CLAIMS

What is claimed is:

1. A DNA-targeting RNA comprising:

(i) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(ii) a second segment that interacts with a site-directed modifying polypeptide.

2. The DNA-targeting RNA of Claim 1, wherein the first segment comprises 8 nucleotides that have 100% complementarity to a sequence in the target DNA.

3. The DNA-targeting RNA of Claim 1, wherein the second segment comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:563-682, or a complement thereof.

4. The DNA-targeting RNA of Claim 1, wherein the second segment comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-562, or a complement thereof.

5. The DNA-targeting RNA of Claim 1, wherein the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

6. A DNA polynucleotide comprising a nucleotide sequence that encodes the DNA-targeting RNA of Claim 1.

7. A recombinant expression vector comprising the DNA polynucleotide of Claim 6.

8. The recombinant expression vector of claim 7, wherein the nucleotide sequence encoding the DNA-targeting RNA is operably linked to a promoter.

9. The recombinant expression vector of claim 8, wherein the promoter is an inducible promoter.

10. The recombinant expression vector of claim 7, wherein the nucleotide sequence encoding the DNA-targeting RNA of Claim 1 further comprises a multiple cloning site.

11. An *in vitro* genetically modified host cell comprising the DNA polynucleotide of Claim 6.

12. A recombinant expression vector comprising:

(i) a nucleotide sequence encoding a DNA-targeting RNA, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a nucleotide sequence encoding the site-directed modifying polypeptide comprising:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

13. A recombinant expression vector comprising:

(i) a nucleotide sequence encoding a DNA-targeting RNA, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a nucleotide sequence encoding the site-directed modifying polypeptide, wherein the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that modulates transcription within the target DNA, wherein the site of modulated transcription within the target DNA is determined by the DNA-targeting RNA.

14. A variant site-directed modifying polypeptide comprising:

(i) an RNA-binding portion that interacts with a DNA-targeting RNA, wherein the DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in a target DNA; and

(ii) an activity portion that exhibits reduced site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

15. The variant site-directed modifying polypeptide of Claim 14, comprising an H840A mutation of the S. pyogenes sequence SEQ ID NO:8 or the corresponding mutation in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

16. The variant site-directed modifying polypeptide of Claim 14, comprising a D10A mutation of the S. pyogenes sequence SEQ ID NO:8 or the corresponding mutation in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

17. The variant site-directed modifying polypeptide of Claim 14, comprising both (i) a D10A mutation of the S. pyogenes sequence SEQ ID NO:8 or the corresponding mutation in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346; and (ii) an H840A mutation of the S. pyogenes sequence SEQ ID NO:8 or the corresponding mutation in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

18. A chimeric site-directed modifying polypeptide comprising:
(i) an RNA-binding portion that interacts with a DNA-targeting RNA, wherein the DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in a target DNA; and
(ii) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

19. The chimeric site-directed modifying polypeptide of Claim 18 comprising an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

20. The chimeric site-directed modifying polypeptide of Claim 18, wherein the DNA-targeting RNA further comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:563-682.

21. The chimeric site-directed modifying polypeptide of Claim 18, wherein the DNA-targeting RNA further comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-562.

22. The chimeric site-directed modifying polypeptide of Claim 18, wherein the enzymatic activity modifies the target DNA.

23. The chimeric site-directed modifying polypeptide of Claim 22, wherein the enzymatic activity is nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity.

24. The chimeric site-directed modifying polypeptide of Claim 23, wherein the enzymatic activity is nuclease activity.

25. The chimeric site-directed modifying polypeptide of Claim 24, wherein the nuclease activity introduces a double strand break in the target DNA.

26. The chimeric site-directed modifying polypeptide of Claim 18, wherein the enzymatic activity modifies a target polypeptide associated with the target DNA.

27. The chimeric site-directed modifying polypeptide of Claim 26, wherein the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity.

28. The chimeric site-directed modifying polypeptide of Claim 26, wherein the target polypeptide is a histone and the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity or deubiquitinating activity.

29. An RNA polynucleotide comprising a nucleotide sequence encoding the chimeric site-directed modifying polypeptide of Claim 18.

30. A DNA polynucleotide comprising a nucleotide sequence encoding the chimeric site-directed modifying polypeptide of Claim 18.

31. A recombinant expression vector comprising the polynucleotide of Claim 30.

32. The recombinant expression vector of Claim 31, wherein the polynucleotide is operably linked to a promoter.

33. The recombinant expression vector of Claim 32, wherein the promoter is an inducible promoter.

34. An *in vitro* genetically modified host cell comprising the polynucleotide of Claim 30.

35. A chimeric site-directed modifying polypeptide comprising:

(i) an RNA-binding portion that interacts with a DNA-targeting RNA, wherein the DNA-targeting RNA comprises a nucleotide sequence that is complementary to a sequence in a target DNA; and

(ii) an activity portion that modulates transcription within the target DNA, wherein the site of modulated transcription within the target DNA is determined by the DNA-targeting RNA.

36. The chimeric site-directed modifying polypeptide of Claim 35, wherein the activity portion increases transcription within the target DNA.

37. The chimeric site-directed modifying polypeptide of Claim 35, wherein the activity portion decreases transcription within the target DNA.

38. A genetically modified cell comprising a recombinant site-directed modifying polypeptide comprising an RNA-binding portion that interacts with a DNA-targeting RNA; and an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

39. The genetically modified cell of Claim 38, wherein the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

40. The genetically modified cell of Claim 38, wherein the cell is selected from the group consisting of: an archaeal cell, a bacterial cell, a eukaryotic cell, a eukaryotic single-cell organism, a somatic cell, a germ cell, a stem cell, a plant cell, an algal cell, an animal cell, in invertebrate cell, a

vertebrate cell, a fish cell, a frog cell, a bird cell, a mammalian cell, a pig cell, a cow cell, a goat cell, a sheep cell, a rodent cell, a rat cell, a mouse cell, a non-human primate cell, and a human cell.

41. A transgenic non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding a recombinant site-directed modifying polypeptide comprising: (i) an RNA-binding portion that interacts with a DNA-targeting RNA; and (ii) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

42. The transgenic organism of Claim 41, wherein the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

43. The transgenic organism of Claim 41, wherein the organism is selected from the group consisting of: an archaea, a bacterium, a eukaryotic single-cell organism, an algae, a plant, an animal, an invertebrate, a fly, a worm, a cnidarian, a vertebrate, a fish, a frog, a bird, a mammal, an ungulate, a rodent, a rat, a mouse, and a non-human primate.

44. A composition comprising:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, the DNA-targeting RNA comprising:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) the site-directed modifying polypeptide, or a polynucleotide encoding the same, the site-directed modifying polypeptide comprising:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

45. The composition of Claim 44, wherein the first segment of the DNA-targeting RNA comprises 8 nucleotides that have at least 100% complementarity to a sequence in the target DNA.

46. The composition of Claim 44, wherein the second segment of the DNA-targeting RNA comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:563-682.

47. The composition of Claim 44, wherein the second segment of the DNA-targeting RNA comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-562.

48. The composition of Claim 44, wherein the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

49. The composition of Claim 44, wherein the enzymatic activity modifies the target DNA.

50. The composition of Claim 49, wherein the enzymatic activity is nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity.

51. The composition of Claim 50, wherein the enzymatic activity is nuclease activity.

52. The composition of Claim 51, wherein the nuclease activity introduces a double strand break in the target DNA.

53. The composition of Claim 44, wherein the enzymatic activity modifies a target polypeptide associated with the target DNA.

54. The composition of Claim 53, wherein the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity.

55. The composition of Claim 53, wherein the target polypeptide is a histone and the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity or deubiquitinating activity.

56. The composition of Claim 44, wherein the DNA-targeting RNA is a double-molecule DNA-targeting RNA and the composition comprises both a targeter-RNA and an activator-RNA, the duplex-forming segments of which are complementary and hybridize to form the second segment of the DNA-targeting RNA.

57. The composition of Claim 56, wherein the duplex-forming segment of the activator-RNA comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-682.

58. A composition comprising:
(i) the DNA-targeting RNA of Claim 44, or a DNA polynucleotide encoding the same; and
(ii) a buffer for stabilizing nucleic acids.

59. A composition comprising:
(i) the site-directed modifying polypeptide of Claim 44, or a polynucleotide encoding the same; and
(ii) a buffer for stabilizing nucleic acids and/or proteins.

60. A composition comprising:
(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, the DNA-targeting RNA comprising:
(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and
(b) a second segment that interacts with a site-directed modifying polypeptide; and
(ii) the site-directed modifying polypeptide, or a polynucleotide encoding the same, the site-directed modifying polypeptide comprising:
(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and
(b) an activity portion that modulates transcription within the target DNA, wherein the site of modulated transcription within the target DNA is determined by the DNA-targeting RNA.

61. The composition of Claim 60, wherein the activity portion increases transcription within the target DNA.

62. The composition of Claim 60, wherein the activity portion decreases transcription within the target DNA.

63. A composition comprising:
(i) the site-directed modifying polypeptide of Claim 60, or a polynucleotide encoding the same; and
(ii) a buffer for stabilizing nucleic acids and/or proteins.

64. A method of site-specific modification of a target DNA, the method comprising:
contacting the target DNA with:
(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises:
(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and
(b) a second segment that interacts with a site-directed modifying polypeptide; and
(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide comprises:
(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and
(b) an activity portion that exhibits site-directed enzymatic activity.

65. The method of Claim 64, wherein the target DNA is extrachromosomal.

66. The method of Claim 64, wherein the target DNA comprises a PAM sequence of the complementary strand that is 5'-CCY-3', wherein Y is any DNA nucleotide and Y is immediately 5' of the target sequence of the complementary strand of the target DNA.

67. The method of Claim 64, wherein the target DNA is part of a chromosome *in vitro*.

68. The method of Claim 64, wherein the target DNA is part of a chromosome *in vivo*.

69. The method of Claim 64, wherein the target DNA is part of a chromosome in a cell.

70. The method of Claim 69, wherein the cell is selected from the group consisting of: an archaeal cell, a bacterial cell, a eukaryotic cell, a eukaryotic single-cell organism, a somatic cell, a germ cell, a stem cell, a plant cell, an algal cell, an animal cell, in invertebrate cell, a vertebrate cell, a fish cell,

a frog cell, a bird cell, a mammalian cell, a pig cell, a cow cell, a goat cell, a sheep cell, a rodent cell, a rat cell, a mouse cell, a non-human primate cell, and a human cell.

71. The method of Claim 64, wherein the DNA-targeting RNA comprises a nucleotide sequence with at least least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:563-682.

72. The method of Claim 64, wherein the DNA-targeting RNA comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-562.

73. The method of Claim 64, wherein the DNA-modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

74. The method of Claim 64, wherein the enzymatic activity modifies the target DNA.

75. The method of Claim 74, wherein the enzymatic activity is nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity or glycosylase activity.

76. The method of Claim 75, wherein the DNA-modifying enzymatic activity is nuclease activity.

77. The method of Claim 76, wherein the nuclease activity introduces a double strand break in the target DNA.

78. The method of Claim 77, wherein the contacting occurs under conditions that are permissive for nonhomologous end joining or homology-directed repair.

79. The method of Claim 78, the method further comprising contacting the target DNA with a donor polynucleotide, wherein the donor polynucleotide, a portion of the donor polynucleotide, a

copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA.

80. The method of Claim 78, wherein the method does not comprise contacting the cell with a donor polynucleotide, wherein the target DNA is modified such that nucleotides within the target DNA are deleted.

81. The method of Claim 64, wherein the enzymatic activity modifies a target polypeptide associated with the target DNA.

82. The method of Claim 81, wherein the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity or demyristoylation activity.

83. The method of Claim 81, wherein the target polypeptide is a histone and the enzymatic activity is methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity or deubiquitinating activity.

84. The method of Claim 64, wherein the complex further comprises an activator-RNA.

85. The method of Claim 84, wherein the activator-RNA comprises a nucleotide sequence with at least 60% identity over a stretch of at least 8 contiguous nucleotides to any one of the nucleotide sequences set forth in SEQ ID NOs:431-682.

86. A method of modulating site-specific transcription within a target DNA, the method comprising contacting the target DNA with:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that modulates transcription,

wherein said contacting results in modulating transcription within the target DNA.

87. The method of Claim 86, wherein transcription within the target DNA is increased.

88. The method of Claim 86, wherein transcription within the target DNA is decreased.

89. A method of site-specific modification at target DNA, the method comprising:

contacting the target DNA with:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that modulates transcription within the target DNA.

90. The method of Claim 86, wherein the site-directed modifying polypeptide increases transcription within the target DNA.

91. The method of Claim 86, wherein the site-directed modifying polypeptide decreases transcription within the target DNA.

92. A method of promoting site-specific cleavage and modification of a target DNA in a cell, the method comprising introducing into the cell:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits nuclease activity that creates a double strand break in the target DNA;

wherein the site of the double strand break is determined by the DNA-targeting RNA, the contacting occurs under conditions that are permissive for nonhomologous end joining or homology-directed repair, and the target DNA is cleaved and rejoined to produce a modified DNA sequence.

93. The method of Claim 92, the method further comprising contacting the target DNA with a donor polynucleotide, wherein the donor polynucleotide, a portion of the donor polynucleotide, a copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA.

94. The method of Claim 92, wherein the method does not comprise contacting the cell with a donor polynucleotide, wherein the target DNA is modified such that nucleotides within the target DNA are deleted.

95. The method of Claim 92, wherein the cell is selected from the group consisting of: an archaeal cell, a bacterial cell, a eukaryotic cell, a eukaryotic single-cell organism, a somatic cell, a germ cell, a stem cell, a plant cell, an algal cell, an animal cell, in invertebrate cell, a vertebrate cell, a fish cell, a frog cell, a bird cell, a mammalian cell, a pig cell, a cow cell, a goat cell, a sheep cell, a rodent cell, a rat cell, a mouse cell, a non-human primate cell, and a human cell.

96. The method of Claim 92, wherein the cell is *in vitro*.

97. The method of Claim 92, wherein the cell is *in vivo*.

98. A method of producing a genetically modified cell in a subject, the method comprising:

(I) introducing into a cell:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) a site-directed modifying polypeptide, or a polynucleotide encoding the same, wherein the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits nuclease activity that creates a double strand break in the target DNA;

wherein the site of the double strand break is determined by the DNA-targeting RNA, the contacting occurs under conditions that are permissive for nonhomologous end joining or homology-directed repair, and the target DNA is cleaved and rejoined to produce a modified DNA sequence;

thereby producing the genetically modified cell; and

(II) transplanting the genetically modified cell into the subject.

99. The method of Claim 98, the method further comprising contacting the cell with a donor polynucleotide, wherein the donor polynucleotide, a portion of the donor polynucleotide, a copy of the donor polynucleotide, or a portion of a copy of the donor polynucleotide integrates into the target DNA.

100. The method of Claim 98, wherein the method does not comprise contacting the cell with a donor polynucleotide, wherein the target DNA is modified such that nucleotides within the target DNA are deleted.

101. The method of Claim 98, wherein the cell is selected from the group consisting of: an archaeal cell, a bacterial cell, a eukaryotic cell, a eukaryotic single-cell organism, a somatic cell, a germ cell, a stem cell, a plant cell, an algal cell, an animal cell, in invertebrate cell, a vertebrate cell, a fish cell, an amphibian cell, a bird cell, a mammalian cell, an ungulate cell, a rodent cell, a non-human primate cell, and a human cell.

102. A method of modifying target DNA in a genetically modified cell that comprises a nucleotide sequence encoding an exogenous site-directed modifying polypeptide, the method comprising introducing into the genetically modified cell a DNA-targeting RNA, or a DNA polynucleotide encoding the same, wherein:

(i) the DNA-targeting RNA comprises:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) the site-directed modifying polypeptide comprises:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits nuclease activity.

103. The method of Claim 102, wherein the site-directed modifying polypeptide comprises an amino acid sequence having at least about 75% amino acid sequence identity to amino acids 7-166 or 731-1003 of the Cas9/Csn1 amino acid sequence depicted in Figure 3, or to the corresponding portions in any of the amino acid sequences set forth as SEQ ID NOs:1-256 and 795-1346.

104. The method of Claim 102, wherein the cell is selected from the group consisting of: an archaeal cell, a bacterial cell, a eukaryotic cell, a eukaryotic single-cell organism, a somatic cell, a germ cell, a stem cell, a plant cell, an algal cell, an animal cell, in invertebrate cell, a vertebrate cell, a fish cell, an amphibian cell, a bird cell, a mammalian cell, an ungulate cell, a rodent cell, a non-human primate cell, and a human cell.

105. The method of Claim 102, wherein the cell is in vivo.

106. The method of Claim 102, wherein the cell is in vitro.

107. The method of Claim 102, wherein the expression of the site-directed modifying polypeptide is under the control of an inducible promoter.

108. The method of Claim 102, wherein the expression of the site-directed modifying polypeptide is under the control of a cell type-specific promoter.

109. A kit comprising:

(i) the DNA-targeting RNA of Claim 1, or a DNA polynucleotide encoding the same; and

(ii) a reagent for reconstitution and/or dilution.

110. The kit of Claim 109, further comprising a reagent selected from the group consisting of: a buffer for introducing into cells the DNA-targeting RNA, a wash buffer, a control reagent, a control expression vector or RNA polynucleotide, a reagent for transcribing the DNA-targeting RNA from DNA, and combinations thereof.

111. A kit comprising:

(i) the site-directed modifying polypeptide of Claim 44, or a polynucleotide encoding the same; and

(ii) a reagent for reconstitution and/or dilution.

112. The kit of Claim 111, further comprising a reagent selected from the group consisting of: a buffer for introducing into cells the site-directed modifying polypeptide, a wash buffer, a control reagent, a control expression vector or RNA polynucleotide, a reagent for *in vitro* production of the site-directed modifying polypeptide from DNA, and combinations thereof.

113. A kit comprising:

(i) the site-directed modifying polypeptide of Claim 60, or a polynucleotide encoding the same; and

(ii) a reagent for reconstitution and/or dilution

114. A kit comprising:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, the DNA-targeting RNA comprising:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) the site-directed modifying polypeptide, or a polynucleotide encoding the same, the site-directed modifying polypeptide comprising:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

115. A kit comprising:

(i) a DNA-targeting RNA, or a DNA polynucleotide encoding the same, comprising:

(a) a first segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA; and

(b) a second segment that interacts with a site-directed modifying polypeptide; and

(ii) the site-directed modifying polypeptide, or a polynucleotide encoding the same, comprising:

(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and

(b) an activity portion that that modulates transcription within the target DNA, wherein the site of modulated transcription within the target DNA is determined by the DNA-targeting RNA.

116. A kit comprising:
(i) the recombinant expression vector of Claim 12; and
(ii) a reagent for reconstitution and/or dilution

117. A kit comprising:
(i) the recombinant expression vector of Claim 13; and
(ii) a reagent for reconstitution and/or dilution

118. A kit comprising:
(i) the recombinant expression vector of Claim 7; and
(ii) a reagent for reconstitution and/or dilution

119. A kit comprising:
(i) the recombinant expression vector of Claim 7; and
(ii) a recombinant expression vector comprising a nucleotide sequence that encodes a site-directed modifying polypeptide, wherein the site-directed modifying polypeptide comprises:
(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and
(b) an activity portion that exhibits site-directed enzymatic activity, wherein the site of enzymatic activity is determined by the DNA-targeting RNA.

120. A kit comprising:
(i) the recombinant expression vector of Claim 7; and
(ii) a recombinant expression vector comprising a nucleotide sequence that encodes a site-directed modifying polypeptide, wherein the site-directed modifying polypeptide comprises:
(a) an RNA-binding portion that interacts with the DNA-targeting RNA; and
(b) an activity portion that modulates transcription within the target DNA, wherein the site of modulated transcription within the target DNA is determined by the DNA-targeting RNA.

121. A kit for targeting target DNA comprising: two or more DNA-targeting RNAs according to Claim 1, or DNA polynucleotides encoding the same, wherein the first segment of at least one of the

two or more DNA-targeting RNAs differs by at least one nucleotide from the first segment of at least one other of the two or more DNA-targeting RNAs.

122. A method of selectively modulating transcription of a target DNA in a host cell, the method comprising introducing into the host cell:

a) a DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding the DNA-targeting RNA, wherein the DNA-targeting RNA comprises:

i) a first segment comprising a nucleotide sequence that is complementary to a target sequence in the target DNA;

ii) a second segment that interacts with a site-directed polypeptide; and

iii) a stability control sequence; and

b) a variant Cas9 site-directed polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the variant Cas9 site-directed polypeptide, wherein the variant Cas9 site-directed polypeptide comprises:

i) an RNA-binding portion that interacts with the DNA-targeting RNA; and

ii) an activity portion that exhibits reduced endodeoxyribonuclease activity,

wherein said DNA-targeting RNA and said variant Cas9 polypeptide form a complex in the cell and wherein said complex selectively modulates transcription of a target DNA in the cell.

123. A method of claim 122, wherein the variant Cas9 site-directed polypeptide can cleave the non-complementary strand of the target DNA but has reduced ability to cleave the complementary strand of the target DNA.

124. The method of claim 123, wherein the variant site-directed polypeptide comprises an H840A mutation of the amino acid sequence depicted in Figure 39A or the corresponding mutation in any of the amino acid sequences set forth in SEQ ID NOs:1-256 and 795-1346.

125. The method of claim 122, wherein the variant site-directed polypeptide can cleave the complementary strand of the target DNA but has reduced ability to cleave the non-complementary strand of the target DNA.

126. The method of claim 125, wherein the variant site-directed polypeptide comprises a D10A mutation of the amino acid sequence depicted in Figure 39A or the corresponding mutation in any of the amino acid sequences set forth in SEQ ID NOs:1-256 and 795-1346.

127. The method of claim 122, wherein the variant site-directed polypeptide has reduced ability to cleave the complementary strand of the target DNA and reduced ability to cleave the non-complementary strand of the target DNA.

128. The method of claim 127, wherein the variant site-directed polypeptide comprises both (i) a D10A mutation of the amino acid sequence depicted in Figure 41 or the corresponding mutation in any of the amino acid sequences set forth in SEQ ID NOs:1-256 and 795-1346; and (ii) an H840A mutation of SEQ ID NO:8 or the corresponding mutation in any of the amino acid sequences set forth in SEQ ID NOs:1-256 and 795-1346.

129. The method of claim 122, wherein the host cell is a prokaryotic cell.

130. The method of claim 122, wherein the host cell is a eukaryotic cell.

131. The method of claim 130, wherein the eukaryotic cell is a mammalian cell.

132. The method of claim 122, wherein transcription of the target DNA is inhibited by at least about 10% compared to the transcription of the target DNA in the absence of the complex.

133. The method of claim 122, wherein the variant Cas9 site-directed polypeptide comprises a heterologous polypeptide.

134. The method of claim 133, wherein the heterologous polypeptide comprises a subcellular localization sequence, a detectable label, a stability control peptide, a transcription modulation sequence, a protein binding sequence, or a combination thereof.

135. The method of claim 134, wherein the heterologous polypeptide comprises a stability control peptide.

136. The method of claim 135, wherein the stability control peptide comprises a degron sequence.

137. The method of claim 133, wherein the heterologous polypeptide is selected from the group consisting of: a transcriptional activator, a transcriptional repressor, a histone lysine methyltransferase,a histone lysine demethylase, a histone lysine acetyltransferase, a histone lysine

deacetylase, a DNA methylase, a DNA demethylase, a boundary element, a periphery recruitment element, a protein docking element, and combinations thereof.

138. The method of claim 133, wherein transcription of the target DNA is increased by at least about 1.2 fold compared to the transcription of the target DNA in the absence of the complex.

139. The method of claim 138, wherein the heterologous polypeptide is selected from the group consisting of: a transcriptional activator, a histone lysine methyltransferase, a histone lysine demethylase, a histone lysine acetyltransferase, a DNA demethylase, a protein docking element, and a combination thereof.

140. The method of claim 122, wherein the DNA-targeting RNA is a single-molecule DNA-targeting RNA.

141. The method of claim 122, wherein the DNA-targeting RNA is a two-molecule DNA-targeting RNA.

142. The method of claim 122, further comprising introducing into the host cell a second DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding the second DNA-targeting RNA, wherein the second DNA-targeting RNA comprises a first segment comprising a nucleotide sequence that is complementary to a second target sequence in the target DNA;

a second segment that interacts with the site-directed polypeptide; and

a stability control sequence.

143. The method of claim 122, wherein the second segment has a length of from about 30 nucleotides to about 60 nucleotides, and comprises a nucleotide sequence having at least about 95% nucleotide sequence identity to a nucleotide sequence selected from:

1) 5'-GUUUUAGAGCUA-(linker)-UAGCAAGUUAAAA-3';

2) 5'-GUUUAGAGCUG-(linker)-CAGCAAGUUAAA-3';

3) 5'-GUUUUAGAGCUG-(linker)-CAGCGAGUUAAA-3';

4) 5'-GUUUUUGUACUCU-(linker)-AGAAGCUACAAAGAU-3';

5) 5'-GUUGUAGCUCC-(linker)-GUUGCUACAAU-3'; and

6) 5'-GUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCCG-3'.

144. An isolated nucleic acid comprising a nucleotide sequence encoding a DNA-targeting RNA comprising:

a) a first segment comprising a nucleotide sequence that is complementary to a target sequence in the target DNA;

b) a second segment that interacts with a site-directed polypeptide; and

c) a stability control sequence.

145. The isolated nucleic acid of claim 144, wherein the nucleotide sequence encoding the DNA-targeting RNA is operably linked to a promoter.

146. The isolated nucleic acid of claim 144, wherein the nucleic acid further comprises a nucleotide sequence encoding a variant Cas9 site-directed polypeptide that exhibits reduced endodeoxyribonuclease activity relative to wild-type Cas9.

147. The isolated nucleic acid of claim 146, wherein the nucleotide sequence encoding the variant Cas9 site-directed polypeptide is operably linked to a promoter.

148. A recombinant expression vector comprising the nucleic acid of claim 24.

149. A genetically modified host cell comprising the isolated nucleic acid of claim 24, or the recombinant expression vector of claim 28.

150. A library of nucleic acids comprising a plurality of members, wherein each member nucleic acid comprises a nucleotide sequence encoding a DNA-targeting RNA comprising:

a) a first segment comprising a nucleotide sequence that is complementary to a target sequence in the target DNA;

b) a second segment that interacts with a site-directed polypeptide; and

c) a stability control sequence,

wherein each member differs from other members of the library in the nucleotide sequence of the first segment.

151. A library of claim 150, wherein the nucleotide sequence encoding a DNA-targeting RNA is operably linked to a promoter.

152. A kit comprising:

a) a DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding the DNA-targeting RNA, wherein the DNA-targeting RNA comprises:

i)) a first segment comprising a nucleotide sequence that is complementary to a target sequence in the target DNA;

ii)) a second segment that interacts with a site-directed polypeptide; and

iii) a stability control sequence; and

b) a buffer.

153. The kit of claim 152, further comprising a variant Cas9 site-directed polypeptide that exhibits reduced endodeoxyribonuclease activity relative to wild-type Cas9.

154. The kit of claim 152, wherein the nucleic acid comprising a nucleotide sequence encoding the DNA-targeting RNA further comprises a nucleotide sequence encoding a variant Cas9 site-directed polypeptide that exhibits reduced endodeoxyribonuclease activity relative to wild-type Cas9.

155. The kit of claim 152, further comprising a nucleic acid comprising a nucleotide sequence encoding a variant Cas9 site-directed polypeptide that exhibits reduced endodeoxyribonuclease activity relative to wild-type Cas9.

CLAIMS

1. A method of modifying a target DNA, the method comprising contacting the target DNA with a complex comprising:

(a) a Cas9 polypeptide and

(b) a single-molecule DNA-targeting RNA comprising:

(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in the target DNA, and

(ii) a protein-binding segment that interacts with said Cas9 polypeptide, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex, wherein said dsRNA duplex comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA),

wherein said two complementary stretches of nucleotides are covalently linked by intervening nucleotides,

wherein said contacting is *in vitro* or in a cell *ex vivo*; and

wherein said modifying is cleavage of the target DNA.

2. The method of claim 1, wherein said dsRNA duplex has a length of from 8 base pairs (bp) to 30 bp.

3. The method of claim 1 or 2, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding segment is greater than 70%.

4. The method of claim 1, 2 or 3, wherein the target DNA is present in a bacterial cell, an archaeal cell, a single-cell eukaryotic organism, a plant cell, a cell from an invertebrate animal, or a cell from a vertebrate animal.

5. The method of claim 1, 2 or 3, wherein the target DNA is chromosomal DNA.

6. The method of any one of the preceding claims wherein the contacting comprises introducing into a cell (a) said Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and (b) said DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA.

7. The method of claim 6 wherein the method further comprises introducing into the cell a donor polynucleotide.

8. The method according to any one of the preceding claims wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide,

wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

9. The method according to any one of claims 1-7 wherein a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

10. A composition comprising:

(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and

(b) a single-molecule DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA, wherein said single-molecule DNA-targeting RNA comprises:

(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, and

(ii) a protein-binding segment that interacts with said Cas9 polypeptide, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex, wherein said dsRNA duplex comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and

wherein said two complementary stretches of nucleotides are covalently linked by intervening nucleotides.

11. The composition of claim 10, wherein said dsRNA duplex has a length of from 8 base pairs (bp) to 30 bp.

12. The composition of claim 10 or 11, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding segment is greater than 70%.

13. The composition of claim 10, 11 or 12, wherein the target DNA is present in a bacterial cell, an archaeal cell, a single-cell eukaryotic organism, a plant cell, a cell from an invertebrate animal, or a cell from a vertebrate animal.

14. The composition of claim 10, 11 or 12, wherein the target DNA is chromosomal DNA.

15. The composition of any one of claims 10-14, wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

16. The composition of any one of claims 10-14, wherein a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein said protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

17. The composition of any one of claims 10-16, wherein the Cas9 polypeptide and the single-molecule DNA-targeting RNA are in a cell *in vitro* or *ex vivo*.

18. A single-molecule DNA-targeting RNA, or a DNA polynucleotide encoding said DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises:

(a) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA, and

(b) a protein-binding segment that interacts with a Cas9 protein, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex, wherein said dsRNA duplex comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and wherein said two complementary stretches of nucleotides are covalently linked by intervening nucleotides.

19. The single-molecule DNA-targeting RNA of claim 18 wherein said dsRNA duplex has a length of from 8 base pairs (bp) to 30 bp.

20. The single-molecule DNA-targeting RNA of claim 18 or 19, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding segment is greater than 70%.

21. The single-molecule DNA-targeting RNA of claim 18, 19 or 20, wherein the target DNA is present in a bacterial cell, an archaeal cell, a single-cell eukaryotic organism, a plant cell, a cell from an invertebrate animal, or a cell from a vertebrate animal.

22. The single-molecule DNA-targeting RNA of claim 18, 19 or 20, wherein the target DNA is chromosomal DNA.

23. One or more nucleic acids comprising:

(a) a first nucleotide sequence encoding a single-molecule DNA-targeting RNA comprising:

(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a target sequence in a target DNA, and

(ii) a protein-binding segment that interacts with a Cas9 polypeptide, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex, wherein said dsRNA duplex comprises complementary nucleotides of a

tracrRNA and a CRISPR RNA (crRNA), and wherein said two complementary stretches of nucleotides are covalently linked by intervening nucleotides;

wherein the first nucleotide sequence encoding said DNA-targeting RNA is operably linked to a promoter; and, optionally,

(b) a second nucleotide sequence encoding a Cas9 polypeptide, wherein the nucleotide sequence encoding said Cas9 polypeptide is operably linked to a promoter.

24. The one or more nucleic acids of claim 23, wherein said nucleic acids are one or more recombinant expression vectors.

25. The one or more nucleic acids of claim 23 or 24, wherein said dsRNA duplex has a length of from 8 base pairs (bp) to 30 bp.

26. The one or more nucleic acids of claim 23, 24 or 25, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding segment is greater than 70%.

27. The one or more nucleic acids of any one of claims 23-26, wherein the target DNA is present in a bacterial cell, an archaeal cell, a single-cell eukaryotic organism, a plant cell, a cell from an invertebrate animal, or a cell from a vertebrate animal.

28. The one or more nucleic acids of any one of claims 23-26, wherein the target DNA is chromosomal DNA.

29. A kit comprising

(a) a Cas9 polypeptide, or a nucleic acid comprising a nucleotide sequence encoding said Cas9 polypeptide; and

(b) a single-molecule DNA-targeting RNA, or a nucleic acid comprising a nucleotide sequence encoding said single-molecule DNA-targeting RNA, wherein the single-molecule DNA-targeting RNA comprises:

(i) a DNA-targeting segment comprising a nucleotide sequence that is complementary to a sequence in a target DNA, and

(ii) a protein-binding segment that interacts with said Cas9 polypeptide, wherein the protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex, wherein said dsRNA duplex comprises complementary nucleotides of a tracrRNA and a CRISPR RNA (crRNA), and,

wherein said two complementary stretches of nucleotides are covalently linked by intervening nucleotides, and

wherein (a) and (b) are in the same or separate containers.

30. The kit of claim 29, wherein the target DNA is present in a bacterial cell, an archaeal cell, a single-cell eukaryotic organism, a plant cell, a cell from an invertebrate animal, or a cell from a vertebrate animal.

31. The kit of claim 29, wherein the target DNA is chromosomal DNA.

32. The composition of any one of claims 10-17, or the single-molecule DNA-targeting RNA of any one of claims 18-22, or the one or more nucleic acids of any one of claims 23-28, for use in a method of therapeutic treatment of a patient.