

WHAT IS CLAIMED IS:

1. A composition for modifying a target nucleic acid, comprising:
 - (a) a targetable nuclease protein; and
 - (b) a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and
 - (iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, andwherein each of the first and the second CDL target sequences are capable of cleavage by the targetable nuclease protein or a complex comprising the targetable nuclease protein, andwherein the composition is formulated for non-viral delivery into a cell.
2. The composition of claim 1, wherein the targetable nuclease protein is an RNA-guided nuclease, optionally wherein the RNA-guided nuclease is a Cas protein, optionally wherein the composition further comprises an RNA comprising at least 17 nucleotides that are complementary to the first and/or second CDL target sequence.
3. The composition of claim 2, wherein the composition further comprises a donor guide RNA (gRNA) configured to form the complex comprising the targetable nuclease protein, and wherein (1) the donor gRNA comprises at least 17 nucleotides that are complementary to each of the first and the second CDL target sequences, and (2) each of the first and the second CDL target sequences are operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the CDL target sequences.
4. The composition of claim 2, wherein the composition further comprises a first donor guide RNA (gRNA) comprising at least 17 nucleotides that are complementary to the first CDL target sequence, a second donor gRNA comprising at least 17 nucleotides that are complementary to the second CDL target sequence, wherein each donor gRNA is configured to form a distinct complex comprising the targetable nuclease protein, and wherein each of the first and the second CDL target sequences are operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the CDL target sequences.

5. The composition of claim 3 or 4, wherein one or more of the donor gRNAs comprises at least 17 nucleotides that are complementary to a genomic target sequence of the cell.
6. A composition for modifying a target nucleic acid, comprising:
 - (a) a CRISPR-CAS RNA-guided nuclease;
 - (b) a donor guide RNA (gRNA); and
 - (c) a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and
 - (iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, andwherein (1) the donor gRNA comprises at least 17 nucleotides that are complementary to each of the first and the second CDL target sequences, and (2) each of the first and the second CDL target sequences are operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the CDL target sequences, andwherein the composition is formulated for non-viral delivery into a cell.
7. The composition of any one of claims 1 to 6, wherein the composition further comprises a second targetable nuclease protein, wherein the second targetable nuclease protein or a complex comprising the second targetable nuclease protein is capable of cleaving a genomic target sequence of the cell, optionally wherein the second targetable nuclease protein is an RNA-guided nuclease, optionally wherein the RNA-guided nuclease is a Cas protein, and optionally wherein the composition further comprises a second RNA comprising at least 17 nucleotides that are complementary to the genomic target sequence.
8. The composition of claim 7, wherein the composition further comprises a target guide RNA (gRNA) configured to form the complex comprising the second targetable nuclease protein, and wherein (1) the target gRNA comprises at least 17 nucleotides that are complementary to the genomic target sequence, and (2) the genomic target sequence is operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the genomic target sequence, optionally wherein the first CDL target sequence, the second

CDL target sequence, and the genomic target sequence comprise the same nucleic acid sequence.

9. The composition of any one of claims 7 or 8, wherein the genomic target sequence comprises a safe-harbor nucleic acid sequence, optionally wherein the safe-harbor nucleic acid sequence comprises the nucleic acid sequence GAGCCATGCTTGGCTTACGA.
10. The composition of any one of claims 3 to 9, wherein one, both, or neither of the PAM sequences are encoded between the CDL target sequences and the HDR template.
11. The composition of any one of claims 1 to 10, wherein the targetable nuclease protein and/or the second targetable nuclease protein is a Cas9 protein or comprises a transcription activator-like (TAL) effector DNA-binding protein and a nuclease or a zinc finger DNA-binding protein and a nuclease.
12. An in-vitro or ex-vivo method for modifying a target nucleic acid in a cell, comprising introducing or having non-virally introduced into the cell the composition of any one of claims 1 to 11, wherein the HDR template is integrated into the target nucleic acid.
13. The method of claim 12, wherein the introducing comprises electroporation.
14. The method of claim 12 or 13, wherein the cell is a primary cell or a primary T cell.
15. A ribonucleoprotein complex for modifying a target nucleic acid, comprising the composition of any one of claims 1 to 11.
16. A ribonucleoprotein complex for modifying a target nucleic acid,
 - (a) a CRISPR-CAS RNA-guided nuclease; and
 - (b) a donor guide RNA (gRNA), wherein the donor gRNA comprises at least 17 nucleotides that are complementary to a co-delivery linearization (CDL) target sequence, and

wherein the composition is formulated for non-viral delivery into a cell.

17. Use of the composition of any one of claims 1 – 11 in the manufacture of a medicament for modifying a target nucleic acid in a cell of a subject in need thereof, wherein the composition is to be introduced or non-virally introduced into the cell, and wherein the HDR template is to be integrated into the target nucleic acid.

18. The use of claim 17, wherein the introduction of the composition into the cell comprises electroporation.
19. The use of claim 17 or claim 18, wherein the cell is a primary cell or a primary T-cell.
20. A method of forming a ribonucleoprotein (RNP) complex, comprising incubating or having incubated (a) a CRISPR-CAS RNA-guided nuclease; and (b) a donor guide RNA (gRNA), wherein the donor gRNA comprises at least 17 nucleotides that are complementary to a co-delivery linearization (CDL) target sequence.
21. A composition comprising a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and
 - (iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, andwherein each of the first and the second CDL target sequences are capable of cleavage by a targetable nuclease protein or a complex comprising the targetable nuclease protein.
22. A method for modifying a target nucleic acid of a cell, the method comprising:
 - providing the cell, and
 - introducing or having introduced into the cell a composition formulated for non-viral delivery, the composition comprising:
 - (a) a targetable nuclease protein; and
 - (b) a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and
 - (iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, and

wherein each of the first and the second CDL target sequences are capable of cleavage by the targetable nuclease protein or a complex comprising the targetable nuclease protein.

23. A method for modifying a target nucleic acid of a cell, the method comprising:

- providing the cell, and
- introducing or having introduced into the cell a composition formulated for non-viral delivery, the composition comprising:
 - (a) a CRISPR-CAS RNA-guided nuclease;
 - (b) a donor guide RNA (gRNA); and
 - (c) a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and
 - (iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, and

wherein (1) the donor gRNA comprises at least 17 nucleotides that are complementary to each of the first and the second CDL target sequences, and (2) each of the first and the second CDL target sequences are operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the CDL target sequences.

24. A method for modifying a genomic target sequence of a cell, the method comprising:

- providing the cell, and
- introducing or having introduced into the cell a composition formulated for non-viral delivery, the composition comprising:
 - (a) a CRISPR-CAS RNA-guided nuclease;
 - (b) a donor guide RNA (gRNA); and
 - (c) a plasmid donor template comprising:
 - (i) a homology directed repair (HDR) template comprising a nucleic acid for insertion flanked by homology arms;
 - (ii) a first co-delivery linearization (CDL) target sequence, wherein the first CDL target sequence is operably linked 5' of the HDR template; and

(iii) a second CDL target sequence, wherein the second CDL target sequence is operably linked 3' of the HDR template, and wherein (1) the donor gRNA comprises at least 17 nucleotides that are complementary to each of the first and the second CDL target sequences, and (2) each of the first and the second CDL target sequences are operably linked to a 3-base pair protospacer adjacent motif (PAM) located 3' of the CDL target sequences

wherein the donor gRNA comprises at least 17 nucleotides that are complementary to the genomic target sequence of the cell, and

wherein the homology arms are complementary to nucleic acid sequences flanking the genomic target sequence of the cell, wherein the nucleic acid for insertion is configured for insertion into the genomic target sequence of the cell.