CLAIMS

1. A method for modifying a chromosomal sequence in a eukaryotic cell, the method comprising:

a) introducing into the eukaryotic cell two RNA-guided nickase systems or nucleic acid encoding said systems, and, optionally, a donor polynucleotide, wherein each RNA-guided nickase system comprises

(i) a RNA-guided endonuclease that is modified to cleave one strand of a double-stranded sequence; and

(ii) a guide RNA comprising a first region having complementarity to a target site in the chromosomal sequence and a second region that interacts with the RNA-guided endonuclease,

wherein each target site is immediately followed by a protospacer adjacent motif (PAM), and the target sites of the two RNA-guided endonucleases are on opposite strands of the chromosomal sequence; and

b) culturing the eukaryotic cell such that the two RNA-guided endonucleases cleave opposite strands of the chromosomal sequence in close enough proximity to introduce a double-stranded break in the chromosomal sequence, and repair of the double-stranded break by a DNA repair process leads to modification of the chromosomal sequence, wherein

the method does not comprise a process for modifying the germ line genetic identity of a human being and, wherein

the method does not comprise a method for treatment of the human or animal body by surgery or therapy.

2. An ex vivo or in vitro method for modifying a chromosomal sequence in a eukaryotic cell, the method comprising:

a) introducing into the eukaryotic cell two RNA-guided nickase systems or nucleic acid encoding said systems, and, optionally, a donor polynucleotide, wherein each RNA-guided nickase system comprises

(i) a RNA-guided endonuclease that is modified to cleave one strand of a double-stranded sequence; and

(ii) a guide RNA comprising a first region having complementarity to a target site in the chromosomal sequence and a second region that interacts with the RNA-guided endonuclease,

wherein each target site is immediately followed by a protospacer adjacent motif (PAM), and the target sites of the two RNA-guided endonucleases are on opposite strands of the chromosomal sequence; and

b) culturing the eukaryotic cell such that the two RNA-guided endonucleases cleave opposite strands of the chromosomal sequence in close enough proximity to introduce a double-stranded break in the chromosomal sequence, and repair of the double-stranded break by a DNA repair process leads to modification of the chromosomal sequence, wherein

the method does not comprise a process for modifying the germ line genetic identity of a human being.

3. The method of claim 1 or claim 2, wherein each RNA-guided endonuclease is derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR/Cas) type II system protein.

4. The method of claim 3, wherein the CRISPR/Cas type II system protein is a Cas9 protein.

5. The method of claim 4, wherein the Cas9 protein comprises a mutation in the RuvC or HNH domain.

6. The method of any previous claim, wherein each RNA-guided endonuclease further comprises at least one additional domain chosen from a nuclear localization signal, a cell-penetrating domain, or a marker domain.

7. The method of any previous claim, wherein the target site is a Rosa26 locus, a HPRT locus, or an AAVS1 locus.

8. The method of any previous claim, wherein the donor polynucleotide comprises a donor sequence that has at least one nucleotide change relative to the chromosomal sequence near the target sites in the chromosomal sequence.

9. The method of claim 8, wherein the donor sequence is flanked by sequences having substantial sequence identity to sequences located upstream and downstream of the target site in the chromosomal sequence.

10. The method of claim 8 or claim 9, wherein the donor sequence is flanked by short overhangs that are compatible with overhangs generated by the RNA-guided endonuclease.

11. The method of any previous claim, wherein the nucleic acid encoding each RNA-guided endonuclease is mRNA, and the nucleic acid encoding each guide RNA is DNA.

12. The method of any of claims 1-10, wherein the nucleic acid encoding each RNA-guided endonuclease is DNA and the nucleic acid encoding each guide RNA is DNA.

13. The method of claim 12, wherein the DNA is part of a vector that further comprises a promoter control sequence that is operably linked to the nucleic acid encoding the RNA-guided endonuclease and a promoter control sequence that is operably linked to the nucleic acid encoding the guide RNA.

14. The method of any of claims 1-13, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, or a non-human mammalian embryo.

15. The method of any of claims 1-13, wherein the eukaryotic cell is an invertebrate cell, an insect cell, a plant cell, a yeast cell or a single cell eukaryotic organism.

16. The method of claim 15, wherein the eukaryotic cell is a plant cell.

17. The method of claim of 14, wherein the human cell or non-human mammalian cell is a T cell.

18. The method of any of claims 1-17, wherein the eukaryotic cell is *in vitro*.

19. The method of any previous claim, wherein the donor polynucleotide is not introduced into the eukaryotic cell, and repair of the double-stranded break by a non-homologous end-joining repair process results in inactivation of the chromosomal sequence.

20. The method of any of claims 1-18, wherein the donor polynucleotide is introduced into the eukaryotic cell, and repair of the double-stranded break results in a change of at least one nucleotide in the chromosomal sequence.

21. The method of claim 20, wherein the change comprises an integration of an exogenous sequence.

22. The method of any one of claims 1-10 or 14-21, wherein the at least one guide RNA is chemically synthesized.

23. A composition comprising at least two RNA-guided nickase systems, each RNA-guided nickase system comprising (i) a RNA-guided endonuclease that is modified to cleave one strand of a double-stranded sequence, and (ii) a guide RNA comprising a first region having complementarity to a target site in a chromosomal sequence and a second region that interacts with the RNA-guided endonuclease, wherein each target site is immediately followed by a protospacer adjacent motif (PAM), and the target sites of two RNA-guided endonucleases are on opposite strands of the chromosomal sequence and in close enough proximity to introduce a double-stranded break in the chromosomal sequence.

24. The composition of claim 23, wherein each RNA-guided endonuclease is derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR/Cas) type II system protein.

25. The composition of claim 24, wherein the CRISPR/Cas type II system protein is a Cas9 protein.

26. The composition of claim 25, wherein the Cas9 protein comprises a mutation in the RuvC or HNH domain.

27. The composition of any of claims 23-26, wherein each RNA-guided endonuclease further comprises at least one additional domain chosen from a nuclear localization signal, a cell-penetrating domain, or a marker domain.

28. The composition of any of claims 23-27, wherein the target site is a Rosa26 locus, a HPRT locus, or an AAVS1 locus.

29. The composition of any of claims 23-28, wherein the at least one guide RNA is chemically synthesized.

30. A nucleic acid encoding the composition of any of claims 23-28.

31. The nucleic acid of claim 30, wherein the nucleic acid encoding each RNA-guided endonuclease is mRNA and the nucleic acid encoding each guide RNA is DNA.

32. The nucleic acid of claim 30, wherein the nucleic acid encoding each RNA-guided endonuclease is DNA and the nucleic acid encoding each guide RNA is DNA.

33. A vector comprising the nucleic acid of claim 32, wherein the nucleic acid encoding each RNA-guided endonuclease is operably linked to a promoter control sequence for expression in a eukaryotic cell, and the nucleic acid encoding each guide RNA is operably linked to a promoter control sequence for expression in a eukaryotic cell.

34. A kit comprising at least two RNA-guided nickase systems or nucleic acid encoding said systems, each RNA-guided nickase system comprising (i) a RNA-guided endonuclease that is modified to cleave one strand of a double-stranded sequence and (ii) a guide RNA comprising a first region having complementarity to a target site in a chromosomal sequence and a second region that interacts with the RNA-guided endonuclease, wherein each target site is immediately followed by a protospacer adjacent motif (PAM), and the target sites of two RNA-guided endonucleases are on opposite strands of the chromosomal sequence and in close enough proximity to introduce a double-stranded break in the chromosomal sequence.

35. The kit of claim 34, further comprising at least one donor polynucleotide.

36. The kit of claim 34 or claim 35, wherein each RNA-guided endonuclease is derived from a clustered regularly interspersed short palindromic repeats (CRISPR)/CRISPR-associated (Cas) (CRISPR/Cas) type II system protein.

37. The kit of claim 36, wherein the CRISPR/Cas type II system protein is a Cas9 protein.

38. The kit of claim 37, wherein the Cas9 protein comprises a mutation in the RuvC or HNH domain.

39. The kit of any of claims 34-38, wherein each RNA-guided endonuclease further comprises at least one additional domain chosen from a nuclear localization signal, a cell-penetrating domain, or a marker domain.

40. The kit of any of claims 34-39, wherein the nucleic acid encoding each RNA-guided endonuclease is mRNA and the nucleic acid encoding each guide RNA is DNA.

41. The kit of any of claims 34-39, wherein the nucleic acid encoding each RNA-guided endonuclease is DNA and the nucleic acid encoding each guide RNA is DNA.

42. The kit of claim 41, wherein the nucleic acid encoding each RNA-guided endonuclease is operably linked to a promoter control sequence for expression in a eukaryotic cell, and the nucleic acid encoding each guide RNA is operably linked to a promoter control sequence for expression in a eukaryotic cell.

43. The kit of claim 35, wherein the donor polynucleotide comprises a donor sequence.

44. The kit of claim 43, wherein the donor sequence is flanked by sequences having substantial sequence identity to sequences on either side of the target sites in the chromosomal sequence.

45. The kit of claim 43, wherein the donor sequence is flanked by short overhangs that are compatible with overhangs generated by the RNA-guided endonuclease.

46. The kit of any of claims 34-39 or 43-45, wherein the at least one guide RNA is chemically synthesized.