We claim:

1. A guide RNA (gRNA) comprising a 5’ end modification or a 3’ end modification and a conserved portion of an gRNA comprising one or more of:

(a) a shortened hairpin 1 region or a substituted and optionally shortened hairpin 1 region, wherein

(i) at least one of the following pairs of nucleotides are substituted in the substituted and optionally shortened hairpin 1 with Watson-Crick pairing nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10, and/or H1-4 and H1-9, and the hairpin 1 region optionally lacks

(aa) any one or two of H1-5 through H1-8,

(bb) one, two, or three of the following pairs of nucleotides: H1-1 and H1-12, H1-2 and H1-11, H1-3 and H1-10 and/or H1-4 and H1-9, and/or

(cc) 1-8 nucleotides of the hairpin 1 region; or

(ii) the shortened hairpin 1 region lacks 6-8 nucleotides, preferably 6 nucleotides; and

(A) one or more of positions H1-1, H1-2, or H1-3 is deleted or substituted relative to SEQ ID NO: 400 and/or

(B) one or more of positions H1-6 through H1-10 is substituted relative to SEQ ID NO: 400; or

(iii) the shortened hairpin 1 region lacks 5-10 nucleotides, preferably 5-6 nucleotides, and one or more of positions N18, H1-12, or n is substituted relative to SEQ ID NO: 400; and/or

(b) a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides and wherein the 6, 7, 8, 9, 10, or 11 nucleotides of the shortened upper stem region include less than or equal to 4 substitutions relative to SEQ ID NO: 400; and/or

(c) a substitution relative to SEQ ID NO: 400 at any one or more of LS6, LS7, US3, US10, B3, N7, N15, N17, H2-2 and H2-14, wherein the substituent nucleotide is neither a pyrimidine that is followed by an adenine, nor an adenine that is preceded by a pyrimidine; and/or

(d) an upper stem region, wherein the upper stem modification comprises a modification to any one or more of US1-US12 in the upper stem region.

2. The gRNA of claim 1, wherein position H1-1 is deleted.

3. The gRNA of claim 1, wherein position H1-1 is substituted.

4. The gRNA of any one of claims 1-3, wherein position H1-2 is deleted.

5. The gRNA of any one of claims 1-3, wherein position H1-2 is substituted.

6. The gRNA of any one of claims 1-5, wherein position H1-3 is deleted.

7. The gRNA of any one of claims 1-5, wherein position H1-3 is substituted.

8. The gRNA of any one of claims 1-7, wherein position H1-4 is deleted.

9. The gRNA of any one of claims 1-7, wherein position H1-5 is deleted.

10. The gRNA of any one of claims 1-9, wherein position H1-6 is deleted.

11. The gRNA of any one of claims 1-9, wherein position H1-6 is substituted.

12. The gRNA of any one of claims 1-11, wherein position H1-7 is deleted.

13. The gRNA of any one of claims 1-11, wherein position H1-7 is substituted.

14. The gRNA of any one of claims 1-13, wherein position H1-8 is deleted.

15. The gRNA of any one of claims 1-13, wherein position H1-8 is substituted.

16. The gRNA of any one of claims 1-15, wherein position H1-9 is deleted.

17. The gRNA of any one of claims 1-15, wherein position H1-9 is substituted.

18. The gRNA of any one of claims 1-17, wherein position H1-10 is deleted.

19. The gRNA of any one of claims 1-17, wherein position H1-10 is substituted.

20. The gRNA of any one of claims 1-19, wherein position H1-11 is deleted.

21. The gRNA of any one of claims 1-20, wherein position H1-12 is deleted.

22. The gRNA of any one of claims 1-21, wherein positions H1-11 and H1-12 are deleted.

23. The gRNA of any one of claims 1-22, wherein positions H1-7 is substituted with a G and/or H1-8 is substituted with a C.

24. The gRNA of any one of claims 1-23, wherein positions H1-6 and/or H1-7 are substituted.

25. The gRNA of any one of claims 1-24, wherein position H1-6 is substituted with a C and/or position H1-7 is substituted with a U.

26. The gRNA of any one of claims 1-25, wherein positions H1-1 and/or H1-12 are substituted.

27. The gRNA of any one of claims 1-26, wherein position H1-1 is substituted with a C and/or position H1-12 is substituted with a G.

28. The gRNA of any one of claims 1-27, wherein position N18 is substituted.

29. The gRNA of claim 28, wherein position N18 is substituted with a C.

30. The gRNA of any one of claims 1-29, wherein position H1-12 is substituted.

31. The gRNA of claim 30, wherein position H1-12 is substituted with a C or an A.

32. The gRNA of any one of claims 1-31, wherein position n is substituted.

33. The gRNA of claim 32, wherein position n is substituted with an A.

34. The gRNA of any one of claims 1-33, comprising a shortened upper stem region, wherein the shortened upper stem region lacks 1-6 nucleotides.

35. The gRNA of any one of claims 1-34, wherein the gRNA is an sgRNA.

36. The gRNA of any one of claims 1-35, wherein the gRNA comprises a 5' end modification.

37. The gRNA of any one of claims 1-36, wherein the gRNA comprises a 3' end modification.

38. The gRNA of any one of claims 1-37, wherein the gRNA comprises a 5' end modification and a 3' end modification.

39. The gRNA of any one of claims 1-38, wherein the gRNA comprises a 3' tail.

40. The gRNA of claim 39, wherein the 3' tail comprises 1-2, 1-3, 1-4, 1-5, 1-7, 1-10 nucleotides or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides.

41. The gRNA of any one of claims 1-38, wherein the gRNA does not comprise a 3' tail.

42. The gRNA of any one of claims 1-41, comprising a modification in the hairpin region.

43. The gRNA of claim 42, further comprising a 3' end modification.

44. The gRNA of claim 42, further comprising a 3' end modification and a 5' end modification.

45. The gRNA of claim 42, further comprising a 5' end modification.

46. The gRNA of any one of claims 1-45, further comprising a guide region.

47. The gRNA of claim 46, wherein the guide region is 17, 18, 19, or 20 nucleotides in length.

48. The gRNA of any one of claims 1-47, wherein the 3' and/or 5' end modification comprises a protective end modification, optionally a modified nucleotide selected from a 2'-O-methyl (2'-OMe) modified nucleotide, a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, an inverted abasic modified nucleotide, or a combination thereof.

49. The gRNA of any one of claims 1-48, comprising a modification in the hairpin region, wherein the modification in the hairpin region comprises a modified nucleotide selected from a 2'-O-methyl (2'-Ome) modified nucleotide, a 2'-fluoro (2'-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or a combination thereof.

50. The gRNA of any one of claims 1-49, wherein the 3’ and/or 5’ end modification comprises or further comprises a 2’-O-methyl (2’-Ome) modified nucleotide.

51. The gRNA of any one of claims 1-50, wherein the 3’ and/or 5’ end modification comprises or further comprises a 2’-fluoro (2’-F) modified nucleotide.

52. The gRNA of any one of claims 1-51, wherein the 3’ and/or 5’ end modification comprises or further comprises a phosphorothioate (PS) linkage between nucleotides.

53. The gRNA of any one of claims 1-52, wherein the 3’ and/or 5’ end modification comprises or further comprises an inverted abasic modified nucleotide.

54. The gRNA of any one of claims 1-53, comprising a modification in the hairpin region, wherein the modification in the hairpin region comprises or further comprises a 2’-O-methyl (2’-Ome) modified nucleotide.

55. The gRNA of any one of claims 1-54, comprising a modification in the hairpin region, wherein the modification in the hairpin region comprises or further comprises a 2’-fluoro (2’-F) modified nucleotide.

56. The gRNA of any one of claims 1-55, wherein the sgRNA comprise a 3’ tail, wherein the 3’ tail comprises a modification of any one or more of the nucleotides present in the 3’ tail.

57. The gRNA of claim 56, wherein the 3’ tail is fully modified.

58. The gRNA of any one of claims 1-57, wherein the upper stem region comprises at least one modification.

59. The gRNA of claim 58, wherein the upper stem modification comprises any one or more of:

i. a modification of any one or more of US1-US12 in the upper stem region; and

ii. a modification of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or all 12 nucleotides in the upper stem region.

60. The gRNA of claim 59, wherein the upper stem modification comprises one or more of:

i. a 2’-OMe modified nucleotide;

ii. a 2’-O-moe modified nucleotide;

iii. a 2’-F modified nucleotide; and

iv. combinations of one or more of (i.) – (iii.).

61. The gRNA of any one of claims 1-60, comprising a nucleotide sequence having at least 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75, or 70% identity to the nucleotide sequence of any one of SEQ ID NOs: 1-98, 201-294, 401-494, 601-698, or 801-875.

62. The gRNA of any one of claims 1-61, comprising a nucleotide sequence having at least 99, 98, 97, 96, 95, 94, 93, 92, 91, 90, 85, 80, 75, or 70% identity to the nucleotide sequence of any one of SEQ ID Nos: 101-198, 301-394, 501-594, 701-798, or 901-975, wherein the modification at each nucleotide of the gRNA that corresponds to a nucleotide of the reference sequence identifier in Table 1A is identical to or equivalent to the modification shown in the reference sequence identifier in Table 1A.

63. A guide RNA comprising any of SEQ ID NOs: 1-98, 201-294, 401-494, 601-698, or 801-875.

64. A guide RNA comprising any of SEQ ID NOs: 101-198, 301-394, 501-594, 701-798, or 901-975, including the modifications of Table 1A.

65. The gRNA of any one of claims 1-64, comprising a YA modification of one or more guide region YA sites.

66. The gRNA of any one of claims 1-65, comprising a YA modification wherein the modification comprises 2'-fluoro, 2'-H, 2'-OMe, ENA, UNA, inosine, or PS modification.

67. The gRNA of any one of claims 1-66, comprising a YA modification of one or more conserved region YA sites.

68. The gRNA of any one of claims 1-67, wherein at least one modified YA site comprises

(i) a 2'-OMe modification, optionally of the pyrimidine of the YA site;

(ii) a 2'-fluoro modification, optionally of the pyrimidine of the YA site; and/or

(iii) a PS modification, optionally of the pyrimidine of the YA site.

69. An LNP composition comprising a gRNA of any one of claims 1-68.

70. A composition comprising a gRNA of any one of claims 1-68 associated with a lipid nanoparticle (LNP).

71. A composition comprising the gRNA of any one of claims 1-68, or the composition of claim 69 or 70, further comprising a nuclease or an mRNA which encodes the nuclease.

72. The composition of claim 71, wherein the nuclease is a Cas protein.

73. The composition of claim 72, wherein the Cas protein is a Cas9.

74. The composition of claim 73, wherein the Cas9 is an *S. pyogenes* Cas9 or an *S. aureus* Cas9.

75. The composition of any one of claims 71-74, wherein the nuclease is a nickase or a dCas.

76. The composition of any one of claims 71-75, wherein the nuclease is modified.

77. The composition of claim 76, wherein the modified nuclease comprises a nuclear localization signal (NLS).

78. The composition of any one of claims 71-77, comprising an mRNA which encodes the nuclease.

79. The composition of claim 78, wherein the mRNA comprises the sequence of any one of SEQ ID NOs: 1099-1127 or 1129-1146.

80. A pharmaceutical formulation comprising the gRNA of any one of claims 1-68 or the composition of any one of claims 69-79 and a pharmaceutically acceptable carrier.

81. A method of modifying a target DNA comprising, delivering a Cas protein or a nucleic acid encoding a Cas protein, and any one or more of the following to a cell:

i. the gRNA of any one of claims 1-68;

ii. the composition of any one of claims 69-79; and

iii. the pharmaceutical formulation of claim 80.

82. The method of claim 81, wherein the method results in an insertion or deletion in a gene.

83. The method of claim 81 or 82, further comprising delivering to the cell a template, wherein at least a part of the template incorporates into a target DNA at or near a double strand break site induced by the Cas protein.

84. The gRNA of any one of claims 1-68, the composition of claims 69-79, or the pharmaceutical formulation of claim 80 for use in preparing a medicament for treating a disease or disorder.

85. Use of the gRNA of any one of claims 1-68, the composition of claims 69-79, or the pharmaceutical formulation of claim 80 in the manufacture of a medicament for treating a disease or disorder.

Claims

1. An invention not claimed in any divisional application.