Claims

1. A composition for cleaving target DNA in human cells comprising a guide RNA specific for target DNA and Cas protein-encoding nucleic acid or Cas protein, wherein the guide RNA is a single-chain guide RNA(sgRNA) comprising 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.

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

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.

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

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

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

13. The composition of claim 15, wherein the Cas protein recognizes the NGG trinucleotide.

14. The composition of claim 1, wherein the Cas protein is Cas9 protein or variant thereof.

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

16. The composition of claim 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.

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

18. The composition of claim 1, wherein the Cas 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.

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

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.

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

22. The method of claim 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.

24. The method of claim 20, wherein the Cas protein is Cas9 protein or variant thereof.

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

26. The method of claim 25, wherein the genus Streptococcus is Streptococcus pyogenes.

27. The method of claim 24, 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.

28. The method of claim 27, wherein the amino acid is an alanine.

29. The method of claim 20, 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.

30. The method of claim 29, 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.

32. A human cell comprising Cas protein and a single-chain guide RNA prepared by the method according to any one of claims 20 to 31.

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.