

CLAIMS

1. A gene modifying polypeptide comprising:
a reverse transcriptase (RT) domain having the sequence of SEQ ID NO: 8,003;
a Cas9 nickase domain, wherein the RT domain is C-terminal of the
Cas9 nickase domain; and
a linker disposed between the RT domain and the Cas9 nickase domain, wherein the
linker comprises the sequence of SEQ ID NO: 15,401.
2. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain is a
SpyCas9 nickase domain.
3. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain comprises
an N863A mutation.
4. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain comprises
the amino acid sequence of SEQ ID NO: 11,096.
5. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain is an
NmeCas9 domain.
6. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain is an
St1Cas9 domain.
7. The gene modifying polypeptide of claim 1, wherein the Cas9 nickase domain is a
SauCas9 domain.
8. The gene modifying polypeptide of claim 1, which comprises a nuclear localization
signal (NLS).

9. The gene modifying polypeptide of claim 1, which comprises a first NLS which is N-terminal of the Cas9 nickase domain.
10. The gene modifying polypeptide of claim 1, which comprises an NLS which is C-terminal of the RT domain.
11. The gene modifying polypeptide of claim 1, which comprises a first NLS which is N-terminal of the Cas9 nickase domain and a second NLS which is C-terminal of the RT domain.
12. The gene modifying polypeptide of claim 1, which comprises a first NLS which is N-terminal of the Cas9 nickase domain, wherein the first NLS comprises the amino acid sequence of PAAKRVKLD (SEQ ID NO: 11,095).
13. The gene modifying polypeptide of claim 1, which comprises an NLS which is C-terminal of the RT domain and comprises the amino acid sequence of KRTADGSEFE (SEQ ID NO: 4650).
14. The gene modifying polypeptide of claim 1, which comprises an NLS which is C-terminal of the RT domain and comprises the amino acid sequence of KRTADGSEFESPKKKAKVE (SEQ ID NO: 4651).
15. The gene modifying polypeptide of claim 1, which comprises the amino acid sequence of SEQ ID NO: 4000 which comprises a first NLS and the Cas9 nickase domain.
16. The gene modifying polypeptide of claim 1, which comprises an NLS having the amino acid sequence according to SEQ ID NO: 4649.
17. The gene modifying polypeptide of claim 1, which comprises a GG amino acid sequence between the Cas9 nickase domain and the linker.

18. The gene modifying polypeptide of claim 11, which comprises an AG amino acid sequence between the RT domain and the second NLS.
19. The gene modifying polypeptide of claim 1, which comprises a GG amino acid sequence between the linker and the RT domain.
20. The gene modifying polypeptide of claim 1, which comprises the amino acid sequence according to SEQ ID NO: 1372.
21. A nucleic acid molecule encoding a gene modifying polypeptide comprising: a reverse transcriptase (RT) domain having the sequence of SEQ ID NO: 8,003; a Cas9 nickase domain, wherein the RT domain is C-terminal of the Cas9 nickase domain; and a linker disposed between the RT domain and the Cas9 nickase domain, wherein the linker comprises a sequence of SEQ ID NO: 15,401.
22. The nucleic acid molecule of claim 21, which comprises RNA.
23. A cell comprising: i) a gene modifying polypeptide comprising: a reverse transcriptase (RT) domain having the sequence of SEQ ID NO: 8,003; a Cas9 nickase domain, wherein the RT domain is C-terminal of the Cas9 nickase domain; and a linker disposed between the RT domain and the Cas9 nickase domain, wherein the linker comprises a sequence of SEQ ID NO: 15,401 or ii) a nucleic acid encoding the gene modifying polypeptide..
24. A system comprising:
- i) a gene modifying polypeptide comprising: a reverse transcriptase (RT) domain having the sequence of SEQ ID NO: 8,003; a Cas9 nickase domain, wherein the RT domain is C-terminal of the Cas9 nickase domain; and a linker disposed between the RT domain and the Cas9 nickase domain, wherein the linker comprises a sequence of SEQ ID NO: 15,401, or a nucleic acid molecule encoding the gene modifying polypeptide, and
 - ii) a template RNA that comprises:

- a) a gRNA spacer that is complementary to a portion of a target nucleic acid sequence;
- b) a gRNA scaffold that binds the Cas9 nickase domain of the gene modifying polypeptide;
- c) a heterologous object sequence; and
- d) a primer binding site sequence.

25. A lipid nanoparticle formulation comprising the system of claim 24.

26. A method for modifying a target nucleic acid molecule in a cell, the method comprising contacting the cell with the system of claim 24, thereby modifying the target nucleic acid molecule.