

THAT WHICH IS CLAIMED:

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 comprising an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 6, 1-5, and 7-12;

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

2. The nucleic acid molecule of claim 1, wherein:

a) said polynucleotide encoding an RGN polypeptide is operably linked to a promoter heterologous to said polynucleotide;

b) said RGN polypeptide is capable of cleaving said target DNA sequence upon binding; optionally wherein said RGN polypeptide is capable of generating a double-stranded break or a single-stranded break;

c) said RGN polypeptide is nuclease inactive or is a nickase;

d) said RGN polypeptide comprises one or more nuclear localization signals;

e) said polynucleotide encoding said RGN polypeptide is codon optimized for expression in a eukaryotic cell;

f) said target DNA sequence is located adjacent to a protospacer adjacent motif (PAM);

g) said nucleic acid molecule is an isolated nucleic acid molecule; and/or

h) said RGN polypeptide is operably linked to an effector domain.

3. The nucleic acid molecule of claim 2, wherein the effector domain is:

a) a prime editing polypeptide; optionally wherein said prime editing polypeptide comprises a DNA polymerase or a reverse transcriptase;

b) a base-editing polypeptide; optionally wherein the base-editing polypeptide is a deaminase; optionally wherein the deaminase is a cytosine deaminase or an adenine deaminase; optionally wherein the deaminase has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 377-448; and/or

c) located at an internal location of the RGN polypeptide or is located at the N-terminus or C-terminus of the RGN polypeptide.

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

5. The vector of claim 4, wherein said vector further comprises at least one nucleotide sequence encoding said gRNA capable of hybridizing to said target DNA sequence; optionally wherein the guide RNA is selected from the group consisting of:

a) a guide RNA comprising:

i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18 or nucleotides 1-24 of SEQ ID NO: 42; and

ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30 or nucleotides 29-98 of SEQ ID NO: 42;

wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6;

b) a guide RNA comprising:

i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13; and

ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25;

wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1;

c) a guide RNA comprising:

i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14; and

ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26;

wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2;

d) a guide RNA comprising:

i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15; and

- ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3;
- e) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4;
- f) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 5;
- g) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 19; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 7;
- h) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 20; and

- ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8;
- i) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 21; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9;
- j) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 22; and
 - ii) a tracrRNA having at least 90% sequence identity to SEQ ID NO: 34;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10;
- k) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35;
 wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11; and
- l) a guide RNA comprising:
 - i) a CRISPR RNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24; and
 - ii) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36;

wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12; optionally wherein said gRNA is a single guide RNA or a dual-guide RNA; optionally wherein said sgRNA further comprises an extension comprising an edit template for prime editing.

6. A cell comprising the nucleic acid molecule of any one of claims 1-3 or the vector of claim 4 or 5.

7. A method for making an RGN polypeptide comprising:

a) culturing the cell of claim 6 under conditions in which the RGN polypeptide is expressed;

or

b) introducing into a cell a heterologous nucleic acid molecule comprising a nucleotide sequence encoding an RNA-guided nuclease (RGN) polypeptide comprising an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 6, 1-5, and 7-12;

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

and culturing said cell under conditions in which the RGN polypeptide is expressed; optionally wherein said method further comprises purifying said RGN polypeptide; and/or

wherein said cell further expresses one or more guide RNAs capable of binding to said RGN polypeptide to form an RGN ribonucleoprotein complex; optionally wherein said method further comprises purifying said RGN ribonucleoprotein complex.

8. An RNA-guided nuclease (RGN) polypeptide, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 6, 1-5, and 7-12; and

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.

9. The RGN polypeptide of claim 8, wherein:

a) said RGN polypeptide is an isolated RGN polypeptide;

b) said RGN polypeptide is capable of cleaving said target DNA sequence upon binding;

optionally wherein cleavage by said RGN polypeptide generates a double-stranded break or a single-stranded break;

- c) said RGN polypeptide is nuclease inactive or a nickase;
- d) said RGN polypeptide is operably linked to an effector domain;
- e) said target DNA sequence is located adjacent to a protospacer adjacent motif (PAM);

and/or

- f) said RGN polypeptide comprises one or more nuclear localization signals.

10. The RGN polypeptide of claim 9, wherein said effector domain is:

a) a prime editing polypeptide; optionally wherein said prime editing polypeptide comprises a DNA polymerase or a reverse transcriptase;

b) a base-editing polypeptide; optionally wherein the base-editing polypeptide is a deaminase; optionally wherein the deaminase is a cytosine deaminase or an adenine deaminase; optionally wherein the deaminase has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 377-448;

and/or

c) located at an internal location of the RGN polypeptide or at the N-terminus or C-terminus of the RGN polypeptide.

11. A ribonucleoprotein (RNP) complex comprising the RGN polypeptide of any one of claims 8-10 and the guide RNA bound to the RGN polypeptide.

12. 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 having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 6, 1-5, and 7-12 or a polynucleotide comprising a nucleotide sequence encoding the RGN polypeptide;

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.

13. The system of claim 12, wherein:

a) at least one of said nucleotide sequences encoding the one or more guide RNAs and said nucleotide sequence encoding the RGN polypeptide is operably linked to a promoter heterologous to said nucleotide sequence;

b) the nucleotide sequences encoding the one or more guide RNAs and the nucleotide sequence encoding the RGN polypeptide are located on one vector;

c) said RGN polypeptide and said one or more guide RNAs are not found complexed to one another in nature;

d) said target DNA sequence is a eukaryotic target DNA sequence;

e) said gRNA is a single guide RNA (sgRNA) or a dual-guide RNA; optionally wherein said sgRNA further comprises an extension comprising an edit template for prime editing;

f) said gRNA is selected from the group consisting of:

i) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18 or nucleotides 1-24 of SEQ ID NO: 42 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30 or nucleotides 29-98 of SEQ ID NO: 42, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6;

ii) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1;

iii) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2;

iv) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3;

xi) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11; and

xii) a gRNA comprising a CRISPR repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36, wherein said RGN polypeptide comprises an amino acid sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12;

g) said target DNA sequence is located adjacent to a protospacer adjacent motif (PAM);

h) the target DNA sequence is within a cell;

i) the one or more guide RNAs is capable of hybridizing to the target DNA sequence and the guide RNA is capable of forming a complex with the RGN polypeptide to direct cleavage of the target DNA sequence; optionally wherein the cleavage generates a double-stranded break or a single-stranded break;

j) said RGN polypeptide is nuclease inactive or is a nickase;

k) said RGN polypeptide is operably linked to an effector domain;

l) the RGN polypeptide comprises one or more nuclear localization signals; and/or

m) said system further comprises one or more donor polynucleotides.

14. The system of claim 13, wherein the effector domain is:

a) a prime editing polypeptide; optionally wherein the prime editing polypeptide comprises a DNA polymerase or a reverse transcriptase;

b) a base-editing polypeptide; optionally wherein the base-editing polypeptide is a deaminase; optionally wherein the deaminase is a cytosine deaminase or an adenine deaminase; optionally wherein the deaminase has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to an amino acid sequence of any one of SEQ ID NOs: 377-448; and/or

c) located at an internal location of the RGN polypeptide or at the N-terminus or C-terminus of the RGN polypeptide.

15. A cell comprising the system of any one of claims 12-14.

16. A pharmaceutical composition comprising the nucleic acid molecule of any one of claims 1-3, the vector of claim 4 or 5, the cell of claim 6 or 15, the RGN polypeptide of any one of claims 8-10, the RNP complex of claim 11, or the system of any one of claims 12-14, and a pharmaceutically acceptable carrier.

17. An *in vitro* or *ex vivo* method for binding a target DNA sequence of a DNA molecule comprising delivering a system according to any one of claims 12-14, to said target DNA sequence or a cell comprising the target DNA sequence, wherein components of said system are delivered simultaneously or sequentially to said target DNA sequence or said cell.

18. The *in vitro* or *ex vivo* method of claim 17, wherein:

a) said RGN polypeptide or said guide RNA further comprises a detectable label, thereby allowing for detection of said target DNA sequence; and/or

b) said guide RNA or said RGN polypeptide further comprises an expression modulator, thereby modulating expression of said target DNA sequence or a gene under transcriptional control by said target DNA sequence.

19. An *in vitro* or *ex vivo* method for cleaving and/or modifying a target DNA sequence of a DNA molecule comprising delivering a system according to any one of claims 12-14 to said target DNA sequence or a cell comprising the DNA molecule, wherein cleavage or modification of said target DNA sequence occurs, and wherein components of said system are delivered simultaneously or sequentially to said target sequence or said cell.

20. An *in vitro* or *ex vivo* method for binding, cleaving, and/or modifying of a target DNA sequence of a DNA molecule comprising:

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

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

and

ii) an RGN polypeptide of any one of claims 8-10;

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 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, cleave said target sequence, and/or modify said target DNA sequence.

21. The *in vitro* or *ex vivo* method of claim 20, wherein said gRNA is a single guide RNA (sgRNA) or a dual-guide RNA; optionally wherein said sgRNA further comprises an extension comprising an edit template for prime editing; optionally wherein:

a) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18 or nucleotides 1-24 of SEQ ID NO: 42 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30 or nucleotides 29-98 of SEQ ID NO: 42;

b) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25;

c) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26;

d) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27;

e) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28;

f) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 5, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29;

g) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 7, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or

100% sequence identity to SEQ ID NO: 19 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31;

h) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 8, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 20 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32;

i) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 9, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 21 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33;

j) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 10, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 22 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 34;

k) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35; or

l) said RGN has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12, said guide RNA comprises a crRNA repeat sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24 and a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36.

22. The *in vitro* or *ex vivo* method of any one of claims 19-21, wherein:

a) said modified target DNA sequence comprises insertion of heterologous DNA into the target DNA sequence, deletion of at least one nucleotide from the target DNA sequence, and/or mutation of at least one nucleotide in the target DNA sequence;

b) said target DNA sequence is located adjacent to a protospacer adjacent motif (PAM);

c) said target DNA sequence is a eukaryotic target DNA sequence; and/or

d) the target DNA sequence is within a cell.

23. The *in vitro* or *ex vivo* method of claim 22, further comprising culturing the cell under conditions in which the RGN polypeptide is expressed and cleaves and modifies the target DNA sequence to produce a DNA molecule comprising a modified target DNA sequence; and selecting a cell comprising said modified target DNA sequence.

24. A cell comprising a modified target DNA sequence according to the method of claim 23.

25. A pharmaceutical composition comprising the cell of any one of claims 6, 15, and 24, and a pharmaceutically acceptable carrier.

26. A pharmaceutical composition of claim 16 or 25 for use in treating a disease, disorder, or condition in a subject in need thereof; optionally wherein said disease is associated with a causal mutation and said effective amount of said pharmaceutical composition corrects said causal mutation; optionally wherein said subject is at risk of developing said disease, disorder, or condition.

27. A nucleic acid molecule comprising a CRISPR RNA (crRNA) or a polynucleotide encoding a 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 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 18, 13-17, and 19-24 or nucleotides 1-24 of SEQ ID NO: 42;

wherein a guide RNA comprising:

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

is capable of hybridizing to a target DNA sequence 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; optionally wherein said polynucleotide encoding a crRNA is operably linked to a promoter heterologous to said polynucleotide.

28. A vector comprising the nucleic acid molecule comprising said polynucleotide encoding said crRNA of claim 27; optionally wherein said vector further comprises a polynucleotide encoding said tracrRNA; optionally wherein said tracrRNA is selected from the group consisting of:

- a) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30 or nucleotides 29-98 of SEQ ID NO: 42, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at

least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18 or nucleotides 1-24 of SEQ ID NO: 42;

b) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13;

c) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14;

d) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15;

e) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 28, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 16;

f) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 29, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17;

g) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 31, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 19;

h) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 32, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 20;

i) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 33, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 21;

j) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 34, wherein said CRISPR repeat

sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 22;

k) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 35, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 23; and

l) a tracrRNA having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24.

29. The vector of claim 28, wherein said vector further comprises a polynucleotide encoding said RGN polypeptide; optionally wherein said RGN polypeptide is selected from the group consisting of:

a) a RGN polypeptide having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 6, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18 or nucleotides 1-24 of SEQ ID NO: 42 and said tracrRNA has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 30 or nucleotides 29-98 of SEQ ID NO: 42;

b) a RGN polypeptide having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 1, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 13 and said tracrRNA has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 25;

c) a RGN polypeptide having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 14 and said tracrRNA has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 26;

d) a RGN polypeptide having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 3, wherein said CRISPR repeat sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 15 and said tracrRNA has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 27;

sequence has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 24 and said tracrRNA has at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 36.

30. A nucleic acid molecule comprising a trans-activating CRISPR RNA (tracrRNA) or a polynucleotide encoding a tracrRNA comprising a nucleotide sequence having at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to any one of SEQ ID NOs: 30, 25-29, and 31-36 or nucleotides 29-98 of SEQ ID NO: 42;

wherein a guide RNA comprising:

- a) said tracrRNA; and
- b) a crRNA comprising a spacer sequence and a CRISPR repeat sequence,

wherein said tracrRNA hybridizes with said CRISPR repeat sequence of said crRNA; is capable of hybridizing to a target DNA sequence 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; optionally wherein said polynucleotide encoding a tracrRNA is operably linked to a promoter heterologous to said polynucleotide.

31. A vector comprising the nucleic acid molecule comprising said polynucleotide encoding said tracrRNA of claim 30.

32. A single guide RNA or a dual guide RNA comprising the nucleic acid molecule comprising said crRNA of claim 27 and the nucleic acid molecule comprising said tracrRNA of claim 30.

33. A cell comprising the nucleic acid molecule of any one of claims 27 and 30, the vector of any one of claims 28, 29, and 31, or the single guide RNA or the dual guide RNA of claim 32.