CLAIMS

What is claimed is:

1. An *in vitro* or *ex vivo* method for site-specific modification of a double-stranded target DNA sequence, the method comprising:

contacting the double-stranded target DNA sequence, which comprises a first strand and a second strand, with a prime editing system, wherein the prime editing system comprises:

(i) a fusion protein comprising a nucleic acid programmable DNA binding protein (napDNAbp) and a reverse transcriptase, wherein the napDNAbp is a Cas9 nickase, a Cas12a nickase, or a Cas12b1 nickase, and

(ii) a prime editing guide RNA (PEgRNA) comprising:

(a) a spacer sequence that comprises a region of complementarity to the first strand of the double-stranded target DNA sequence;

(b) an extension arm that comprises a DNA synthesis template and a primer binding site in a 5′ to 3′ orientation, wherein the primer binding site comprises a region of complementarity to a region upstream of a nick site in the second strand of the double-stranded target DNA sequence, and wherein the DNA synthesis template encodes one or more nucleotide changes compared to a region downstream of the nick site in the second strand of the double-stranded target DNA sequence, and

(c) a gRNA core that interacts with the napDNAbp; wherein the contacting occurs in a cell and results in:

nicking the second strand of the double-stranded target DNA sequence to form a free 3′ end at the nick site;

annealing the primer binding site with the region of the second strand of the double-stranded target DNA sequence upstream of the nick site;

synthesizing a single strand of DNA encoded by the DNA synthesis template from the free 3′ end of the second strand of the double-stranded target DNA sequence by the reverse transcriptase; and

replacing the region downstream of the nick site in the second strand of the double- stranded target DNA sequence with the single strand of DNA, thereby modifying the sequence of the double-stranded target DNA sequence.

2. The method of claim 1, wherein the one or more nucleotide changes are 1-150 bases downstream of the nick site in the second strand.

3. The method of claim 1 or 2, wherein the DNA synthesis template comprises a region of complementarity to the second strand of the double-stranded target DNA sequence.

4. The method of any one of claims 1-3, wherein the one or more nucleotide changes comprise a nucleotide substitution as compared to the region of the second strand downstream of the nick site in the double-stranded target DNA sequence.

5. The method of any one of claims 1-4, wherein the one or more nucleotide changes comprise a nucleotide deletion as compared to the region of the second strand downstream of the nick site in the double-stranded target DNA sequence.

6. The method of any one of claims 1-5, wherein the one or more nucleotide changes comprise a nucleotide insertion as compared to the region of the second strand downstream of the nick site in the double-stranded target DNA sequence.

7. The method of any one of claims 1-6, wherein the one or more nucleotide changes comprise two or more nucleotide insertions, nucleotide substitutions, nucleotide deletions, or a combination of two or more of a nucleotide insertion, a nucleotide substitution, or a nucleotide deletion, as compared to the region of the second strand downstream of the nick site in the double-stranded target DNA sequence.

8. The method of any one of claims 1-7, wherein the napDNAbp is a Cas9 nickase (nCas9).

9. The method of claim 8, wherein the Cas9 nickase is a Cas9 nickase (nCas9) comprising a substitution in the HNH domain.

10. The method of claim 9, wherein the substitution in the HNH domain is H840A relative to SEQ ID NO: 18.

11. The method of any one of claims 1-10, wherein the reverse transcriptase is a retrovirus reverse transcriptase.

12. The method of any one of claims 1-11, wherein the reverse transcriptase is a Moloney Murine Leukemia reverse transcriptase (M-MLV RT).

13. The method of any one of claims 1-12 further comprising contacting the double-stranded target DNA sequence with a guide RNA, wherein the guide RNA directs the napDNAbp to generate a second nick in the first strand of the double-stranded target DNA sequence.

14. The method of claim 13, wherein the first nick and the second nick are at least 14 nucleotides apart.

15. The method of any one of claims 1-6 or 8-14, wherein the one or more nucleotide changes comprise a single nucleotide substitution, a single nucleotide insertion, a single nucleotide deletion, or combinations thereof, as compared to the region of the second strand downstream of the nick site in the double-stranded target DNA sequence.

16. The method of any one of claims 1-15, wherein the one or more nucleotide changes are 4-6 bases downstream of the position of the nick site in the second strand in the double-stranded target DNA sequence.

17. The method of any one of claims 1-15, wherein the one or more nucleotide changes are at least 7 base pairs downstream of the position of the nick site in the second strand in the double-stranded target DNA sequence, and wherein the DNA synthesis template is at least 12 bases in length.

18. The method of claim 8, wherein the Cas9 nickase is derived from a *Streptococcus pyogenes* Cas9, a *Staphylococcus aureus* Cas9, a *Neisseria meningitis* Cas9, a *Streptococcus thermophilis* Cas9, or a *Treponema denticola* Cas9.

19. The method of any one of claims 1-18, wherein the DNA synthesis template is from 3 nucleotides to 500 nucleotides in length.

20. The method of any one of claims 1-19, wherein the DNA synthesis template is from 8 nucleotides to 31 nucleotides in length.

21. The method of any one of claims 1-20, wherein the primer binding site is from 3 nucleotides to 500 nucleotides in length.

22. The method of any one of claims 1-21, wherein the primer binding site is from 8 nucleotides to 15 nucleotides in length.

23. The method of any one of claims 1-22, wherein the spacer sequence is about 20 nucleotides in length.