Claims

1. A composition comprising a Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system for cleavage of a target nucleic acid in a eukaryotic cell, said CRISPR/Cas system comprising (i) a Cas9 polypeptide and a nuclear localization signal, or a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal, and (ii) a guide RNA that hybridizes to the target nucleic acid in a eukaryotic cell, wherein the guide RNA is a chimeric guide RNA comprising a CRISPR RNA (crRNA) sequence fused to a trans activating crRNA (tracrRNA) sequence, wherein the crRNA sequence is at the 5' end relative to the tracrRNA sequence.

2. The composition of claim 1, wherein said Cas9 polypeptide is a *Streptococcus* Cas9 polypeptide.

3. The composition of claim 2, wherein said Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 polypeptide.

4. The composition of any one of claims 1-3, which comprises in item (i) a Cas9 polypeptide and a nuclear localization signal.

5. The composition of any one of claims 1-3, which comprises in item (i) a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal.

6. The composition of claim 5, wherein said nucleic acid encoding a Cas9 polypeptide is codon-optimized for expression in eukaryotic cells.

7. The composition of claim 5, wherein said nucleic acid encoding a Cas9 polypeptide is codon-optimized for expression in mammalian cells.

8. The composition of any one of claims 1-7, wherein said nuclear localization signal is located at the C terminus or N terminus of the Cas9 polypeptide.

9. The composition of claim 8, wherein said nuclear localization signal is located at the C terminus of the Cas9 polypeptide.

10. The composition of any one of claims 1-9, wherein the target nucleic acid is a genomic sequence located at its endogenous site in the genome of the eukaryotic cell.

11. The composition of any one of claims 1-10, wherein the guide RNA is a synthetic guide RNA.

12. The composition of any one of claims 1-11, wherein the guide RNA is *in vitro* transcribed RNA.

13. The composition of any one of claims 1-12, wherein the guide RNA comprises one or more additional nucleic acid residues 5' to the crRNA sequence.

14. The composition of claim 13, wherein the one or more additional nucleic residues are two nucleic acid residues, and wherein there are no additional nucleic acid residues between the two nucleic acid residues and the crRNA sequence.

15. The composition of claim 14, wherein the two nucleic acid residues at the 5' end are both guanine residues.

16. The composition of any one of claims 1-15, wherein the target nucleic acid sequence comprises a sequence having a trinucleotide protospacer adjacent motif (PAM), wherein the PAM is a trinucleotide of 5'-NGG-3' selected from the group consisting of 5'-AGG-3', 5'-GGG-3', 5'-TGG-3' and 5'-CGG-3'.

17. A composition comprising a Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system for cleavage of a target nucleic acid in a eukaryotic cell, said CRISPR/Cas system comprising (i) a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal, wherein said nucleic acid comprises SEQ ID NO:1, or a Cas9 polypeptide and a nuclear localization signal encoded by the nucleic acid, and (ii) a guide RNA that hybridizes to the target nucleic acid in a eukaryotic cell, wherein the guide RNA

is a chimeric guide RNA comprising a CRISPR RNA (crRNA) sequence fused to a trans activating crRNA (tracrRNA) sequence, wherein the crRNA sequence is at the 5' end relative to the tracrRNA sequence.

18. The composition of claim 17, wherein the target nucleic acid sequence comprises a sequence having a trinucleotide protospacer adjacent motif (PAM), wherein the PAM is a trinucleotide of 5'-NGG-3' selected from the group consisting of 5'-AGG-3', 5'-GGG-3', 5'-TGG-3' and 5'-CGG-3'.

19. The composition of claim 17 or 18, which comprises in item (i) a Cas9 polypeptide and a nuclear localization signal encoded by the nucleic acid.

20. The composition of claim 17 or 18, which comprises in item (i) a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal, wherein said nucleic acid comprises SEQ ID NO:1.

21. The composition of any one of claims 17-20, wherein said nuclear localization signal is located at the C terminus or N terminus of the Cas9 polypeptide.

22. The composition of claim 21, wherein said nuclear localization signal is located at the C terminus of the Cas9 polypeptide

23. The composition of any one of claims 17-22, wherein the target nucleic acid is a genomic sequence located at its endogenous site in the genome of the eukaryotic cell.

24. The composition of any one of claims 17-23, wherein the guide RNA is a synthetic guide RNA.

25. The composition of any one of claims 17-24, wherein the guide RNA is *in vitro* transcribed RNA.

26. The composition of any one of claims 17-25, wherein the guide RNA comprises one or more additional nucleic acid residues 5' to the crRNA sequence.

27. The composition of claim 26, wherein the one or more additional nucleic acid residues are two nucleic acid residues, and wherein there are no additional nucleic acid residues between the two nucleic acid residues and the crRNA sequence.

28. The composition of claim 27, wherein the two nucleic acid residues at the 5' end are both guanine residues.

29. An *in vitro* method of introducing a site-specific, double-stranded break at a target nucleic acid sequence in a eukaryotic cell, the method comprising introducing into the eukaryotic cell a Type II Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system, wherein the CRISPR/Cas system comprises:

    a) a Cas9 polypeptide and a nuclear localization signal, or a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal, and

    b) a guide RNA that hybridizes to the target nucleic acid, wherein the guide RNA is a chimeric guide RNA comprising a CRISPR RNA (crRNA) sequence fused to a trans activating crRNA (tracrRNA) sequence, wherein the crRNA sequence is at the 5' end relative to the tracrRNA sequence; wherein the target nucleic acid sequence comprises a sequence that binds to the crRNA sequence,

    and whereby a site-specific, double stranded break at the target nucleic acid sequence is introduced.

30. The method of claim 29, wherein the Cas9 polypeptide is a *Streptococcus* Cas9 polypeptide.

31. The method of claim 30, wherein the Cas9 polypeptide is a *Streptococcus pyogenes* Cas9 polypeptide.

32. The method of any one of claims 29-31, wherein the CRISPR/Cas system comprises in item (a) a Cas9 polypeptide and a nuclear localization signal.

33. The method of any one of claims 29-31, wherein the CRISPR/Cas system comprises in item (a) a nucleic acid encoding a Cas9 polypeptide and a nuclear localization signal.

34. The method of claim 33, wherein said nucleic acid encoding a Cas9 polypeptide is codon-optimized for expression in eukaryotic cells.

35. The method of claim 33, wherein the nucleic acid encoding the Cas 9 polypeptide is codon-optimized for expression in mammalian cells.

36. The method of any one of claims 33-35, wherein the nucleic acid encoding the Cas9 polypeptide is introduced into the eukaryotic cell before introducing the guide RNA into the eukaryotic cell.

37. The method of any one of claims 33-35, wherein the nucleic acid encoding the Cas9 polypeptide is co-transfected into the eukaryotic cell with the guide RNA.

38. The method of any one of claims 29-37, wherein the nuclear localization signal is located at the C terminus or N terminus of the Cas9 polypeptide.

39. The method of claim 38, wherein the nuclear localization signal is located at the C terminus of the Cas9 polypeptide.

40. The method of any one of claims 29-39, wherein the eukaryotic cell is a mammalian cell.

41. The method of claim 40, wherein the mammalian cell is a human cell.

42. The method of any one of claims 29-39, wherein the eukaryotic cell is a non-human eukaryotic cell.

43. The method of any one of claims 29-42, wherein the target nucleic acid sequence is a genomic sequence located at its endogenous site in the genome of the eukaryotic cell.

44. The method of any one of claims 29-43, wherein the guide RNA is a synthetic guide RNA.

45. The method of any one of claims 29-44, wherein the guide RNA is in vitro transcribed

RNA.

46. The method of any one of claims 29-45, wherein the guide RNA comprises one or more additional nucleic acid residues 5’ to the crRNA sequence.

47. The method of claim 46, wherein the one or more additional nucleic acid residues are two nucleic acid residues, and wherein there are no additional nucleic acid residues between the two nucleic acid residues and the crRNA sequence.

48. The method of claim 47, wherein the two nucleic acid residues at the 5’ end are both guanine residues.

49. The method of any one of claims 29-48, wherein the target nucleic acid sequence comprises a sequence having a trinucleotide protospacer adjacent motif (PAM), wherein the PAM is a trinucleotide of 5’-NGG-3’ selected from the group consisting of 5’-AGG-3’, 5’-GGG-3’, 5’-TGG-3’ and 5’-CGG-3’.