

CLAIMS

1. A prime editor comprising (i) a nucleic acid programmable DNA binding protein (napDNAbp) and (ii) a DNA polymerase, wherein the napDNAbp is a Cas9 nickase (nCas9) comprising a R221K amino acid substitution, a N394K amino acid substitution, and an amino acid substitution that inactivates HNH domain nuclease activity, or corresponding amino acid substitutions thereof, relative to a wild type Cas9 as set forth in SEQ ID NO: 2.
2. The prime editor of claim 1, wherein the nCas9 is connected to the DNA polymerase in a fusion protein.
3. The prime editor of claim 2, wherein the fusion protein comprises the structure: NH₂-[napDNAbp]-[DNA polymerase]-COOH, wherein “[]-[” indicates an optional linker sequence.
4. The prime editor of any one of claims 1-3, wherein the nCas9 comprises a R221K, a N394K, and a H840X amino acid substitution compared to a wild type Cas9 as set forth in SEQ ID NO: 2, wherein X is any amino acid other than histidine.
5. The prime editor of claim 4, wherein the nCas9 comprises a H840A amino acid substitution compared to a wild type Cas9 as set forth in SEQ ID NO: 2.
6. The prime editor of any one of claims 1-5, wherein the nCas9 comprises SEQ ID NO: 104 or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 104.
7. The prime editor of any one of claims 2-6, wherein the nCas9 and the DNA polymerase are connected by a linker.
8. The prime editor of claim 7, wherein the linker comprises the sequence of SEQ ID NO: 105.

9. The prime editor of claim 7 or 8, wherein the prime editor further comprises (i) a SV40 NLS at the N terminus, and (ii) a SV40 NLS and/or a c-Myc NLS at the C terminus.
10. The prime editor of claim 9, wherein the N terminus SV40 NLS comprises SEQ ID NO: 101, the C terminus SV40 NLS comprises SEQ ID NO: 140, and the c-Myc NLS comprises SEQ ID NO: 135.
11. The prime editor of any one of claims 1-10, wherein the DNA polymerase is a reverse transcriptase.
12. The prime editor of claim 11, wherein the reverse transcriptase is a retroviral reverse transcriptase.
13. The prime editor of claim 11 or 12, wherein the reverse transcriptase is a Moloney-Murine Leukemia Virus reverse transcriptase (MMLV-RT) comprising an amino acid sequence of any one of SEQ ID NOs: 81-98 or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with any one of SEQ ID NOs: 81-98.
14. The prime editor of claim 13, wherein the MMLV-RT comprises a D200N, a T330P, a T306K, a W313F, and a L603W amino acid substitution relative to MMLV-RT as set forth in SEQ ID NO: 81.
15. The prime editor of any one of claims 2-14, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 99, or an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 99.
16. A prime editor system comprising the prime editor of any one of claims 1-15 and a prime editing guide RNA (pegRNA).

17. The prime editor system of claim 16, wherein the prime editor system further comprises a second strand nicking single guide RNA (sgRNA).
18. One or more polynucleotides encoding the prime editor of any one of claims 1-15 or the prime editor system of claim 16 or 17.
19. The one or more polynucleotides of claim 18, wherein the one or more polynucleotides are DNA or mRNA.
20. A polynucleotide encoding the prime editor of claim 16 or 17.
21. The polynucleotide of claim 20, wherein the polynucleotide comprises SEQ ID NO: 5660, or a sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity with SEQ ID NO: 5660.
22. An in vitro or ex-vivo method of editing a nucleic acid molecule comprising contacting the nucleic acid molecule with the prime editor system of claim 16 or 17.
23. The prime editor of any one of claims 1-15, the prime editor system of claim 16 or 17, the one or more polynucleotides of claim 18 or 19, or the polynucleotide of claim 20 or 21 for use as a medicament.