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Auxiliary Request 10

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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 DNA-targeting RNA comprising:

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

20 wherein the target DNA is present in a single-cell eukaryotic organism, an animal cell, or a plant cell,

wherein said modifying is cleavage of the target DNA,

wherein:

(A) said contacting is in vitro or in a cell ex vivo; and/or

25 ~~(B) said method is not a method for treatment of the human or animal body by therapy, and wherein said method is not a process for modifying the germline genetic identity of human beings.~~

2. The method of claim 1, wherein the DNA-targeting RNA comprises one or more
30 of a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, or a Peptide Nucleic Acid.

3. The method of claim 1 or claim 2, wherein said dsRNA duplex has:

(i) a length of from 8 base pairs (bp) to 15 bp;

35 (ii) a length of from 8 base pairs (bp) to 30 bp; or

(iii) a length of from 15 base pairs (bp) to 18 bp.

4. The method of any one of claims 1-3, wherein the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding

segment is greater than 70%.

5. The method of any one of claims 1-4, wherein the contacting comprises introducing into a cell (a) the Cas9 polypeptide, or a polynucleotide encoding the Cas9 polypeptide, and (b) the DNA-targeting RNA, or one or more DNA polynucleotides encoding the DNA-targeting RNA; optionally wherein the method further comprises introducing into the cell a donor polynucleotide.

6. ~~The method of any one of claims 1-5, wherein the nucleotide sequence that is complementary to a sequence in the target DNA is 15 to 18 nucleotides (nt) long, 18 to 20 nt long, or 20 to 25 nt long.~~

67. The method of any one of claims 1-56, wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein the protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

78. The method of any one of claims 1-56, wherein a protein transduction domain is covalently linked to the carboxyl terminus of the Cas9 polypeptide, wherein the protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

89. The method of any one of claims 1-78, wherein the DNA-targeting RNA is a two-molecule DNA-targeting RNA and comprises two separate RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA duplex.

910. The method of any one of claims 1-89, wherein the target DNA is chromosomal DNA.

1011. The method of any one of claims 1 to 910, wherein the target DNA is present in a cell from an invertebrate animal, a cell from a vertebrate animal, a cell from a mammal, a cell from a rodent, or a cell from a human.

1112. A composition comprising:
- (a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide, and
 - (b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein the DNA-targeting RNA comprises:
 - 5 (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;
- 10 wherein the DNA-targeting RNA is a two-molecule DNA-targeting RNA and comprises two separate RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA duplex, and wherein the target DNA is present in a single-cell eukaryotic organism, an animal cell, or a plant cell.
- 15 1213. One or more nucleic acids comprising:
- (a) a first nucleotide sequence encoding a 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
- 20 protein-binding segment comprises two complementary stretches of nucleotides that hybridize to form a double stranded RNA (dsRNA) duplex;
- wherein the first nucleotide sequence encoding said DNA-targeting RNA is operably linked to a promoter and wherein the DNA-targeting RNA is a two-molecule DNA-targeting RNA and comprises two separate RNA molecules, each of which comprises
- 25 one of the two complementary stretches of nucleotides that hybridize to form the dsRNA duplex; 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;
- wherein the target DNA is present in a single-cell eukaryotic organism, an animal cell, or
- 30 a plant cell.
1314. The one or more nucleic acids of claim 1213, wherein said nucleic acids are one or more recombinant expression vectors, optionally wherein the one or more recombinant expression vectors are one or more viral vectors.

1415. The one or more nucleic acids of claim 1213 or claim 1314, wherein the nucleotide sequence encoding said DNA-targeting RNA is operably linked to a promoter that is functional in a eukaryotic cell, and/or the nucleotide sequence encoding a Cas9 polypeptide is operably linked to a promoter that is functional in a eukaryotic cell.

1516. A kit comprising
(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide; and
(b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein the 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 the DNA-targeting RNA is a two-molecule DNA-targeting RNA and comprises two separate RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA duplex, wherein (a) and (b) are in the same or separate containers, and
wherein the target DNA is present in a single-cell eukaryotic organism, an animal cell, or a plant cell.

17. — A genetically modified host cell, which is a eukaryotic cell, comprising:
(a) a Cas9 polypeptide, or a polynucleotide encoding said Cas9 polypeptide; and/or
(b) a DNA-targeting RNA, or one or more DNA polynucleotides encoding said DNA-targeting RNA, wherein the 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;
and wherein
(A) the genetically modified host cell is in vitro or ex vivo; and/or

(B) the genetically modified host cell is not a human cell.

5 1618. The composition of claim 1112, the one or more nucleic acids of any one of claims 13-15, or the kit of claim 1516, or the genetically modified host cell of claim 17, wherein the DNA-targeting RNA comprises one or more of a modified nucleobase, a modified backbone or non-natural internucleoside linkage, a modified sugar moiety, a Locked Nucleic Acid, or a Peptide Nucleic Acid.

10 1719. The composition of claim 1112, or 1618, the one or more nucleic acids of any one of claims 1213-1415, or the kit of claim 1516, or the genetically modified host cell of claim 17 wherein:

- (i) said dsRNA duplex has a length of from 8 base pairs (bp) to 15 bp;
- (ii) said dsRNA duplex has a length of from 8 base pairs (bp) to 30 bp; or
- (iii) said dsRNA duplex has a length of from 15 base pairs (bp) to 18 bp.

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1820. The composition of any one of claims 1112 and 1618-1719, the one or more nucleic acids of any one of claims 1213-1415 and 1718-19, or the kit of any one of claims 1516 and 1618-1719, or the genetically modified host cell of any one of claims 17-19, wherein:

- 20 (i) the percent complementarity between the nucleotides that hybridize to form the dsRNA duplex of the protein-binding segment is greater than 70%; and/or
- (ii) the target DNA is chromosomal DNA.

25 1921. The composition of any one of claims 1112 and 1618-1820, the one or more nucleic acids of any one of claims 1213-1415 and 17198-1820, or the kit of any one of claims 1516 and 1618-1820, or the genetically modified host cell of any one of claims 17-20, wherein a protein transduction domain is covalently linked to the amino terminus of the Cas9 polypeptide, wherein the protein transduction domain facilitates the traversal of the Cas9 polypeptide from the cytosol to within an organelle of a cell.

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2022. The composition of any one of claims 1112 and 1618-1820, the one or more nucleic acids of any one of claims 1213-1415 and 181719-1820, or the kit of any one of claims 1516 and 1618-1820, or the genetically modified host cell of any one of claims 17-20, wherein a protein transduction domain is covalently linked to the carboxyl

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

5 ~~23. The composition of any one of claims 12 and 18-22, the one or more nucleic acids of any one of claims 13-15 and 18-22, or the kit of any one of claims 16 and 18-22, or the genetically modified host cell of any one of claims 17-22, wherein the DNA-targeting RNA is a two-molecule DNA-targeting RNA and comprises two separate RNA molecules, each of which comprises one of the two complementary stretches of nucleotides that hybridize to form the dsRNA duplex.~~

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2124. The composition of any one of claims 1112 and 1618-2023, the one or more nucleic acids of any one of claims 1213-1415 and 1718-2023, or the kit of any one of claims 1516 and 1618-2023, for use in a method of therapeutic treatment of a patient.

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2225. The composition of any one of claims 1112 and 1618-2023, the one or more nucleic acids of any one of claims 1213-1415 and 1718-2023, or the kit of any one of claims 1516 and 1618-2023, wherein the target DNA is present in a cell from an invertebrate animal, a cell from a vertebrate animal, a cell from a mammal, a cell from a rodent, or a cell from a human.