CLAIMS

1. A nucleotide molecule comprising an engineered immunoglobulin heavy chain diversity (D_H) region ("engineered D_H region") comprising:

(i) an engineered D_H gene segment comprising a D_H gene segment immediately adjacent to a 23-mer RSS ("engineered D_H gene segment") and

(ii) an unrearranged D_H gene segment flanked on its 5' side by a first 12-mer RSS and on its 3' side by a second 12-mer RSS ("unrearranged D_H gene segment"),

wherein (i) the engineered D_H gene segment and (ii) the unrearranged D_H gene segment are operably linked such that (i) the engineered D_H gene segment and (ii) the unrearranged D_H gene segment are able to join in a D_H-D_H recombination event according to the 12/23 rule,

wherein the nucleotide molecule is suitable for obtaining rodents having the engineered D_H region, and

optionally wherein the engineered D_H region comprises only human D_H gene segments.

2. The nucleotide molecule of claim 1, wherein the nucleotide molecule further comprises:

(a) at least one unrearranged immunoglobulin heavy chain variable (V_H) gene segment ("unrearranged V_H gene segment") that is upstream of and operably linked to the engineered D_H region;

(b) at least one unrearranged immunoglobulin heavy chain joining (J_H) gene segment ("unrearranged J_H gene segment") that is downstream of and operably linked to the engineered D_H region; or

(c) a combination of (a) and (b).

3. The nucleotide molecule of claim 2, wherein

(a) the at least one unrearranged V_H gene segment comprises an unrearranged human V_H6-1 gene segment, the at least one unrearranged J_H gene segment comprises an unrearranged human J_H6 gene segment, or a combination thereof;

(b) the at least one unrearranged J_H gene segment comprises an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment;

(c) the at least one unrearranged J_H gene segment comprises an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment, optionally wherein the unrearranged human J_H1 gene segment, the unrearranged human J_H2 gene segment, the unrearranged human J_H3 gene segment, the unrearranged human J_H4 gene segment, the unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene segment are in germline configuration; and/or

(d) the at least one unrearranged V_H gene segment comprises the full repertoire of functional unrearranged human V_H gene segments spanning between and including an unrearranged human V_H3-74 gene segment and an unrearranged human V_H1-6 gene segment ("full repertoire of functional unrearranged human V_H gene segments"), optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration.

4. The nucleotide molecule of any one of the preceding claims, wherein:

(a) the nucleotide molecule further comprises one or more functional rodent Adam6 genes, optionally wherein the one or more functional rodent Adam6 genes is located between a human V_H1-2 gene segment and a human V_H6-1 gene segment and/or replaces a human Adam6 gene;

(b) the engineered D_H gene segment comprises the 23-mer RSS immediately adjacent to the 5'-end of the D_H gene segment;

(c) the engineered D_H gene segment comprises the 23-mer RSS immediately adjacent to the 3'-end of the D_H gene segment;

(d) the engineered D_H gene segment comprises:

(i) a human D_H3-3 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 5'-end of the D_H3-3 gene segment;

(ii) a human D_H2-2 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-2 gene segment;

(iii) a human D_H2-8 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-8 gene segment;

(iv) a human D_H2-15 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-15 gene segment; or

(v) any combination of (i)-(iv);

(e) the nucleotide molecule comprises the nucleotide sequence set forth as SEQ ID NO:52 or comprises the nucleotide sequence set forth as SEQ ID NO:61;

(f) the nucleotide molecule comprises the nucleotide sequence set forth as SEQ ID NO:70;

(g) the nucleotide molecule comprises the nucleotide sequence set forth as SEQ ID NO:71;

(h) the nucleotide molecule comprises the nucleotide sequence set forth as SEQ ID NO:72.

5. The nucleotide molecule of any one of claims 1-4, comprising in operable linkage from 5' to 3':

(a) at least one unrearranged human V_H gene segment;

(b) the engineered D_H region, wherein the engineered D_H gene segment comprises a human D_H3-3 gene segment immediately adjacent to the 23-mer RSS, wherein the 23-mer RSS is immediately adjacent to the 5' end of the human D_H3-3 gene segment, and

(c) at least one unrearranged human J_H gene segment.

6. The nucleotide molecule of any one of claims 1-4, comprising in operable linkage from 5' to 3':

(a) at least one unrearranged human V_H gene segment,

(b) the engineered D_H region, wherein the engineered D_H gene segment comprises a human D_H2 gene segment immediately adjacent to the 23-mer RSS, wherein the 23-mer RSS is immediately adjacent to the 3'end of the human D_H2 gene segment, optionally wherein:

(i) the engineered D_H gene segment comprises a human D_H2-2 gene segment immediately adjacent to 23-mer RSS;

(ii) the engineered D_H gene segment comprises a human D_H2-8 gene segment immediately adjacent to 23-mer RSS;

(iii) the engineered D_H gene segment comprises a human D_H2-15 gene segment immediately adjacent to 23-mer RSS; or

(iv) any combination thereof, and

(c) at least one unrearranged human J_H gene segment.

7. The nucleotide molecule of claim 5 or claim 6, wherein:

(a) the at least one unrearranged human V_H gene segment comprises from 5' to 3' an unrearranged human V_H1-2 gene segment and an unrearranged human V_H6-1 gene segment, wherein the nucleotide further comprises one or more functional rodent Adam6 genes between the unrearranged human V_H1-2 gene segment and the unrearranged human V_H6-1 gene segment, and wherein the unrearranged human V_H1-2 gene segment, the one or more functional rodent Adam6 genes, and the unrearranged human V_H6-1 gene segment are contiguous;

(b) the at least one unrearranged human V_H gene segment comprises the full repertoire of functional unrearranged human V_H gene segments, optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration; and/or

(c) the at least one unrearranged human J_H gene segment comprises an unrearranged human J_H6 gene segment, optionally wherein:

(i) the at least one unrearranged human J_H gene segment comprises an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene segment; and/or

(ii) wherein the at least one unrearranged human J_H gene segment comprises an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment, further optionally wherein the unrearranged human J_H1 gene segment, the unrearranged J_H2 gene segment, the unrearranged J_H3 gene segment, the unrearranged J_H4 gene segment, the unrearranged J_H5 gene segment, and the unrearranged J_H6 gene segment are in germline configuration.

8. The nucleotide molecule of any one of claims 1-7, further comprising:

(a) at its 3'-end, a heavy chain immunoglobulin constant (C_H) region or a portion thereof;

(b) at its 3'-end, a rodent C_H region or a portion thereof;

(c) at its 3’ end, a rodent C_H region or a portion thereof comprising a rodent intronic enhancer region and a rodent IgM gene; and/or

(d) a drug selection cassette located upstream of the engineered D_H gene segment, optionally wherein the drug selection cassette is flanked by one or more site-specific recombination sites.

9. A targeting vector comprising a nucleotide molecule of any one of claims 1-8, comprising 5’- and 3’- homology arms configured to allow homologous recombination with an immunoglobulin heavy chain sequence at an endogenous rodent immunoglobulin heavy chain locus, optionally wherein the immunoglobulin heavy chain sequence comprises a human or humanized immunoglobulin heavy chain variable region.

10. A method of modifying an immunoglobulin heavy chain variable region to engineer D_H-D_H recombination, the method comprising:

(a) obtaining an immunoglobulin heavy chain variable chain sequence comprising a D_H region comprising one or more unrearranged D_H gene segments, each of which unrearranged D_H gene segment is flanked on its 5’ side by a first 12-mer RSS and on its 3’ side by a second 12-mer RSS,

optionally wherein the one or more unrearranged D_H gene segments comprises at least one unrearranged human D_H gene segment, further optionally wherein the at least one unrearranged human D_H gene segment comprises all the functional human D_H gene segments spanning between and including an unrearranged human D_H1-1 gene segment and an unrearranged human D_H 7-27 gene segment, optionally wherein all the functional human D_H gene segments are in germline configuration, and

(b) engineering the D_H region to further comprise at least one engineered D_H gene segment, wherein the engineered D_H gene segment comprises a D_H gene segment immediately adjacent to a 23-mer RSS,

wherein, in the resulting engineered D_H region, the engineered D_H gene segment and at least one of the one or more unrearranged D_H gene segments are operably linked and able to join in a D_H-D_H recombination event according to the 12/23 rule,

optionally wherein the resulting engineered D_H region comprises only human D_H gene segments.

11. The method of claim 10, wherein the D_H region of claim 10(a) comprises two or more unrearranged D_H gene segments, each of which is flanked on its 5' side by a first 12-mer RSS and on its 3' side by a second 12-mer RSS, and

wherein engineering comprises

(a) replacing at least one of the two or more unrearranged D_H gene segments with the engineered D_H gene segment as defined in claim 10(b),

(b) replacing the first 12-mer RSS or the second 12-mer RSS of at least one of the two or more unrearranged D_H gene segments with a 23-mer RSS, or

(c) a combination of (a) and (b),

optionally, wherein the immunoglobulin heavy chain variable chain sequence further comprises a J_H region comprising at least one unrearranged J_H gene segment,

wherein the J_H region is operably linked to the D_H region, and

wherein replacing at least one of the two or more unrearranged D_H gene segments with the engineered D_H gene segment as defined in claim 10(b) comprises deleting the at least one unrearranged J_H gene segment,

further optionally wherein the J_H region comprises a full repertoire of human germline J_H gene segments comprising an unrearranged human J_H1 gene segment, an unrearranged human J_H2 gene segment, an unrearranged human J_H3 gene segment, an unrearranged human J_H4 gene segment, an unrearranged human J_H5 gene segment, and an unrearranged human J_H6 gene segment, optionally wherein the unrearranged human J_H1 gene segment, the unrearranged human J_H2 gene segment, the unrearranged human J_H3 gene segment, the unrearranged human J_H4 gene segment, the unrearranged human J_H5 gene segment, and the unrearranged human J_H6 gene segment are in germline configuration, and

wherein deleting at least one germline J_H gene segment comprises deleting the unrearranged human J_H1 gene segment, the unrearranged human J_H2 gene segment, and the unrearranged human J_H3 gene segment, and optionally further deleting the unrearranged human J_H4 gene segment and the unrearranged human J_H5 gene segment.

12. The method of claim 10 or 11, wherein:

(a) the engineered D_H gene segment as defined in claim 10(b) comprises

(i) a human D_H3-3 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 5'-end of the D_H3-3 gene segment,

(ii) a human D_H2-2 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-2 gene segment,

(iii) a human D_H2-8 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-8 gene segment,

(iv) a human D_H2-15 gene segment immediately adjacent to the 23-mer RSS, optionally wherein the 23-mer RSS is immediately adjacent to the 3'-end of the D_H2-15 gene segment, or

(v) any combination of (i)-(iv);

(b) engineering comprises replacing the most 3' unrearranged D_H gene segment of the D_H region as defined in claim 10(a) with the engineered D_H gene segment as defined in claim 10(b); and/or

(c) engineering comprises replacing at least one of the one or more unrearranged D_H gene segments that corresponds to the engineered D_H gene segment as defined in claim 10(b) with the engineered D_H gene segment as defined in claim 10(b).

13. An immunoglobulin heavy chain locus comprising the nucleotide molecule of any one of claims 1-8, the targeting vector of claim 9, or the immunoglobulin heavy chain variable region modified according to the method of any one of claims 10-12.

14. A rodent genome comprising the nucleotide molecule of any one of claims 1-8, the targeting vector of claim 9, the immunoglobulin heavy chain variable region modified according to the method of any one of claims 10-12, or the immunoglobulin heavy chain locus of claim 13, wherein the rodent genome is optionally a rodent germline genome.

15. The rodent genome of claim 14, wherein the rodent genome comprises an immunoglobulin heavy chain variable region that comprises:

(a) unrearranged V_H gene segments,

(b) the engineered D_H region, optionally wherein the engineered D_H region comprises only human D_H gene segments, and

(c) unrearranged J_H gene segments,

wherein (a)-(c) are in operable linkage such that, upon recombination, the immunoglobulin heavy chain variable region comprises a rearranged heavy chain variable region sequence encoding an immunoglobulin heavy chain variable domain, optionally wherein the rearranged heavy chain variable region sequence is formed after a $V_H(D_H\text{-}D_H)J_H$ recombination event,

optionally wherein:

(aa) the engineered D_H gene segment comprises a D_H segment immediately adjacent to a 3' 23-mer RSS;

(bb) the engineered D_H gene segments comprises a D_H gene segment immediately adjacent to a 5' 23-mer RSS;

(cc) the immunoglobulin heavy chain variable region is a human immunoglobulin heavy chain variable region comprising only human V_H gene segments, human D_H gene segments, and human J_H gene segments, optionally wherein the human immunoglobulin heavy chain variable region is operably linked to a C_H region, further optionally wherein the C_H region is an endogenous C_H region, optionally at an endogenous immunoglobulin heavy chain locus;

(dd) the unrearranged V_H gene segments comprise the full repertoire of functional unrearranged human V_H gene segments, optionally wherein the full repertoire of functional unrearranged human V_H gene segments is in germline configuration;

(ee) the unrearranged J_H gene segments comprise a human J_H6 gene segment; and/or

(ff) (i) the rodent genome lacks an endogenous Adam6 gene; or

(ii) the rodent genome further comprises one or more rodent Adam6 genes, optionally wherein:

the one or more rodent Adam6 genes comprises an endogenous rodent Adam6 gene;

the one or more rodent Adam6 genes are inserted between two human V_H gene segments,

optionally wherein the one or more rodent Adam6 genes are inserted between an unrearranged human $V_H1\text{-}2$ gene segment and an unrearranged human $V_H6\text{-}1$ gene segment

the one or more one or more rodent Adam6 genes are inserted in the place of a human Adam6 pseudogene; and/or

the one or more one or more rodent Adam6 genes are inserted between an unrearranged human V_H gene segment and an unrearranged human D_H gene segment.

16. The rodent genome of claim 14 or 15, wherein:

(a) the rodent genome further comprises a terminal deoxynucleotidyl transferase gene;

(b) the rodent genome is characterized in that it is homozygous for the engineered D_H region or the rodent genome is characterized in that it is heterozygous for the engineered D_H region; and/or

(c) the rodent genome is a rat genome or a mouse genome.

17. A rodent cell comprising the rodent genome of any one of claims 14 to 16, optionally wherein the rodent cell is a rat cell or a mouse cell.

18. The rodent cell of claim 17, wherein the rodent cell is a rodent embryonic stem cell.

19. A rodent generated from the rodent embryonic stem cell of claim 18, wherein the rodent comprises the rodent genome in its germline.

20. The rodent of claim 19, wherein:

(a) the rodent further comprises a somatic genome that comprises a rearranged heavy chain $V_H(D_HA\text{-}D_HB)J_H$ coding sequence,

optionally wherein the rearranged heavy chain $V_H(D_HA\text{-}D_HB)J_H$ coding sequence is operably linked to an IgM constant region gene sequence and/or wherein D_HA or D_HB is derived from the engineered D_H gene segment or a portion thereof and at least 9 base pairs of D_HA or D_HB is identical to at least 9 base pairs of the engineered D_H gene segment; and/or

(b) at least 95% of all rearranged heavy chain VDJ coding sequences in the rodent have a CDR3 length of at least 10 amino acids, optionally wherein at least 70% of all rearranged heavy chain VDJ gene sequences in the rodent have a CDR3 length of at least 11 amino acids, optionally wherein at least 15% of all rearranged heavy chain VDJ gene sequences in the rodent have a CDR3 length of at least 14 amino acids, further optionally characterized in that the rodent exhibits at least a at least a 3-fold increased frequency of D_H-D_H recombination as compared to the frequency of D_H-D_H recombination in a reference rodent.

21. A method of making a rodent whose genome comprises an engineered D_H region, the method comprising

(A) generating the rodent from the embryonic stem cell of claim 18, or

(B)

(a) modifying the genome of a rodent embryonic stem cell to comprise a DNA fragment comprising one or more engineered D_H gene segments, each comprising a D_H gene segment immediately adjacent to a 23-mer RSS, optionally wherein the DNA fragment comprises the nucleotide molecule of any one of claims 1-8, the targeting vector of claim 9, the immunoglobulin heavy chain variable region modified according to the method of any one of claims 10-12, or the immunoglobulin heavy chain of claim 13, and

(b) generating the rodent using the modified rodent embryonic stem cell of (B)(a).

22. The method of claim 21, wherein the rodent is a rat or a mouse.

23. A method of producing an antibody or obtaining a nucleic acid encoding same, the method comprising

immunizing a rodent with an antigen, which rodent comprises a germline genome comprising an engineered D_H region that comprises one or more engineered D_H gene segments that are each immediately adjacent to a 23-mer RSS, optionally wherein the rodent is the rodent according to any one of claims 19 to 20 or made according to claim 21, and

allowing the rodent to produce an immune response to the antigen including an antibody, or nucleic acid encoding same, that binds the antigen,

optionally wherein:

(a) the method is one further comprising recovering the antibody, or nucleic acid encoding same, from the rodent or a rodent cell, wherein the rodent cell is a B cell or a hybridoma;

(b) one or more engineered D_H gene segments that are each immediately adjacent to a 23-mer RSS comprises a D_H gene segment operably linked to a 5' 23-mer RSS;

(c) one or more engineered D_H gene segments that are each immediately adjacent to a 23-mer RSS comprises a D_H gene segment operably linked to a 3' 23-mer RSS; and/or

(d) the rodent is a rat or a mouse.