CLAIMS

1. A method for making a mouse XY embryonic stem (ES) cell line capable of producing a fertile XY female mouse in an F0 generation, comprising:

(a) modifying a mouse XY ES cell to comprise a genetic modification comprising a deletion that inactivates an endogenous *Sry* gene, wherein the mouse XY ES cell comprises a Y chromosome from a 129S6 strain; and

(b) culturing the modified mouse XY ES cell under conditions to produce the mouse XY ES cell line capable of producing the fertile XY female non-human mammal in the F0 generation.

2. The method of claim 1, wherein the genetic modification is generated by introducing into the mouse XY ES cell a nuclease agent that induces a nick or a double-strand break at a recognition site on the Y chromosome or a polynucleotide encoding the nuclease agent.

3. The method of claim 2, wherein the nuclease agent is a zinc finger nuclease (ZFN), a Transcription Activator-Like Effector Nuclease (TALEN), a meganuclease, or a Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) protein and a guide RNA (gRNA).

4. The method of claim 3, wherein the nuclease agent is the Cas protein and the gRNA, wherein the Cas protein is a Cas9 protein, and wherein the gRNA comprises:

(a) a CRISPR RNA (crRNA) that targets the recognition site, wherein the recognition site is immediately flanked by a Protospacer Adjacent Motif (PAM) sequence; and

(b) a trans-activating CRISPR RNA (tracrRNA),

optionally wherein the gRNA targets a sequence comprising SEQ ID NO: 10, SEQ ID NO: 11, or SEQ ID NO: 12.

5. The method of claim 3, wherein the nuclease agent is the TALEN,

optionally wherein the TALEN targets a sequence comprising SEQ ID NO: 72 and a sequence comprising SEQ ID NO: 73.

6. The method of any one of claims 1-5, wherein the genetic modification is generated by introducing into the mouse XY ES cell a targeting vector comprising an insert

polynucleotide flanked by first and second homology arms corresponding to first and second target sites at a target genomic locus on the Y chromosome, wherein the mouse XY ES cell is modified to comprise the insert polynucleotide at the target genomic locus.

7. The method of claim 6, wherein the first homology arm is from about 400-1000 base pairs, and the second homology arm is from about 400-1000 base pairs.

8. The method of claim 6, wherein the sum total of the first homology arm and the second homology arm is at least 10 kb.

9. The method of any one of claims 6-8, wherein the genetic modification is generated by introducing into the mouse XY ES cell:

(i) a nuclease agent that induces the nick or a double-strand break at the recognition site on the Y chromosome or a polynucleotide encoding the nuclease agent; and

(ii) the targeting vector comprising the insert polynucleotide flanked by first and second homology arms corresponding to first and second target sites at the target genomic locus on the Y chromosome,

wherein the mouse XY ES cell is modified to comprise the insert polynucleotide at the target genomic locus.

10. The method of any one of claims 1-9, wherein the mouse XY ES cell is not cultured in a KO-DMEM-like low osmotic strength growth medium.

11. The method of any one of claims 1-9, wherein the culturing step comprises culturing the modified mouse XY ES cell in a medium comprising: (1) a base medium comprising about 3 mg/mL sodium chloride and about 2.2 mg/mL sodium bicarbonate and having an osmolality of about 218 mOsm/kg; and (2) supplements that maintain the modified mouse XY ES cell in culture.

12. The method of any one of claims 1-11, wherein upon introduction of the modified mouse XY ES cell into a mouse host embryo and following gestation of the mouse host embryo to produce F0 mice, at least 60% of the F0 mice are XY females which upon attaining sexual maturity are fertile.

13. A method for making a fertile XY female mouse in an F0 generation, comprising:

(a) introducing a mouse XY embryonic stem (ES) cell into a mouse host embryo, wherein the mouse XY ES cell comprises a Y chromosome from a 129S6 strain and a genetic modification comprising a deletion that inactivates an endogenous mouse *Sry* gene, and optionally wherein the mouse host embryo is a pre-morula stage embryo;

(b) gestating the mouse host embryo; and

(c) obtaining F0 mice following gestation of the mouse host embryo, wherein at least 60% of the F0 mice are XY females which upon attaining sexual maturity are fertile.

14. The method of claim 13, wherein the F0 XY female mouse is fertile when crossed to a wild-type mouse, optionally wherein the wild-type mouse is a C57BL/6 mouse.

15. The method of any one of claims 1-14, wherein the mouse XY ES cell is isolated from a mouse that is a cross between a 129S6 strain and a C57BL/6 strain.

16. The method of claim 15, wherein the mouse XY ES cell is isolated from a hybrid embryo that has been produced by crossing a female C57BL/6NTac mouse to a male 129S6/SvEvTac mouse.

17. The method of claim 16, wherein the mouse XY ES cell is a VGF1 mouse ES cell.

18. The method of any one of claims 1-17, wherein the genetic modification further comprises an insertion of one or more nucleotides, a substitution of one or more nucleotides, or a combination thereof, optionally wherein:

(I) the genetic modification further comprises a knockout; a knockin; a replacement of an endogenous nucleic acid sequence with a homologous, orthologous, or heterologous sequence; or a combination thereof;

(II) the genetic modification further comprises an insertion of a nucleic acid encoding a selectable marker and/or a nucleic acid encoding a reporter gene operably linked to a promoter active in the mouse XY ES cell; or

(III) the genetic modification further comprises an insertion of a nucleic acid encoding a reporter gene operably linked to an endogenous *Sry* promoter.

19. The method of any one of claims 1-18, further comprising modifying the mouse XY ES cell to comprise at least one additional targeted genetic modification of a polynucleotide of interest.

20. The method of any one of claims 12-19, wherein upon introduction of the modified mouse XY ES cell into the mouse host embryo and following gestation of the mouse host embryo, at least 80% of the F0 mice are XY females which upon attaining sexual maturity are fertile, optionally wherein at least 90% of the F0 mice are XY females which upon attaining sexual maturity are fertile, and optionally wherein at least 95% of the F0 mice are XY females which upon attaining sexual maturity are fertile.