CLAIMS

What is claimed is:

1. A method for modifying a target genomic locus in a mouse embryonic stem (ES) cell, comprising:

(a) introducing into the mouse ES cell:

(i) a zinc finger nuclease (ZFN) that makes a single or double-strand break at or near the target genomic locus; and

(ii) a large targeting vector (LTVEC) comprising an insert nucleic acid flanked by an upstream homology arm and a downstream homology arm, wherein the insert nucleic acid ranges from about 5 kb to about 30 kb in length, and the sum total of the upstream and downstream homology arms is at least 10 kb; and

(b) selecting a targeted mouse ES cell comprising the insert nucleic acid in the target genomic locus, wherein integration of the insert nucleic acid into the target genomic locus results in deletion of an endogenous nucleic acid sequence at the target genomic locus and replacement with the insert nucleic acid, wherein combined use of the LTVEC with the ZFN results in an increased targeting efficiency compared to use of the LTVEC alone.

2. The method of claim 1, wherein the targeting efficiency of the LTVEC is increased at least 2-fold compared to use of the LTVEC alone.

3. The method of claim 1 or 2, wherein an expression construct comprising a nucleic acid sequence encoding the ZFN is introduced into the mouse ES cell, and wherein the nucleic acid sequence is operably linked to a promoter active in the mouse ES cell.

4. The method of claim 1 or 2, wherein an mRNA encoding the ZFN is introduced into the mouse ES cell.

5. The method of any one of claims 1-4, wherein a target sequence of the ZFN is located in an intron, exon, a promoter, a promoter regulatory region, or an enhancer region in the target genomic locus.

6. The method of any one of claims 1-5, wherein the insert nucleic acid

comprises a selection cassette, a reporter gene, a nucleic acid flanked by site-specific recombination target sequences, or a conditional allele.

7. The method of any one of claims 1-6, wherein the insert nucleic acid comprises a nucleic acid sequence from a different species.

8. The method of claim 7, wherein the insert nucleic acid comprises a human nucleic acid sequence.

9. The method of claim 8, wherein the insert nucleic acid comprises a genomic nucleic acid sequence that encodes a human immunoglobulin heavy chain variable region amino acid sequence.

10. The method of claim 8, wherein the insert nucleic comprises a genomic nucleic acid sequence that encodes a human immunoglobulin light chain variable region amino acid sequence.

11. The method of claim 10, wherein the genomic nucleic acid sequence comprises an unrearranged human λ and/or κ light chain variable region nucleic acid sequence.

12. The method of claim 10, wherein the genomic nucleic acid sequence comprises a rearranged human λ and/or κ light chain variable region nucleic acid sequence.

13. The method of claim 8, wherein the insert nucleic acid comprises a human disease allele.

14. The method of claim 9, wherein the insert nucleic acid comprises a rearranged human immunoglobulin heavy chain variable region nucleic acid sequence operably linked to an immunoglobulin heavy chain constant region nucleic acid sequence.

15. The method of claim 9, wherein the insert nucleic acid comprises an unrearranged human immunoglobulin heavy chain variable region nucleic acid sequence operably linked to an immunoglobulin heavy chain constant region nucleic acid sequence.

16. The method of claim 14 or 15, wherein the immunoglobulin heavy chain

constant region sequence is a mouse immunoglobulin heavy chain constant region sequence, a human immunoglobulin heavy chain constant region sequence, or a combination thereof.

17. The method of any one of claims 14-16, wherein the immunoglobulin heavy chain constant region nucleic acid sequence is selected from a C_H1, a hinge, a C_H2, a C_H3, and a combination thereof.

18. The method of claim 11, wherein the insert nucleic acid comprises an unrearranged human λ or κ light chain variable region nucleic acid sequence operably linked to a mouse or human immunoglobulin light chain constant region nucleic acid sequence selected from a λ light chain constant region nucleic acid sequence and a κ light chain constant region nucleic acid sequence.

19. The method of claim 12, wherein the insert nucleic acid comprises a rearranged human λ or κ light chain variable region nucleic acid sequence operably linked to a mouse or human immunoglobulin light chain constant region nucleic acid sequence selected from a λ light chain constant region nucleic acid sequence and a κ light chain constant region nucleic acid sequence.

20. The method of any one of claims 1-19, wherein the insert nucleic acid comprises a nucleic acid sequence homologous or orthologous to the replaced nucleic acid sequence at the target genomic locus of the mouse ES cell.

21. The method of any one of claims 1-20, wherein integration of the insert nucleic acid into the target genomic locus results in a knockout, a knock-in, a point mutation, a domain swap, an exon swap, an intron swap, a regulatory sequence swap, a gene swap, or a combination thereof.

22. The method of any one of claims 1-21, wherein selecting step (b) is carried out via a modification of allele (MOA) assay.

23. The method of any one of claims 1-22, wherein the LTVEC is from about 50 kb to about 300 kb.

24. The method of any one of claims 1-23, wherein the sum total of the

upstream and the downstream homology arms is from 10 kb to about 200 kb.

25. The method of any one of claims 1-24, wherein each of the upstream and downstream homology arms is between about 5 kb and about 200 kb in length.

26. The method of any one of claims 1-25, wherein each of the upstream and downstream homology arms is between about 5 kb and about 200 kb in length, and wherein the LTVEC ranges from about 50 kb to about 300 kb in length.

27. The method of any one of claims 1-26, wherein integration of the insert nucleic acid into the target genomic locus results in a deletion of an endogenous gene at the target genomic locus.

28. The method of any one of claims 1-27, further comprising:

(a) introducing the modified mouse ES cell into a pre-morula stage embryo;

(b) incubating the embryo until the blastocyst stage and implanting the embryo into a surrogate mother to produce an F0 mouse; and

(c) identifying a mouse bearing the genetically modified genomic locus.

29. A modified mouse embryonic stem (ES) cell produced by the method of any one of claims 1 to 27.

30. A mouse bearing a genetically modified genomic locus produced by the method of claim 28.