## Claims

1. A composition for modifying a target DNA in human cells comprising 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.

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, 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. 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.

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

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

10. The composition of claim 9, wherein the Streptococcus is Streptococcus pyogenes.

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

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

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

14. The composition of claim 12, 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. The composition of claim 14, wherein the amino acid is an alanine.

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

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.

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

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

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

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

23. The method of claim 22, wherein the Streptococcus is Streptococcus pyogenes.

24. The method of claim 21, 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.

25. The method of claim 24, wherein the amino acid is an alanine.

26. The method of claim 18, 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.

27. The method of claim 26, wherein the two nicks are separated by at least 100 bps.

28. A human cell comprising Cas9 protein and a single-chain guide RNA prepared by the method according to any one of claims 18 to 27.