**THAT WHICH IS CLAIMED IS:**

1. A modified *Lachnospiraceae bacterium* CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) Cas12a (LbCas12a) polypeptide, wherein the modified LbCas12a polypeptide comprises an amino acid sequence having at least 80% identity to the amino acid sequence of **SEQ ID NO:1** (LbCas12a) and a mutation at one or more than one of the following positions of K116, K120, K121, D122, E125, T148, T149, T152, D156, E159, Q529, D535, K538, D541, Y542, L585, K591, M592, K595, V596, S599, K600, K601, Y616, Y646, W649 with reference to position numbering of **SEQ ID NO:1**, optionally a mutation at one or more than one of the following positions of K116, K120, K121, D122, E125, T152, D156, E159, G532, D535, K538, D541, and/or K595 with reference to position numbering of **SEQ ID NO:1**.

2. The modified LbCas12a polypeptide of claim 1, wherein the modified LbCas12a polypeptide comprises one or more than one or the amino acid mutations of K116R, K116N, K120R, K120H, K120N, K120T, K120Y, K120Q, K121S, K121T, K121H, K121R, K121G, K121D, K121Q, D122R, D122K, D122H, D122E, D122N, E125R, E125K, E125Q, E125Y, T148H, T148S, T148A, T148C, T149A, T149C, T149S, T149G, T149H, T149P, T149F, T149N, T149D, T149V, T152R, T152K, T152W, T152Y, T152H, T152Q, T152E, T152L, T152F, D156R, D156K, D156Y, D156W, D156Q, D156H, D156I, D156V, D156L, D156E, E159K, E159R, E159H, E159Y, E159Q, Q529N, Q529T, Q529H, Q529A, Q529F, Q529G, Q529G, Q529S, Q529P, Q529W, Q529D, G532D, G532N, G532S, G532H, G532F, G532K, G532R, G532Q, G532A, G532L, G532C, D535N, D535H, D535V, D535T, D535,S D535A, D535W, D535K, K538R K538V, K538Q, K538W, K538Y, K538F, K538H, K538L, K538M, K538C, K538G, K538A, K538P, D541N, D541H, D541R, D541K, D541Y, D541I, D541A, D541S, D541E, Y542R, Y542K, Y542H, Y542Q, Y542F, Y542L, Y542M, Y542P, Y542V, Y542N, Y542T, L585G, L585H, L585F, K591W, K591F, K591Y, K591H, K591R, K591S, K591A, K591G, K591P, M592R, M592K, M592Q, M592E, M592A, K595R, K595Q, K595Y, K595L, K595W, K595H, K595E, K595S, K595D, K595M, V596T, V596H, V596G, V596A, S599G, S599H, S599N, S599D, K600R, K600H, K600G, K601R, K601H, K601Q, K601T, Y616K, Y616R, Y616E, Y616F, Y616H, Y646R, Y646E, Y646K, Y646H, Y646Q, Y646W, Y646N, W649H, W649K, W649Y, W649R, W649E, W649S, W649V, and/or W649T with reference to position numbering of **SEQ ID NO:1**, optionally comprises one or more than one of the amino acid mutations of K116R, K116N, K120Y, K121S,

K121R, D122H, D122N, E125K, T152R, T152K, T152Y, T152Q, T152E, T152F, D156R, D156W, D156Q, D156H, D156I, D156V, D156L, D156E, E159K, E159R, G532N, G532S, G532H, G532K, G532R, G532L, D535N, D535H, D535T, D535,S D535A, D535W, K538R K538V, K538Q, K538W, K538Y, K538F, K538H, K538L, K538M, K538C, K538G, K538A, D541E, K595R, K595Q, K595Y, K595W, K595H, K595S, and/or K595M with reference to position numbering of **SEQ ID NO:1**.

3. The modified LbCas12a polypeptide of claim 1 or claim 2, wherein the modified LbCas12a polypeptide has an altered PAM (protospacer adjacent motif) specificity.

4. The modified LbCas12a polypeptide of claim 1 or claim 2, further comprising a mutation in the nuclease active site (e.g., RuvC domain) (e.g., deadLbCas12a, dLbCas12a).

5. A fusion protein comprising the modified LbCas12a polypeptide of any one of claims 1 to 4, and a linker, optionally a peptide linker.

6. The fusion protein of claim 5, wherein the peptide linker is linked to the C-terminus and/or the N-terminus of the modified LbCas12a polypeptide.

7. A fusion protein comprising the modified LbCas12a polypeptide of any one of claims 1 to 4, and a polypeptide of interest.

8. The fusion protein of claim 7, wherein the polypeptide of interest is linked to the modified LbCas12a polypeptide.

9. The fusion protein of claim 7 or claim 8, wherein the polypeptide of interest is linked to the C-terminus and/or the N-terminus of the modified LbCas12a polypeptide.

10. The fusion protein of any one of claims 7 to 9, wherein the polypeptide of interest is linked to the modified LbCas12a polypeptide via a linker, optionally a peptide linker.

11. The fusion protein of any one of claims 7 to 9, wherein the polypeptide of interest comprises at least one polypeptide or protein domain having deaminase (deamination) activity, nickase activity, recombinase activity, transposase activity, methylase activity,

glycosylase (DNA glycosylase) activity, glycosylase inhibitor activity (e.g., uracil-DNA glycosylase inhibitor (UGI)), demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, single-strand RNA cleavage activity, double-strand RNA cleavage activity, restriction endonuclease activity (e.g., Fok1), nucleic acid binding activity, methyltransferase activity, DNA repair activity, DNA damage activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, polymerase activity, ligase activity, helicase activity, and/or photolyase activity.

12. The fusion protein of any one of claims 7 to 11, wherein the polypeptide of interest comprises at least one polypeptide or protein domain having deaminase activity.

13. The fusion protein of claim 12, wherein the at least one polypeptide or protein domain having deaminase activity is a cytosine deaminase domain or an adenine deaminase domain.

14. The fusion protein of claim 13, wherein the cytosine deaminase domain is an apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) domain.

15. The fusion protein of claim 13, wherein the adenine deaminase domain is TadA (tRNA-specific adenosine deaminase) and/or TadA* (evolved tRNA-specific adenosine deaminase).

16. The fusion protein of any one of claims 7 to 14, wherein the at least one polypeptide has glycosylase inhibitor activity, optionally wherein the at least one polypeptide is a uracil-DNA glycosylase inhibitor (UGI).

17. A polynucleotide encoding the modified LbCas12a polypeptide of any one of claims 1 to 4, or the fusion protein of any one of claims 5 to 16.

18. The polynucleotide of claim 17, wherein the polynucleotide encoding the modified LbCas12a polypeptide or the polynucleotide encoding the fusion protein is operatively associated with a promoter, optionally wherein the promoter is a promoter region comprising an intron.

19. The polynucleotide of claim 17 or claim 18, wherein the polynucleotide is codon optimized for expression in an organism.

20. The polynucleotide of any one of claims 17 to 19, wherein the organism is an animal, a plant, a fungus, an archaeon, or a bacterium.

21. A complex comprising the modified LbCas12a polypeptide any one of claims 1 to 4, or the fusion protein of any one of claims 5 to 16, and a guide nucleic acid (e.g., CRISPR RNA, CRISPR DNA, crRNA, crDNA).

22. A nucleic acid construct encoding the complex of claim 21.

23. A composition comprising (a) the modified LbCas12a polypeptide of any one of claims 1 to 4, or the fusion protein of any one of claims 5 to 16 and (b) a guide nucleic acid.

24. An expression cassette or vector comprising the polynucleotide of any one of claims 17 to 20, or the nucleic acid construct of claim 22.

25. A Type V Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) - associated (Cas) (CRISPR-Cas) system comprising:

(a) a fusion protein comprising (i) the modified LbCas12a polypeptide of any one of claims 1 to 4 or a nucleic acid encoding the modified LbCas12a polypeptide of any one of claims 1 to 4, and (ii) a polypeptide of interest or a nucleic acid encoding the polypeptide of interest; and

(b) a guide nucleic acid (CRISPR RNA, CRISPR DNA, crRNA,crDNA) comprising a spacer sequence and a repeat sequence, wherein the guide nucleic acid is capable of forming a complex with the modified LbCas12a polypeptide or the fusion protein and the spacer sequence is capable of hybridizing to a target nucleic acid, thereby guiding the modified LbCas12a polypeptide and the polypeptide of interest to the target nucleic acid, whereby the target nucleic acid is modified (e.g., cleaving or editing) or modulated (e.g., modulating transcription).

26. The system of claim 25, wherein the modified LbCas12a polypeptide comprises a mutation in the nuclease active site (e.g., RuvC domain) (e.g., deadCas12a, dCas12a).

27. The system of claim 25 or claim 26, wherein the polypeptide of interest is linked to the C-terminus and/or the N-terminus of the modified LbCas12a polypeptide.

28. The system of any one of claims 25 to 27, wherein the polypeptide of interest is linked to the C-terminus and/or the N-terminus of the modified LbCas12a polypeptide via a linker, optionally a peptide linker.

29. The system of any one of claims 25 to 28, wherein the polypeptide of interest comprises at least one polypeptide or protein domain having deaminase (deamination) activity, nickase activity, recombinase activity, transposase activity, methylase activity, glycosylase (DNA glycosylase) activity, glycosylase inhibitor activity (e.g., uracil-DNA glycosylase inhibitor (UGI)). demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, nuclease activity, single-strand RNA cleavage activity, double-strand RNA cleavage activity, restriction endonuclease activity (e.g., Fok1), nucleic acid binding activity, methyltransferase activity, DNA repair activity, DNA damage activity, dismutase activity, alkylation activity, depurination activity, oxidation activity, pyrimidine dimer forming activity, integrase activity, transposase activity, polymerase activity, ligase activity, helicase activity, and/or photolyase activity.

30. The system of any one of claims 25 to 29, wherein the polypeptide of interest comprises at least one polypeptide or protein domain having deaminase activity.

31. The system of claim 30, wherein the at least one polypeptide or protein domain having deaminase activity is a cytosine deaminase domain or an adenine deaminase domain.

32. The system of claim 31, wherein the cytosine deaminase domain is an apolipoprotein B mRNA editing catalytic polypeptide-like (APOBEC) domain.

33. The system of claim 31, wherein the adenine deaminase domain is TadA (tRNA-specific adenosine deaminase) and/or TadA* (evolved tRNA-specific adenosine deaminase).

34. The system of any one of claims 25 to 32, wherein the polypeptide of interest comprises a uracil-DNA glycosylase inhibitor (UGI).

35. The system of any one of claims 25 to 34, wherein one or both of (a) and (b) are comprised in one or more expression cassettes and/or vectors.

36. A cell comprising the polynucleotide of any one of claims 17 to 20, the nucleic acid construct of claim 22, the expression cassette or vector of claim 24, or the system of any one of claims 25 to 35.

37. A method of modifying a target nucleic acid, comprising:

contacting the target nucleic acid with:

(a)(i) the modified LbCas12a polypeptide of any one of claims 1 to 4, or the fusion protein of any one of claims 5 to 16, and (ii) a guide nucleic acid (e.g., CRISPR RNA, CRISPR DNA, crRNA,crDNA);

(b) the complex of claim 21 and a guide nucleic acid;

(c) a composition comprising (i) the modified LbCas12a polypeptide of any one of claims 1 to 4, or the fusion protein of any one of claims 5 to 16, and (ii) a guide nucleic acid; and/or

(d) the system of any one of claims 25 to 35, thereby modifying the target nucleic acid.

38. A method of modifying a target nucleic acid, comprising

contacting a cell or a cell free system comprising the target nucleic acid with:

(a)(i) the polynucleotide of any one of claims 17 to 20, or an expression cassette or vector comprising the same, and (ii) a guide nucleic acid, or an expression cassette or vector comprising the same; and/or

(b) the nucleic acid construct of claim 22, or an expression cassette or vector comprising the same, thereby modifying the target nucleic acid.

39. A method of editing a target nucleic acid, comprising:

contacting the target nucleic acid with:

(a)(i) the fusion protein of any one of claims 7 to 16, and (a)(ii) a guide nucleic acid;

(b) a complex comprising the fusion protein of any one of claims 7 to 16, and a guide nucleic acid;

(c) a composition comprising the fusion protein of any one of claims 7 to 16 and a guide nucleic acid; and/or,

(d) the system of any one of claims 25 to 35, thereby editing the target nucleic acid.

40. A method of editing a target nucleic acid, comprising contacting a cell or a cell free system comprising the target nucleic acid with:

(a)(i) a polynucleotide encoding the fusion protein of any one of claims 7 to 16, or an expression cassette or vector comprising the same, and (a)(ii) a guide nucleic acid, or an expression cassette or vector comprising the same; and/or

(b) a nucleic acid construct encoding a complex comprising the fusion protein of any one of claims 7 to 16, and a guide nucleic acid, or an expression cassette or vector comprising the same; and/or

(c) the system of any one of claims 25 to 35, thereby editing the target nucleic acid.

41. A method of constructing a randomized DNA library comprising double stranded nucleic acid molecules for determining protospacer adjacent motif (PAM) requirements/specificity of a CRISPR-Cas nuclease having a PAM recognition site at the 5' end of the protospacer, the method comprising:

preparing two or more double stranded nucleic acid molecules comprising the following steps:

(a) synthesizing a non-target oligonucleotide (first) strand and a target oligonucleotide (second) strand for each of the two or more double stranded nucleic acid molecules, wherein the non-target oligonucleotide strand comprises, 5' to 3':

(i) a first sequence having about 5 to about 15 nucleotides,

(ii) a second sequence having at least four randomized nucleotides,

(iii) a protospacer sequence comprising about 16 to about 25 nucleotides, and

(iv) a third sequence having about 5 to about 20 nucleotides, wherein the first sequence having about 5 to 15 nucleotides of (i) is immediately adjacent to the 5' end of the second sequence of (ii), the second sequence of (ii) is immediately adjacent to the 5' end of

the protospacer sequence of (iii), and the protospacer sequence is immediately adjacent to the 5' end of the third sequence of (iv); and the target oligonucleotide (second) strand is complementary to the non-target oligonucleotide strand; and

(b) annealing the non-target oligonucleotide strand to the complementary target oligonucleotide strand to produce a double stranded nucleic acid molecule, wherein the first sequence comprises a restriction site (at its 5' end) and the third sequence comprises a restriction site (at its 3' end), wherein the first sequence (i), the protospacer sequence (iii) and the third sequence (iv) of each of the two or more double stranded nucleic acid molecules is identical, thereby constructing the randomized DNA library comprising double stranded nucleic acid molecules.

42. A method of constructing a randomized DNA library comprising double stranded nucleic acid molecules for determining protospacer adjacent motif (PAM) requirements/specificity of a CRISPR-Cas nuclease having a PAM recognition site at the 3' end of the protospacer, the method comprising: preparing two or more double stranded nucleic acid molecules comprising the following steps:

(a) synthesizing a non-target oligonucleotide (first) strand and a target oligonucleotide (second) strand for each of the two or more double stranded nucleic acid molecules, wherein the non-target oligonucleotide strand comprises, 5' to 3':

(i) a first sequence having about 5 to about 20 nucleotides,

(ii) a protospacer sequence comprising about 16 to about 25 nucleotides,

(iii) a second sequence having at least four randomized nucleotides, and

(iv) a third sequence having about 5 to about 15 nucleotides, wherein the first sequence having about 5 to 20 nucleotides of (i) is immediately adjacent to the 5' end of the protospacer sequence of (ii), the second sequence of (iii) is immediately adjacent to the 3' end of the protospacer sequence of (iii), and the third sequence of (iv) is immediately adjacent to the 3' end of the second sequence of (iii); and the target oligonucleotide (second) strand is complementary to the non-target oligonucleotide strand; and

(b) annealing the non-target oligonucleotide strand to the complementary target oligonucleotide strand to produce a double stranded nucleic acid molecule, wherein the first sequence (i) comprises a restriction site (at its 5' end) and the third sequence (iv) comprises a restriction site (at its 3' end), wherein the first sequence (i), the protospacer sequence (ii) and the third sequence (iv) of each of the two or more double stranded nucleic acid molecules is

identical, thereby constructing the randomized DNA library comprising double stranded nucleic acid molecules.

43. The method of claim 41 or claim 42, further comprising ligating the double stranded nucleic acid molecules into a vector to produce a vector comprising the randomized DNA library.

44. The method of any one of claims 41 to 43, further comprising amplifying the randomized DNA library.

45. The method of claim 44, wherein amplifying comprises introducing the vector comprising the randomized DNA library into one or more bacterial cells and culturing the one or more bacterial cells.

46. The method of claim 45, further comprising isolating the vector comprising the randomized DNA library from the one or more bacterial cells.

47. The method of claim 46, further comprising linearizing the vector to provide the randomized DNA library.

48. The method of claim 47, wherein the linearizing comprises contacting the vector with a restriction enzyme.

49. The method of claim 48, wherein the restriction enzyme is ScaI or PfoI.

50. A randomized DNA library produced by the method of any one of claims 41 to 49.

51. A randomized DNA library may be provided for determining protospacer adjacent motif (PAM) requirements/specificity of a CRISPR-Cas nuclease having a PAM recognition site on the 5' end of protospacer, the randomized DNA library comprising two or more double stranded nucleic acid molecules each of which comprises:

(a) a non-target oligonucleotide (first) strand and a target oligonucleotide (second) strand, wherein the non-target oligonucleotide strand comprises, 5' to 3':

(i) a first sequence having about 5 to about 15 nucleotides (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 nucleotides, and any range or value therein),

(ii) a second sequence having at least four randomized nucleotides (e.g., at least 4, 5, 6, 7, 8, 9, 10, or more, and any range or value therein),

(iii) a protospacer sequence comprising about 16 to about 25 nucleotides e.g., about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides, and

(iv) a third sequence having about 5 to about 20 nucleotides (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides, and any range or value therein), wherein the first sequence having about 5 to 15 nucleotides of (i) is immediately adjacent to the 5' end of the second sequence of (ii), the second sequence of (ii) is immediately adjacent to the 5' end of the protospacer sequence of (iii), and the protospacer sequence is immediately adjacent to the 5' end of the third sequence of (iv); and the target oligonucleotide (second) strand is complementary to the non-target oligonucleotide strand; and

(b) the non-target oligonucleotide strand is annealed to the complementary target oligonucleotide strand to produce a double stranded nucleic acid molecule, wherein the first sequence comprises a restriction site (at its 5' end) and the third sequence comprises a restriction site (at its 3' end), wherein the first sequence (i), the protospacer sequence (iii) and the third sequence (iv) of each of the two or more double stranded nucleic acid molecules are identical.

52. A randomized DNA library may be provided for determining protospacer adjacent motif (PAM) requirements/specificity of a CRISPR-Cas nuclease having a PAM recognition site on the 3' end of protospacer, the randomized DNA library comprising two or more double stranded nucleic acid molecules each of which comprises:

(a) a non-target oligonucleotide (first) strand and a target oligonucleotide (second) strand, wherein the non-target oligonucleotide strand comprises, 5' to 3':

(i) a first sequence having about 5 to about 20 nucleotides (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 nucleotides, and any range or value therein)

(ii) a protospacer sequence comprising about 16 to about 25 nucleotides e.g., about 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides,

(iii) a second sequence having at least four randomized nucleotides (e.g., at least 4, 5, 6, 7, 8, 9, 10, or more, and any range or value therein), and

(iv) a third sequence having about 5 to about 15 nucleotides (e.g., about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 nucleotides, and any range or value therein), wherein the first sequence

having about 5 to 20 nucleotides of (i) is immediately adjacent to the 5' end of the protospacer sequence of (ii), the second sequence of (iii) is immediately adjacent to the 3' end of the protospacer sequence of (iii), and the third sequence of (iv) is immediately adjacent to the 3' end of the second sequence of (iii); and the target oligonucleotide (second) strand is complementary to the non-target oligonucleotide strand; and

(b) the non-target oligonucleotide strand is annealed to the complementary target oligonucleotide strand to produce a double stranded nucleic acid molecule, wherein the first sequence comprises a restriction site (at its 5' end) and the third sequence comprises a restriction site (at its 3' end), wherein the first sequence (i), the protospacer sequence (ii) and the third sequence (iv) of each of the two or more double stranded nucleic acid molecules are identical

53. A method of determining the protospacer adjacent motif (PAM) specificity of a CRISPR-Cas nuclease, the method comprising:

contacting the CRISPR-Cas nuclease with the randomized DNA library of claim 51 or 52; and

sequencing the double stranded nucleic acid molecules of the randomized DNA library before (control) and after contact with the nuclease, wherein the double stranded nucleic acid molecules present in the randomized DNA library prior to contact with the nuclease but that are absent in the randomized DNA library after contact with the nuclease identifies the PAM recognition sequence of the CRISPR-Cas nuclease, there by determining the PAM specificity of the CRISPR-Cas nuclease.

54. The method of claim 53, wherein the sequencing comprises next generation sequencing.

55. A kit comprising the polynucleotide of any one of claims 17 to 20 or 22, and/or an expression cassette or vector comprising the same, and optionally instructions for the use thereof.

56. The kit of claim 55, further comprising a CRISPR-Cas12a guide nucleic acid and/or expression cassette or vector comprising the same.

57. The kit of claim 56, wherein the guide nucleic acid comprises a cloning site for cloning of a nucleic acid sequence that is identical or complementary to a target nucleic acid sequence into the backbone of the guide nucleic acid.

58. The kit of claim 55 to 57, wherein the polynucleotide further encodes one or more nuclear localization signals, wherein the one or more nuclear localization signals are fused to the CRISPR-Cas12a nuclease.

59. The kit of claim 55 to 58, wherein the polynucleotide, expression cassette or vector further encodes one or more selectable markers.

60. The kit of claim 55 to 59, wherein the polynucleotide is an mRNA and encodes one or more introns within the encoded CRISPR-Cas12a nuclease.