CLAIMS

1. A guide RNA (gRNA) which is a short-single guide RNA (short-sgRNA) comprising a guide region and a conserved portion of an sgRNA, wherein the conserved portion comprises, from 5’ to 3’:

i. a lower stem region portion being nucleotides LS1 to LS6;

ii. a bulge region portion being nucleotides B1 to B2;

iii. an upper stem region being nucleotides US1 to US12;

iv. a bulge region portion being nucleotides B3 to B6;

v. a lower stem region portion being nucleotides LS7 to LS12;

vi. a nexus region being nucleotides N1 to N18; and

vii. a hairpin region, wherein the hairpin region comprises, from 5’ to 3’:

a. a hairpin 1 region being nucleotides H1-1 to H1-12;

b. a nucleotide; and

c. a hairpin 2 region being nucleotides H2-1 to H2-15,

wherein

I. the hairpin region lacks 5-10 of the nucleotides of (vii)(a)-(c);

II. the short-sgRNA comprises a 5’ end modification and/or a 3’ end modification; and

III. at least 10 nucleotides of the upper stem region comprise 2’-O-methyl (2’-O-Me) modifications.

2. The gRNA claim 1, wherein:

i. the short-sgRNA comprises a 3’ tail;

ii. the short-sgRNA comprises a 3’ tail and the 3’ tail comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides;

iii. the short-sgRNA does not comprise a 3’ tail;

iv. the short-sgRNA comprises a 3’ tail and the 3’ tail comprises a modification of any one or more of the nucleotides present in the 3’ tail; or

v. the short-sgRNA comprises a 3’ tail and the 3’ tail is fully modified.

3. The gRNA of claim 1 or claim 2, wherein the 5-10 nucleotides:

i. are consecutive;

ii. are within the hairpin 1 region;

iii. are within the hairpin 1 region and the nucleotide between the hairpin 1 region and the hairpin 2 region;

iv. are within the hairpin 1 region and the two nucleotides immediately 3’ of the hairpin 1 region;

v. include at least a portion of the hairpin 1 region;

vi. comprise nucleotides H1-6 to H1-12 and the nucleotide between H1-12 and H2-1, nucleotides H1-5 to H1-12, nucleotides H1-6 to H1-10, or nucleotides H1-5 to H1-8; or

vii comprise nucleotides H1-6 to H1-12 and the nucleotide between H1-12 and H2-1, nucleotides H1-5 to H1-12, nucleotides H1-6 to H1-10, or nucleotides H1-5 to H1-8 and wherein the short-sgRNA comprises modifications at at least nucleotides H1-1 to H1-5 and H2-1 to H2-12.

4. The gRNA of any one of claims 1-3, wherein:

i. the gRNA comprises a modification in the hairpin region comprising a modified nucleotide selected from a 2’-O-methyl (2’-O-Me) modified nucleotide, a 2’-fluoro (2’-F) modified nucleotide, a phosphorothioate (PS) linkage between nucleotides, or combinations thereof;

ii. the 3’ and/or 5’ end modification comprises or further comprises a 2’-O-methyl (2’-O-Me) modified nucleotide;

iii. the 3’ and/or 5’ end modification comprises or further comprises a 2’-O-(2-methoxyethyl) (2’-O-moe) modified nucleotide;

iv. the 3’ and/or 5’ end modification comprises or further comprises a 2’-fluoro (2’-F) modified nucleotide;

v. the 3’ and/or 5’ end modification comprises or further comprises a phosphorothioate (PS) linkage between nucleotides;

vi. the 3’ and/or 5’ end modification comprises or further comprises an inverted abasic modified nucleotide;

vii. the gRNA comprises a modification in the hairpin region comprising or further comprising a 2’-O-methyl (2’-O-Me) modified nucleotide; and/or

viii. the gRNA comprises a modification in the hairpin region comprising or further comprising a 2’-fluoro (2’-F) modified nucleotide.

5. A guide RNA which is a short-sgRNA comprising from 5’ to 3’, a guide region and a conserved portion, wherein the gRNA comprises a modified nucleotide sequence having at least 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identity to any one of:

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 206);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 207);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 228);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmA*mG*mU*mG (SEQ ID NO: 231);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAfCfUfUfGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 241);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmGfCmAfCmCfGmAfGmUfCmGfG*mU*fG*mC (SEQ ID NO: 242);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 306);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 307);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 328);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmA*mG*mU*mG (SEQ ID NO: 331);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAfCfUfUfGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 341);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmGfCmAfCmCfGmAfGmUfCmGfG*mU*fG*mC (SEQ ID NO: 342); or

mN*mN*mN*NNN*N*fN*fN*fN*fNNfNfNNNfNf$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 354);

wherein $(N)_x$ denotes x contiguous nucleotides, where x is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, m denotes a nucleotide that has been modified with 2'-O-Me, f denotes a nucleotide that has been modified with 2'-F, and * denotes a nucleotide that is linked to the next nucleotide with a phosphorothioate (PS) bond.

6. The gRNA of claim 5, comprising the modified nucleic acid sequence of any of:

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 206);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 207);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 228);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmA*mG*mU*mG (SEQ ID NO: 231);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAfCfUfUfGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 241);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmGfCmAfCmCfGmAfGmUfCmGfG*mU*fG*mC (SEQ ID NO: 242);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 306);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 307);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 328);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmA*mG*mU*mG (SEQ ID NO: 331);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAfCfUfUfGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 341);

mN*mN*mN*$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmGfCmAfCmCfGmAfGmUfCmGfG*mU*fG*mC (SEQ ID NO: 342); or

mN*mN*mN*NNN*N*fN*fN*fN*fNNfNfNNNfNf$(N)_x$GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 354);

wherein $(N)_x$ denotes x contiguous nucleotides, where x is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25, m denotes a nucleotide that has been modified with 2'-O-Me, f denotes a nucleotide that has been modified with 2'-F, and * denotes a nucleotide that is linked to the next nucleotide with a phosphorothioate (PS) bond.

7. The gRNA of any one of claims 1-6, wherein the guide region comprises at least 20 nucleotides and comprises modifications and/or unmodified nucleotides at at least 15, 16, 17, 18, 19 or all of nucleotides 1-20 from the 5' end of the 5' terminus that match the modification pattern selected from:

i. mN*mN*mN*NNNNNNNNNNNNNNNNN;

ii. (invd)mNmNmNNNNNNNNNNNNNNNNN;

iii. (invd)mN*mN*mN*NNNNNNNNNNNNNNNNN;

iv. mN*mN*mN*NNN*N*fN*fN*fN*fNNfNfNNNfNfNNN;

v. moeN*moeN*moeN*NNNNNNNNNNNNNNNNN; or

vi. (invd)NNNNNNNNNNNNNNNNNNN,

wherein N is a natural or non-natural nucleotide, mN denotes a nucleotide that has been substituted with a 2'-O-Me modified nucleotide, fN denotes a nucleotide that has been substituted with a 2'-fluoro (2'-F) modified nucleotide, moeN denotes a nucleotide that has been substituted with a 2'-O-(2-methoxyethyl) (2'-O-moe) modified nucleotide, * denotes a phosphorothioate (PS) bond between the two adjacent nucleotides, and (invd) denotes an inverted abasic modified nucleotide.

8. The gRNA of any one of claims 1-7, wherein the gRNA comprises a modified sequence having at least 90% identity to any one of:

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGGCACCGAGUCGG*mU*mG*mC (SEQ ID NO: 206);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAACUUGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 207);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 228);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAmAmCmUmUmGmAmAmAmAmA*mG*mU*mG (SEQ ID NO: 231);

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAfCfUfUfGmGmCmAmCmCmGmAmGmUmCmGmG*mU*mG*mC (SEQ ID NO: 241); or

GUUUUAGAmGmCmUmAmGmAmAmAmUmAmGmCAAGUUAAAAUAAGGCUAGUCCGUUAUCAfAmCfUmUfGmGfCmAfCmCfGmAfGmUfCmGfG*mU*fG*mC (SEQ ID NO: 242);

wherein m denotes a nucleotide that has been modified with 2'-O-Me, f denotes a nucleotide that has been modified with 2'-F, and * denotes a nucleotide that is linked to the next nucleotide with a phosphorothioate (PS) bond.

9. A lipid nanoparticle (LNP) composition comprising the gRNA of any one of claims 1-8.

10. A composition comprising the gRNA of any one of claims 1-8 further comprising a lipid nanoparticle (LNP).

11. A composition or lipid nanoparticle (LNP) composition comprising the gRNA of any one of claims 1-8, or the composition or LNP composition of claim 9 or 10:

i. further comprising a nuclease or a nucleic acid which encodes the nuclease;

i. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas protein;

ii. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas protein and the Cas protein is a Cas9;

iii. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a Cas protein, the Cas protein is a Cas9, and the Cas9 is an *S. pyogenes* Cas9 or an *S. aureus* Cas9;

iv. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is a nickase or a dCas;

v. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is modified;

vi. further comprising a nuclease or a nucleic acid which encodes the nuclease, wherein the nuclease is modified and the modified nuclease comprises a nuclear localization signal (NLS); or

vii. further comprising a nucleic acid which encodes a nuclease, wherein the nucleic acid comprises the sequence of any one of SEQ ID NOs: 3499-3527 and 3529-3546.

12. A pharmaceutical formulation comprising the gRNA of any one of claims 1-8 or the composition or LNP composition of any one of claims 9-11 and a pharmaceutically acceptable carrier.

13. 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 1-8;

ii. the composition or LNP composition of any one of claims 9-11; and

iii. the pharmaceutical formulation of claim 12.

14. The method of claim 13:

i. wherein the method results in an insertion or deletion in a gene; or

ii. 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.

15. Use of the gRNA of any one of claims 1-8, the composition or LNP composition of any one of claims 9-11, or the pharmaceutical formulation of claim 12 in the manufacture of a medicament for treating a disease or disorder.