CLAIMS

1. A nucleic acid molecule comprising a polynucleotide encoding an RNA-guided nuclease (RGN) polypeptide, wherein said polynucleotide comprises a nucleotide sequence encoding an RGN polypeptide selected from the group consisting of:

a) an RGN polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 117, 30, 75, 1, 9, 16, 23, 38, 46, 61, 69, 82, 89, 95, 103, or 110; and

b) an RGN polypeptide comprising the amino acid sequence set forth as SEQ ID NO: 54;

wherein said RGN polypeptide is capable of binding a target DNA sequence of a DNA molecule in an RNA-guided sequence specific manner when bound to a guide RNA (gRNA) capable of hybridizing to said target DNA sequence, and

wherein said polynucleotide encoding an RGN polypeptide is operably linked to a promoter heterologous to said polynucleotide.

2. The nucleic acid molecule of claim 1, wherein said RGN polypeptide is nuclease inactive or is capable of functioning as a nickase.

3. The nucleic acid molecule of claim 1 or 2, wherein the RGN polypeptide is operably fused to a base-editing polypeptide.

4. A vector comprising the nucleic acid molecule of any one of claims 1-3.

5. The vector of claim 4, further comprising at least one nucleotide sequence encoding said gRNA capable of hybridizing to said target DNA sequence, and wherein the guide RNA comprises a CRISPR RNA comprising a CRISPR repeat sequence having at least 95% sequence identity to SEQ ID NO: 118, 31, 76, 2, 10, 17, 24, 39, 47, 55, 62, 70, 83, 90, 96, 104, or 111.

6. The vector of claim 5, wherein said gRNA comprises a tracrRNA having at least 95% sequence identity to SEQ ID NO: 119, 32, 77, 3, 11, 18, 25, 40, 48, 56, 63, 71, 84, 91, 97, 105, or 112.

7. A cell comprising the nucleic acid molecule of any one of claims 1-3 or the vector of any one of claims 4-6.

8. A nucleic acid molecule comprising a polynucleotide encoding a CRISPR RNA (crRNA), wherein said crRNA comprises a spacer sequence and a CRISPR repeat sequence, wherein said CRISPR repeat sequence comprises a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 118, 31, 76, 2, 10, 17, 24, 39, 47, 55, 62, 70, 83, 90, 96, 104, or 111;

wherein a guide RNA comprising:

a) said crRNA; and optionally,

b) a trans-activating CRISPR RNA (tracrRNA) capable of hybridizing to said CRISPR repeat sequence of said crRNA;

is capable of hybridizing to a target DNA sequence of a DNA molecule in a sequence specific manner through the spacer sequence of said crRNA when said guide RNA is bound to an RNA-guided nuclease (RGN) polypeptide, and

wherein said polynucleotide encoding a crRNA is operably linked to a promoter heterologous to said polynucleotide.

9. A vector comprising the nucleic acid molecule of claim 8.

10. The vector of claim 9, wherein said vector further comprises a polynucleotide encoding said tracrRNA, and wherein said tracrRNA comprises a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 119, 32, 77, 3, 11, 18, 25, 40, 48, 56, 63, 71, 84, 91, 97, 105, or 112.

11. A nucleic acid molecule comprising a polynucleotide encoding a trans-activating CRISPR RNA (tracrRNA) comprising a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 119, 32, 77, 3, 11, 18, 25, 40, 48, 56, 63, 71, 84, 91, 97, 105, or 112;

wherein a guide RNA comprising:

a) said tracrRNA; and

b) a crRNA comprising a spacer sequence and a CRISPR repeat sequence, wherein said tracrRNA is capable of hybridizing with said CRISPR repeat sequence of said crRNA;

is capable of hybridizing to a target DNA sequence of a DNA molecule in a sequence specific manner through the spacer sequence of said crRNA when said guide RNA is bound to an RNA-guided nuclease (RGN) polypeptide, and

wherein said polynucleotide encoding a tracrRNA is operably linked to a promoter heterologous to said polynucleotide.

12. A vector comprising the nucleic acid molecule of claim 11.

13. The vector of claim 9, wherein said vector further comprises a polynucleotide encoding said crRNA, and wherein the CRISPR repeat sequence of said crRNA comprises a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 118, 31, 76, 2, 10, 17, 24, 39, 47, 55, 62, 70, 83, 90, 96, 104, or 111.

14. A system for binding a target DNA sequence of a DNA molecule, said system comprising:

a) one or more guide RNAs capable of hybridizing to said target DNA sequence or one or more polynucleotides comprising nucleotide sequences encoding the one or more guide RNAs (gRNAs); and

b) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence selected from the group consisting of:

i) an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 117, 30, 75, 1, 9, 16, 23, 38, 46, 61, 69, 82, 89, 95, 103, or 110; and

ii) the amino acid sequence set forth as SEQ ID NO: 54;

or a polynucleotide comprising a nucleotide sequence encoding the RGN polypeptide;

wherein said nucleotide sequences encoding the one or more guide RNAs and encoding the RGN polypeptide are each operably linked to a promoter heterologous to said nucleotide sequence; and

wherein the one or more guide RNAs are capable of forming a complex with the RGN polypeptide, in order to direct said RGN polypeptide to bind to said target DNA sequence of the DNA molecule.

15. The system of claim 14, wherein the target DNA sequence is within a cell.

16. The system of claim 14 or 15, wherein said RGN polypeptide is nuclease inactive or is capable of functioning as a nickase, and wherein the RGN polypeptide is operably linked to a base-editing polypeptide.

17. The system of claim 14 or 15, wherein said system further comprises one or more donor polynucleotides or one or more nucleotide sequences encoding the one or more donor polynucleotides.

18. A method for binding a target DNA sequence of a DNA molecule comprising delivering a system according to any one of claims 14-17, to said target DNA sequence or a cell comprising the target DNA sequence.

19. A method for cleaving or modifying a target DNA sequence of a DNA molecule comprising delivering a system according to any one of claims 14-17, to said target DNA sequence or a cell comprising the DNA molecule and cleavage or modification of said target DNA sequence occurs.

20. A method for binding a target DNA sequence of a DNA molecule, said method comprising:

a) assembling an RNA-guided nuclease (RGN) ribonucleotide complex *in vitro* by combining:

i) one or more guide RNAs capable of hybridizing to the target DNA sequence; and

ii) an RGN polypeptide comprising an amino acid sequence selected from the group consisting of:

A) an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 117, 30, 75, 1, 9, 16, 23, 38, 46, 61, 69, 82, 89, 95, 103, or 110; and

B) the amino acid sequence set forth as SEQ ID NO: 54;

under conditions suitable for formation of the RGN ribonucleotide complex; and

b) contacting said target DNA sequence or a cell comprising said target DNA sequence with the *in vitro*-assembled RGN ribonucleotide complex;

wherein the one or more guide RNAs hybridize to the target DNA sequence, thereby directing said RGN polypeptide to bind to said target DNA sequence.

21. A method for cleaving and/or modifying a target DNA sequence of a DNA molecule, comprising contacting the DNA molecule with:

a) an RNA-guided nuclease (RGN) polypeptide, wherein said RGN comprises an amino acid sequence selected from the group consisting of:

i) an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 117, 30, 75, 1, 9, 16, 23, 38, 46, 61, 69, 82, 89, 95, 103, or 110; and

ii) the amino acid sequence set forth as SEQ ID NO: 54;

b) one or more guide RNAs capable of targeting the RGN of (a) to the target DNA sequence;

wherein the one or more guide RNAs hybridize to the target DNA sequence, thereby directing said RGN polypeptide to bind to said target DNA sequence and cleavage and/or modification of said target DNA sequence occurs.

22. The method of claim 21, wherein said modified target DNA sequence comprises deletion or mutation of at least one nucleotide in the target DNA sequence.

23. The method of claim 21 or 22, wherein said RGN polypeptide is nuclease inactive or functions as a nickase, and wherein the RGN polypeptide is operably linked to a base-editing polypeptide.

24. The method of claim 21, wherein said modified target DNA sequence comprises insertion of heterologous DNA into the target DNA sequence.

25. The method of any one of claims 21-24, wherein the target DNA sequence is within a cell.

26. The method of claim 25, further comprising culturing the cell under conditions in which the RGN polypeptide is expressed and cleaves the target DNA sequence to produce a DNA molecule comprising a modified DNA sequence; and selecting a cell comprising said modified target DNA sequence.

27. A cell comprising a modified target DNA sequence according to the method of claim 26.

28. A system for binding a target DNA sequence of a DNA molecule, said system comprising:

a) one or more guide RNAs capable of hybridizing to said target DNA sequence or one or more polynucleotides comprising one or more nucleotide sequences encoding the one or more guide RNAs (gRNAs); and

b) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence selected from the group consisting of:

i) an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 117, 30, 75, 1, 9, 16, 23, 38, 46, 61, 69, 82, 89, 95, 103, or 110; and

ii) the amino acid sequence set forth as SEQ ID NO: 54;

wherein the one or more guide RNAs are capable of hybridizing to the target DNA sequence, and wherein the one or more guide RNAs are capable of forming a complex with the RGN polypeptide, in order to direct said RGN polypeptide to bind to said target DNA sequence of the DNA molecule.

29. A method of detecting a target DNA sequence of a DNA molecule in a sample, the method comprising:

a) contacting the sample with:

i) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 54 or 137, wherein said RGN polypeptide is capable of binding said target DNA sequence of a DNA molecule in an RNA-guided sequence specific manner when bound to a guide RNA capable of hybridizing to said target DNA sequence;

ii) said guide RNA; and

iii) a detector single-stranded DNA (ssDNA) that does not hybridize with the guide RNA; and

b) measuring a detectable signal produced by cleavage of the detector ssDNA by the RGN, thereby detecting the target DNA.

30. A kit for detecting a target DNA sequence of a DNA molecule in a sample, the kit comprising:

a) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 54 or 137, wherein said RGN polypeptide is capable of binding said target DNA sequence of a DNA molecule in an RNA-guided sequence specific manner when bound to a guide RNA capable of hybridizing to said target DNA sequence;

b) said guide RNA; and

c) a detector single-stranded DNA (ssDNA) that does not hybridize with the guide RNA.

31. The method of claim 29 or the kit of claim 30, wherein said detector ssDNA comprises a fluorophore/quencher pair or a fluorescence resonance energy transfer (FRET) pair.

32. A method of cleaving single-stranded DNAs, the method comprising contacting a population of nucleic acids, wherein said population comprises a DNA molecule comprising a target DNA sequence and a plurality of non-target ssDNAs with:

a) an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 54 or 137, wherein said RGN polypeptide is capable of binding said target DNA sequence in an RNA-guided sequence specific manner when bound to a guide RNA capable of hybridizing to said target DNA sequence; and

b) said guide RNA;

wherein the RGN polypeptide cleaves non-target ssDNAs of said plurality.

33. A nucleic acid molecule comprising a polynucleotide encoding a CRISPR RNA (crRNA), wherein said crRNA comprises a spacer sequence and a CRISPR repeat sequence, wherein said CRISPR repeat sequence comprises a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 240;

wherein a guide RNA comprising:

a) said crRNA; and optionally

b) a trans-activating CRISPR RNA (tracrRNA) capable of hybridizing to said CRISPR repeat sequence of said crRNA;

is capable of hybridizing to a target DNA sequence of a DNA molecule in a sequence specific manner through the spacer sequence of said crRNA when said guide RNA is bound to an RNA-guided nuclease (RGN) polypeptide, and

wherein said polynucleotide encoding a crRNA is operably linked to a promoter heterologous to said polynucleotide.

34. A nucleic acid molecule comprising a polynucleotide encoding a trans-activating CRISPR RNA (tracrRNA) comprising a nucleotide sequence having at least 95% sequence identity to SEQ ID NO: 241;

wherein a guide RNA comprising:

a) said tracrRNA; and

b) a crRNA comprising a spacer sequence and a CRISPR repeat sequence, wherein said tracrRNA is capable of hybridizing with said CRISPR repeat sequence of said crRNA;

is capable of hybridizing to a target DNA sequence of a DNA molecule in a sequence specific manner through the spacer sequence of said crRNA when said guide RNA is bound to an RNA-guided nuclease (RGN) polypeptide, and

wherein said polynucleotide encoding a tracrRNA is operably linked to a promoter heterologous to said polynucleotide.