Claims

1. A composition for modifying~~cleaving~~ a target DNA in human cells comprising a guide RNA specific for the target DNA and a nucleic acid encoding a Cas9 protein ~~encoding nucleic acid~~ and a nuclear localization signal, or Cas9 protein and a nuclear localization signal, wherein the guide RNA is a single-chain guide RNA(sgRNA) comprising a CRISPR RNA (crRNA) sequence fused to a trans activating crRNA (tracrRNA) sequence, wherein the crRNA sequence is at the 5' end relative to the tracrRNA sequence ~~a portion of a crRNA and a portion of a tracrRNA~~, and wherein the target DNA sequence comprises ~~at least substantially~~ 20 nucleotides complementary to the crRNA ~~portion~~ of the single-chain guide RNA and a protospacer adjacent motif(PAM), and wherein the PAM comprises ~~at least~~ three nucleotides.

2. The composition of claim 1, wherein the target DNA is an endogenous target DNA.

3. The composition of claim 1, wherein the guide RNA further comprises one or more additional nucleotides at the 5' end.

4. The composition of claim 1, wherein the guide RNA further comprises 2 additional guanine nucleotides at the 5' end.

5. The composition of claim 1, which induces targeted mutagenesis in human cells.

~~6. The composition of claim 1, for use in the genotyping of a genome in human cells.~~

6~~7~~. The composition of claim 1, wherein the guide RNA and the Cas9 protein function as a pair, and wherein the pair comprises two guide RNAs which induce two nicks on different strands.

7~~8~~. The composition of claim 1, wherein the guide RNA is in the form of an isolated RNA, or is encoded in a vector, wherein the vector is a viral vector, plasmid

vector, or agrobacterium vector.

~~9. The composition of claim 1, comprising a guide RNA specific for the target DNA and Cas9 protein.~~

8~~10~~. The composition of claim 1, for modifying~~cleaving~~ the target DNA in human cells ex vivo or in vivo.

9~~11~~. The composition of claim 1, wherein the Cas9 protein-encoding nucleic acid or the Cas9 protein is derived from ~~the genus~~ Streptococcus.

10~~12~~. The composition of claim 9~~14~~, wherein the ~~genus~~ Streptococcus is Streptococcus pyogenes.

11~~13~~. The composition of claim 1~~15~~, wherein the Cas9 protein recognizes the NGG trinucleotide.

12~~14~~. The composition of claim 1, wherein the Cas9 protein is ~~Cas9 protein or~~ a variant thereof in which at least one residue is changed to any other amino acid.

13~~15~~. The composition of claim 1, wherein the Cas9 protein is linked to a protein transduction domain.

14~~16~~. The composition of claim 12~~14~~, wherein the variant of Cas9 protein is a mutant form of Cas9 in which the catalytic aspartate residue is changed to any other amino acid.

15~~17~~. The composition of claim 14~~16~~, wherein the amino acid is an alanine.

16~~18~~. The composition of claim 1, wherein the Cas9 protein-encoding nucleic acid comprises a nucleotide sequence of SEQ ID No. 1 or a nucleotide sequence having at least 50% homology to SEQ ID NO. 1.

17~~19~~. A kit for modifying~~cleaving~~ a target DNA in human cells comprising the composition according to any one of claims 1 to 16~~18~~.

18. An in vitro or ex vivo method of modifying a target DNA in human cell, the method comprising introducing into the human cell a composition of CRISPR/Cas9 system, wherein the composition comprises a guide RNA specific for the target DNA and a nucleic acid encoding a Cas9 protein and a nuclear localization signal, or Cas9 protein and a nuclear localization signal, wherein the guide RNA is a single-chain guide RNA (sgRNA) comprising a CRISPR RNA (crRNA) sequence fused to a trans activating crRNA (tracrRNA) sequence, wherein the crRNA sequence is at the 5' end relative to the tracrRNA sequence, and wherein the target DNA sequence comprises 20 nucleotides complementary to the crRNA of the single-chain guide RNA and a protospacer adjacent motif (PAM), and wherein the PAM comprises three nucleotides.

~~20. A method for preparing a human cell comprising Cas protein and a single-chain guide RNA comprising a step of co-transfecting or serial• transfecting the human cell with a Cas~~

~~protein• encoding nucleic acid or a Cas protein, and a singlechain guide RNA, wherein the single-chain RNA comprises a portion of a crRNA and a portion of a tracrRNA.~~

19~~21~~. The method of claim 18~~20~~, wherein the guide RNA further comprises one or more additional nucleotides at the 5' end of the single-chain guide RNA.

20~~22~~. The method of claim 18~~20~~, wherein the guide RNA further comprises 2 additional guanine nucleotides at the 5' end of the single-chain guide RNA.

~~23. The method of claim 20, wherein the serial-transfection is performed by transfection with Cas protein-encoding nucleic acid first, followed by second transfection with naked single-chain guide RNA.~~

21~~24~~. The method of claim 18~~20~~, wherein the Cas9 protein is ~~Cas9 protein or a~~ variant thereof in which at least one residue is changed to any other amino acid.

22~~25~~. The method of claim 18~~20~~, wherein the Cas9 protein-encoding nucleic acid or the Cas9 protein is derived from ~~the genus~~ Streptococcus.

2326. The method of claim 2225, wherein the ~~genus~~ Streptococcus is Streptococcus pyogenes.

2427. The method of claim 2124, wherein the variant of Cas9 protein is a mutant form of Cas9 in which the catalytic aspartate residue is changed to any other amino acid.

2528. The method of claim 2427, wherein the amino acid is an alanine.

2629. The method of claim 1820, wherein the single-chain guide RNA and Cas protein function as a pair, and wherein the pair comprises two guide RNAs which induce two nicks on different DNA strands.

2730. The method of claim 2629, wherein the two nicks are separated by at least 100 bps.

~~31. The method of claim 20, wherein the transfection is performed by the method selected from the group consisting of microinjection, electroporation, DEAE-dextran treatment, lipofection, nanoparticle-mediated transfection, protein transduction domain mediated transduction, virus• mediated gene delivery, and PEG-mediated transfection in protoplast.~~

2832. A human cell comprising Cas9 protein and a single-chain guide RNA prepared by the method according to any one of claims 1820 to 2731.

~~33. A composition for genotyping mutations or variations in an isolated biological sample, comprising a guide RNA specific for the target DNA sequence Cas protein.~~

~~34. The composition of claim 33, wherein the mutations or variations are induced by engineered nucleases in cells.~~

~~35. The composition of claim 33, wherein the mutations or variations are a naturally- occurring mutations or variations.~~

~~36. A composition for genotyping nucleic acid sequences in pathogenic microorganisms in an isolated biological sample, comprising a guide RNA specific for the target DNA sequence and Cas protein.~~

~~37. A kit for genotyping mutations or variations in an isolated biological sample, comprising the composition of any one of claims 33 to 36.~~

~~38. A method of genotyping mutations or variations in an isolated biological sample, using the composition of any one of claims 33 to 36.~~