CLAIMS

What is claimed is:

1. A guide RNA comprising a spacer sequence, a gRNA core, and an extension arm, wherein the extension arm comprises (i) a primer binding site, (ii) an RT edit template, and (iii) a homology arm;

wherein the spacer sequence comprises from 10 to 40 nucleotides which contain a nucleotide sequence that is complementary to a protospacer sequence in a target strand of a target DNA sequence;

wherein the gRNA core is capable of binding a nucleic acid programmable DNA binding protein (napDNAbp);

wherein the extension arm is capable of serving as a template for reverse transcription synthesis of a single strand of DNA and comprises the homology arm, the RT edit template, and the primer binding site in a 5' to 3' direction;

wherein the primer binding site is 8-20 nucleotides in length and is capable of annealing to a 3'-ended ssDNA flap that is formed after napDNAbp nicking of a non-target strand of the target DNA sequence and which serves as a primer sequence to prime reverse transcription of the single strand of DNA;

wherein the RT edit template encodes a desired edit in the single strand of DNA; and

wherein the homology arm encodes a portion of the single strand of DNA that is complementary to the target strand of the target DNA sequence.

2. The guide RNA of claim 1, wherein the protospacer comprises about 20 nucleotides.

3. The guide RNA of claim 1 or 2, wherein the extension arm is between approximately 7 and approximately 500 nucleotides in length.

4. The guide RNA of claim 1 or 2, wherein the extension arm is at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16

nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, at least 30 nucleotides, at least 31 nucleotides, at least 32 nucleotides, at least 33 nucleotides, at least 34 nucleotides, at least 35 nucleotides, at least 36 nucleotides, at least 37 nucleotides, at least 38 nucleotides, at least 39 nucleotides, at least 40 nucleotides, or at least 100 nucleotides in length.

5. The guide RNA of any one of claims 1-4, wherein the extension arm is a 5' extension arm.

6. The guide RNA of any one of claims 1-4, wherein the extension arm is a 3' extension arm.

7. The guide RNA of any one of claims 1-6, wherein the edit template is at least 1 nucleotides, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, at least 30 nucleotides, at least 31 nucleotides, at least 32 nucleotides, at least 33 nucleotides, at least 34 nucleotides, at least 35 nucleotides, at least 36 nucleotides, at least 37 nucleotides, at least 38 nucleotides, at least 39 nucleotides, at least 40 nucleotides or, at least 100 nucleotides in length.

8. The guide RNA of any one of claims 1-7, wherein the desired edit comprises one or more single-base nucleotide changes, one or more deletions, one or more insertions, or a combination thereof.

9. The guide RNA of any one of claims 1-8, wherein the homology arm is at least 1 nucleotides, at least 2 nucleotides, at least 3 nucleotides, at least 4 nucleotides, at least 5 nucleotides, at least 6 nucleotides, at least 7 nucleotides, at least 8 nucleotides, at least 9 nucleotides, at least 10 nucleotides, at least 11 nucleotides, at least 12 nucleotides, at least 13 nucleotides, at least 14 nucleotides, at least 15 nucleotides, at least 16 nucleotides, at least 17 nucleotides, at least 18 nucleotides, at least 19 nucleotides, at least 20 nucleotides, at least 21 nucleotides, at least 22 nucleotides, at least 23 nucleotides, at least 24 nucleotides, at least 25 nucleotides, at least 26 nucleotides, at least 27 nucleotides, at least 28 nucleotides, at least 29 nucleotides, or at least 30 nucleotides.

10. The guide RNA of any one of claims 1-9, further comprising a 5′ end modifier region comprising a hairpin sequence, a stem/loop sequence, or a toeloop sequence.

11. The guide RNA of any one of claims 1-10, further comprising a 3′ end modifier region comprising a hairpin sequence, a stem/loop sequence, or a toeloop sequence.

12. The guide RNA of any one of claims 1-11, wherein the spacer sequence comprises a nucleotide sequence at least 90% identical to any one of SEQ ID NOs: 135515 – 271028 or 880463 – 947840.

13. The guide RNA of any one of claims 1-11, wherein the spacer sequence comprises the nucleotide sequence of any one of SEQ ID NOs: 135515 – 271028 or 880463 – 947840.

14. The guide RNA of any one of claims 1-13, wherein the gRNA core comprises the nucleotide sequence of SEQ ID Nos: 1361579 or 1361580.

15. The guide RNA of any one of claims 1-14, wherein the primer binding site comprises a nucleotide sequence at least 90% identical to SEQ ID NOs: 406543 – 542056 or 1015219 – 1082596.

16. The guide RNA of any one of claims 1-14, wherein the primer binding site comprises the nucleotide sequence of any one of SEQ ID NOs: 406543 – 542056 or 1015219 – 1082596.

17. The guide RNA of any one of claims 1-16, wherein the RT edit template comprises a nucleotide sequence at least 90% identical to SEQ ID NOs: 542057 – 677570 or 1082597-1149974.

18. The guide RNA of any one of claims 1-16, wherein the RT edit template comprises the nucleotide sequence of any one of SEQ ID NOs: 542057 – 677570 or 1082597-1149974.

19. The guide RNA of any one of claims 1-18, wherein the homology arm comprises a nucleotide sequence at least 90% identical to any one of SEQ ID NOs: 677,571 – 813,084 or 1149975-1217352.

20. The guide RNA of any one of claims 1-18, wherein the homology arm comprises the nucleotide sequence of any one of SEQ ID NOs: 677571 – 813084 or 1149975-1217352.

21. The guide RNA of any one of claims 1-20, wherein the extension arm comprises a nucleotide sequence at least 90% identical to any one of SEQ ID NOs: 271029 – 406542 or 947841 – 1015218.

22. The guide RNA of any one of claims 1-20, wherein the extension arm comprises the nucleotide sequence of any one of SEQ ID NOs: 271029 – 406542 or 947841 – 1015218.

23. The guide RNA of any one of claims 1-22, comprising a sequence at least 90% identical to any one of SEQ ID NOs: 1-135514 or 813085-880462.

24. The guide RNA of any one of claims 1-22, comprising the sequence of any one of SEQ ID NOs: 1-135514 or 813085-880462.

25. A polynucleotide encoding the guide RNA of any one of claims 1-24.

26. A vector comprising the polynucleotide of claim 25.

27. A pharmaceutical composition comprising: (a) the guide RNA of any one of claims 1-24, the polynucleotide of claim 25, or the vector of claim 26 and (b) a pharmaceutically acceptable excipient.

28. A prime editing complex comprising a napDNAbp, a reverse transcriptase, and any one of the guide RNAs of claims 1-24.

29. The prime editing complex of claim 28, wherein the napDNAbp and the reverse transcriptase are formed as a fusion protein.

30. The prime editing complex of claim 28 or 29, wherein the napDNAbp is Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute, or a variant of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute.

31. The prime editing complex of any one of claims 28-30, wherein the napDNAbp comprises nickase activity.

32. The prime editing complex of claim 28 or 29, wherein the napDNAbp is a Cas9 nickase.

33. One or more polynucleotides encoding the prime editing complex of any one of claims 28-32.

34. One or more vectors comprising the one or more polynucleotides of claim 33.

35. A pharmaceutical composition comprising the one or more vectors of claim 34 and a pharmaceutically acceptable excipient.

36. A composition comprising the guide RNA of any one of claims 1-24 and one or more polynucleotides encoding a prime editor comprising a napDNAbp and a reverse transcriptase.

37. A composition comprising the polynucleotide of claim 25 and one or more polynucleotides encoding a prime editor comprising a napDNAbp and a reverse transcriptase.

38. The composition of claim 36 or 37, wherein the prime editor is a fusion protein.

39. The composition of any one of claims 36-38, wherein the napDNAbp is Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute, or a variant of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, or Argonaute.

40. The composition of any one of claims 36-38, wherein the napDNAbp comprises nickase activity.

41. The composition of any one of claims 36-38, wherein the napDNAbp is a Cas9 nickase.

42. The composition of any one of claims 36-41, wherein the reverse transcriptase is a Moloney Murine Leukemia Virus reverse transcriptase (MMLV-RT) or a variant MMLV-RT.

43. The composition of claim 42, wherein the reverse transcriptase is a variant MMLV-RT comprising
one or more of the following mutations: P51X, S67X, E69X, L139X, T197X, D200X, H204X, F209X, E302X, T306X, F309X, W313X, T330X, L345X, L435X, N454X, D524X, E562X, D583X, H594X, L603X, E607X, and D653X relative to the wild type M-MLV RT of SEQ ID NO: 1361485 or at a corresponding amino acid position in another wild type M-MLV RT polypeptide sequence, and wherein X is any amino acid.

44. The composition of claim 42, wherein the variant M-MLV RT comprises one or more of the following mutations: P51L, S67K, E69K, L139P, T197A, D200N, H204R, F209N, E302K, E302R, T306K, F309N, W313F, T330P, L345G, L435G, N454K, D524G, E562Q, D583N,

H594Q, L603W, E607K, and D653N relative to the wild type M-MLV RT of SEQ ID NO: 1361485 or at a corresponding amino acid position in another wild type M-MLV RT polypeptide sequence.

45. The composition of claim 42, wherein the variant M-MLV RT comprises amino acid substitutions D200N, T330P and/or L603W relative to the wild type M-MLV RT of SEQ ID NO: 1361485 or at a corresponding amino acid position in another wild type M-MLV RT polypeptide sequence.

46. The composition of claim 42, wherein the variant M-MLV RT comprises amino acid substitutions D200N, T306K, W313F, T330P, and L603W relative to the wild type M-MLV RT of SEQ ID NO: 1361485 or at a corresponding amino acid position in another wild type M-MLV RT polypeptide sequence

47. The composition of claim 42, wherein the variant M-MLV RT comprises the sequence of SEQ ID NO: 1361514.

48. The composition of claim 38, wherein the fusion protein comprises the sequence of SEQ ID NO: 1361516.