CLAIMS:

1. A lipid nanoparticle ("LNP") composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises:

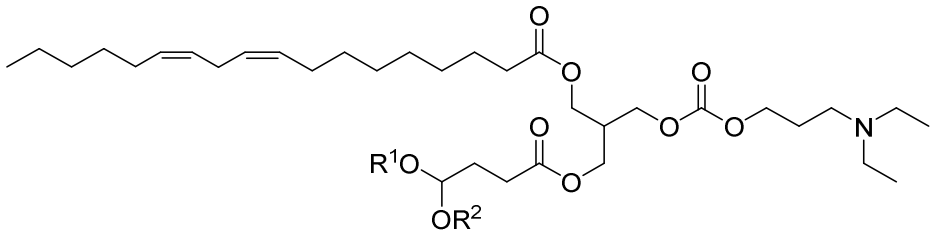
50-60 mol-% amine lipid;

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is about 6.

2. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent;

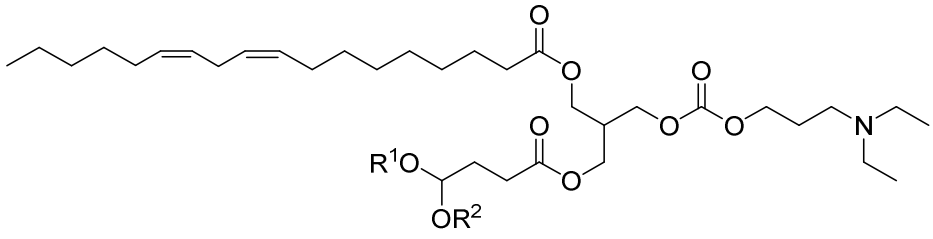
50-60 mol-% amine lipid;

27-39.5 mol-% helper lipid;

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is 5-7.

3. The LNP composition of claim 2, wherein the N/P ratio is about 6.

4. The~~An~~ LNP composition of claim 7 ~~comprising:~~

~~an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and~~

~~a lipid component~~, wherein the lipid component comprises

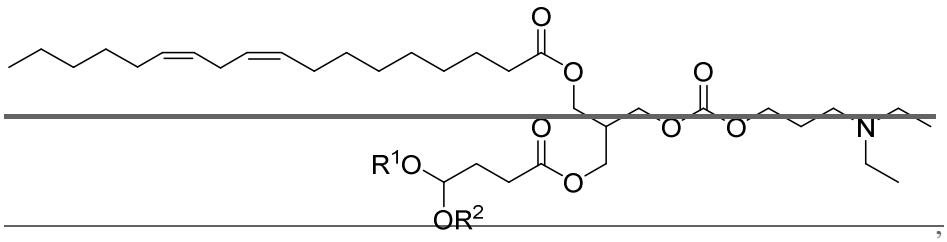
50-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid~~,~~

~~the amine lipid is represented by the following structural formula~~



,

~~wherein R^1 and R^2 are each independently a C4-C12 alkyl, and~~

~~the N/P ratio of the LNP composition is 3-10~~.

5. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises

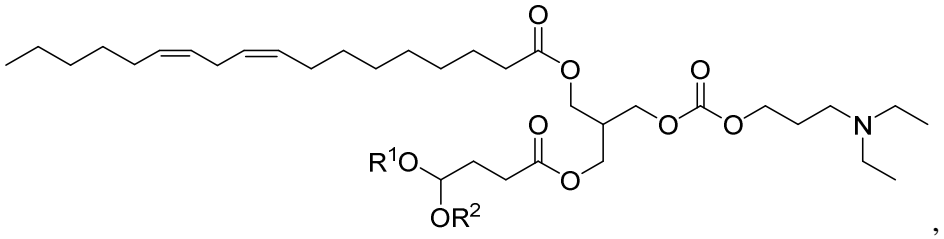
40-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is about 6.

6. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises

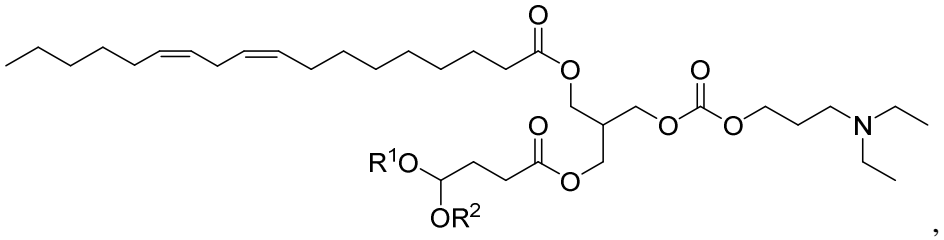
50-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

1.5-10 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is about 6.

7. A lipid nanoparticle~~An~~ (LNP) composition comprising:

an RNA component, wherein the RNA component comprises (i) an mRNA encoding~~es~~ an RNA-guided DNA-binding agent and (ii) a gRNA nucleic acid; and

a lipid component, wherein the lipid component comprises

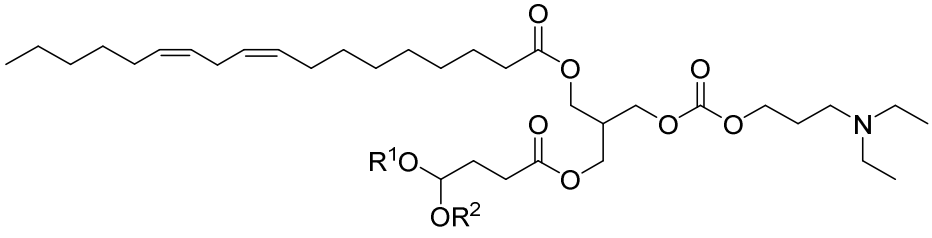
40-60 mol-% amine lipid;

0-10 mol-% neutral lipid; and

1.5-10 mol-% PEG lipid, wherein

the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is 5~~3~~-7.

8. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises:

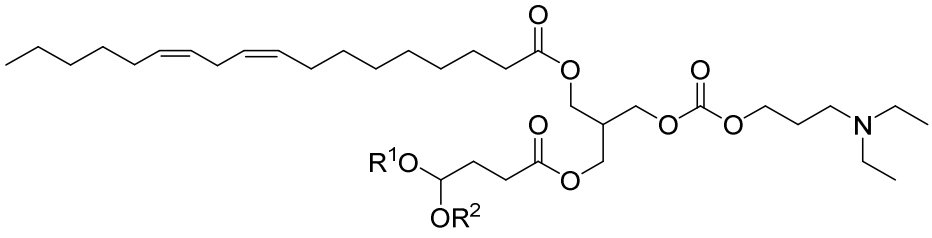
40-60 mol-% amine lipid;

less than 1 mol-% neutral lipid; and

1.5-10 mol-% PEG lipid, wherein

the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is 3-10.

9. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

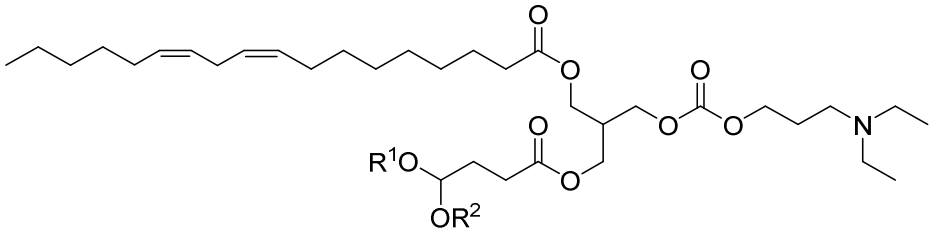
a lipid component, wherein the lipid component comprises:

40-60 mol-% amine lipid; and

1.5-10 mol-% PEG lipid, wherein

the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl,

the N/P ratio of the LNP composition is 3-10, and

the LNP composition is essentially free of or free of neutral phospholipid.

10. An LNP composition comprising:

an RNA component, wherein the RNA component encodes an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises:

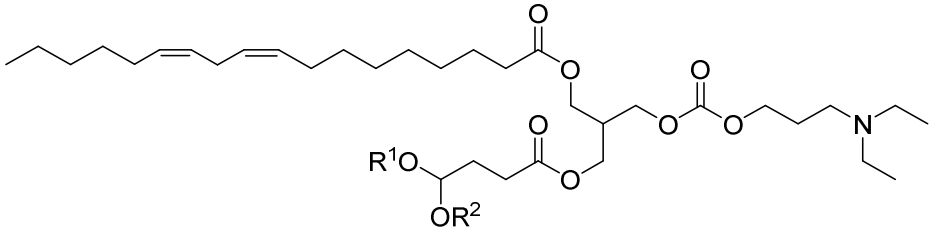
50-60 mol-% amine lipid;

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid, wherein

the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is 3-7.

11. The LNP composition of any one of claims ~~1~~–4 and 7-10, wherein the N/P ratio of the LNP composition is about 6.

12. The LNP composition of any one of claims 1-3, 5, 6, and 8-11, wherein the RNA component comprises an mRNA.

13. The LNP composition of any one of claims 1-12, wherein the RNA component comprises a Class 2 Cas nuclease mRNA.

14. The LNP composition of any one of claims 1-13, wherein the RNA component comprises a Cas9 nuclease mRNA.

15. The LNP composition of any one of claims 11-14, wherein the mRNA is a modified mRNA.

16. The LNP composition of any one of claims ~~1~~4, 7, and 12-15, wherein the ~~RNA component comprises an~~ mRNA ~~comprising~~ comprises an open reading frame encoding the RNA-guided DNA-binding agent, wherein the open reading frame has a uridine content from its minimum uridine content to 150% of the minimum uridine content.

17. The LNP composition of any one of claims ~~1~~4, 7, and 12-16, wherein the ~~RNA component comprises an~~ mRNA ~~comprising~~ comprises an open reading frame encoding the RNA-guided DNA-binding agent, wherein the open reading frame has a uridine dinucleotide content from its minimum uridine dinucleotide content to 150% of the minimum uridine dinucleotide content.

18. The LNP composition of any one of claims ~~1~~4, 7, and 12-17, wherein the ~~RNA component comprises an~~ mRNA ~~comprising~~ comprises a sequence with at least 90% identity to any one of SEQ ID NO: 1, 4, 7, 9, 10, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 50, 52, 54, 65, or 66, wherein the mRNA comprises an open reading frame encoding the RNA-guided DNA-binding agent.

19. The LNP composition of any one of claims 1-3, 5, 6, and 8-18, wherein the RNA component further comprises a gRNA nucleic acid.

20. The LNP composition of ~~claim 19~~any one of claims 1-19, wherein the gRNA nucleic acid is a gRNA.

21. The LNP composition of claim 19 or 20, wherein the gRNA nucleic acid is or encodes a dual-guide RNA (dgRNA).

22. The LNP composition of claim 19 or 20, wherein the gRNA nucleic acid is or encodes a single guide RNA (sgRNA).

23. The LNP composition of any one of claims 20-22, wherein the gRNA is modified.

24. The LNP composition of claim 23, wherein the gRNA comprises a modification selected from 2'-O-methyl (2'-O-Me) modified nucleotide, a phosphorothioate (PS) bond between nucleotides; and a 2'-fluoro (2'-F) modified nucleotide.

25. The LNP composition of claim 23 or 24, wherein the gRNA comprises a modification at one or more of the first five nucleotides at the 5' end.

26. The LNP composition of any one of claims 23-25, wherein the gRNA comprises a modification at one or more of the last five nucleotides at the 3' end.

27. The LNP composition of any one of claims 23-26, wherein the gRNA comprises PS bonds between the first four nucleotides at the 5' end.

28. The LNP composition of any one of claims 23-27, wherein the gRNA comprises PS bonds between the last four nucleotides at the 3' end.

29. The LNP composition of any one of claims 23-28, wherein the gRNA further compris~~ing~~es 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

30. The LNP composition of any one of claims 23-29, wherein the gRNA further compris~~ing~~es 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

31. The LNP composition of any one of claims 1-30, further comprising at least one template nucleic acid.

32. The LNP composition of any one of claims 1-31, wherein the lipid component comprises about 3 mol-% PEG lipid.

33. The LNP composition of any one of claims 1-32, wherein the lipid component comprises about 2.5 mol-% PEG lipid.

34. The LNP composition of any one of claims 1-33, wherein the lipid component comprises about 50 mol-% amine lipid.

35. The LNP composition of any one of claims 1-34, wherein the lipid component comprises about 55 mol-% amine lipid.

~~36. The LNP composition of any one of claims 1-35, wherein the mol-% amine lipid is ± 3 mol % of a target mol % of amine lipid.~~

~~37. The LNP composition of any one of claims 1-36, wherein the mol-% amine lipid is ± 2 mol % of a target mol % of amine lipid.~~

~~38~~36. The LNP composition of any one of claims 1-~~37~~35, wherein the lipid component comprises 47-53 mol-% mol-% amine lipid.

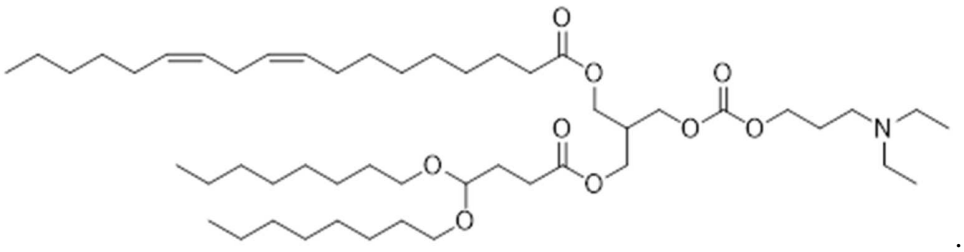
~~39~~37. The LNP composition of any one of claims 1-~~38~~36, wherein the lipid component comprises 48-53 mol-%~~ mol-%~~ amine lipid.

~~40~~38. The LNP composition of any one of claims 1-~~37~~39, wherein the lipid component comprises 53-57 mol-%~~ mol-%~~ amine lipid.

~~41~~39. The LNP composition of any one of claims 1-~~40~~38, wherein the N/P ratio is 6 ± 1.

~~42~~40. The LNP composition of any one of claims 1-~~41~~39, wherein the N/P ratio is 6 ± 0.5.

~~43~~41. The LNP composition of any one of claims 1-~~42~~40, wherein the amine lipid is Lipid A, wherein Lipid A is represented by the following structural formula:



.

~~44~~42. The LNP composition of any one of claims 1-~~42~~40, wherein R^1 and R^2 is each independently a C5-C12 alkyl.

~~45~~43. The LNP composition of any one of claims 1-~~42~~40, wherein R^1 and R^2 is each independently a C5-C10 alkyl.

~~46~~44. The LNP composition of any one of claims 1-~~42~~40, wherein R^1 and R^2 are each independently selected from a C4, C5, C6, C7, C8, C9, C10, C11, and C12 alkyl.

~~47~~45. The LNP composition of any one of claims 1-~~46~~44, wherein the helper lipid is a steroid, sterol, or alkyl resorcinol.

46. The LNP composition of any one of claims 1-44, wherein the helper lipid is selected from cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate.

~~48~~47. The LNP composition of any one of claims 1-~~47~~44, wherein the helper lipid is cholesterol.

~~49~~48. The LNP composition of any one of claims 1-~~48~~47, wherein the neutral lipid is a neutral phospholipid.

49. The LNP composition of any one of claims 1-47, wherein the neutral lipid is selected from dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC),

dioleylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC), 1,2-diarachidoyl-*sn*-glycero-3-phosphatidylcholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauroylphosphatidylcholine (DLPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (DBPC), 1-stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-*sn*-glycero-3-phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine, distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine, and combinations thereof.

50. The LNP composition of any one of claims 1-~~49~~47, wherein the neutral lipid is DSPC.

51. The LNP composition of any one of claims 1-~~49~~47, wherein the neutral lipid is DPPC.

52. The LNP composition of any one of claims 1-47, wherein the neutral lipid is dimyristoyl phosphatidylethanolamine (DMPE).

~~52~~53. The LNP composition of any one of claims 1-~~51~~52, wherein the PEG lipid comprises dimyristoylglycerol (DMG).

~~53~~54. The LNP composition of any one of claims 1-~~52~~53, wherein the PEG lipid comprises a PEG-2k.

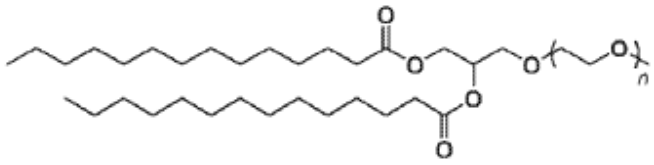
55. The LNP composition of any one of claims 1-52, wherein the PEG lipid is selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE), PEG-dilaurylglycamide, PEG-

dimyristylglycamide, PEG-dipalmitoylglycamide, PEG-distearoylglycamide, PEG-cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl-[omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]-methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DMG), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE), 1,2-distearoyl-*sn*-glycerol, methoxypolyethylene glycol (PEG2k-DSG), poly(ethylene glycol)-2000-dimethacrylate (PEG2k-DMA), and 1,2-distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA).

~~54~~56. The LNP composition of any one of claims 1-~~53~~52, wherein the PEG lipid is a PEG-DMG.

~~55~~57. The LNP composition of claim ~~54~~56, wherein the PEG-DMG is a PEG2k-DMG.

58. The LNP composition of any one of claims 1-52, wherein the PEG lipid has the following structural formula:



DMG-PEG, wherein n represents the number average degree of polymerization of ethylene oxide subunits, and n is 40-50

~~56~~59. The LNP composition of any one of claims 1-~~55~~58, wherein the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA.

~~57~~60. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 10:1 to 1:10 by weight.

~~58~~61. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 5:1 to 1:5 by weight.

~~59~~62. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 3:1 to 1:1 by weight.

~~60~~63. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 2:1 to 1:1 by weight.

~~61~~64. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio of about 2:1 by weight

~~62~~65. The LNP composition of claim ~~56~~59, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio of about 1:1 by weight.

~~63~~66. An in vitro method of gene editing, comprising contacting a cell with at least one LNP composition of any one of claims ~~1~~59-~~62~~65~~, wherein the RNA-guided DNA-binding agent is a Class 2 Cas nuclease mRNA~~.

~~64~~67. An in vitro method of gene editing, comprising delivering a Class 2 Cas nuclease mRNA and a guide RNA nucleic acid to a cell, wherein the Class 2 Cas nuclease mRNA and the guide RNA nucleic acid are formulated as at least one LNP composition of any one of claims ~~56~~59-~~62~~65.

~~65~~68. An in vitro method of producing a genetically engineered cell, comprising contacting a cell with at least one LNP composition of any one of claims ~~1~~59-~~62~~65.

~~66~~69. The method of any one of claims ~~63~~66-~~65~~68, wherein the LNP composition is administered at least two times.

~~67~~70. The method of claim ~~66~~69, wherein the LNP composition is administered 2-5 times.

~~68~~71. The method of claim ~~66-~~69 or ~~67~~70, wherein editing improves upon readministration.

~~69~~72. The method of any one of claims ~~63~~66-~~68~~71, further comprising introducing at least one template nucleic acid to the cell.

~~70. The method of any one of claims 63-69, comprising contacting the cell with a first LNP composition and a second LNP composition, wherein the first LNP composition is an LNP composition of any one of claims 1-55, and the second LNP composition comprises a gRNA.~~

~~71. The method of claim 70, wherein the cell is contacted with the first and second LNP compositions simultaneously.~~

~~72. The method of claim 70, wherein the cell is contacted with the first and second LNP compositions sequentially.~~