

AMENDED CLAIMS**received by the International Bureau on 30 August 2013 (30.08.2013)**

1. A genetically modified non-human animal comprising in its germline an immunoglobulin locus comprising a substitution or an insertion in an immunoglobulin variable locus of at least one non-histidine codon with a histidine codon, wherein the histidine codon is not encoded by a corresponding wild-type germline gene segment.
2. The non-human animal of claim 1, wherein the non-human animal comprises a first and a second variable locus, wherein at least the first or the second variable locus comprises an insertion of at least one histidine codon or a substitution of at least one non-histidine codon with a histidine codon.
3. The non-human animal of claim 2, wherein both the first and the second variable locus each comprise a substitution or insertion of at least one non-histidine codon with a histidine codon.
4. The non-human animal of claim 3, wherein the first variable locus comprises at least a functional portion of an unrearranged heavy chain variable locus (unrearranged V, D, J segments).
5. The non-human animal of claim 4, wherein the unrearranged heavy chain variable locus comprises at least a portion of a human locus (unrearranged V, D, J segments).
6. The non-human animal of claim 4, wherein the unrearranged heavy chain locus is a human locus comprising unrearranged V segments, a synthetic D segment that comprises a linker, and a human J segment.
7. The non-human animal of claim 6, wherein the synthetic D segment comprises at least one histidine codon.
8. The non-human animal of claim 3, wherein the second variable locus comprises at least a functional portion of an unrearranged light chain locus (unrearranged V, J segments).
9. The non-human animal of claim 4, wherein the second variable locus comprises a rearranged immunoglobulin light chain variable gene sequence (rearranged VJ sequence).
10. The non-human animal of claim 1, wherein the substitution of a non-histidine codon with a histidine codon and/or the insertion of a histidine codon is in

a nucleic acid sequence that encodes a variable domain and the histidine is in a region selected from an N-terminal region of an immunoglobulin chain, a loop 4 region of an immunoglobulin chain, a CDR1 of a heavy chain, a CDR2 of a heavy chain, a CDR3 of a heavy chain, a CDR1 of a light chain, a CDR2 of a light chain, a CDR3 of a light chain, and a combination thereof.

11. The non-human animal of claim 2, wherein at least one of the first variable locus or the second variable locus is operably linked to an endogenous non-human constant region nucleic acid sequence at an endogenous non-human immunoglobulin locus.

12. The non-human animal of claim 5, wherein the first variable locus (unrearranged human V, D, J segments) is operably linked to an endogenous non-human immunoglobulin heavy chain constant region nucleic acid sequence.

13. The non-human animal of claim 12, wherein the first variable locus (unrearranged human V, D, J segments) is operably linked to the endogenous non-human immunoglobulin heavy chain constant region nucleic acid sequence at an endogenous non-human immunoglobulin locus.

14. The non-human animal of claim 6, wherein the second variable locus (unrearranged V, J segments) is operably linked to an endogenous non-human immunoglobulin light chain constant region sequence.

15. The non-human animal of claim 14, wherein the endogenous non-human immunoglobulin light chain constant region sequence is at an endogenous non-human immunoglobulin locus.

16. A genetically modified non-human animal, comprising

at least a portion of a human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence (unrearranged V, D, J segments) operably linked to a constant region gene sequence, wherein one or more of the V, D, and J gene segments comprise at least one substitution of a non-histidine codon for a histidine codon, or at least one histidine codon insertion, wherein the histidine codon of the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment;

at least a portion of a human unrearranged immunoglobulin light chain variable region nucleic acid sequence (unrearranged V, J segments) operably

linked to a constant region gene sequence, wherein one or more of the V and J gene segments comprise at least one substitution of a non-histidine codon for a histidine codon, or at least one histidine codon insertion, wherein the histidine codon of the human unrearranged immunoglobulin light chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment;

wherein the non-human animal expresses an immunoglobulin heavy chain variable domain and/or an immunoglobulin light chain variable domain that comprises a histidine derived from a histidine substitution or insertion in the germline of the mouse.

17. The genetically modified non-human animal of claim 16, wherein the non-human animal is a mammal.

18. The genetically modified mammal of claim 17, wherein the mammal is a rodent.

19. The rodent of claim 18, wherein the rodent is selected from the group consisting of a mouse, a rat, and a hamster.

20. The genetically modified non-human animal of claim 16, wherein the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is operably linked to a non-human constant region sequence.

21. The genetically modified non-human animal of claim 20, wherein the non-human constant region nucleic acid sequence operably linked to the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin locus in the germline of the non-human animal.

23. The genetically modified non-human animal of claim 16, wherein the non-human constant region nucleic acid sequence operably linked to the human unrearranged immunoglobulin light chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin locus in the germline of the non-human animal.

24. A genetically modified non-human animal, comprising
at least a portion of a human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence (unrearranged V, D, J segments) operably

linked to a constant region gene sequence, wherein one or more of the unarranged V, D, and J gene segments comprise at least one substitution of a non-histidine codon for a histidine codon, or at least one histidine codon insertion wherein the histidine codon of the human unarranged immunoglobulin heavy chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment;

a human rearranged immunoglobulin light chain variable region nucleic acid sequence (rearranged VJ sequence) operably linked to a light chain constant region gene sequence, wherein the rearranged VJ sequence comprises at least one substitution of a non-histidine codon for a histidine codon, or at least one histidine codon insertion, wherein the histidine codon of the human rearranged immunoglobulin light chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment;

wherein the non-human animal expresses an immunoglobulin heavy chain variable domain and/or an immunoglobulin light chain variable domain that comprises a histidine derived from a histidine substitution or insertion in the germline of the mouse.

25. The genetically modified non-human animal of claim 24, which is a mammal.
26. The genetically modified mammal of claim 25, which is a rodent.
27. The rodent of claim 26, selected from the group consisting of a mouse, a rat, and a hamster.
28. The genetically modified non-human animal of claim 24, wherein the human unarranged immunoglobulin heavy chain variable region nucleic acid sequence is operably linked to a non-human constant region sequence.
29. The genetically modified non-human animal of claim 28, wherein the non-human constant region sequence operably linked to the human unarranged immunoglobulin heavy chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin locus in the germline of the non-human animal.
30. The genetically modified non-human animal of claim 29, wherein the non-human constant region sequence operably linked to the human rearranged immunoglobulin light chain variable region nucleic acid sequence is at an

endogenous non-human immunoglobulin locus in the germline of the non-human animal.

31. A genetically modified non-human animal, wherein the animal comprises in its germline

at least one insertion, or at least one substitution, of a non-histidine codon for a histidine codon at an immunoglobulin heavy chain locus, wherein the histidine codon of the immunoglobulin heavy chain locus is not encoded by a corresponding germline gene segment; and

at least one insertion, or at least one substitution, of a non-histidine codon for a histidine codon at an immunoglobulin light chain locus, wherein the histidine codon of the immunoglobulin light chain locus is not encoded by a corresponding germline gene segment; and

a B cell population that is characterized by an enhanced presence of histidine residues in immunoglobulin heavy and light chains of the B cell population as compared with a wild-type non-human animal.

32. The genetically modified non-human animal of claim 31, wherein the enhancement is about 2-4 fold.

33. The genetically modified non-human animal of claim 31, wherein the enhancement is about 2-10 fold.

34. A genetically modified non-human animal that expresses immunoglobulin light and heavy chains that comprise histidines encoded by substitutions and/or insertions in germline immunoglobulin sequences of the non-human animal.

35. The genetically modified non-human animal of claim 1, wherein the variable region sequence comprises a cluster of 2, 3, 4, or 5 histidines that are substitutions of a histidine codon for a non-histidine codon and/or insertions of histidine codons.

36. The genetically modified non-human animal of claim 5, wherein the unrearranged heavy chain locus comprises D gene segments that are inverted with respect to the direction of orientation of the heavy chain locus.

37. A method for making a non-human animal that makes antibody variable domains with histidines encoded by germline histidine codons, comprising:

modifying the non-human animal in its germline to comprise at least one substitution of histidine codon for a non-histidine codon, or insertion of a histidine

codon, in an unrearranged immunoglobulin heavy chain variable (unrearranged V, D, J segments) locus, wherein the histidine codon of the unrearranged immunoglobulin heavy chain variable locus is not encoded by a corresponding wild-type germline gene segment; and,

modifying the non-human animal in its germline to comprise at least one substitution of a histidine codon for a non-histidine codon, or insertion of a histidine codon, in an unrearranged immunoglobulin light chain variable (unrearranged V, J segments) locus, wherein the histidine codon of the unrearranged immunoglobulin light chain variable locus is not encoded by a corresponding wild-type germline gene segment.

38. The method of claim 37, wherein the method comprises genetically modifying the germline of the mouse to comprise at least a portion of a human unrearranged immunoglobulin heavy chain variable (V, D, J segments) locus, and making the histidine substitution or insertion in the unrearranged immunoglobulin heavy chain variable (unrearranged V, D, J segments) human locus.

39. The method of claim 37, wherein the method comprises genetically modifying the germline of the mouse to comprise at least a portion of a human unrearranged immunoglobulin light chain (unrearranged V, J segments) locus, and making the histidine substitution or insertion in the unrearranged human immunoglobulin light chain locus.

40. The method of claim 37, wherein the non-human animal is a rodent.

41. The method of claim 40, wherein the rodent is selected from a mouse, a rat, and a hamster.

42. A method for making a non-human animal that makes antibody variable domains with histidines encoded by germline histidine codons, comprising:

modifying the non-human animal to comprise at least one substitution of histidine codon for a non-histidine codon, or insertion of a histidine codon, in an unrearranged immunoglobulin heavy chain variable (unrearranged V, D, J segments) locus, wherein the histidine codon of the unrearranged immunoglobulin heavy chain variable locus is not encoded by a corresponding wild-type germline gene segment; and,

modifying the non-human animal to comprise at least one substitution of a histidine codon for a non-histidine codon, or insertion of a histidine codon, in a

rearranged immunoglobulin light chain variable sequence (rearranged VJ sequence) in the germline, wherein the histidine codon comprised in the rearranged immunoglobulin light chain variable sequence is not encoded by a corresponding wild-type germline gene segment.

43. The method of claim 42, wherein the non-human animal is a rodent.

44. The method of claim 43, wherein the rodent is selected from a mouse, a rat, and a hamster.

The claims defining the invention are as follows:

1. A genetically modified non-human animal comprising in its germline an immunoglobulin locus that comprises an unrearranged immunoglobulin variable gene
5 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 substituted or inserted histidine codon is not encoded by a corresponding wild-type gene segment, wherein the non-human animal expresses a nucleotide sequence encoding an immunoglobulin variable domain that comprises a histidine amino acid derived from the substitution of the at least one non-histidine
10 codon or insertion of the at least one histidine codon in the unrearranged immunoglobulin variable gene sequence.
2. The non-human animal of claim 1, wherein the non-human animal comprises a first and a second immunoglobulin variable locus, wherein at least the first or the second
15 immunoglobulin variable locus comprises an unrearranged immunoglobulin variable gene sequence comprising the insertion of the at least one histidine codon or the substitution of the at least one non-histidine codon with a histidine codon.
3. The non-human animal of claim 2, wherein both the first and the second
20 immunoglobulin variable locus each comprise an unrearranged immunoglobulin variable gene sequence comprising the substitution of the at least one non-histidine codon with a histidine codon or the insertion of the at least one histidine codon.
4. The non-human animal of claim 2, wherein the first immunoglobulin variable locus
25 comprises at least a functional portion of an unrearranged immunoglobulin heavy chain variable gene sequence (unrearranged V, D, J segments).
5. The non-human animal of claim 4, wherein the unrearranged immunoglobulin heavy
30 chain variable gene sequence comprises at least a portion of a human immunoglobulin heavy chain gene sequence (unrearranged V, D, J segments).
6. The non-human animal of claim 4, wherein the unrearranged immunoglobulin heavy
35 chain variable gene sequence is a human immunoglobulin heavy chain gene sequence comprising unrearranged V segments, a synthetic D segment that comprises a linker, and a human J segment.

7. The non-human animal of claim 6, wherein the synthetic D segment comprises at least one histidine codon.

8. The non-human animal of claim 2, wherein the second immunoglobulin variable locus comprises at least a functional portion of an unrearranged immunoglobulin light chain variable gene sequence (unrearranged V, J segments).

9. The non-human animal of claim 2, wherein the second immunoglobulin variable locus comprises a rearranged immunoglobulin light chain variable gene sequence (rearranged VJ sequence).

10. The non-human animal of claim 1, wherein the substitution of the at least one non-histidine codon with a histidine codon and/or the insertion of the at least one histidine codon is in a nucleic acid sequence that encodes an immunoglobulin variable domain and the inserted or substituted histidine is in a region selected from an N-terminal region of an immunoglobulin chain, a loop 4 region of an immunoglobulin chain, a CDR1 of an immunoglobulin heavy chain, a CDR2 of an immunoglobulin heavy chain, a CDR3 of an immunoglobulin heavy chain, a CDR1 of an immunoglobulin light chain, a CDR2 of an immunoglobulin light chain, a CDR3 of an immunoglobulin light chain, and a combination thereof.

11. The non-human animal of claim 2, wherein at least one of the first immunoglobulin variable locus or the second immunoglobulin variable locus is operably linked to an endogenous non-human immunoglobulin constant region nucleic acid sequence at an endogenous non-human immunoglobulin locus.

12. The non-human animal of claim 5, wherein the unrearranged immunoglobulin heavy chain variable gene sequence (unrearranged human V, D, J segments) is operably linked to an endogenous non-human immunoglobulin heavy chain constant region nucleic acid sequence.

13. The non-human animal of claim 12, wherein the unrearranged immunoglobulin heavy chain variable gene sequence (unrearranged human V, D, J segments) is operably linked to the endogenous non-human immunoglobulin heavy chain constant region nucleic acid sequence at an endogenous non-human immunoglobulin locus.

14. The non-human animal of claim 8, wherein the immunoglobulin light chain variable gene sequence (unrearranged V, J segments) is operably linked to an endogenous non-human immunoglobulin light chain constant region sequence.

15. The non-human animal of claim 14, wherein the endogenous non-human immunoglobulin light chain constant region sequence is at an endogenous non-human immunoglobulin locus.

16. A genetically modified non-human animal, comprising

at least a portion of a human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence (unrearranged V, D, J segments) operably linked to an immunoglobulin heavy chain constant region gene sequence, wherein one or more of the V, D, and J segments comprise at least one substitution of a non-histidine codon with a histidine codon, or at least one histidine codon insertion, wherein the histidine codon substituted or inserted into the one or more of the V, D and J segments of the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment; and;

at least a portion of a human unrearranged immunoglobulin light chain variable region nucleic acid sequence (unrearranged V, J segments) operably linked to an immunoglobulin light chain constant region gene sequence, wherein one or more of the V and J segments comprise at least one substitution of a non-histidine codon with a histidine codon, or at least one histidine codon insertion, wherein the histidine codon substituted or inserted into one or more of the V and J segments of the human unrearranged immunoglobulin light chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment;

wherein the non-human animal expresses an immunoglobulin heavy chain variable domain and/or an immunoglobulin light chain variable domain that comprises a histidine derived from the at least one histidine codons substituted or inserted into the one or more of the V, D and J segments of the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence and/or one or more of the V and J segments of the human unrearranged immunoglobulin light chain variable region nucleic acid sequence, respectively.

17. The genetically modified non-human animal of claim 16, wherein the non-human animal is a mammal.

18. The genetically modified mammal of claim 17, wherein the mammal is a rodent.

19. The rodent of claim 18, wherein the rodent is selected from the group consisting of a mouse, a rat, and a hamster.

20. The genetically modified non-human animal of claim 16, wherein the human
5 unarranged immunoglobulin heavy chain variable region nucleic acid sequence is operably linked to a non-human immunoglobulin heavy chain constant region sequence.

21. The genetically modified non-human animal of claim 20, wherein the non-human
10 immunoglobulin heavy chain constant region nucleic acid sequence operably linked to the human unarranged immunoglobulin heavy chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin heavy chain locus in the germline of the non-human animal.

22. The genetically modified non-human animal of claim 16, wherein a non-human
15 immunoglobulin light chain constant region nucleic acid sequence is operably linked to the human unarranged immunoglobulin light chain variable region nucleic acid sequence.

23. The genetically modified non-human animal of claim 22, wherein the non-human
20 immunoglobulin light chain constant region nucleic acid sequence operably linked to the human unarranged immunoglobulin light chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin locus in the germline of the non-human animal.

24. A genetically modified non-human animal, comprising
at least a portion of a human unarranged immunoglobulin heavy chain variable
25 region nucleic acid sequence (unarranged V, D, J segments) operably linked to an immunoglobulin heavy chain constant region gene sequence, wherein one or more of the unarranged V, D, and J segments comprise at least one substitution of a non-histidine codon with a histidine codon, or at least one histidine codon insertion, wherein the histidine codon substituted or inserted into the one or more of the V, D and J segments of the human
30 unarranged immunoglobulin heavy chain variable region nucleic acid sequence is not encoded by a corresponding human germline gene segment; and;
a human rearranged immunoglobulin light chain variable region nucleic acid sequence (rearranged VJ sequence) operably linked to an immunoglobulin light chain constant region
35 gene sequence, wherein the rearranged VJ sequence comprises at least one substitution of a non-histidine codon with a histidine codon, or at least one histidine codon insertion, wherein the histidine codon substituted or inserted the rearranged VJ sequence is not encoded by a corresponding human germline gene segment;

wherein the non-human animal expresses an immunoglobulin heavy chain variable domain and/or an immunoglobulin light chain variable domain that comprises a histidine derived from the at least one histidine codons substituted or inserted into the one or more of the V, D and J segments of the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence and/or the rearranged VJ sequence, respectively.

25. The genetically modified non-human animal of claim 24, which is a mammal.

26. The genetically modified mammal of claim 25, which is a rodent.

27. The rodent of claim 26, selected from the group consisting of a mouse, a rat, and a hamster.

28. The genetically modified non-human animal of claim 24, wherein the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is operably linked to a non-human immunoglobulin heavy chain constant region sequence.

29. The genetically modified non-human animal of claim 28, wherein the non-human immunoglobulin heavy chain constant region sequence operably linked to the human unrearranged immunoglobulin heavy chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin heavy chain locus in the germline of the non-human animal.

30. The genetically modified non-human animal of claim 29, wherein a non-human immunoglobulin light chain constant region sequence is operably linked to the human rearranged immunoglobulin light chain variable region nucleic acid sequence.

31. A genetically modified non-human animal, wherein the non-human animal comprises a B cell population that is characterized by an enhanced presence of histidine residues in immunoglobulin chains of the B cell population as compared with a wild-type non-human animal, wherein the enhanced histidine residues are encoded by substitutions and/or insertions of histidine codons not encoded by corresponding gene sequences.

32. The genetically modified non-human animal of claim 31, wherein the enhancement is about 2-4 fold.

33. The genetically modified non-human animal of claim 31, wherein the enhancement is about 2-10 fold.

34. A genetically modified non-human animal that expresses immunoglobulin chains that
5 comprise histidines encoded by substitutions and/or insertions in germline endogenous immunoglobulin sequences of the non-human animal.

35. The genetically modified non-human animal of claim 1, wherein an immunoglobulin variable region sequence in the immunoglobulin variable locus comprises 2, 3, 4, or 5
10 histidines that are substitutions of a histidine codon for a non-histidine codon and/or insertions of histidine codons.

36. The genetically modified non-human animal of claim 5, wherein the unrearranged immunoglobulin heavy chain gene sequence comprises D gene segments that are inverted
15 with respect to the direction of orientation of the immunoglobulin heavy chain gene sequence.

37. A method for making a non-human animal that makes antibody variable domains with histidines encoded by germline histidine codons, comprising:

modifying the non-human animal in its germline to comprise at least one substitution
20 of histidine codon for a non-histidine codon, or insertion of a histidine codon, in an unrearranged immunoglobulin heavy chain variable (unrearranged V, D, J segments) gene sequence, wherein the histidine codon of the unrearranged immunoglobulin heavy chain variable gene segment is not encoded by a corresponding wild-type germ line gene segment; and,

25 modifying the non-human animal in its germline to comprise at least one substitution of a histidine codon for a non-histidine codon, or insertion of a histidine codon, in an unrearranged immunoglobulin light chain variable (unrearranged V, J segments) gene sequence, wherein the histidine codon of the unrearranged immunoglobulin light chain variable gene segment is not encoded by a corresponding wild-type germ line gene segment.

30 38. The method of claim 37, wherein the method comprises genetically modifying the germline of the mouse to comprise at least a portion of a human unrearranged immunoglobulin heavy chain variable (V, D, J segments) gene sequence, and making the at least one histidine substitution or insertion in the unrearranged immunoglobulin heavy chain
35 variable (unrearranged V, D, J segments) human gene sequence.

39. The method of claim 37, wherein the method comprises genetically modifying the germline of the mouse to comprise at least a portion of a human unrearranged immunoglobulin light chain (unrearranged V, J segments) gene sequence, and making the at least one histidine substitution or insertion in the unrearranged human immunoglobulin light chain gene sequence.

40. The method of claim 37, wherein the non-human animal is a rodent.

41. The method of claim 40, wherein the rodent is selected from a mouse, a rat, and a hamster.

42. A method for making a non-human animal that makes antibody variable domains with histidines encoded by germline histidine codons, comprising:

modifying the non-human animal to comprise at least one substitution of histidine codon for a non-histidine codon, or insertion of a histidine codon, in an unrearranged immunoglobulin heavy chain variable (unrearranged V, D, J segments) gene sequence, wherein the histidine codon of the unrearranged immunoglobulin heavy chain variable gene sequence is not encoded by a corresponding wild-type germline gene segment; and,

modifying the non-human animal to comprise at least one substitution of a histidine codon for a non-histidine codon, or insertion of a histidine codon, in a rearranged immunoglobulin light chain variable sequence (rearranged VJ sequence) in the germline, wherein the histidine codon comprised in the rearranged immunoglobulin light chain variable sequence is not encoded by a corresponding wild-type germline gene segment.

43. The method of claim 42, wherein the non-human animal is a rodent.

44. The method of claim 43, wherein the rodent is selected from a mouse, a rat, and a hamster.

45. The non-human animal of claim 8, wherein the unrearranged light chain gene sequence is a human unrearranged light chain gene sequence (unrearranged V, J segments).

46. The non-human animal of claim 9, wherein the rearranged immunoglobulin light chain variable gene sequence is a human rearranged immunoglobulin light chain variable gene sequence (rearranged VJ sequence).

47. The genetically modified non-human animal of claim 30, wherein the non-human immunoglobulin light chain constant region sequence operably linked to the human rearranged immunoglobulin light chain variable region nucleic acid sequence is at an endogenous non-human immunoglobulin light chain locus in the germline of the non-human animal.

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