant region gene.
12. A mouse comprising in its germline a replacement at an endogenous immunoglobulin heavy chain locus of all or substantially all endogenous V_H , D_H , and J_H gene segments with single human V_H gene segment and/or polymorphic variants thereof, one or more human D_H gene segments and one or more human J_H gene segments.
13. The mouse of claim 12, further comprising a replacement at an endogenous immunoglobulin light chain locus of all or substantially all endogenous V_L and J_L gene segments with one or more human V_L and one or more human J_L gene segments.
14. The mouse of claim 12, wherein the single human V_H gene segment is selected from a human V_H1-69 and a human V_H1-2 gene segment.
15. A cell or tissue derived from the mouse of claim 1 or 12.
16. A method of making a nucleic acid sequence encoding a human V_H domain comprising
 - (a) immunizing a mouse of claim 1 or 12 with an antigen of interest;
 - (b) allowing said mouse to mount an immune response to the antigen of interest; and,
 - (c) obtaining a nucleic acid sequence encoding a human V_H domain from said mouse.
17. The method of claim 16, wherein the immunoglobulin V_H region sequence is at least 75% identical to SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56 or SEQ ID NO: 58.
18. The method of claim 16, wherein the immunoglobulin V_H region sequence comprises SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, or a polymorphic variant thereof.
19. The method of claim 16, wherein the immunoglobulin V_H region sequence encodes a protein that is at least 75% identical with SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 or SEQ ID NO: 59.

20. The method of claim 16, wherein the immunoglobulin V_H region sequence is at least 95% identical to SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66 or SEQ ID NO: 68.
21. The method of claim 16, wherein the immunoglobulin V_H region sequence comprises SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, or a polymorphic variant thereof.
22. The method of claim 16, wherein the immunoglobulin V_H region sequence encodes a protein that is at least 95% identical with SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67 or SEQ ID NO: 69.
23. Use of a mouse according to claims 1 or 12 to make a nucleic acid sequence encoding a human heavy chain variable domain.
24. Use according to claim 23, wherein the human heavy chain variable domain is characterized by having human FR1-CDR1-FR2-CDR2-FR3 sequences derived from a polymorphic human V_H gene segment.
25. Use according to claim 24, wherein the human V_H gene segment is selected from a human V_H1-2, V_H1-69, V_H2-26, V_H2-70, or V_H3-23 gene segment.
26. Use according to claim 25, wherein the human V_H gene segment is V_H1-2.
27. Use according to claim 25, wherein the human V_H gene segment is V_H1-69.
28. Use of a mouse according to claim 1 or 12 to make a human antibody, wherein the human antibody comprises a heavy chain variable domain derived from a rearranged human V_H1-2 gene segment, human V_H1-69 gene segment, or polymorphic variant thereof.
29. Use according to claim 28, wherein the rearranged human V_H1-69 gene segment is at least 75% identical to SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56 or SEQ ID NO: 58.

30. Use according to claim 28, wherein the rearranged human V_H1-2 gene segment is at least 95% identical to SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66 or SEQ ID NO: 68.

CLAIMS

1. A mouse having in its germline genome a restricted immunoglobulin heavy chain locus characterized by the presence of a single unrearranged human V_H gene segment, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a non-human constant region gene sequence,
wherein the mouse further comprises a diverse repertoire of rearranged human immunoglobulin heavy chain variable region genes, each of which is linked to the non-human constant region gene sequence and derived from the restricted immunoglobulin heavy chain locus.
2. The mouse of claim 1, wherein the mouse comprises a deletion of all endogenous V_H, D_H, and J_H gene segments.
3. The mouse of claim 1 or claim 2, wherein the single unrearranged human V_H gene segment is selected from V_H1-2, V_H1-69, V_H2-26, V_H2-70, and V_H3-23 or a polymorphic variant thereof capable of rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.
4. The mouse of any one of claims 1 to 3, wherein the single unrearranged human V_H gene segment is V_H1-69 or a polymorphic variant thereof capable for rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.
5. The mouse of claim 3, wherein the single unrearranged human V_H gene segment is V_H1-2 or a polymorphic variant thereof capable for rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.
6. The mouse of any one of claims 1 to 5, wherein the single unrearranged human V_H gene segment is operably linked to a rodent immunoglobulin constant region gene.
7. The mouse of any one of claims 1 to 6, wherein the immunoglobulin constant region gene is a mouse constant region gene.

8. The mouse of any one of claims 1 to 7, further comprising one or more unrearranged human immunoglobulin V_L gene segments operably linked to one or more unrearranged human J_L gene segments.
9. The mouse of claim 8, wherein the one or more unrearranged human V_L gene segments and/or the one or more unrearranged human J_L gene segments are selected from human κ and human λ gene segments.
10. The mouse of claim 8 or claim 9, wherein the one or more unrearranged human immunoglobulin V_L gene segments and one or more unrearranged human J_L gene segments are operably linked to a non-human light chain constant gene.
11. The mouse of claim 10, wherein the non-human light chain constant gene is selected from a mouse or rat κ or λ constant region gene.
12. The mouse of any one of claims 1 to 11, wherein all or substantially all endogenous V_H, D_H, and J_H gene segments at an endogenous immunoglobulin heavy chain locus is replaced with the restricted immunoglobulin heavy chain locus characterized by the presence of the single unrearranged human V_H gene segment, the one or more unrearranged human D_H gene segments and the one or more unrearranged human J_H gene segments operably linked to a non-human constant region gene sequence.
13. The mouse of claim 12, further comprising a replacement at an endogenous immunoglobulin light chain locus of all or substantially all endogenous V_L and J_L gene segments with one or more unrearranged human V_L and one or more unrearranged human J_L gene segments.
14. A cell or tissue derived from the mouse of any one of claims 1 to 13.
15. A method of making a nucleic acid sequence that encodes a human immunoglobulin heavy chain variable domain comprising
 - (a) amplifying a nucleic acid comprising a rearranged human immunoglobulin heavy chain variable region gene from a lymphocyte of a mouse of any one of claims 1 to 13, or a hybridoma produced from the lymphocyte,

wherein the lymphocyte comprises one of the diverse repertoire of rearranged human immunoglobulin heavy chain variable region genes.

16. The method of claim 15, wherein the rearranged human immunoglobulin heavy chain variable region gene is derived from a human V_H1-69 gene segment that comprises a sequence at least 75% identical to SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56 or SEQ ID NO: 58.

17. The method of claim 16, wherein the rearranged human immunoglobulin heavy chain variable region gene is derived from a human V_H1-69 gene segment that comprises a sequence comprising SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, or a polymorphic variant thereof, wherein the polymorphic variant is capable for rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.

18. The method of claim 15, wherein the rearranged human immunoglobulin heavy chain variable region gene comprises a sequence that encodes a protein that is at least 75% identical with SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57 or SEQ ID NO: 59.

19. The method of claim 15, wherein the rearranged human immunoglobulin heavy chain variable region gene is derived from a human V_H1-2 gene segment that comprises a sequence at least 95% identical to SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66 or SEQ ID NO: 68.

20. The method of claim 19, wherein the rearranged human immunoglobulin heavy chain variable region gene is derived from a human V_H1-2 gene segment that comprises a sequence comprising SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68., or a polymorphic variant thereof, wherein the polymorphic variant is capable for rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.

21. The method of claim 15, wherein the rearranged human immunoglobulin heavy chain variable region gene comprises a sequence that encodes a protein that is at least 95% identical with SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67 or SEQ ID NO: 69.
22. Use of a mouse according to any one of claims 1-13 to make a nucleic acid sequence encoding a human heavy chain variable domain.
23. Use according to claim 22, wherein the human heavy chain variable domain is characterized by having human FR1-CDR1-FR2-CDR2-FR3 sequences derived from a polymorphic human V_H gene segment capable for rearranging and forming a rearranged heavy chain variable domain with one or more D_H segments and one or more J_H segments.
24. Use according to claim 22 or claim 23, wherein the human V_H gene segment is selected from a human V_H1-2 , V_H1-69 , V_H2-26 , V_H2-70 , or V_H3-23 gene segment.
25. Use of a mouse according to any one of claims 1-13 to make a human antibody, wherein the human antibody comprises a heavy chain variable domain derived from a rearranged human V_H1-2 gene segment, human V_H1-69 gene segment, or polymorphic variant thereof, wherein polymorphic variant is capable for rearranging and forming a rearranged heavy chain variable domain with the one or more D_H segments and the one or more J_H segments.
26. Use according to claim 25, wherein the rearranged human V_H1-69 gene segment is at least 75% identical to SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56 or SEQ ID NO: 58, wherein the rearranged human V_H1-69 gene segment is capable for rearranging and forming a rearranged heavy chain variable domain with the one or more D_H segments and the one or more J_H segments.
27. Use according to claim 25, wherein the rearranged human V_H1-2 gene segment is at least 95% identical to SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66 or SEQ ID NO: 68, wherein the rearranged human V_H1-2 gene segment is capable for rearranging and forming a rearranged heavy chain variable domain with the one or more D_H segments and the one or more J_H segments.

28. The cell of claim 14, wherein the cell is a lymphocyte or a hybridoma produced from the lymphocyte.

29. The method of any one of claims 15-21, further comprising immunizing the mouse with an antigen of interest before amplifying the nucleic acid.

30. The method of any one of claims 15-21 and 29, wherein the rearranged human immunoglobulin V_H region gene sequence comprises at least one somatic hypermutation.

31. The method of any one of claims 15-21 and 28-30, wherein the lymphocyte specifically binds an antigen of interest.

32. A vector comprising a nucleic acid sequence made according to the method of any one of claims 15-21 and 28-31.

33. The vector according to claim 32 wherein the nucleic acid sequence is linked to a sequence that encodes a human heavy chain constant domain.

34. A totipotent mouse cell for making a mouse that produces a human heavy chain variable domain comprising in its genome a restricted immunoglobulin heavy chain locus characterized by the presence of a single unrearranged human V_H gene segment, one or more unrearranged human D_H gene segments, and one or more unrearranged human J_H gene segments operably linked to a non-human heavy chain constant region gene sequence.

35. The totipotent mouse cell of claim 34, wherein the non-human heavy chain constant region gene sequence is a mouse heavy chain constant region gene sequence.

36. The totipotent mouse cell of claim 34 or claim 35, wherein the totipotent mouse cell is an embryonic stem cell.

37. A method of making a human immunoglobulin heavy chain variable domain comprising expressing in a host cell the nucleic acid made according to the method of any one of claims 15-21 and 28-31 or the nucleic acid of any one of claims 32 or 33.

38. A host cell comprising the nucleic acid made according to the method of any one of claims 15-21 and 28-31 or the nucleic acid of any one of claims 32 or 33.

39. The host cell of claim 38, wherein the cell is a mammalian cell.

40. A method of obtaining a cell that expresses a human immunoglobulin heavy chain variable domain comprising

harvesting a lymphocyte from a non-human animal that comprises in its germline genome a restricted immunoglobulin heavy chain locus characterized by the presence of a single human unrearranged V_H gene segment, one or more human unrearranged D_H gene segments, and one or more human unrearranged J_H gene segments operably linked to a non-human immunoglobulin constant region,

wherein the lymphocyte expresses a rearranged human immunoglobulin V_H region gene derived from the restricted immunoglobulin heavy chain locus.

41. The method of claim 40, further comprising as last steps

obtaining from the lymphocyte, or a hybridoma produced from the lymphocyte, a first nucleotide sequence that comprises the rearranged human immunoglobulin V_H region gene sequence; and

expressing in a host cell a first nucleic acid comprising a sequence identical to or substantially identical to the first nucleotide sequence.

42. The method of claim 41, wherein the first nucleic acid is operably linked to a human heavy chain constant region gene.

43. The method of claim 41 or claim 42, further comprising expressing in the cell a second nucleic acid operably linked to a human light chain constant region gene, wherein the second nucleic acid comprises a sequence identical to or substantially identical to a second nucleotide sequence that encodes a human light chain variable (V_L) domain that is cognate to the human immunoglobulin V_H domain.

44. The method of any one of claims 41-43, further comprising expressing in the cell a third nucleic acid operably linked to a human heavy chain constant region gene, wherein the third

nucleic acid encodes a second human immunoglobulin V_H domain that is cognate to the human immunoglobulin V_L domain.

45. The method of any one of claims 41-44, wherein the host cell is selected from a HeLa cell, a DU145 cell, a Lncap cell, a MCF-7 cell, a MDA-MB-438 cell, a PC3 cell, a T47D cell, a THP-1 cell, a U87 cell, a SHSY5y cell, a Saos-2 cell, a Vero cell, a CHO cell, a GH3 cell, a PC12 cell, a human retinal cell, and a MC3T3 cell.

46. The method of claim 40, further comprising producing a hybridoma from the harvested lymphocyte.

47. The method of any one of the preceding claims, wherein the single human unrearranged V_H gene segment the one or more human unrearranged D_H gene segments, and the one or more human unrearranged J_H gene segments are operably linked to the non-human immunoglobulin constant region at an endogenous heavy chain locus of the non-human animal.

48. The method of any one of the preceding claims, wherein the non-human animal further comprises one or more human immunoglobulin V_L and one or more human immunoglobulin J_L gene segments operably linked to a non-human light chain constant region.

49. The method of any one of the preceding claims, wherein the non-human animal comprises

- (a) a deletion of an endogenous immunoglobulin heavy chain variable locus and a deletion of an endogenous κ light chain variable locus, or
- (b) a deletion of an endogenous immunoglobulin heavy chain variable locus and a deletion of an endogenous λ light chain variable locus.

50. The method of any one of the preceding claims, wherein the non-human animal is a rodent selected from the group consisting of a hamster, a rat and a mouse.

51. A method of making a human immunoglobulin heavy chain variable domain comprising culturing the cell obtained according to of any one of claims 40-50 or the hybridoma of claim 51.