We claim:

1. A method for modifying a genome at a genomic locus of interest in a mouse cell or a human cell, comprising contacting the genome with a Cas protein, a CRISPR RNA that hybridizes to a target sequence at the genomic locus of interest, and a tracrRNA in the presence of a large targeting vector (LTVEC),

wherein the LTVEC is at least 10 kb and comprises a first nucleic acid flanked by a 5' homology arm that is homologous to a 5' target sequence at the genomic locus of interest and a 3' homology arm that is homologous to a 3' target sequence at the genomic locus of interest,

wherein following contacting with the Cas protein, the CRISPR RNA, and the tracrRNA in the presence of the LTVEC, the genome is modified to comprise a targeted genetic modification comprising deletion of a region of the genomic locus of interest and insertion of the first nucleic acid at the genomic locus of interest,

wherein:

(i) the region of the genomic locus of interest deleted is at least 30 kb, and/or

(ii) the inserted first nucleic acid is at least 30 kb.

2. The method of claim 1, wherein the Cas protein, the CRISPR RNA, the tracrRNA, and the LTVEC are introduced into the mouse cell or the human cell.

3. The method of claim 1 or 2, further comprising identifying the modified mouse cell or the modified human cell comprising the targeted genetic modification at the genomic locus of interest.

4. The method of claim 2 or 3, wherein the CRISPR RNA and the tracrRNA are introduced together in the form of a single transcript.

5. The method of claim 2 or 3, wherein the CRISPR RNA and the tracrRNA

are introduced separately.

6. The method of any one of claims 2-5, wherein:

(a) the Cas protein is introduced into the mouse cell or the human cell in the form of a protein, a messenger RNA (mRNA) encoding the Cas protein, or a DNA encoding the Cas protein;

(b) the CRISPR RNA is introduced into the mouse cell or the human cell in the form of an RNA or a DNA encoding the CRISPR RNA; and

(c) the tracrRNA is introduced into the mouse cell or the human cell in the form of an RNA or a DNA encoding the tracrRNA.

7. The method of claim 6, wherein the Cas protein, the CRISPR RNA, and the tracrRNA are introduced into the mouse cell or the human cell as a protein-RNA complex.

8. The method of claim 6, wherein:

(a) the DNA encoding the Cas protein is in the form of a first expression construct comprising a first promoter operably linked to a second nucleic acid encoding the Cas protein;

(b) the DNA encoding the CRISPR RNA is in the form of a second expression construct comprising a second promoter operably linked to a third nucleic acid encoding the CRISPR RNA; and

(c) the DNA encoding the tracrRNA is in the form of a third expression construct comprising a third promoter operably linked to a fourth nucleic acid encoding the tracrRNA;

wherein the first, second, and third promoters are active in the mouse cell or the human cell.

9. The method of claim 8, wherein the first, second, and/or third expression

constructs are on a single nucleic acid molecule.

10. The method of claim 6, wherein:

(a) the DNA encoding the Cas protein is in the form of a first expression construct comprising a first promoter operably linked to a second nucleic acid encoding the Cas protein; and

(b) the DNA encoding the CRISPR RNA and the DNA encoding the tracrRNA are in the form of a second expression construct comprising a second promoter operably linked to a third nucleic acid encoding a gRNA comprising the CRISPR RNA and the tracrRNA in a single transcript;

wherein the first and second promoters are active in the mouse cell or the human cell.

11. The method of claim 10, wherein the first and the second expression constructs are on a single nucleic acid molecule.

12. The method of any preceding claim, wherein the targeted genetic modification comprises simultaneous deletion of an endogenous nucleic acid sequence at the genomic locus of interest and insertion of the first nucleic acid at the genomic locus of interest in a single step.

13. The method of claim 12, wherein the deleted endogenous nucleic acid sequence is from about 30 kb to about 110 kb, and the inserted first nucleic acid is from about 40 kb to about 140 kb.

14. The method of any preceding claim, wherein the targeted genetic modification is a biallelic genetic modification.

15. The method of claim 14, wherein the biallelic genetic modification comprises deletion of an endogenous nucleic acid sequence and insertion of the first nucleic acid at the genomic locus of interest in two homologous chromosomes.

16. The method of claim 14, wherein the modified mouse cell or the modified human cell is compound heterozygous at the genomic locus of interest.

17. The method of claim 14, wherein the modified mouse cell or the modified human cell is hemizygous at the genomic locus of interest.

18. The method of claim 16, wherein the targeted genetic modification at the genomic locus of interest in one chromosome comprises deletion of an endogenous nucleic acid sequence and insertion of the first nucleic acid.

19. The method of claim 16, wherein the targeted genetic modification comprises: (1) deletion of an endogenous nucleic acid sequence at the genomic locus of interest in first and second homologous chromosomes; and (2) insertion of the first nucleic acid into the genomic locus of interest in the first homologous chromosome and disruption of the genomic locus of interest in the second homologous chromosome.

20. The method of any preceding claim, wherein the LTVEC is at least 15 kb, at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, or at least 90 kb.

21. The method of any preceding claim, wherein the LTVEC is at least 100 kb, at least 150 kb, or at least 200 kb.

22. The method of any preceding claim, wherein the first nucleic acid is at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, at least 200 kb, at least 250 kb, or at least 300 kb.

23. The method of any preceding claim, wherein the first nucleic acid is about 40 kb to about 140 kb.

24. The method of any preceding claim, wherein the mouse cell or the human cell is a primary cell or an immortalized cell.

25. The method of any one of claims 1-23, wherein the mouse cell or the human cell is a pluripotent cell.

26. The method of claim 25, wherein the mouse pluripotent cell is a mouse embryonic stem (ES) cell.

27. The method of claim 25, wherein the human pluripotent cell is a human embryonic stem (ES) cell, a human adult stem cell, a developmentally restricted human progenitor cell, or a human induced pluripotent stem (iPS) cell.

28. The method of claim 27, wherein the human iPS cells is being maintained in a medium comprising a base medium and supplements, wherein the medium comprises:

(a) a leukemia inhibitory factor (LIF) polypeptide;

(b) a glycogen synthase kinase (GSK3) inhibitor; and

(c) a MEK inhibitor;

wherein the medium has an osmolality of about 175 mOsm/kg to about 280 mOsm/kg.

29. The method of any preceding claim, wherein the Cas protein is Cas9.

30. The method of any preceding claim, wherein the target sequence is immediately flanked by a Protospacer Adjacent Motif (PAM) sequence.

31. The method of any preceding claim, wherein the sum total of the 5' and the 3' homology arms of the LTVEC is from about 10 kb to about 150 kb.

32. The method of any preceding claim, wherein the sum total of the 5' and the 3' homology arms of the LTVEC is from about 10 kb to about 20 kb, from about 20 kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 120 kb, or from about 120 kb to 150 kb.

33. The method of any preceding claim, wherein the targeted genetic

modification comprises:

(a) a replacement of an endogenous nucleic acid sequence with a homologous or an orthologous nucleic acid sequence;

(b) a deletion of an endogenous nucleic acid sequence;

(c) a deletion of an endogenous nucleic acid sequence,

wherein the deletion ranges from about 5 kb to about 10 kb, from about 10 kb to about 20 kb, from about 20 kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 150 kb, or from about 150 kb to about 200 kb, from about 200 kb to about 300 kb, from about 300 kb to about 400 kb, from about 400 kb to about 500 kb, from about 500 kb to about 1 Mb, from about 1 Mb to about 1.5 Mb, from about 1.5 Mb to about 2 Mb, from about 2 Mb to about 2.5 Mb, or from about 2.5 Mb to about 3 Mb;

(d) insertion of an exogenous nucleic acid sequence;

(e) insertion of an exogenous nucleic acid sequence ranging from about 5 kb to about 10 kb, from about 10 kb to about 20 kb, from about 20 kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 150 kb, from about 150 kb to about 200 kb, from about 200 kb to about 250 kb, from about 250 kb to about 300 kb, from about 300 kb to about 350 kb, or from about 350 kb to about 400 kb;

(f) insertion of an exogenous nucleic acid sequence comprising a homologous or an orthologous nucleic acid sequence;

(g) insertion of a chimeric nucleic acid sequence comprising a human and a non-human nucleic acid sequence;

(h) insertion of a conditional allele flanked by site-specific recombinase target sequences;

(i) insertion of a selectable marker or a reporter gene operably linked to a promoter active in the pluripotent cell; or

(j) a combination thereof.

34. The method of any preceding claim, wherein the 5' target sequence and the 3' target sequence are separated by at least 5 kb but less than 3 Mb.

35. The method of any preceding claim, wherein the 5' target sequence and the 3' target sequence are separated by at least 5 kb but less than 10 kb, at least 10 kb but less than 20 kb, at least 20 kb but less than 40 kb, at least 40 kb but less than 60 kb, at least 60 kb but less than 80 kb, at least about 80 kb but less than 100 kb, at least 100 kb but less than 150 kb, or at least 150 kb but less than 200 kb, at least about 200 kb but less than about 300 kb, at least about 300 kb but less than about 400 kb, at least about 400 kb but less than about 500 kb, at least about 500 kb but less than about 1Mb, at least about 1 Mb but less than about 1.5 Mb, at least about 1.5 Mb but less than about 2 Mb, at least about 2 Mb but less than about 2.5 Mb, or at least about 2.5 Mb but less than about 3 Mb.

36. The method of any preceding claim, wherein the 5' target sequence and the 3' target sequence are separated by at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 110 kb, at least 120 kb, at least 130 kb, at least 140 kb, at least 150 kb, at least 160 kb, at least 170 kb, at least 180 kb, at least 190 kb, or at least 200 kb.

37. The method of any preceding claim, wherein the 5' target sequence and the 3' target sequence are separated by from about 30 kb to about 110 kb.

38. The method of any preceding claim, wherein the genomic locus of interest comprises the Interleukin-2 receptor gamma locus, the ApoE locus, the Rag1 locus, the Rag2 locus, both of the Rag1 and the Rag2 loci, the Adamts5 locus, the Trpa1 locus, the Folh1 locus, the Erbb4 locus, the Lrp5 locus, the C5 (Hc) locus, the Ror1 locus, or the Dpp4 locus.

39. The method of any preceding claim, wherein the genomic locus of interest

comprises extrachromosomal DNA.

40. A method for producing an F0 generation mouse that comprises a targeted genetic modification at a genomic locus of interest, comprising:

(a) contacting the genome in a mouse ES cell with a Cas protein, a CRISPR RNA that hybridizes to a target sequence at the genomic locus of interest, and a tracrRNA in the presence of a large targeting vector (LTVEC) to form a modified mouse ES cell,

wherein the LTVEC is at least 10 kb and comprises a first nucleic acid flanked by a 5' homology arm that is homologous to a 5' target sequence at the genomic locus of interest and a 3' homology arm that is homologous to a 3' target sequence at the genomic locus of interest,

wherein following contacting with the Cas protein, the CRISPR RNA, and the tracrRNA in the presence of the LTVEC, the genome is modified to comprise the targeted genetic modification, wherein the targeted genetic modification comprises deletion of a region of the genomic locus of interest and insertion of the first nucleic acid at the genomic locus of interest,

wherein:

(i) the region of the genomic locus of interest deleted is at least 30 kb, and/or

(ii) the inserted first nucleic acid is at least 30 kb;

(b) identifying the modified mouse ES cell comprising the targeted genetic modification at the genomic locus of interest;

(c) introducing the modified mouse ES cell into a mouse host embryo; and

(d) gestating the mouse host embryo in a surrogate mother,

wherein the surrogate mother produces the F0 generation mouse comprising the

targeted genetic modification at the genomic locus of interest.

41. The method of claim 40, wherein the CRISPR RNA and the tracrRNA are introduced together in the form of a single transcript.

42. The method of claim 40, wherein the CRISPR RNA and the tracrRNA are introduced separately.

43. The method of any one of claims 40-42, wherein:

(a) the Cas protein is introduced into the mouse ES cell in the form of a protein, a messenger RNA (mRNA) encoding the Cas protein, or a DNA encoding the Cas protein;

(b) the CRISPR RNA is introduced into the mouse ES cell in the form of an RNA or a DNA encoding the CRISPR RNA; and

(c) the tracrRNA is introduced into the mouse ES cell in the form of an RNA or a DNA encoding the tracrRNA.

44. The method of claim 43, wherein the Cas protein, the CRISPR RNA, and the tracrRNA are introduced into the mouse ES cell as a protein-RNA complex.

45. The method of claim 43, wherein:

(a) the DNA encoding the Cas protein is in the form of a first expression construct comprising a first promoter operably linked to a second nucleic acid encoding the Cas protein;

(b) the DNA encoding the CRISPR RNA is in the form of a second expression construct comprising a second promoter operably linked to a third nucleic acid encoding the CRISPR RNA; and

(c) the DNA encoding the tracrRNA is in the form of a third expression construct comprising a third promoter operably linked to a fourth nucleic acid encoding the

tracrRNA;

wherein the first, second, and third promoters are active in the mouse ES cell.

46. The method of claim 45, wherein the first, second, and third expression constructs are on a single nucleic acid molecule.

47. The method of claim 43, wherein:

(a) the DNA encoding the Cas protein is in the form of a first expression construct comprising a first promoter operably linked to a second nucleic acid encoding the Cas protein; and

(b) the DNA encoding the CRISPR RNA and the DNA encoding the tracrRNA are in the form of a second expression construct comprising a second promoter operably linked to a third nucleic acid encoding a gRNA comprising the CRISPR RNA and the tracrRNA in a single transcript;

wherein the first and second promoters are active in the mouse ES cell.

48. The method of claim 47, wherein the first and the second expression constructs are on a single nucleic acid molecule.

49. The method of any one of claims 40-48, wherein the targeted genetic modification comprises simultaneous deletion of an endogenous nucleic acid sequence at the genomic locus of interest and insertion of the first nucleic acid at the genomic locus of interest.

50. The method of any one of claims 40-49, wherein the targeted genetic modification is a biallelic genetic modification.

51. The method of claim 50, wherein the biallelic genetic modification comprises deletion of an endogenous nucleic acid sequence and insertion of the first nucleic acid at the genomic locus of interest in two homologous chromosomes.

52. The method of claim 50, wherein the modified mouse ES cell is

compound heterozygous at the genomic locus of interest.

53. The method of claim 50, wherein the modified mouse ES cell is hemizygous at the genomic locus of interest.

54. The method of claim 52, wherein the targeted genetic modification at the genomic locus of interest in one chromosome comprises deletion of an endogenous nucleic acid sequence and insertion of the first nucleic acid.

55. The method of claim 52, wherein the targeted genetic modification comprises: (1) deletion of an endogenous nucleic acid sequence at the genomic locus of interest in first and second homologous chromosomes; and (2) insertion of the first nucleic acid into the genomic locus of interest in the first homologous chromosome and disruption of the genomic locus of interest in the second homologous chromosome.

56. The method of any one of claims 40-55, wherein the Cas protein is Cas9.

57. A method for modifying a genome at a genomic locus of interest in a eukaryotic cell, comprising contacting the genome with a Cas protein, a CRISPR RNA that hybridizes to a target sequence at the genomic locus of interest, and a tracrRNA in the presence of a large targeting vector (LTVEC),

wherein the LTVEC is at least 10 kb and comprises a first nucleic acid flanked by a 5' homology arm that is homologous to a 5' target sequence at the genomic locus of interest and a 3' homology arm that is homologous to a 3' target sequence at the genomic locus of interest,

wherein the eukaryotic cell is not a rat cell, and

wherein following contacting with the Cas protein, the CRISPR RNA, and the tracrRNA in the presence of the LTVEC, the genome is modified to comprise a targeted genetic modification comprising deletion of a region of the genomic locus of interest and insertion of the first nucleic acid at the genomic locus of interest,

wherein:

(i) the region of the genomic locus of interest deleted is at least 30 kb, and/or

(ii) the inserted first nucleic acid is at least 30 kb.

58. The method of claim 57, wherein the non-rat eukaryotic cell is a pluripotent cell, a non-pluripotent cell, a mammalian cell, a human cell, a non-human mammalian cell, a rodent cell, a mouse cell, a hamster cell, or a fibroblast.

59. The method of claim 57, wherein the non-rat eukaryotic cell is a primary cell or an immortalized cell.

CLAIMS

1. An *in vitro* method for modifying a genome at a genomic locus of interest in a mouse embryonic stem (ES) cell, comprising:

contacting the mouse ES cell with a Cas9 protein, a CRISPR RNA that hybridizes to a CRISPR target sequence at the genomic locus of interest, a tracrRNA, and a large targeting vector (LTVEC) that is at least 10 kb in size and comprises an insert nucleic acid flanked by:

(i) a 5' homology arm that is homologous to a 5' target sequence at the genomic locus of interest; and

(ii) a 3' homology arm that is homologous to a 3' target sequence at the genomic locus of interest,

wherein following contacting the mouse ES cell with the Cas9 protein, the CRISPR RNA, and the tracrRNA in the presence of the LTVEC, the genome of the mouse ES cell is modified to comprise a targeted genetic modification comprising deletion of a region of the genomic locus of interest wherein the deletion is at least 30 kb and/or insertion of the insert nucleic acid at the genomic locus of interest wherein the insertion is at least 30 kb.

2. The method of claim 1, wherein the CRISPR RNA and the tracrRNA are introduced as a single nucleic acid molecule comprising the CRISPR RNA and the tracrRNA.

3. The method of claim 2, wherein the single nucleic acid molecule comprises the CRISPR RNA and the tracrRNA fused together in the form of a single guide RNA (sgRNA).

4. The method of claim 1, wherein the CRISPR RNA and the tracrRNA are introduced separately.

5. The method of any preceding claim, wherein:

(a) the Cas9 protein is introduced in the form of a protein, a messenger RNA (mRNA) encoding the Cas9 protein, or a DNA encoding the Cas9 protein;

(b) the CRISPR RNA is introduced in the form of an RNA or a DNA encoding the CRISPR RNA; and

(c) the tracrRNA is introduced in the form of an RNA or a DNA encoding the tracrRNA.

6. The method of claim 5, wherein the Cas9 protein, the CRISPR RNA, and the tracrRNA are introduced as a protein-RNA complex.

7. The method of claim 5, wherein:

(a) the DNA encoding the Cas9 protein is in the form of a first expression construct comprising a first promoter operably linked to a first nucleic acid encoding the Cas9 protein;

(b) the DNA encoding the CRISPR RNA is in the form of a second expression construct comprising a second promoter operably linked to a second nucleic acid encoding the CRISPR RNA; and

(c) the DNA encoding the tracrRNA is in the form of a third expression construct comprising a third promoter operably linked to a third nucleic acid encoding the tracrRNA;

wherein the first, second, and third promoters are active in the mouse ES cell, and

wherein the first, second, and third expression constructs are on a single nucleic acid molecule or on multiple nucleic acid molecules.

8. The method of claim 5, wherein:

(a) the DNA encoding the Cas9 protein is in the form of a first expression construct comprising a first promoter operably linked to a first nucleic acid encoding the Cas9 protein; and

(b) the DNA encoding the CRISPR RNA and the DNA encoding the tracrRNA are in the form of a second expression construct comprising a second promoter operably linked to a second nucleic acid encoding a gRNA comprising the CRISPR RNA and the tracrRNA;

wherein the first and second promoters are active in the mouse ES cell, and

wherein the first and second expression constructs are on a single nucleic acid molecule or on separate nucleic acid molecules.

9. The method of any preceding claim, wherein the targeted genetic modification comprises simultaneous deletion of an endogenous nucleic acid sequence at the genomic locus of interest and insertion of the insert nucleic acid at the genomic locus of interest.

10. The method of claim 9, wherein the deleted endogenous nucleic acid sequence is from 30 kb to about 110 kb, and the insert nucleic acid is from about 40 kb to about 140 kb.

11. The method of any preceding claim, wherein the targeted genetic modification is a biallelic genetic modification.

12. The method of claim 11, wherein the biallelic genetic modification comprises deletion of an endogenous nucleic acid sequence and insertion of the insert nucleic acid at the genomic locus of interest in two homologous chromosomes.

13. The method of claim 11, wherein the modified mouse ES cell is compound heterozygous or hemizygous at the genomic locus of interest.

14. The method of claim 13, wherein the targeted genetic modification at the genomic locus of interest in one chromosome comprises deletion of an endogenous nucleic acid sequence and insertion of the insert nucleic acid.

15. The method of claim 14, wherein the targeted genetic modification comprises: (1) deletion of an endogenous nucleic acid sequence at the genomic locus of interest in first and second homologous chromosomes; and (2) insertion of the insert nucleic acid into the genomic locus of interest in the first homologous chromosome and disruption of the genomic locus of interest in the second homologous chromosome.

16. The method of any preceding claim, wherein the LTVEC is at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, or at least 200 kb; or

wherein the targeted genetic modification comprises deletion of a region of the genomic locus of interest wherein the deletion is at least 30 kb, and the LTVEC is at least 15 kb, at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, or at least 200 kb.

17. The method of any preceding claim, wherein targeted genetic modification comprises insertion of the insert nucleic acid, wherein the insert nucleic acid is at least 40 kb, at

least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, at least 200 kb, at least 250 kb, or at least 300 kb; or

wherein the targeted genetic modification further comprises deletion of a region of the genomic locus of interest wherein the deletion is at least 30 kb, and the insert nucleic acid is at least 10 kb, at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, at least 200 kb, at least 250 kb, or at least 300kb.

18. The method of any preceding claim, wherein the insert nucleic acid is from about 40 kb to about 140 kb.

19. The method of any preceding claim, wherein the CRISPR target sequence is immediately flanked by a Protospacer Adjacent Motif (PAM) sequence.

20. The method of any preceding claim, wherein the sum total of the 5' and the 3' homology arms of the LTVEC is from 10 kb to about 20 kb, from about 20 kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 120 kb, or from about 120 kb to 150 kb.

21. The method of any preceding claim, wherein the targeted genetic modification comprises:

(a) replacement of an endogenous nucleic acid sequence with a homologous or an orthologous nucleic acid sequence;

(b) deletion of an endogenous nucleic acid sequence;

(c) deletion of an endogenous nucleic acid sequence,

wherein the deletion ranges from at least 30 kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 150 kb, from about 150 kb to about 200 kb, from about 200 kb to about 300 kb, from about 300 kb to about 400 kb, from about 400 kb to about 500 kb, from about 500 kb to about 1 Mb, from about 1 Mb to about 1.5 Mb, from about 1.5 Mb to about 2 Mb, from about 2 Mb to about 2.5 Mb, or from about 2.5 Mb to about 3 Mb;

(d) insertion of an exogenous nucleic acid sequence;

(e) insertion of an exogenous nucleic acid sequence ranging from at least 30

kb to about 40 kb, from about 40 kb to about 60 kb, from about 60 kb to about 80 kb, from about 80 kb to about 100 kb, from about 100 kb to about 150 kb, from about 150 kb to about 200 kb, from about 200 kb to about 250 kb, from about 250 kb to about 300 kb, from about 300 kb to about 350 kb, or from about 350 kb to about 400 kb;

(f) insertion of an exogenous nucleic acid sequence comprising a homologous or an orthologous nucleic acid sequence;

(g) insertion of a chimeric nucleic acid sequence comprising a human and a non-human nucleic acid sequence;

(h) insertion of a conditional allele flanked by site-specific recombinase target sequences;

(i) insertion of a selectable marker or a reporter gene operably linked to a promoter active in the mouse ES cell; or

(j) a combination thereof.

22. The method of any preceding claim, wherein the targeted genetic modification comprises deletion of a region of the genomic locus of interest,

wherein the deletion is at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least about 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, at least 200 kb, at least 250 kb, or at least 300 kb; or

wherein the targeted genetic modification further comprises insertion of the insert nucleic acid at the genomic locus of interest wherein the insertion is at least 30 kb, and the deletion is at least 10 kb, at least 20 kb, at least 30 kb, at least 40 kb, at least 50 kb, at least 60 kb, at least 70 kb, at least 80 kb, at least 90 kb, at least 100 kb, at least 150 kb, at least 200 kb, at least 250 kb, or at least 300 kb.

23. The method of any preceding claim, wherein the region of the genomic locus being deleted is from about 30 kb to about 110 kb.

24. The method of any preceding claim, wherein the targeted genetic modification comprises deletion of a region of the genomic locus of interest wherein the deletion is at least 30 kb and insertion of the insert nucleic acid at the genomic locus of interest wherein the insertion is at least 30 kb.

25. The method of any preceding claim, wherein the genomic locus of interest is endogenous to the mouse ES cell.

26. The method of any one of claims 1-24, wherein the genomic locus of interest comprises a heterologous or exogenous segment of DNA that was integrated into the genome of the mouse ES cell.

27. The method of any preceding claim, wherein the genomic locus of interest is an immunoglobulin locus.

28. The method of claim 27, wherein the immunoglobulin locus encodes a mouse immunoglobulin heavy chain variable region amino acid sequence.

29. The method of claim 27, wherein the immunoglobulin locus encodes a mouse immunoglobulin light chain variable region amino acid sequence.

30. The method of claim 29, wherein the immunoglobulin locus comprises an unrearranged mouse λ and/or κ light chain variable region nucleic acid sequence.

31. The method of claim 29, wherein the immunoglobulin locus comprises a rearranged mouse λ and/or κ light chain variable region nucleic acid sequence.

32. The method of any one of claims 1-26, wherein the genomic locus of interest is a T cell receptor locus.

33. The method of claim 32, wherein the T cell receptor locus is a T cell receptor alpha locus.

34. The method of any one of claims 1-26, wherein the genomic locus of interest comprises an Interleukin-2 receptor gamma locus, an *ApoE* locus, a *Rag1* locus, a *Rag2* locus, both of the *Rag1* and the *Rag2* loci, an *Adamts5* locus, a *Trpa1* locus, a *Folh1* locus, an *Erbb4* locus, a *Lrp5* locus, a *C5* (*Hc*) locus, a *Ror1* locus, or a *Dpp4* locus.

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

36. The method of claim 35, wherein the insert nucleic acid comprises one or more functional human V_H gene segments comprising V_H1-2, V_H1-3, V_H1-8, V_H1-18, V_H1-24, V_H1-45, V_H1-46, V_H1-58, V_H1-69, V_H2-5, V_H2-26, V_H2-70, V_H3-7, V_H3-9, V_H3-11, V_H3-13, V_H3-15, V_H3-16, V_H3-20, V_H3-21, V_H3-23, V_H3-30, V_H3-30-3, V_H3-30-5, V_H3-33, V_H3-35, V_H3-38, V_H3-43, V_H3-48, V_H3-49, V_H3-53, V_H3-64, V_H3-66, V_H3-72, V_H3-73, V_H3-74, V_H4-4, V_H4-28, V_H4-30-1, V_H4-30-2, V_H4-30-4, V_H4-31, V_H4-34, V_H4-39, V_H4-59, V_H4-61, V_H5-51, V_H6-1, V_H7-4-1, V_H7-81, or a combination thereof.

37. The method of claim 35 or 36, wherein the insert nucleic acid comprises one or more functional human D gene segments comprising D1-1, D1-7, D1-14, D1-20, D1-26, D2-2, D2-8, D2-15, D2-21, D3-3, D3-9, D3-10, D3-16, D3-22, D4-4, D4-11, D4-17, D4-23, D5-12, D5-5, D5-18, D5-24, D6-6, D6-13, D6-19, D6-25, D7-27, or a combination thereof.

38. The method of any one of claims 35-37, wherein the insert nucleic acid comprises one or more functional J_H gene segments comprising J_H1, J_H2, J_H3, J_H4, J_H5, J_H6, or a combination thereof.

39. The method of any one of claims 1-34, wherein the insert nucleic acid comprises a genomic nucleic acid sequence that encodes a human immunoglobulin light chain variable region amino acid sequence.

40. The method of claim 39, wherein the insert nucleic acid comprises one or more human Vκ gene segments comprising Vκ4-1, Vκ5-2, Vκ 7-3, Vκ 2-4, Vκ1-5, Vκ1-6, Vκ3-7, Vκ1-8, Vκ1-9, Vκ2-10, Vκ3-11, Vκ1-12, Vκ1-13, Vκ2-14, Vκ3-15, Vκ1-16, Vκ1-17, Vκ2-18, Vκ2-19, Vκ3-20, Vκ6-21, Vκ1-22, Vκ1-23, Vκ2-24, Vκ3-25, Vκ2-26, Vκ1-27, Vκ2-28, Vκ2-29, Vκ2-30, Vκ3-31, Vκ1-32, Vκ1-33, Vκ3-34, Vκ1-35, Vκ2-36, Vκ1-37, Vκ2-38, Vκ1-39, Vκ2-40, or a combination thereof.

41. The method of claim 39 or 40, wherein the insert nucleic acid comprises one or more human V_λ gene segments comprising V_λ3-1, V_λ4-3, V_λ2-8, V_λ3-9, V_λ3-10, V_λ2-11,

Vλ3-12, Vλ2-14, Vλ3-16, Vλ2-18, Vλ3-19, Vλ3-21, Vλ3-22, Vλ2-23, Vλ3-25, Vλ3-27, or a combination thereof.

42. The method of any one of claims 39-41, wherein the insert nucleic acid comprises one or more human Jκ gene segments comprising Jκ1, Jκ2, Jκ3, Jκ4, Jκ5, or a combination thereof.

43. The method of any one of claims 1-34, wherein the insert nucleic acid comprises a polynucleotide encoding at least a region of a human T cell receptor.

44. The method of claim 43, wherein the T cell receptor is a T cell receptor alpha.

45. The method of any one of claims 1-34, wherein the insert nucleic acid comprises at least one disease allele.

46. The method of claim 45, wherein the insert nucleic acid comprises at least one human disease allele of a human gene.

47. The method of any preceding claim, wherein the targeted genetic modification results in a humanized genomic locus comprising: (a) an insertion of a homologous or orthologous human nucleic acid sequence; (b) a replacement of an endogenous nucleic acid sequence with a homologous or orthologous nucleic acid sequence; or (c) a combination thereof.

48. The method of claim 47, wherein the targeted genetic modification comprises a replacement of an endogenous mouse nucleic acid sequence with a homologous or orthologous human nucleic acid sequence.

49. The method of any preceding claim, further comprising:

(b) identifying the modified mouse ES cell comprising the targeted genetic modification at the genomic locus of interest;

(c) introducing the modified mouse ES cell into a mouse host embryo; and

(d) gestating the mouse host embryo in a surrogate mother,

wherein the surrogate mother produces an F0 generation mouse comprising the targeted genetic modification at the genomic locus of interest.