

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 (peq1-1 G5C (mut1)), SEQ ID NO: 221 (peq1-1 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.