CLAIMS

What is claimed is:

1. An isolated endonuclease comprising at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage.

2. The endonuclease of claim 1, wherein the endonuclease is derived from a Cas9 protein.

3. The isolated endonuclease of either claim 1 or claim 2, wherein the endonuclease is modified to lack at least one functional nuclease domain.

4. The isolated endonuclease of any one of claims 1 to 3, further comprising a cell-penetrating domain, a marker domain, or both.

5. The isolated endonuclease of any one of claim 1 to 4, which is part of a protein-RNA complex comprising the guide RNA.

6. The isolated endonuclease of claim 5, wherein the guide RNA is a single molecule comprising a 5' region that is complementary to a target site.

7. An isolated nucleic acid encoding the endonuclease of any one of claims 1 to 4.

8. The isolated nucleic acid of claim 7, wherein the nucleic acid is codon optimized for translation in mammalian cells.

9. The isolated nucleic acid of claim 7, wherein the nucleic acid is codon optimized for translation in human cells.

10. The isolated nucleic acid of any one of claims 7 to 9, which is operably linked to a promoter control sequence.

11. A vector comprising the isolated nucleic acid of claim 10.

12. The vector of claim 11, further comprising a sequence encoding a guide RNA that is operably linked to a promoter control sequence.

13. A method for modifying a chromosomal sequence in a eukaryotic cell or embryo, the method comprising:
    a) introducing into the eukaryotic cell or embryo (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide; and
    b) culturing the eukaryotic cell or embryo such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified.

14. The method of claim 13, wherein the RNA-guided endonuclease is derived from a Cas9 protein.

15. The method of either claim 13 or claim 14, wherein the nucleic acid encoding the RNA-guided endonuclease is mRNA.

16. The method of either claim 13 or claim 14, wherein the nucleic acid encoding the RNA-guided endonuclease is DNA.

17. The method of claim 16, wherein the DNA is part of a vector that further comprises a sequence encoding the guide RNA.

18. The method of any one of claims 13 to 17, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, a stem cell, a non-mammalian

vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism.

19. The method of any one of claims 13 to 17, wherein the embryo is a non-human one cell animal embryo.

20. A fusion protein comprising a CRISPR/Cas-like protein or fragment thereof and an effector domain.

21. The fusion protein of claim 20, wherein the CRISPR/Cas-like protein is derived from a Cas9 protein.

22. The fusion protein of claim 21, wherein the Cas9 protein is modified to lack at least one functional nuclease domain.

23. The fusion protein of claim 21, wherein the Cas9 protein is modified to lack all nuclease activity.

24. The fusion protein of any one of claims 20 to 23, wherein the effector domain is a cleavage domain.

25. The fusion protein of claim 24, wherein the cleavage domain is a FokI endonuclease domain or a modified FokI cleavage domain.

26. The fusion protein of claim 25, wherein the fusion protein forms a dimer with a second fusion protein.

27. The fusion protein of claim 25, wherein the fusion protein forms a heterodimer with a zinc finger nuclease comprising a FokI endonuclease domain or a modified FokI cleavage domain.

28. The fusion protein of claim 20, wherein the CRISPR/Cas-like protein is derived from a Cas9 protein modified to lack all nuclease activity, and the effector domain is a FokI endonuclease domain or a modified FokI cleavage domain.

29. The fusion protein of any one of claims 20 to 23, wherein the effector domain is chosen from a transcriptional activation domain, a transcriptional repressor domain, and an epigenetic modification domain.

30. The fusion protein of any one of claims 20 to 29, wherein the fusion protein further comprises at least one additional domain chosen from a nuclear localization signal, a cell-penetrating domain, and a marker domain.

31. An isolated nucleic acid encoding the fusion protein of any one claims 20 to 30.

32. A method for modifying a chromosomal sequence or regulating expression of a chromosomal sequence in a cell or embryo, the method comprising introducing into the cell or embryo (a) at least one fusion protein or nucleic acid encoding at least one fusion protein, wherein the fusion protein comprises a CRISPR/Cas-like protein or a fragment thereof and an effector domain, and (b) at least one guide RNA or DNA encoding at least one guide RNA, wherein the one guide RNA guides the CRISPR/Cas-like protein of the fusion protein to a targeted site in the chromosomal sequence and the effector domain of the fusion protein modifies the chromosomal sequence or regulates expression of the chromosomal sequence.

33. The method of claim 32, wherein the CRISPR/Cas-like protein is derived from a Cas9 protein.

34. The method of claim 33, wherein the cas9 protein is modified to lack at least one functional nuclease domain.

35. The method of claim 33, wherein the cas9 protein is modified to lack all nuclease activity.

36. The method of any one of claims 32 to 35, wherein fusion protein further comprises at least one additional domain chosen from a nuclear localization signal, a cell-penetrating domain, and a marker domain.

37. The method of any one of claims 32 to 36, wherein the effector domain is a cleavage domain.

38. The method of claim 37, wherein one fusion protein or nucleic acid encoding one fusion protein and two guide RNAs or DNA encoding two guide RNAs are introduced into the cell or embryo.

39. The method of claim 38, wherein the fusion protein comprises a Cas9 protein or fragment thereof and a FokI cleavage domain or a modified FokI cleavage domain.

40. The method of claim 37, wherein two fusion proteins or nucleic acid encoding two fusion proteins and two guide RNAs or DNA encoding two guide RNAs are introduced into the cell or embryo.

41. The method of claim 40, wherein each fusion protein comprises a different Cas9 protein or fragment thereof and/or a FokI cleavage domain or a modified FokI cleavage domain.

42. The method of claim 37, wherein one fusion protein or nucleic acid encoding one fusion protein, one guide RNA or DNA encoding one guide RNA, and one zinc finger nuclease or nucleic acid encoding one zinc finger nuclease are introduced into the cell or embryo.

43. The method of claim 42, wherein the fusion protein comprises a Cas9 protein or fragment thereof and a FokI cleavage domain or derivative thereof, and the zinc finger nuclease comprises a FokI cleavage domain or a modified FokI cleavage domain.

44. The method of any one of claims 37 to 43, further comprising introducing into the cell or embryo at least one donor polynucleotide.

45. The method of any one of claims 32 to 36, wherein the effector domain is chosen from an epigenetic modification domain, a transcriptional activation domain, or a transcriptional repressor domain.

46. The method of claim 45, wherein one fusion protein or nucleic acid encoding one fusion protein, and one guide RNA or DNA encoding one guide RNA are introduced into the cell or embryo.

47. The method of any one of claims 32 to 46, wherein the cell is a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism.

48. The method of any one of claims 32 to 46, wherein the embryo is a non-human one cell animal embryo.

CLAIMS

What is claimed is:

1. An isolated endonuclease comprising at least one nuclear localization signal, at least one nuclease domain, and at least one domain that interacts with a guide RNA to target the endonuclease to a specific nucleotide sequence for cleavage, wherein the endonuclease is derived from a Cas9 protein and comprises a RuvC-like nuclease domain and a HNH-like nuclease domain.

2. The isolated endonuclease of claim 1, wherein the endonuclease is modified to lack one functional nuclease domain.

3. The isolated endonuclease of any one of claims 1 to 2, further comprising a marker.

4. The isolated endonuclease of any one of claim 1 to 3, which is part of a protein-RNA complex comprising the guide RNA.

5. The isolated endonuclease of claim 4, wherein the guide RNA is a single molecule comprising a 5′ region that is complementary to a target site.

6. An isolated nucleic acid encoding the endonuclease of any one of claims 1 to 3.

7. The isolated nucleic acid of claim 6, wherein the nucleic acid is codon optimized for translation in mammalian cells.

8. The isolated nucleic acid of claim 6, wherein the nucleic acid is codon optimized for translation in human cells.

9. The isolated nucleic acid of any one of claims 6 to 8, which is operably linked to a promoter control sequence.

10. A vector comprising the isolated nucleic acid of claim 9.

11. The vector of claim 10, further comprising a sequence encoding a guide RNA that is operably linked to a promoter control sequence.

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

a) introducing into the eukaryotic cell (i) at least one RNA-guided endonuclease comprising at least one nuclear localization signal or nucleic acid encoding at least one RNA-guided endonuclease comprising at least one nuclear localization signal, wherein the RNA-guided endonuclease is derived from a Cas9 protein, (ii) at least one guide RNA or DNA encoding at least one guide RNA, and, optionally, (iii) at least one donor polynucleotide; and

b) culturing the eukaryotic cell such that each guide RNA directs an RNA-guided endonuclease to a targeted site in the chromosomal sequence where the RNA-guided endonuclease introduces a double-stranded break in the targeted site, and the double-stranded break is repaired by a DNA repair process such that the chromosomal sequence is modified.

13. The method of claim 12, wherein the nucleic acid encoding the RNA-guided endonuclease is mRNA.

14. The method of claim 12, wherein the nucleic acid encoding the RNA-guided endonuclease is DNA.

15. The method of claim 14, wherein the DNA is part of a vector that further comprises a sequence encoding the guide RNA.

16. The method of any one of claims 12 to 15, wherein the eukaryotic cell is a human cell, a non-human mammalian cell, a stem cell, a non-mammalian vertebrate cell, an invertebrate cell, a plant cell, or a single cell eukaryotic organism.