We claim:

1. A method for targeted modification of a genomic locus of interest in a pluripotent rat cell, comprising:

(a) introducing into the pluripotent rat cell a large targeting vector (LTVEC) comprising an insert nucleic acid flanked by a 5’ homology arm complementary to a first nucleic acid sequence at the genomic locus of interest and a 3’ homology arm complementary to a second nucleic acid sequence at the genomic locus of interest, wherein the 5’ homology arm is at least 5 kb and the 3’ homology arm is at least 5 kb; and

(b) identifying a genetically modified pluripotent rat cell comprising a targeted genetic modification at the genomic locus of interest,

wherein the targeted genetic modification is capable of being transmitted through the germline, and

wherein the pluripotent rat cell to be used for targeted modification of the genomic locus of interest is obtainable by culturing isolated rat embryonic stem cells on a feeder cell layer that is not modified to express leukemia inhibitory factor (LIF) with a medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of a MEK pathway inhibitor and a GSK3 inhibitor.

2. The method of claim 1, wherein the pluripotent rat cell is a rat embryonic stem (ES) cell, optionally wherein the rat ES cell is derived from a DA strain or an ACI strain.

3. The method of claim 1 or 2, wherein the pluripotent rat cell:

(i) is characterized by expression of at least one pluripotency marker selected from the group consisting of Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, and Utf1;

(ii) lacks expression of one or more of the pluripotency markers c-Myc, Ecat1, and Rexo1;

(iii) lacks expression of one or more of the mesodermal markers Brachyury and Bmpr2;

(iv) lacks expression of one or more of the endodermal markers Gata6, Sox17, and Sox7; or

(v) lacks expression of one or more of the neural markers Nestin and Pax6.

4. The method of any one of claims 1-3, wherein the concentration of LIF in the medium is between about 75 U/mL to about 125 U/mL, preferably between about 90 U/mL to about 110 U/mL, and more preferably about 100 U/mL.

5. The method of any one of claims 1-4, wherein the MEK pathway inhibitor is MEK inhibitor PD0325901 and/or the GSK3 inhibitor is CHIR99021.

6. The method of claim 5, wherein the MEK pathway inhibitor is MEK inhibitor PD0325901 at a concentration of 0.8 μM to 1.2 μM, and the GSK3 inhibitor is CHIR99021 at a concentration of 2.5 μM to 3.5 μM.

7. The method of claim 6, wherein the concentration of LIF in the medium is about 100 U/mL, the MEK pathway inhibitor is MEK inhibitor PD0325901 at a concentration of about 1 μM, and the GSK3 inhibitor is CHIR99021 at a concentration of about 3 μM.

8. The method of claim 7, wherein the medium is a 2i medium that comprises: DMEM/F12 basal medium at a concentration of lx; neurobasal medium at a concentration of lx; penicillin/streptomycin at a concentration of 1%; L-Glutamine at a concentration of 4 mM; 2-mercaptoethanol at a concentration of 0.1 mM; N2 supplement at a concentration of 1x; B27 supplement at a concentration 1x; LIF at a concentration of 100U/mL; PD0325901 at a concentration of 1 μM, and CHIR99021 at a concentration of 3 μM.

9. The method of any one of claims 1-8, wherein the target genomic locus:

(i) is an Interleukin-2 receptor gamma locus, an ApoE locus, a Rag1 locus, a Rag2 locus, or a Rag2/Rag1 locus;

(ii) is an immunoglobulin locus,

optionally wherein the target genomic locus encodes a mammalian immunoglobulin heavy chain variable region amino acid sequence, or

optionally wherein the target genomic locus comprises a genomic DNA sequence that encodes a mammalian immunoglobulin light chain variable region amino acid sequence, optionally wherein the genomic DNA sequence comprises an unrearranged

mammalian λ and/or κ light chain variable region nucleic acid sequence; or

(iii) is a T cell receptor locus, optionally wherein the T cell receptor locus is a T cell receptor alpha locus.

10. The method of any one of claims 1-9, wherein:

(i) the LTVEC is from about 20 kb to about 400 kb;

(ii) the 5’ homology arm ranges from about 5 kb to about 100 kb or the 3’ homology arm ranges from about 5 kb to about 100 kb;

(iii) the sum total of the 5’ and the 3’ homology arms of the LTVEC is from about 10 kb to about 150 kb; or

(iv) the sum total of the 5’ and the 3’ homology arms is at least 10 kb.

11. The method of any one of claims 1-10, wherein the insert nucleic acid:

(i) comprises a polynucleotide of interest comprising a genomic nucleic acid sequence that encodes a human immunoglobulin heavy chain variable region amino acid sequence;

(ii) comprises a polynucleotide of interest comprising a genomic nucleic acid sequence that encodes a human immunoglobulin light chain variable region amino acid sequence;

(iii) comprises a polynucleotide of interest comprising a polynucleotide encoding at least a region of a T cell receptor, optionally wherein the T cell receptor is a T cell receptor alpha; or

(iv) comprises a polynucleotide of interest comprising at least one disease allele.

12. The method of any one of claims 1-11, wherein the insert nucleic acid:

(i) is from a human;

(ii) comprises a knock-in allele of at least one exon of an endogenous gene;

(iii) comprises a regulatory element;

(iv) comprises a conditional allele;

(v) comprises a nucleic acid flanked by site-specific recombination target sequences, optionally wherein the site-specific recombination target sequences flank a

polynucleotide encoding a selection marker and/or a reporter gene;

(vi) comprises a polynucleotide encoding a selection marker, optionally wherein the insert nucleic acid comprises a self-deleting selection cassette; or

(vii) comprises a reporter gene operably linked to a promoter.

13. The method of any one of claims 1-12, wherein the targeted genetic modification is biallelic.

14. The method of any one of claims 1-13, wherein the targeted genetic modification comprises:

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

(ii) deletion of an endogenous rat nucleic acid sequence, optionally wherein the deletion ranges from about 5 kb to about 3 Mb;

(iii) an exogenous nucleic acid sequence ranging from about 5 kb to about 400 kb;

(iv) an exogenous nucleic acid sequence comprising a nucleic acid sequence that is homologous or orthologous to an endogenous rat nucleic acid sequence;

(v) a chimeric nucleic acid sequence comprising a human nucleic acid sequence and a rat nucleic acid sequence;

(vi) a conditional allele flanked by site-specific recombinase target sequences; or

(vii) a reporter gene operably linked to a promoter active in a rat cell.

15. The method of any one of claims 1-14, wherein the targeted genetic modification comprises:

(i) insertion of a human nucleic acid sequence that is homologous or orthologous to a rat nucleic acid sequence at an endogenous genomic locus, optionally wherein the size of the insertion is from about 5 kb to about 500 kb;

(ii) replacement of the rat nucleic acid sequence at the endogenous genomic locus with the homologous or orthologous human nucleic acid sequence, optionally wherein the size of the replacement is from about 5 kb to about 500 kb;

(iii) a chimeric nucleic acid sequence comprising a human nucleic acid sequence and a rat nucleic acid sequence; or

(iv) a combination thereof.

16. The method of any one of claims 1-15, wherein introducing step (a) further comprises introducing a nucleic acid encoding a nuclease agent that promotes a homologous recombination between the LTVEC and the genomic locus of interest in the pluripotent rat cell.

17. The method of claim 16, wherein the nuclease agent comprises:

(i) a zinc finger nuclease; or

(ii) a Transcription Activator-Like Effector Nuclease (TALEN).

18. The method of claim 16, wherein the nuclease agent comprises a CRISPR/Cas system comprising a Cas9 nuclease and a guide RNA (gRNA) comprising a fused crRNA-tracrRNA, optionally wherein:

(i) the gRNA comprises:

(I) a chimeric RNA of the nucleic acid sequence of SEQ ID NO: 2; or

(II) a chimeric RNA of the nucleic acid sequence of SEQ ID NO: 3;

(ii) the crRNA comprises SEQ ID NO: 4; SEQ ID NO: 5; or SEQ ID NO: 6; or

(iii) the tracrRNA comprises SEQ ID NO: 7 or SEQ ID NO: 8.

19. The method of any one of claims 1-18, wherein the introducing step is mediated by electroporation.

20. The method of any one of claims 1-19, wherein the identification step employs a quantitative assay for assessing a modification of allele (MOA) at the genomic locus of interest.

21. The method of any one of claims 1-20, wherein steps (a) and (b) are sequentially repeated to allow for the targeted integration of at least two insert nucleic acids into the genomic locus of interest.

22. A method for targeted modification of a genomic locus of interest in a pluripotent rat cell, comprising:

(a) targeting a genomic locus of interest in a pluripotent rat cell according to the method of any one of claims 1-21 to form a genetically modified pluripotent rat cell, wherein the insert nucleic acid comprises a human nucleic acid; and

(b) introducing the genetically modified pluripotent rat cell into a host rat embryo.