

CLAIMS

1. A method of obtaining a cell that expresses an antigen-specific immunoglobulin variable domain with histidines encoded by germline histidine codons, comprising harvesting a lymphocyte from a non-human animal; wherein the non-human animal comprises in its germline genome either or both (i) an unrearranged immunoglobulin heavy chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon or an insertion of at least one histidine codon, and (ii) an unrearranged immunoglobulin light chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon or an insertion of at least one histidine codon; wherein the non-human animal further comprises *in vivo* a plurality of diverse antigen-binding proteins that display high affinity for an antigen of interest and comprise an immunoglobulin heavy chain variable domain that retains at least one histidine amino acid derived from the substituted or inserted histidine codon in the germline genome and/or an immunoglobulin light chain variable domain that retains at least one histidine amino acid derived from the substituted or inserted histidine codon in the germline genome; and wherein the lymphocyte expresses one of the plurality of diverse antigen-binding proteins.
2. The method of claim 1, wherein the unrearranged immunoglobulin heavy chain variable region gene sequence is an unrearranged human immunoglobulin heavy chain variable region nucleic sequence comprising (i) unrearranged human V_H, unrearranged human D_H, and unrearranged human J_H gene segments, and (ii) the substituted or inserted histidine codon, and/or wherein the unrearranged immunoglobulin light chain gene sequence is an unrearranged human immunoglobulin light chain variable region nucleic acid sequence comprising (i) unrearranged human V_L and unrearranged human J_L gene segments, and (ii) the substituted or inserted histidine codon.
3. The method of claim 1, wherein the unrearranged immunoglobulin heavy chain variable region gene sequence is an unrearranged human immunoglobulin heavy chain variable region nucleic acid sequence comprising (i) unrearranged human V_H gene segments, a synthetic D segment that comprises a linker, an unrearranged human J_H gene segment, and (ii) the substituted or inserted histidine codon, and/or wherein the unrearranged immunoglobulin light chain gene sequence is an unrearranged human light chain variable region nucleic acid sequence comprising (i) unrearranged human V_L and unrearranged human J_L gene segments, and (ii) the substituted or inserted histidine codon.
4. The method of any one of claims 1 to 3, wherein an endogenous non-human immunoglobulin heavy chain variable region gene is replaced with the unrearranged immunoglobulin heavy chain variable region gene sequence, which is optionally operably linked to an endogenous non-human immunoglobulin heavy chain constant region gene sequence, and/or wherein an endogenous non-human immunoglobulin light chain variable region gene is replaced with the unrearranged immunoglobulin light chain variable region gene sequence, which is optionally operably linked to an endogenous non-human immunoglobulin light chain constant region gene sequence.

5. The method of any one of claims 1 to 4, wherein the substituted or inserted histidine codon is in a CDR1 encoding sequence, a CDR2 encoding sequence, a CDR3 encoding sequence, an N terminal encoding sequence or a loop encoding sequence; optionally a CDR3 encoding sequence.
6. The method of any one of claims 1 to 5, wherein the non-human animal is a rodent; optionally a rat, a mouse, or a hamster; optionally a mouse.
7. The method of any one of claims 1 to 6, further comprising the step of producing a hybridoma from the harvested lymphocyte.
8. The method of any one of claims 1 to 7, further comprising as last steps obtaining from the harvested lymphocyte, or the hybridoma produced therefrom, a first nucleotide sequence that encodes the immunoglobulin heavy chain variable domain that retains at least one histidine amino acid derived from the substituted or inserted histidine codon in the germline genome and/or a second nucleotide sequence that encodes the immunoglobulin light chain variable domain that retains at least one histidine amino acid derived from the substituted or inserted histidine codon in the germline genome; and expressing in a cell a first nucleic acid operably linked to a human heavy chain constant region gene and/or a second nucleic acid operably linked to a human light chain constant region gene, wherein the first nucleic acid comprises a sequence identical to or substantially identical to the first nucleotide sequence, and wherein the second nucleic acid comprises a sequence identical to or substantially identical to the second nucleotide sequence.
9. A hybridoma produced according to the method of claim 7.
10. A nucleic acid comprising a sequence identical to or substantially identical to the first nucleotide sequence or the second nucleotide sequence obtained according to the method of claim 8.
11. A cell comprising the nucleic acid of claim 10.
12. An immunoglobulin variable domain made by the cell obtained by the method of any one of claims 1 - 8, the hybridoma of claim 9, or the cell of claim 11.
13. A method of obtaining a cell that expresses an antigen-specific immunoglobulin variable domain with histidines encoded by germline histidine codons, comprising harvesting a lymphocyte from a non-human animal; wherein the non-human animal comprises in its germline genome both (i) an unrearranged immunoglobulin heavy chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon or an insertion of at least one histidine codon, and (ii) a rearranged immunoglobulin light chain variable region gene sequence comprising a substitution of at least one non-histidine codon with a histidine codon or an insertion of at least one

histidine codon; wherein the non-human animal further comprises in vivo a plurality of diverse antigen-binding proteins that display high affinity for an antigen of interest and comprises an immunoglobulin light chain variable domain that is encoded by the rearranged immunoglobulin light chain variable region gene sequence and retains at least one histidine amino acid derived from substituted or inserted histidine codon in the germline genome; and wherein the lymphocyte expresses one of the plurality of diverse antigen-binding proteins.

14. The method of claim 13, wherein the unrearranged immunoglobulin heavy chain variable region gene sequence is an unrearranged human immunoglobulin heavy chain variable region nucleic acid sequence comprising (i) unrearranged human V_H, unrearranged human D_H, and unrearranged human J_H gene segments, and the substituted or inserted histidine codon, or (ii) unrearranged human V_H gene segments, a synthetic D segment that comprises a linker, an unrearranged human J_H gene segment, and the substituted or inserted histidine codon; and wherein the rearranged immunoglobulin light chain variable region gene sequence is a rearranged human immunoglobulin light chain variable region nucleic acid sequence comprising a human V_L gene segment rearranged with a human J_L gene segment and the substituted or inserted histidine codon.
15. The method of claim 13 or claim 14, wherein an endogenous non-human immunoglobulin heavy chain variable region gene is replaced with the unrearranged immunoglobulin heavy chain variable region gene sequence, which is optionally operably linked to an endogenous non-human immunoglobulin heavy chain constant region gene sequence, and wherein an endogenous non-human immunoglobulin light chain variable region gene is replaced with the rearranged immunoglobulin light chain variable region gene sequence, which is optionally operably linked to an endogenous non-human immunoglobulin light chain constant region gene sequence.
16. The method of any one of claims 13 to 15, wherein the rearranged immunoglobulin light chain variable region gene sequence is derived from a human Vk1-39 gene segment or a human Vk3-20 gene segment.
17. The method of any one of claim 13 to 16, wherein the substituted or inserted histidine codon is in a CDR1 encoding sequence, a CDR2 encoding sequence, a CDR3 encoding sequence, an N terminal encoding sequence or a loop encoding sequence; optionally a CDR3 encoding sequence.
18. The method of any one of claims 13 to 17, wherein the non-human animal is a rodent; optionally a rat, a mouse, or a hamster; optionally a mouse.
19. The method of any one of claims 13 to 18, further comprising the step of producing a hybridoma from the harvested lymphocyte.

20. The method of any one claims 13 to 19, further comprising as last steps obtaining from the harvested lymphocyte, or the hybridoma produced therefrom, a first nucleotide sequence that encodes an immunoglobulin heavy chain variable domain that is cognate to the immunoglobulin light chain variable domain, and optionally comprises at least one histidine amino acid that is derived from the substituted or inserted histidine codon in the germline; and expressing in a cell a first nucleic acid operably linked to a human heavy chain constant region gene and a second nucleic acid operably linked to a human light chain constant region gene, wherein the first nucleic acid comprises a sequence identical to or substantially identical to the first nucleotide sequence, and wherein the second nucleic acid comprises a sequence identical to or substantially identical to the rearranged immunoglobulin light chain variable region gene sequence.
21. The method of claim 20, wherein the cell further comprises a third nucleic acid operably linked to a human heavy chain constant region gene, wherein the third nucleic acid encodes a different immunoglobulin heavy chain variable domain that is cognate to the immunoglobulin light chain variable domain, and wherein the cell expresses the first, second and third nucleic acids as a bi-specific antigen-binding protein.
22. The method of claim 21, wherein one of the human heavy chain constant region genes is modified to omit a Protein A-binding determinant.
23. A hybridoma formed according to the method of claim 19.
24. A nucleic acid comprising a sequence identical to or substantially identical to the first nucleotide sequence obtained according to the method of claim 20.
25. A cell comprising the nucleic acid of claim 24.
26. An antigen-specific antibody made by a cell obtained according to the method of any one of claims 13-22, the hybridoma of claim 23, or the cell of claim 25.