CLAIMS

1. A method for making a targeted genetic modification in rat embryonic stem (ES) cells, comprising:

(a) culturing a population of rat ES cells under conditions comprising a layer of feeder cells that are not modified to express leukemia inhibitory factor (LIF) and a medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of a MEK inhibitor and a GSK3 inhibitor; and

(b) modifying the rat ES cells to comprise the targeted genetic modification, wherein the rat ES cells are capable of transmitting the targeted genetic modification through the germline.

2. The method of claim 1, further comprising generating the population of rat ES cells in step (a), comprising:

(i) culturing *in vitro* a first layer of feeder cells that are not modified to express leukemia inhibitory factor (LIF) and a morula or a blastocyst stage rat embryo,

wherein the zona pellucida of the morula or blastocyst stage rat embryo has been removed, and

wherein the culture conditions maintain pluripotency of a rat ES cell and comprise a medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of a MEK inhibitor and a GSK3 inhibitor;

(ii) transferring an outgrowth of an amorphous undifferentiated mass of rat ES cells obtained from step (i) to an *in vitro* culture well comprising a second layer of the feeder cells that are not modified to express LIF; and

(iii) culturing the outgrowth under conditions comprising the medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of a MEK inhibitor and a GSK3 inhibitor.

3. The method of claim 1 or 2, wherein the rat ES cells:

(ii) lack expression of c-Myc; and/or

(iii) have a normal karyotype; and/or

(iv) form spherical, free-floating colonies in culture.

4. The method of any one of claims 1-3, further comprising:

(c) introducing at least one of the modified rat ES cells from step (b) into a rat host embryo to produce an F0 embryo;

(d) implanting the F0 embryo into a surrogate mother;

(e) gestating the F0 embryo in the surrogate mother to term; and

(f) identifying an F0 rat having the targeted genetic modification.

5. The method of claim 4, wherein the modified rat ES cell introduced into the rat host embryo is from the same rat strain as the rat host embryo.

6. The method of claim 4, wherein the modified rat ES cell introduced into the rat host embryo is from a different rat strain than the rat host embryo.

7. A method of making a genetically modified rat, comprising:

(a) providing a rat ES cell line comprising a population of rat ES cells obtained by culturing isolated rat ES cells on a layer of feeder cells that are 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 MEK inhibitor PD0325901 and GSK3 inhibitor CHIR99021, and wherein the population of rat ES cells:

lacks expression of c-Myc;

forms spherical, free-floating colonies in culture;

is diploid; and

is germline competent;

(b) obtaining a rat ES cell clone comprising a targeted modification, wherein the obtaining consists of:

(i) modifying the population of rat ES cells to comprise the targeted genetic modification; and

(ii) identifying in a single cloning step a rat ES cell clone comprising the targeted genetic modification;

(c) introducing the rat ES cell clone into a rat host embryo, wherein the rat ES cell clone is from a different rat strain than the rat host embryo; and

(d) gestating the rat host embryo comprising the rat ES cell clone in a surrogate mother, wherein the surrogate mother produces F0 progeny comprising the targeted genetic modification, wherein the targeted genetic modification is transmitted through the germline.

8. The method of claim 7, wherein the providing step (a) comprises:

(i) culturing *in vitro* a first layer of the feeder cells that are not modified to express leukemia inhibitory factor (LIF) and a morula or a blastocyst stage rat embryo,

wherein the zona pellucida of the morula or blastocyst-stage rat embryo has been removed, and

wherein the culture conditions maintain pluripotency of a rat ES cell and comprise a medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of MEK inhibitor PD0325901 and GSK3 inhibitor CHIR99021; and

(ii) transferring an outgrowth of an amorphous undifferentiated mass of rat ES cells to an *in vitro* culture well comprising a second layer of the feeder cells that are not modified to express LIF; and

(iii) culturing the outgrowth under conditions comprising the medium comprising about 50 U/mL to about 150 U/mL LIF and a combination of inhibitors consisting of MEK inhibitor PD0325901 and GSK3 inhibitor CHIR99021, thereby maintaining pluripotency of the rat ES cells.

9. The method of any one of claims 4-8, further comprising breeding a male F0 rat comprising the targeted genetic modification with a wild type female rat to produce an F1 progeny that is heterozygous for the targeted genetic modification;, and optionally breeding a male rat of the F1 progeny with a female rat of the F1 progeny to obtain an F2 progeny that is homozygous for the targeted genetic modification.

10. The method of claim 9, wherein at least 3% of the F0 rats having the targeted genetic modification transmit the targeted genetic modification to the F1 progeny,

optionally wherein at least 10% of the F0 rats having the targeted genetic modification transmit the targeted genetic modification to the F1 progeny, and

optionally wherein at least 60% of the F0 rats having the targeted genetic modification transmit the targeted genetic modification to the F1 progeny.

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

(I) the rat ES cells are derived from an ACI rat or derived from a Dark Agouti (DA) rat; and/or

(II) the rat ES cells are derived from a morula stage or a blastocyst stage embryo from a superovulated rat.

12. The method of any one of claims 1-11, wherein the targeted genetic modification comprises an insertion, a deletion, a knockout, a knockin, a point mutation, or a combination thereof.

13. The method of claim 12, wherein the targeted genetic modification comprises an insertion of a heterologous polynucleotide into the genome of the rat ES cells.

14. The method of any one of claims 1-13, wherein the modifying step comprises modifying the rat ES cells to comprise two or more targeted modifications, wherein the rat ES cells can transmit the two or more targeted genetic modifications through the germline.

15. The method of any one of claims 1-14, wherein the rat ES cells are male (XY) rat ES cells or female (XX) rat ES cells.

16. The method of any one of claims 1-15, wherein the rat ES cells have one or more of the following characteristics:

(a) the rat ES cells express at least one pluripotency marker selected from Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, and Utf1;

(b) the rat ES cells do not express one or more pluripotency markers selected from c-Myc, Ecat1, and Rexo1;

(c) the rat ES cells do not express one or more mesodermal markers selected from Brachyury and Bmpr2;

(d) the rat ES cells do not express one or more endodermal markers selected from Gata6, Sox17, and Sox7;

(e) the rat ES cells do not express one or more neural markers selected from Nestin and Pax6;

(f) the rat ES cells express one or more pluripotency markers selected from Oct-4, Sox2, and alkaline phosphatase; and

(g) the rat ES cells express of one or more rat ESC-specific genes selected from Adherens Junctions Associated Protein 1 (Ajap1), Claudin 5 (Cldn5), Cdc42 guanine nucleotide exchange factor 9 (Arhgef9), Calcium/calmodulin-dependent protein kinase IV (Camk4), ephrin-A1 (Efna1), EPH receptor A4 (Epha4), gap junction protein beta 5 (Gjb5), Insulin-like growth factor binding protein-like 1 (Igfbpl1), Interleukin 36 beta (Il1f8), Interleukin 28 receptor, alpha (Il28ra), left-right determination factor 1 (Lefty1), Leukemia inhibitory factor receptor alpha (Lifr), Lysophosphatidic acid receptor 2 (Lpar2), Neuronal pentraxin receptor (Ntm), Protein tyrosine phosphatase non-receptor type 18 (Ptpn18), Caudal type homeobox 2 (Cdx2), Fibronectin type III and ankyrin repeat domains 1 (Fank1), Forkhead box E1 (thyroid transcription factor 2) (Foxe1), Hairy/enhancer-of-split related with YRPW motif 2 (Hey2), Forkhead box E1 (thyroid transcription factor 2) (Foxe1), Hairy/enhancer-of-split related with YRPW motif 2 (Hey2), Lymphoid enhancer-binding factor 1 (Lef1), Sal-like 3 (Drosophila) (Sall3), SATB homeobox 1 (Satb1), and miR-632.

17. The method of any one of claims 1-16, wherein the medium comprises about 75 U/mL to about 125 U/mL LIF, optionally wherein the medium comprises about 90 U/mL to about 110 U/mL LIF.

18. The method of claim 17, wherein the medium comprises about 100 U/mL LIF.

19. The method of any one of claims 1-18, wherein the feeder cells comprise a monolayer of mitotically inactivated mouse embryonic fibroblasts (MEFs).

20. The method of any one of claims 1-19, wherein the LIF in the medium is a mouse LIF or has at least 91% sequence identity to SEQ ID NO: 1.

21. The method of any one of claims 1-20, wherein the MEK inhibitor comprises PD0325901 and/or wherein the GSK3 inhibitor comprises CHIR99021.

22. The method of claim 21, wherein the MEK inhibitor is PD0325901 at a concentration of 0.8 µM to about 1.2 µM, and the GSK3 inhibitor is CHIR99021 at a concentration of about 2.5 µM to about 3 µM or 3 µMto about 3.5 µM,

optionally, wherein the concentration of the MEK inhibitor is about 1 µM, and the concentration of the GSK3 inhibitor is about 3 µM.

23. The method of claim 22, wherein the concentration of LIF in the medium is about 100 U/mL, the MEK inhibitor is PD0325901 at a concentration of about 1 µM, and the GSK3 inhibitor is CHIR99021 at a concentration of about 3 µM.

24. The method of any one of claims 1-23, wherein the medium further comprises DMEM/F12 basal medium and Neurobasal medium.

25. The method of claim 24, wherein the medium comprises: 1x DMEM/F12 basal medium; 1x neurobasal medium; 1% penicillin/streptomycin; 4 mM L-glutamine; 0.1 mM 2-mercaptoethanol; 1x N2 supplement; 1x B27 supplement; 100 U/mL LIF; 1 µM PD0325901; and 3 µM CHIR99021.

26. The method of any one of claims 1-25, wherein the modifying step comprises one or more rounds of electroporation.

27. The method of any one of claims 1-26, wherein the targeted genetic modification is generated via a homologous recombination event.

28. The method of any one of claims 1-27, wherein the targeted genetic modification is generated by employing a targeting vector comprising upstream and downstream homology arms flanking an insert polynucleotide, wherein the homology arms correspond to genomic regions within a targeted genomic locus.

29. The method of any one of claims 1-28, wherein the targeted genetic modification is generated using a nuclease agent that generates a single or double strand break at a targeted genomic locus.

30. The method of claim 29, wherein the nuclease agent is a transcription activator-like effector nuclease (TALEN), a zinc-finger nuclease (ZFN), a meganuclease, or a CRISPR/Cas system.

31. The method of any one of claims 1-30, wherein the modifying step comprises:

(i) introducing into the rat ES cells a heterologous polynucleotide comprising a selection marker operably linked to a promoter active in the rat ES cells; and

(ii) culturing *in vitro* the rat ES cells in alternating first and second culture media, wherein the first culture medium comprises an effective amount of a selection agent for a first time period and the second culture medium does not comprise the selection agent, and wherein the *in vitro* culture conditions are sufficient to maintain pluripotency, thereby selecting the rat ES cells having the heterologous polynucleotide stably integrated into the genome.

32. The method of claim 31, wherein the first and second culture media are alternated every 24 hours, and wherein the selection marker imparts resistance to an antibiotic,

optionally wherein the selection marker comprises one or more of neomycin phosphotransferase (neo^r), hygromycin B phosphotransferase (hyg^r), puromycin-N-acetyltransferase ($puro^r$), and blasticidin S deaminase (bsr^r); and

optionally wherein the antibiotic is G418.

33. The method of claim 31 or 32, wherein the selection marker has one or more of the following characteristics:

(a) the selection marker comprises a non-attenuated selection marker gene;

(b) the selection marker has an increased activity compared to the wild type selection marker; and

(c) the rat ES cells comprise multiple copies of the selection marker stably incorporated into the genome.

34. An *in vitro* culture system for culturing and maintaining pluripotency of rat ES cells, comprising:

(a) a feeder cell layer not modified to express leukemia inhibitory factor (LIF);

(b) a medium comprising about 50 U/mL to about 150 U/mL LIF, and a combination of inhibitors consisting of a MEK inhibitor and a GSK3 inhibitor; and

(c) a population of one or more rat ES cells comprising a targeted genetic modification, wherein the rat ES cells are capable of transmitting the targeted genetic modification through the germline.

35. The *in vitro* culture system of claim 34, wherein the rat ES cells:

(i) lack expression of c-Myc; and/or

(ii) have a normal karyotype; and/or

(iii) form spherical, free-floating colonies in culture.

36. The *in vitro* culture system of claim 34 or 35, wherein the medium comprises about 75 U/mL to about 125 U/mL LIF, optionally wherein the medium comprises about 90 U/mL to about 110 U/mL LIF.

37. The *in vitro* culture system of claim 36, wherein the medium comprises about 100 U/mL LIF.

38. The *in vitro* culture system of any one of claims 34-37, wherein the feeder cell layer comprises a monolayer of mitotically inactivated mouse embryonic fibroblasts (MEFs).

39. The *in vitro* culture system of any one of claims 34-38, wherein the LIF in the medium is a mouse LIF or has at least 91% sequence identity to SEQ ID NO: 1.

40. The *in vitro* culture system of any one of claims 34-39, wherein the MEK inhibitor comprises PD0325901 and/or wherein the GSK3 inhibitor comprises CHIR99021.

41. The *in vitro* culture system of claim 40, wherein the MEK inhibitor is PD0325901 at a concentration of 0.8 µM to about 1.2 µM, and the GSK3 inhibitor is CHIR99021 at a concentration of about 2.5 µM to about 3 µM or 3 µM to about 3.5 µM,

optionally, wherein the concentration of the MEK inhibitor is about 1 μM, and the concentration of the GSK3 inhibitor is about 3 μM.

42. The *in vitro* culture system of claim 41, wherein the concentration of LIF in the medium is about 100 U/mL, the MEK inhibitor is PD0325901 at a concentration of about 1 μM, and the GSK3 inhibitor is CHIR99021 at a concentration of about 3 μM.

43. The *in vitro* culture system of any one of claims 34-42, wherein the medium further comprises DMEM/F12 basal medium and Neurobasal medium.

44. The *in vitro* culture system of claim 43, wherein the medium comprises: 1x DMEM/F12 basal medium; 1x neurobasal medium; 1% penicillin/streptomycin; 4 mM L-glutamine; 0.1 mM 2-mercaptoethanol; 1x N2 supplement; 1x B27 supplement; 100 U/mL LIF; 1 μM PD0325901; and 3 μM CHIR99021.

45. The *in vitro* culture system of any one of claims 34-44, wherein the targeted genetic modification comprises an insertion, a deletion, a knockout, a knockin, a point mutation, or a combination thereof.

46. The *in vitro* culture system of claim 45, wherein the targeted genetic modification comprises an insertion of a heterologous polynucleotide into the genome of the rat ES cells.

47. The *in vitro* culture system of claim 46, wherein the heterologous polynucleotide comprises a selection marker, and wherein the selection marker has one or more of the following characteristics:

(a) the selection marker comprises a non-attenuated selection marker gene operably linked to a promoter;

(b) the selection marker has increased activity compared to a wild type selection marker; and

(c) the rat ES cells comprise multiple copies of the selection marker stably incorporated into the genome.

48. The *in vitro* culture system of any one of claims 34-47, wherein:

(a) the rat ES cells are derived from an ACI rat or derived from a Dark Agouti (DA) rat; and/or

(b) the rat ES cells are derived from a morula stage or a blastocyst stage embryo.

49. The *in vitro* culture system of any one of claims 34-48, wherein the rat ES cells are male (XY) rat ES cells or female (XX) rat ES cells.

50. The *in vitro* culture system of any one of claims 34-49, wherein the rat ES cells have one or more of the following characteristics:

(a) the rat ES cells express at least one pluripotency marker selected from Dnmt3L, Eras, Err-beta, Fbxo15, Fgf4, Gdf3, Klf4, Lef1, LIF receptor, Lin28, Nanog, Oct4, Sox15, Sox2, and Utf1;

(b) the rat ES cells do not express one or more pluripotency markers selected from c-Myc, Ecat1, and Rexo1;

(c) the rat ES cells do not express one or more mesodermal markers selected from Brachyury and Bmpr2;

(d) the rat ES cells do not express one or more endodermal markers selected from Gata6, Sox17, and Sox7;

(e) the rat ES cells do not express one or more neural markers selected from Nestin and Pax6;

(f) the rat ES cells express one or more pluripotency markers selected from Oct-4, Sox2, and alkaline phosphatase; and

(g) the rat ES cells express of one or more rat ESC-specific genes selected from Adherens Junctions Associated Protein 1 (Ajap1), Claudin 5 (Cldn5), Cdc42 guanine nucleotide exchange factor 9 (Arhgef9), Calcium/calmodulin-dependent protein kinase IV (Camk4), ephrin-A1 (Efna1), EPH receptor A4 (Epha4), gap junction protein beta 5 (Gjb5), Insulin-like growth factor binding protein-like 1 (Igfbpl1), Interleukin 36 beta (Il1f8), Interleukin 28 receptor, alpha (Il28ra), left-right determination factor 1 (Lefty1), Leukemia inhibitory factor receptor alpha (Lifr), Lysophosphatidic acid receptor 2 (Lpar2), Neuronal pentraxin receptor (Ntm), Protein tyrosine phosphatase non-receptor type 18 (Ptpn18), Caudal type homeobox 2 (Cdx2), Fibronectin type III and ankyrin repeat domains 1 (Fank1), Forkhead box E1 (thyroid

transcription factor 2) (Foxe1), Hairy/enhancer-of-split related with YRPW motif 2 (Hey2), Forkhead box E1 (thyroid transcription factor 2) (Foxe1), Hairy/enhancer-of-split related with YRPW motif 2 (Hey2), Lymphoid enhancer-binding factor 1 (Lef1), Sal-like 3 (Drosophila) (Sall3), SATB homeobox 1 (Satb1), and miR-632.