

CLAIMS

What is claimed is:

1. A fusion protein comprising a nuclease and an engineered zinc finger domain, wherein the engineered zinc finger domain binds to a target site specified in any of SEQ ID NOs:28-30 and 75-90.
2. The fusion protein of claim 1, wherein the nuclease comprises a cleavage domain or cleavage-half-domain.
3. The fusion protein of claim 1 or 2 wherein the nuclease comprises a Type IIS restriction endonuclease cleavage domain or cleavage half-domain.
4. The fusion protein of claim 3, wherein the cleavage domain or cleavage half-domain is naturally occurring or engineered.
5. A polynucleotide encoding a fusion protein according to any of claims 1 to 4.
6. A cell comprising a fusion protein according to any of claims 1-4 or a polynucleotide according to claim 5.
7. The cell of claim 6, wherein the cell is an embryo cell.
8. A composition comprising a fusion protein according to any of claims 1-4 or a polynucleotide according to claim 5 and a pharmaceutically acceptable excipient.
9. Use of one or more fusion proteins according to any of claims 1-4 or one or more polynucleotides according to claim 5 in the manufacture of a medical composition for cleaving one or more *Rosa* genes in a cell, wherein the use comprises:
 - introducing, into the cell, the one or more fusion proteins according to any of claims 1-4 or one or more polynucleotides according to claim 5, such that the one or more *Rosa* gene are cleaved.

10. Use of an exogenous polynucleotide sequence in the manufacture of a medical composition for introducing the exogenous polynucleotide sequence into the genome of a cell, wherein the use comprises:

cleaving one or more *Rosa* genes according to claim 9; and
contacting the cell with the exogenous polynucleotide sequence;
wherein cleavage of the one or more genes stimulates integration of the exogenous polynucleotide sequence into the genome by homologous recombination.

11. The use of claim 10, wherein the exogenous polynucleotide sequence is physically into the genome.

12. The use of claim 11, wherein the exogenous polynucleotide sequence is integrated into the genome via nucleic acid replication processes.

13. The use of claim 11, wherein the exogenous polynucleotide sequence is integrated into the genome via non-homology dependent targeted integration.

14. Use of a first ZFN and a second ZFN in the manufacture of a medical composition for modifying a *Rosa* gene sequence in the genome of cell, wherein the use comprises:

cleaving one or more *Rosa* genes according to claim 9, wherein
(i) the first ZFN cleaves at a first cleavage site and the second ZFN cleaves at a second cleavage site;
(ii) the *Rosa* gene sequence is located between the first cleavage site and the second cleavage site;
(iii) cleavage of the first and second cleavage sites results in a modification of the gene sequence by non-homologous end joining or homology directed repair.

15. The use of claim 14, wherein the modification comprises a deletion.

16. The use of claim 15, wherein the modification comprises insertion of an exogenous sequence.

17. Use of a first ZFN and a second ZFN as defined in any one of claims 14 to 16 in the manufacture of a medical composition for generating a transgenic animal, wherein the use comprises;

modifying a *Rosa* gene sequence in an embryo cell as described in any of claims 14 to 16; and

~~allowing the embryo to develop into an animal.~~

18. The use of claim 17, wherein the modification comprises one or more random mutations at defined positions.

19. The use of claim 17, wherein the modification comprises insertion of a humanized gene.

20. The use of claim 19, wherein the humanized gene is associated with drug metabolism.

21. The use of any of claims 17 to 20, wherein the animal is a sexually mature animal and the modified gene sequences are present in at least a portion of gametes of the sexually mature animal.

22. A method of creating one or more heritable mutant alleles in at least one *Rosa* locus of interest, the method comprising
generating a transgenic animal according to any of claims 17 to 21, wherein the embryo is raised to sexual maturity; and
allowing the sexually mature animal to produce offspring; wherein at least some of the offspring comprise the mutant alleles.

23. A kit comprising a fusion protein according to any of claims 1-4 or a polynucleotide according to claim 5.

24. The kit of claim 23, further comprising additional components selected from the group consisting of one or more exogenous sequences, instructions for use, and combinations thereof.

25. An *in vitro* method for cleaving one or more *Rosa* genes in a cell, the method comprising:

introducing, into the cell, one or more fusion proteins according to any of claims 1-4 or one or more polynucleotides according to claim 5 into the cell, such that the one or more *Rosa* gene are cleaved.

26. An *in vitro* method of introducing the exogenous polynucleotide sequence into the genome of a cell, the method comprising:
cleaving one or more *Rosa* genes according to claim 9; and
contacting the cell with an exogenous polynucleotide sequence;
wherein cleavage of the one or more genes stimulates integration of the exogenous polynucleotide sequence into the genome by homologous recombination.

27. An *in vitro* method of modifying a *Rosa* gene sequence in the genome of a cell, the method comprising:
cleaving one or more *Rosa* genes according to claim 9, wherein
(i) a first ZFN cleaves at a first cleavage site and a second ZFN cleaves at a second cleavage site;
(ii) the *Rosa* gene sequence is located between the first cleavage site and the second cleavage site;
(iii) cleavage of the first and second cleavage sites results in a modification of the gene sequence by non-homologous end joining or homology directed repair.

28. An *in vitro* method of claim 15, wherein the modification comprises insertion of an exogenous sequence.

CLAIMS

What is claimed is:

1. An isolated fusion protein comprising a nuclease and an engineered zinc finger domain, wherein the engineered zinc finger domain binds to a target site in a *Rosa* gene and the zinc finger domain comprises four, five or six zinc finger recognition regions designated and ordered F1 to F4, or F1 to F5 or F1 to F6 from N-terminus to C-terminus and further wherein the zinc finger DNA-binding domains comprise recognition regions selected from the group consisting of:

- (i) F1: DRSDLSR (SEQ ID NO:38);
F2: RSDDLTR (SEQ ID NO:39);
F3: TSGHLSR (SEQ ID NO:40);
F4: RSDNLSV (SEQ ID NO:41); and
F5: RSANLTR (SEQ ID NO:42);
- (ii) F1: QSDHLTK (SEQ ID NO:43);
F2: NSSNLSR (SEQ ID NO:44);
F3: RSDHLTK (SEQ ID NO:45);
F4: NSDHLSR (SEQ ID NO:46); and
F5: RSDHLSR (SEQ ID NO:47);
- (iii) F1: RSDHLSE (SEQ ID NO:48);
F2: RSAALAR (SEQ ID NO:49);
F3: RSDHLST (SEQ ID NO:50);
F4: QNAHRIT(SEQ ID NO:51); and
F5: RSAVLSE (SEQ ID NO:52);
- (iv) F1: QSGDLTR (SEQ ID NO:17);
F2: TSGSLTR (SEQ ID NO:18);
F3: RSANLTR (SEQ ID NO:42);
F4: RSDHLTK (SEQ ID NO:45); and
F5: NSDHLSR (SEQ ID NO:46);
- (v) F1: RSANLTR (SEQ ID NO:42);
F2: QSGDLTR (SEQ ID NO:17);
F3: QSGDLTR (SEQ ID NO:17);
F4: RSANLAR (SEQ ID NO:53); and

- F5: RSDNLRE (SEQ ID NO:54);
- (vi) F1: RSDHLST (SEQ ID NO:50);
F2: DNRDRIK (SEQ ID NO:55);
F3: RSDTLSE (SEQ ID NO:56);
F4: QSSHLAR (SEQ ID NO:57); and
F5: QNAHRKT (SEQ ID NO:22);
- (vii) F1: QSGDLTR (SEQ ID NO:17);
F2: QSGDLTR (SEQ ID NO:17);
F3: RSDNLTR (SEQ ID NO:58);
F4: RSDNLSE (SEQ ID NO:21); and
F5: QNAHRKT (SEQ ID NO:22);
- (viii) F1: DRSDLSR (SEQ ID NO:38);
F2: RSDHLST (SEQ ID NO:50);
F3: DNRDRIK (SEQ ID NO:55);
F4: RSDTLSE (SEQ ID NO:56); and
F5: QSSHLAR (SEQ ID NO:57);
- (ix) F1: QSGDLTR (SEQ ID NO:17);
F2: RSDNLTR (SEQ ID NO:58);
F3: RSDNLSE (SEQ ID NO:21);
F4: QNAHRKT (SEQ ID NO:22);
F5: RSDHLSE (SEQ ID NO:48); and
F6: TSSTRKT (SEQ ID NO:59);
- (x) F1: TSGNLTR (SEQ ID NO:60);
F2: QSGNLAR (SEQ ID NO:61);
F3: RSDALSV (SEQ ID NO:62);
F4: DSSHRTR (SEQ ID NO:63); and
F5: RSDVLSE (SEQ ID NO:64)
- (xi) F1: RSDNLSE (SEQ ID NO:21);
F2: QNAHRKT (SEQ ID NO:22);
F3: RSDHLSE (SEQ ID NO:48);
F4: TSSTRKT (SEQ ID NO:59); and
F5: TSGHLR (SEQ ID NO:40);
- (xii) F1: TSGNLTR (SEQ ID NO:60);
F2: QSGNLAR (SEQ ID NO:61);

- F3: RSDALSV (SEQ ID NO:62); and
 F4: DSSHRTR (SEQ ID NO:63);
- (xiii) F1: QRSNLVR (SEQ ID NO:65);
 F2: RSDHLTQ (SEQ ID NO:66);
 F3: QSGHLQR (SEQ ID NO:67); and
 F4: DRSHLAR (SEQ ID NO:68);
- (xiv) F1: RSDVLSE (SEQ ID NO:64);
 F2: QRNHRTT (SEQ ID NO:69);
 F3: TKRSLIE (SEQ ID NO:70);
 F4: TSSNLSR (SEQ ID NO:71);
 F5: RSDDLK (SEQ ID NO:25); and
 F6: DNRDRIK (SEQ ID NO:55);
- (xv) F1: RSDHLSA (SEQ ID NO:72);
 F2: QSGHLSR (SEQ ID NO:24);
 F3: RSDHLSR (SEQ ID NO:47);
 F4: QNDNRIK (SEQ ID NO:73); and
 F5: QSGNLAR (SEQ ID NO:61); and
- (xvi) F1: NNRDLIN (SEQ ID NO:74);
 F2: TSSNLSR (SEQ ID NO:71);
 F3: RSDVLSE (SEQ ID NO:64);
 F4: QRNHRTT (SEQ ID NO:69);
 F5: TKRSLIE (SEQ ID NO:70); and
 F6: TSSNLSR (SEQ ID NO:71).

2. The isolated fusion protein of claim 1, wherein the nuclease comprises a cleavage domain or cleavage-half-domain.

3. The isolated fusion protein of claim 1 or 2 wherein the nuclease comprises a Type IIS restriction endonuclease cleavage domain or cleavage half-domain.

4. The isolated fusion protein of claim 3, wherein the cleavage domain or cleavage half-domain is naturally occurring or engineered.

5. A polynucleotide encoding an isolated fusion protein according to any one of claims 1 to 4.

6. An isolated cell comprising a fusion protein according to any one of claims 1-4 or a polynucleotide according to claim 5.

7. The isolated cell of claim 6, wherein the isolated cell is an embryo cell.

8. A composition comprising a fusion protein according to any one of claims 1-4 or a polynucleotide according to claim 5 and a pharmaceutically acceptable excipient.

9. A kit comprising an isolated fusion protein according to any one of claims 1-4 or a polynucleotide according to claim 5.

10. The kit of claim 9, further comprising additional components selected from the group consisting of one or more exogenous sequences, instructions for use, and combinations thereof.

11. An *in vitro* method for cleaving one or more *Rosa* genes in an isolated cell, the method comprising:

introducing, into the isolated cell, one or more fusion proteins according to any one of claims 1-4 or one or more polynucleotides according to claim 5 into the isolated cell, such that the one or more *Rosa* gene are cleaved.

12. An *in vitro* method of introducing the exogenous polynucleotide sequence into the genome of an isolated cell, the method comprising:

cleaving one or more *Rosa* genes as defined in claim 11; and

contacting the isolated cell with an exogenous polynucleotide sequence;

wherein cleavage of the one or more genes stimulates integration of the exogenous polynucleotide sequence into the genome by homologous recombination.

13. The method of claim 12, wherein the exogenous polynucleotide sequence is physically integrated into the genome.

14. The method of claim 13, wherein the exogenous polynucleotide sequence is integrated into the genome via nucleic acid replication processes.

15. The method of claim 13, wherein the exogenous polynucleotide sequence is integrated into the genome via non-homology dependent targeted integration.

16. An *in vitro* method of modifying a *Rosa* gene sequence in the genome of an isolated cell, the method comprising:

cleaving one or more *Rosa* genes as defined in claim 11, wherein

(i) a first ZFN cleaves at a first cleavage site and a second ZFN cleaves at a second cleavage site;

(ii) the *Rosa* gene sequence is located between the first cleavage site and the second cleavage site;

(iii) cleavage of the first and second cleavage sites results in a modification of the gene sequence by non-homologous end joining or homology directed repair.

17. The method of claim 16, wherein the modification comprises a deletion.

18. The method of claim 16, wherein the modification comprises insertion of an exogenous sequence.

19. A method for generating a non-human transgenic animal, wherein the method comprises:

modifying a *Rosa* gene sequence in a non-human embryo cell as described in any one of claims 16 to 18; and allowing the non-human embryo to develop into an animal.

20. The method of claim 19, wherein the modification comprises one or more random mutations at defined positions.

21. The method of claim 19, wherein the modification comprises insertion of a humanized gene.

22. The method of claim 21, wherein the humanized gene is associated with drug metabolism.

23. The method of any one of claims 19 to 22, wherein the animal is a sexually mature animal and the modified gene sequences are present in at least a portion of gametes of the sexually mature animal.

24. A method of creating one or more heritable mutant alleles in at least one *Rosa* locus of interest, the method comprising
generating a non-human transgenic animal according to any one of claims 19 to 23, wherein the non-human embryo is raised to sexual maturity; and
allowing the sexually mature non-human animal to produce offspring; wherein at least some of the offspring comprise the mutant alleles.