CLAIMS

WHAT IS CLAIMED IS:

1. A prime editing system comprising:

(A) a first prime editing guide RNA (PEgRNA) or one or more polynucleotides encoding the first PEgRNA, wherein the first PEgRNA comprises from 5' to 3' in a contiguous sequence in a single molecule:

(i) a first spacer that is complementary to a first search target sequence on a first strand of a TRAC gene,

(ii) a first gRNA core capable of binding to a Cas9 protein, and

(iii) a first extension arm comprising

(a) a first editing template encoding a recombinase recognition site (RRS) or its reverse complement, and

(b) a first primer binding site (PBS),

wherein the first spacer is SEQ ID NO: 61 and the first PBS is SEQ ID NO: 71, or wherein the first spacer is SEQ ID NO: 4 and the first PBS is SEQ ID NO: 15; and

(B) a second PEgRNA or one or more polynucleotides encoding the second PEgRNA, wherein the second PEgRNA comprises, from 5' to 3' in a contiguous sequence in a single molecule:

(i) a second spacer that is complementary to a second search target sequence on a second strand of the TRAC gene complementary to the first strand,

(ii) a second gRNA core capable of binding to the Cas9 protein, and

(iii) a second extension arm comprising

(a) a second editing template that is completely complementary to the first editing template, and

(b) a second PBS,

wherein the second spacer is SEQ ID NO: 177 and the second PBS is SEQ ID NO: 186, or wherein the second spacer is SEQ ID NO: 522 and the second PBS is SEQ ID NO: 531.

2. The prime editing system of claim 1, wherein the first spacer is SEQ ID NO: 61 and the first PBS is SEQ ID NO: 71.

3. The prime editing system of claim 1 or 2, wherein the second spacer is SEQ ID NO: 177 and the second PBS is SEQ ID NO: 186.

4. The prime editing system of claim 1, wherein or the first spacer is SEQ ID NO: 4 and the first PBS is SEQ ID NO: 15.

5. The prime editing system of claim 1 or 4, wherein the second spacer is SEQ ID NO: 522 and the second PBS is SEQ ID NO: 531.

6. The prime editing system of any one of claims 1-5, wherein the first gRNA core and the second gRNA core comprise the same sequence.

7. The prime editing system of claim 6, wherein the first gRNA core, the second gRNA core, or both comprise SEQ ID NO: 590.

8. The prime editing system of any one of claims 1-7, wherein the first spacer, the first gRNA core, the first editing template, and the first PBS form a contiguous sequence in a single molecule.

9. The prime editing system of claim 8, wherein the first PEgRNA comprises from 5' to 3' the first spacer, the first gRNA core, the first editing template, and the first PBS.

10. The prime editing system of any one of claims 1-9, wherein the second spacer, the second gRNA core, the second editing template, and the second PBS form a contiguous sequence in a single molecule.

11. The prime editing system of claim 10, wherein the second pegRNA comprises from 5' to 3' the second spacer, the second gRNA core, the second editing template, and the second PBS.

12. The prime editing system of any one of claims 1-11, wherein the first editing template encodes the RRS.

13. The prime editing system of any one of claims 1-11, wherein the second editing template encodes the RRS.

14. The prime editing system of any one of claims 1-13, wherein the RRS is an attB sequence recognized by a Bxb1 recombinase.

15. The prime editing system of any one of claims 1-13, wherein the RRS is an attP sequence recognized by a Bxb1 recombinase.

16. The prime editing system of any one of claims 1-15, wherein the first PEgRNA and/or the second PEgRNA further comprises a 3' motif.

17. The prime editing system of claim 16, wherein the first PEgRNA and the second PEgRNA comprise the 3' motif that is UUUU.

18. The prime editing system of any one of claims 1-17, wherein the first PEgRNA and/or the second PEgRNA further comprises 5'mN*mN*mN* and 3' mN*mN*mN*N modifications, where m indicates that the nucleotide contains a 2'-O-Me modification and a * indicates the presence of a phosphorothioate bond.

19. The prime editing system of any one of claims 1-18, further comprising a prime editor or one or more polynucleotides encoding the prime editor, wherein the prime editor comprises (a) a Cas9 nickase having a nuclease inactivating mutation in a HNH domain and (b) a reverse transcriptase.

20. The prime editing system of claim 19, wherein the prime editor is a fusion protein.

21. The prime editing system of claim 19 or 20, further comprising a recombinase that recognizes the RRS, or one or more polynucleotides encoding the recombinase.

22. The prime editing system of claim 21, further comprising a DNA polynucleotide that comprises (a) a donor sequence and (b) a second RRS recognized by the recombinase.

23. The prime editing system of claim 22, wherein (i) the RRS comprises a Bxb1 attB sequence provided in Table 5, and the second RRS comprises a corresponding attP sequence provided in Table 5, or (ii) the RRS comprises a Bxb1 attP sequence provided in Table 5, and the second RRS comprises a corresponding attB sequence provided in Table 5.

24. The prime editing system of claim 23, wherein the donor sequence comprises an open reading frame that encodes a polypeptide.

25. The prime editing system of claim 24, wherein the donor sequence encodes a chimeric antigen receptor (CAR).

26. An in vitro or ex vivo method of making a modified cell, the method comprising contacting a cell with the prime editing system of any one of claims 19-25.

27. The method of claim 26, wherein the cell is a mammalian cell.

28. The method of claim 26, wherein the cell is a human cell.

29. The method of any one of claims 26-28, wherein the cell is an immune cell, optionally wherein the cell is a T cell.

30. A cell generated by the method of any one of claims 26-29.

31. Use of a population of modified cells generated according to the method of any one of claims 26-29 in the manufacture of a medicament for treating a disease or disorder in a subject.

32. The use of claim 31, wherein the disease or disorder is a cancer, a microbial infection, an autoimmune disorder, a T cell malignancy, or a graft versus host disorder.