CRISPR-CAS EFFECTOR POLYPEPTIDES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/952,909, filed December 23, 2019, which application is incorporated herein by reference in its entirety.

INTRODUCTION

[0002] CRISPR-Cas systems include Cas proteins, which are involved in acquisition, targeting and cleavage of foreign DNA or RNA, and a guide RNA(s), which includes a segment that binds Cas proteins and a segment that binds to a target nucleic acid. For example, Class 2 CRISPR-Cas systems comprise a single Cas protein bound to a guide RNA, where the Cas protein binds to and cleaves a targeted nucleic acid. The programmable nature of these systems has facilitated their use as a versatile technology for use in modification of target nucleic acid.

SUMMARY

[0003] The present disclosure provides RNA-guided CRISPR-Cas effector proteins, nucleic acids encoding same, and compositions comprising same. The present disclosure provides ribonucleoprotein complexes comprising: an RNA-guided CRISPR-Cas effector protein of the present disclosure; and a guide RNA. The present disclosure provides methods of modifying a target nucleic acid, using an RNA-guided CRISPR-Cas effector protein of the present disclosure and a guide RNA.

[0003a] In one aspect, the present disclosure refers to a composition comprising:

a) a Cas12L polypeptide, or a nucleic acid molecule encoding the Cas12L polypeptide; and

b) a Cas12L guide RNA, or one or more DNA molecules encoding the Cas12L guide RNA.

[0003b] In one aspect, the present disclosure refers to a Cas12L fusion polypeptide comprising: a Cas12L polypeptide fused to a heterologous polypeptide.

[0003c] In one aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding any one of the Cas12L fusion polypeptides disclosed herein.

[0003d] In one aspect, the present disclosure refers to a eukaryotic cell comprising one or more of:

a) a Cas12L polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L polypeptide,

b) a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, and

c) a Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.

[0003e] In one aspect, the present disclosure refers to a cell comprising a comprising a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide.

[0003f] In one aspect, the present disclosure refers to a method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the Cas12L polypeptide.

[0003g] In one aspect, the present disclosure refers to a method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L fusion polypeptide comprising a Cas12L polypeptide fused to a heterologous polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid.

[0003h] In one aspect, the present disclosure refers to a system comprising:

a) a Cas12L polypeptide and a Cas12L guide RNA;

b) a Cas12L polypeptide, a Cas12L guide RNA, and a DNA donor template;

c) a Cas12L fusion polypeptide and a Cas12L guide RNA;

d) a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

e) an mRNA encoding a Cas12L polypeptide, and a Cas12L guide RNA;

f) an mRNA encoding a Cas12L polypeptide; a Cas12L guide RNA, and a DNA donor template;

g) an mRNA encoding a Cas12L fusion polypeptide, and a Cas12L guide RNA;

h) an mRNA encoding a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA; and

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and a DNA donor template.

[0003i] In one aspect, the present disclosure refers to a kit comprising the Cas12L system disclosed herein.

[0003j] In one aspect, the present disclosure refers to an implantable device comprising the Cas12L system disclosed herein.

[0003k]In one aspect, the present disclosure refers to a method of detecting a target DNA in a sample, the method comprising:

(a) contacting the sample with:

(i) a Cas12L polypeptide;

(ii) a guide RNA comprising: a region that binds to the Cas12L polypeptide, and a guide sequence that hybridizes with the target DNA; and

(iii) a detector nucleic acid that is single stranded and does not hybridize with the guide sequence of the guide RNA; and

(b) measuring a detectable signal produced by cleavage of the single stranded detector nucleic acid by the Cas12L polypeptide, thereby detecting the target DNA.

BRIEF DESCRIPTION OF THE DRAWINGS

[0004] **FIG. 1** presents a maximum Likelihood phylogenetic tree of the Cas12 superfamily, produced from a multiple sequence alignment with 1000 iterations. Cas12L forms a distinct clade separate from other Cas12 proteins.

[0005] **FIG. 2A-2Z, 2AA-2ZZ, and 2AAA-2FFF** provide amino acid sequences of Cas12L polypeptides.

[0006] **FIG. 3A-3B** provide the locations of active site residues of the RuvC-I, RuvC-II, and RuvC-III domains of Cas12L polypeptides.

[0007] **FIG. 4** depicts CRISPR locus architecture with active sites annotated.

CLAIMS

What is claimed is:

1. A composition comprising:

a) a Cas12L polypeptide, or a nucleic acid molecule encoding the Cas12L polypeptide; and

b) a Cas12L guide RNA, or one or more DNA molecules encoding the Cas12L guide RNA.

2. The composition of claim 1, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

3. The composition of claim 1 or claim 2, wherein the Cas12L guide RNA comprises a nucleotide sequence having 80%, 90%, 95%, 98%, 99%, or 100%, nucleotide sequence identity with any one of the crRNA sequences depicted in FIG. 7, FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

4. The composition of claim 1 or claim 2, wherein the Cas12L polypeptide is fused to a nuclear localization signal (NLS).

5. The composition of any one of claims 1-4, wherein:

a) the composition comprises a lipid.;

~~6. The composition of any one of claims 1-4, wherein~~b) components (a) and (b) are within a liposome.; or

~~7. The composition of any one of claims 1-4, wherein~~c) components (a) and (b) are within a particle.

~~8. The composition of any one of claims 1-7, comprising one or more of: a buffer, a nuclease inhibitor, and a protease inhibitor.~~

~~9. The composition of any one of claims 1-8, wherein the Cas12L polypeptide comprises an amino acid sequence having 85% or more identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~10~~6. The composition of any one of claims 1-~~9~~5, wherein the Cas12L polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule.

~~11~~7. The composition of any one of claims 1-~~9~~5, wherein the Cas12L polypeptide is a catalytically inactive Cas12L polypeptide (dCas12L).

~~12~~8. The composition of any one of claims 1-~~11~~7, wherein the Cas12L polypeptide has a length of from 700 amino acids to 800 amino acids.

~~13~~9. The composition of any one of claims 1-~~12~~8, further comprising a DNA donor template.

~~14~~10. A Cas12L fusion polypeptide comprising: a Cas12L polypeptide fused to a heterologous polypeptide.

~~15~~11. The Cas12L fusion polypeptide of Claim ~~14~~10, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

~~16. The Cas12L fusion polypeptide of Claim 14, wherein the Cas12L polypeptide comprises an amino acid sequence having 85% or more identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~17~~12. The Cas12L fusion polypeptide of ~~any one of claims 14-16~~claim 10 or claim 11, wherein the Cas12L polypeptide is a nickase that can cleave only one strand of a double-stranded target nucleic acid molecule~~.~~, or

~~18. The Cas12L fusion polypeptide of any one of claims 14-17,~~ wherein the Cas12L polypeptide is a catalytically inactive Cas12L polypeptide (dCas12L).

~~19. The Cas12L fusion polypeptide of any one of claims 14-18, wherein the Cas12L polypeptide has a length of from 700 amino acids to 800 amino acids.~~

~~20. The Cas12L fusion polypeptide of any one of claims 14-19, wherein the heterologous polypeptide is fused to the N-terminus and/or the C-terminus of the Cas12L polypeptide.~~

~~21~~13. The Cas12L fusion polypeptide of any one of claims ~~14~~10-~~20~~12, comprising a nuclear localization signal (NLS).

~~22. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide is a targeting polypeptide that provides for binding to a cell surface moiety on a target cell or target cell type.~~

~~23~~14. The Cas12L fusion polypeptide of any one of claims ~~14-21~~10-13, wherein the heterologous polypeptide exhibits enzymatic activity.

~~24. The Cas12L fusion polypeptide of claim 23, wherein the heterologous polypeptide exhibits an one or more enzymatic activities selected from: nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity and glycosylase activity.~~

~~25~~15. The Cas12L fusion polypeptide of claim ~~23~~14, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: reverse transcriptase activity, nuclease activity, methyltransferase activity, demethylase activity, deamination activity, depurination activity, integrase activity, transposase activity, and recombinase activity.

~~26. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid.~~

~~27. The Cas12L fusion polypeptide of claim 26, wherein the heterologous polypeptide exhibits histone modification activity.~~

~~28. The Cas12L fusion polypeptide of claim 26 or claim 27, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity,~~

~~demyristoylation activity, glycosylation activity (e.g., from O-GlcNAc transferase) and deglycosylation activity.~~

~~29. The Cas12L fusion polypeptide of claim 28, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, and deacetylase activity.~~

~~30. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide is an endosomal escape polypeptide.~~

~~31. The Cas12L fusion polypeptide of claim 30, wherein the endosomal escape polypeptide comprises an amino acid sequence selected from: GLFXALLXLLXSLWXLLLXA (SEQ ID NO:72), and GLFHALLHLLHSLWHLLLHA (SEQ ID NO:73), wherein each X is independently selected from lysine, histidine, and arginine.~~

~~32. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide is a chloroplast transit peptide.~~

~~33. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide comprises a protein transduction domain.~~

~~34~~16. The Cas12L fusion polypeptide of any one of claims ~~14-21~~10-13, wherein the heterologous polypeptide is a protein that increases or decreases transcription.

~~35. The Cas12L fusion polypeptide of claim 34, wherein the heterologous polypeptide is a transcriptional repressor domain.~~

~~36. The Cas12L fusion polypeptide of claim 34, wherein the heterologous polypeptide is a transcriptional activation domain.~~

~~37. The Cas12L fusion polypeptide of any one of claims 14-21, wherein the heterologous polypeptide is a protein binding domain.~~

~~38~~17. A nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide of any one of claims ~~14-37~~10-16.

39. The nucleic acid of Claim 38, wherein the nucleotide sequence encoding the Cas12L fusion polypeptide is operably linked to a promoter.

40. The nucleic acid of Claim 39, wherein the promoter is functional in a eukaryotic cell.

41. The nucleic acid of Claim 40, wherein the promoter is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell.

42. The nucleic acid of any one of Claims 39-41, wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.

43. The nucleic acid of any one of Claims 38-42, wherein the nucleic acid is a recombinant expression vector.

44. The nucleic acid of Claim 43, wherein the recombinant expression vector is a recombinant adenoassociated viral vector, a recombinant retroviral vector, or a recombinant lentiviral vector.

45. The nucleic acid of Claim 39, wherein the promoter is functional in a prokaryotic cell.

46. The nucleic acid of Claim 38, wherein the nucleic acid molecule is an mRNA.

47. One or more nucleic acids comprising:

(a) a nucleotide sequence encoding a Cas12L guide RNA; and

(b) a nucleotide sequence encoding a Cas12L polypeptide.

48. The one or more nucleic acids of claim 47, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

49. The one or more nucleic acids of claim 47, wherein the Cas12L polypeptide comprises an amino acid sequence having 85% or more identity to the amino acid depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

~~50. The one or more nucleic acids of any one of claims 47-49, wherein the Cas12L guide RNA comprises a nucleotide sequence having 80% or more nucleotide sequence identity with any one of the crRNA sequences set forth in FIG. 7, FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~51. The one or more nucleic acids of any one of claims 47-50, wherein the Cas12L polypeptide is fused to a nuclear localization signal (NLS).~~

~~52. The one or more nucleic acids of any one of claims 47-51, wherein the nucleotide sequence encoding the Cas12L guide RNA is operably linked to a promoter.~~

~~53. The one or more nucleic acids of any one of claims 47-52, wherein the nucleotide sequence encoding the Cas12L polypeptide is operably linked to a promoter.~~

~~54. The one or more nucleic acids of Claim 52 or Claim 53, wherein the promoter operably linked to the nucleotide sequence encoding the Cas12L guide RNA, and/or the promoter operably linked to the nucleotide sequence encoding the Cas12L polypeptide, is functional in a eukaryotic cell.~~

~~55. The one or more nucleic acids of Claim 54, wherein the promoter is functional in one or more of: a plant cell, a fungal cell, an animal cell, cell of an invertebrate, a fly cell, a cell of a vertebrate, a mammalian cell, a primate cell, a non-human primate cell, and a human cell.~~

~~56. The one or more nucleic acids of any one of Claims 53-55, wherein the promoter is one or more of: a constitutive promoter, an inducible promoter, a cell type-specific promoter, and a tissue-specific promoter.~~

~~57. The one or more nucleic acids of any one of Claims 47-56, wherein the one or more nucleic acids is one or more recombinant expression vectors.~~

~~58. The one or more nucleic acids of Claim 57, wherein the one or more recombinant expression vectors are selected from: one or more adenoassociated viral vectors, one or more recombinant retroviral vectors, or one or more recombinant lentiviral vectors.~~

~~59. The one or more nucleic acids of Claim 53, wherein the promoter is functional in a prokaryotic cell.~~

~~60~~18. A eukaryotic cell comprising one or more of:

a) a Cas12L polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L polypeptide,

b) a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, and

c) a Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.

~~61. The eukaryotic cell of claim 60, comprising the nucleic acid encoding the Cas12L polypeptide, wherein said nucleic acid is integrated into the genomic DNA of the cell.~~

~~62. The eukaryotic cell of claim 60 or claim 61, wherein the eukaryotic cell is a plant cell, a mammalian cell, an insect cell, an arachnid cell, a fungal cell, a bird cell, a reptile cell, an amphibian cell, an invertebrate cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, or a human cell.~~

~~63~~19. A cell comprising a comprising a Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide.

~~64. The cell of claim 63, wherein the cell is a prokaryotic cell.~~

~~65. The cell of claim 63 or claim 64, comprising the nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, wherein said nucleic acid molecule is integrated into the genomic DNA of the cell.~~

~~66~~20. A method of modifying a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid,

wherein said contacting results in modification of the target nucleic acid by the Cas12L polypeptide.

~~67. The method of claim 66, wherein said modification is cleavage of the target nucleic acid.~~

68. The method of claim 66 or claim 67, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA.

69. The method of any of claims 66-68, wherein said contacting takes place *in vitro* outside of a cell.

70. The method of any of claims 66-68, wherein said contacting takes place inside of a cell in culture.

71. The method of any of claims 66-68, wherein said contacting takes place inside of a cell *in vivo*.

72. The method of claim 70 or claim 71, wherein the cell is a eukaryotic cell.

73. The method of claim 72, wherein the cell is selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell.

74. The method of claim 70 or claim 71, wherein the cell is a prokaryotic cell.

75. The method of any one of claims 66-74, wherein said contacting results in genome editing.

76. The method of any one of claims 66-75, wherein said contacting comprises: introducing into a cell: (a) the Cas12L polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L polypeptide, and (b) the Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.

77. The method of claim 76, wherein said contacting further comprises: introducing a DNA donor template into the cell.

78. The method of any one of claims 66-77, wherein the Cas12L guide RNA comprises a nucleotide sequence having 80% or more nucleotide sequence identity with any one of the crRNA sequences set forth in FIG. 7, FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

79. The method of any one of claims 66-78, wherein the Cas12L polypeptide is fused to a nuclear localization signal.

~~80~~21. A method of modulating transcription from a target DNA, modifying a target nucleic acid, or modifying a protein associated with a target nucleic acid, the method comprising contacting the target nucleic acid with:

a) a Cas12L fusion polypeptide comprising a Cas12L polypeptide fused to a heterologous polypeptide; and

b) a Cas12L guide RNA comprising a guide sequence that hybridizes to a target sequence of the target nucleic acid.

~~81. The method of claim 80, wherein the Cas12L guide RNA comprises a nucleotide sequence having 80% or more nucleotide sequence identity with any one of the crRNA sequences set forth in FIG. 7, FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~82. The method of claim 80 or claim 81, wherein the Cas12L fusion polypeptide comprises nuclear localization signal.~~

~~83. The method of any of claims 80-82, wherein said modification is not cleavage of the target nucleic acid.~~

~~84. The method of any of claims 80-83, wherein the target nucleic acid is selected from: double stranded DNA, single stranded DNA, RNA, genomic DNA, and extrachromosomal DNA.~~

~~85. The method of any of claims 80-84, wherein said contacting takes place *in vitro* outside of a cell.~~

~~86. The method of any of claims 80-84, wherein said contacting takes place inside of a cell in culture.~~

~~87. The method of any of claims 80-84, wherein said contacting takes place inside of a cell *in vivo*.~~

~~88. The method of claim 86 or claim 87, wherein the cell is a eukaryotic cell.~~

~~89. The method of claim 88, wherein the cell is selected from: a plant cell, a fungal cell, a mammalian cell, a reptile cell, an insect cell, an avian cell, a fish cell, a parasite cell, an arthropod cell, a cell of an invertebrate, a cell of a vertebrate, a rodent cell, a mouse cell, a rat cell, a primate cell, a non-human primate cell, and a human cell.~~

~~90. The method of claim 86 or claim 87, wherein the cell is a prokaryotic cell.~~

~~91. The method of any one of claims 80-90, wherein said contacting comprises: introducing into a cell: (a) the Cas12L fusion polypeptide, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L fusion polypeptide, and (b) the Cas12L guide RNA, or a nucleic acid comprising a nucleotide sequence encoding the Cas12L guide RNA.~~

~~92. The method of any one of claims 80-91, wherein the Cas12L polypeptide is a catalytically inactive Cas12L polypeptide (dCas12L).~~

~~93. The method of any one of claims 80-92, wherein the Cas12L polypeptide has a length of from 700 amino acids to 800 amino acids.~~

~~94. The method of any one of claims 80-93, wherein the heterologous polypeptide exhibits an enzymatic activity.~~

~~95. The method of claim 94, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: nuclease activity, methyltransferase activity, demethylase activity, DNA repair activity, DNA damage activity, deamination activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, recombinase activity, polymerase activity, ligase activity, helicase activity, photolyase activity and glycosylase activity.~~

~~96. The method of claim 94, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: reverse transcriptase activity, nuclease activity, methyltransferase activity, demethylase activity, deamination activity, depurination activity, integrase activity, transposase activity, and recombinase activity.~~

~~97. The method of any one of claims 80-93, wherein the heterologous polypeptide exhibits an enzymatic activity that modifies a target polypeptide associated with a target nucleic acid.~~

~~98. The method of claim 97, wherein the heterologous polypeptide exhibits histone modification activity.~~

99. The method of claim 97 or claim 98, wherein the heterologous polypeptide exhibits an one or more enzymatic activities selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, demyristoylation activity, glycosylation activity (e.g., from O-GlcNAc transferase) and deglycosylation activity.

100. The method of claim 99, wherein the heterologous polypeptide exhibits one or more enzymatic activities selected from: methyltransferase activity, demethylase activity, acetyltransferase activity, and deacetylase activity.

101. The method of any one of claims 80-93, wherein the heterologous polypeptide is protein that increases or decreases transcription.

102. The method of claim 101, wherein the heterologous polypeptide is a transcriptional repressor domain.

103. The method of claim 101, wherein the heterologous polypeptide is a transcriptional activation domain.

104. The method of any one of claims 80-93, wherein the heterologous polypeptide is a protein biding domain.

105. A transgenic, multicellular, non-human organism whose genome comprises a transgene comprising a nucleotide sequence encoding one or more of:
a) a Cas12L polypeptide,
b) a Cas12L fusion polypeptide, and
c) a Cas12L guide RNA.

106. The transgenic, multicellular, non-human organism of claim 105, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence set forth in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.

~~107. The transgenic, multicellular, non-human organism of claim 105, wherein the Cas12L polypeptide comprises an amino acid sequence having 85% or more amino acid sequence identity to the amino acid sequence set forth in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~108. The transgenic, multicellular, non-human organism of any one of claims 105-107, wherein the organism is a plant, a monocotyledon plant, a dicotyledon plant, an invertebrate animal, an insect, an arthropod, an arachnid, a parasite, a worm, a cnidarian, a vertebrate animal, a fish, a reptile, an amphibian, an ungulate, a bird, a pig, a horse, a sheep, a rodent, a mouse, a rat, or a non-human primate.~~

~~109~~22. A system comprising:

a) a Cas12L polypeptide and a Cas12L guide RNA;

b) a Cas12L polypeptide, a Cas12L guide RNA, and a DNA donor template;

c) a Cas12L fusion polypeptide and a Cas12L guide RNA;

d) a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

e) an mRNA encoding a Cas12L polypeptide, and a Cas12L guide RNA;

f) an mRNA encoding a Cas12L polypeptide; a Cas12L guide RNA, and a DNA donor template;

g) an mRNA encoding a Cas12L fusion polypeptide, and a Cas12L guide RNA;

h) an mRNA encoding a Cas12L fusion polypeptide, a Cas12L guide RNA, and a DNA donor template;

i) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA;

j) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and iii) a DNA donor template;

k) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; and ii) a nucleotide sequence encoding a Cas12L guide RNA; and

l) one or more recombinant expression vectors comprising: i) a nucleotide sequence encoding a Cas12L fusion polypeptide; ii) a nucleotide sequence encoding a Cas12L guide RNA; and a DNA donor template.

~~110. The Cas12L system of claim 109, wherein the Cas12L polypeptide comprises an amino acid sequence having 50% or more amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~111. The Cas12L system of claim 109, wherein the Cas12L polypeptide comprises an amino acid sequence having 85% or more amino acid sequence identity to the amino acid sequence depicted in any one of FIG. 2A-2Z, FIG. 2AA-2ZZ, and FIG. 2AAA-2FFF.~~

~~112. The Cas12L system of any of claims 109-111, wherein the donor template nucleic acid has a length of from 8 nucleotides to 1000 nucleotides.~~

~~113. The Cas12L system of any of claims 109-111, wherein the donor template nucleic acid has a length of from 25 nucleotides to 500 nucleotides.~~

~~114~~23. A kit comprising the Cas12L system of ~~any one of claims 109-113~~claim 22.

~~115. The kit of claim 114, wherein the components of the kit are in the same container.~~

~~116. The kit of claim 114, wherein the components of the kit are in separate containers.~~

~~117. A sterile container comprising the Cas12L system of any one of claims 109-116.~~

~~118. The sterile container of claim 117, wherein the container is a syringe.~~

~~119~~24. An implantable device comprising the Cas12L system of ~~any one of claims 109-116~~claim 22.

~~120. The implantable device of claim 119, wherein the Cas12L system is within a matrix.~~

~~121. The implantable device of claim 119, wherein the Cas12L system is in a reservoir.~~

~~122~~25. A method of detecting a target DNA in a sample, the method comprising:

(a) contacting the sample with:

(i) a Cas12L polypeptide;

(ii) a guide RNA comprising: a region that binds to the Cas12L polypeptide, and a guide sequence that hybridizes with the target DNA; and

(iii) a detector nucleic acid that is single stranded and does not hybridize with the guide sequence of the guide RNA; and

(b) measuring a detectable signal produced by cleavage of the single stranded detector nucleic acid by the Cas12L polypeptide, thereby detecting the target DNA.

~~123~~26. The method of claim ~~122~~25, wherein the target DNA is bacterial DNA~~.~~.

~~124. The method of claim 122, wherein the target DNA is~~ viral DNA~~.~~, or

~~125. The method of claim 124, wherein the target DNA is papovavirus, human papillomavirus (HPV), hepadnavirus, Hepatitis B Virus (HBV), herpesvirus, varicella zoster virus (VZV), Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus, adenovirus, poxvirus, or parvovirus DNA.~~

~~126. The method of claim 122, wherein the target~~ DNA ~~is~~ from a human cell.

~~127. The method of claim 122, wherein the target DNA is human fetal or cancer cell DNA.~~

~~128. The method of claim 122, wherein the sample comprises a cell lysate.~~

~~129. The method of claim 122, wherein the sample comprises cells.~~

~~130. The method of claim 122, wherein the sample is a blood, serum, plasma, urine, aspirate, or biopsy sample.~~

~~131. The method of any one of claims 122-130, further comprising determining an amount of the target DNA present in the sample.~~

~~132. The method of claim 131, wherein said measuring a detectable signal comprises one or more of: visual based detection, sensor based detection, color detection, gold nanoparticle based detection, fluorescence polarization, colloid phase transition/dispersion, electrochemical detection, and semiconductor based sensing.~~

~~133. The method of any one of claims 122-132, wherein the labeled detector nucleic acid is DNA.~~

~~134. The method of any one of claims 122-132, wherein the labeled detector nucleic acid is RNA.~~

~~135. The method of any one of claims 122-134, wherein the labeled detector nucleic acid comprises a modified nucleobase, a modified sugar moiety, and/or a modified nucleic acid linkage.~~

~~136. The method of any one of claims 122-135, wherein the detectable signal is detectable in less than 45 minutes.~~

~~137. The method of any one of claims 122-135, wherein the detectable signal is detectable in less than 30 minutes.~~

~~138~~27. The method of ~~any one of claims 122-137~~claim 25, further comprising amplifying the target DNA in the sample by loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), recombinase polymerase amplification (RPA), strand displacement amplification (SDA), nucleic acid sequence-based amplification (NASBA), transcription mediated amplification (TMA), nicking enzyme amplification reaction (NEAR), rolling circle amplification (RCA), multiple displacement amplification (MDA), Ramification (RAM), circular helicase-dependent amplification (cHDA), single primer isothermal amplification (SPIA), signal mediated amplification of RNA technology (SMART), self-sustained sequence replication (3SR), genome exponential amplification reaction (GEAR), or isothermal multiple displacement amplification (IMDA).

~~139~~28. The method of any one of claims ~~122-138~~25-27, wherein target DNA in the sample is present at a concentration of less than 10 aM.

~~140. The method according to any one of claim 122-139, wherein the label of the labeled detector nucleic acid comprises a fluorescence-emitting dye pair.~~

~~141. The method according to claim 140, wherein the fluorescence-emitting dye pair is a fluorescence resonance energy transfer (FRET) pair.~~

~~142. The method according to claim 140, wherein the fluorescence-emitting dye pair is a quencher/fluor pair.~~

~~143. The method according to any one of claims 142-147, wherein the labeled detector nucleic acid comprises two or more fluorescence-emitting dye pairs.~~

~~144. The method according to claim 143, wherein said two or more fluorescence-emitting dye pairs include a fluorescence resonance energy transfer (FRET) pair and a quencher/fluor pair.~~