

THAT WHICH IS CLAIMED IS:

1. A method of modifying a target nucleic acid, the method comprising:
contacting the target nucleic acid with

5 (a) a Type V CRISPR-Cas effector protein or a Type II CRISPR-Cas effector protein;

(b) a reverse transcriptase, and

(c) an extended guide nucleic acid (e.g., extended Type II or Type V CRISPR RNA, extended Type II or Type V CRISPR DNA, extended Type II or Type V crRNA, extended Type II or Type V crDNA), wherein the extended guide nucleic acid further comprises a structured RNA motif, thereby modifying the target nucleic acid.

2. ~~The method of claim 1, wherein the Type V CRISPR-Cas effector protein or the Type II CRISPR-Cas effector protein, the reverse transcriptase, and the extended guide nucleic acid form a complex or are comprised in a complex.~~

3. ~~The method of claim 1 or claim 2, wherein the extended guide nucleic acid comprises:~~

(i) ~~a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid (Type II or Type V CRISPR RNA, Type II or Type V CRISPR DNA, Type II or Type V crRNA, Type II or Type V crDNA) and/or a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid and a traer nucleic acid (e.g., Type II or Type V traerRNA, Type II or Type V traerDNA); and~~

(ii) ~~an extended portion comprising a primer binding site and a reverse transcriptase template (RT template) (RTT).~~

4. ~~The method of claim 3, wherein the extended portion is fused to either the 5' end or 3' end of the CRISPR nucleic acid (e.g., 5' to 3': repeat-spacer-extended portion, or extended portion-repeat-spacer) and/or to the 5' or 3' end of the traer nucleic acid.~~

5. ~~The method of claim 3 or claim 4, wherein the extended portion of the extended guide nucleic acid comprises, 5' to 3', an RT template and a primer binding site.~~

~~6. — The method of any one of claims 1-5, wherein the extended guide nucleic acid further comprises a structured RNA motif.~~

~~72. The method of claim 61, wherein the structured RNA motif is located at the 3' end of the extended guide nucleic acid.~~

~~83. The method of claim 61 or claim 72, wherein the structured RNA motif is AsCpf1BB (SEQ ID NO:189), BoxB (SEQ ID NO:190), pseudoknot (decoy) (SEQ ID NO:95, SEQ ID NO:203), pseudoknot (tEvoPreQ1) (SEQ ID NO:191), fmpknot (SEQ ID NO:192), mpknot (SEQ ID NO:193), MS2 (SEQ ID NO:194), PP7 (SEQ ID NO:195), SLBP (SEQ ID NO:196), TAR (SEQ ID NO:197), and/or ThermoPh (SEQ ID NO:198).~~

~~94. The method of any one of claims 61-83, wherein the structured RNA motif is a pseudoknot.~~

~~105. The method of claim 94, wherein the pseudoknot is a tEvoPreQ1 pseudoknot comprising the nucleic acid sequence of SEQ ID NO:158 or an EvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:191.~~

~~116. The method of claim 94, wherein the pseudoknot comprises the nucleic acid sequenced of SEQ ID NO:95 or SEQ ID NO:203.~~

~~12. — The method of any one of claims 5-11, wherein the target nucleic acid is double stranded and comprises a first strand and a second strand and the primer binding site binds to the second strand (non-target, top strand) of the target nucleic acid.~~

~~13. — The method of any one of claims 5-11, wherein the target nucleic acid is double stranded and comprises a first strand and a second strand and the primer binding site binds to the first strand (e.g., binds to the target strand, same strand to which the CRISPR-Cas effector protein is recruited, bottom strand) of the target nucleic acid.~~

14. — The method of any one of claims 5-11, wherein the target nucleic acid is double stranded and comprises a first strand and a second strand and the primer binding site binds to the second strand (non target strand, opposite strand from that to which the CRISPR-Cas effector protein is recruited) of the target nucleic acid.

15. — The method of any one of claims 3-14, wherein the primer binding site (PBS) is length of about one nucleotide to about 100 nucleotides, optionally wherein the PBS is about 4 nucleotides to about 80 nucleotides, about 30 nucleotides to about 80 nucleotides, about 40 to about 60 nucleotides in length, or about 8, 16, 24, 32, 40, 48, 56, 64, 72, or 80 nucleotides in length.

16. — The method of any one of claims 3-15, wherein the RT template (RTT) is a length of about one to about 100 nucleotides, optionally wherein the RTT is a length of about 35 to about 100, about 35 nucleotides to about 80 nucleotides, optionally about 35 nucleotides to about 75 nucleotides, about 40 nucleotides to about 75 nucleotides, about 45 nucleotides to about 75 nucleotides, about 45 nucleotides to about 60 nucleotides, or a length of about 36, 40, 44, 47, 50, 52, 55, 63, 72 or 74 nucleotides.

17. The method of any one of claims 3-1 to 166, wherein the extended guide nucleic acid further comprises:

(i) a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid (crRNA, crDNA) and/or a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid and a tracr nucleic acid;
and

(ii) an extended portion comprising a primer binding site and a reverse transcriptase template (RT template) (RTT) and the RTT is a length of about 35 nucleotides to about 75 nucleotides and the PBS is a length of about 30 nucleotides to about 80 nucleotides, optionally wherein the PBS is a length of about 8, 16, 24, 32, 40, 48, 56, 64, 72, or 80 nucleotides and the RTT is a length of about 36, 40, 44, 47, 50, 52, 55, 63, 72 or 74 nucleotides.

18. — The method of any one of claims 3 to 17, wherein the extended portion of the extended guide RNA is linked to the CRISPR nucleic acid and/or the tracrRNA via a linker.

19. — The method of claim 18, wherein the linker is 1 to 100 nucleotides in length.

20. — The method of any one of claims 3 to 19, wherein when the extended portion is located 5' of the crRNA, the Type V CRISPR-Cas effector protein is modified to reduce (or eliminate) self-processing RNase activity.

21.8. The method of ~~any of the preceding claims~~ claim 7, wherein the Type V CRISPR-Cas effector protein or the Type II CRISPR-Cas effector protein is a fusion protein and/or the reverse transcriptase is a fusion protein, wherein the Type V CRISPR-Cas fusion protein or Type II CRISPR-Cas fusion protein, the reverse transcriptase fusion protein and/or the extended guide nucleic acid is fused to one or more components that recruit the reverse transcriptase to the Type V CRISPR-Cas effector protein or Type II CRISPR-Cas effector protein, optionally the one or more components recruit via protein-protein interactions, protein-RNA interactions, and/or chemical interactions, wherein the Type V CRISPR-Cas fusion protein or Type II CRISPR-Cas fusion protein is fused to a chromatin modulating peptide.

22. — The method of any of the preceding claims, wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas effector fusion protein comprising a Type V CRISPR-Cas effector protein domain fused (linked) to a peptide tag (e.g., an epitope or a multimerized epitope) and the reverse transcriptase is a reverse transcriptase fusion protein comprising a reverse transcriptase domain fused (linked) to an affinity polypeptide that binds to the peptide tag, optionally wherein the target nucleic acid is contacted with two or more reverse transcriptase fusion proteins, or the Type II CRISPR-Cas effector protein is a Type II CRISPR-Cas effector fusion protein comprising a Type II CRISPR-Cas effector protein domain fused (linked) to a peptide tag (e.g., an epitope or a multimerized epitope) and the reverse transcriptase is a reverse transcriptase fusion protein comprising a reverse transcriptase domain fused (linked) to an affinity polypeptide that binds to the peptide tag, optionally wherein the target nucleic acid is contacted with two or more reverse transcriptase fusion protein.

23. — The method of claim 22, wherein the peptide tag comprises a GCN4 peptide tag (e.g., Sun-Tag), a c-Myc affinity tag, an HA affinity tag, a His affinity tag, an S affinity tag, a

methionine His affinity tag, an RGD His affinity tag, a FLAG octapeptide, a strep tag or strep tag II, a V5 tag, and/or a VSV-G epitope.

24. — The method of claim 22 or claim 23, wherein the peptide tag comprises two or more copies of the peptide tag.

25. — The method of any one of claims 22-24, wherein the affinity polypeptide is an antibody, an affibody, an anticalin, a monobody and/or a DARPin.

26. — The method of claim 25, wherein the antibody is an scFv antibody.

27. — The method of any one of the preceding claims, wherein the extended guide nucleic acid is linked to an RNA recruiting motif, and the reverse transcriptase is a reverse transcriptase fusion protein comprising a reverse transcriptase domain fused (linked) to an affinity polypeptide that binds to the RNA recruiting motif, optionally wherein the target nucleic acid is contacted with two or more reverse transcriptase fusion proteins, optionally wherein the extended guide RNA is linked to two or more RNA recruiting motifs, optionally wherein the two or more RNA recruiting motifs are the same RNA recruiting motif or different RNA recruiting motifs.

28. — The method of claim 27 wherein the recruiting motif is located on the 3' end of the extended portion of the extended guide nucleic acid or is embedded in the extended portion.

29. — The method of claim 27 or claim 28, wherein the RNA recruiting motif and corresponding affinity polypeptide are a telomerase Ku binding motif (e.g., Ku binding hairpin) and the affinity polypeptide of Ku (e.g., Ku heterodimer); a telomerase Sm7 binding motif and the affinity polypeptide of Sm7; an MS2 phage operator stem-loop and the affinity polypeptide MS2 Coat Protein (MCP), a PP7 phage operator stem-loop and the affinity polypeptide PP7 Coat Protein (PCP); an SfMu phage Com stem-loop and the affinity polypeptide Com RNA binding protein; a PUF binding site (PBS) and the affinity polypeptide Pumilio/fem 3 mRNA binding factor (PUF); and/or a synthetic RNA aptamer and the corresponding aptamer ligand.

30. — The method of any one of claims 27-29, wherein the RNA recruiting motif and corresponding affinity polypeptide are an MS2 phage operator stem-loop and the affinity polypeptide MS2 Coat Protein (MCP), and/or a PUF binding site (PBS) and the affinity polypeptide Pumilio/fem-3 mRNA binding factor (PUF).

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31. — The method of claim 21, wherein the one or more components that recruit chemical interactions are rapamycin-inducible dimerization of FRB-FKBP; Biotin-streptavidin; SNAP tag; Halo tag; CLIP tag; DmrA-DmrC heterodimer induced by a compound; bifunctional ligand (e.g., fusion of two protein-binding chemicals together, e.g., dihydrofolate reductase (DHFR).

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32. — The method of any one of the preceding claims, further comprising contacting the target nucleic acid with

(a) a CRISPR-Cas effector protein; and

(b) a guide nucleic acid, wherein (i) the CRISPR-Cas effector protein nicks or cuts a site

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on the first strand of the target nucleic acid that is located about 10 to about 125 base pairs

(either 5' or 3') from a site on the second strand that has been nicked by the Type II or Type V CRISPR-Cas effector protein, or (ii) the CRISPR-Cas effector protein nicks or cuts a site on the second strand of the target nucleic acid that is located about 10 to about 125 base pairs (either 5' or 3') from a site on the first strand that has been nicked by the Type II or Type V CRISPR-Cas

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effector protein, thereby improving mismatch repair, wherein the CRISPR-Cas effector protein is a Type I, Type II, Type III, Type IV, or Type V CRISPR-Cas effector protein.

33. — The method of any one of claims 21-32, wherein the Type V CRISPR-Cas fusion protein or Type II CRISPR-Cas fusion protein is fused to a chromatin-modulating peptide.

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349. The method of any one of claims 21-33 claim 8, wherein the chromatin modulating peptide is fused to the C-terminus and/or the N-terminus of the Type V CRISPR-Cas fusion protein or Type II CRISPR-Cas fusion protein.

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3510. The method of any one of claims 21-34 claim 8 or claim 9, wherein the Type V CRISPR-Cas fusion protein or Type II CRISPR-Cas fusion protein is fused at its N-terminus to a reverse

transcriptase and the chromatin modulating peptide is fused to the N-terminus of the reverse transcriptase.

3611. The method of any one of claims ~~338-3510~~, wherein the chromatin modulating peptide is CHD1 (e.g., ~~SEQ ID NO:199~~), H1G (e.g., ~~SEQ ID NO:200~~), HB1 (e.g., ~~SEQ ID NO:201~~), and HN1 (e.g., ~~SEQ ID NO:202~~).

3712. The method of ~~any one of the preceding claims~~ claim 11, wherein CHD1 is SEQ ID NO:199, H1G is SEQ ID NO:200, HB1 is SEQ ID NO:201, and HN1 is SEQ ID NO:202 further comprising contacting the target nucleic acid with a Dna2 polypeptide and/or a 5' flap endonuclease (FEN), optionally an FEN1 polypeptide.

38. — The method of claim 37, wherein the FEN and/or Dna2 polypeptide is overexpressed (in the presence of the target nucleic acid).

39. — The method of claim 37 or claim 38, wherein the FEN is a fusion protein comprising an FEN domain fused to the Type II or Type V CRISPR-Cas effector protein or domain and/or wherein the Dna2 polypeptide is a fusion protein comprising an Dna2 domain fused to the Type II or Type V CRISPR-Cas effector protein or domain.

40. — The method of any one of the preceding claims, wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas fusion protein comprising a Type V CRISPR-Cas effector protein domain fused (linked) to a peptide tag (e.g., an epitope or a multimerized epitope) and the FEN is an FEN fusion protein comprising an FEN domain fused to an affinity polypeptide that binds to the peptide tag, and/or wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas fusion protein comprising a Type V CRISPR-Cas effector protein domain fused to a peptide tag and the Dna2 polypeptide is an Dna2 fusion protein comprising an Dna2 domain fused to an affinity polypeptide that binds to the peptide tag, optionally wherein the target nucleic acid is contacted with two or more FEN fusion proteins and/or two or more Dna2 fusion proteins, thereby recruiting the FEN and/or Dna2 to the Type V CRISPR-Cas effector protein domain, and the target nucleic acid.

41. — The method of any one of the claims 1-39, wherein the Type II CRISPR-Cas effector protein is a Type II CRISPR-Cas fusion protein comprising a Type II CRISPR-Cas effector protein domain fused (linked) to a peptide tag (e.g., an epitope or a multimerized epitope) and the FEN is an FEN fusion protein comprising an FEN domain fused to an affinity polypeptide that binds to the peptide tag, and/or wherein the Type II CRISPR-Cas effector protein is a Type II CRISPR-Cas fusion protein comprising a Type II CRISPR-Cas effector protein domain fused to a peptide tag and the Dna2 polypeptide is an Dna2 fusion protein comprising an Dna2 domain fused to an affinity polypeptide that binds to the peptide tag, optionally wherein the target nucleic acid is contacted with two or more FEN fusion proteins and/or two or more Dna2 fusion proteins, thereby recruiting the FEN and/or Dna2 to the Type II CRISPR-Cas effector protein domain, and the target nucleic acid.

42. — The method of any one of the preceding claims, wherein the Type V CRISPR-Cas effector protein is a Cas12a (Cpf1) domain, Cas12b (C2c1) domain, Cas12c (C2c3) domain, Cas12d (CasY) domain, Cas12e (CasX) domain, Cas12g domain, Cas12h domain, Cas12i domain, C2c4 domain, C2c5 domain, C2c8 domain, C2c9 domain, C2c10 domain, Cas14a domain, Cas14b domain, and/or Cas14c domain, or the Type II CRISPR-Cas effector protein is a Cas9 domain, optionally wherein the Type V CRISPR-Cas effector protein and/or or the Type II CRISPR-Cas effector protein comprises one or more mutations as described herein.

43. — The method of claim 42, wherein the Type V CRISPR-Cas effector protein is an LbCas12a nickase comprising a R1138A mutation with reference to the amino acid position numbering of SEQ ID NO:9, an AsCas12a nickase comprising an R1226A mutation with reference to the amino acid position numbering of SEQ ID NO:2), a FnCas12a comprising an R1228A with reference to the amino acid position numbering of SEQ ID NO:6, or a PdCas12a nickase comprising an R1241A mutation with reference to the amino acid position numbering of SEQ ID NO:14.

44. — The method of claim 42, wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas effector protein from *Acidaminococcus* sp. (AsCas12a) or from Lachnospiraceae bacterium (e.g., LbCas12a) or a modified Type V CRISPR-Cas effector protein therefrom.

45. — The method of claim 42, wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas effector protein from *Butyrivibrio hungatei* (BhCas12) or a modified Type V CRISPR-Cas effector protein therefrom.

46. — The method of claim 42, wherein the Type II CRISPR-Cas effector protein is a Cas9 effector protein/domain, optionally from *Streptococcus*.

47. — The method of any one of claims 1-40 or 42-46, wherein the Type V CRISPR-Cas effector protein comprises reduced single-stranded DNA cleavage activity (ssDNAse activity) or is modified (mutated) to reduce (or eliminate) ssDNAse activity.

48. — The method of any one of claims 1-40 or 42-47, wherein the Type V CRISPR-Cas effector protein is modified (mutated) to reduce (or eliminate) self-processing RNAse activity.

49. — The method of claim 48, wherein the Type V CRISPR-Cas effector protein is a Cas12a CRISPR-Cas effector protein having a H759A mutation with reference to amino acid position numbering of SEQ ID NO:1.

50. — The method of claim 49, wherein the Cas12a CRISPR-Cas effector protein having a H759A mutation comprises a sequence having at least 80% sequence identity to the amino acid sequence of SEQ ID NO:148.

51. — The method of claim 50, wherein the Cas12a CRISPR-Cas effector protein having a H759A mutation is a LbCas12a CRISPR-Cas effector protein having at least 90% sequence identity to the amino acid sequence of SEQ ID NO:148.

52. — The method of any one of the preceding claims, wherein the Type V CRISPR-Cas effector protein is modified to reduce or eliminate nuclease activity (e.g., a mutation in a nuclease active site (e.g., in the RuvC domain), or the Type II CRISPR-Cas effector protein is modified to reduce or eliminate nuclease activity (e.g., a mutation in a nuclease active site (e.g., in the RuvC or HNH domain), to produce a deactivated Type V CRISPR-Cas effector protein or deactivated Type II CRISPR-Cas effector protein (e.g., deadCas (dCas, dCas12a, dCas9).

53. — The method of claim 52, wherein the deactivated Type V CRISPR-Cas effector protein or the deactivated Type II CRISPR-Cas effector protein comprises nickase activity.

54. — The method of claim 52 or claim 53, wherein the deactivated Type V CRISPR-Cas effector protein or the deactivated Type II CRISPR-Cas effector protein is fused to a nicking enzyme (e.g., FokI, BfiI, e.g., an engineered FokI or BfiI).

55. — The method of any one of the preceding claims, wherein Type V CRISPR-Cas effector protein comprises an amino acid modification to reduce DNA binding affinity of the Type V CRISPR-Cas effector protein.

56. — The method of claim 55, wherein the modification is an amino acid substitution.

57. — The method of claim 55 or claim 56, wherein the modification is an amino acid substitution to an alanine, optionally wherein the alanine replaces a positively charged amino acid residue.

58. — The method of claim 57, wherein the amino acid substitution is K167A, K272A, and/or K349A with reference to the amino acid position numbering of SEQ ID NO:148.

59. — The method of claim 58, wherein Type V CRISPR-Cas effector protein is a Cas12a CRISPR-Cas effector protein comprising an amino acid substitution of K167A, K272A, K349A, K167A+K272A, K167A+K349A, K272A+K349A, or K167A+K272A+K349A with

reference to the amino acid position numbering of ~~SEQ ID NO:148~~, optionally wherein the Type V CRISPR-Cas effector protein is an LbCas12a.

60. — The method of any one of the preceding claims, wherein reverse transcriptase comprises an amino acid modification.

61. — The method of claim 60, wherein amino acid modification is an amino acid substitution.

62. — The method of claim 61, wherein the amino acid substitution is L139P, D200N, W388R, E607K, T306K, W313F, F155Y, H638G, Q221R, V223M and/or D524N with reference to the amino acid position numbering of ~~SEQ ID NO:172~~.

63. — The method of claim 61 or claim 62, wherein reverse transcriptase comprises an amino acid substitution of L139P, D200N, W388R, and E607K; L139P, D200N, T306K, W313F, W388R, and E607K; 5M (T355A/Q357M/K358R/A359G/S360A), F155Y, and H638G; 5M (T355A/Q357M/K358R/A359G/S360A), Q221R, and V223M; or 5M T355A/Q357M/K358R/A359G/S360A) and D524N with reference to the amino acid position numbering of ~~SEQ ID NO:172~~.

64. — The method of any one of the preceding claims, wherein the Type V CRISPR-Cas effector protein is a Type V CRISPR-Cas fusion protein comprising a Type V CRISPR-Cas effector protein domain fused to the reverse transcriptase or the Type II CRISPR-Cas effector protein is a Type II CRISPR-Cas fusion protein comprising a Type II CRISPR-Cas effector protein domain fused to the reverse transcriptase.

65. — The method of claim 64, wherein the reverse transcriptase is fused to the C-terminus of the Type V CRISPR-Cas effector polypeptide or the Type II CRISPR-Cas effector polypeptide.

66. — The method of claim 65, wherein the reverse transcriptase is fused to the N-terminus of the Type V CRISPR-Cas effector polypeptide or the Type II CRISPR-Cas effector polypeptide.

~~67~~13. The method of any of the preceding claims, wherein the reverse transcriptase is fused to one or more single stranded RNA binding domains (RBDs), thereby improving the thermostability, processivity and template affinity of the reverse transcriptase.

5 ~~68~~14. The method of claim ~~67~~13, wherein the one or more single stranded RNA binding domains are fused to the N-terminus of the reverse transcriptase, optionally wherein the reverse transcriptase is further fused at its C-terminus to the N-terminus of the Type II CRISPR-Cas effector protein and/or Type V CRISPR-Cas effector protein.

10 ~~69.~~—The method of any one of the preceding claims, further comprising contacting the target nucleic acid with a 5'-3' exonuclease.

15 ~~70.~~—The method of claim ~~69~~, wherein the 5'-3' exonuclease is fused to a Type V CRISPR-Cas effector protein, optionally to a Type V CRISPR-Cas fusion protein or the 5'-3' exonuclease is fused to a Type II CRISPR-Cas effector protein, optionally to a Type II CRISPR-Cas fusion protein.

20 ~~71.~~—The method of claim ~~70~~, wherein the 5'-3' exonuclease is fused to the C-terminus of the Type V CRISPR-Cas effector protein, optionally to the C-terminus of the Type V CRISPR-Cas fusion protein, or is fused to the C-terminus of the Type II CRISPR-Cas effector protein, optionally to the C-terminus of the Type II CRISPR-Cas fusion protein.

25 ~~72.~~—The method of claim ~~69~~, wherein the 5'-3' exonuclease is a fusion protein comprising the 5'-3' exonuclease fused to a peptide tag and the Type II or Type V CRISPR-Cas effector protein is a fusion protein comprising a Type II or Type V CRISPR-Cas effector protein domain fused to an affinity polypeptide that is capable of binding to the peptide tag.

30 ~~73.~~—The method of claim ~~69~~, wherein the 5'-3' exonuclease is a fusion protein comprising the 5'-3' exonuclease fused to an affinity polypeptide that is capable of binding to the peptide tag and the Type II or Type V CRISPR-Cas effector protein is a fusion protein comprising a Type II or Type V CRISPR-Cas effector protein domain fused to a peptide tag.

74. ~~The method of claim 69, wherein the 5' 3' exonuclease is a fusion protein comprising the 5' 3' exonuclease fused to an affinity polypeptide that is capable of binding to an RNA recruiting motif and the extended guide nucleic acid is linked to an RNA recruiting motif.~~

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75. ~~The method of any one of claims 69 74, wherein the 5' 3' exonuclease is a RecE exonuclease (RecE), a RecJ exonuclease (RecJ), a T5 exonuclease (T5_Exo), a T7 exonuclease (T7_Exo), a lambda exonuclease (Lambda_exo), a sbcB (exodeoxyribonuclease), or an hExo1 (human exonuclease 1).~~

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~~76~~15. The method of any one of the preceding claims, further comprising contacting the target nucleic acid with a single stranded DNA binding protein (ssDNA binding protein).

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~~77~~16. The method of claim ~~76~~15, wherein the ssDNA binding protein is fused to the Type II V CRISPR-Cas effector protein or Type V CRISPR-Cas effector protein.

~~78~~17. The method of claim ~~77~~16, wherein the ssDNA binding protein is fused to the C-terminus of the Type II or Type V CRISPR-Cas effector protein.

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~~79~~18. The method of claim ~~77~~16, wherein the ssDNA binding protein is fused to the N-terminus of the Type II or Type V CRISPR-Cas effector protein.

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~~80.~~ ~~The method of any of claims 76 to 79, wherein the ssDNA binding protein is from a human, a bacterium or a phage.~~

~~81~~19. The method of any of claims ~~76-15~~ to ~~80~~18, wherein the ssDNA binding protein is hRad51 (optionally, hRad51_S208E_A209D), hRad52, BsRecA, EcRecA, T4ssB and/or Brex27.

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~~82~~20. The method of any one of the preceding claims, further comprising reducing double strand breaks by introducing a chemical inhibitor of non-homologous end joining (NHEJ), by introducing a CRISPR guide nucleic acid or an siRNA targeting an NHEJ protein to transiently

knock-down expression of the NHEJ protein, or by introducing a polypeptide that prevents NHEJ, wherein the polypeptide that prevents NHEJ is a Gam protein and the Gam protein is fused to the reverse transcriptase and/or the CRISPR-Cas effector protein, optionally the Gam protein is fused to the N-terminus of the reverse transcriptase and/or the N-terminus of the

5 CRISPR-Cas effector protein.

83. — ~~The method of claim 82, wherein the polypeptide that prevents NHEJ is fused to the reverse transcriptase and/or the CRISPR-Cas effector protein, optionally the polypeptide that prevents NHEJ is fused to the N-terminus of the reverse transcriptase and/or the CRISPR-Cas effector protein.~~

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84. — ~~The method of claim 82 or claim 83, wherein the polypeptide that prevents NHEJ is a Gam protein.~~

15 ~~85~~21. The method of claim ~~84~~20, wherein the Gam protein is *Escherichia* phage Mu Gam protein, optionally the Gam protein comprise the amino acid sequence of **SEQ ID NO:147**.

86. — ~~The method of any one of the preceding claims, wherein the Type II or Type V CRISPR-Cas effector protein is encoded by a polynucleotide and/or the reverse transcriptase is encoded by a polynucleotide.~~

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87. — ~~The method of any one of claims 32 to 86, wherein the CRISPR-Cas effector protein is encoded by a polynucleotide.~~

25 88. — ~~The method of any one of claims 37-87, wherein the 5' flap endonuclease (FEN) is encoded by a polynucleotide.~~

89. — ~~The method of any one of claims 69-88, wherein the 5' 3' exonuclease is encoded by a polynucleotide.~~

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90. — The method of any one of claims 1-89, wherein the target nucleic acid is in a cell, in a cell free system or in a virus.

91. — The method of claim 90, wherein the cell is a eukaryotic cell or a prokaryotic cell.

92. — The method of claim 90 or claim 91, wherein the cell is from an animal, a plant, a fungus, an archaeon, a bacterium or a virus.

93. — The method of any one of claims 86 to 92, wherein the polynucleotide encoding the Type II or Type V CRISPR-Cas effector protein and/or the polynucleotide encoding the reverse transcriptase are comprised in the same or separate expression cassettes, optionally when the polynucleotide encoding the Type II or Type V CRISPR-Cas effector protein and the polynucleotide encoding the reverse transcriptase are comprised in the same expression cassette, the polynucleotide encoding the Type II or Type V CRISPR-Cas effector protein and the polynucleotide encoding the reverse transcriptase are operably linked to a single promoter or to two or more separate promoters in any combination.

94. — The method of any one of claims 87 to 93, wherein the polynucleotide encoding the CRISPR-Cas effector protein is comprised in an expression cassette, optionally wherein the CRISPR-Cas effector protein is operably linked to a promoter.

95. — The method of claim 94, wherein the same or separate expression cassettes are comprised in the same or separate vectors.

96. — The method of claim 94, wherein the expression cassette is comprised a vector.

97. — The method of any one of claims 88 to 96, wherein the polynucleotide encoding the 5' flap endonuclease is comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.

98.— ~~The method of any one of claims 83 to 91, wherein the polynucleotide encoding the 5' 3' exonuclease is comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.~~

99.— ~~The method of any one of the preceding claims, wherein the extended guide nucleic acid is comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.~~

100.— ~~The method of any one of claims 32 to 99, wherein the guide nucleic acid is comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.~~

101.— ~~The method of any one of claims 85 to 100, wherein the polynucleotides encoding the Type II or Type V CRISPR-Cas effector proteins, the CRISPR-Cas effector proteins, the reverse transcriptases, the 5' flap endonucleases, the 5' 3' exonucleases and expression cassettes and/or vectors comprising the same are codon optimized for expression in an organism, optionally in a prokaryote or a eukaryote, optionally in an animal, a plant, a fungus, an archaeon, a bacterium or a virus.~~

102.— ~~The method of claim 101, wherein the codon optimization is for expression in a dicot plant.~~

103.— ~~The method of claim 101, wherein the codon optimization is for expression in a monocot plant.~~

104.— ~~A method of modifying a target nucleic acid, the method comprising:
contacting the target nucleic acid at a first site with
(a)(i) a first CRISPR-Cas effector protein; and
—(ii) a first extended guide nucleic acid (e.g., extended CRISPR RNA, extended CRISPR DNA, extended crRNA, extended crDNA); and
(b)(i) a second CRISPR-Cas effector protein;
—(ii) a first reverse transcriptase; and~~

—(ii) a first guide nucleic acid, thereby modifying the target nucleic acid.

105. — The method of claim 104, wherein the first extended guide nucleic acid comprises
(a) a CRISPR nucleic acid (CRISPR RNA, CRISPR DNA, crRNA, crDNA); and
5 (b) an extended portion comprising a primer binding site and a reverse transcriptase
template (RT template).

106. — The method of claim 104 or claim 105, wherein the CRISPR nucleic acid of the first
extended guide nucleic acid comprises a spacer sequence capable of binding to (having
10 substantial homology to) a first site on the first strand of the target nucleic acid.

107. — The method of any one of claims 104–106, wherein the first guide nucleic acid comprises
a CRISPR nucleic acid (CRISPR RNA, CRISPR DNA, crRNA, crDNA).

108. — The method of claim 107, wherein the CRISPR nucleic acid of the first guide nucleic acid
comprises a spacer sequence that binds to a second site on the first strand of the target nucleic
acid that is upstream of the first site on the first strand of the target nucleic acid.

109. — The method of any one of claims 104–108, wherein the second CRISPR–Cas effector
20 protein is a CRISPR–Cas fusion protein comprising a CRISPR–Cas effector protein domain fused
to the reverse transcriptase.

110. — The method of any one of claims 104–108, wherein the second CRISPR–Cas effector
protein is a CRISPR–Cas fusion protein comprising a CRISPR–Cas effector protein domain fused
25 to a peptide tag and the first reverse transcriptase is a reverse transcriptase fusion protein
comprising a reverse transcriptase domain fused to an affinity polypeptide capable of binding the
peptide tag.

111. — The method of any one of claims 104–108, wherein the first guide nucleic acid is linked
30 to an RNA recruiting motif and the first reverse transcriptase is a reverse transcriptase fusion

protein comprising a reverse transcriptase domain fused to an affinity polypeptide capable of binding the RNA-recruiting motif.

112. — The method of any one of claims 104–111, further comprising contacting the target nucleic acid with

(a) a third CRISPR-Cas effector protein; and

(b) a second guide nucleic acid,

wherein the third CRISPR-Cas effector protein nicks a site on the first strand of the target nucleic acid that is located about 10 to about 125 base pairs (either 5' or 3') from the second site on the second strand that has been nicked by the second CRISPR-Cas effector protein, thereby improving mismatch repair.

113. — The method of any one of claims 104–112, further comprising contacting the target nucleic acid with a 5'-3' exonuclease.

114. — The method of claim 113, wherein the 5'-3' exonuclease is fused to the first CRISPR-Cas effector protein.

115. — The method of claim 114, wherein the 5'-3' exonuclease is fused to the C-terminus of the first CRISPR-Cas effector protein.

116. — The method of claim 113, wherein the 5'-3' exonuclease is a fusion protein comprising the 5'-3' exonuclease fused to a peptide tag and the first CRISPR-Cas effector protein is a fusion protein comprising a CRISPR-Cas effector protein domain fused to an affinity polypeptide that is capable of binding to the peptide tag.

117. — The method of claim 113, wherein the 5'-3' exonuclease is a fusion protein comprising the 5'-3' exonuclease fused to an affinity polypeptide that is capable of binding to the peptide tag and the first CRISPR-Cas effector protein is a fusion protein comprising a CRISPR-Cas effector protein domain fused to a peptide tag.

118. — The method of claim 113, wherein the 5' 3' exonuclease is a fusion protein comprising the 5' 3' exonuclease fused to an affinity polypeptide that is capable of binding to an RNA recruiting motif and extended guide nucleic acid is linked to an RNA recruiting motif.

119. — The method of any one of claims 113–118, wherein the 5' 3' exonuclease is a RecE exonuclease (RecE), a RecJ exonuclease (RecJ), a T5 exonuclease (T5_Exo), a T7 exonuclease (T7_Exo), a lambda exonuclease (Lambda_exo), a sbcB (exodeoxyribonuclease), or an hExo1 (human exonuclease 1).

120. — The method of any one of claims 104–119, further comprising contacting the target nucleic acid with

(a) a fourth CRISPR-Cas effector protein;

(b) a second reverse transcriptase, and

(c) a second extended guide nucleic acid (e.g., extended CRISPR RNA, extended CRISPR DNA, extended crRNA, extended crDNA);

wherein the second extended guide nucleic acid targets (spacer is substantially complementary to/binds to) a site on the first strand of the target nucleic acid, thereby modifying the target nucleic acid.

121. — The method of any one of claims 104–120, wherein the first CRISPR-Cas effector protein, the second CRISPR-Cas effector protein, the third CRISPR-Cas effector protein and/or the fourth CRISPR-Cas effector protein are a Type I, Type II, Type III, Type IV, or Type V CRISPR-Cas effector protein, in any combination.

122. — The method of any one of claims 1 to 103, further comprising contacting the target nucleic acid with

(a) a second CRISPR-Cas effector protein, optionally a Type II or Type V CRISPR-Cas effector protein;

(b) a second reverse transcriptase, and

(c) a second extended guide nucleic acid (e.g., extended CRISPR RNA, extended CRISPR DNA, extended crRNA, extended crDNA);

wherein the second extended guide nucleic acid targets (spacer is substantially complementary to/binds to) a site on the first strand (target strand) or the second strand (non-target strand) of the target nucleic acid, thereby modifying the target nucleic acid.

5 123. — The method of claim 104-122, wherein the first and/or second extended guide nucleic acid further comprises a pseudoknot.

124. — The method of claim 123, wherein the pseudoknot is located at the 3' end of the extended guide nucleic acid.

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125. — The method of claim 123 or claim 124, wherein the pseudoknot is a tEvoPreQ1 Pseudoknot comprising the nucleic acid sequence of **SEQ ID NO:158** or an EvoPreQ1 Pseudoknot comprising the nucleic acid sequence of **SEQ ID NO:191**.

15 126. — The method of claim 123 or claim 124, wherein the pseudoknot comprises the nucleic acid sequenced of **SEQ ID NO:95** or **SEQ ID NO:203**.

127. — The method of any one of claims 104-126, further comprising contacting the target nucleic acid with a single stranded DNA binding protein (ssDNA binding protein).

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128. — The method of claim 127, wherein the ssDNA binding protein is fused to the first, second, third and/or fourth CRISPR-Cas effector protein.

25 129. — The method of claim 127 or claim 128, wherein the ssDNA binding protein is fused to the C terminus of the first, second, third and/or fourth CRISPR-Cas effector protein.

130. — The method of claim 127 or claim 128, wherein the ssDNA binding protein is fused to the N terminus of the first, second, third and/or fourth CRISPR-Cas effector protein.

30 131. — The method of any of claims 127-130, wherein the ssDNA binding protein is from a human, a bacterium or a phage.

132. — The method of any of claims 127-131, wherein the ssDNA binding protein is hRad51 (optionally, hRad51_S208E_A209D), hRad52, BsRecA, EcRecA, T4ssB and/or Brex27.

133. — The method of any one of claims 104-132, further comprising reducing double strand breaks by introducing a chemical inhibitor of non homologous end joining (NHEJ), by introducing a CRISPR guide nucleic acid or an siRNA targeting an NHEJ protein to transiently knock down expression of the NHEJ protein, or by introducing a polypeptide that prevents NHEJ.

134. — The method of claim 133, wherein the polypeptide that prevents NHEJ is fused to the first and/or second reverse transcriptase and/or the first, second, third and/or fourth CRISPR-Cas effector protein, optionally the polypeptide that prevents NHEJ is fused to the N terminus of the first and/or second reverse transcriptase and/or the first, second, third and/or fourth CRISPR-Cas effector protein.

135. — The method of claim 133 or claim 134, wherein the polypeptide that prevents NHEJ is a Gam protein.

136. — The method of claim 135, wherein the Gam protein is *Escherichia* phage Mu Gam protein, optionally the Gam protein comprise the amino acid sequence of SEQ ID NO:147.

137. — An extended guide nucleic acid comprising (i) a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid (Type II or Type V CRISPR RNA, Type II or Type V CRISPR DNA, Type II or Type V crRNA, Type II or Type V crDNA) and/or a Type V CRISPR nucleic acid or Type II CRISPR nucleic acid and a tracr nucleic acid (e.g., Type II or Type V tracrRNA, Type II or Type V tracrDNA); and (ii) an extended portion comprising a primer binding site and a reverse transcriptase template (RT template) (RTT).

138. — The extended guide nucleic acid of claim 137, wherein the extended guide nucleic acid further comprises a pseudoknot.

139. — ~~The extended guide nucleic acid of claim 138, wherein the pseudoknot is located at the 3' end of the extended guide nucleic acid.~~

140. — ~~The extended guide nucleic acid of claim 138 or claim 139, wherein the pseudoknot is a tEvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:158 or an EvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:191.~~

141. — ~~The extended guide nucleic acid of claim 138 or claim 139, wherein the pseudoknot comprises the nucleic acid sequenced of SEQ ID NO:95 or SEQ ID NO:203.~~

142. — ~~The extended guide nucleic acid of any one of claims 137-141, comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.~~

143. — ~~A complex comprising:~~
~~(a) a Type V CRISPR-Cas effector protein or a Type II CRISPR-Cas effector protein;~~
~~(b) a reverse transcriptase, and~~
~~(c) an extended guide nucleic acid (e.g., extended CRISPR RNA, extended CRISPR DNA, extended crRNA, extended crDNA).~~

144. — ~~The complex of claim 143, wherein the extended guide nucleic acid further comprises a pseudoknot.~~

145. — ~~The complex of claim 144, wherein the pseudoknot is located at the 3' end of the extended guide nucleic acid.~~

146. — ~~The complex of claim 144 or claim 145, wherein the pseudoknot is a tEvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:158 or an EvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:191.~~

147. — The complex of claim 144 or claim 145, wherein the pseudoknot comprises the nucleic acid sequence of ~~SEQ ID NO:95~~ or ~~SEQ ID NO:203~~.

148. — The complex of claim 143–147, wherein the Type II or Type V CRISPR–Cas effector protein is a fusion protein comprising a Type II or Type V CRISPR–Cas effector protein domain fused to a peptide tag.

149. — The complex of claim 143–147, wherein the Type II or Type V CRISPR–Cas effector protein is a fusion protein comprising a Type V CRISPR–Cas effector protein domain fused to an affinity polypeptide that is capable of binding a peptide tag.

150. — The complex of claim 143–147, wherein the Type II or Type V CRISPR–Cas effector protein is a fusion protein comprising a Type II or Type V CRISPR–Cas effector protein domain fused to an affinity polypeptide that is capable of binding an RNA recruiting motif.

151. — The complex of any one of claims 143–150, wherein the reverse transcriptase is a fusion protein comprising reverse transcriptase domain fused to a peptide tag.

152. — The complex of any one of claims 143–150, wherein the reverse transcriptase is a fusion protein comprising reverse transcriptase domain fused to an affinity polypeptide that is capable of binding a peptide tag.

153. — The complex of any one of claims 143–150, wherein the reverse transcriptase is a fusion protein comprising reverse transcriptase domain fused to an affinity polypeptide that is capable of binding an RNA recruiting polypeptide.

154. — The complex of any one of claims 143–153, comprised in an expression cassette, optionally wherein the expression cassette is comprised in a vector.

155. — An expression cassette codon optimized for expression in an organism, comprising 5' to 3': (a) polynucleotide encoding a promoter sequence;

(b) a polynucleotide encoding a Type V CRISPR-Cas nuclease (e.g., Cpf1 (Cas12a), dCas12a and the like) or a Type II CRISPR-Cas nuclease (e.g., Cas9, dCas9 and the like) that is codon-optimized for expression in the organism;

(c) a linker sequence; and

(d) a polynucleotide encoding a reverse transcriptase that is codon-optimized for expression in the organism.

156. The expression cassette of claim 155, wherein the organism is an animal, a plant, a fungus, an archaeon, a bacterium or a virus.

157. The expression cassette of claim 156, wherein the organism is a plant, the polynucleotide encoding a Type V CRISPR-Cas nuclease is codon-optimized for expression in a plant, and the promoter sequence is a plant-specific promoter sequence (e.g., ZmUbi1, MtUbi2, RNA polymerase II (Pol II)).

158. The expression cassette of any one of claims 155-157, wherein the reverse transcriptase is fused to one or more ssRNA binding domains (RBDs).

159. The method of claim 158, wherein the one or more single-stranded RNA binding domains are fused to the N-terminus of the reverse transcriptase, optionally wherein the reverse transcriptase is further fused at its C-terminus to the N-terminus of the Type II CRISPR-Cas effector protein and/or Type V CRISPR-Cas effector protein.

160. The expression cassette of any one of claims 155 to 159, wherein the linker is an amino acid or peptide linker.

161. The expression cassette of claim 160 wherein the peptide linker is about 2 to about 100 amino acids (residues) in length.

162. The expression cassette of claim 160 or claim 161, wherein the peptide linker is a GS linker.

163. — ~~An expression cassette codon optimized for expression in a plant, comprising:~~
~~(a) a polynucleotide encoding a plant specific promoter sequence (e.g., ZmUbi1, MtUb2),~~
and

5 ~~(b) an extended guide nucleic acid, wherein the extended guide nucleic acid comprises an~~
~~extended portion comprising at its 3' end a primer binding site and an edit to be incorporated into~~
~~the target nucleic acid (e.g., reverse transcriptase template), optionally wherein the extended~~
~~guide nucleic acid is comprised in an expression cassette, optionally wherein the extended guide~~
~~nucleic acid is operably linked to a Pol II promoter.~~

10 164. — ~~The expression cassette of claim 163, wherein the extended guide nucleic acid further~~
~~comprises a pseudoknot.~~

15 165. — ~~The expression cassette of claim 164, wherein the pseudoknot is located at the 3' end of~~
~~the extended guide nucleic acid.~~

20 166. — ~~The expression cassette of claim 164 or claim 165, wherein the pseudoknot is a~~
~~tEvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:158 or an~~
~~EvoPreQ1 Pseudoknot comprising the nucleic acid sequence of SEQ ID NO:191.~~

25 167. — ~~The expression cassette of claim 164 or claim 165, wherein the pseudoknot comprises the~~
~~nucleic acid sequence of SEQ ID NO:95 or SEQ ID NO:203.~~

30 168. — ~~The expression cassette of any one of claims 157–167, wherein the plant specific~~
~~promoter is associated with an intron or is a promoter region comprising an intron (e.g., ZmUbi1~~
~~comprising an intron; MtUb2 comprising an intron).~~

 169. — ~~The expression cassette of any one of claims 156–168, wherein the codon optimization is~~
~~for expression in a dicot plant.~~

170.—~~The expression cassette of any one of claims 156–168, wherein the codon optimization is for expression in a monocot plant.~~

171.—~~A method of modifying a target nucleic acid in a plant or plant cell, comprising
5 introducing the expression cassette of any one of claims 155–170 into the plant or plant cell,
thereby modifying the target nucleic acid in the plant or plant cell.~~

172.—~~The method of claim 171, further comprising regenerating the plant cell comprising the
modified target nucleic acid to produce a plant comprising the modified target nucleic acid.~~

173.—~~The method of any one of claims 1 to 132, 171, or 172, wherein the contacting is carried
10 out at a temperature of about 20°C to 42°C.~~