CLAIMS

1. A pegRNA for prime editing comprising a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template and a primer binding site, wherein the extension arm comprises a nucleic acid moiety attached thereto selected from the group consisting of a toe-loop, hairpin, stem-loop, pseudoknot, aptamer, G-quadraplex, tRNA, riboswitch, or ribozyme.

2. The pegRNA of claim 1, wherein the nucleic acid moiety is attached to the 3′ end of the extension arm.

3. The pegRNA of claim 1, wherein the nucleic acid moiety is attached to the 5′ end of the extension arm.

4. The pegRNA of claim 1, wherein the pseudoknot is a Mpknot1 moiety having a nucleotide sequence selected from the group consisting of: SEQ ID NO: 195 (Mpknot1), SEQ ID NO: 196 (Mpknot1 3′ trimmed), SEQ ID NO: 197 (Mpknot1 with 5′ extra), SEQ ID NO: 198 (Mpknot1 U38A), SEQ ID NO: 199 (Mpknot1 U38A A29C), SEQ ID NO: 200 (MMLC A29C), SEQ ID NO: 201 (Mpknot1 with 5′ extra and U38A), SEQ ID NO: 202 (Mpknot1 with 5′ extra and U38A A29C), and SEQ ID NO: 203 (Mpknot1 with 5′ extra and A29C), or a nucleotide sequence having at least 80% sequence identity therewith.

5. The pegRNA of claim 1, wherein the G-quadruplex has a nucleotide sequence selected from the group consisting of: SEQ ID NO: 204 (tns1), SEQ ID NO: 205 (stk40), SEQ ID NO: 206 (apc2), SEQ ID NO: 207 (ceacam4), SEQ ID NO: 208 (pitpnm3), SEQ ID NO: 209 (rlf), SEQ ID NO: 210 (erc1), SEQ ID NO: 211 (ube3c), SEQ ID NO: 212(taf15), SEQ ID NO: 213 (stard3), and SEQ ID NO: 214 (g2), or a nucleotide sequence having at least 80% sequence identity therewith.

6. The pegRNA of claim 1, wherein the evopreq1 has a nucleotide sequence selected from the group consisting of: SEQ ID NO: 215 (evopreq1), SEQ ID NO: 216 (evopreq1motif1), SEQ ID NO: 217 (evopreq1motif2), SEQ ID NO: 218 (evopreq1motif3), SEQ ID NO: 219 (shorter preq1-1), SEQ ID NO: 220 (preq1-1 G5C (mut1)), and SEQ ID NO: 221 (preq1-1 G15C (mut2)), or a nucleotide sequence having at least 80% sequence identity therewith.

7. The pegRNA of claim 1, wherein the tRNA moiety has a nucleotide sequence of SEQ ID NO: 222, or a nucleotide sequence having at least 80% sequence identity therewith.

8. The pegRNA of claim 1, wherein the nucleic acid moiety has a nucleotide sequence of SEQ ID NO: 223 (xrn1), or a nucleotide sequence having at least 80% sequence identity therewith.

9. The pegRNA of claim 1, wherein the nucleic acid moiety has a nucleotide sequence of SEQ ID NO: 224 (grp1 intron P4P6), or a nucleotide sequence having at least 80% sequence identity therewith.

10. The pegRNA of any of claims 1-9, wherein the nucleic acid moiety is attached to the pegRNA by a linker.

11. The pegRNA of claim 10, wherein the linker has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 225-236.

12. The pegRNA of claim 10, wherein the linker is 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 in length, wherein the linker is no longer than 50 nucleotides.

13. The pegRNA of claim 10, wherein the linker is 8 nucleotides in length.

14. The pegRNA of claim 1, wherein the extension arm is positioned at the 3´ or 5´ end of the guide RNA, and wherein the nucleic acid extension arm is DNA or RNA.

15. The pegRNA of claim 1, wherein the pegRNA is capable of binding to a napDNAbp and directing the napDNAbp to a target DNA sequence.

16. The pegRNA of claim 15, wherein the target DNA sequence comprises a target strand and a complementary non-target strand.

17. The pegRNA of claim 16, wherein the guide RNA hybridizes to the target strand to form an RNA-DNA hybrid and an R-loop.

18. The pegRNA of claim 1, wherein the nucleic acid extension arm is 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, at least 41 nucleotides, at least 42 nucleotides, at least 43 nucleotides, at least 44 nucleotides, at least 45 nucleotides, at least 46 nucleotides, at least 47 nucleotides, at least 48 nucleotides, at least 49 nucleotides, or at least 50 nucleotides.

19. The pegRNA of claim 1, wherein the DNA synthesis template is 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, or at least 15 nucleotides in length.

20. The pegRNA of claim 1, wherein the DNA synthesis template encodes a desired edit.

21. The pegRNA of claim 1, wherein the primer binding site is 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, or at least 15 nucleotides in length.

22. A complex for prime editing comprising:

(a) fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a domain comprising an RNA-dependent DNA polymerase activity; and

(b) a pegRNA of any one of claims 1-21.

23. The complex of claim 22, wherein the napDNAbp has a nickase activity.

24. The complex of claim 22, wherein the napDNAbp is a Cas9 protein or variant thereof.

25. The complex of claim 22, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

26. The complex of claim 22, wherein the napDNAbp is Cas9 nickase (nCas9).

27. The complex of claim 22, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute and optionally has a nickase activity.

28. The complex of claim 22, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NOs: 32, 34, 36, 102-128, and 132.

29. The complex of claim 22, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 32, 34, 36, 102-128, and 132.

30. The complex of claim 22, wherein the domain comprising an RNA-dependent DNA polymerase activity is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

31. A nucleic acid molecule encoding the pegRNA of any one of claim 1-19.

32. An expression vector comprising the nucleic acid molecule of claim 31, wherein the nucleic acid molecule is under the control of a promoter.

33. The expression vector of claim 32, wherein the promoter is a polIII promoter.

34. The expression vector of claim 32, wherein the promoter is a U6 promoter.

35. The expression vector of claim 32, wherein the promoter is a U6, U6v4, U6v7, or U6v9 promoter, or a fragment thereof.

36. A cell comprising the pegRNA of any one of claims 1-21.

37. A cell comprising the complex of any one of claims 22-30.

38. A cell comprising the nucleic acid molecule of claim 31.

39. A cell comprising the expression vector of any one of claims 32-35.

40. A pharmaceutical composition comprising: (i) a pegRNA of any one of claims 1-21, a complex of any one of claims 22-30, a nucleic acid molecule of claim 31, an expression vector of any one of claims 32-35, or a cell of any one of claims 36-39, and (ii) a pharmaceutically acceptable excipient.

41. A kit composition comprising: (i) a pegRNA of any one of claims 1-21, a complex of any one of claims 22-30, a nucleic acid molecule of claim 31, an expression vector of any one of claims 32-35, or a cell of any one of claims 36-39, and (ii) a set of instructions for conducting prime editing.

42. A method of prime editing comprising contacting a target DNA sequence with a pegRNA of any of claims 1-21 and a prime editor comprising a napDNAbp and a domain having an RNA-dependent DNA polymerase activity, wherein the editing efficiency is increased as compared to the same method using a pegRNA not comprising the modification.

43. The method of claim 42, wherein the editing efficiency is increased by at least 1.5 fold.

44. The method of claim 42, wherein the editing efficiency is increased by at least 2 fold.

45. The method of claim 42, wherein the editing efficiency is increased by at least 3 fold.

46. The method of claim 42, wherein the napDNAbp has a nickase activity.

47. The method of claim 42, wherein the napDNAbp is a Cas9 protein or variant thereof.

48. The method of claim 47, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

49. The method of claim 48, wherein the napDNAbp is Cas9 nickase (nCas9).

50. The method of claim 42, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute and optionally has a nickase activity.

51. The method of claim 42, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NOs: 32, 34, 36, 102-128, and 132.

52. The method of claim 42, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 32, 34, 36, 102-128, and 132.

53. The method of claim 42, wherein the domain comprising an RNA-dependent DNA polymerase activity is a naturally-occurring reverse transcriptase from a retrovirus or a retrotransposon.

54. The pegRNA of claim 10, wherein the linker is designed by a computational method of claim 56.

55. A method for precisely installing a nucleotide edit in a double stranded target DNA sequence, the method comprising: contacting the double stranded target DNA sequence with a prime editor comprising a nucleic acid programmable DNA binding protein (napDNAbp), a DNA polymerase, and a prime editing guide RNA (PEgRNA), wherein the PEgRNA comprises:

(a) a spacer that hybridizes to a first strand of the double stranded target DNA sequence;

(b) an extension arm that hybridizes to a second strand of the double stranded target DNA sequence;

(c) a DNA synthesis template comprising the nucleotide edit;

(d) a gRNA core that interacts with the napDNAbp;

(e) a nucleic acid moiety attached thereto selected from the group consisting of a toe-loop, hairpin, stem-loop, pseudoknot, aptamer, G-quadraplex, tRNA, riboswitch, or ribozyme; and

(f) a linker that couples the nucleic acid moiety to the pegRNA,

wherein the linker is designed by a computational model; and

wherein the PEgRNA directs the prime editor to install the nucleotide edit in the double stranded target DNA sequence.

56. A method for identifying at least one nucleic acid linker for coupling a prime editing guide RNA (pegRNA) to a nucleic acid moiety, the method comprising:

using at least one computer hardware processor to perform:

generating a plurality of nucleic acid linker candidates including a first nucleic acid linker candidate;

identifying the at least one nucleic acid linker from among the plurality of nucleic acid linker candidates at least in part by:

calculating multiple scores for each of at least some of the plurality of nucleic acid linker candidates, the calculating comprising calculating a first set of scores for the first nucleic acid linker candidate, the first set of scores comprising:

a first score indicative of a degree of interaction between the first nucleic acid linker candidate and a first region of the pegRNA;

a second score indicative of a degree of interaction between the first nucleic acid linker candidate and a second region of the pegRNA; and

identifying the at least one nucleic acid linker from among the at least some of the plurality of nucleic acid linker candidates using the calculated multiple scores; and

outputting information indicative of the at least one nucleic acid linker.

57. The method of claim 56, wherein the first score is indicative of a degree to which the first nucleic acid linker candidate is predicted to avoid interaction with the first region of the pegRNA, and wherein the second score is indicative of a degree to which the first nucleic acid linker candidate is predicted to avoid interaction with the second region of the pegRNA.

58. The method of claim 57, wherein the first region comprises a primer binding site (PBS) of the pegRNA.

59. The method of claim 58, wherein the second region comprises a spacer of the pegRNA.

60. The method of claim 57, wherein the first set of scores further comprises a third score indicative of a degree to which the first nucleic acid linker candidate is predicted to avoid interaction with a third region of the pegRNA and a fourth score indicative of a degree to which the first nucleic acid linker candidate is predicted to avoid interaction with a fourth region of the pegRNA.

61. The method of claim 60, wherein the third region comprises a DNA synthesis template.

62. The method of claim 61, wherein the fourth region comprises a gRNA core that interacts with a nucleic acid programmable DNA binding protein (napDNAbp).

63. The method of claim 60,

wherein the pegRNA is for installing a nucleotide edit in a double stranded target DNA sequence,

wherein the pegRNA comprises:

a spacer that hybridizes to a first strand of the double stranded target DNA sequence,

an extension arm that hybridizes to a second strand of the double stranded target DNA sequence, the extension arm comprising a primer binding site (PBS) and a DNA synthesis template comprising the nucleotide edit, and

a gRNA core that interacts with a nucleic acid programmable DNA binding protein napDNAbp, and

wherein the first region comprises the PBS, the second region comprises the spacer, the

third region comprises the DNA synthesis template, and the fourth region comprises the gRNA core.

64. The method of claim 56, wherein the plurality of nucleic acid linker candidates comprises a second nucleic acid linker candidate, and wherein identifying the at least one nucleic acid linker from among the at least some of the plurality of nucleic acid linker candidates using the calculated multiple scores comprises:

comparing the first set of scores for the first nucleic acid linker candidate with a second set of scores for the second nucleic acid linker candidate.

65. The method of claim 64, wherein:

the first region comprises a primer binding site (PBS),

the first score in the first set of scores is indicative of a degree to which the first nucleic acid linker candidate is predicted to avoid interaction with the first region of the pegRNA,

a third score in the second set of scores is indicative of a degree to which the second nucleic acid linker candidate is predicted to avoid interaction with the first region of the pegRNA, and

comparing the first set of scores with the second set of scores comprises:

comparing the first score with the third score.

66. The method of claim 65, wherein when the first score is equal to or is within a threshold distance of the third score, comparing the first set of scores with the second set of scores further comprises:

comparing a score, other than the first score, in the first set of scores with another score, other than the third score, in the second set of scores.

67. A PEgRNA for prime editing comprising (i) a guide RNA comprising a spacer and (ii) at least one nucleic acid extension arm comprising a DNA synthesis template, a primer binding site, a toehold motif, and an additional nucleic acid moiety.

68. The PEgRNA of claim 67, wherein the toehold motif and the additional nucleic acid moiety are attached to the 3′ end of the extension arm.

69. The PEgRNA of claim 67 or 68, wherein the toehold motif is attached to the 3′ end of the extension arm, and the additional nucleic acid moiety is attached to the 3′ end of the toehold motif.

70. The PEgRNA of any one of claims 67-69, wherein the toehold motif is attached to the PEgRNA by a linker.

71. The PEgRNA of claim 70, wherein the linker is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 nucleotides in length.

72. The PEgRNA of any one of claims 67-71, wherein the PEgRNA is capable of binding to a nucleic acid programmable DNA binding protein (napDNAbp) of a prime editor and directing the napDNAbp to a target DNA sequence.

73. A prime editing system for site specific genome modification comprising (a) a PEgRNA of any one of claims 67-72, and (b) a prime editor comprising (i) a napDNAbp, (ii) a DNA polymerase, and (iii) a portion that binds to the toehold motif of the PEgRNA.

74. The system of claim 73, wherein the portion of the prime editor that binds to the toehold motif of the PEgRNA is fused to the N-terminal end of the prime editor.

75. The system of claim 73, wherein the portion of the prime editor that binds to the toehold motif of the PEgRNA is fused to the C-terminal end of the prime editor.

76. The system of any one of claims 73-75, wherein the portion of the prime editor that binds to the toehold motif of the PEgRNA is fused to the prime editor by a linker.

77. The system of claim 76, wherein the linker is at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at

least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, or more than 30 amino acids in length.

78. The system of claim 76 or 77, wherein the linker comprises an xten linker.

79. The system of any one of claims 73-78, wherein the portion of the prime editor that binds to the toehold motif of the PEgRNA comprises an MS2 bacteriophage coat protein.

80. The system of any one of claims 73-79, wherein the napDNAbp has a nickase activity.

81. The system of any one of claims 73-80, wherein the napDNAbp is a Cas9 protein or a variant thereof.

82. The system of any one of claims 73-79, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

83. The system of any one of claims 73-79, wherein the napDNAbp is a Cas9 nickase (nCas9).

84. The system of any one of claims 73-79, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute, and optionally has a nickase activity.

85. A polynucleotide comprising the PEgRNA of any one of claims 67-72.

86. A vector comprising the polynucleotide of claim 85.

87. A cell comprising the PEgRNA of any one of claims 67-72, the system of any one of claims 73-84, the polynucleotide of claim 85, or the vector of claim 86.

88. A pharmaceutical composition comprising (i) the PEgRNA of any one of claims 67-72, the system of any one of claims 73-84, the polynucleotide of claim 85, or the vector of claim 86, and (ii) a pharmaceutically acceptable excipient.

89. A kit comprising the PEgRNA of any one of claims 67-72, the system of any one of claims 73-84, the polynucleotide of claim 85, the vector of claim 86, or the cell of claim 87.

90. A method of prime editing comprising providing a target DNA sequence to the system of any one of claims 73-84, wherein the target DNA sequence is contacted with the PEgRNA and the prime editor of the system.

91. A pair of PEgRNAs for prime editing comprising

(i) a first PEgRNA comprising a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template and a primer binding site, wherein the extension arm comprises a nucleic acid moiety attached thereto selected from the group consisting of a toe-loop, hairpin, stem-loop, pseudoknot, aptamer, G-quadraplex, tRNA, riboswitch, or ribozyme; and

(ii) a second PEgRNA comprising a second strand nicking guide RNA, wherein the second strand nicking guide RNA comprises at least one nucleic acid extension arm comprising a DNA synthesis template and a primer binding site.

92. The pair of PEgRNAs of claim 91, wherein the first PEgRNA and the second PEgRNA are each capable of binding to a nucleic acid programmable DNA binding protein (napDNAbp) of a prime editor and directing the napDNAbp to a target DNA sequence.

93. A prime editing system for site specific genome modification comprising (a) a pair of PEgRNAs of claim 91 or 92, and (b) at least one prime editor comprising a napDNAbp and a DNA polymerase.

94. The system of claim 93, wherein the system comprises a first prime editor and second prime editor, each comprising a napDNAbp and a DNA polymerase.

95. The system of claim 94, wherein the napDNAbp of the first prime editor binds to the first PEgRNA of the pair of PEgRNAs, and wherein the napDNAbp of the second prime editor binds to the second PEgRNA of the pair of PEgRNAs.

96. The system of any one of claims 93-95, wherein the napDNAbp has a nickase activity.

97. The system of any one of claims 93-95, wherein the napDNAbp is a Cas9 protein or a variant thereof.

98. The system of any one of claims 93-95, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

99. The system of any one of claims 93-95, wherein the napDNAbp is a Cas9 nickase (nCas9).

100. The system of any one of claims 93-95, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute, and optionally has a nickase activity.

101. A polynucleotide comprising the PEgRNA of claim 91 or 92.

102. A vector comprising the polynucleotide of claim 101.

103. A cell comprising the PEgRNA of claim 91 or 92, the system of any one of claims 93-100, the polynucleotide of claim 101, or the vector of claim 102.

104. A pharmaceutical composition comprising (i) the PEgRNA of claim 91 or 92, the system of any one of claims 93-100, the polynucleotide of claim 101, or the vector of claim 102, and (ii) a pharmaceutically acceptable excipient.

105. A kit comprising the PEgRNA of claim 91 or 92, the system of any one of claims 93-100, the polynucleotide of claim 101, the vector of claim 102, or the cell of claim 103.

106. A method of prime editing comprising providing a target DNA sequence to the system of any one of claims 93-100, wherein the target DNA sequence is contacted with the pair of PEgRNAs and the one or more prime editors of the system.

107. A PEgRNA comprising (i) a guide RNA comprising a spacer and (ii) at least one nucleic acid extension arm comprising a DNA synthesis template and a primer binding site, wherein the primer binding site comprises one or more modified nucleotideswhich result in a greater reduction in binding affinity of the primer binding site to the spacer than of the primer binding site to a protospacer sequence on a target DNA molecule.

108. The PEgRNA of claim 107, wherein the one or more modified nucleotides comprise genetic mutations.

109. The PEgRNA of claim 107, wherein the one or more modified nucleotides comprise chemically-modified nucleotides.

110. A prime editing system for site specific genome modification comprising (a) a pair of PEgRNAs of any one of claims 107-109, and (b) at least one prime editor comprising a napDNAbp and a DNA polymerase.

111. The system of claim 110, wherein the system comprises a first prime editor and second prime editor, each comprising a napDNAbp and a DNA polymerase.

112. The system of claim 111, wherein the napDNAbp of the first prime editor binds to the first PEgRNA of the pair of PEgRNAs, and wherein the napDNAbp of the second prime editor binds to the second PEgRNA of the pair of PEgRNAs.

113. The system of any one of claims 110-112, wherein the napDNAbp has a nickase activity.

114. The system of any one of claims 110-112, wherein the napDNAbp is a Cas9 protein or a variant thereof.

115. The system of any one of claims 110-112, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

116. The system of any one of claims 110-112, wherein the napDNAbp is a Cas9 nickase (nCas9).

117. The system of any one of claims 110-112, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute, and optionally has a nickase activity.

118. A polynucleotide comprising the PEgRNA of any one of claims 107-109.

119. A vector comprising the polynucleotide of claim 118.

120. A cell comprising the PEgRNA of any one of claims 107-109, the system of any one of claims 110-117, the polynucleotide of claim 118, or the vector of claim 119.

121. A pharmaceutical composition comprising (i) the PEgRNA of any one of claims 107-109, the system of any one of claims 110-117, the polynucleotide of claim 118, or the vector of claim 119, and (ii) a pharmaceutically acceptable excipient.

122. A kit comprising the PEgRNA of any one of claims 107-109, the system of any one of claims 110-117, the polynucleotide of claim 118, the vector of claim 119, or the cell of claim 120.

123. A method of prime editing comprising providing a target DNA sequence to the system of any one of claims 110-117, wherein the target DNA sequence is contacted with the pair of PEgRNAs and the one or more prime editors of the system.

124. A method of correcting one or more mutations in a *CDKL5* gene by prime editing using a single pegRNA comprising contacting a target DNA sequence with a prime editor comprising (i) a napDNAbp and (ii) a domain having an RNA-dependent DNA polymerase activity, and a pegRNA, wherein the pegRNA targets the prime editor to a *CDKL5* gene comprising one or more mutations.

125. The method of claim 124, wherein the pegRNA is provided in FIG. 146.

126. The method of claim 124, wherein the pegRNA is provided in FIG. 148.

127. The method of claim 124, wherein the mutation in the *CDKL5* gene comprises a *1412delA* mutation.

128. The method of claim 124, wherein the one or more mutations encodes a V172I, A173D, R175S, W176G, W176R, Y177C, R178P, P180L, E181A, or L182P substitution.

129. The method of claim 124, wherein the napDNAbp has a nickase activity.

130. The method of claim 124, wherein the napDNAbp is a Cas9 protein or variant thereof.

131. The method of claim 124, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

132. The method of claim 124, wherein the napDNAbp is Cas9 nickase (nCas9).

133. The method of claim 124, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute, and optionally has a nickase activity.

134. The method of claim 124, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase.

135. A method of treating a plurality of subjects having CDKL5 deficiency disorder caused by different mutations in the *CDKL5* gene comprising contacting a target DNA sequence with a prime editor comprising (i) a napDNAbp and (ii) a domain having an RNA-dependent DNA polymerase activity, and a singular pegRNA, wherein the singular pegRNA is capable

of targeting the prime editor to the *CDKL5* gene in any of the plurality of subjects to result in a repaired *CDKL5* gene in a mutation-agnostic manner.

136. The method of claim 135, wherein the pegRNA is provided in FIG. 148.

137. The method of claim 135, wherein the pegRNA is provided in FIG. 150.

138. The method of claim 135, wherein the mutation in the *CDKL5* gene comprises a *1412delA* mutation.

139. The method of claim 135, wherein the one or more mutations encodes a V172I, A173D, R175S, W176G, W176R, Y177C, R178P, P180L, E181A, or L182P substitution.

140. The method of claim 135, wherein the napDNAbp has a nickase activity.

141. The method of claim 135, wherein the napDNAbp is a Cas9 protein or variant thereof.

142. The method of claim 135, wherein the napDNAbp is a nuclease active Cas9, a nuclease inactive Cas9 (dCas9), or a Cas9 nickase (nCas9).

143. The method of claim 135, wherein the napDNAbp is Cas9 nickase (nCas9).

144. The method of claim 135, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12d, Cas12e, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute, and optionally has a nickase activity.

145. The method of claim 135, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase.

CLAIMS

1. A pegRNA for prime editing comprising:

a guide RNA and at least one nucleic acid extension arm comprising a DNA synthesis template and a primer binding site,

wherein the extension arm comprises a nucleic acid moiety attached thereto comprising a prequeosin1-1 riboswitch aptamer (evopreQ1-1) or a variant thereof.

2. The pegRNA of claim 1, wherein the nucleic acid moiety is attached to the 3′ end of the extension arm.

3. The pegRNA of claim 1 or 2, wherein the evopreQ1-1 has a nucleotide sequence selected from the group consisting of: SEQ ID NO: 215 (evopreq1), SEQ ID NO: 216 (evopreq1motif1), SEQ ID NO: 217 (evopreq1motif2), SEQ ID NO: 218 (evopreq1motif3), SEQ ID NO: 219 (shorter preq1-1), SEQ ID NO: 220 (preq1-1 G5C (mut1)), SEQ ID NO: 221 (preq1-1 G15C (mut2)), and nucleotide sequences having at least 80% sequence identity therewith.

4. The pegRNA of any one of claims 1-3, wherein the evopreQ1 comprises a nucleotide sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 219.

5. The pegRNA of any one of claims 1-3, wherein the evopreQ1 comprises SEQ ID NO: 219.

6. The pegRNA of any of claims 1-5, wherein the nucleic acid moiety is attached to the extension arm by a linker.

7. The pegRNA of claim 6, wherein the linker lacks base pairing with the primer binding site, the DNA synthesis template, or the guide RNA of a pegRNA as predicted by RNA-to-RNA interaction probability with ViennaRNA.

8. The pegRNA of claim 7, wherein the probability of lacking base pairing with the primer binding site, the DNA synthesis template, or the gRNA is at least 80% or at least 90%.

9. The pegRNA of any one of claims 6-8, wherein the linker lacks four consecutive uridines.

10. The pegRNA of claim 6, wherein the linker has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 225-236.

11. The pegRNA of any one of claims 6-10, wherein the linker is 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 in length, wherein the linker is no longer than 50 nucleotides in length.

12. The pegRNA of any one of claims 6-11, wherein the linker consists of 4 nucleotides, 8 nucleotides, 15 nucleotides, 4-32 nucleotides, or 8-16 nucleotides.

13. The pegRNA of any one of claims 6-12, wherein the linker is 8 nucleotides in length.

14. The pegRNA of any one of claims 1-13, wherein the extension arm is positioned at the 3′ end of the guide RNA.

15. The pegRNA of any one of claims 1-14, wherein the nucleic acid extension arm is RNA.

16. The pegRNA of any one of claims 1-15, wherein the guide RNA comprises a gRNA core capable of interacting with a nucleic acid programmable DNA binding protein (napDNAbp)

selected from the group consisting of Cas9, Cas12a (Cpf1), Cas12e (CasX), Cas12d (CasY), Cas12b1 (C2c1), Cas13a (C2c2), Cas12c (C2c3), GeoCas9, CjCas9, Cas12g, Cas12h, and Cas12i proteins.

17. A system for prime editing comprising:

(a) a nucleic acid programmable DNA binding protein (napDNAbp) and a domain comprising an RNA-dependent DNA polymerase activity; and

(b) a pegRNA of any one of claims 1-16.

18. The system of claim 17, wherein the napDNAbp has a nickase activity.

19. The system of claim 17 or 18, wherein the napDNAbp is a Cas9 protein or a variant thereof.

20. The system of claim 17 or 18, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas12b2, Cas13a, Cas12c, Cas12h, Cas12i, Cas12g, Cas12f (Cas14), Cas12f1, Cas12j (CasΦ), and Argonaute.

21. The system of any one of claims 17-20, wherein the napDNAbp has a nickase activity.

22. The system of any one of claims 17-21, wherein the domain comprising an RNA-dependent DNA polymerase activity is a reverse transcriptase comprising any one of the amino acid sequences of SEQ ID NOs: 32, 34, 36, 102-128, and 132, or an amino acid sequence having at least 80%, 85%, 90%, 95%, 98%, or 99% sequence identity with the amino acid sequence of any one of SEQ ID NOs: 32, 34, 36, 102-128, and 132.

23. A nucleic acid molecule encoding the pegRNA of any one of claims 1-16.

24. An expression vector comprising the nucleic acid molecule of claim 23, wherein the nucleic acid molecule is under the control of a promoter.

25. The expression vector of claim 24, wherein the promoter is a polIII promoter, a U6 promoter, aU6v4 promoter, a U6v7 promoter, a U6v9 promoter, or a fragment thereof.

26. A cell comprising the pegRNA of any one of claims 1-16, the system of any one of claims 17-22, the nucleic acid molecule of claim 23, or the expression vector of claim 24 or 25.

27. The pegRNA of any one of claims 1-16, the system of any one of claims 17-22, the nucleic acid molecule of claim 23, the expression vector of claim 24 or 25, or the cell of claim 26, for use as a medicament.