

CLAIMS:

1. A prime editing guide RNA (PEgRNA) comprising:
 - (a) a spacer that comprises a region of complementarity to a search target sequence in target strand of a double stranded target DNA;
 - (b) an extension arm comprising:
 - (i) an editing template that comprises an intended edit compared to the double stranded target DNA, and
 - (ii) a primer binding site (PBS) that comprises a region of complementarity to a region upstream of a nick site in a non-target strand of the double stranded target DNA; and
 - (c) a guide RNA (gRNA) core comprising at least 80% identity to SEQ REF NO: 16 and containing one or more modifications relative to SEQ REF NO: 16, the one or more modifications comprising:
 - i. a first insertion between nucleotides 12 and 13 and a second insertion between nucleotides 16 and 17, wherein the first insertion is the reverse complement of the second insertion;
 - ii. a first insertion between nucleotides 52 and 53 and a second insertion between nucleotides 56 and 57, wherein the first insertion is the reverse complement of the second insertion;
 - iii. complementary substitutions of nucleotides 2 and 29, 3 and 28, 4 and 27, 51 and 58, or combinations thereof;
 - iv. replacement of nucleotides 11-12 with a replacement sequence 1 and replacement of nucleotides 17-18 with a replacement sequence 2, wherein the replacement sequences 1 is at least 3 nucleotides in length and wherein the replacement sequence 2 is the reverse compliment of replacement sequence 1;
 - v. a T to G or T to C substitution at nucleotide 5 and a complementary substitution at nucleotide 26; or
 - vi. any combination thereof.

2. The PEGRNA of claim 1, wherein the gRNA core comprises the first insertion between nucleotides 12 and 13 and the second insertion between nucleotides 16 and 17, wherein:

(a) the first insertion is 1 to 6 nucleotides in length, wherein the first insertion comprises the sequence UGCUG; or

(b) the first insertion is 1 to 3 nucleotides in length, wherein the first insertion comprises a sequence selected from the group consisting of C, CC, CA, CG, A, AC, AA, AG, CCC, CCAC, CCAAC, and CCACAC, or

(c) wherein the second insertion comprises the sequence CAGCA.

3. The PEGRNA of claim 1, wherein the gRNA core comprises the first insertion between nucleotides 52 and 53 and the second insertion between nucleotides 56 and 57, wherein the first insertion is 1-8 nucleotides in length; or

wherein the gRNA core comprises the complementary substitutions of nucleotides 2 and 29, 3 and 28, 4 and 27, 11 and 18, 12 and 17, 51 and 58, or combinations thereof, wherein

- i) the gRNA core comprises a U to A substitution at nucleotide 2,
- ii) the gRNA core comprises a U to A substitution at nucleotide 3, or
- iii) the gRNA core comprises a U to A substitution at nucleotide 4.

4. The PEGRNA of claim 3, wherein the gRNA core comprises:

(a) a U to G substitution at nucleotide 51 and optionally an A to C substitution at nucleotide 58; or

(b) the replacement of nucleotides 11-12 with the replacement sequence 1 and the replacement of nucleotides 17-18 with the replacement sequence 2, wherein the replacement sequence 1 is 3 to 5 nucleotides in length and wherein the replacement sequence 1 comprises a sequence selected from the group consisting of CAGC, CCGC, GGAC, UGC, UCC, GAGGC, AGC, GGC, CGCA, GCACA, GGUC, and GGG.

5. The PEGRNA of any one of claims 1-4, wherein the gRNA core comprises:

- (i) a U to A substitution at nucleotide 5 and an A to U substitution at nucleotide 26;
- (ii) complementary substitutions at nucleotides 52 and 57; wherein the complementary substitutions at nucleotides 52 and 57 are a U to G substitution at nucleotide 52 and an A to C

substitution at nucleotide 57, or a U to C substitution at nucleotide 52 and an A to G substitution at nucleotide 57;

(iii) complementary substitutions at nucleotides 49 and 60, optionally wherein the complementary substitutions at 49 and 60 are an A to G substitution at nucleotide 49 and a U to C substitution at nucleotide 60; or

(iv) nucleotides 62-76 of SEQ REF NO: 16.

6. A prime editing guide RNA (PEgRNA) comprising:

(a) a spacer that comprises a region of complementarity to a search target sequence in target strand of a double stranded target DNA;

(b) an extension arm comprising:

(i) an editing template that comprises an intended edit compared to the double stranded target DNA, and

(ii) a primer binding site (PBS) that comprises a region of complementarity to a region upstream of a nick site in a non-target strand of the double stranded target DNA; and

(c) a guide RNA (gRNA) core comprising a sequence selected from the group consisting of SEQ REF NO:s 17-61, 3860-4253, 4255-4349, 4351-4359, and 4452.

7. The PEgRNA of claim 6, wherein the gRNA core comprises:

(i) a sequence selected from the group consisting of SEQ REF NOs: 4352, 3860, 3862, 3865, 3908, 3915, 3982, 3991, 4035, 4261, 4262, 4263, 4264, 4265, 4266, 4268, 4277, 4278, 4280, 4283, 4284, 4285, 4286, 4269, 4287, 4288, 4289, 4290, 4291, 4270, 4271, 4272, 4274, 4275, 4276, 4292, 4301, 4302, 4304, 4305, 4306, 4309, 4293, 4311, 4312, 4313, 4315, 4316, 4317, 4319, 4320, 4294, 4321, 4322, 4323, 4295, 4296, 4297, 4299, 4324, 4333, 4334, 4338, 4339, 4341, 4342, 4343, 4345, 4346, 4348, 4349, 4328, 4329, 4330, and 4332,

(ii) a sequence selected from the group consisting of SEQ REF NOs: 4294, 4319, 4322, 4286, 4290, 4346, 4271, 4264, 4317, 4330, 4312, 4356, 4280, and 4452, or

(iii) SEQ REF NO: 4354.

8. A prime editing system comprising:

(a) the PEgRNA of any one of claims 1-7 or one or more polynucleotides encoding the PEgRNA, wherein the one or more polynucleotides are mRNA; and

(b) a prime editor comprising a Cas protein and a DNA polymerase or one or more polynucleotides encoding the prime editor, wherein the Cas protein:

(i) has nickase activity;

(ii) is a Cas9 comprising a mutation in an HNH domain, wherein the Cas9 comprises at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to compared to SEQ REF NO: 4442; or

(iv) is a Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, Cas14h, Cas14u, or Casφ.

9. The prime editing system of claim 8, wherein the DNA polymerase is a reverse transcriptase, wherein the reverse transcriptase is a retrovirus reverse transcriptase, optionally wherein the retrovirus reverse transcriptase comprises at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ REF NO: 4444.

10. The prime editing system of any one of claims 8-9, wherein the Cas protein and the DNA polymerase are fused or linked in a fusion protein, wherein the fusion protein comprises the sequence of SEQ REF NO: 4440.

11. The prime editing system of any one of claims 8-10, wherein the one or more polynucleotides comprise (a) a first sequence encoding an N-terminal portion of the Cas protein and an intein-N and (b) a second sequence encoding an intein-C, a C-terminal portion of the Cas protein and the DNA polymerase.

12. The prime editing system of any one of claims 8-11, comprising one or more vectors that comprise the one or more polynucleotide encoding the PEgRNA and the one or more polynucleotides encoding the prime editor, wherein the one or more vectors are AAV vectors.

13. A lipid nanoparticle (LNP) or ribonucleoprotein (RNP) comprising the prime editing system of any one of claims 8-11.

14. An *in vitro* or *ex vivo* method for editing a double stranded target DNA, the method comprising contacting the target DNA with (a) the PEGRNA of any one of claims 1-7 and a prime editor comprising a Cas9 nickase and a reverse transcriptase, (b) the prime editing system of any one of claims 8-12, or (c) the LNP or RNP of claim 13, wherein the double stranded target DNA is in a cell .