

CLAIMS

What is claimed is:

1. A system for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited, wherein the system comprises a first prime editor complex and a second prime editor complex, wherein each of the first and second prime editor complexes comprises (1) a prime editor comprising (i) a nucleic acid programmable DNA binding protein (napDNAbp), and (ii) a polypeptide having an RNA-dependent DNA polymerase activity; and (2) a pegRNA comprising a spacer sequence, gRNA core, a DNA synthesis template, and a primer binding site, wherein the DNA synthesis template of the pegRNA of the first prime editor complex encodes a first single-stranded DNA sequence and the DNA synthesis template of the pegRNA of the second prime editor complex encodes a second single-stranded DNA sequence, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence each comprises a region of complementarity to the other, and wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence form a duplex comprising an edited portion as compared to the DNA sequence at the target site to be edited, which integrates into the target site to be edited.
2. The system of claim 1, wherein the prime editor of the first prime editor complex, the second prime editor complex, or both the first prime editor complex and the second prime editor complex is a fusion protein comprising the napDNAbp and the polypeptide having an RNA-dependent DNA polymerase activity.
3. The system of claim 1 or 2, wherein the first prime editor complex and the second prime editor complex comprise the same prime editor.
4. The system of claim 1 or 2, wherein the first prime editor complex and the second prime editor complex comprise different prime editors.
5. The system of any one of claims 1-4, wherein the napDNAbp is a Cas9 domain or variant thereof.
6. The system of any one of claims 1-5, wherein the napDNAbp is a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, a Cas9 nickase domain, or variant thereof.
7. The system of any one of claims 1-4, wherein the napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, CasX, CasY, and

Argonaute.

8. The system of any one of claims 1-7, wherein the napDNAbp has a nickase activity.
9. The system of any one of claims 1-4, wherein the napDNAbp comprises an amino acid sequence of any one of SEQ ID NOs: 18-88, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 18-88.
10. The system of any one of claims 1-9, wherein the polypeptide having an RNA-dependent DNA polymerase activity is a reverse transcriptase.
11. The system of any one of claims 1-10, wherein the polypeptide having an RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, and 700-766 or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 89-100, 105-122, 128-129, and 132.
12. The system of any one of claims 1-11, wherein the prime editor further comprises a linker that joins the napDNAbp and the polypeptide having the RNA-dependent DNA polymerase activity.
13. The system of claim 12, wherein the linker comprises an amino acid sequence of any one of SEQ ID NOs: 166-177, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 166-177.
14. The system of claim 12 or 13, wherein the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.
15. The system of any one of claims 1-14, wherein the pegRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244.
16. The system of any one of claims 1-15, wherein the spacer sequence of the pegRNA of the first prime editor complex binds to a first binding site on a first strand of the double-stranded

DNA sequence upstream from the target site to be edited.

17. The system of any one of claims 1-16, wherein the spacer sequence of the pegRNA of the second prime editor complex binds to a second binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.
18. The system of claim 17, whereby the binding of the spacer sequence of the pegRNAs of the first and second prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequences on each strand by the napDNAbps of each of the first and second prime editor complex.
19. The system of claim 18, wherein the first and second binding sites define two ends of a contiguous region of double-stranded DNA comprising the target site to be edited.
20. The system of claim 19, wherein the contiguous region between the first and second binding sites is at least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29 nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34 nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78

nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

21. The system of claim 19, wherein the contiguous region becomes replaced by the duplex comprising the edited portion.
22. The system of any one of claims 1-21, wherein the DNA synthesis template is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89

nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

23. The system of any one of claims 1-22, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have the same lengths.
24. The system of any one of claims 1-22, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have different lengths.
25. The system of any one of claims 1-24, wherein the region of complementarity is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence or the second single-stranded DNA sequence.
26. The system of claim 25, wherein the region of complementarity 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least

70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length, and is either less than or equal to the length of the desired DNA sequence and complement thereof.

27. The system of claim 25, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence form the duplex comprising the edited portion.
28. The system of any one of claims 1-27, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are each single-stranded DNA strands having 3' ends.
29. The system of claim 21, wherein the replacement of the contiguous region by the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.
30. A system for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited, said system comprising:
 - a) a first prime editor complex comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (first napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEGRNA) that binds to a first binding site on a first strand of the double-stranded DNA sequence upstream of the target site to be edited;
 - b) a second prime editor complex comprising:
 - iii. a second prime editor comprising a second nucleic acid programmable DNA binding

protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and

- iv. a second prime editing guide RNA (second PEgRNA) that binds to a second binding site on a second strand of the double-stranded DNA sequence downstream of the target site to be edited;

wherein the first PEgRNA comprises a first DNA synthesis template encoding a first single-stranded DNA sequence and the second PEgRNA comprises a second DNA synthesis template encoding a second single-stranded DNA sequence, and wherein the first strand and the second strand of the double-stranded DNA sequence are complementary to each other.

- 31. The system of claim 30, wherein the prime editor of the first prime editor complex, the second prime editor complex, or both the first prime editor complex and the second prime editor complex is a fusion protein comprising the napDNAbp and the polypeptide having an RNA-dependent DNA polymerase activity.
- 32. The system of claim 30, wherein the first and the second single-stranded DNA sequence each comprise a region of complementarity to the other.
- 33. The system of claim 30, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence form a duplex comprising the edited portion which integrates into the target site to be edited.
- 34. The system of claim 30, wherein the first single-stranded DNA sequence and/or the second single stranded sequence does not comprise sequence homology as compared to the DNA sequence at the target site.
- 35. The system of claim 30, wherein the first PEgRNA further comprises a first spacer sequence, a first gRNA core, and a first primer binding site, and the second PEgRNA further comprises a second spacer sequence, a second gRNA core, and a second primer binding site.
- 36. The system of any one of claims 30-35, wherein the first prime editor complex and the second prime editor complex comprise the same prime editor.
- 37. The system of any one of claims 30-36, wherein the first polypeptide comprising an RNA-dependent DNA polymerase activity and the second polypeptide comprising an RNA-dependent DNA polymerase activity are the same.

38. The system of any one of claims 30-37, wherein the first and/or second napDNAbp is a Cas9 domain or variant thereof.
39. The system of any one of claims 30-38, wherein the first and/or second napDNAbp is a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, a Cas9 nickase domain, or a variant thereof.
40. The system of any one of claims 30-39, wherein the first and/or second napDNAbp is selected from the group consisting of Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, CasX, CasY, and Argonaute.
41. The system of any one of claims 30-40, wherein the napDNAbp has a nickase activity.
42. The system of any one of claims 30-41, wherein the first and/or second napDNAbp comprises an amino acid sequence of any one of SEQ ID NOs: 18-88, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 18-88.
43. The system of any one of claims 30-42, wherein the first and/or second polypeptide having an RNA-dependent DNA polymerase activity is a reverse transcriptase.
44. The system of any one of claims 30-43, wherein the first and/or second polypeptide having an RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, and 700-766 or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 89-100, 105-122, 128-129, and 132.
45. The system of any one of claims 30-44, wherein the first prime editor further comprises a linker that joins the first napDNAbp and the first polypeptide having the RNA-dependent DNA polymerase activity; and/or the second prime editor further comprises a linker that joins the second napDNAbp and the second polypeptide having the RNA-dependent DNA polymerase activity.
46. The system of claim 45, wherein the linker comprises an amino acid sequence of any one of SEQ ID NOs: 166-177, or an amino acid sequence having at least an 80%, 85%, 90%, 95%,

or 99% sequence identity with any one of SEQ ID NOs: 166-177.

47. The system of claim 45, wherein the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acid in length.
48. The system of any one of claims 30-47, wherein the first and/or second pegRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244.
49. The system of any one of claims 30-48, wherein the first spacer sequence binds to a first binding site on a first strand of the double-stranded DNA sequence upstream from the target site to be edited.
50. The system of claim 35, wherein the second spacer sequence of the second prime editor complex binds to a second binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.
51. The system of claim 35, whereby the binding of the spacer sequence of the first and second prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequences on each strand by the first and/or second napDNAbps of each of the first and second prime editor complexes.
52. The system of claim 30, wherein the first and second binding sites define two ends of a contiguous region of double-stranded DNA comprising the target site to be edited.
53. The system of claim 52, wherein the contiguous region between the first and second binding sites is least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29 nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34

nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

54. The system of claim 52, wherein the contiguous region becomes replaced by the duplex comprising the edited portion.
55. The system of any one of claims 30-54, wherein the first and/or second DNA synthesis template is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

56. The system of any one of claims 30-55, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have the same lengths.
57. The system of any one of claims 30-56, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have different lengths.
58. The system of any one of claims 30-57, wherein the region of complementarity is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence or the second single-stranded DNA sequence.
59. The system of any one of claims 30-58, wherein the region of complementarity 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

60. The system of any one of claims 30-59, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence form a duplex.
61. The system of any one of claims 30-60, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are each single-stranded DNA strands having 3' ends.
62. The system of claim 54, wherein the replacement of the contiguous region by the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.
63. A polynucleotide encoding the system of any of claims 1-62.

64. A polynucleotide encoding the first and second prime editors of any one of claims 1-62.
65. A polynucleotide encoding the first and/or second pegRNA of any one of claims 1-62.
66. A polynucleotide encoding the first and/or second pegRNA of any one of claims 30-62.
67. A polynucleotide encoding the first and second pegRNA of any one of claims 1-29.
68. A polynucleotide encoding the first and second pegRNA of any one of claims 30-62.
69. A vector comprising a polynucleotide of any one of claims 63-68.
70. A cell comprising a polynucleotide of any one of claims 63-68 or a vector of claim 69.
71. A pharmaceutical composition comprising a polynucleotide of any one of claims 63-68, a vector of claim 69, or a cell of claim 70, and a pharmaceutical excipient.
72. A kit comprising a polynucleotide of any of claims 63-68 or a vector of claim 69 and instructions for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited.
73. A method for simultaneously editing a first and a second complementary strands of a double-stranded DNA sequence at a target site, said method comprising contacting the double-stranded DNA sequence with a pair of prime editor complexes, said pair comprising:
- a. a first prime editor complex, comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEGRNA) that binds to a first binding site on the first strand of the genomic DNA sequence upstream of the target site;
 - b. a second prime editor complex, comprising:
 - i. a second prime editor comprising a second nucleic acid programmable DNA binding protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a second prime editing guide RNA (second PEGRNA) that binds to a second binding

site on the second strand of the genomic DNA sequence downstream of the target site;

wherein the first prime editor complex causes a first nick at a sequence complementary to the first binding site and the subsequent polymerization of a first single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the first nick;

wherein the second prime editor complex causes a second nick at a sequence complementary to the second binding site and the subsequent polymerization of a second single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the second nick;

wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are reverse complements over at least a region of complementarity and form a duplex comprising an edit; and

wherein the the duplex replaces the nicked first and second complementary strands of the double-stranded DNA sequence.

74. The method of claim 73, wherein the prime editor of the first prime editor complex, the second prime editor complex, or both the first prime editor complex and the second prime editor complex is a fusion protein comprising the napDNAbp and the polypeptide having an RNA-dependent DNA polymerase activity
75. The method of claim 73, wherein the first prime editor complex and the second prime editor complex comprise the same prime editor.
76. The method of claim 73, wherein the first prime editor complex and the second prime editor complex comprise a different prime editor.
77. The method of any one of claims 73-76, wherein the first and/or second napDNAbp is a Cas9 domain or variant thereof.
78. The method of any one of claims 73-77, wherein the first and/or second napDNAbp is a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, a Cas9 nickase domain, or variant thereof.
79. The method of any one of claims 73-78, wherein the first and/or second napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, CasX, CasY, and Argonaute.

80. The method of any one of claims 73-79, wherein the first and/or second napDNABp has a nickase activity.
81. The method of any one of claims 73-80, wherein the first and/or second napDNABp comprises an amino acid sequence of any one of SEQ ID NOs: 18-88, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 18-88.
82. The method of any one of claims 73-81, wherein the first and/or second polypeptide having an RNA-dependent DNA polymerase activity is a reverse transcriptase.
83. The method of any one of claims 73-82, wherein the first and/or second polypeptide having an RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, and 700-766 or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 89-100, 105-122, 128-129, and 132.
84. The method of any one of claims 73-83, wherein the first prime editor further comprises a linker that joins the first napDNABp and the first polypeptide having the RNA-dependent DNA polymerase activity, and/or the second prime editor further comprises a linker that joins the second napDNABp and the second polypeptide having the RNA-dependent DNA polymerase activity.
85. The method of claim 84, wherein the linker comprises an amino acid sequence of any one of SEQ ID NOs: 166-177, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 166-177.
86. The method of claim 84 or 85, wherein the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.
87. The method of any one of claims 73-86, wherein the first and/or second pegRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244.

88. The method of any one of claims 73-87, wherein the first and/or second PEGRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 325-330, 499-505, 101-104, 181-183, and 223-244.
89. The method of any one of claims 73-88, wherein the first spacer sequence of the first prime editor complex binds to a first binding site on a first strand of the double-stranded DNA sequence upstream from the target site to be edited.
90. The method of any one of claims 73-89, wherein the second spacer sequence of the second prime editor complex binds to a second binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.
91. The method of claim 90, wherein the binding of the first and second spacer sequences of the first and second prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequence on each strand by the napDNABps of each first and second prime editor complex.
92. The method of claim 91, wherein the first and second binding sites define two ends of a contiguous region of double-stranded DNA comprising the target site to be edited.
93. The method of claim 92, wherein the contiguous region between the first and second binding sites is least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29 nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34 nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54

nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

94. The method of claim 92 or 93, wherein the contiguous region becomes replaced by the duplex comprising the edited portion.
95. The method of any one of claims 73-94, wherein the first and/or second DNA synthesis template is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68

nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

96. The method of any one of claims 73-95, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have the same lengths.
97. The method of any one of claims 73-96, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have different lengths.
98. The method of any one of claims 73-97, wherein the region of complementarity is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence or the second single-stranded DNA sequence.
99. The method of any one of claims 73-98, wherein the region of complementarity 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length, and is either less than or equal to the length of the desired DNA sequence and complement thereof.

100. The method of any one of claims 73-99, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence form the duplex.
101. The method of any one of claims 73-100, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are each single-stranded DNA strands having 3' ends.
102. The method of claim 94, wherein the replacement of the contiguous region by the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.
103. The method of any one of claims 73-102, wherein the duplex comprising an edit comprising one or more recombinase sites.
104. The method of claim 103 further comprising providing a recombinase to promote recombinase-mediated editing of the DNA sequence at the target site to be edited.
105. A pair of pegRNAs for use in dual prime editing, wherein the pair comprises:

- a. a first prime editing guide RNA (first PEgRNA) that binds to a first binding site on a first strand of a double-stranded DNA sequence upstream of a target site to be edited, wherein the first PEgRNA comprises a first spacer sequence, a first gRNA core, a first DNA synthesis template, and a first primer binding site, wherein the first DNA synthesis template encodes a first single-stranded DNA sequence comprising an edited portion;
- b. a second prime editing guide RNA (second PEgRNA) that binds to a second binding site on a second strand of the double-stranded DNA sequence downstream of the target site to be edited, wherein the second PEgRNA comprises a second spacer sequence, a second gRNA core, a second DNA synthesis template, and a second primer binding site, wherein the second DNA synthesis template encodes a second single-stranded DNA sequence comprising the edited portion, wherein the second single-stranded DNA sequence comprises a region of complementarity to the first single-stranded DNA sequence.
106. The pair of pegRNAs of claim 105, wherein the first PEgRNA comprises the structure:
5'-[first gRNA spacer sequence]-[first gRNA core]-[first DNA synthesis template]-[first primer binding site]-3'; and
wherein the second PEgRNA comprises the structure:
5'-[second gRNA spacer sequence]-[second gRNA core]-[second DNA synthesis template]-[second primer binding site]-3'.
107. The pair of pegRNAs of claim 105 or 106, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence form a duplex that integrates into the double-stranded DNA sequence at the target site to be edited.
108. The pair of pegRNAs of claim 107, wherein the integration of the duplex results in an insertion, deletion, or replacement of DNA at the target site.
109. The pair of pegRNAs of claim 108, wherein the insertion is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

110. The pair of pegRNAs of claim 108, wherein the deletion is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66

nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides, or more in length.

111. The pair of pegRNAs of claim 108, wherein the replacement is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94

- nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides, or more in length.
112. A polynucleotide encoding a pair of pegRNAs of any one of claims 105-111.
113. A vector comprising the polynucleotide of claim 112 for expressing the pair of pegRNAs in a cell.
114. A cell comprising the vector of claim 113.
115. A pharmaceutical composition comprising a pair of pegRNAs of any one of claims 105-111, a vector of claim 113, or a cell of claim 114, and a pharmaceutical excipient.
116. A pair of prime editor complexes, said pair comprises:
- a. a first prime editor complex, comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEgRNA) that binds to a first target sequence on the strand of the genomic DNA sequence upstream of the target site;
 - b. a second prime editor complex, comprising:
 - i. a second prime editor comprising a second nucleic acid programmable DNA binding protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a second prime editing guide RNA (second PEgRNA) that binds to a second target sequence on the second strand of the genomic DNA sequence downstream of the target site.
117. The pair of prime editor complexes of claim 116, wherein the prime editor of the first prime editor complex, the second prime editor complex, or both the first prime editor complex and the second prime editor complex is a fusion protein comprising the napDNAbp and the polypeptide having an RNA-dependent DNA polymerase activity.
118. A polynucleotide encoding a pair of prime editor complexes of claim 116 or 117.

119. A vector comprising the polynucleotide of claim 118 for expressing the pair of prime editor complexes in a cell.
120. A cell comprising the vector of claim 119.
121. A pharmaceutical composition comprising a pair of prime editor complexes of claim 116 or 117, a vector of claim 119, or a cell of claim 120, and a pharmaceutical excipient.
122. The system of any one of claims 1-62, or the method of any one of claims 73-104, wherein integration of the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.
123. The system or method of claim 122, wherein the insertion is a peptide tag.
124. The system or method of claim 123, wherein the peptide tag is a poly-histidine (e.g., HHHHHH) (SEQ ID NO: 252-262), FLAG (e.g., DYKDDDDK) (SEQ ID NO: 2), V5 (e.g., GKPIPNLLGLDST) (SEQ ID NO: 3), GCN4, HA (e.g., YPYDVPDYA) (SEQ ID NO: 5), Myc (e.g. EQKLISEED) (SEQ ID NO: 6), or GST.
125. The system or method of claim 123, wherein the peptide tag has an amino acid sequence selected from the group consisting of SEQ ID NO: 1-6, 245-249, 252-262, 264-273, 275-276, 281, 278-288, and 622.
126. The system or method of claim 122, wherein the insertion is an immunoepitope tag.
127. The system or method of claim 126, wherein the immunoepitope tag is selected from the group consisting of: tetanus toxoid (SEQ ID NO: 396); diphtheria toxin mutant CRM197 (SEQ ID NO: 630); mumps immunoepitope 1 (SEQ ID NO: 400); mumps immunoepitope 2 (SEQ ID NO: 402); mumps immunoepitope 3 (SEQ ID NO: 404); rubella virus (SEQ ID NO: 406); hemagglutinin (SEQ ID NO: 408); neuraminidase (SEQ ID NO: 410); TAP1 (SEQ ID NO: 412); TAP2 (SEQ ID NO: 414); hemagglutinin epitopes toward class I HLA (SEQ ID NO: 416); neuraminidase epitopes toward class I HLA (SEQ ID NO: 418); hemagglutinin epitopes toward class II HLA (SEQ ID NO: 420); neuraminidase epitopes toward class II HLA (SEQ ID NO: 422); hemagglutinin epitope H5N1-bound class I and class II HLA (SEQ ID NO: 424); and neuraminidase epitope H5N1-bound class I and class II HLA (SEQ ID NO: 426).

128. The system or method of claim 122, wherein the insertion is a dimerization domain.
129. The system or method of claim 128, wherein the dimerization domain is small molecule binding domain of FKBP12 of SEQ ID NO: 488, FKBP12-F36V of SEQ ID NO: 489, or cyclophilin of SEQ ID NOs: 490 or 493-494.
130. A system for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited, the system comprising:
- a) a first prime editor complex comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (first napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEGRNA) that binds to a first binding site on a first strand of the double-stranded DNA sequence upstream of the target site to be edited;
 - b) a second prime editor complex comprising:
 - i. a second prime editor comprising a second nucleic acid programmable DNA binding protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a second prime editing guide RNA (second PEGRNA) that binds to a second binding site on a second strand of the double-stranded DNA sequence upstream of the target site to be edited;
 - c) a third prime editor complex comprising:
 - i. a third prime editor comprising a third nucleic acid programmable DNA binding protein (third napDNAbp) and a third polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a third prime editing guide RNA (third PEGRNA) that binds to a third binding site on the first strand of the double-stranded DNA sequence downstream of the target site to be edited;
 - d) a fourth prime editor complex comprising:
 - i. a fourth prime editor comprising a fourth nucleic acid programmable DNA binding protein (fourth napDNAbp) and a fourth polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a fourth prime editing guide RNA (fourth PEGRNA) that binds to a fourth binding site on the second strand of the double-stranded DNA sequence downstream of the target site to be edited;
- wherein the first PEGRNA comprises a first DNA synthesis template encoding a first

single-stranded DNA sequence, the second PEGRNA comprises a second DNA synthesis template encoding a second single-stranded DNA sequence, the third PEGRNA comprises a third DNA synthesis template encoding a third single-stranded DNA sequence, and the fourth PEGRNA comprises a fourth DNA synthesis template encoding a fourth single-stranded DNA sequence;

wherein the first and the third single-stranded DNA sequence each comprise a region of complementarity to the other; and wherein, wherein the second and the fourth single-stranded DNA sequence each comprise a region of complementarity to the other.

131. A system for editing one or more double-stranded DNA sequences, the system comprising:

- a) a first prime editor complex comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (first napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEGRNA) that binds to a first binding site on a first strand of a first double-stranded DNA sequence at a first target site to be edited;
- b) a second prime editor complex comprising:
 - i. a second prime editor comprising a second nucleic acid programmable DNA binding protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a second prime editing guide RNA (second PEGRNA) that binds to a second binding site on a second strand of the first double-stranded DNA sequence at the first target site to be edited;
- c) a third prime editor complex comprising:
 - i. a third prime editor comprising a third nucleic acid programmable DNA binding protein (third napDNAbp) and a third polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a third prime editing guide RNA (third PEGRNA) that binds to a first binding site on a first strand of a second double-stranded DNA sequence at a second target site to be edited;
- d) a fourth prime editor complex comprising:
 - i. a fourth prime editor comprising a fourth nucleic acid programmable DNA binding protein (fourth napDNAbp) and a fourth polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a fourth prime editing guide RNA (fourth PEGRNA) that binds to a second binding site on a second strand of the second double-stranded DNA sequence at

the second target site to be edited;

wherein the first PEGRNA comprises a first DNA synthesis template encoding a first single-stranded DNA sequence, the second PEGRNA comprises a second DNA synthesis template encoding a second single-stranded DNA sequence, the third PEGRNA comprises a third DNA synthesis template encoding a third single-stranded DNA sequence, and the fourth PEGRNA comprises a fourth DNA synthesis template encoding a fourth single-stranded DNA sequence;

wherein the first and the third single-stranded DNA sequence each comprise a region of complementarity to the other; and wherein, wherein the second and the fourth single-stranded DNA sequence each comprise a region of complementarity to the other.

132. The system of claim 131, wherein the prime editor of the first prime editor complex, the second prime editor complex, the third prime editor complex, an/or the fourth prime editor complex is a fusion protein comprising the napDNAAbp and the polypeptide having an RNA-dependent DNA polymerase activity

133. The system of any one of claims 130-132, wherein

(i) the first PEGRNA further comprises a first spacer sequence, first gRNA core, and a first primer binding site;

(ii) the second PEGRNA further comprises a second spacer sequence, a second gRNA core, and a second primer binding site;

(iii) the third PEGRNA further comprises a third spacer sequence, a third gRNA core, and a third primer binding site; and

(iv) the fourth PEGRNA comprises a fourth spacer sequence, a fourth gRNA core, a fourth DNA synthesis template, and a fourth primer binding site.

134. The system of claim 131, wherein the region of complementarity of the first and third single-stranded DNA sequence, and/or the region of complementarity of the second and fourth single-stranded DNA sequence, form a duplex.

135. The system of claim 134, wherein the first spacer sequence binds to the first binding site of the first double stranded DNA sequence upstream of the first target site to be edited and the second spacer sequence binds to the second binding site on the first double stranded DNA sequence downstream of the first target site to be edited.

136. The system of claim 134 or 135, wherein the third spacer sequence binds to the first binding site of the second double stranded DNA sequence upstream of the second target site to be edited and

the forth spacer sequence binds to the second binding site on the second double stranded DNA sequence downstream of the second target site to be edited.

137. The system of any one of claims 134-136, wherein the first and second binding sites define two ends of a first contiguous region of the first double-stranded DNA.

138. The system of any one of claims 134-137, wherein the third and fourth binding sites define two ends of a second contiguous region of the second double-stranded DNA.

139. The system of claim 134, wherein the contiguous region between the first and second binding sites or the third and fourth binding sites is least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29 nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34 nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110

nucleotides in length.

140. The system of any one of claims 134-139, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the region of complementarity of the second single-stranded DNA sequence and the fourth single-stranded DNA sequence, form a duplex.

141. The system of claim 140, wherein the duplex is incorporated in the first double stranded DNA and/or the second double stranded DNA.

142. The system of any one of claims 134-141, wherein the system results in replacement of the first contiguous region by the second contiguous region into the first double-stranded DNA.

143. The system of any one of claims 134-142, wherein the system results in exchange of the first contiguous region and the second contiguous region between the first double-stranded DNA and the second double-stranded DNA.

144. The system of any one of claims 134-143, wherein the first prime editor complex, the second prime editor complex, the third prime editor complex, and the fourth prime editor complex comprise the same prime editor.

145. The system of any one of claims 134-144, wherein the first, second, third, and fourth polypeptide comprising an RNA-dependent DNA polymerase activity are the same.

146. The system of any one of claims 134-145, wherein the first, second, third, and/or fourth napDNAbp is a Cas9 domain or variant thereof.

147. The system of any one of claims 134-146, wherein the first, second, third, and/or fourth napDNAbp is a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, or a Cas9 nickase domain or a variant thereof.

148. The system of any one of claims 134-147, wherein the first, second, third, and/or fourth napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c, and Argonaute.

149. The system of any one of claims 134-148, wherein the first, second, third, and/or fourth napDNAbp has nickase activity.

150. The system of any one of claims 134-149, wherein the first, second, third, and/or fourth napDNAbp comprises an amino acid sequence of any one of SEQ ID NOs: 2-65, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 2-65.

151. The system of any one of claims 134-150, wherein the first, second, third, and/or fourth polypeptide having an RNA-dependent DNA polymerase activity is a reverse transcriptase.

152. The system of any one of claims 134-151, wherein the first, second, third, and/or fourth polypeptide having an RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 37, 68-79, 82-98, 81, 98, and 110 or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 37, 68-79, 82-98, 81, 98, and 110.

153. The system of any one of claims 134-152, wherein

- (i) the first prime editor further comprises a linker that joins the first napDNAbp and the first polypeptide having the RNA-dependent DNA polymerase activity;
- (ii) the second prime editor further comprises a linker that joins the second napDNAbp and the second polypeptide having the RNA-dependent DNA polymerase activity;
- (iii) the third prime editor further comprises a linker that joins the third napDNAbp and the third polypeptide having the RNA-dependent DNA polymerase activity; and/or
- (iv) the fourth prime editor further comprises a linker that joins the fourth napDNAbp and the fourth polypeptide having the RNA-dependent DNA polymerase activity.

154. The system of claim 153, wherein the linker comprises an amino acid sequence of any one of SEQ ID NOs: 119-128, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 119-128.

155. The system of claim 153 or 154, wherein the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.

156. The system of any one of claims 134-155, wherein the first, second, third, and/or fourth PEGRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 192-203, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 192-203.

157. The system of any one of claims 134-156, wherein the first spacer sequence binds to a first binding site on a first strand of the double-stranded DNA sequence upstream from the target site to be edited.

158. The system of any one of claims 134-157, wherein the second spacer sequence of the second prime editor complex binds to a second binding site on a second strand of the double-stranded DNA sequence upstream from the target site to be edited.

159. The system of any one of claims 134-158, wherein the third spacer sequence binds to a third binding site on a first strand of the double-stranded DNA sequence downstream from the target site to be edited.

160. The system of any one of claims 134-159, wherein the fourth spacer sequence of the fourth prime editor complex binds to a fourth binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.

161. The system of claim 160, wherein the binding of the spacer sequence of the first and second prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequences on each strand by the first and/or second napDNAbps of each first and second prime editor complexes.

162. The system of claim 161, wherein the binding of the spacer sequence of the third and fourth prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequences on each strand by the third and/or fourth napDNAbps of each third and fourth prime editor complexes.

163. The system of claim 133, wherein the first and third binding sites, or the second and fourth binding sites, define two ends of a contiguous region of double-stranded DNA.

164. The system of claim 163, wherein the contiguous region between the first and third binding sites or the second and fourth binding sites is least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29

nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34 nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

165. The system of any one of claims 130-164, wherein the region of complementarity of the first single-stranded DNA sequence and the third single-stranded DNA sequence, and/or the region of complementarity of the second single-stranded DNA sequence and the fourth single-stranded DNA sequence, form a duplex.

166. The system of claim 164, wherein the contiguous region becomes replaced by the duplex.

167. The system of claim 166, wherein the duplex is incorporated in the double-stranded DNA at the target site.

168. The system of claim any one of claims 122-167, wherein the first, second, third, and/or fourth DNA synthesis template is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

169. The system of any one of claims 122-168, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded DNA sequence and the fourth single-stranded DNA sequence, have the same lengths.

170. The system of any one of claims 122-169, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded DNA sequence and the fourth single-stranded DNA sequence, have different lengths.

171. The system of any one of claims 122-170, wherein the region of complementarity of the first, second, third, and/or fourth single-stranded DNA sequence is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least

50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence and the third single-stranded DNA sequence, or the second single-stranded DNA sequence and the fourth single-stranded DNA sequence.

172. The system of any one of claims 122-171, wherein the region of complementarity of the first, second, third, and/or fourth single-stranded DNA sequence 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length, and is either less than or equal to the length of the first single-stranded DNA sequence and the third single-stranded DNA sequence, and the second single-stranded DNA sequence and the fourth single-stranded DNA sequence.

173. The system of any one of claims 130-172, wherein the first, second, third, and fourth single-stranded DNA sequence are each single-stranded DNA strands having 3' ends.
174. The system of claim 173, wherein the incorporation of the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.
175. A polynucleotide encoding the first, second, third, and fourth prime editors of any one of claims 130-174.
176. A polynucleotide encoding the first, second, third, and fourth PEGRNA of any of claims 130-175.
177. A vector comprising a polynucleotide of claim 175 or 176.
178. A cell comprising a polynucleotide of claim 175 or 176 or a vector of claim 177.
179. A pharmaceutical composition comprising a polynucleotide of claim 175 or 176, a vector of claim 177, or a cell of claim 178, and a pharmaceutical excipient.
180. A kit comprising a polynucleotide of claim 175 or 176 or a vector of claim 177 and instructions for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited.
181. A method for simultaneously editing first and second complementary strands of a double-stranded DNA sequence at a target site, the method comprising contacting the double-stranded DNA sequence with a pair of prime editor complexes, the pair comprising:
- (a) a first prime editor complex, comprising:
 - i. a first prime editor comprising a first nucleic acid programmable DNA binding protein (first napDNAbp) and a first polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a first prime editing guide RNA (first PEGRNA) that binds to a first target sequence on the first strand of the genomic DNA sequence upstream of the target site;
 - (b) a second prime editor complex, comprising:
 - i. a second prime editor comprising a second nucleic acid programmable DNA binding protein (second napDNAbp) and a second polypeptide comprising an RNA-dependent DNA polymerase activity; and
 - ii. a second prime editing guide RNA (second PEGRNA) that binds to a second target

sequence on the second strand of the genomic DNA sequence upstream of the target site;

(c) a third prime editor complex, comprising:

- i. a third prime editor comprising a third nucleic acid programmable DNA binding protein (third napDNAbp) and a third polypeptide comprising an RNA-dependent DNA polymerase activity; and
- ii. a third prime editing guide RNA (third PEGRNA) that binds to a third target sequence on the first strand of the genomic DNA sequence downstream of the target site;

(d) a fourth prime editor complex, comprising:

- i. a fourth prime editor comprising a second nucleic acid programmable DNA binding protein (fourth napDNAbp) and a fourth polypeptide comprising an RNA-dependent DNA polymerase activity; and
- ii. a fourth prime editing guide RNA (fourth PEGRNA) that binds to a fourth target sequence on the second strand of the genomic DNA sequence downstream of the target site;

wherein the first prime editor complex causes a first nick at the first target sequence and the subsequent polymerization of a first single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the first nick;

wherein the second prime editor complex causes a second nick at the second target sequence and the subsequent polymerization of a second single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the second nick;

wherein the third prime editor complex causes a third nick at the third target sequence and the subsequent polymerization of a third single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the third nick;

wherein the fourth prime editor complex causes a fourth nick at the fourth target sequence and the subsequent polymerization of a fourth single-stranded DNA sequence having a 3'-end from the available 5'-end formed by the fourth nick;

wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are reverse complements over at least a region of complementarity and form a duplex, wherein the duplex replaces the nicked first and second complementary strands of the double-stranded DNA sequence; and

wherein the third single-stranded DNA sequence and the fourth single-stranded DNA sequence are reverse complements over at least a region of complementarity and form a duplex, wherein the duplex replaces the nicked first and second complementary strands of the double-stranded DNA sequence.

182. The method of claim 181, wherein the prime editor of the first prime editor complex, the

second prime editor complex, the third prime editor complex, and/or the fourth prime editor complex is a fusion protein comprising the napDNAbp and the polypeptide having an RNA-dependent DNA polymerase activity.

183. The method of claim 181 or 182, wherein the first prime editor complex, the second prime editor complex, the third prime editor complex, and the fourth prime editor complex comprise the same prime editor.

184. The method of any one of claims 181-183, wherein the first, second, third, and/or fourth prime editor complex comprise a different prime editor.

185. The method of any one of claims 181-184, wherein first, second, third, and/or fourth napDNAbp is a Cas9 domain or variant thereof.

186. The method of any one of claims 181-185, wherein first, second, third, and/or fourth napDNAbp is a nuclease active Cas9 domain, a nuclease inactive Cas9 domain, or a Cas9 nickase domain or variant thereof.

187. The method of any one of claims 181-184, wherein the first, second, third, and/or fourth napDNAbp is selected from the group consisting of: Cas9, Cas12e, Cas12d, Cas12a, Cas12b1, Cas13a, Cas12c.

188. The method of any one of claims 181-187, wherein the first, second, third, and/or fourth napDNAbp has a nickase activity.

189. The method of any one of claims 181-188, wherein the first, second, third, and/or fourth napDNAbp comprises an amino acid sequence of any one of SEQ ID NOs: 2-65, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 2-65.

190. The method of any one of claims 181-189, wherein the first, second, third, and/or fourth polypeptide having an RNA-dependent DNA polymerase activity is a reverse transcriptase.

191. The method of any one of claims 181-190, wherein the first, second, third, and/or fourth polypeptide having an RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 37, 68-79, 82-98, 81, 98, and 110 or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 37, 68-79, 82-

98, 81, 98, and 110.

192. The method of any one of claims 181-191, wherein

(i) the first prime editor further comprises a linker that joins the first napDNAbp and the first polypeptide having the RNA-dependent DNA polymerase activity;

(ii) the second prime editor further comprises a linker that joins the second napDNAbp and the second polypeptide having the RNA-dependent DNA polymerase activity;

(iii) the third prime editor further comprises a linker that joins the third napDNAbp and the third polypeptide having the RNA-dependent DNA polymerase activity; and/or

(iv) the fourth prime editor further comprises a linker that joins the fourth napDNAbp and the fourth polypeptide having the RNA-dependent DNA polymerase activity.

193. The method of claim 192, wherein the linker comprises an amino acid sequence of any one of SEQ ID NOs: 119-128, or an amino acid sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 119-128.

194. The method of claim 192 or 193, wherein the linker is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length.

195. The method of any one of claims 192-194, wherein the first, second, third, and/or fourth PEGRNA comprises a nucleotide sequence of any one of SEQ ID NOs: 192-203, or a nucleotide sequence having at least an 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 192-203.

196. The method of any one of claims 192-195, wherein the first spacer sequence of the first prime editor complex binds to a first binding site on a first strand of the double-stranded DNA sequence upstream from the target site to be edited.

197. The method of any one of claims 192-196, wherein the second spacer sequence of the second prime editor complex binds to a second binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.

198. The method of any one of claims 192-197, wherein the third spacer sequence of the third prime editor complex binds to a third binding site on a first strand of the double-stranded DNA sequence upstream from the target site to be edited.

199. The method of any one of claims 192-198, wherein the fourth spacer sequence of the fourth prime editor complex binds to a fourth binding site on a second strand of the double-stranded DNA sequence downstream from the target site to be edited.

200. The method of claim 199, wherein the binding of the first and second spacer sequences of the first and second prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequence on each strand by the napDNAbps of each first and second prime editor complex.

201. The method of claim 200, wherein the binding of the third and fourth spacer sequences of the third and fourth prime editor complexes in the presence of a PAM sequence results in the nicking of the first and second strands, respectively, at a nick site proximal to the PAM sequence on each strand by the napDNAbps of each third and fourth prime editor complex.

202. The method of claim 200 or 201, wherein the first and second binding sites, and/or the third and fourth binding sites, define two ends of a contiguous region of double-stranded DNA comprising the target site to be edited.

203. The method of claim 202, wherein the contiguous region between the first and second binding sites, and/or the contiguous region between the third and fourth binding sites, is least 10 nucleobase pairs, at least 11 nucleobase pairs, at least 12 nucleobase pairs, at least 13 nucleobase pairs, at least 14 nucleobase pairs, at least 15 nucleobase pairs, at least 16 nucleobase pairs, at least 17 nucleobase pairs, at least 18 nucleobase pairs, at least 19 nucleobase pairs, at least 20 nucleobase pairs, at least 21 nucleobase pairs, at least 22 nucleobase pairs, at least 23 nucleobase pairs, at least 24 nucleobase pairs, at least 25 nucleobase pairs, at least 26 nucleobase pairs, at least 27 nucleobase pairs, at least 28 nucleobase pairs, at least 29 nucleobase pairs, at least 30 nucleobase pairs, at least 31 nucleobase pairs, at least 32 nucleobase pairs, at least 33 nucleobase pairs, at least 34 nucleobase pairs, at least 35 nucleobase pairs, at least 36 nucleobase pairs, at least 37 nucleobase pairs, at least 37 nucleobase pairs, at least 38 nucleobase pairs, at least 39 nucleobase pairs, or at least 40 nucleobase pairs, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69

nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

204. The method of claim 202 or 203, wherein the contiguous region becomes replaced by the duplex.

205. The method of any one of claims 181-204, wherein the first, second, third, and/or fourth DNA synthesis template is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85

nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotides, at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

206. The method of any one of claims 181-205, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded-DNA sequence and the fourth single-stranded DNA sequence, have the same lengths.

207. The method of any one of claims 181-206, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded-DNA sequence and the fourth single-stranded DNA sequence, have different lengths.

208. The method of any one of claims 181-207, wherein the region of complementarity is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence and the second single-stranded DNA sequence, or the third single-stranded DNA sequence and the fourth single-stranded DNA sequence.

209. The method of any one of claims 181-208, wherein the region of complementarity 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58

nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

210. The method of any one of claims 188-209, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded-DNA sequence and the fourth single-stranded DNA sequence, form the duplex.

211. The method of any one of claims 188-210, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded-DNA sequence and the fourth single-stranded DNA sequence, are each single-stranded DNA strands having 3' ends.

212. The method of claim 204, wherein the replacement of the contiguous region by the duplex comprising the edited portion results in an insertion, deletion, or replacement of DNA at the target site.

213. The method of any one of claims 181-212, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are on ends of a target DNA sequence, and wherein the third single-stranded DNA sequence and the fourth single-stranded DNA sequence are on opposite ends of the same target DNA sequence.

214. The method of claim 213, wherein the target DNA sequence is inverted.

215. The method of any one of claims 181-214 further comprising providing a circular DNA donor.

216. The method of claim 215, wherein the first single-stranded DNA sequence and the third single-stranded DNA sequence are on opposite ends of the target DNA sequence, and wherein the second single-stranded DNA sequence and the fourth single-stranded DNA sequence are on the circular DNA donor.
217. The method of claim 216, wherein a portion of the circular DNA donor between the second single-stranded DNA sequence and the fourth single-stranded DNA sequence replaces the target sequence between the first single-stranded DNA sequence and the third single-stranded DNA sequence.
218. The method of any one of claims 181-217, wherein the first single-stranded DNA sequence and the third single-stranded DNA sequence are on a first nucleic acid molecule, and wherein the second single-stranded DNA sequence and the fourth single-stranded DNA sequence are on a second nucleic acid molecule.
219. The method of claim 218, wherein a portion of the first nucleic acid molecule between the first single-stranded DNA sequence and the third single-stranded DNA sequence is incorporated into the second nucleic acid molecule, and wherein a portion of the second nucleic acid molecule between the second single-stranded DNA sequence and the fourth single-stranded DNA sequence is incorporated into the first nucleic acid molecule.
220. The method of claim 218 or 219, wherein the first nucleic acid molecule is a first chromosome, and the second nucleic acid molecule is a second chromosome.
221. A set of PEgRNAs for use in multi-flap prime editing, wherein the PEgRNAs comprise:
- (a) a first prime editing guide RNA (first PEgRNA) that binds to a first binding site on a first strand of a double-stranded DNA sequence upstream of a target site to be edited, wherein the first PEgRNA comprises a first spacer sequence, first gRNA core, a first DNA synthesis template, and a first primer binding site, wherein the first DNA synthesis template encodes a first single-stranded DNA sequence;
 - (b) a second prime editing guide RNA (second PEgRNA) that binds to a second binding site on a second strand of the double-stranded DNA sequence upstream of the target site to be edited, wherein the second PEgRNA comprises a second spacer sequence, second gRNA core, a second DNA synthesis template, and a second primer binding site, wherein the second DNA synthesis template encodes a second single-stranded DNA sequence comprising the edited portion, wherein the second single-stranded DNA sequence is complementary to the first single-stranded DNA sequence.

- (c) a third prime editing guide RNA (third PEGRNA) that binds to a third binding site on a first strand of a double-stranded DNA sequence downstream of a target site to be edited, wherein the third PEGRNA comprises a third spacer sequence, third gRNA core, a third DNA synthesis template, and a third primer binding site, wherein the third DNA synthesis template encodes a third single-stranded DNA sequence;
- (d) a fourth prime editing guide RNA (fourth PEGRNA) that binds to a fourth binding site on a second strand of the double-stranded DNA sequence downstream of the target site to be edited, wherein the fourth PEGRNA comprises a fourth spacer sequence, fourth gRNA core, a fourth DNA synthesis template, and a fourth primer binding site, wherein the fourth DNA synthesis template encodes a fourth single-stranded DNA sequence, wherein the fourth single-stranded DNA sequence comprises a region of complementarity to the third single-stranded DNA sequence.
222. The set of PEGRNAs of claim 221, wherein the first PEGRNA comprises the structure:
5'-[first gRNA spacer sequence]-[first gRNA core]-[first DNA synthesis template]-[first primer binding site]-3';
the second PEGRNA comprises the structure:
5'-[second gRNA spacer sequence]-[second gRNA core]-[second DNA synthesis template]-[second primer binding site]-3';
the third PEGRNA comprises the structure:
5'-[third gRNA spacer sequence]-[third gRNA core]-[third DNA synthesis template]-[third primer binding site]-3'; and
the fourth PEGRNA comprises the structure:
5'-[fourth gRNA spacer sequence]-[fourth gRNA core]-[fourth DNA synthesis template]-[fourth primer binding site]-3'.
223. The set of PEGRNAs of claim 221 or 222, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence, and/or the third single-stranded DNA sequence and the fourth single-stranded DNA sequence, form a duplex that becomes integrated into the double-stranded DNA sequence at the target site to be edited.
224. The set of PEGRNAs of any one of claims 221-223, wherein the integration of the duplex results in an insertion, deletion, or replacement of DNA at the target site.
225. The set of PEGRNAs of claim 224, wherein the insertion is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

226. The set of PEGRNAs of claim 224, wherein the deletion is at least 1 nucleotide, at least 2 nucleotides, 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 is 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides, or more in length.

227. The set of PEGRNAs of claim 224, wherein the replacement is at least 1 nucleotide, at least 2 nucleotides, 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, 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67

nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides, or more in length.

228. A polynucleotide encoding a set of PEgRNAs of any of claims 221-227.
229. A vector comprising the polynucleotide of claim 228 for expressing the plurality of PEgRNAs in a cell.
230. A cell comprising the vector of claim 229.
231. A pharmaceutical composition comprising the plurality of PEgRNAs of any one of claims 221-227, a vector of claim 229, or a cell of claim 230, and a pharmaceutical excipient.
232. A method for modifying a genome in a cell, comprising:
- (i) contacting a genome with a dual-flap prime editing system, said system comprising a first prime editor/pegRNA complex capable of installing a first 3' nucleic acid flap at a first nick site on a first strand of a target site and a second prime editor/pegRNA complex capable of installing a second 3' nucleic acid flap at a second nick site on a second strand of a target site,
 - wherein the first 3' nucleic acid flap and the second 3' nucleic acid flap are reverse complement sequences that form a duplex, and
 - wherein the duplex comprises a first recombinase site, and
 - wherein the cell installs the duplex comprising the first recombinase site at the target site in place of an endogenous duplex positioned between the first and second nick sites;
 - (ii) contacting the first recombinase site with a donor nucleic acid comprising a second recombinase site; and

- (iii) contacting the genome with a recombinase capable recombination between the first and second recombinase sites, thereby integrating the donor nucleic acid at the target site in the genome.

CLAIMS

1. A system for simultaneously editing both strands of a double-stranded DNA sequence at a target site to be edited, said system comprising:

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

(b) a first prime editing guide RNA (first PEGRNA) that comprises

(i) a first spacer sequence that binds to a first binding site on a first strand of the double-stranded DNA sequence upstream of the target site relative to the second strand,

(ii) a first gRNA core that is capable of complexing with the prime editor, and

(iii) a first DNA synthesis template that encodes a first single-stranded DNA sequence,

and

(c) a second prime editing guide RNA (second PEGRNA) that comprises

(i) a second spacer sequence that binds to a second binding site on a second strand of the double-stranded DNA sequence downstream of the target site relative to the second strand;

(ii) a second gRNA core that is capable of complexing with the prime editor, and

(iii) a second DNA synthesis template that encodes a second single-stranded DNA sequence,

wherein the prime editor is capable of cleaving the first strand at a first cut site when complexed with the second PEGRNA and cleaving the second strand at a second cut site when complexed with the first PEGRNA, and

wherein the first single-stranded DNA sequence comprises a first edit compared to the second strand of the target site that starts at a position no more than 3 nucleotides from the first cut site.

2. The system of claim 1, wherein the second single-stranded DNA sequence comprises a second edit compared to the first strand of the target site that starts at a position no more than 3 nucleotides from the second cut site.
3. The system of any one of claims 1 or 2, wherein the first PEGRNA comprises a first primer binding site complementary to a region of the second strand upstream of the first cut site.
4. The system of any one of claims 1-3, wherein the second PEGRNA comprises a second primer binding site complementary to a region of the first strand upstream of the second cut site.
5. The system of any one of claims 1-4, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence are reverse complements over at least a region of complementarity.
6. The system of claim 5, wherein the region of complementarity is at least 5 nucleotides in length.
7. The system of claim 5, wherein a portion of 3' ends of the first and second single-stranded DNA sequence are reverse complements of each other.
8. The system of claim 5, wherein the first single-stranded DNA sequence is the reverse complement of the second single-stranded DNA sequence.
9. The system of any one of claims 5-8, wherein the region of complementarity of the first single-stranded DNA sequence and the second single-stranded DNA sequence forms a duplex comprising the first edit and the second edit.
10. The system of any one of claims 5-9, wherein the region of complementarity is at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, at least 50%, at least 45%, at least 40%, at least 35%, at

least 30%, at least 25%, at least 20%, at least 15%, or at least 10% of the total length of the first single-stranded DNA sequence or the second single-stranded DNA sequence.

11. The system of any one of claims 1-10, wherein the distance between the first and second binding sites is 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, at least 50 nucleotides, at least 51 nucleotides, at least 52 nucleotides, at least 53 nucleotides, at least 54 nucleotides, at least 55 nucleotides, at least 56 nucleotides, at least 57 nucleotides, at least 58 nucleotides, at least 59 nucleotides, at least 60 nucleotides, at least 61 nucleotides, at least 62 nucleotides, at least 63 nucleotides, at least 64 nucleotides, at least 65 nucleotides, at least 66 nucleotides, at least 67 nucleotides, at least 68 nucleotides, at least 69 nucleotides, at least 70 nucleotides, at least 71 nucleotides, at least 72 nucleotides, at least 73 nucleotides, at least 74 nucleotides, at least 75 nucleotides, at least 76 nucleotides, at least 77 nucleotides, at least 78 nucleotides, at least 79 nucleotides, at least 80 nucleotides, at least 81 nucleotides, at least 82 nucleotides, at least 83 nucleotides, at least 84 nucleotides, at least 85 nucleotides, at least 86 nucleotides, at least 87 nucleotides, at least 88 nucleotides, at least 89 nucleotides, at least 90 nucleotides, at least 91 nucleotides, at least 92 nucleotides, at least 93 nucleotides, at least 94 nucleotide at least 95 nucleotides, at least 96 nucleotides, at least 97 nucleotides, at least 98 nucleotides, at least 99 nucleotides, at least 100 nucleotides, at least 101 nucleotides, at least 102 nucleotides, at least 103 nucleotides, at least 104 nucleotides, at least 105 nucleotides, at least 106 nucleotides, at least 107 nucleotides, at least 108 nucleotides, at least 109 nucleotides, or at least 110 nucleotides in length.

12. The system of any one of claims 1-11, wherein the first edit comprises an insertion, a deletion, a substitution, or a combination thereof.

13. The system of any one of claims 1-12, wherein the second edit comprises an insertion, a deletion, a substitution, or a combination thereof.

14. The system of any one of claims 1-13, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have the same lengths.

15. The system of any one of claims 1-14, wherein the first single-stranded DNA sequence and the second single-stranded DNA sequence have different lengths.
16. The system of any one of claims 1-15, wherein the first or second edit comprises one or more recombinase sites.
17. The system of claim 16, further comprising a recombinase.
18. The system of claim 17, wherein the recombinase is a serine recombinase.
19. The system of claim 18, wherein the recombinase is Bxb1.
20. The system of any one of claims 1-19, wherein the first edit starts at a position no more than 2 nucleotides from the first cut site.
21. The system of any one of claims 1-20, wherein the second edit starts at a position no more than 2 nucleotides from the second cut site.
22. The system of any one of claims 1-21, wherein the first edit starts at a position that is 0 nucleotides from the first cut site.
23. The system of any one of claims 1-22, wherein the second edit starts at a position that is 0 nucleotides from the first cut site.
24. The system of any one of claims 1-23, wherein the first single-stranded DNA sequence and/or the second single-stranded DNA sequence does not comprise sequence homology as compared to the DNA sequence at the target site.
25. The system of any one of claims 1-24, wherein the napDNAbp has a nickase activity.
26. The system of any one of claims 1-22, wherein the napDNAbp comprises an amino acid sequence of any one of SEQ ID NOs: 18-88, or an amino acid sequence having at least 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 18-88.

27. The system of any one of claims 1-26, wherein the polypeptide comprising the RNA-dependent DNA polymerase activity is a reverse transcriptase.
28. The system of any one of claims 1-27, wherein the polypeptide comprising the RNA-dependent DNA polymerase activity comprises an amino acid sequence of any one of SEQ ID NOs: 89-100, 105-122, 128-129, 132, 139, 143, 149, 154, 159, and 700-766, or an amino acid sequence having at least 80%, 85%, 90%, 95%, or 99% sequence identity with any one of SEQ ID NOs: 89-100, 105-122, 128-129, and 132.
29. One or more polynucleotides encoding the system of any one of claims 1-28.
30. An *in vitro* method for simultaneously editing both strands of a double-stranded DNA sequence at a target site comprising contacting the double-stranded DNA sequence with the system of any one of claims 1-28.
31. The system of any one of claims 1-28, or the one or more polynucleotides of claim 29, for use as a medicament.
32. Use of the system of any one of claims 1-28, or the one or more polynucleotides of claim 29, in the manufacture of a pharmaceutical composition for treating a disease or a disorder selected from the group consisting of adenosine deaminase (ADA) deficiency, alpha-1 antitrypsin deficiency, cystic fibrosis, Duchenne muscular dystrophy, galactosemia, hemochromatosis, Huntington's disease, maple syrup urine disease, Marfan syndrome, neurofibromatosis type 1, pachyonychia congenital, phenylketonuria, severe combined immunodeficiency, sickle cell disease, Smith-Lemli-Opitz syndrome, and Tay-Sachs disease, heart disease, high blood pressure, Alzheimer's disease, arthritis, diabetes, cancer, obesity, prion disease, trinucleotide repeat expansion disease, and cyclin-dependent kinase-like (CDKL) 5 deficiency disorder.