(b) a tracr mate sequence comprised of RNA, and (c) a tracr RNA sequence comprised of RNA, wherein the tracr mate sequence hybridizes to the tracr sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation; and (III) creating one or more nicks or cuts or base edits in the target DNA, wherein the M-SmallCas9 polypeptide is directed to the target DNA by the sgRNA in its processed or unprocessed form.

In a further aspect, provided herein is use of a composition comprising

(I) a M-SmallCas9 polypeptide as described herein or a nucleic acid encoding the same; and

(II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA in situ, each comprising:

(a) an engineered DNA targeting segment comprised of RNA and capable of hybridizing to a target sequence in a polynucleotide locus,

(b) a tracr mate sequence comprised of RNA, and

(c) a tracr RNA sequence comprised of RNA,

wherein the tracr mate sequence hybridizes to the tracr sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation;

for targeting, editing, modifying, or manipulating a target DNA at one or more locations in a cell *ex vivo* or *in vitro*.

In one example, provided herein is use of a composition comprising (I) a M-SmallCas9 polypeptide disclosed herein or a nucleic acid encoding the same; and/or (II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA in situ, each comprising: (a) an engineered DNA targeting segment comprised of RNA and capable of hybridizing to such target sequence in a polynucleotide locus, (b) a tracr mate sequence comprised of RNA, and (c) a tracr RNA sequence comprised of RNA, wherein the tracr mate sequence hybridizes to the tracr sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation; for targeting, editing, modifying, or manipulating a target DNA at one or more locations in a cell or in vitro.

In another aspect, provided herein is a cell comprising (I) a M-SmallCas9 polypeptide as disclosed herein, or a nucleic acid encoding a M-SmallCas9 polypeptide disclosed herein; and (II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA in situ, each comprising: (a) an engineered DNA targeting segment that can hybridize to a target sequence in a polynucleotide locus, (b) a tracr mate sequence, and (c) a tracr RNA sequence, wherein the tracr mate sequence that can hybridize to the tracr RNA sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation.

In yet another aspect, provided herein is a kit comprising (I) a nucleic acid sequence encoding a

CLAIMS

1. A M-SmallCas9 polypeptide comprising an amino acid sequence according to SEQ ID NO. 7 (M-SluCas9-R414A).

2. A composition comprising
   (I) a M-SmallCas9 polypeptide according to claim 1; and
   (II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) that allow the generation of such one or more sgRNA(s) *in situ*, each sgRNA or DNA encoding an sgRNA comprising:
      (a) an engineered DNA targeting segment that can hybridize to a target sequence in a polynucleotide locus,
      (b) a tracr mate sequence, and
      (c) a tracr RNA sequence,

   wherein the tracr mate sequence can hybridize to the tracr RNA sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation.

3. A method of targeting, editing, modifying, or manipulating a target DNA at one or more locations in a cell *ex vivo* or *in vitro*, the method comprising
   (I) introducing a heterologous M-SmallCas9 polypeptide according to claim 1 or a nucleic acid encoding a M-SmallCas9 of claim 1 into the cell *ex vivo* or into the *in vitro* environment; and
   (II) introducing one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) encoding such one or more sgRNA(s) in the cell *ex vivo* or the *in vitro* environment, each sgRNA or DNA encoding the sgRNA comprising:
      (a) an engineered DNA targeting segment comprising an RNA and capable of hybridizing to a target sequence in a polynucleotide locus,
      (b) a tracr mate sequence comprised of RNA, and
      (c) a tracr RNA sequence comprised of RNA,

   wherein the tracr mate sequence hybridizes to the tracr RNA sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation; and

(III) creating one or more nicks or cuts or base edits in the target DNA, wherein the M-SmallCas9 polypeptide is directed to the target DNA by the sgRNA in its processed or unprocessed form.

4. Use of a composition comprising

(I) a M-SmallCas9 polypeptide according to claim 1 or a nucleic acid encoding the same; and

(II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA in situ, each comprising:

(a) an engineered DNA targeting segment comprised of RNA and capable of hybridizing to a target sequence in a polynucleotide locus,

(b) a tracr mate sequence comprised of RNA, and

(c) a tracr RNA sequence comprised of RNA,

wherein the tracr mate sequence hybridizes to the tracr sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation;

for targeting, editing, modifying, or manipulating a target DNA at one or more locations in a cell *ex vivo* or in vitro.

5. A cell comprising

(I) a M-SmallCas9 polypeptide according to claim 1, or a nucleic acid encoding a M-SmallCas9 polypeptide according to claim 1; and

(II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA *in situ*, each comprising:

(a) an engineered DNA targeting segment that can hybridize to a target sequence in a polynucleotide locus,

(b) a tracr mate sequence, and

(c) a tracr RNA sequence,

wherein the tracr mate sequence that can hybridize to the tracr RNA sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation.

6. A kit comprising

   (I) a nucleic acid sequence encoding a M-SmallCas9 polypeptide according to claim 1, wherein the nucleic acid sequence encoding the M-SmallCas9 is operably linked to a promoter; and

   (II) one or more single heterologous guide RNA(s) (sgRNA) or DNA(s) suitable for the generation of such one or more sgRNA *in situ*, each sgRNA comprising:

   (a) an engineered DNA targeting segment that can hybridize to a target sequence in a polynucleotide locus,

   (b) a tracr mate sequence, and

   (c) a tracr RNA sequence,

   wherein the tracr mate sequence can hybridize to the tracr RNA sequence, and wherein (a), (b), and (c) are arranged in a 5' to 3' orientation.

7. A composition comprising a M-SmallCas9 polypeptide according to claim 1.

8. The composition according to claim 2 or 7, wherein the composition is a pharmaceutical composition.

9. The composition according to claim 8, wherein the pharmaceutical composition is for use in gene therapy.

10. The M-SmallCas9 polypeptide of claim 1, further comprising a nuclear localization signal.

11. A polynucleotide encoding a M-SmallCas9 polypeptide according to claim 1.

12. The polynucleotide of claim 11, wherein the polynucleotide is codon optimized.