

CLAIMS

What is claimed is:

1. A genetically modified differentiated somatic cell of a non-human animal comprising a self-excisable, recombinase expression cassette having a site-specific recombinase gene operably linked to an embryonic stem (ES) cell-specific promoter,

wherein the site-specific recombinase cassette is flanked upstream and downstream by first and second recombination sites that are oriented in the same direction with respect to each other in such a way that the cassette can be excised in the presence of the site-specific recombinase, and

wherein the ES cell-specific promoter drives transcription of the site-specific recombinase gene in undifferentiated pluripotent stem cells but not in the genetically modified differentiated somatic cells, and wherein the ES cell-specific promoter is selected from the group consisting of Oct-3/4 promoter, Sox2 promoter, Nanog promoter, and a combination thereof.

2. The genetically modified differentiated somatic cell of claim 1, wherein:

(a) the somatic cell is selected from the group consisting of a skin cell, blood cell, nerve cell, muscle cell, bone cell, kidney cell, liver cell, and a fat cell; or

(b) the somatic cell is a fibroblast.

3. The genetically modified differentiated somatic cell of claim 2, wherein the fibroblast is derived from a pig or cow.

4. The genetically modified differentiated somatic cell of claim 3, wherein the pig is a mini pig.

5. The genetically modified differentiated somatic cell of claim 1, wherein:

(a) the ES cell-specific promoter drives transcription of the site-specific recombinase gene in ES cells of a blastocyst-stage embryo; or

(b) the self-excisable, recombinase expression cassette further comprises a selective marker gene between the first and second recombination sites, wherein the selective marker gene is operably linked to a promoter, preferably wherein the promoter operably linked to the selective marker gene is a constitutive promoter.

6. The genetically modified differentiated somatic cell of claim 1, wherein:

(a) the self-excisable, recombinase expression cassette does not comprise a selective marker gene, and the selective marker gene is located in another locus in the genome of the somatic cell, and wherein the selective marker gene is flanked upstream and downstream by third and fourth recombination sites that are oriented in the same direction with respect to each other such that the selective marker can be removed in the presence of the site-specific recombinase;

(b) the genetically modified genome of the somatic cell comprises a conditional knockout allele, and wherein the conditional knockout allele is flanked upstream and downstream by the first and second recombination sites such that the conditional allele can be removed in the presence of the site-specific recombinase;

(c) the recombinase expression cassette comprises a nucleotide sequence homologous to at least one exon of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites;

(d) the recombinase expression cassette comprises a nucleotide sequence homologous to at least one intron of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites;

(e) the self-excisable, recombinase expression cassette is located in a transcriptionally-active locus in the genome of the somatic cell; or

(f) the site-specific recombinase is selected from the group consisting of Cre, Flp, and Dre recombinases.

7. A method for producing a genetically modified and cloned non-human animal that is free of a selective marker gene and a recombinase gene, the method comprising:

(a) introducing a nucleic acid construct into a differentiated somatic cell of a non-human animal to create a genetically modified genome

wherein the nucleic acid construct comprises a self-excisable, recombinase expression cassette comprising a site-specific recombinase gene operably linked to an ES cell-specific promoter,

wherein the recombinase expression cassette is flanked upstream and downstream by first and second recombination sites that are oriented in the same direction such that the cassette can be excised in the presence of the site-specific recombinase, and

wherein the ES cell-specific promoter drives transcription of the site-specific recombinase gene in undifferentiated pluripotent stem cells but not in the differentiated somatic cell and wherein the ES cell-specific promoter is selected from the group consisting of Oct-3/4 promoter, Sox2 promoter, Nanog promoter, and a combination thereof;

(b) transferring the genetically modified genome of (a) into an enucleated host oocyte;

(c) fusing and activating the oocyte of (b) to form an artificial zygote;

(d) culturing the artificial zygote of (c) until the zygote reaches a blastocyst embryonic stage; and

(e) implanting the blastocyst of (d) into a uterus of a surrogate mother to form the genetically modified, cloned non-human animal that is free of the selective marker gene and the recombinase gene.

8. The method according to claim 7, wherein

the self-excisable, recombinase expression cassette comprises a selective marker gene located between the first and second recombination sites, and wherein the selective marker

gene is operably linked to a promoter, preferably wherein the promoter operably linked to the selective marker gene is a constitutive promoter.

9. The method according to claim 7, wherein:

(a) the self-excisable, recombinase expression cassette does not comprise a selective marker gene, and the selective marker gene is located in another locus in the genome of the somatic cell, and wherein the selective marker gene is flanked upstream and downstream by third and fourth recombination sites that are oriented in the same direction with respect to each other such that the selective marker can be removed in the presence of the site-specific recombinase;

(b) the somatic cell comprises a conditional knockout allele in the genome, and wherein the conditional knockout allele is flanked upstream and downstream by the first and second recombination sites such that the conditional allele can be removed in the presence of the site-specific recombinase;

(c) the nucleic acid construct comprises a nucleotide sequence homologous to at least one exon of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites, preferably wherein the promoter is a constitutive promoter;

(d) the nucleic acid construct comprises a nucleotide sequence homologous to at least one intron of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites;

(e) the nucleic acid construct comprises a selective marker gene operably linked to a promoter; or

(f) the site-specific recombinase is selected from the group consisting of Cre, Flp, and Dre recombinases.

10. A cloned oocyte of a non-human animal comprising a genetically modified genome from a differentiated somatic cell, wherein the genetically modified genome comprises a self-excisable, recombinase expression cassette having a site-specific recombinase

gene operably linked to an ES cell-specific promoter, wherein the ES cell-specific promoter is selected from the group consisting of Oct-3/4 promoter, Sox2 promoter, Nanog promoter, and a combination thereof, and wherein the self-excisable, recombinase expression cassette is flanked by first and second recombination sites oriented in the same direction with respect to each other such that the cassette can be excised in the presence of the site-specific recombinase.

11. The cloned oocyte according to claim 10, wherein:

(a) the non-human animal is selected from the group consisting of a mouse, a rat, a rabbit, a bird, a cow, a pig, a sheep, a goat, a horse, and a donkey;

(b) the differentiated somatic cell is selected from the group consisting of a skin cell, a blood cell, a nerve cell, a muscle cell, a bone cell, a liver cell, and a fat cell; or

(c) the differentiated somatic cell is a fibroblast.

12. The cloned oocyte according to claim 11, wherein the fibroblast is derived from a pig or a cow.

13. The cloned oocyte according to claim 12, wherein the pig is a mini pig.

14. The cloned oocyte according to claim 10, wherein:

(a) the ES cell-specific promoter drives transcription of the site-specific recombinase gene in ES cells of a blastocyst-stage embryo; or

(b) the self-excisable, recombinase expression cassette comprises a selective marker gene between the first and second recombination sites, and wherein the selective marker gene is operably linked to a promoter, preferably wherein the promoter operably linked to the selective marker gene is a constitutive promoter.

15. The cloned oocyte according to claim 10, wherein:

(a) the self-excisable, recombinase expression cassette does not comprise a selective marker gene, and the selective marker gene is located in another locus in the somatic cell genome, and wherein the selective marker gene is flanked upstream and downstream by

third and fourth recombination sites that are oriented in the same direction with respect to each other such that the selective marker can be removed in the presence of the site-specific recombinase;

(b) the somatic cell comprises a conditional knockout allele in a genome, and wherein the conditional knockout allele is flanked upstream and downstream by the first and the second recombination sites such that the conditional allele can be removed in the presence of the site-specific recombinase;

(c) the self-excisable, recombinase expression cassette comprises a nucleotide sequence homologous to at least one exon of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites;

(d) the self-excisable, recombinase expression cassette comprises a nucleotide sequence homologous to at least one intron of an endogenous gene being targeted, and wherein the nucleotide sequence is flanked upstream and downstream by the first and second recombination sites;

(e) the self-excisable, recombinase expression cassette is located in a transcriptionally-active locus in the somatic cell genome; or

(f) the site-specific recombinase is selected from the group consisting of Cre, Flp, and Dre recombinases.