

CLAIMS

1. A composition comprising:
 - (a) a programmable DNA modification protein or nucleic acid encoding the programmable DNA modification protein, wherein the programmable DNA modification protein is a RNA-guided clustered regularly interspersed short palindromic repeats (CRISPR) nuclease system or the programmable DNA modification protein is a fusion protein comprising a catalytically inactive CRISPR system linked to a non-nuclease domain; and
 - (b) at least one programmable DNA-binding protein or nucleic acid encoding the at least one programmable DNA-binding protein, wherein the at least one programmable DNA-binding protein is a CRISPR system lacking nuclease activity;wherein each CRISPR system comprises a CRISPR protein and a guide RNA;
wherein the programmable DNA modification protein is targeted to a target chromosomal sequence and each of the at least one programmable DNA-binding protein is targeted to a site within about 250 base pairs on either side of the target chromosomal sequence; and
wherein each CRISPR protein is a type II CRISPR protein or a type V CRISPR protein.
2. The composition of claim 1, wherein the at least one programmable DNA-binding protein is targeted to a site within about 100 base pairs on either side of the target chromosomal sequence.
3. The composition of claim 2, wherein the at least one programmable DNA-binding protein is targeted to a site within about 75 base pairs on either side of the target chromosomal sequence.
4. The composition of claim 3, wherein the at least one programmable DNA-binding protein is targeted to a site within about 50 base pairs on either side of the target chromosomal sequence.
5. The composition of claim 4, wherein the at least one programmable DNA-binding protein is targeted to a site within about 25 base pairs on either side of the target chromosomal sequence.

6. The composition of any one of claims 1 to 5 wherein the CRISPR system lacking nuclease activity is a catalytically inactive CRISPR protein.
7. The composition of any one of claims 1 to 6, wherein the non-nuclease domain of the fusion protein has cytosine deaminase activity, histone acetyltransferase activity, transcriptional activation activity, or transcriptional repressor activity.
8. The composition of any one of claims 1 to 7, wherein nucleic acid encoding each CRISPR protein is mRNA or DNA.
9. The composition of any one of claims 1 to 8, wherein nucleic acid encoding each CRISPR protein and/or nucleic acid encoding each guide RNA is part of a plasmid vector or a viral vector.
10. The composition of any one of claims 1 to 9, wherein the guide RNA of each CRISPR system is at least partially chemically synthesized.
11. The composition of any one of claims 1 to 10, wherein the guide RNA of each CRISPR system is enzymatically synthesized.
12. A kit comprising the composition of any one of claims 1 to 11.
13. An in vitro or ex vivo method for targeted genome modification in a eukaryotic cell, the method comprising introducing into the eukaryotic cell:
 - (a) a programmable DNA modification protein or nucleic acid encoding the programmable DNA modification protein, wherein the programmable DNA modification protein is a RNA-guided clustered regularly interspersed short palindromic repeats (CRISPR) nuclease system or the programmable DNA modification protein is a fusion protein comprising a catalytically inactive CRISPR system linked to a non-nuclease domain; and
 - (b) at least one programmable DNA-binding protein or nucleic acid encoding the at least one programmable DNA-binding protein, wherein the at least one programmable DNA-binding protein is a CRISPR system lacking nuclease activity;

wherein each CRISPR system comprises a CRISPR protein and a guide RNA;

wherein the programmable DNA modification protein is targeted to a target chromosomal sequence and each of the at least one programmable DNA-binding protein is targeted to a site proximal to the target chromosomal sequence;

wherein binding of the at least one programmable DNA-binding protein to the site proximal to the target chromosomal sequence increases accessibility of the programmable DNA modification protein to the target chromosomal sequence, thereby increasing targeted genome modification efficiency and/or specificity;

wherein each CRISPR protein is a type II CRISPR protein or a type V CRISPR protein; and

wherein the site proximal to the target chromosomal sequence is located within about 250 base pairs on either side of the target chromosomal sequence.

14. The method of claim 13, wherein the site proximal to the target chromosomal sequence is located within about 100 base pairs on either side of the target chromosomal sequence.
15. The method of claim 14, wherein the site proximal to the target chromosomal sequence is located within about 75 base pairs on either side of the target chromosomal sequence.
16. The method of claim 15, wherein the site proximal to the target chromosomal sequence is located within about 50 base pairs on either side of the target chromosomal sequence.
17. The method of claim 16, wherein the site proximal to the target chromosomal sequence is located within about 25 base pairs on either side of the target chromosomal sequence.
18. The method of any one of claims 13 to 17, wherein the CRISPR system lacking nuclease activity is a catalytically inactive CRISPR protein.
19. The method of any one of claims 13 to 18, wherein the non-nuclease domain of the fusion protein has cytosine deaminase activity, histone acetyltransferase activity, transcriptional activation activity, or transcriptional repressor activity.

20. The method of any one of claims 13 to 19, wherein the eukaryotic cell is *in vitro*.
21. The method of any one of claims 13 to 20, wherein the eukaryotic cell is a mammalian cell.
22. The method of claim 21, wherein the mammalian cell is a human cell.
23. The composition of any of claims 1-11, the kit of claim 12, or the method of any of claims 13-22, wherein the programmable DNA modification protein and the at least one programmable DNA binding protein are of a different CRISPR protein type or subtype to each other.
24. The composition of any of claims 1-11, the kit of claim 12, or the method of any of claims 13-22, wherein
 - (i) the programmable DNA modification protein is a type II CRISPR protein and the at least one programmable DNA binding protein is a type V CRISPR protein,
 - (ii) the programmable DNA modification protein is a type V CRISPR protein and the at least one programmable DNA binding protein is a type II CRISPR protein, or
 - (iii) the programmable DNA modification protein is a type V CRISPR protein and the at least one programmable DNA binding protein is a type V CRISPR protein.
25. The composition of any of claims 1-11, the kit of claim 12, or the method of any of claims 13-22, wherein
 - (i) the programmable DNA modification protein is a type IIA CRISPR protein and the at least one programmable DNA binding protein is a type IIB CRISPR protein,
 - (ii) the programmable DNA modification protein is a type IIB CRISPR protein and the at least one programmable DNA binding protein is a type IIA CRISPR protein,
 - (iii) the programmable DNA modification protein is a type IIC CRISPR protein and the at least one programmable DNA binding protein is a type IIA CRISPR protein, or
 - (iv) the programmable DNA modification protein is a type V CRISPR protein and the at least one programmable DNA binding protein is a type IIA CRISPR protein.

26. The composition, kit or method according to claim 24 or claim 25, wherein (i) the type II CRISPR protein is selected from *Francisella novicida* CRISPR-Cas9 (FnCas9), *Campylobacter jejuni* CRISPR-Cas9 (CjCas9) and *Streptococcus pyogenes* CRISPR-Cas9 (SpCas9); and/or (ii) the type V CRISPR protein is *Francisella novicida* CRISPR-Cpf1 (FnCpf1).
27. The composition, kit or method according to claim 24, claim 25 or claim 26, wherein the at least one programmable DNA binding protein is a Cas9 type II CRISPR protein, wherein the Cas9 protein has one or more mutations in each of the RuvC-like domain and the HNH-like domain.
28. The composition, kit or method according to claim 27, wherein (i) the one or more mutations in the RuvC-like domain are D10A, D8A, E762A, and/or D986A; and/or (ii) the one or more mutations in the HNH-like domain are H840A, H559A, N854A, N856A, and/or N863A.