

We Claim:

1. A polynucleotide comprising:
 - a nucleotide sequence encoding an *N. meningitidis* (Nme) Cas9 polypeptide, wherein the nucleotide sequence comprises the sequence of SEQ ID NO: 264; and
 - a nucleotide sequence encoding a first nuclear localization signal (NLS), a nucleotide sequence encoding a second NLS, and a nucleotide sequence encoding a third NLS, optionally wherein (a) the first, second, and third NLS are N-terminal to the nucleotide sequence encoding the Nme Cas9, or (b) the first and second NLS are N-terminal to the nucleotide sequence encoding the NmeCas9 and the third NLS is C-terminal to the nucleotide sequence encoding the Nme Cas9.
2. The polynucleotide of claim 1, wherein the polynucleotide further comprises a poly-A sequence or a polyadenylation signal sequence, optionally wherein the poly-A sequence comprises non-adenine nucleotides, optionally wherein the poly-A sequence comprises 100-400 nucleotides.
3. The polynucleotide of claim 2, wherein the poly-A sequence comprises a sequence of SEQ ID NO: 409.
4. The polynucleotide of any one of claims 1-3, wherein the first and second NLS are independently selected from SEQ ID NOs: 383, 422, 384-386, 388 and 410-421.
5. The polynucleotide of any one of claims 1-4, wherein the polynucleotide further comprises (i) a nucleotide sequence encoding a linker sequence between the first NLS and the second NLS, and/or (ii) a nucleotide sequence encoding a linker sequence between the Nme Cas9 coding sequence and the NLS proximal to the Nme Cas9 coding sequence, optionally wherein the linker comprises at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids.
6. The polynucleotide of claim 5, wherein the linker sequence comprises an amino acid sequence GGG or GGGS, optionally wherein the GGG or GGGS sequence is at the N-terminus of the linker sequence.
7. The polynucleotide of claim 5, wherein the linker sequence comprises an amino acid sequence of any one of SEQ ID NOs: 61-122.
8. The polynucleotide of claim 1, wherein the polynucleotide further comprises one or more additional heterologous functional domains.
9. The polynucleotide of claim 1, wherein the polynucleotide comprises
 - 1) a 5' cap, optionally wherein the 5' cap is Cap0, Cap1, or Cap2;

- 2) a 5' UTR (i) with at least 90% identity to any one of SEQ ID NOs: 393, 391, 392, 398; or (ii) comprising any one of SEQ ID NOs: 391-398; or
 - 3) a 3' UTR (i) with at least 90% identity to any one of SEQ ID NOs: 401, 399-406; or (ii) comprising any one of SEQ ID NOs: 401, 399, 400, 402-406,
- optionally wherein the polynucleotide comprises a 5' UTR and a 3' UTR from the same source.
10. The polynucleotide of claim 1, wherein the polynucleotide is an mRNA.
 11. The polynucleotide of claim 10, wherein at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99%, or 100% of the uridine in the mRNA is substituted with a modified uridine.
 12. A composition comprising the polynucleotide according to claim 1, and at least one guide RNA (gRNA).
 13. A composition comprising the polynucleotide according to claim 1, further comprising a single guide RNA, wherein the single guide RNA comprises a guide region and a conserved region, (A) wherein the conserved region comprises one or more of:
 - (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 2-24 nucleotides, wherein
 - (i) one or more of nucleotides 37-48 and 53-64 is deleted and optionally one or more of nucleotides 37-64 is substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 36 is linked to nucleotide 65 by at least 2 nucleotides; and/or
 - (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2-10, optionally 2-8 nucleotides, wherein
 - (i) one or more of nucleotides 82-86 and 91-95 is deleted and optionally one or more of positions 82-96 is substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 81 is linked to nucleotide 96 by at least 4 nucleotides; and/or
 - (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 2-18, optionally 2-16 nucleotides, wherein
 - (i) one or more of nucleotides 113-121 and 126-134 is deleted and optionally one or more of nucleotides 113-134 is substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 112 is linked to nucleotide 135 by at least 4 nucleotides;

wherein one or both nucleotides 144-145 are optionally deleted relative to SEQ ID NO: 500;

wherein at least 10 nucleotides are modified nucleotides; and/or

- (B) wherein the conserved region comprises one or more of:
- (a) a shortened repeat/anti-repeat region, wherein the shortened repeat/anti-repeat region lacks 2-24 nucleotides, wherein
 - (i) one or more of nucleotides 37-64 is deleted and optionally substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 36 is linked to nucleotide 65 by (i) a first internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides; and/or
 - (b) a shortened hairpin 1 region, wherein the shortened hairpin 1 lacks 2-10, optionally 2-8 nucleotides, wherein
 - (i) one or more of nucleotides 82-95 is deleted and optionally substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 81 is linked to nucleotide 96 by (i) a second internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides; and/or
 - (c) a shortened hairpin 2 region, wherein the shortened hairpin 2 lacks 2-18, optionally 2-16 nucleotides, wherein
 - (i) one or more of nucleotides 113-134 is deleted and optionally substituted relative to SEQ ID NO: 500; and
 - (ii) nucleotide 112 is linked to nucleotide 135 by (i) a third internal linker that alone or in combination with nucleotides substitutes for 4 nucleotides, or (ii) at least 4 nucleotides;
- wherein one or both nucleotides 144-145 are optionally deleted as compared to SEQ ID NO: 500;
- wherein the gRNA comprises at least one of the first internal linker, the second internal linker, and the third internal linker.
14. A polypeptide encoded by the polynucleotide of claim 1.
 15. A vector comprising the polynucleotide of claim 1.
 16. An expression construct, or a plasmid comprising the expression construct comprising a promoter operably linked to a sequence encoding the polynucleotide of claim 1, optionally wherein the promoter is an RNA polymerase promoter, optionally a bacterial RNA polymerase promoter.

17. The expression construct of claim 16, further comprising (i) a poly-A tail sequence, optionally wherein the poly-A tail sequence is an encoded poly-A tail sequence; and/or (ii) a polyadenylation signal sequence.
18. A host cell comprising the vector of claim 15 or the expression construct of claim 16.
19. A pharmaceutical composition comprising the polynucleotide, composition, or polypeptide of any one of claims 1 and 12-14, and a pharmaceutically acceptable carrier.
20. A kit comprising the polynucleotide, composition, or polypeptide of any one of claims 1 and 12-14.
21. The polynucleotide, composition, or polypeptide of any one of claims 1 and 12-14 for use as a medicament.
22. Use of the polynucleotide, composition, or polypeptide of any one of claims 1 and 12-14 in the manufacture of a medicament for modifying a target gene in a cell.
23. The polynucleotide or composition of any one of claims 1 and 12-13, wherein the polynucleotide or composition is formulated as a lipid nanoparticle (LNP) composition.
24. An *in vitro* method of modifying a target gene comprising delivering to a cell the polynucleotide, polypeptide, or composition of any one of claims 1 and 12-14.
25. An *in vitro* method of modifying a target gene, comprising delivering to the cell one or more lipid nanoparticle (LNP) compositions, comprising the polynucleotide according to claim 1, and one or more guide RNAs.
26. A method of producing a polynucleotide of any one of claims 1-25, comprising contacting the expression construct of claim 16 with an RNA polymerase and NTPs that comprise at least one modified nucleotide.