

CLAIMS

What is claimed is:

1. A prime editor comprising: (a) a nucleic acid-programmable DNA-binding protein (napDNAbp); and (b) an MMLV reverse transcriptase variant comprising:
 - a sequence at least 80%, at least 90%, or at least 95% identical to SEQ ID NO: 33, or a truncation of SEQ ID NO: 33 lacking an RNaseH domain, and further comprising one or more mutations relative to SEQ ID NO: 33 selected from the group consisting of T13I, V19I, A32T, G38V, S60Y, P111L, K120R, H126Y, T128N, T128F, T128H, V129S, P132S, G138R, C157F, P175Q, P175S, D200S, D200Y, D200C, Y222F, V223A, V223M, V223T, V223W, V223Y, L234I, T246I, N249S, T287A, P292T, E302A, E302K, G316R, E346K, K373N, W388C, V402A, K445N, M457I, and A462S.

2. The prime editor of claim 1, wherein the MMLV reverse transcriptase variant comprises any one of the following groups of mutations relative to the amino acid sequence of SEQ ID NO: 33:
 - D200Y and E302A;
 - D200Y, V223A, and M457I;
 - V223M, T306K, and A462S;
 - D200N and E302K;
 - D200Y and E302K;
 - T128N and V223A;
 - V19I, A32T, and D200Y;
 - D200S, V223A, E346K, and W388C;
 - S60Y, V223A, and N249S;
 - P111L, V223A, T287A, and G316R;
 - S60Y, G138R, and V223A;
 - S60Y, Y222F, V223A, and K445N; or
 - S60Y, C157F, V223A, and T246I.

3. The prime editor of any preceding claim, wherein the one or more mutations comprise:

(i) T13I, G38V, K120R, H126Y, P132S, P175Q, P175S, L234I, P292T, G316R, K373N, V402A, or M457I; or

(ii) T128F, T128H, T128N, V129S, D200C, V223M, V223T, V223W, or V223Y.

4. The prime editor of any preceding claim, wherein the MMLV reverse transcriptase variant comprises the amino acid sequence of any one of SEQ ID NOs: 35-42, 172-177, 183, and 184.

5. The prime editor of any preceding claim, wherein the napDNAbp:

(i) is a Cas protein;

(ii) is a Cas9 nickase (nCas9);

(iii) comprises the amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11, or an amino acid sequence at least 80%, at least 90%, or at least 95% identical to SEQ ID NO: 9, SEQ ID NO: 10, or SEQ ID NO: 11; and/or

(iv) comprises a Cas9 variant comprising one or more mutations relative to SEQ ID NO: 9 or SEQ ID NO: 11 selected from the group consisting of D23G, H99Q, H99R, E102K, E102S, E102R, N175K, D177G, K218R, N309D, I312V, E471K, G485S, K562N, D608N, I632V, D645N, D645E, R654C, G687D, G715E, H721Y, R753K, R753G, H754R, K775R, E790K, T804A, K918A, K1003R, M1021Y, E1071K, and E1260D; and optionally wherein the Cas9 variant comprises the amino acid sequence of any one of SEQ ID NOs: 178-180.

6. An MMLV reverse transcriptase variant comprising:

a sequence at least 80%, at least 90%, or at least 95% identical to SEQ ID NO: 33, or a truncation of SEQ ID NO: 33 lacking an RNaseH domain, and further comprising one or more mutations relative to SEQ ID NO: 33 selected from the group consisting of T13I, V19I, A32T, G38V, S60Y, P111L, K120R, H126Y, T128N, T128F, T128H, V129S, P132S, G138R, C157F, P175Q, P175S, D200S, D200Y, D200C, Y222F, V223A, V223M, V223T, V223W, V223Y, L234I, T246I, N249S, T287A, P292T, E302A, E302K, G316R, E346K, K373N, W388C, V402A, K445N, M457I, and A462S.

7. The MMLV reverse transcriptase variant of claim 6, wherein the MMLV reverse transcriptase variant comprises any one of the following groups of mutations relative to the amino acid sequence of SEQ ID NO: 33:

D200Y and E302A;

D200Y, V223A, and M457I;
 V223M, T306K, and A462S;
 D200N and E302K;
 D200Y and E302K;
 T128N and V223A;
 V19I, A32T, and D200Y;
 D200S, V223A, E346K, and W388C;
 S60Y, V223A, and N249S;
 P111L, V223A, T287A, and G316R;
 S60Y, G138R, and V223A;
 S60Y, Y222F, V223A, and K445N; or
 S60Y, C157F, V223A, and T246I.

8. The MMLV reverse transcriptase variant of claim 6 or 7, wherein the one or more mutations comprise:
 - (i) T13I, G38V, K120R, H126Y, P132S, P175Q, P175S, L234I, P292T, G316R, K373N, V402A, or M457I; or
 - (ii) T128F, T128H, T128N, V129S, D200C, V223M, V223T, V223W, or V223Y.
9. The MMLV reverse transcriptase variant of any one of claims 6-8, wherein the MMLV reverse transcriptase variant comprises the amino acid sequence of any one of SEQ ID NOs: 35-42, 172-177, 183, and 184.
10. A complex comprising the prime editor of any one of claims 1-5 and a prime editing guide RNA (PEgRNA).
11. One or more polynucleotides encoding the prime editor of any one of claims 1-5.
12. A vector comprising the one or more polynucleotides of claim 11.
13. A pharmaceutical composition comprising the prime editor of any of claims 1-5, the complex of claim 10, the one or more polynucleotides of claim 11, or the vector of claim 12.

14. An *in vitro* or *ex vivo* method comprising contacting a nucleic acid molecule with the prime editor of any one of claims 1-5 and a prime editing guide RNA (pegRNA).
15. The prime editor of any of claims 1-5, the complex of claim 10, the one or more polynucleotides of claim 11, the vector of claim 12, or the pharmaceutical composition of claim 13 for use as a medicament.