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(54) Title: PROGRAMMABLE NUCLEASES AND METHODS OF USE

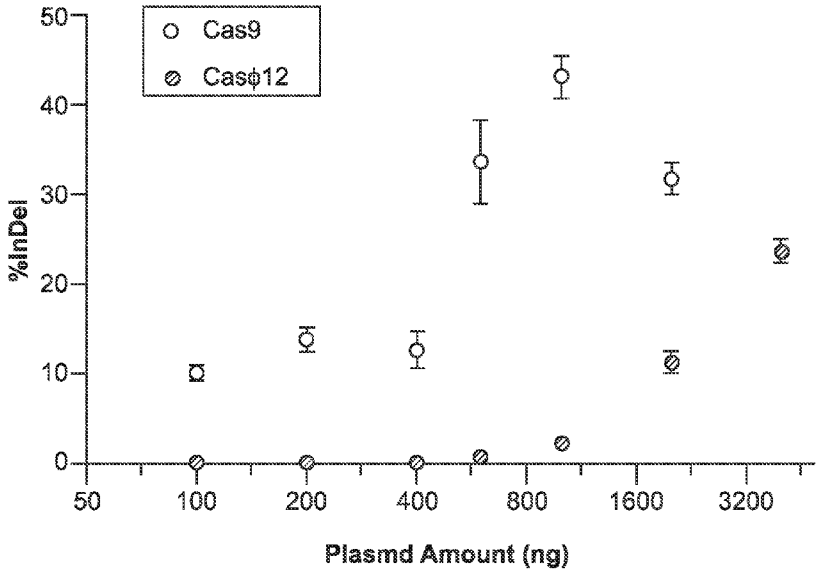


FIG. 17

(57) Abstract: Provided herein, in certain embodiments, are programmable nucleases, guide nucleic acids, and complexes thereof. Certain programmable nucleases provided herein comprise a RuvC domain. Also provided herein are nucleic acids encoding said programmable nucleases and guide nucleic acids. Also provided herein are methods of genome editing, methods of regulating gene expression, and methods of detecting nucleic acids with said programmable nucleases and guide nucleic acids.

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PROGRAMMABLE NUCLEASES AND METHODS OF USE

CROSS-REFERENCE

[0001] The present application claims priority to and benefit from U.S. Provisional Application No.: 63/034,346, filed on June 3, 2020, U.S. Provisional Application No.: 63/037,535, filed on June 10, 2020, U.S. Provisional Application No.: 63/040,998, filed on June 18, 2020, U.S. Provisional Application No.: 63/092,481, filed on October 15, 2020, U.S. Provisional Application No.: 63/116,083, filed on November 19, 2020, U.S. Provisional Application No.: 63/124,676, filed on December 11, 2020, U.S. Provisional Application No.: 63/156,883, filed on March 4, 2021, and U.S. Provisional Application No.: 63/178,472, filed on April 22, 2021, the entire contents of each of which are herein incorporated by reference.

BACKGROUND

[0002] Certain programmable nucleases can be used for genome editing of nucleic acid sequences or detection of nucleic acid sequences. There is a need for high efficiency, programmable nucleases that are capable of working under various sample conditions and can be used for both genome editing and diagnostics.

SUMMARY

[0003] In various aspects, the present disclosure provides a composition comprising: a) a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and b) a guide nucleic acid or a nucleic acid encoding said guide nucleic acid, wherein said guide nucleic acid comprises a region comprising a nucleotide sequence that is complementary to a target nucleic acid sequence and an additional region, wherein said region and said additional region are heterologous to each other.

[0004] In some aspects, the additional region of the guide nucleic acid comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the programmable CasΦ nuclease comprises nickase activity. In some aspects, the programmable CasΦ nuclease comprises double-strand cleavage activity. In

some aspects, the programmable CasΦ nuclease comprises at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

[0005] In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises at least 98% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the guide nucleic acid does not comprise a tracrRNA. In some aspects, the programmable CasΦ nuclease does not require a tracrRNA. In some aspects, the programmable CasΦ nuclease comprises greater nickase activity when complexed with the guide nucleic acid at a temperature from about 20°C to about 25°C, as compared with complex formation at a temperature of about 37°C. In some aspects, the guide nucleic acid comprises at least 98% sequence identity to SEQ ID NO: 54. In some aspects, the guide nucleic acid comprises at least 98% sequence identity to SEQ ID NO: 57. In some aspects, the programmable CasΦ nuclease comprises greater nickase activity when complexed with the guide nucleic acid comprising a sequence comprising at least 98% sequence identity to SEQ ID NO: 57, as compared to when complexed with a guide nucleic acid comprising SEQ ID NO: 49.

[0006] In some aspects, the programmable CasΦ nuclease exhibits greater nicking activity as compared to double stranded cleavage activity. In some aspects, the programmable CasΦ nuclease exhibits greater double stranded cleavage activity as compared to nicking activity. In some aspects, the programmable CasΦ nuclease comprises a single active site in a RuvC domain that is capable of catalyzing pre-crRNA processing and nicking or cleaving of nucleic acids. In some aspects, the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TBN-3', wherein B is one or more of C, G, or, T. In some aspects, the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTTN-3'.

[0007] In various aspects, the present disclosure provides a method of modifying a target nucleic acid sequence, the method comprising: contacting a target nucleic acid sequence with a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and a guide nucleic acid, wherein the programmable CasΦ nuclease cleaves the target nucleic acid sequence, thereby modifying the target nucleic acid sequence.

[0008] In some aspects, the programmable CasΦ nuclease introduces a double-stranded break in the target nucleic acid sequence. In some aspects, the programmable CasΦ nuclease comprises double-strand cleavage activity. In some aspects, the programmable CasΦ nuclease cleaves a single-strand of the target nucleic acid sequence. In some aspects, the programmable CasΦ nuclease comprises nickase activity. In some aspects, the programmable CasΦ nuclease exhibits greater nicking activity as compared to double stranded cleavage activity. In some aspects, the programmable CasΦ nuclease exhibits greater double stranded cleavage activity as compared to nicking activity. In some aspects, the target nucleic acid is DNA. In some aspects, the target nucleic acid is double-stranded DNA. In some aspects, the programmable CasΦ nuclease cleaves a non-target strand of the double-stranded DNA, wherein the non-target strand is non-complementary to the guide nucleic acid. In some aspects, the programmable CasΦ nuclease does not cleave a target strand of the double-stranded DNA, wherein the target strand is complementary to the guide nucleic acid.

[0009] In some aspects, the programmable CasΦ nuclease comprises at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises at least 98% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the guide nucleic acid comprises a sequence comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

[0010] In some aspects, the guide nucleic acid does not comprise a tracrRNA. In some aspects, the target nucleic acid sequence comprises a mutated sequence or a sequence associated with a disease. In some aspects, the mutated sequence is removed after the programmable CasΦ nuclease cleaves the target nucleic acid sequence. In some aspects, the target nucleic acid sequence is in a human cell. In some aspects, the method is performed in vivo. In some aspects, the method is performed ex vivo. In some aspects, the method further comprises inserting a donor polynucleotide into the target nucleic acid sequence at the site of cleavage.

[0011] In various aspects, the present disclosure provides a method of introducing a break in a target nucleic acid, the method comprising: contacting the target nucleic acid with: (a) a first guide nucleic acid comprising a region that binds to a first programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107; and (b) a second guide nucleic acid comprising a region that binds to a second programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, wherein the first guide nucleic acid comprises a first additional region that binds to the target nucleic acid and wherein the second guide nucleic acid comprises a second additional region that binds to the target nucleic acid and wherein the first additional region of the first guide nucleic acid and the second additional region of the second guide nucleic acid bind opposing strands of the target nucleic acid. In some aspects, the first programmable nickase, the second programmable nickase, or both comprise at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

[0012] In some aspects, the first programmable nickase, the second programmable nickase, or both comprise at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the first programmable nickase, the second programmable nickase, or both comprise a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

[0013] In some aspects, the first programmable nickase and the second programmable nickase exhibit greater nicking activity as compared to double stranded cleavage activity. In some aspects, the first programmable nickase and the second programmable nickase nick the target nucleic acid at two different sites. In some aspects, the target nucleic acid comprises double stranded DNA. In some aspects, the two different sites are on opposing strands of the double stranded DNA. In some aspects, the target nucleic acid comprises a mutated sequence or a sequence is associated with a disease. In some aspects, the mutated sequence is removed after

the first programmable nickase and the second programmable nickase nick the target nucleic acid. In some aspects, the target nucleic acid is in a cell. In some aspects, the method is performed in vivo. In some aspects, the method is performed ex vivo. In some aspects, the first programmable nickase and the second programmable nickase are the same. In some aspects, the first programmable nickase and the second programmable nickase are different.

[0014] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising contacting a sample comprising a target nucleic acid with (a) a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107; (b) a guide RNA comprising a region that binds to the programmable CasΦ nuclease and an additional region that binds to the target nucleic acid; and (c) a labeled single stranded DNA reporter that does not bind the guide RNA; cleaving the labeled single stranded DNA reporter by the programmable CasΦ nuclease to release a detectable label; and detecting the target nucleic acid by measuring a signal from the detectable label.

[0015] In some aspects, the target nucleic acid is single stranded DNA. In some aspects, the target nucleic acid is double stranded DNA. In some aspects, the target nucleic acid is a viral nucleic acid. In some aspects, the target nucleic acid is bacterial nucleic acid. In some aspects, the target nucleic acid is from a human cell. In some aspects, the target nucleic acid is a fetal nucleic acid. In some aspects, the sample is derived from a subject's saliva, blood, serum, plasma, urine, aspirate, or biopsy sample. In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

[0016] In some aspects, the guide RNA comprises at least about 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide RNA comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the sample comprises a phosphate buffer, a Tris buffer, or a HEPES buffer. In some aspects, the sample comprises a pH of 7 to 9. In some aspects, the sample comprises a pH of 7.5 to 8. In some aspects, the sample comprises a salt concentration of 25 nM to 200 mM. In some aspects, the single stranded DNA reporter comprises an ssDNA- fluorescence quenching DNA reporter. In some aspects, the ssDNA- fluorescence quenching DNA reporter is a universal

ssDNA- fluorescence quenching DNA reporter. In some aspects, the programmable CasΦ nuclease exhibits PAM-independent cleaving.

[0017] In various aspects, the present disclosure provides a method of modulating transcription of a gene in a cell, the method comprising: introducing into a cell comprising a target nucleic acid sequence: (i) a fusion polypeptide or a nucleic acid encoding the fusion polypeptide, wherein the fusion polypeptide comprises: (a) a dCasΦ polypeptide comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, wherein the dCasΦ polypeptide is enzymatically inactive; and (b) a polypeptide comprising transcriptional regulation activity; and (ii) a guide nucleic acid, or a nucleic acid comprising a nucleotide sequence encoding the guide nucleic acid, wherein the guide nucleic acid comprises a region that binds to the dCasΦ polypeptide and an additional region that binds to the target nucleic acid; wherein transcription of the gene is modulated through the fusion polypeptide acting on the target nucleic acid sequence.

[0018] In some aspects, the dCasΦ polypeptide comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107. In some aspects, the guide nucleic acid comprises at least about 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some aspects, the polypeptide comprising transcriptional regulation activity polypeptide comprises transcription activation activity.

[0019] In some aspects, the polypeptide comprising transcriptional regulation activity polypeptide comprises transcription repressor activity. In some aspects, the polypeptide comprising transcriptional regulation activity polypeptide comprises an activity selected from the group consisting of transcription activation activity, transcription repression activity, nuclease activity, transcription release factor activity, histone modification activity, histone acetyltransferase activity, nucleic acid association activity, DNA methylase activity, direct or indirect DNA demethylase activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, deaminase activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, and demyristoylation activity.

[0020] In various aspects, the present disclosure provides a composition comprising: a) a Cas nuclease or nucleic acid encoding said Cas nuclease, and b) a guide nucleic acid or a nucleic acid encoding said guide nucleic acid, wherein said guide nucleic acid comprises a region comprising a nucleotide sequence that is complementary to a target nucleic acid sequence and an additional region, wherein said region and said additional region are heterologous to each other; wherein the Cas nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving a target nucleic acid. In some aspects, the same active site in the RuvC domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid. In some aspects, the Cas nuclease is the programmable CasΦ nuclease as disclosed herein. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TBN-3', wherein B is one or more of C, G, or, T. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTTN-3'. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTN-3'. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTB-3', wherein B is C, G, or T. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTK-3', 5'-VTTK-3', 5'-VTTS-3', 5'-TTTS-3' or 5'-VTTN-3', where K is G or T, V is A, C or G, and S is C or G. In some aspects, the composition is used in any of the above methods.

[0021] In various aspects, the present disclosure provides the use of a programmable CasΦ nuclease to modify a target nucleic acid sequence according to any one of the above methods. In various aspects, the present disclosure provides the use of a first programmable nickase and a second programmable nickase to introduce a break in a target nucleic acid according to any one of the above methods. In various aspects, the present disclosure provides the use of a programmable CasΦ nuclease to detect a target nucleic acid in a sample according to any one of the above methods. In various aspects, the present disclosure provides the use of a dCasΦ polypeptide to modulate transcription of a gene in a cell according to any one of the above methods. In some aspects, the region is a spacer region and the additional region is a repeat region. In some aspects, the region is a repeat region and the additional region is a spacer region. In some aspects, the repeat region comprises a GAC sequence, optionally wherein the GAC sequence is at the 3' end of the repeat region. In some aspects, the repeat region comprises a hairpin, optionally wherein the hairpin is in the 3' portion of the repeat region. In some aspects, the hairpin comprises a double-stranded stem portion and a single-stranded loop portion. In some aspects, a strand of the stem portion comprises a CYC sequence and the other strand of the stem portion comprises a GRG sequence, wherein Y and R are complementary. In some aspects, the G of the GAC sequence is in the stem portion of the hairpin. In some aspects, each strand of the

stem portion comprises 3, 4 or 5 nucleotides. In some aspects, the loop portion comprises between 2 and 8 nucleotides, optionally wherein the loop portion comprises 4 nucleotides. In some aspects, the guide nucleic acid comprises at least 98% sequence identity to SEQ ID NO: 54.

[0022] In some aspects, the repeat region is between 15 and 50 nucleotides in length, preferably, wherein the repeat region is between 19 and 37 nucleotides in length. In some aspects, the spacer region is between 15 and 50 nucleotides in length, between 15 and 40 nucleotides in length, or between 15 and 35 nucleotides in length, preferably wherein the spacer region is between 16 and 30 nucleotides in length. In some aspects, the spacer region is between 16 and 20 nucleotides in length. In some aspects, the programmable CasΦ nuclease forms a complex with a divalent metal ion, preferably wherein the divalent metal ion is Mg2+.

[0023] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0024] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0025] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, or SEQ ID NO. 107, and wherein a) the programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516; b) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; c) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; d) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0026] In some aspects, the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid. In some aspects, the programmable CasΦ nuclease is fused or linked to one or more NLS. In some aspects, the one or more NLS are fused or linked to the N-terminus of the programmable CasΦ nuclease; the one or more NLS are fused or linked to the C-terminus of the programmable CasΦ nuclease; or the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable CasΦ nuclease. In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

[0027] In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a cell, preferably wherein the cell is a eukaryotic cell. In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell. In some cases, an aspect comprises a eukaryotic cell comprising the programmable CasΦ nuclease or a nucleic acid described herein.

[0028] In some aspects, the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

[0029] In some cases, an aspect comprises a vector comprising a nucleic acid described herein. In some aspects, the vector is a viral vector.

[0030] In some aspects, the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTN-3'. In some aspects, the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTB-3', wherein B is C, G, or T. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTN-3', optionally wherein the PAM is 5'-TTN-3'. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTK-3', 5'-VTTK-3', 5'-VTTS-3', 5'-TTTS-3' or 5'-VTTN-3', where K is G or T, V is A, C or G, and S is C or G. In some aspects, the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTB-3', wherein B is C, G, or T.

[0031] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0032] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5'

overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0033] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0034] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0035] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to

the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0036] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0037] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0038] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid

sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0039] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0040] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0041] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0042] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0043] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a

staggered cut with a 5' overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0044] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0045] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0046] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second

region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0047] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0048] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0049] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group

consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0050] In various aspects, the present disclosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0051] In various aspects, the present disclosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable

of processing a pre-crRNA and cleaving the target nucleic acid; the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid. In some aspects the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid.

[0052] In some aspects, the programmable CasΦ nuclease is fused or linked to one or more NLS. In some aspects, the one or more NLS are fused or linked to the N-terminus of the programmable CasΦ nuclease; the one or more NLS are fused or linked to the C-terminus of the programmable CasΦ nuclease; or the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable CasΦ nuclease.

[0053] In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides. In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a cell, preferably wherein the cell is a eukaryotic cell.

[0054] In some cases, an aspect comprises the programmable CasΦ nuclease or a nucleic acid described herein and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides.

[0055] In some aspects, a eukaryotic cell comprises the programmable CasΦ nuclease or a nucleic acid described herein. In some aspects, the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides. In some aspects, a vector comprises a nucleic acid described herein. In some aspects, the vector is a viral vector.

[0056] In various aspects, the present disclosure provides a guide nucleic acid, or a nucleic acid encoding said guide nucleic acid, comprising a sequence that is the same as or differs by no more than 5, 4, 3, 2, or 1 nucleotides from: a sequence from Tables A to AH; or a sequence comprising a repeat sequence from Table 2 and a spacer sequence from Tables A to H. In some aspects, the guide nucleic acid comprises a sequence from Tables A to AH; or a sequence comprising a repeat sequence from Table 2 and a spacer sequence from Tables A to H. In some aspects, the guide nucleic acid comprises RNA and/or DNA. In some aspects, the guide nucleic acid is a guide RNA. Some aspects further comprise a complex comprising the guide nucleic acid and a programmable CasΦ nuclease. Some aspects comprise a eukaryotic cell comprising the guide nucleic acid. In some aspects, the eukaryotic cell further comprises a programmable CasΦ nuclease. Some aspects further comprise a vector encoding the guide nucleic acid. In some aspects, the vector is a viral vector.

[0057] In various aspects, the present discosure provides a method of introducing a first modification in a first gene and a second modification in a second gene, the method comprising contacting a cell with a CasΦ nuclease; a first guide RNA that is at least partially complementary to an equal length portion of the first gene; and a second guide RNA that is at least partially complementary to an equal length portion of the second gene. In some aspects, the CasΦ nuclease is a CasΦ12 nuclease. In some aspects, the CasΦ12 nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 12. In some aspects, the first and/or second modification comprises an insertion of a nucleotide, a deletion of a nucleotide or a combination thereof. In some aspects, the first and/or second modification comprises an epigenetic modification. In some aspects, the first and/or second mutation results in a reduction in the expression of the first gene and/or second gene, respectively. In some aspects, the reduction in the expression is at least about a 10% reduction, at least about a 20% reduction, at least about a 30% reduction, at least about a 40% reduction, at least about a 50% reduction, at least about a 60% reduction, at least about a 70% reduction, at least about an 80% reduction, or at least about a 90% reduction. In some aspects, the method comprises contacting the cell with three different guide RNAs targeting three different genes.

[0058] In various aspects, the present discosure provides a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 12. In some aspects, the programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 12. In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 12. In some aspects, the programmable CasΦ nuclease comprises at least 98%

sequence identity to SEQ ID NO: 12. In some aspects, the programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 12. In some aspects, the programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 18. In some aspects, the programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 18. In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 18. In some aspects, the programmable CasΦ nuclease comprises at least 98% sequence identity to SEQ ID NO: 18. In some aspects, the programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 18. In some aspects, the programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease comprises at least 98% sequence identity to SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 32. In some aspects, the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence. In some aspects, the programmable CasΦ nuclease does not require a tracrRNA to cleave a target nucleic acid. In some aspects, the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving a target nucleic acid.

[0059] In various aspects, the present discosure provides a composition comprising the programmable CasΦ nuclease disclosed herein or a nucleic acid encoding said programmable nuclease, and a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides. In some aspects, the composition comprises the programmable CasΦ nuclease or a nucleic acid encoding said programmable nuclease and a cell, preferably wherein the cell is a eukaryotic cell. In various aspects, the present discosure provides a programmable CasΦ nuclease disclosed herein or a nucleic acid encoding said programmable nuclease, and a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a

eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides.

[0060] In various aspects, the present discosure provides a eukaryotic cell comprising the programmable CasΦ nuclease disclosed herein or a nucleic acid encoding said programmable nuclease. In some asepcts, the cell further comprises a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides.

[0061] In various aspects, the present discosure provides a vector comprising the nucleic acid encoding a programmable nuclease as disclosed herein. In some aspects, the vector is a viral vector. In some aspects, the vector further comprises a nucleic acid encoding a guide nucleic acid, wherein the guide nucleic acid comprises a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease. In some aspects, the guide nucleic acid is a guide RNA. In some aspects, the vector further comprises a donor polynucleotide. In some aspects, the guide nucleic acid is a guide RNA.

[0062] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the programmable nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0063] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the

programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0064] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0065] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0066] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is

complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0067] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

[0068] In various aspects, the present discosure provides a programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides; a complex comprising the programmable nuclease and the guide RNA binds to the target sequence; the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; the programmable nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid. In some aspects, the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the

target nucleic acid. In some aspects, the programmable nuclease is fused or linked to one or more NLS.

[0069] In various aspects, the programmable nuclease disclosed herein or the nucleic acid encoding said programmable nuclease is fused to one or more NLS. In some aspects, the one or more NLS are fused or linked to the N-terminus of the programmable nuclease. In some aspects, the one or more NLS are fused or linked to the C-terminus of the programmable nuclease; or the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable nuclease.

[0070] In various aspects, the present discosure provides a composition comprising a programmable nuclease disclosed herein or a nucleic acid encoding the programmable nuclease; and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides. In some aspects, the programmable nuclease or a nucleic acid disclosed herein is comprised in a cell, preferably wherein the cell is a eukaryotic cell.In some aspects, the composition comprising the programmable nuclease or a nucleic acid disclosed herein further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease and a cell, preferably wherein the cell is a eukaryotic cell. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides.

[0071] In various aspects, the present discosure provides a eukaryotic cell comprising a programmable nuclease disclosed herein or a nucleic acid molecule encoding said programmable nuclease. In some aspects, the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease. In some aspects, the first region comprises a seed region comprising between 10 and 16 nucleosides. In some aspects, the seed region comprises 16 nucleosides. In some aspects, the nucleic acid disclosed herein is comprised in a vector. In some aspects, the vector is a viral vector.

[0072] In some aspects, the present disclosure provides a complex comprising a first programmable CasΦ nuclease and a second programmable CasΦ nuclease. In some aspects, the first programmable CasΦ nuclease and the second programmable CasΦ nuclease are the same programmable CasΦ nuclease. In some aspects, the dimer comprises a first programmable CasΦ

nuclease and a second programmable CasΦ nuclease. In some aspects, the composition comprises a first programmable CasΦ nuclease and a second programmable CasΦ nuclease.

[0073] In various aspects, the present discosure provides a method of modifying a cell comprising a target nucleic acid, comprising introducing a composition comprising a programmable CasΦ nuclease, programmable nuclease or a cas nuclease to a cell, wherein the programmable CasΦ nuclease, programmable nuclease or the cas nuclease cleaves the target nucleic acid, thereby modifying the cell.

[0074] In various aspects, the disclosure provides a method of modifying a cell comprising a target nucleic acid, comprising introducing to the cell (i) the programmable CasΦ nuclease or programmable nuclease disclosed herein and (ii) a guide nucleic acid, wherein the programmable CasΦ nuclease or programmable Cas nuclease cleaves the target nucleic acid, thereby modifying the cell.In some aspects, the guide nucleic acid is a guide RNA. In some aspects, the method further comprises introducing a donor polynucleotide to the cell. In some aspects, the method comprises inserting the donor polynucleotide into the target nucleic acid at the site of cleavage. In some aspects, the cell is a eukaryotic cell, preferably a human cell. In some aspects, the cell is a T cell. In some aspects, the cell is a CAR-T cell. In some aspects, the cell is a stem cell. In some aspects, the cell is a hematopoietic stem cell. In some aspects, the stem cell is a pluripotent stem cell, preferably an induced pluripotent stem cell. In some aspects, the modified cell obtained or obtainable by the method disclosed herein. In some aspect, the disclosure provides a modified human cell obtained or obtainable by the methods herein. In some aspects, the modified cell is a eukaryotic cell, preferably a human cell. In some aspects, the cell is a T cell. In some aspects, the T cell is a CAR-T cell. In some aspects, the cell is a stem cell. In some aspects, the cell is a hematopoietic stem cell. In some aspects, the cell is a pluripotent stem cell, preferably an induced pluripotent stem cell.

[0075] In some aspects, the method comprises the use of a CasΦ nuclease to introduce a first modification in a first gene and a second modification in a gene according to the methods disclosed herein. In some aspects, the method comprises the use of a programmable CasΦ nuclease, programmable nuclease or a cas nuclease to modify a cell according to the methods disclosed herein. In some aspects, the method comprises lipid nanoparticle delivery of a nucleic acid encoding the programmable CasΦ nuclease, programmable nuclease or cas nuclease, and the guide nucleic acid. In some aspects, the nucleic acid further comprises a donor polynucleotide. In some aspects, the nucleic acid is a viral vector. In some aspects, the viral vector is an AAV vector.

INCORPORATION BY REFERENCE

[0076] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0077] 42256-779_601_SLThe patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0078] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0079] **FIG. 1** illustrates results of a cis-cleavage assay on CasΦ polypeptides to assess programmable nickase activity. The results showed that CasΦ orthologs comprise programmable nickase activity. The assay was performed on five CasΦ polypeptides, designated CasΦ.2, CasΦ.11, CasΦ.17, CasΦ.18, and CasΦ.12, in **FIG. 1**. For the assay, each of the CasΦ polypeptides was complexed with a guide nucleic acid at room temperature for 20 minutes to form a ribonucleoprotein (RNP) complex. The RNP complexes for each of the CasΦ polypeptides were separately incubated at 37°C for 60 minutes with plasmid DNA targeted by the guide nucleic acids. The graph shows the percentage of plasmids that developed nicks (single-stranded breaks) or linearized (double-stranded breaks) during the 60 minute incubation, as measured by gel-electrophoresis. The data showed that CasΦ.2, CasΦ.11, CasΦ.17, and CasΦ.18 acted as programmable nickases. CasΦ.17 and CasΦ.18 produced only nicked product. CasΦ.2 and CasΦ.11 generated some linearized product but primarily nicked intermediate. CasΦ.12 generated almost entirely linearized product.

[0080] **FIG. 2A and FIG. 2B** illustrate results of a cis-cleavage assay on CasΦ polypeptides to assess the effect of crRNA repeat sequence and RNP complexing temperature on the programmable nickase activity of CasΦ polypeptides. Each of three proteins (designated CasΦ.11, CasΦ.17 and CasΦ.18 in **FIG. 2A** and **FIG. 2B**) was tested for its ability to nick plasmid DNA when complexed with one of four crRNAs comprising the repeat sequences of CasΦ.2, CasΦ.7, CasΦ.10 and CasΦ.18 (abbreviated j2, j7, j10, and j18, respectively, in **FIG. 2A** and **FIG. 2B**). **FIG. 2C** illustrates the alignment of CasΦ.2, CasΦ.7, CasΦ.10, and CasΦ.18 repeat sequences showing conserved (highlighted in black) and diverged nucleotides. For the assay, the RNP complex formation of each of the CasΦ polypeptides with the guide nucleic acid was performed at either room temperature or at 37°C. The incubation of the RNP complex with the input plasmid DNA that comprised the target sequence for the guide nucleic acids was carried out for 60 minutes at 37°C. **FIG. 2A** shows the percentage of input plasmid DNA that

was nicked by RNP complexes assembled at room temperature. The data showed that crRNAs comprising repeat sequences from all tested CasΦ polypeptides supported nickase activity by CasΦ.11, CasΦ.17, and CasΦ.18; the only exception was the CasΦ.17/CasΦ.2-repeat pairing. **FIG. 2B** shows the percentage of input plasmid DNA that was nicked by RNP complexes assembled at 37°C. The data showed that the activity of each protein is completely abolished when complexed with crRNAs comprising a repeat sequence from CasΦ.2 or CasΦ.10. **FIG. 2D** shows corresponding data for CasΦ.2, CasΦ.4, CasΦ.6, CasΦ.9, CasΦ.10, CasΦ.12 and CasΦ.13 for the experiment shown in **FIG. 2A** and **FIG. 2B**. **FIG. 2D** also shows the percentage of input plasmid DNA that was linearized by CasΦ.2, CasΦ.4, CasΦ.6, CasΦ.9, CasΦ.10, CasΦ.11, CasΦ.12, CasΦ.13, CasΦ.17 and CasΦ.18 when complexed with one of four crRNAs J2, j7, j10 and j18, as described above.

[0081] FIG. 3 illustrates results of a cis-cleavage assay and sequencing run demonstrating that CasΦ nickases cleave the non-target strand of a double-stranded DNA target. A cis-cleavage assay was performed with four CasΦ polypeptides, CasΦ.12, CasΦ.2, CasΦ.11, and CasΦ.18, and a control comprising no CasΦ polypeptide, on a super-coiled plasmid DNA comprising a protospacer immediately downstream of a TTTN PAM sequence. The resulting DNA from the assay was Sanger sequenced using forward and reverse primers. The forward primer comprised the sequence of the target strand (TS) of the DNA sequence, while the reverse primer comprised the sequence of the non-target strand (NTS). If a strand had been cleaved by the CasΦ polypeptide being assayed, the sequencing signal would drop off from the cleavage site. **Fig. 3A** illustrates the cleavage pattern for the control that comprised no CasΦ polypeptide. In the absence of CasΦ polypeptide, the target DNA remained uncut and resulted in complete sequencing of both target and non-target strands. **FIG. 3B** illustrates the cleavage pattern for CasΦ.12 protein, which comprises double-stranded DNA cleavage activity. As shown in the figure, the sequencing signal dropped off on both the target and the non-target strands (as shown by arrows) demonstrating cleavage of both strands. **Fig. 3C** illustrates the cleavage pattern for CasΦ.2, which predominantly nicks DNA as illustrated in **FIG. 1**. The sequencing signal dropped off only on the non-target strand (bottom arrow) demonstrating nicking of the non-target strand. **Figure 3D** illustrates the cleavage pattern for CasΦ.11. As illustrated in FIG. 1, CasΦ.11 only nicks DNA after 60 minutes of incubation with plasmid DNA. The sequencing signal dropped off on the non-target strand (bottom arrow), thus demonstrating that CasΦ.11 nicks the non-target strand. **Figure 3E** illustrates the cleavage pattern for CasΦ.18. As illustrated in FIG. 1, CasΦ.18 only nicks DNA after 60 minutes of incubation with plasmid DNA. The sequencing signal dropped off on the non-target strand (bottom arrow), thus demonstrating that CasΦ.18 nicks the non-target strand.

[0082] FIG. 4 illustrates results of a cis-cleavage assay on CasΦ polypeptides to assess the effect of crRNA repeat and target sequence the programmable nickase and double strand DNA

cleavage activity of CasΦ polypeptides. The heat map in **FIG.4A** cleavage products for 60 minute *in vitro* plasmid cleavage reactions of 12 CasΦ orthologs paired with 10 crRNA repeat sequences. Except for 0, all Repeat and CasΦ axis labels refer Cas12Φ system numbers. Repeat 0 is a negative control including the CasΦ.18 crRNA repeat sequence and a non-targeting spacer sequence. With rare exceptions, preference for nicking or linearizing target DNA is not affected by crRNA repeat or target DNA sequence. Raw data for CasΦ.12 and CasΦ.18 targeting spacer 1 (boxes) are shown in B. **FIG.4B** shows the raw gel data used to generate a subset of the heat map from FIG.4A. CasΦ.12 predominantly linearizes plasmid DNA (i.e. cleaves both strands of a double strand DNA target) whereas CasΦ.18 primarily does not proceed beyond the first strand nicking.

[0083] FIG.5 illustrates the structural conservation of CasΦ crRNA repeats. **FIG. 5A** shows the structure of the crRNA repeats for CasΦ.1, CasΦ.2, CasΦ.7, CasΦ.11, CasΦ.12, CasΦ.13, CasΦ.18, and CasΦ.32. These structures were calculated using an online RNA prediction tool (https://rna.urmc.rochester.edu/RNAstructureWeb/Servers/Predict1/Predict1.html) using default parameters at 37 °C. The sequences of these repeats are provided in **TABLE 2**. **FIG.5B** shows the consensus structure of the crRNA as determined by the LocaRNA tool using the crRNA repeats from CasΦ.1, CasΦ.2, CasΦ.4, CasΦ.7, CasΦ.10, CasΦ.11, CasΦ.12, CasΦ.13, Cas12Φ.17, CasΦ.18, CasΦ.19, CasΦ.21, CasΦ.22, CasΦ.23, CasΦ.24, CasΦ.25, CasΦ.26, CasΦ.27, CasΦ.28, CasΦ.29, CasΦ.30, CasΦ.31, CasΦ.32, CasΦ.33, CasΦ.35 and CasΦ.41. **FIG.5C** shows a further refined consensus structure of the crRNA determined by the LocaRNA tool. The LocaRNA tool aligns RNA sequences while considering consensus secondary structure of the RNA sequence.

[0084] FIG.6 illustrates the optimal PAM preferences for CasΦ.2, CasΦ.4, CasΦ.11, CasΦ.12 and CasΦ.18. An *in vitro* cleavage assay was performed using a linear DNA target. Starting with a TTTA PAM, each position was varied one by one to the other 3 nucleotides for a total of 12 variants in addition to parental TTTA. **FIG.6A** shows a heat map which illustrates the absolute levels of double strand cleavage (or nicking for CasΦ.18). **FIG.6B** shows the data from FIG.6A after normalization to the parental TTTA PAM as 100%. **FIG.6C** shows the optimal PAM preferences of these CasΦ polypeptides with a summary of the data shown in FIG.6A and FIG.6B.

[0085] FIG.7 illustrates that CasΦ polypeptides rapidly nick supercoiled DNA. CasΦ polypeptides where assembled with their native repeat crRNAs targeting one of two targets (S1, TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 108), or S2, CACAGCTTGTCTGTAAGCGGATGCCATATG (SEQ ID NO: 109)) immediately downstream of a GTTG or TTTG PAM. Reactions were initiated with the addition of supercoiled target DNA and stopped after 1, 3, 6, 15, 30 and 60 mins. The cleavage was quantified by agarose gel

analysis as nicked (left column) or linear (right column). Error bars are +/- SEM of duplicate time courses.

[0086] FIG.8 illustrates that CasΦ polypeptides prefer full-length repeats and spacers from 16 to 20 nucleotides. crRNA panels varying in repeat and spacer length were tested for their ability to support CasΦ polypeptides spacer cleavage. Two different CasΦ repeats that function across CasΦ orthologs were utilized. **FIG.8A** shows results of the assay for nicking (top) or linearization (bottom) as influenced by the length of the crRNA repeat. 19 nucleotides was the shortest repeat still supporting cleaving activity. **FIG.8B** shows results for nicking (top) or linearization (bottom) as influenced by the length of the crRNA spacer. The optimal spacer length varied by target but is generally 16 to 20 nucleotides.

[0087] FIG.9 illustrates CasΦ.12 cleavage in HEK293T cells and the effect of changing the spacer length on this cleavage. **FIG.9A** provides a schematic of how CasΦ.12 cleavage activity was assessed in HEK293T cells. An Ac-GFP- expressing HEK293T cell line was transfected with a plasmid expressing CasΦ.12 and its crRNA targeting the Ac-GFP gene. CasΦ.12 cleavage was assessed by the reduction in Ac-GFP-expressing cells as assessed by flow cytometry. As shown in **FIG.9B**, varying the spacer length varied the degree of CasΦ.12 cleavage. CasΦ.12 has a preference for a spacer length of 17 to 22 nucleotides in HEK293T cells, but longer spacers (up to 30 nucleotides was tested) also supported CasΦ.12 cleavage.

[0088] FIG.10 illustrates that the CasΦ disclosed herein are a novel family of Cas nucleases. As shown in **FIG.10A**, the InterPro database did not recognize CasΦ.2 as a protein family member. As a positive control, the InterPro database identified *Acidaminococcus sp.* (strain BV3L6) as a Cas12a protein family member, as shown in **FIG.10B**.

[0089] FIG.11 illustrates the raw HMM for PF07282.

[0090] FIG.12 illustrates the raw HMM for PF18516.

[0091] FIG.13 illustrates the cleavage activity of CasΦ.19-CasΦ.48.

[0092] FIG.14 illustrates the PAM requirement of CasΦ polypeptides. **FIG.14A** shows the PAM requirement of CasΦ.2, CasΦ.4, CasΦ.11 and CasΦ.12. **FIG. 14B** shows the PAM requirement of CasΦ.20, CasΦ.26, CasΦ.32, CasΦ.38 and CasΦ.45. **FIG.14C** shows the cleavage products from the assessment of the PAM requirement for CasΦ.20, CasΦ.24 and CasΦ.25. **FIG.14D** shows the quantification of the raw data shown in **FIG.14C**.

[0093] FIG.15 illustrates endogenous gene editing in HEK293T cells.

[0094] FIG.16 illustrates endogenous gene editing in CHO cells. **FIG.16A** shows CasΦ.12 mediated generation of insertion or deletion mutations (indel) in the endogenous *Bak1, Bax* and *Fut8* genes. **FIG.16B** shows the DNA donor oligos used to assess CasΦ.12 mediated gene

editing via the homology directed repair pathway. **FIG.16C** shows the detection of indels following delivery of CasΦ.12. **FIG.16D** shows the sequence analysis for the data in FIG.15C. **FIG.16E** shows the detection of incorporated donor template following delivery of CasΦ.12 and a donor oligo. Further examples of CasΦ.12 mediated generation of indel mutations are shown in **FIG.16F**, **FIG.16G** and **FIG.16H** for *Bak1, Bax* and *Fut8* genes, respectively. **FIG.16I** shows the DNA donor oligos used to assess CasΦ.12 mediated gene editing via the homology directed repair pathway. **FIG.16J** shows the frequency of HDR in CHO cells following delivery of either Cas9 and a gRNA targeting *Bax*, CasΦ.12 and a gRNA targeting *Bax* or CasΦ.12 and a gRNA targeting *Fut8*. **FIG.16K** and **FIG.16L** show the frequency of indel mutations and HDR, respectively, detected in CHO cells following delivery of CasΦ.12 and AAV6 DNA donors at the indicated number of viral genomes per cell (1x10^5, 3x10^5, or 1x10^6).

[0095] **FIG.17** illustrates endogenous gene editing in K562 cells.

[0096] **FIG.18** illustrates endogenous gene editing in primary cells. **FIG.18A** shows a flow cytometry analysis of T cells that have received CasΦ.12 with or without a gRNA targeting the beta-2 microglobulin gene. **FIG.18B** shows the modification detected in K562 cells and T cells following delivery of CasΦ.12 and a gRNA targeting the beta-2 microglobulin gene. **FIG.18C** shows the sequence analysis of the T cell population which received CasΦ.12 and the gRNA targeting the beta-2 microglobulin gene. **FIG.18D** shows a flow cytometry analysis of T cells that have received CasΦ.12 with a gRNA targeting the T Cell Receptor Alpha Constant gene. **FIG.18E** shows the sequence analysis of cell populations that received CasΦ.12 with a gRNA targeting the T Cell Receptor Alpha Constant gene. **FIG.18F** shows the quantification of indels detected by sequence analysis.

[0097] **FIG.19** illustrates the cleavage of the second DNA strand by CasΦ nucleases in a separable reaction step to the cleavage of the first DNA strand.

[0098] **FIG.20** illustrates the trans cleavage of ssDNA by CasΦ nucleases in a detection assay.

[0099] **FIG.21** illustrates the CasΦ.12-mediated efficiency is comparable to that of Cas9. **FIG21A** shows the frequency of indel mutations and quantification of B2M knockout cells from flow cytometry panels in **FIG21B**.

[0100] **FIG.22** illustrates the identification of optimized gRNAs for genome editing with CasΦ.12 in CHO cells. **FIG.22A** shows the frequency of indel mutations induced by CasΦ.12 polypeptides complexed with a 2'fluoro modified gRNA. **FIG.22B** shows further CasΦ.12 RNP complexes that can mediate genome editing in CHO cells.

[0101] **FIG.23** illustrates minimal off-target CasΦ.12-mediated genome editing in CHO and HEK293 cells. **FIG23.A-F** are off-target analysis InDel validation from a list of potential off-target sites based on in-silico computational predictions. **FIG.23A** shows Casϕ.12 targeting

Fut8, **FIG.23B** shows Casϕ.12 targeting *BAX*, **FIG.23C** shows Cas9 targeting *BAX*, **FIG.23D** shows Cas9 targeting *Fut8*, **FIG.23E** shows Cas9 targeting *Bak1* and **FIG.23F** shows Casϕ.12 targeting *Bak1*. **FIG.23G** shows off-target analysis using unbiased guide-seq procedure, using Casϕ.12 and guides targeting human *Fut8* in HEK293 cells. **FIG.23H** shows off-target analysis using unbiased guide-seq procedure, using Cas9 and guides targeting human *Fut8* in HEK293 cells.

[0102] FIG.24 illustrates CasΦ.12-mediated genome editing via homology directed repair (HDR). **FIG.24A** shows CasΦ.12-mediated gene editing via the HDR pathway. **FIG.24B** shows a schematic of the donor oligonucleotide

[0103] FIG.25 illustrates the ability of CasΦ.12 to target multiple genes. **FIG. 25A** shows the percentage of B2M and TRAC knockout after CasΦ.12-mediated genome editing with gRNAs with a repeat length of 20 nucleotides and a spacer length of 20 nucleotides. **FIG. 25B** shows the percentage of B2M and TRAC knockout after CasΦ.12-mediated genome editing with gRNAs with a repeat length of 20 nucleotides and a spacer length of 17 nucleotides. **FIG.25C** shows corresponding flow cytometry panels for B2M and TRAC knockout with different gRNAs. **FIG.25D** shows the percentage of TRAC knockout after CasΦ.12-mediated genome editing with modified gRNAs of different spacer lengths (repeat length of 20 nucleotides and a spacer length of 17 or 20 nucleotides). **FIG.25E** shows a corresponding flow cytometry panel for TRAC knockout after CasΦ.12-mediated genome editing.

[0104] FIG. 26 illustrates the extended seed region of CasΦ.12. **FIG.26A** and **FIG.26B** show no indel mutations or CD3 knockout occurs when there is a single or double mismatch in the first 1-16 nucleotides from the 5' end of the spacer. **FIG.26C** and **FIG.26D** provide schematics of the gRNAs with mismatches.

[0105] FIG.27 illustrates the ability of CasΦ.12 to mediate genome editing in CHO cells with modified gRNAs.

[0106] FIG.28 illustrates the ability of CasΦ.12 to mediate genome editing with gRNAs with variations in repeat and spacer length. **FIG.28A** shows the frequency of CasΦ.12-mediated indel mutations using gRNA of different repeat lengths. **FIG.28B** shows the frequency of CasΦ.12-mediated indel mutations using gRNA of different spacer lengths.

[0107] FIG.29A-E illustrate exemplary gRNAs for targeting CD3, B2M and PD1 with CasΦ.12 in human primary T cells. **FIG.29F** shows the screening of gRNAs targeting TRAC. **FIG.29H** shows the screening of gRNAs targeting B2M. **FIG.29G** and **FIG.29I** show flow cytometry panels of exemplary gRNAs targeting TRAC and B2M, respectively.

[0108] FIG.30 illustrates delivery of CasΦ.12 RNPs or CasΦ.12 mRNA both lead to efficient genome editing. **FIG30A** and **FIG.30B** show flow cytometry panels of CasΦ.12 RNP complexes

targeting B2M and TRAC in T cells, and are quantified in **FIG30C** and **FIG.30D. FIG30E** and **FIG.30F** show the quantification of indels detected by sequence analysis with delivery of CasΦ.12 RNPs. **FIG.30G** and **FIG.30I** show the frequency of indel mutations after delivery of CasΦ.12 mRNA and the quantification of B2M knockout cells shown in **FIG.30H** is an exemplary FACS panel for two data points in FIG. 30G. **FIG.30J** shows the distribution of the size of indel mutations induced by CasΦ.12 or Cas9.

[0109] FIG.31 illustrates CasΦ.12 can process its own guide RNA in mammalian cells.

[0110] FIG. 32 illustrates CasΦ polypeptide-induced cleavage patterns. **FIG.32A**, shows CasΦ polypeptides generated nicked and linearized plasmid DNA. **FIG32B** shows a schematic of the cut sites on the target and non-target strand. **FIG.32C** shows sequence analysis of the non-target stand target strand and is represented in **FIG.32D**. **FIG.32E** shows a table of cut sites and overhangs of the different CasΦ polypeptides.

[0111] FIG.33 illustrates the ability of CasΦ RNP complexes to knockout multiple genes simultaneously. T cells were nucleofected with RNP complexes of CasΦ.12 and gRNAs targeting B2M, TRAC or PDCD1 and the percentage knockout was measured using flow cytometry.

[0112] FIG.34 illustrates the ability of CasΦ.12 RNP complexes to mediate high efficiency genome editing of PCKS9 in mouse Hepa1-6 cells. 95 CasΦ gRNAs were used along with Cas9, as a control. CasΦ.12 RNP complexes induced a maximum indel frequency of 48%, whereas Cas9 RNP complexed induced a maximum indel frequency of 22%.

[0113] FIG.35 illustrates the ability of a CasΦ.12 all-in-one vector to mediate genome editing in Hepa1-6 mouse hepatoma cells. **FIG35A** shows a plasmid map of the AAV encoding the CasΦ polypeptide sequence and gRNA sequence. **FIG.35B** illustrates repeat truncations. **FIG.35C** shows efficient transfection with AAV. **FIG.35D** shows the frequency of CasΦ.12 induced indel mutations. **FIG.35E** and **FIG35.F** show the frequency of CasΦ.12 induced indel mutations with different gRNA containing repeat and spacer sequences of different lengths.

[0114] FIG.36 illustrates the optimization of LNP delivery of mRNA encoding CasΦ and gRNA. A range of N/P ratios were tested and the frequency of indel mutations was determined.

[0115] FIG.37 illustrates CasΦ-mediated genome editing of $CD34^+$ hematopoietic stem cells. Cells were nucleofected with either RNP complexes containing CasΦ.12 polypeptides and a B2M-targeting guide, or a mixture of CasΦ.12 mRNA and B2M-targeting guide and the frequency of indel mutations was determined.

[0116] FIG.38 illustrates CasΦ-mediated genome editing of induced pluripotent stem cells. Cells were nucleofected with RNP complexes (CasΦ.12 polypeptides and gRNAs targeting

either the B2M locus or targeting a CIITA locus) and the frequency of indel mutations was determined.

[0117] FIG.39 illustrates CasΦ-mediated genome editing of the CIITA locus in K562 cells. Cells were nucleofected with RNP complexes (CasΦ polypeptides and gRNAs targeting CIITA) and the frequency of indel mutations was determined by NGS.

DETAILED DESCRIPTION

[0118] The present disclosure provides methods, compositions, systems, and kits comprising programmable CasΦ nucleases. An illustrative composition comprises a programmable CasΦ nuclease or a nucleic acid encoding the programmable CasΦ nuclease, wherein the programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105. In some embodiments, the composition further comprises a guide nucleic acid or a nucleic acid encoding the guide nucleic acid, wherein the guide nucleic acid comprises a region comprising a nucleotide sequence that is complementary to a target nucleic acid sequence and an additional region, wherein the region and the additional region are heterologous to each other. As used herein, the term "heterologous" may be used to describe or indicate that a first sequence is different from a second sequence and do not naturally occur together. As used herein, the term "heterologous" may be used to describe that a first moiety (e.g., a first sequence) is different from a second moiety (e.g., a second sequence) and, as such, the two moieties do not naturally occur together and are engineered to be a part of one entity. For example, a guide nucleic acid sequence comprising a region and an additional region that are heterologous to each other may indicate that the guide nucleic acid sequence is engineered to include the region and the additional region. The programmable CasΦ nuclease and the guide nucleic acid may be complexed together in a ribonucleoprotein complex. Alternatively, compositions consistent with the present disclosure include nucleic acids encoding for the programmable CasΦ nuclease and the guide nucleic acid. In some embodiments, the guide nucleic acid comprises a sequence with at least about 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86. In some embodiments, the programmable CasΦ nuclease is SEQ ID NO: 12 or SEQ ID NO: 105. In some embodiments, the programmable CasΦ nuclease comprises nickase activity. In some embodiments, the programmable CasΦ nuclease comprises double-strand cleavage activity. As used herein, CasΦ may be referred to as Cas12j or Cas14u.

[0119] Also disclosed herein are compositions, methods, and systems for modifying a target nucleic acid sequence. An illustrative method for modifying a target nucleic acid sequence comprises contacting a target nucleic acid sequence with a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105, and a guide nucleic acid, wherein the programmable CasΦ nuclease cleaves the target nucleic acid sequence, thereby modifying the target nucleic

acid sequence. In some embodiments, the programmable CasΦ nuclease introduces a double-stranded break in the target nucleic acid. In some embodiments, the programmable CasΦ nuclease introduces a single-stranded break.

[0120] Also disclosed herein are compositions, methods, and systems for modifying a target nucleic acid sequence comprising use of two or more programmable CasΦ nickases. An illustrative method for introducing a break in a target nucleic acid comprises contacting the target nucleic acid with: (a) a first guide nucleic acid comprising a region that binds to a first programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105; and (b) a second guide nucleic acid comprising a region that binds to a second programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105, wherein the first guide nucleic acid comprises an additional region that binds to the target nucleic acid and wherein the second guide nucleic acid comprises an additional region that binds to the target nucleic acid and wherein the additional region of the first guide nucleic acid and the additional region of the second guide nucleic acid bind opposing strands of the target nucleic acid.

[0121] Also disclosed herein are compositions, methods, and systems for detecting a target nucleic acid in a sample. An illustrative method for detecting a target nucleic acid in a sample comprises contacting the sample comprising the target nucleic acid with (a) a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105; (b) a guide RNA comprising a region that binds to the programmable CasΦ nuclease and an additional region that binds to the target nucleic acid; and (c) a labeled, single stranded DNA reporter that does not bind the guide RNA; cleaving the labeled single stranded DNA reporter by the programmable CasΦ nuclease to release a detectable label; and detecting the target nucleic acid by measuring a signal from the detectable label.

[0122] Also disclosed herein are compositions, methods, and systems for modulating transcription of a gene in a cell. An illustrative method of modulating transcription of a gene in a cell comprises introducing into a cell comprising a target nucleic acid sequence: (i) a fusion polypeptide or a nucleic acid encoding the fusion polypeptide, wherein the fusion polypeptide comprises: (a) a dCasΦ polypeptide comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47 and SEQ ID NO. 105, wherein the dCasΦ polypeptide is enzymatically inactive; and (b) a polypeptide comprising transcriptional regulation activity; and (ii) a guide nucleic acid, or a nucleic acid comprising a nucleotide sequence encoding the guide nucleic acid, wherein the guide nucleic acid comprises a region that binds to the dCasΦ polypeptide and an additional region that binds to the target nucleic acid;

wherein transcription of the gene is modulated through the fusion polypeptide acting on the target nucleic acid sequence.

[0123] Also disclosed is use of a programmable CasΦ nuclease to modify a target nucleic acid sequence according to any of the methods described herein. Also disclosed is use of a first programmable nickase and a second programmable nickase to introduce a break in a target nucleic acid according to any of the methods described herein. Also disclosed is use of a programmable CasΦ nuclease to detect a target nucleic acid in a sample according to any of the methods described herein. Also disclosed is use of a dCasΦ polypeptide to modulate transcription of a gene in a cell according to any of the methods described herein.

Programmable Nucleases

[0124] The present disclosure provides methods and compositions comprising programmable nucleases. The programmable nucleases can be complexed with a guide nucleic acid of the disclosure for targeting a target nucleic acid for detection, editing, modification, or regulation of the target nucleic acid.

[0125] The programmable nuclease can be used for detecting a target nucleic acid. For example, in certain embodiments, when the programmable nuclease is complexed with the guide nucleic acid and the target nucleic acid hybridizes to the guide nucleic acid, trans-cleavage of a single stranded DNA (ssDNA), such as an ssDNA reporter, by the programmable nuclease is activated. Detection of trans-cleavage of ssDNA can be used to determine a target nucleic acid in a sample.

[0126] The programmable nuclease can be used for editing or modifying a target nucleic acid, for example, by site-specific cleavage of a target sequence, donor nucleic acid insertion, or a combination thereof.

[0127] The programmable nuclease can be used for gene regulation of a target nucleic acid, for example, using a catalytically inactive programmable nuclease in combination with a polypeptide comprising gene regulation activity.

[0128] In some embodiments, the programmable nuclease is a programmable nuclease comprising site-specific nucleic acid cleavage activity. In some embodiments, the programmable nuclease is a programmable nuclease comprising double-strand DNA cleavage activity. In some embodiments, the programmable nuclease is a programmable nickase. In some embodiments, the programmable nuclease is a programmable DNA nickase. In some embodiments, the programmable nuclease is a programmable nuclease comprising a catalytically inactive nuclease domain. In some embodiments, the programmable nuclease comprising a catalytically inactive nuclease domain can include at least 1, at least 2, at least 3, at least 4, or at least 5 mutations relative to a wild type nuclease domain. Said mutations may be present within the cleaving or active site of the nuclease.

[0129] In some embodiments, the programmable nuclease is a programmable DNA nuclease. In some embodiments, the programmable nuclease is a Type V CRISPR/Cas enzyme, wherein a Type V CRISPR/Cas enzyme comprises a single active site or catalytic domain in a single RuvC domain. The RuvC domain is typically near the C-terminus of the enzyme. A single RuvC domain may comprise RuvC subdomains, for example RuvCI, RuvCII and RuvCIII. As used herein a "Type V CRISPR/Cas enzyme" or "Type V cas nuclease" or "Type V cas effector" may be used to describe a family of enzymes or a member thereof having diverse N-terminal structures and often comprising a conserved single catalytic RuvC-like endonuclease domain that is C-terminal of the N-terminal structures, derived from the TnpB protein encoded by autonomous or non-autonomous transposons. The terms "RuvC domain" and "RuvC-like domain" are used interchangeably for Type V CRISPR/Cas enzymes, Type V cas nucleases and Type V cas effectors. In some embodiments, the Type V CRISPR/Cas enzyme is a CasΦ nuclease. A CasΦ polypeptide can function as an endonuclease that catalyzes cleavage at a specific sequence in a target nucleic acid. A programmable CasΦ nuclease of the present disclosure may have a single active site in a RuvC domain that is capable of catalyzing pre-crRNA processing and nicking or cleaving of nucleic acids. This compact catalytic site may render the programmable CasΦ nuclease especially advantageous for genome engineering and new functionalities for genome manipulation.

[0130] In some embodiments, the RuvC domain is a RuvC-like domain. Various RuvC-like domains are known in the art and are easily identified using online tools such as InterPro (https://www.ebi.ac.uk/interpro/). For example, a RuvC-like domain may be a domain which shares homology with a region of TnpB proteins of the IS605 and other related families of transposons, as described in review articles such as Shmakov *et al.* (Nature Reviews Microbiology volume 15, pages 169–182(2017)) and Koonin E.V. and Makarova K.S. (2019, Phil. Trans. R. Soc., B 374:20180087). In some embodiments, the RuvC-like domain shares homology with the transposase IS605, OrfB, C-terminal. A transposase IS605, OrfB, C-terminal is easily identified by the skilled person using bioinformatics tools, such as PFAM (Finn *et al.* (Nucleic Acids Res. 2014 Jan 1; 42(Database issue): D222–D230); El-Gebali *et al.* (2019) Nucleic Acids Res. doi:10.1093/nar/gky995). PFAM is a database of protein families in which each entry is composed of a seed alignment which forms the basis to build a profile hidden Markov model (HMM) using the HMMER software (hmmer.org). It is readily accessible via pfam.xfam.org, maintained by EMBL-EBI, which easily allows an amino acid sequence to be analyzed against the current release of PFAM (*e.g.* version 33.1 from May 2020), but local builds can also be implemented using publicly- and freely-available database files and tools. A transposase IS605, OrfB, C-terminal is easily identified by the skilled person using the HMM PF07282. PF07282 is reproduced for reference in Figure 11 (accession number PF07282.12). The skilled person would also be able to identify a RuvC domain, for example with the HMM PF18516, using the PFAM tool. PF18516 is reproduced for reference in Figure 12 (accession

number PF18516.2). In some embodiments, the programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 but does not match PFAM family PF18516, as assessed using the PFAM tool (*e.g.* using PFAM version 33.1, and the HMM accession numbers PF07282.12 and PF18516.2). PFAM searches should ideally be performed using an E-value cut-off set at 1.0.

[0131] In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 21%, at least 22%, at least 23%, at least 24%, at least 25%, at least 26%, at least 27%, at least 28%, at least 29%, at least 30%, at least 31%, at least 32%, at least 33%, at least 34%, at least 35%, at least 36%, at least 37%, at least 38%, at least 39%, at least 40%, at least 41%, at least 42%, at least 43%, at least 44%, at least 45%, at least 46%, at least 47%, at least 48%, at least 49%, at least 50%, at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 20%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 25%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 30%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 35%. In some embodiments, , a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 40%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 45%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 50%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 55%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and

guide RNA combination described herein – has an editing efficiency of at least 60%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 65%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 70%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 75%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 80%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 85%. In some , a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 90%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 95%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of at least 100%. In some embodiments, a programmable nuclease described herein - or a programmable nuclease and guide RNA combination described herein – has an editing efficiency of 42%. In some embodiments, said editing efficiency is determined by analyzing the frequency of indel mutations in a nucleic acid or gene knockout.

[0132] In some embodiments, a programmable nuclease described herein has a primary amino acid sequence length of less than 1500 amino acids, less than 1450 amino acids, less than 1400 amino acids, less than 1350 amino acids, less than 1300 amino acids, less than 1250 amino acids, less than 1200 amino acids, less than 1150 amino acids, less than 1100 amino acids, less than 1050 amino acids, less than 1000 amino acids, less than 950 amino acids, less than 900 amino acids, less than 850 amino acids, or less than 800 amino acids.

[0133] In some examples, a programmable nuclease described herein is a Type V cas nuclease. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 20%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 25%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 30%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 35%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 40%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 45%.

In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 50%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 55%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 60%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 65%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 70%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 75%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 80%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 85%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 90%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of at least 95%. In some examples, the Type V cas nuclease, or a composition comprising the Type V cas nuclease, has an editing efficiency of 100%.

[0134] In some examples, a programmable nuclease described herein has a primary amino acid sequence length of less than 850 amino acids. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 20%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 25%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 30%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 35%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 40%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 45%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 50%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 55%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 60%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 65%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 70%. In some examples,

the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 75%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 80%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 85%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 90%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of at least 95%. In some examples, the programmable nuclease having a primary amino acid sequence length of less than 850 amino acids has an editing efficiency of 100%.

[0135] **TABLE 1** provides amino acid sequences of illustrative CasΦ polypeptides that can be used in compositions and methods of the disclosure.

TABLE 1 – CasΦ Amino Acid Sequences

Name	SEQ ID NO	Amino Acid Sequence
CasΦ.1	1	MADTPTLFTQFLRHHLPGQRFRKDILKQAGRILANKGEDATI AFLRGKSEESPPDFQPPVKCPIIACSRPLTEWPIYQASVAIQGY VYGQSLAEFEASDPGCSKDGLLGWFDKTGVCTDYFSVQGLN LIFQNARKRYIGVQTKVTNRNEKRHKKLKRINAKRIAEGLPE LTSDEPESALDETGHLIDPPGLNTNIYCYQQVSPKPLALSEVN QLPTAYAGYSTSGDDPIQPMVTKDRLSISKGQPGYIPEHQRA LLSQKKHRRMRGYGLKARALLVIVRIQDDWAVIDLRSLLRN AYWRRIVQTKEPSTITKLLKLVTGDPVLDATRMVATFTYKPG IVQVRSAKCLKNKQGSKLFSERYLNETVSVTSIDLGSNNLVA VATYRLVNGNTPELLQRFTLPSHLVKDFERYKQAHDTLEDSI QKTAVASLPQGQQTEIRMWSMYGFREAQERVCQELGLADG SIPWNVMTATSTILTDLFLARGGDPKKCMFTSEPKKKKNSKQ VLYKIRDRAWAKMYRTLLSKETREAWNKALWGLKRGSPDY ARLSKRKEELARRCVNYTISTAEKRAQCGRTIVALEDLNIGFF HGRGKQEPGWVGLFTRKKENRWLMQALHKAFLELAHHRG YHVIEVNPAYTSQTCPVCRHCDPDNRDQHNREAFHCIGCGFR GNADLDVATHNIAMVAITGESLKRARGSVASKTPQPLAAE
CasΦ.2	2	MPKPAVESEFSKVLKKHFPGERFRSSYMKRGGKILAAQGEE AVVAYLQGKSEEEPPNFQPPAKCHVVTKSRDFAEWPIMKAS EAIQRYIYALSTTERAACKPGKSSESHAAWFAATGVSNHGYS HVQGLNLIFDHTLGRYDGVLKKVQLRNEKARARLESINASR ADEGLPEIKAEEEEVATNETGHLLQPPGINPSFYVYQTISPQA YRPRDEIVLPPEYAGYVRDPNAPIPLGVVRNRCDIQKGCPGYI PEWQREAGTAISPKTGKAVTVPGLSPKKNKRMRRYWRSEKE KAQDALLVTVRIGTDWVVIDVRGLLRNARWRTIAPKDISLN

		ALLDLFTGDPVIDVRRNIVTFTYTLDACGTYARKWTLKGKQ TKATLDKLTATQTVALVAIDLGQTNPISAGISRVTQENGALQ CEPLDRFTLPDDLLKDISAYRIAWDRNEEELRARSVEALPEA QQAEVRALDGVSKETARTQLCADFGLDPKRLPWDKMSSNT TFISEALLSNSVSRDQVFFTPAPKKGAKKKAPVEVMRKDRT WARAYKPRLSVEAQKLKNEALWALKRTSPEYLKLSRRKEEL CRRSINYVIEKTRRRTQCQIVIPVIEDLNVRFFHGSGKRLPGW DNFFTAKKENRWFIQGLHKAFSDLRTHRSFYVFEVRPERTSIT CPKCGHCEVGNRDGEAFQCLSCGKTCNADLDVATHNLTQV ALTGKTMPKREEPRDAQGTAPARKTKKASKSKAPPAEREDQ TPAQEPSQTS
CasΦ.3	3	MYILEMADLKSEPSLLAKLLRDRFPGKYWLPKYWKLAEKKR LTGGEEAACEYMADKQLDSPPPNFRPPARCVILAKSRPFEDW PVHRVASKAQSFVIGLSEQGFAALRAAPPSTADARRDWLRS HGASEDDLMALEAQLLETIMGNAISLHGGVLKKIDNANVKA AKRLSGRNEARLNKGLQELPPEQEGSAYGADGLLVNPPGLN LNIYCRKSCCPKPVKNTARFVGHYPGYLRDSDSILISGTMDR LTIIEGMPGHIPAWQREQGLVKPGGRRRRLSGSESNMRQKVD PSTGPRRSTRSGTVNRSNQRTGRNGDPLLVEIRMKEDWVLL DARGLLRNLRWRESKRGLSCDHEDLSLSGLLALFSGDPVIDP VRNEVVFLYGEGIIPVRSTKPVGTRQSKKLLERQASMGPLTLI SCDLGQTNLIAGRASAISLTHGSLGVRSSVRIELDPEIIKSFERL RKDADRLETEILTAAKETLSDEQRGEVNSHEKDSPQTAKASL CRELGLHPPSLPWGQMGPSTTFIADMLISHGRDDDAFLSHGE FPTLEKRKKFDKRFCLESRPLLSSETRKALNESLWEVKRTSSE YARLSQRKKEMARRAVNFVVEISRRKTGLSNVIVNIEDLNVR IFHGGGKQAPGWDGFFRPKSENRWFIQAIHKAFSDLAAHHGI PVIESDPQRTSMTCPECGHCDSKNRNGVRFLCKGCGASMDA DFDAACRNLERVALTGKPMPKPSTSCERLLSATTGKVCSDHS LSHDAIEKAS
CasΦ.4	4	MEKEITELTKIRREFPNKKFSSTDMKKAGKLLKAEGPDAVRD FLNSCQEIIGDFKPPVKTNIVSISRPFEEWPVSMVGRAIQEYYF SLTKEELESVHPGTSSEDHKSFFNITGLSNYNYTSVQGLNLIF KNAKAIYDGTLVKANNKNKKLEKKFNEINHKRSLEGLPIITP DFEEPFDENGHLNNPPGINRNIYGYQGCAAKVFVPSKHKMV SLPKEYEGYNRDPNLSLAGFRNRLEIPEGEPGHVPWFQRMDI PEGQIGHVNKIQRFNFVHGKNSGKVKFSDKTGRVKRYHHSK YKDATKPYKFLEESKKVSALDSILAIITIGDDWVVFDIRGLYR NVFYRELAQKGLTAVQLLDLFTGDPVIDPKKGVVTFSYKEG VVPVFSQKIVPRFKSRDTLEKLTSQGPVALLSVDLGQNEPVA ARVCSLKNINDKITLDNSCRISFLDDYKKQIKDYRDSLDELEI KIRLEAINSLETNQQVEIRDLDVFSADRAKANTVDMFDIDPN LISWDSMSDARVSTQISDLYLKNGGDESRVYFEINNKRIKRS DYNISQLVRPKLSDSTRKNLNDSIWKLKRTSEEYLKLSKRKL ELSRAVVNYTIRQSKLLSGINDIVIILEDLDVKKKFNGRGIRDI GWDNFFSSRKENRWFIPAFHKAFSELSSNRGLCVIEVNPAWT SATCPDCGFCSKENRDGINFTCRKCGVSYHADIDVATLNIAR VAVLGKPMSGPADRERLGDTKKPRVARSRKTMKRKDISNST VEAMVTA

CasΦ.5	5	MDMLDTETNYATETPAQQQDYSPKPPKKAQRAPKGFSKKA RPEKKPPKPITLFTQKHFSGVRFLKRVIRDASKILKLSESRTITF LEQAIERDGSAPPDVTPPVHNTIMAVTRPFEEWPEVILSKALQ KHCYALTKKIKIKTWPKKGPGKKCLAAWSARTKIPLIPGQVQ ATNGLFDRIGSIYDGVEKKVTNRNANKKLEYDEAIKEGRNPA VPEYETAYNIDGTLINKPGYNPNLYITQSRTPRLITEADRPLVE KILWQMVEKKTQSRNQARRARLEKAAHLQGLPVPKFVPEK VDRSQKIEIRIIDPLDKIEPYMPQDRMAIKASQDGHVPYWQRP FLSKRRNRRVRAGWGKQVSSIQAWLTGALLVIVRLGNEAFL ADIRGALRNAQWRKLLKPDATYQSLFNLFTGDPVVNTRTNH LTMAYREGVVNIVKSRSFKGRQTREHLLTLLGQGKTVAGVS FDLGQKHAAGLLAAHFGLGEDGNPVFTPIQACFLPQRYLDSL TNYRNRYDALTLDMRRQSLLALTPAQQQEFADAQRDPGGQ AKRACCLKLNLNPDEIRWDLVSGISTMISDLYIERGGDPRDV HQQVETKPKGKRKSEIRILKIRDGKWAYDFRPKIADETRKAQ REQLWKLQKASSEFERLSRYKINIARAIANWALQWGRELSG CDIVIPVLEDLNVGSKFFDGKGKWLLGWDNRFTPKKENRWF IKVLHKAVAELAPHRGVPVYEVMPHRTSMTCPACHYCHPTN REGDRFECQSCHVVKNTDRDVAPYNILRVAVEGKTLDRWQ AEKKPQAEPDRPMILIDNQES
CasΦ.6	6	MDMLDTETNYATETPAQQQDYSPKPPKKAQRAPKGFSKKA RPEKKPPKPITLFTQKHFSGVRFLKRVIRDASKILKLSESRTITF LEQAIERDGSAPPDVTPPVHNTIMAVTRPFEEWPEVILSKALQ KHCYALTKKIKIKTWPKKGPGKKCLAAWSARTKIPLIPGQVQ ATNGLFDRIGSIYDGVEKKVTNRNANKKLEYDEAIKEGRNPA VPEYETAYNIDGTLINKPGYNPNLYITQSRTPRLITEADRPLVE KILWQMVEKKTQSRNQARRARLEKAAHLQGLPVPKFVPEK VDRSQKIEIRIIDPLDKIEPYMPQDRMAIKASQDGHVPYWQRP FLSKRRNRRVRAGWGKQVSSIQAWLTGALLVIVRLGNEAFL ADIRGALRNAQWRKLLKPDATYQSLFNLFTGDPVVNTRTNH LTMAYREGVVDIVKSRSFKGRQTREHLLTLLGQGKTVAGVS FDLGQKHAAGLLAAHFGLGEDGNPVFTPIQACFLPQRYLDSL TNYRNRYDALTLDMRRQSLLALTPAQQQEFADAQRDPGGQ AKRACCLKLNLNPDEIRWDLVSGISTMISDLYIERGGDPRDV HQQVETKPKGKRKSEIRILKIRDGKWAYDFRPKIADETRKAQ REQLWKLQKASSEFERLSRYKINIARAIANWALQWGRELSG CDIVIPVLEDLNVGSKFFDGKGKWLLGWDNRFTPKKENRWF IKVLHKAVAELAPHKGVPVYEVMPHRTSMTCPACHYCHPTN REGDRFECQSCHVVKNTDRDVAPYNILRVAVEGKTLDRWQ AEKKPQAEPDRPMILIDNQES
CasΦ.7	7	MSSLPTPLELLKQKHADLFKGLQFSSKDNKMAGKVLKKDGE EAALAFLSERGVSRGELPNFRPPAKTLVVAQSRPFEEFPIYRV SEAIQLYVYSLSVKELETVPSGSSTKKEHQRFFQDSSVPDFGY TSVQGLNKIFGLARGIYLGVITRGENQLQKAKSKHEALNKKR RASGEAETEFDPTPYEYMTPERKLAKPPGVNHSIMCYVDISV DEFDFRNPDGIVLPSEYAGYCREINTAIEKGTVDRLGHLKGG PGYIPGHQRKESTTEGPKINFRKGRIRRSYTALYAKRDSRRVR QGKLALPSYRHHMMRLNSNAESAILAVIFFGKDWVVFDLRG LLRNVRWRNLFVDGSTPSTLLGMFGDPVIDPKRGVVAFCYK EQIVPVVSKSITKMVKAPELLNKLYLKSEDPLVLVAIDLGQT

		NPVGVGVYRVMNASLDYEVVTRFALESELLREIESYRQRTN AFEAQIRAETFDAMTSEEQEEITRVRAFSASKAKENVCHRFG MPVDAVDWATMGSNTIHIAKWVMRHGDPSLVEVLEYRKDN EIKLDKNGVPKKVKLTDKRIANLTSIRLRFSQETSKHYNDTM WELRRKHPVYQKLSKSKADFSRRVVNSIIRRVNHLVPRARIV FIIEDLKNLGKVFHGSGKRELGWDSYFEPKSENRWFIQVLHK AFSETGKHKGYYIIECWPNWTSCTCPKCSCCDSENRHGEVFR CLACGYTCNTDFGTAPDNLVKIATTGKGLPGPKKRCKGSSK GKNPKIARSSETGVSVTESGAPKVKKSSPTQTSQSSSQSAP
CasΦ.8	8	MNKIEKEKTPLAKLMNENFAGLRFPFAIIKQAGKKLLKEGEL KTIEYMTGKGSIEPLPNFKPPVKCLIVAKRRDLKYFPICKASC EIQSYVYSLNYKDFMDYFSTPMTSQKQHEEFFKKSGLNIEYQ NVAGLNLIFNNVKNTYNGVILKVKNRNEKLKKKAIKNNYEF EEIKTFNDDGCLINKPGINNVIYCFQSISPKILKNITHLPKEYND YDCSVDRNIIQKYVSRLDIPESQPGHVPEWQRKLPEFNNTNN PRRRRKWYSNGRNISKGYSVDQVNQAKIEDSLLAQIKIGED WIILDIRGLLRDLNRRELISYKNKLTIKDVLGFFSDYPIIDIKKN LVTFCYKEGVIQVVSQKSIGNKKSKQLLEKLIENKPIALVSID LGQTNPVSVKISKLNKINNKISIESFTYRFLNEEILKEIEKYRK DYDKLELKLINEA
CasΦ.9	9	MDMLDTETNYATETPSQQQDYSPKPPKKDRRAPKGFSKKAR PEKKPPKPITLFTQKHFSGVRFLKRVIRDASKILKLSESRTITFL EQAIERDGSAPPDVTPPVHNTIMAVTRPFEEWPEVILSKALQK HCYALTKKIKIKTWPKKGPGKKCLAAWSARTKIPLIPGQVQA TNGLFDRIGSIYDGVEKKVTNRNANKKLEYDEAIKEGRNPAV PEYETAYNIDGTLINKPGYNPNLYITQSRTPRLITEADRPLVEK ILWQMVEKKTQSRNQARRARLEKAAHLQGLPVPKFVPEKV DRSQKIEIRIIDPLDKIEPYMPQDRMAIKASQDGHVPYWQRPF LSKRRNRRVRAGWGKQVSSIQAWLTGALLVIVRLGNEAFLA DIRGALRNAQWRKLLKPDATYQSLFNLFTGDPVVNTRTNHL TMAYREGVVDIVKSRSFKGRQTREHLLTLLGQGKTVAGVSF DLGQKHAAGLLAAHFGLGEDGNPVFTPIQACFLPQRYLDSLT NYRNRYDALTLDMRRQSLLALTPAQQQEFADAQRDPGGQA KRACCLKLNLNPDEIRWDLVSGISTMISDLYIERGGDPRDVH QQVETKPKGKRKSEIRILKIRDGKWAYDFRPKIADETRKAQR EQLWKLQKASSEFERLSRYKINIARAIANWALQWGRELSGC DIVIPVLEDLNVGSKFFDGKGKWLLGWDNRFTPKKENRWFI KVLHKAVAELAPHRGVPVYEVMPHRTSMTCPACHYCHPTN REGDRFECQSCHVVKNTDRDVAPYNILRVAVEGKTLDRWQ AEKKPQAEPDRPMILIDNQES
CasΦ.10	10	MDMLDTETNYATETPSQQQDYSPKPPKKDRRAPKGFSKKAR PEKKPPKPITLFTQKHFSGVRFLKRVIRDASKILKLSESRTITFL EQAIERDGSAPPDVTPPVHNTIMAVTRPFEEWPEVILSKALQK HCYALTKKIKIKTWPKKGPGKKCLAAWSARTKIPLIPGQVQA TNGLFDRIGSIYDGVEKKVTNRNANKKLEYDEAIKEGRNPAV PEYETAYNIDGTLINKPGYNPNLYITQSRTPRLITEADRPLVEK ILWQMVEKKTQSRNQARRARLEKAAHLQGLPVPKFVPEKV DRSQKIEIRIIDPLDKIEPYMPQDRMAIKASQDGHVPYWQRPF LSKRRNRRVRAGWGKQVSSIQAWLTGALLVIVRLGNEAFLA

		DIRGALRNAQWRKLLKPDATYQSLFNLFTGDPVVNTRTNHL TMAYREGVVNIVKSRSFKGRQTREHLLTLLGQGKTVAGVSF DLGQKHAAGLLAAHFGLGEDGNPVFTPIQACFLPQRYLDSLT NYRNRYDALTLDMRRQSLLALTPAQQQEFADAQRDPGGQA KRACCLKLNLNPDEIRWDLVSGISTMISDLYIERGGDPRDVH QQVETKPKGKRKSEIRILKIRDGKWAYDFRPKIADETRKAQR EQLWKLQKASSEFERLSRYKINIARAIANWALQWGRELSGC DIVIPVLEDLNVGSKFFDGKGKWLLGWDNRFTPKKENRWFI KVLHKAVAELAPHRGVPVYEVMPHRTSMTCPACHYCHPTN REGDRFECQSCHVVKNTDRDVAPYNILRVAVEGKTLDRWQ AEKKPQAEPDRPMILIDNQES
CasΦ.11	11	MSNKTTPPSPLSLLLRAHFPGLKFESQDYKIAGKKLRDGGPE AVISYLTGKGQAKLKDVKPPAKAFVIAQSRPFIEWDLVRVSR QIQEKIFGIPATKGRPKQDGLSETAFNEAVASLEVDGKSKLNE ETRAAFYEVLGLDAPSLHAQAQNALIKSAISIREGVLKKVEN RNEKNLSKTKRRKEAGEEATFVEEKAHDERGYLIHPPGVNQ TIPGYQAVVIKSCPSDFIGLPSGCLAKESAEALTDYLPHDRMT IPKGQPGYVPEWQHPLLNRRKNRRRRDWYSASLNKPKATCS KRSGTPNRKNSRTDQIQSGRFKGAIPVLMRFQDEWVIIDIRGL LRNARYRKLLKEKSTIPDLLSLFTGDPSIDMRQGVCTFIYKAG QACSAKMVKTKNAPEILSELTKSGPVVLVSIDLGQTNPIAAK VSRVTQLSDGQLSHETLLRELLSNDSSDGKEIARYRVASDRL RDKLANLAVERLSPEHKSEILRAKNDTPALCKARVCAALGL NPEMIAWDKMTPYTEFLATAYLEKGGDRKVATLKPKNRPE MLRRDIKFKGTEGVRIEVSPEAAEAYREAQWDLQRTSPEYLR LSTWKQELTKRILNQLRHKAAKSSQCEVVVMAFEDLNIKMM HGNGKWADGGWDAFFIKKRENRWFMQAFHKSLTELGAHK GVPTIEVTPHRTSITCTKCGHCDKANRDGERFACQKCGFVAH ADLEIATDNIERVALTGKPMPKPESERSGDAKKSVGARKAAF KPEEDAEAAE
CasΦ.12	12	MIKPTVSQFLTPGFKLIRNHSRTAGLKLKNEGEEACKKFVRE NEIPKDECPNFQGGPAIANIIAKSREFTEWEIYQSSLAIQEVIFT LPKDKLPEPILKEEWRAQWLSEHGLDTVPYKEAAGLNLIIKN AVNTYKGVQVKVDNKNKNNLAKINRKNEIAKLNGEQEISFE EIKAFDDKGYLLQKPSPNKSIYCYQSVSPKPFITSKYHNVNLP EEYIGYYRKSNEPIVSPYQFDRLRIPIGEPGYVPKWQYTFLSK KENKRRKLSKRIKNVSPILGIICIKKDWCVFDMRGLLRTNHW KKYHKPTDSINDLFDYFTGDPVIDTKANVVRFRYKMENGIV NYKPVREKKGKELLENICDQNGSCKLATVDVGQNNPVAIGL FELKKVNGELTKTLISRHPTPIDFCNKITAYRERYDKLESSIKL DAIKQLTSEQKIEVDNYNNNFTPQNTKQIVCSKLNINPNDLP WDKMISGTHFISEKAQVSNKSEIYFTSTDKGKTKDVMKSDY KWFQDYKPKLSKEVRDALSDIEWRLRRESLEFNKLSKSREQ DARQLANWISSMCDVIGIENLVKKNNFFGGSGKREPGWDNF YKPKKENRWWINAIHKALTELSQNKGKRVILLPAMRTSITCP KCKYCDSKNRNGEKFNCLKCGIELNADIDVATENLATVAITA QSMPKPTCERSGDAKKPVRARKAKAPEFHDKLAPSYTVVLR EAV

CasΦ.13	13	MRQPAEKTAFQVFRQEVIGTQKLSGGDAKTAGRLYKQGKM EAAREWLLKGARDDVPPNFQPPAKCLVVAVSHPFEEWDISK TNHDVQAYIYAQPLQAEGHLNGLSEKWEDTSADQHKLWFE KTGVPDRGLPVQAINKIAKAAVNRAFGVVRKVENRNEKRRS RDNRIAEHNRENGLTEVVREAPEVATNADGFLLHPPGIDPSIL SYASVSPVPYNSSKHSFVRLPEEYQAYNVEPDAPIPQFVVED RFAIPPGQPGYVPEWQRLKCSTNKHRRMRQWSNQDYKPKA GRRAKPLEFQAHLTRERAKGALLVVMRIKEDWVVFDVRGL LRNVEWRKVLSEEAREKLTLKGLLDLFTGDPVIDTKRGIVTF LYKAEITKILSKRTVKTKNARDLLLRLTEPGEDGLRREVGLV AVDLGQTHPIAAAIYRIGRTSAGALESTVLHRQGLREDQKEK LKEYRKRHTALDSRLRKEAFETLSVEQQKEIVTVSGSGAQIT KDKVCNYLGVDPSTLPWEKMGSYTHFISDDFLRRGGDPNIV HFDRQPKKGKVSKKSQRIKRSDSQWVGRMRPRLSQETAKAR MEADWAAQNENEEYKRLARSKQELARWCVNTLLQNTRCIT QCDEIVVVIEDLNVKSLHGKGAREPGWDNFFTPKTENRWFIQ ILHKTFSELPKHRGEHVIEGCPLRTSITCPACSYCDKNSRNGE KFVCVACGATFHADFEVATYNLVRLATTGMPMPKSLERQG GGEKAGGARKARKKAKQVEKIVVQANANVTMNGASLHSP
CasΦ.14	14	MSSLPTPLELLKQKHADLFKGLQFSSKDNKMAGKVLKKDGE EAALAFLSERGVSRGELPNFRPPAKTLVVAQSRPFEEFPIYRV SEAIQLYVYSLSVKELETVPSGSSTKKEHQRFFQDSSVPDFGY TSVQGLNKIFGLARGIYLGVITRGENQLQKAKSKHEALNKKR RASGEAETEFDPTPYEYMTPERKLAKPPGVNHSIMCYVDISV DEFDFRNPDGIVLPSEYAGYCREINTAIEKGTVDRLGHLKGG PGYIPGHQRKESTTEGPKINFRKGRIRRSYTALYAKRDSRRVR QGKLALPSYRHHMMRLNSNAESAILAVIFFGKDWVVFDLRG LLRNVRWRNLFVDGSTPSTLLGMFGDPVIDPKRGVVAFCYK EQIVPVVSKSITKMVKAPELLNKLYLKSEDPLVLVAIDLGQT NPVGVGVYRVMNASLDYEVVTRFALESELLREIESYRQRTN AFEAQIRAETFDAMTSEEQEEITRVRAFSASKAKENVCHRFG MPVDAVDWATMGSNTIHIAKWVMRHGDPSLVEVLEYRKDN EIKLDKNGVPKKVKLTDKRIANLTSIRLRFSQETSKHYNDTM WELRRKHPVYQKLSKSKADFSRRVVNSIIRRVNHLVPRARIV FIIEDLKNLGKVFHGSGKRELGWDSYFEPKSENRWFIQVLHK AFSETGKHKGYYIIECWPNWTSCTCPKCSCCDSENRHGEVFR CLACGYTCNTDFGTAPDNLVKIATTGKGLPGPKKRCKGSSK GKNPKIARSSETGVSVTESGAPKVKKSSPTQTSQSSSQSAP
CasΦ.15	15	MIKPTVSQFLTPGFKLIRNHSRTAGLKLKNEGEEACKKFVRE NEIPKDECPNFQGGPAIANIIAKSREFTEWEIYQSSLAIQEVIFT LPKDKLPEPILKEEWRAQWLSEHGLDTVPYKEAAGLNLIIKN AVNTYKGVQVKVDNKNKNNLAKINRKNEIAKLNGEQEISFE EIKAFDDKGYLLQKPSPNKSIYCYQSVSPKPFITSKYHNVNLP EEYIGYYRKSNEPIVSPYQFDRLRIPIGEPGYVPKWQYTFLSK KENKRRKLSKRIKNVSPILGIICIKKDWCVFDMRGLLRTNHW KKYHKPTDSINDLFDYFTGDPVIDTKANVVRFRYKMENGIV NYKPVREKKGKELLENICDQNGSCKLATVDVGQNNPVAIGL FELKKVNGELTKTLISRHPTPIDFCNKITAYRERYDKLESSIKL DAIKQLTSEQKIEVDNYNNNFTPQNTKQIVCSKLNINPNDLP WDKMISGTHFISEKAQVSNKSEIYFTSTDKGKTKDVMKSDY

		KWFQDYKPKLSKEVRDALSDIEWRLRRESLEFNKLSKSREQ DARQLANWISSMCDVIGIENLVKKNNFFGGSGKREPGWDNF YKPKKENRWWINAIHKALTELSQNKGKRVILLPAMRTSITCP KCKYCDSKNRNGEKFNCLKCGIELNADIDVATENLATVAITA QSMPKPTCERSGDAKKPVRARKAKAPEFHDKLAPSYTVVLR EAV
CasΦ.16	16	MSNKTTPPSPLSLLLRAHFPGLKFESQDYKIAGKKLRDGGPE AVISYLTGKGQAKLKDVKPPAKAFVIAQSRPFIEWDLVRVSR QIQEKIFGIPATKGRPKQDGLSETAFNEAVASLEVDGKSKLNE ETRAAFYEVLGLDAPSLHAQAQNALIKSAISIREGVLKKVEN RNEKNLSKTKRRKEAGEEATFVEEKAHDERGYLIHPPGVNQ TIPGYQAVVIKSCPSDFIGLPSGCLAKESAEALTDYLPHDRMT IPKGQPGYVPEWQHPLLNRRKNRRRRDWYSASLNKPKATCS KRSGTPNRKNSRTDQIQSGRFKGAIPVLMRFQDEWVIIDIRGL LRNARYRKLLKEKSTIPDLLSLFTGDPSIDMRQGVCTFIYKAG QACSAKMVKTKNAPEILSELTKSGPVVLVSIDLGQTNPIAAK VSRVTQLSDGQLSHETLLRELLSNDSSDGKEIARYRVASDRL RDKLANLAVERLSPEHKSEILRAKNDTPALCKARVCAALGL NPEMIAWDKMTPYTEFLATAYLEKGGDRKVATLKPKNRPE MLRRDIKFKGTEGVRIEVSPEAAEAYREAQWDLQRTSPEYLR LSTWKQELTKRILNQLRHKAAKSSQCEVVVMAFEDLNIKMM HGNGKWADGGWDAFFIKKRENRWFMQAFHKSLTELGAHK GVPTIEVTPHRTSITCTKCGHCDKANRDGERFACQKCGFVAH ADLEIATDNIERVALTGKPMPKPESERSGDAKKSVGARKAAF KPEEDAEAAE
CasΦ.17	17	MYSLEMADLKSEPSLLAKLLRDRFPGKYWLPKYWKLAEKK RLTGGEEAACEYMADKQLDSPPPNFRPPARCVILAKSRPFED WPVHRVASKAQSFVIGLSEQGFAALRAAPPSTADARRDWLR SHGASEDDLMALEAQLLETIMGNAISLHGGVLKKIDNANVK AAKRLSGRNEARLNKGLQELPPEQEGSAYGADGLLVNPPGL NLNIYCRKSCCPKPVKNTARFVGHYPGYLRDSDSILISGTMD RLTIIEGMPGHIPAWQREQGLVKPGGRRRRLSGSESNMRQKV DPSTGPRRSTRSGTVNRSNQRTGRNGDPLLVEIRMKEDWVL LDARGLLRNLRWRESKRGLSCDHEDLSLSGLLALFSGDPVID PVRNEVVFLYGEGIIPVRSTKPVGTRQSKKLLERQASMGPLT LISCDLGQTNLIAGRASAISLTHGSLGVRSSVRIELDPEIIKSFE RLRKDADRLETEILTAAKETLSDEQRGEVNSHEKDSPQTAKA SLCRELGLHPPSLPWGQMGPSTTFIADMLISHGRDDDAFLSH GEFPTLEKRKKFDKRFCLESRPLLSSETRKALNESLWEVKRTS SEYARLSQRKKEMARRAVNFVVEISRRKTGLSNVIVNIEDLN VRIFHGGGKQAPGWDGFFRPKSENRWFIQAIHKAFSDLAAH HGIPVIESDPQRTSMTCPECGHCDSKNRNGVRFLCKGCGASM DADFDAACRNLERVALTGKPMPKPSTSCERLLSATTGKVCS DHSLSHDAIEKAS
CasΦ.18	18	MEKEITELTKIRREFPNKKFSSTDMKKAGKLLKAEGPDAVRD FLNSCQEIIGDFKPPVKTNIVSISRPFEEWPVSMVGRAIQEYYF SLTKEELESVHPGTSSEDHKSFFNITGLSNYNYTSVQGLNLIF KNAKAIYDGTLVKANNKNKKLEKKFNEINHKRSLEGLPIITP DFEEPFDENGHLNNPPGINRNIYGYQGCAAKVFVPSKHKMV

		SLPKEYEGYNRDPNLSLAGFRNRLEIPEGEPGHVPWFQRMDI PEGQIGHVNKIQRFNFVHGKNSGKVKFSDKTGRVKRYHHSK YKDATKPYKFLEESKKVSALDSILAIITIGDDWVVFDIRGLYR NVFYRELAQKGLTAVQLLDLFTGDPVIDPKKGVVTFSYKEG VVPVFSQKIVPRFKSRDTLEKLTSQGPVALLSVDLGQNEPVA ARVCSLKNINDKITLDNSCRISFLDDYKKQIKDYRDSLDELEI KIRLEAINSLETNQQVEIRDLDVFSADRAKANTVDMFDIDPN LISWDSMSDARVSTQISDLYLKNGGDESRVYFEINNKRIKRS DYNISQLVRPKLSDSTRKNLNDSIWKLKRTSEEYLKLSKRKL ELSRAVVNYTIRQSKLLSGINDIVIILEDLDVKKKFNGRGIRDI GWDNFFSSRKENRWFIPAFHKTFSELSSNRGLCVIEVNPAWT SATCPDCGFCSKENRDGINFTCRKCGVSYHADIDVATLNIAR VAVLGKPMSGPADRERLGDTKKPRVARSRKTMKRKDISNST VEAMVTA
CasΦ.19	19	MLVRTSTLVQDNKNSRSASRAFLKKPKMPKNKHIKEPTELA KLIRELFPGQRFTRAINTQAGKILKHKGRDEVVEFLKNKGIDK EQFMDFRPPTKARIVATSGAIEEFSYLRVSMAIQECCFGKYKF PKEKVNGKLVLETVGLTKEELDDFLPKKYYENKKSRDRFFL KTGICDYGYTYAQGLNEIFRNTRAIYEGVFTKVNNRNEKRRE KKDKYNEERRSKGLSEEPYDEDESATDESGHLINPPGVNLNI WTCEGFCKGPYVTKLSGTPGYEVILPKVFDGYNRDPNEIISC GITDRFAIPEGEPGHIPWHQRLEIPEGQPGYVPGHQRFADTGQ NNSGKANPNKKGRMRKYYGHGTKYTQPGEYQEVFRKGHRE GNKRRYWEEDFRSEAHDCILYVIHIGDDWVVCDLRGPLRDA YRRGLVPKEGITTQELCNLFSGDPVIDPKHGVVTFCYKNGLV RAQKTISAGKKSRELLGALTSQGPIALIGVDLGQTEPVGARAF IVNQARGSLSLPTLKGSFLLTAENSSSWNVFKGEIKAYREAID DLAIRLKKEAVATLSVEQQTEIESYEAFSAEDAKQLACEKFG VDSSFILWEDMTPYHTGPATYYFAKQFLKKNGGNKSLIEYIP YQKKKSKKTPKAVLRSDYNIACCVRPKLLPETRKALNEAIRI VQKNSDEYQRLSKRKLEFCRRVVNYLVRKAKKLTGLERVII AIEDLKSLEKFFTGSGKRDNGWSNFFRPKKENRWFIPAFHKA FSELAPNRGFYVIECNPARTSITDPDCGYCDGDNRDGIKFECK KCGAKHHTDLDVAPLNIAIVAVTGRPMPKTVSNKSKRERSG GEKSVGASRKRNHRKSKANQEMLDATSSAAE
CasΦ.20	20	MPKIKKPTEISLLRKEVFPDLHFAKDRMRAASLVLKNEGREA AIEYLRVNHEDKPPNFMPPAKTPYVALSRPLEQWPIAQASIAI QKYIFGLTKDEFSATKKLLYGDKSTPNTESRKRWFEVTGVPN FGYMSAQGLNAIFSGALARYEGVVQKVENRNKKRFEKLSEK NQLLIEEGQPVKDYVPDTAYHTPETLQKLAENNHVRVEDLG DMIDRLVHPPGIHRSIYGYQQVPPFAYDPDNPKGIILPKAYAG YTRKPHDIIEAMPNRLNIPEGQAGYIPEHQRDKLKKGGRVKR LRTTRVRVDATETVRAKAEALNAEKARLRGKEAILAVFQIEE DWALIDMRGLLRNVYMRKLIAAGELTPTTLLGYFTETLTLDP RRTEATFCYHLRSEGALHAEYVRHGKNTRELLLDLTKDNEKI ALVTIDLGQRNPLAAAIFRVGRDASGDLTENSLEPVSRMLLP QAYLDQIKAYRDAYDSFRQNIWDTALASLTPEQQRQILAYE AYTPDDSKENVLRLLLGGNVMPDDLPWEDMTKNTHYISDR YLADGGDPSKVWFVPGPRKRKKNAPPLKKPPKPRELVKRSD HNISHLSEFRPQLLKETRDAFEKAKIDTERGHVGYQKLSTRK

		DQLCKEILNWLEAEAVRLTRCKTMVLGLEDLNGPFFNQGKG KVRGWVSFFRQKQENRWIVNGFRKNALARAHDKGKYILEL WPSWTSQTCPKCKHVHADNRHGDDFVCLQCGARLHADAEV ATWNLAVVAIQGHSLPGPVREKSNDRKKSGSARKSKKANES GKVVGAWAAQATPKRATSKKETGTARNPVYNPLETQASCP AP
CasΦ.21	21	MTPSPQIARLVETPLAAALKAHHPGKKFRSDYLKKAGKILKD QGVEAAMAHLDGKDQAEPPNFKPPAKCRIVARSREFSEWPI VKASVEIQKYIYGLTLEERKACDPGKSSASHKAWFAKTGVN TFGYSSVQGFNLIFGHTLGRYDGVLVKTENLNKKRAEKNER FRAKALAEGRAEPVCPPLVTATNDTGQDVTLEDGRVVRPGQ LLQPPGINPNIYAYQQVSPKAYVPGIIELPEEFQGYSRDPNAVI LPLVPRDRLSIPKGQPGYVPEPHREGLTGRKDRRMRRYYETE RGTKLKRPPLTAKGRADKANEALLVVVRIDSDWVVMDVRG LLRNARWRRLVSKEGITLNGLLDLFTGDPVLNPKDCSVSRDT GDPVNDPRHGVVTFCYKLGVVDVCSKDRPIKGFRTKEVLER LTSSGTVGMVSIDLGQTNPVAAAVSRVTKGLQAETLETFTLP DDLLGKVRAYRAKTDRMEEGFRRNALRKLTAEQQAEITRYN DATEQQAKALVCSTYGIGPEEVPWERMTSNTTYISDHILDHG GDPDTVFFMATKRGQNKPTLHKRKDKAWGQKFRPAISVETR LARQAAEWELRRASLEFQKLSVWKTELCRQAVNYVMERTK KRTQCDVIIPVIEDLPVPLFHGSGKRDPGWANFFVHKRENRW FIDGLHKAFSELGKHRGIYVFEVCPQRTSITCPKCGHCDPDNR DGEKFVCLSCQATLNADLDVATTNLVRVALTGKVMPRSERS GDAQTPGPARKARTGKIKGSKPTSAPQGATQTDAKAHLSQT GV
CasΦ.22	22	MTPSPQIARLVETPLAAALKAHHPGKKFRSDYLKKAGKILKD QGVEAAMAHLDGKDQAEPPNFKPPAKCRIVARSREFSEWPI VKASVEIQKYIYGLTLEERKACDPGKSSASHKAWFAKTGVN TFGYSSVQGFNLIFGHTLGRYDGVLVKTENLNKKRAEKNER FRAKALAEGRAEPVCPPLVTATNDTGQDVTLEDGRVVRPGQ LLQPPGINPNIYAYQQVSPKAYVPGIIELPEEFQGYSRDPNAVI LPLVPRDRLSIPKGQPGYVPEPHREGLTGRKDRRMRRYYETE RGTKLKRPPLTAKGRADKANEALLVVVRIDSDWVVMDVRG LLRNARWRRLVSKEGITLNGLLDLFTGDPVLNPKDCSVSRDT GDPVNDPRHGVVTFCYKLGVVDVCSKDRPIKGFRTKEVLER LTSSGTVGMVSIDLGQTNPVAAAVSRVTKGLQAETLETFTLP DDLLGKVRAYRAKTDRMEEGFRRNALRKLTAEQQAEITRYN DATEQQAKALVCSTYGIGPEEVPWERMTSNTTYISDHILDHG GDPDTVFFMATKRGQNKPTLHKRKDKAWGQKFRPAISVETR LARQAAEWELRRASLEFQKLSVWKTELCRQAVNYVMERTK KRTQCDVIIPVIEDLPVPLFHGSGKRDPGWANFFVHKRENRW FIDGLHKAFSELGKHRGIYVFEVCPQRTSITCPKCGHCDPDNR DGEKFVCLSCQATLHADLDVATTNLVRVALTGKVMPRSERS GDAQTPGPARKARTGKIKGSKPTSAPQGATQTDAKAHLSQT GV
CasΦ.23	23	MKTEKPKTALTLLREEVFPGKKYRLDVLKEAGKKLSTKGRE ATIEFLTGKDEERPQNFQPPAKTSIVAQSRPFDQWPIVQVSLA VQKYIYGLTQSEFEANKKALYGETGKAISTESRRAWFEATGV

		DNFGFTAAQGINPIFSQAVARYEGVIKKVENRNEKKLKKLTK KNLLRLESGEEIEDFEPEATFNEEGRLLQPPGANPNIYCYQQIS PRIYDPSDPKGVILPQIYAGYDRKPEDIISAGVPNRLAIPEGQP GYIPEHQRAGLKTQGRIRCRASVEAKARAAILAVVHLGEDW VVLDLRGLLRNVYWRKLASPGTLTLKGLLDFFTGGPVLDAR RGIATFSYTLKSAAAVHAENTYKGKGTREVLLKLTENNSVA LVTVDLGQRNPLAAMIARVSRTSQGDLTYPESVEPLTRLFLP DPFLEEVRKYRSSYDALRLSIREAAIASLTPEQQAEIRYIEKFS AGDAKKNVAEVFGIDPTQLPWDAMTPRTTYISDLFLRMGGD RSRVFFEVPPKKAKKAPKKPPKKPAGPRIVKRTDGMIARLREI RPRLSAETNKAFQEARWEGERSNVAFQKLSVRRKQFARTVV NHLVQTAQKMSRCDTVVLGIEDLNVPFFHGRGKYQPGWEG FFRQKKENRWLINDMHKALSERGPHRGGYVLELTPFWTSLR CPKCGHTDSANRDGDDFVCVKCGAKLHSDLEVATANLALV AITGQSIPRPPREQSSGKKSTGTARMKKTSGETQGKGSKACV SEALNKIEQGTARDPVYNPLNSQVSCPAP
CasΦ.24	24	VYNPDMKKPNNIRRIREEHFEGLCFGKDVLTKAGKIYEKDGE EAAIDFLMGKDEEDPPNFKPPAKTTIVAQSRPFDQWPIYQVS QAVQERVFAYTEEEFNASKEALFSGDISSKSRDFWFKTNNIS DQGIGAQGLNTILSHAFSRYSGVIKKVENRNKKRLKKLSKKN QLKIEEGLEILEFKPDSAFNENGLLAQPPGINPNIYGYQAVTPF VFDPDNPGDVILPKQYEGYSRKPDDIIEKGPSRLDIPKGQPGY VPEHQRKNLKKKGRVRLYRRTPPKTKALASILAVLQIGKDW VLFDMRGLLRSVYMREAATPGQISAKDLLDTFTGCPVLNTR TGEFTFCYKLRSEGALHARKIYTKGETRTLLTSLTSENNTIAL VTVDLGQRNPAAIMISRLSRKEELSEKDIQPVSRRLLPDRYLN ELKRYRDAYDAFRQEVRDEAFTSLCPEHQEQVQQYEALTPE KAKNLVLKHFFGTHDPDLPWDDMTSNTHYIANLYLERGGDP SKVFFTRPLKKDSKSKKPRKPTKRTDASISRLPEIRPKMPEDA RKAFEKAKWEIYTGHEKFPKLAKRVNQLCREIANWIEKEAK RLTLCDTVVVGIEDLSLPPKRGKGKFQETWQGFFRQKFENR WVIDTLKKAIQNRAHDKGKYVLGLAPYWTSQRCPACGFIHK SNRNGDHFKCLKCEALFHADSEVATWNLALVAVLGKGITNP DSKKPSGQKKTGTTRKKQIKGKNKGKETVNVPPTTQEVEDII AFFEKDDETVRNPVYKPTGT
CasΦ.25	25	MKKPNNIRRIREEHFEGLCFGKDVLTKAGKIYEKDGEEAAID FLMGKDEEDPPNFKPPAKTTIVAQSRPFDQWPIYQVSQAVQE RVFAYTEEEFNASKEALFSGDISSKSRDFWFKTNNISDQGIGA QGLNTILSHAFSRYSGVIKKVENRNKKRLKKLSKKNQLKIEE GLEILEFKPDSAFNENGLLAQPPGINPNIYGYQAVTPFVFDPD NPGDVILPKQYEGYSRKPDDIIEKGPSRLDIPKGQPGYVPEHQ RKNLKKKGRVRLYRRTPPKTKALASILAVLQIGKDWVLFDM RGLLRSVYMREAATPGQISAKDLLDTFTGCPVLNTRTGEFTF CYKLRSEGALHARKIYTKGETRTLLTSLTSENNTIALVTVDL GQRNPAAIMISRLSRKEELSEKDIQPVSRRLLPDRYLNELKRY RDAYDAFRQEVRDEAFTSLCPEHQEQVQQYEALTPEKAKNL VLKHFFGTHDPDLPWDDMTSNTHYIANLYLERGGDPSKVFF TRPLKKDSKSKKPRKPTKRTDASISRLPEIRPKMPEDARKAFE KAKWEIYTGHEKFPKLAKRVNQLCREIANWIEKEAKRLTLC DTVVVGIEDLSLPPKRGKGKFQETWQGFFRQKFENRWVIDT

		LKKAIQNRAHDKGKYVLGLAPYWTSQRCPACGFIHKSNRNG DHFKCLKCEALFHADSEVATWNLALVAVLGKGITNPDSKKP SGQKKTGTTRKKQIKGKNKGKETVNVPPTTQEVEDIIAFFEK DDETVRNPVYKPTGT
CasΦ.26	26	VIKTHFPAGRFRKDHQKTAGKKLKHEGEEACVEYLRNKVSD YPPNFKPPAKGTIVAQSRPFSEWPIVRASEAIQKYVYGLTVAE LDVFSPGTSKPSHAEWFAKTGVENYGYRQVQGLNTIFQNTV NRFKGVLKKVENRNKKSLKRQEGANRRRVEEGLPEVPVTVE SATDDEGRLLQPPGVNPSIYGYQGVAPRVCTDLQGFSGMSV DFAGYRRDPDAVLVESLPEGRLSIPKGERGYVPEWQRDPERN KFPLREGSRRQRKWYSNACHKPKPGRTSKYDPEALKKASAK DALLVSISIGEDWAIIDVRGLLRDARRRGFTPEEGLSLNSLLG LFTEYPVFDVQRGLITFTYKLGQVDVHSRKTVPTFRSRALLES LVAKEEIALVSVDLGQTNPASMKVSRVRAQEGALVAEPVHR MFLSDVLLGELSSYRKRMDAFEDAIRAQAFETMTPEQQAEIT RVCDVSVEVARRRVCEKYSISPQDVPWGEMTGHSTFIVDAV LRKGGDESLVYFKNKEGETLKFRDLRISRMEGVRPRLTKDTR DALNKAVLDLKRAHPTFAKLAKQKLELARRCVNFIEREAKR YTQCERVVFVIEDLNVGFFHGKGKRDRGWDAFFTAKKENR WVIQALHKAFSDLGLHRGSYVIEVTPQRTSMTCPRCGHCDK GNRNGEKFVCLQCGATLHADLEVATDNIERVALTGKAMPKP PVRERSGDVQKAGTARKARKPLKPKQKTEPSVQEGSSDDGV DKSPGDASRNPVYNPSDTLSI
CasΦ.27	27	MAKAKTLAALLRELLPGQHLAPHHRWVANKLLMTSGDAAA FVIGKSVSDPVRGSFRKDVITKAGRIFKKDGPDAAAAFLDGK WEDRPPNFQPPAKAAIVAISRSFDEWPIVKVSCAIQQYLYALP VQEFESSVPEARAQAHAAWFQDTGVDDCNFKSTQGLNAIFN HGKRTYEGVLKKAQNRNDKKNLRLERINAKRAEAGQAPLV AGPDESPTDDAGCLLHPPGINANIYCYQQVSPRPYEQSCGIQL PPEYAGYNRLSNVAIPPMPNRLDIPQGQPGYVPEHHRHGIKK FGRVRKRYGVVPGRNRDADGKRTRQVLTEAGAAAKARDSV LAVIRIGDDWTVVDLRGLLRNAQWRKLVPDGGITVQGLLDL FTGDPVIDPRRGVVTFIYKADSVGIHSEKVCRGKQSKNLLER LCAMPEKSSTRLDCARQAVALVSVDLGQRNPVAARFSRVSL AEGQLQAQLVSAQFLDDAMVAMIRSYREEYDRFESLVREQA KAALSPEQLSEIVRHEADSAESVKSCVCAKFGIDPAGLSWDK MTSGTWRIADHVQAAGGDVEWFFFKTCGKGKEIKTVRRSDF NVAKQFRLRLSPETRKDWNDAIWELKRGNPAYVSFSKRKSE FARRVVNDLVHRARRAVRCDEVVFAIEDLNISFFHGKGQRQ MGWDAFFEVKQENRWFIQALHKAFVERATHKGGYVLEVAP ARTSTTCPECRHCDPESRRGEQFCCIKCRHTCHADLEVATFNI EQVALTGVSLPKRLSSTLL
CasΦ.28	28	MSKEKTPPSAYAILKAKHFPDLDFEKKHKMMAGRMFKNGA SEQEVVQYLQGKGSESLMDVKPPAKSPILAQSRPFDEWEMV RTSRLIQETIFGIPKRGSIPKRDGLSETQFNELVASLEVGGKPM LNKQTRAIFYGLLGIKPPTFHAMAQNILIDLAINIRKGVLKKV DNLNEKNRKKVKRIRDAGEQDVMVPAEVTAHDDRGYLNHP PGVNPTIPGYQGVVIPFPEGFEGLPSGMTPVDWSHVLVDYLP HDRLSIPKGSPGYIPEWQRPLLNRHKGRRHRSWYANSLNKPR

		KSRTEEAKDRQNAGKRTALIEAERLKGVLPVLMRFKEDWLII DARGLLRNARYRGVLPEGSTLGNLIDLFSDSPRVDTRRGICTF LYRKGRAYSTKPVKRKESKETLLKLTEKSTIALVSIDLGQTNP LTAKLSKVRQVDGCLVAEPVLRKLIDNASEDGKEIARYRVA HDLLRARILEDAIDLLGIYKDEVVRARSDTPDLCKERVCRFL GLDSQAIDWDRMTPYTDFIAQAFVAKGGDPKVVTIKPNGKP KMFRKDRSIKNMKGIRLDISKEASSAYREAQWAIQRESPDFQ RLAVWQSQLTKRIVNQLVAWAKKCTQCDTVVLAFEDLNIG MMHGSGKWANGGWNALFLHKQENRWFMQAFHKALTELS AHKGIPTIEVLPHRTSITCTQCGHCHPGNRDGERFKCLKCEFL ANTDLEIATDNIERVALTGLPMPKGERSSAKRKPGGTRKTKK SKHSGNSPLAAE
CasΦ.29	29	MEKAGPTSPLSVLIHKNFEGCRFQIDHLKIAGRKLAREGEAA AIEYLLDKKCEGLPPNFQPPAKGNVIAQSRPFTEWAPYRASV AIQKYIYSLSVDERKVCDPGSSSDSHEKWFKQTGVQNYGYT HVQGLNLIFKHALARYDGVLKKVDNRNEKNRKKAERVNSF RREEGLPEEVFEEEKATDETGHLLQPPGVNHSIYCYQSVRPK PFNPRKPGGISLPEAYSGYSLKPQDELPIGSLDRLSIPPGQPGY VPEWQRSQLTTQKHRRKRSWYSAQKWKPRTGRTSTFDPDR LNCARAQGAILAVVRIHEDWVVFDVRGLLRNALWRELAGK GLTVRDLLDFFTGDPVVDTKRGVVTFTYKLGKVDVHSLRTV RGKRSKKVLEDLTLSSDVGLVTIDLGQTNVLAADYSKVTRSE NGELLAVPLSKSFLPKHLLHEVTAYRTSYDQMEEGFRRKALL TLTEDQQVEVTLVRDFSVESSKTKLLQLGVDVTSLPWEKMS SNTTYISDQLLQQGADPASLFFDGERDGKPCRHKKKDRTWA YLVRPKVSPETRKALNEALWALKNTSPEFESLSKRKIQFSRR CMNYLLNEAKRISGCGQVVFVIEDLNVRVHHGRGKRAIGWD NFFKPKRENRWFMQALHKAASELAIHRGMHIIEACPARSSIT CPKCGHCDPENRCSSDREKFLCVKCGAAFHADLEVATFNLR KVALTGTALPKSIDHSRDGLIPKGARNRKLKEPQANDEKACA
CasΦ.30	30	MKEQSPLSSVLKSNFPGKKFLSADIRVAGRKLAQLGEAAAVE YLSPRQRDSVPNFRPPAFCTVVAKSRPFEEWPIYKASVLLQE QIYGMTGQEFEERCGSIPTSLSGLRQWASSVGLGAAMEGLH VQGMNLMVKNAINRYKGVLVKVENRNKKLVEANEAKNSS REERGLPPLRPPELGSAFGPDGRLVNPPGIDKSIRLYQGVSPV PVVKTTGRPTVHRLDIPAGEKGHVPLWQREAGLVKEGPRRR RMWYSNSNLKRSRKDRSAEASEARKADSVVVRVSVKEDWV DIDVRGLLRNVAWRGIERAGESTEDLLSLFSGDPVVDPSRDS VVFLYKEGVVDVLSKKVVGAGKSRKQLEKMVSEGPVALVS CDLGQTNYVAARVSVLDESLSPVRSFRVDPREFPSADGSQGV VGSLDRIRADSDRLEAKLLSEAEASLPEPVRAEIEFLRSERPSA VAGRLCLKLGIDPRSIPWEKMGSTTSFISEALSAKGSPLALHD GAPIKDSRFAHAARGRLSPESRKALNEALWERKSSSREYGVI SRRKSEASRRMANAVLSESRRLTGLAVVAVNLEDLNMVSKF FHGRGKRAPGWAGFFTPKMENRWFIRSIHKAMCDLSKHRGI TVIESRPERTSISCPECGHCDPENRSGERFSCKSCGVSLHADFE VATRNLERVALTGKPMPRRENLHSPEGATASRKTRKKPREA TASTFLDLRSVLSSAENEGSGPAARAG

CasΦ.31	31	MLPPSNKIGKSMSLKEFINKRNFKSSIIKQAGKILKKEGEEAV KKYLDDNYVEGYKKRDFPITAKCNIVASNRKIEDFDISKFSSF IQNYVFNLNKDNFEEFSKIKYNRKSFDELYKKIANEIGLEKPN YENIQGEIAVIRNAINIYNGVLKKVENRNKKIQEKNQSKDPPK LLSAFDDNGFLAERPGINETIYGYQSVRLRHLDVEKDKDIIVQ LPDIYQKYNKKSTDKISVKKRLNKYNVDEYGKLISKRRKERI NKDDAILCVSNFGDDWIIFDARGLLRQTYRYKLKKKGLCIKD LLNLFTGDPIINPTKTDLKEALSLSFKDGIINNRTLKVKNYKK CPELISELIRDKGKVAMISIDLGQTNPISYRLSKFTANNVAYIE NGVISEDDIVKMKKWREKSDKLENLIKEEAIASLSDDEQREV RLYENDIADNTKKKILEKFNIREEDLDFSKMSNNTYFIRDCLK NKNIDESEFTFEKNGKKLDPTDACFAREYKNKLSELTRKKIN EKIWEIKKNSKEYHKISIYKKETIRYIVNKLIKQSKEKSECDDII VNIEKLQIGGNFFGGRGKRDPGWNNFFLPKEENRWFINACH KAFSELAPHKGIIVIESDPAYTSQTCPKCENCDKENRNGEKFK CKKCNYEANADIDVATENLEKIAKNGRRLIKNFDQLGERLPG AEMPGGARKRKPSKSLPKNGRGAGVGSEPELINQSPSQVIA
CasΦ.32	32	VPDKKETPLVALCKKSFPGLRFKKHDSRQAGRILKSKGEGAA VAFLEGKGGTTQPNFKPPVKCNIVAMSRPLEEWPIYKASVVI QKYVYAQSYEEFKATDPGKSEAGLRAWLKATRVDTDGYFN VQGLNLIFQNARATYEGVLKKVENRNSKKVAKIEQRNEHRA ERGLPLLTLDEPETALDETGHLRHRPGINCSVFGYQHMKLKP YVPGSIPGVTGYSRDPSTPIAACGVDRLEIPEGQPGYVPPWDR ENLSVKKHRRKRASWARSRGGAIDDNMLLAVVRVADDWA LLDLRGLLRNTQYRKLLDRSVPVTIESLLNLVTNDPTLSVVK KPGKPVRYTATLIYKQGVVPVVKAKVVKGSYVSKMLDDTT ETFSLVGVDLGVNNLIAANALRIRPGKCVERLQAFTLPEQTV EDFFRFRKAYDKHQENLRLAAVRSLTAEQQAEVLALDTFGP EQAKMQVCGHLGLSVDEVPWDKVNSRSSILSDLAKERGVD DTLYMFPFFKGKGKKRKTEIRKRWDVNWAQHFRPQLTSETR KALNEAKWEAERNSSKYHQLSIRKKELSRHCVNYVIRTAEK RAQCGKVIVAVEDLHHSFRRGGKGSRKSGWGGFFAAKQEG RWLMDALFGAFCDLAVHRGYRVIKVDPYNTSRTCPECGHC DKANRDRVNREAFICVCCGYRGNADIDVAAYNIAMVAITGV SLRKAARASVASTPLESLAAE
CasΦ.33	33	MSKTKELNDYQEALARRLPGVRHQKSVRRAARLVYDRQGE DAMVAFLDGKEVDEPYTLQPPAKCHILAVSRPIEEWPIARVT MAVQEHVYALPVHEVEKSRPETTEGSRSAWFKNSGVSNHG VTHAQTLNAILKNAYNVYNGVIKKVENRNAKKRDSLAAKN KSRERKGLPHFKADPPELATDEQGYLLQPPSPNSSVYLVQQH LRTPQIDLPSGYTGPVVDPRSPIPSLIPIDRLAIPPGQPGYVPLH DREKLTSNKHRRMKLPKSLRAQGALPVCFRVFDDWAVVDG RGLLRHAQYRRLAPKNVSIAELLELYTGDPVIDIKRNLMTFR FAEAVVEVTARKIVEKYHNKYLLKLTEPKGKPVREIGLVSID LNVQRLIALAIYRVHQTGESQLALSPCLHREILPAKGLGDFDK YKSKFNQLTEEILTAAVQTLTSAQQEEYQRYVEESSHEAKAD LCLKYSITPHELAWDKMTSSTQYISRWLRDHGWNASDFTQIT KGRKKVERLWSDSRWAQELKPKLSNETRRKLEDAKHDLQR ANPEWQRLAKRKQEYSRHLANTVLSMAREYTACETVVIAIE NLPMKGGFVDGNGSRESGWDNFFTHKKENRWMIKDIHKAL

		SDLAPNRGVHVLEVNPQYTSQTCPECGHRDKANRDPIQRERF CCTHCGAQRHADLEVATHNIAMVATTGKSLTGKSLAPQRLQ EAAE
CasΦ.41	34	VLLSDRIQYTDPSAPIPAMTVVDRRKIKKGEPGYVPPFMRKN LSTNKHRRMRLSRGQKEACALPVGLRLPDGKDGWDFIIFDG RALLRACRRLRLEVTSMDDVLDKFTGDPRIQLSPAGETIVTC MLKPQHTGVIQQKLITGKMKDRLVQLTAEAPIAMLTVDLGE HNLVACGAYTVGQRRGKLQSERLEAFLLPEKVLADFEGYRR DSDEHSETLRHEALKALSKRQQREVLDMLRTGADQARESLC YKYGLDLQALPWDKMSSNSTFIAQHLMSLGFGESATHVRYR PKRKASERTILKYDSRFAAEEKIKLTDETRRAWNEAIWECQR ASQEFRCLSVRKLQLARAAVNWTLTQAKQRSRCPRVVVVV EDLNVRFMHGGGKRQEGWAGFFKARSEKRWFIQALHKAYT ELPTNRGIHVMEVNPARTSITCTKCGYCDPENRYGEDFHCRN PKCKVRGGHVANADLDIATENLARVALSGPMPKAPKLK
CasΦ.34	35	MTPSFGYQMIIVTPIHHASGAWATLRLLFLNPKTSGVMLGMT KTKSAFALMREEVFPGLLFKSADLKMAGRKFAKEGREAAIE YLRGKDEERPANFKPPAKGDIIAQSRPFDQWPIVQVSQAIQK YIFGLTKAEFDATKTLLYGEGNHPTTESRRRWFEATGVPDFG FTSAQGLNAIFSSALARYEGVIQKVENRNEKRLKKLSEKNQR LVEEGHAVEAYVPETAFHTLESLKALSEKSLVPLDDLMDKID RLAQPPGINPCLYGYQQVAPYIYDPENPRGVVLPDLYLGYCR KPDDPITACPNRLDIPKGQPGYIPEHQRGQLKKHGRVRRFRY TNPQAKARAKAQTAILAVLRIDEDWVVMDLRGLLRNVYFRE VAAPGELTARTLLDTFTGCPVLNLRSNVVTFCYDIESKGALH AEYVRKGWATRNKLLDLTKDGQSVALLSVDLGQRHPVAVM ISRLKRDDKGDLSEKSIQVVSRTFADQYVDKLKRYRVQYDA LRKEIYDAALVSLPPEQQAEIRAYEAFAPGDAKANVLSVMFQ GEVSPDELPWDKMNTNTHYISDLYLRRGGDPSRVFFVPQPST PKKNAKKPPAPRKPVKRTDENVSHMPEFRPHLSNETREAFQ KAKWTMERGNVRYAQLSRFLNQIVREANNWLVSEAKKLTQ CQTVVWAIEDLHVPFFHGKGKYHETWDGFFRQKKEDRWFV NVFHKAISERAPNKGEYVMEVAPYRTSQRCPVCGFVDADNR HGDHFKCLRCGVELHADLEVATWNIALVAVQGHGIAGPPRE QSCGGETAGTARKGKNIKKNKGLADAVTVEAQDSEGGSKK DAGTARNPVYIPSESQVNCPAP
CasΦ.35	36	MKPKTPKPPKTPVAALIDKHFPGKRFRASYLKSVGKKLKNQ GEDVAVRFLTGKDEERPPNFQPPAKSNIVAQSRPIEEWPIHKV SVAVQEYVYGLTVAEKEACSDAGESSSSHAAWFAKTGVENF GYTSVQGLNKIFPPTFNRFDGVIKKVENRNEKKRQKATRINE AKRNKGQSEDPPEAEVKATDDAGYLLQPPGINHSVYGYQSIT LCPYTAEKFPTIKLPEEYAGYHSNPDAPIPAGVPDRLAIPEGQ PGHVPEEHRAGLSTKKHRRVRQWYAMANWKPKPKRTSKPD YDRLAKARAQGALLIVIRIDEDWVVVDARGLLRNVRWRSLG KREITPNELLDLFTGDPVLDLKRGVVTFTYAEGVVNVCSRST TKGKQTKVLLDAMTAPRDGKKRQIGMVAVDLGQTNPIAAE YSRVGKNAAGTLEATPLSRSTLPDELLREIALYRKAHDRLEA QLREEAVLKLTAEQQAENARYVETSEEGAKLALANLGVDTS TLPWDAMTGWSTCISDHLINHGGDTSAVFFQTIRKGTKKLET

		IKRKDSSWADIVRPRLTKETREALNDFLWELKRSHEGYEKLS KRLEELARRAVNHVVQEVKWLTQCQDIVIVIEDLNVRNFHG GGKRGGGWSNFFTVKKENRWFMQALHKAFSDLAAHRGIPV LEVYPARTSITCLGCGHCDPENRDGEAFVCQQCGATFHADLE VATRNIARVALTGEAMPKAPAREQPGGAKKRGTSRRRKLTE VAVKSAEPTIHQAKNQQLNGTSRDPVYKGSELPAL
CasΦ.43	37	MSEITDLLKANFKGKTFKSADMRMAGRILKKSGAQAVIKYL SDKGAVDPPDFRPPAKCNIIAQSRPFDEWPICKASMAIQQHIY GLTKNEFDESSPGTSSASHEQWFAKTGVDTHGFTHVQGLNLI FQHAKKRYEGVIKKVENYNEKERKKFEGINERRSKEGMPLL EPRLRTAFGDDGKFAEKPGVNPSIYLYQQTSPRPYDKTKHPY VHAPFELKEITTIPTQDDRLKIPFGAPGHVPEKHRSQLSMAKH KRRRAWYALSQNKPRPPKDGSKGRRSVRDLADLKAASLAD AIPLVSRVGFDWVVIDGRGLLRNLRWRKLAHEGMTVEEML GFFSGDPVIDPRRNVATFIYKAEHATVKSRKPIGGAKRAREEL LKATASSDGVIRQVGLISVDLGQTNPVAYEISRMHQANGELV AEHLEYGLLNDEQVNSIQRYRAAWDSMNESFRQKAIESLSM EAQDEIMQASTGAAKRTREAVLTMFGPNATLPWSRMSSNTT CISDALIEVGKEEETNFVTSNGPRKRTDAQWAAYLRPRVNPE TRALLNQAVWDLMKRSDEYERLSKRKLEMARQCVNFVVAR AEKLTQCNNIGIVLENLVVRNFHGSGRRESGWEGFFEPKREN RWFMQVLHKAFSDLAQHRGVMVFEVHPAYSSQTCPACRYV DPKNRSSEDRERFKCLKCGRSFNADREVATFNIREIARTGVG LPKPDCERSRGVQTTGTARNPGRSLKSNKNPSEPKRVLQSKT RKKITSTETQNEPLATDLKT
CasΦ.44	38	MTPKTESPLSALCKKHFPGKRFRTNYLKDAGKILKKHGEDA VVAFLSDKQEDEPANFCPPAKVHILAQSRPFEDWPINLASKAI QTYVVYGLTADERKTCEPGTSKESHDRWFKETGVDHHGFTSV QGLNLIFKHTLNRYDGVIKKVETRNEKRRSSVVRINEKKAAE GLPLIAAEAEETAFGEDGRLLQPPGVNHSIYCFQQVSPQPYSS KKHPQVVLPHAVQGVDPDAPIPVGRPNRLDIPKGQPGYVPE WQRPHLSMKCKRVRMWYARANWRRKPGRRSVLNEARLKE ASAKGALPIVLVIGDDWLVMDARGLLRSVFWRRVAKPGLSL SELLNVTPTGLFSGDPVIDPKRGLVTFTSKLGVVAVHSRKPTR GKKSKDLLLKMTKPTDDGMPRHVGMVAIDLGQTNPVAAEY SRVVQSDAGTLKQEPVSRGVLPDDLLKDVARYRRAYDLTEE SIRQEAIALLSEGHRAEVTKLDQTTANETKRLLVDRGVSESLP WEKMSSNTTYISDCLVALGKTDDVFFVPKAKKGKKETGIAV KRKDHGWSKLLRPRTSPEARKALNENQWAVKRASPEYERLS RRKLELGRRCVNHIIQETKRWTQCEDIVVVLEDLNVGFFHGS GKRPDGWDNFFVSKRENRWFIQVLHKAFGDLATHRGTHVIE VHPARTSITCIKCGHCDAGNRDGESFVCLASACGDRRHADLE VATRNVARVAITGERMPPSEQARDVQKAGGARKRKPSARN VKSSYPAVEPAPASP
CasΦ.36	39	MSDNKMKKLSKEEKPLTPLQILIRKYIDKSQYPSGFKTTIIKQ AGVRIKSVKSEQDEINLANWIISKYDPTYIKRDFNPSAKCQIIA TSRSVADFDIVKMSNKVQEIFFASSHLDKNVFDIGKSKSDHD SWFERNNVDRGIYTYSNVQGMNLIFSNTKNTYLGVAVKAQN KFSSKMKRIQDINNFRITNHQSPLPIPDEIKIYDDAGFLLNPPG

		VNPNIFGYQSCLLKPLENKEIISKTSFPEYSRLPADMIEVNYKI SNRLKFSNDQKGFIQFKDKLNLFKINSQELFSKRRRLSGQPIL LVASFGDDWVVLDGRGLLRQVYYRGIAKPGSITISELLGFFT GDPIVDPIRGVVSLGFKPGVLSQETLKTTSARIFAEKLPNLVL NNNVGLMSIDLGQTNPVSYRLSEITSNMSVEHICSDFLSQDQI SSIEKAKTSLDNLEEEIAIKAVDHLSDEDKINFANFSKLNLPED TRQSLFEKYPELIGSKLDFGSMGSGTSYIADELIKFENKDAFY PSGKKKFDLSFSRDLRKKLSDETRKSYNDALFLEKRTNDKYL KNAKRRKQIVRTVANSLVSKIEELGLTPVINIENLAMSGGFFD GRGKREKGWDNFFKVKKENRWVMKDFHKAFSELSPHHGVI VIESPPYCTSVTCTKCNFCDKKNRNGHKFTCQRCGLDANAD LDIATENLEKVAISGKRMPGSERSSDERKVAVARKAKSPKGK AIKGVKCTITDEPALLSANSQDCSQSTS
CasΦ.37	40	MALSLAEVRERHFKGLRFRSSYLKRAGKILKKEGEAACVAY LTGKDEESPPNFKPPAKCDVVAQSRPFEEWPIVQASVAVQSY VYGLTKEAFEAFNPGTTKQSHEACLAATGIDTCGYSNVQGL NLIFRQAKNRYEGVITKVENRNKKAKKKLTRKNEWRQKNG HSELPEAPEELTFNDEGRLLQPPGINPSLYTYQQISPTPWSPKD SSILPPQYAGYERDPNAPIPFGVAKDRLTIASGCPGYIPEWMR TAGEKTNPRTQKKFMHPGLSTRKNKRMRLPRSVRSAPLGAL LVTIHLGEDWLVLDVRGLLRNARWRGVAPKDISTQGLLNLF TGDPVIDTRRGVVTFTYKPETVGIHSRTWLYKGKQTKEVLEK LTQDQTVALVAIDLGQTNPVSAAASRVSRSGENLSIETVDRF FLPDELIKELRLYRMAHDRLEERIREESTLALTEAQQAEVRAL EHVVRDDAKNKVCAAFNLDAASLPWDQMTSNTTYLSEAIL AQGVSRDQVFFTPNPKKGSKEPVEVMRKDRAWVYAFKAKL SEETRKAKNEALWALKRASPDYARLSKRREELCRRSVNMVI NRAKKRTQCQVVIPVLEDLNIGFFHGSGKRLPGWDNFFVAK KENRWLMNGLHKSFSDLAVHRGFYVFEVMPHRTSITCPACG HCDSENRDGEAFVCLSCKRTYHADLDVATHNLTQVAGTGLP MPEREHPGGTKKPGGSRKPESPQTHAPILHRTDYSESADRLG S
CasΦ.45	41	QAVIKYLSDKGAVDPPDFRPPAKCNIIAQSRPFDEWPICKASM AIQQHIYGLTKNEFDESSPGTSSASHEQWFAKTGVDTHGFTH VQGLNLIFQHAKKRYEGVIKKVENYNEKERKKFEGINERRSK EGMPLLEPRLRTAFGDDGKFAEKPGVNPSIYLYQQTSPRPYD KTKHPYVHAPFELKEITTIPTQDDRLKIPFGAPGHVPEKHRSQ LSMAKHKRRRAWYALSQNKPRPPKDGSKGRRSVRDLADLK AASLADAIPLVSRVGFDWVVIDGRGLLRNLRWRKLAHEGMT VEEMLGFFSGDPVIDPRRNVATFIYKAEHATVKSRKPIGGAK RAREELLKATASSDGVIRQVGLISVDLGQTNPVAYEISRMHQ ANGELVAEHLEYGLLNDEQVNSIQRYRAAWDSMNESFRQK AIESLSMEAQDEIMQASTGAAKRTREAVLTMFGPNATLPWS RMSSNTTCISDALIEVGKEEETNFVTSNGPRKRTDAQWAAYL RPRVNPETRALLNQAVWDLMKRSDEYERLSKRKLEMARQC VNFVVARAEKLTQCNNIGIVLENLVVRNFHGSGRRESGWEG FFEPKRENRWFMQVLHKAFSDLAQHRGVMVFEVHPAYSSQ TCPACRYVDPKNRSSEDRERFKCLKCGRSFNADREVATFNIR

		EIARTGVGLPKPDCERSRDVQTPGTARKSGRSLKSQDNLSEP KRVLQSKTRKKITSTETQNEPLATDLKT
CasΦ.38	42	MIKEQSELSKLIEKYYPGKKFYSNDLKQAGKHLKKSEHLTAK ESEELTVEFLKSCKEKLYDFRPPAKALIISTSRPFEEWPIYKAS ESIQKYIYSLTKEELEKYNISTDKTSQENFFKESLIDNYGFANV SGLNLIFQHTKAIYDGVLKKVNNRNNKILKKYKRKIEEGIEID SPELEKAIDESGHFINPPGINKNIYCYQQVSPTIFNSFKETKIICP FNYKRNPNDIIQKGVIDRLAIPFGEPGYIPDHQRDKVNKHKK RIRKYYKNNENKNKDAILAKINIGEDWVLFDLRGLLRNAYW RKLIPKQGITPQQLLDMFSGDPVIDPIKNNITFIYKESIIPIHSESI IKTKKSKELLEKLTKDEQIALVSIDLGQTNPVAARFSRLSSDL KPEHVSSSFLPDELKNEICRYREKSDLLEIEIKNKAIKMLSQEQ QDEIKLVNDISSEELKNSVCKKYNIDNSKIPWDKMNGFTTFIA DEFINNGGDKSLVYFTAKDKKSKKEKLVKLSDKKIANSFKPK ISKETREILNKITWDEKISSNEYKKLSKRKLEFARRATNYLIN QAKKATRLNNVVLVVEDLNSKFFHGSGKREDGWDNFFIPKK ENRWFIQALHKSLTDVSIHRGINVIEVRPERTSITCPKCGCCD KENRKGEDFKCIKCDSVYHADLEVATFNIEKVAITGESMPKP DCERLGGEESIG
CasΦ.39	43	VAFLDGKEVDEPYTLQPPAKCHILAVSRPIEEWPIARVTMAV QEHVYALPVHEVEKSRPETTEGSRSAWFKNSGVSNHGVTHA QTLNAILKNAYNVYNGVIKKVENRNAKKRDSLAAKNKSRER KGLPHFKADPPELATDEQGYLLQPPSPNSSVYLVQQHLRTPQ IDLPSGYTGPVVDPRSPIPSLIPIDRLAIPPGQPGYVPLHDREKL TSNKHRRMKLPKSLRAQGALPVCFRVFDDWAVVDGRGLLR HAQYRRLAPKNVSIAELLELYTGDPVIDIKRNLMTFRFAEAV VEVTARKIVEKYHNKYLLKLTEPKGKPVREIGLVSIDLNVQR LIALAIYRVHQTGESQLALSPCLHREILPAKGLGDFDKYKSKF NQLTEEILTAAVQTLTSAQQEEYQRYVEESSHEAKADLCLKY SITPHELAWDKMTSSTQYISRWLRDHGWNASDFTQITKGRK KVERLWSDSRWAQELKPKLSNETRRKLEDAKHDLQRANPE WQRLAKRKQEYSRHLANTVLSMAREYTACETVVIAIENLPM KGGFVDGNGSRESGWDNFFTHKKENRWMIKDIHKALSDLAP NRGVHVLEVNPQYTSQTCPECGHRDKANRDPIQRERFCCTH CGAQRHADLEVATHNIAMVATTGKSLTGKSLAPQRLQ
CasΦ.42	44	LEIPEGEPGHVPWFQRMDIPEGQIGHVNKIQRFNFVHGKNSG KVKFSDKTGRVKRYHHSKYKDATKPYKFLEESKKVSALDSI LAIITIGDDWVVFDIRGLYRNVFYRELAQKGLTAVQLLDLFT GDPVIDPKKGIITFSYKEGVVPVFSQKIVSRFKSRDTLEKLTSQ GPVALLSVDLGQNEPVAARVCSLKNINDKIALDNSCRIPFLD DYKKQIKDYRDSLDELEIKIRLEAINSLDVNQQVEIRDLDVFS ADRAKASTVDMFDIDPNLISWDSMSDARFSTQISDLYLKNGG DESRVYFEINNKRIKRSDYNISQLVRPKLSDSTRKNLNDSIWK LKRTSEEYLKLSKRKLELSRAVVNYTIRQSKLLSGINDIVIILE DLDVKKKFNGRGIRDIGWDNFFSSRKENRWFIPAFHKSFSEL SSNRGLCVIEVNPAWTSATCPDCGFCSKENRDGINFTCRKCG VSYHADIDVATLNIARVAVLGKPMSGPADRERLGGTKKPRV ARSRKDMKRKDISNGTVEVMVTA

CasΦ.46	45	IPSFGYLDRLKIAKGQPGYIPEWQRETINPSKKVRRYWATNH EKIRNAIPLVVFIGDDWVIIDGRGLLRDARRRKLADKNTTIEQ LLEMVSNDPVIDSTRGIATLSYVEGVVPVRSFIPIGEKKGREY LEKSTQKESVTLLSVDIGQINPVSCGVYKVSNGCSKIDFLDKF FLDKKHLDAIQKYRTLQDSLEASIVNEALDEIDPSFKKEYQNI NSQTSNDVKKSLCTEYNIDPEAISWQDITAHSTLISDYLIDNNI TNDVYRTVNKAKYKTNDFGWYKKFSAKLSKEAREALNEKI WELKIASSKYKKLSVRKKEIARTIANDCVKRAETYGDNVVV AMESLTKNNKVMSGRGKRDPGWHNLGQAKVENRWFIQAIS SAFEDKATHHGTPVLKVNPAYTSQTCPSCGHCSKDNRSSKD RTIFVCKSCGEKFNADLDVATYNIAHVAFSGKKLSPPSEKSSA TKKPRSARKSKKSRKS
CasΦ.47	46	SPIEKLLNGLLVKITFGNDWIICDARGLLDNVQKGIIHKSYFT NKSSLVDLIDLFTCNPIVNYKNNVVTFCYKEGVVDVKSFTPI KSGPKTQENLIKKLKYSRFQNEKDACVLGVGVDVGVTNPFA INGFKMPVDESSEWVMLNEPLFTIETSQAFREEIMAYQQRTD EMNDQFNQQSIDLLPPEYKVEFDNLPEDINEVAKYNLLHTLN IPNNFLWDKMSNTTQFISDYLIQIGRGTETEKTITTKKGKEKIL TIRDVNWFNTFKPKISEETGKARTEIKRDLQKNSDQFQKLAK SREQSCRTWVNNVTEEAKIKSGCPLIIFVIEALVKDNRVFSGK GHRAIGWHNFGKQKNERRWWVQAIHKAFQEQGVNHGYPVI LCPPQYTSQTCPKCNHVDRDNRSGEKFKCLKYGWIGNADLD VGAYNIARVAITGKALSKPLEQKKIKKAKNKT
CasΦ.48	47	LLDNVQKGIIHKSYFTNKSSLVDLIDLFTCNPIVNYKNNVVTF CYKEGVVDVKSFTPIKSGPKTQENLIKKLKYSRFQNEKDACV LGVGVDVGVTNPFAINGFKMPVDESSEWVMLNEPLFTIETSQ AFREEIMAYQQRTDEMNDQFNQQSIDLLPPEYKVEFDNLPED INEVAKYNLLHTLNIPNNFLWDKMSNTTQFISDYLIQIGRGTE TEKTITTKKGKEKILTIRDVNWFNTFKPKISEETGKARTEIKR DLQKNSDQFQKLAKSREQSCRTWVNNVTEEAKIKSGCPLIIF VIEALVKDNRVFSGKGHRAIGWHNFGKQKNERRWWVQAIH KAFQEQGVNHGYPVILCPPQYTSQTCPKCNHVDRDNRSGEK FKCLKYGWIGNADLDVGAYNIARVAITGKALSKPLEQKKIK KAKNKT
CasΦ.49	105	MIKPTVSQFLTPGFKLIRNHSRTAGLKLKNEGEEACKKFVRE NEIPKDECPNFQGGPAIANIIAKSREFTEWEIYQSSLAIQEVIFT LPKDKLPEPILKEEWRAQWLSEHGLDTVPYKEAAGLNLIIKN AVNTYKGVQVKVDNKNKNNLAKINRKNEIAKLNGEQEISFE EIKAFDDKGYLLQKPSPNKSIYCYQSVSPKPFITSKYHNVNLP EEYIGYYRKSNEPIVSPYQFDRLRIPIGEPGYVPKWQYTFLSK KENKRRKLSKRIKNVSPILGIICIKKDWCVFDMRGLLRTNHW KKYHKPTDSINDLFDYFTGDPVIDTKANVVRFRYKMENGIV NYKPVREKKGKELLENICDQNGSCKLATVDVGQNNPVAIGL FELKKVNGELTKTLISRHPTPIDFCNKITAYRERYDKLESSIKL DAIKQLTSEQKIEVDNYNNNFTPQNTKQIVCSKLNINPNDLP WDKMISGTHFISEKAQVSNKSEIYFTSTDKGKTKDVMKSDY KWFQDYKPKLSKEVRDALSDIEWRLRRESLEFNKLSKSREQ DARQLANWISSMCDVIGIENLVKKNNFFGGSGKREPGWDNF YKPKKENRWWINAIHKALTELSQNKGKRVILLPAMRTSITCP

		KCKYCDSKNRNGEKFNCLKCGIELNADIDVATENLATVAITA QSMPKPTCERSGDAKKPVRARKAKAPEFHDKLAPSYTVVLR EAV**KRPAATKKAGQAKKKKEF** **(Underlined sequence is Nuclear Localization Signal; SEQ ID NO: 106)**
CasΦ.12 with NLS Signals	107	SNA**PKKKRKVGIHGVPAA**MIKPTVSQFLTPGFKLIRNHSRT AGLKLKNEGEEACKKFVRENEIPKDECPNFQGGPAIANIIAKS REFTEWEIYQSSLAIQEVIFTLPKDKLPEPILKEEWRAQWLSE HGLDTVPYKEAAGLNLIIKNAVNTYKGVQVKVDNKNKNNL AKINRKNEIAKLNGEQEISFEEIKAFDDKGYLLQKPSPNKSIY CYQSVSPKPFITSKYHNVNLPEEYIGYYRKSNEPIVSPYQFDR LRIPIGEPGYVPKWQYTFLSKKENKRRKLSKRIKNVSPILGIICI KKDWCVFDMRGLLRTNHWKKYHKPTDSINDLFDYFTGDPVI DTKANVVRFRYKMENGIVNYKPVREKKGKELLENICDQNGS CKLATVDVGQNNPVAIGLFELKKVNGELTKTLISRHPTPIDFC NKITAYRERYDKLESSIKLDAIKQLTSEQKIEVDNYNNNFTPQ NTKQIVCSKLNINPNDLPWDKMISGTHFISEKAQVSNKSEIYF TSTDKGKTKDVMKSDYKWFQDYKPKLSKEVRDALSDIEWR LRRESLEFNKLSKSREQDARQLANWISSMCDVIGIENLVKKN NFFGGSGKREPGWDNFYKPKKENRWWINAIHKALTELSQNK GKRVILLPAMRTSITCPKCKYCDSKNRNGEKFNCLKCGIELN ADIDVATENLATVAITAQSMPKPTCERSGDAKKPVRARKAK APEFHDKLAPSYTVVLREAV**KRPAATKKAGQAKKKKEF** **(Underlined sequences Nuclear Localization Signals; SEQ ID NO: 112 and 106)**

[0136] In some embodiments, any of the programmable CasΦ nucleases of the present disclosure (e.g., any one of SEQ ID NO: 1 to 47, 105, or 107, or fragments or variants thereof) may include a nuclear localization signal (NLS). In some cases, one or more NLS are fused or linked to the N-terminus of the programmable CasΦ nuclease. In some embodiments, one or more NLS are fused or linked to the C-terminus of the programmable CasΦ nuclease. In some embodiments, one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable CasΦ nuclease. In some embodiments, the link between the NLS and the programmable CasΦ nuclease comprises a tag. In some cases, said NLS may have a sequence of KRPAATKKAGQAKKKKEF (SEQ ID NO: 106). The NLS can be selected to match the cell type of interest, for example several NLSs are known to be functional in different types of eukaryotic cell *e.g.* in mammalian cells. Suitable NLSs include the SV40 large T antigen NLS (PKKKRKV, SEQ ID NO: 110) and the c-Myc NLS (PAAKRVKLD,SEQ ID NO: 111). In some embodiments, an NLS may be the SV40 large T antigen NLS or the c-Myc NLS. NLSs that are functional in plant cells are described in Chang *et al.*, (*Plant Signal Behav*. 2013 Oct; 8(10):e25976). In some embodiments, an NLS sequence can be selected from the following consensus sequences: KR(K/R)R, K(K/R)RK; (P/R)XXKR(˜DE)(K/R); KRX(W/F/Y)XXAF

(SEQ ID NO: 2489); (R/P)XXKR(K/R)(^DE); LGKR(K/R)(W/F/Y) (SEQ ID NO: 2490); KRX10–12K(KR)(KR) or KRX10–12K(KR)X(K/R).

[0137] In some embodiments, the nucleoplasmin NLS (KRPAATKKAGQAKKKKEF (SEQ ID NO: 106)) is linked or fused to the C-terminus of the programmable CasΦ nuclease. In some embodiments, the SV40 NLS (PKKKRKVGIHGVPAA) (SEQ ID NO: 112) is linked or fused to the N-terminus of the programmable CasΦ nuclease. In preferred embodiments, the nucleoplasmin NLS (SEQ ID NO: 106) is linked or fused to the C-terminus of the programmable CasΦ nuclease and the SV40 NLS (SEQ ID NO: 112) is linked or fused to the N-terminus of the programmable CasΦ nuclease.

[0138] In some embodiments, the CasΦ nuclease comprises more than 200 amino acids, more than 300 amino acids, more than 400 amino acids. In some embodiments, the CasΦ nuclease comprises less than 1500 amino acids, less than 1000 amino acids or less than 900 amino acids. In some embodiments, the CasΦ nuclease comprises between 200 and 1500 amino acids, between 300 and 1000 amino acids, or between 400 and 900 amino acids. In preferred embodiments, the CasΦ nuclease comprises between 400 and 900 amino acids.

[0139] "Percent identity" and "% identity" can refer to the extent to which two sequences (nucleotide or amino acid) have the same residue at the same positions in an alignment. For example, "an amino acid sequence is X% identical to SEQ ID NO: Y" can refer to % identity of the amino acid sequence to SEQ ID NO: Y and is elaborated as X% of residues in the amino acid sequence are identical to the residues of sequence disclosed in SEQ ID NO: Y. Generally, computer programs can be employed for such calculations. Illustrative programs that compare and align pairs of sequences, include ALIGN (Myers and Miller, Comput Appl Biosci. 1988 Mar;4(1):11-7), FASTA (Pearson and Lipman, Proc Natl Acad Sci U S A. 1988 Apr;85(8):2444-8; Pearson, Methods Enzymol. 1990;183:63-98) and gapped BLAST (Altschul et al., Nucleic Acids Res. 1997 Sep 1;25(17):3389-40), BLASTP, BLASTN, or GCG (Devereux et al., Nucleic Acids Res. 1984 Jan 11;12(1 Pt 1):387-95).

[0140] A CasΦ polypeptide or a variant thereof can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 to SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107.

[0141] A programmable nuclease or nickase of the present disclosure can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 to SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107.

[0142] Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 2.

[0143] Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 4.

[0144] Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 11.

[0145] Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 17.

[0146] Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 18.

[0147] Compositions and methods of the disclosure can comprise a programmable polypeptide or nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 12.

[0148] Compositions and methods of the disclosure can comprise a programmable polypeptide or nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 105.

[0149] Compositions and methods of the disclosure can comprise a programmable polypeptide or nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to SEQ ID NO: 107.

[0150] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to

SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 2. . In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 2. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 2.

[0151] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 4. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 4.

[0152] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 11. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 11.

[0153] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 12. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 12.

[0154] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 17. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 17.

[0155] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 18. In

some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 18. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 18.

[0156] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 92% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 97% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 105.

[0157] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to the N-terminal 717 amino acid residues of SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence of the N-terminal 717 amino acid residues of SEQ ID NO: 105.

[0158] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises

a sequence with 75% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 105. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 106. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 106.

[0159] In some embodiments, the programmable nuclease comprises a sequence with at least 70% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 75% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 80% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 85% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 90% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 95% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 98% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence with at least 99% identity to SEQ ID NO: 107. In some embodiments, the programmable nuclease comprises a sequence of SEQ ID NO: 107.

[0160] The programmable nucleases disclosed herein can be codon optimized for expression in a specific cell, for example, a bacterial cell, a plant cell, a eukaryotic cell, an animal cell, a mammalian cell, or a human cell. In some embodiments, the programmable nuclease is codon optimized for a human cell.

[0161] The programmable nucleases presented in **TABLE 1** or variants or fragments thereof comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107 can comprise nicking activity. Compositions and methods of the disclosure can comprise a programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107. Compositions and methods of the disclosure can comprise a programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 2. Compositions and methods of the disclosure can comprise a

programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 4. Compositions and methods of the disclosure can comprise a programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 11. Compositions and methods of the disclosure can comprise a programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 17. Compositions and methods of the disclosure can comprise a programmable nuclease comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 18.

[0162] The programmable nucleases presented in **TABLE 1** or variants thereof comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107 can comprise double-strand DNA cleavage activity. Compositions and methods of the disclosure can comprise a programmable nuclease capable of introducing a double-strand break in a target DNA sequence and comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107. Compositions and methods of the disclosure can comprise a programmable nuclease with double-strand DNA cleaving activity and comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 12. Compositions and methods of the disclosure can comprise a programmable nuclease with double-strand DNA cleaving activity and comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 2. Compositions and methods of the disclosure can comprise a programmable nickase comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 4. Compositions and methods of the disclosure can comprise a programmable nuclease with double-strand DNA cleaving activity and comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 11.

[0163] The programmable nucleases presented in **TABLE 1** or variants thereof comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47 and SEQ ID NO. 105 can comprise nickase activity and double-strand DNA cleavage

activity. The ratio of the nickase activity and double-strand DNA cleavage activity can be modulated depending on the reaction conditions including for example, RNP complexing temperature, the crRNA repeat sequence in the guide nucleic acid. In some embodiments, nickase activity is reduced when RNP complexing temperature is room temperature, for example 20 to 22 °C, compared to when RNP complexing temperature is 37°C. In some embodiments, the double-strand DNA cleavage activity is insensitive to RNP complexing at 37°C compared to room temperature, or the double-strand DNA cleavage activity is reduced by 10%, 20% or 30% when complexed with a guide RNA at room temperature as compared to when complexed at 37°C. In a preferred embodiment, double-strand cleavage activity is similar when the RNP complexing temperature is room temperature and 37°C.

[0164] The programmable nucleases presented in **TABLE 1** or variants thereof comprising at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO: 107 can comprise reduced or substantially no nucleic acid cleavage activity.

[0165] In some embodiments, the N-terminal amino acid sequence of the programmable nuclease is not MISKMIKPTV (SEQ ID NO: 113). In some embodiments, the programmable nuclease does not include the amino acid sequence MISKMIKPTV (SEQ ID NO: 114).

[0166] In some embodiments, the N-terminal amino acid sequence of the programmable nuclease is not MISK (SEQ ID NO: 115). In some embodiments, the programmable nuclease does not include the amino acid sequence MISK (SEQ ID NO: 115).

[0167] In some embodiments, a composition comprises a first programmable nuclease described herein and a second programmable nuclease described herein. In some embodiments, a complex comprises a first programmable nuclease described herein and a second programmable nuclease described herein. In preferred embodiments, a complex comprises a first programmable nuclease described herein and a second programmable nuclease described herein, wherein the first and second programmable nucleases are the same programmable nuclease. In some embodiments, the first and second programmable nucleases form a dimer. In some preferred embodiments, the first and second programmable nucleases form a homodimer.

[0168] In some embodiments, a dimer comprises a first programmable nuclease described herein and a second programmable nuclease described herein. In preferred embodiments, the dimer is a homodimer wherein the first and second programmable nucleases are the same.

[0169] In some embodiments, a programmable nuclease may be a programmable nickase. The present disclosure provides compositions of programmable nickases, capable of introducing a break in a single strand of a double stranded DNA (dsDNA) (“nicking”). In some embodiments the programmable nickase is a programmable DNA nickase. Said programmable nickases can be

coupled to a guide nucleic acid that targets a particular region of interest in the dsDNA. In some embodiments, two programmable nickases are combined and delivered together to generate two strand breaks. For example, a first programmable nickase can be targeted to and nicks a first region of dsDNA and a second programmable nickase can be targeted to and nicks a second region of the same dsDNA on the opposing strand. When combined and delivered together to generate nicks on opposing strands of the dsDNA, two strand breaks in the dsDNA can be generated. The strand breaks can be repaired and rejoined by non-homologous end joining (NHEJ) or homology directed repair (HDR). Thus, two programmable nickases disclosed herein can be combined to selectively edit nucleic acid sequences. This can be useful in any genome editing method, for example, used for therapeutic applications to treat a disease or disorder, or for agricultural applications.

[0170] In some embodiments, a programmable nuclease as disclosed herein can be used for genome editing purposes to generate strand breaks in order to excise a region of DNA or to subsequently introduce a region of DNA (e.g., donor DNA).

[0171] In some embodiments, the programmable nucleases (e.g., nickases) disclosed herein can be used in DNA Endonuclease Targeted CRISPR TransReporter (DETECTR) assays. In some embodiments, the programmable nuclease is a programmable nickase. A DETECTR assay can utilize the trans-cleavage abilities of some programmable nucleases to achieve fast and high-fidelity detection of a target nucleic acid in a sample. The target nucleic acid can be DNA or RNA. For example, following target DNA extraction from a biological sample, crRNA comprising a portion that is complementary to the target DNA of interest can bind to the target DNA sequence, initiating indiscriminate ssDNase activity by the programmable nuclease. In some embodiments, the extracted DNA is amplified by PCR or isothermal amplification reactions before contacting the DNA to the programmable nuclease complexed with a guide RNA. Upon hybridization with the target DNA, the trans-cleavage activity of the programmable nuclease is activated, which can then cleave an ssDNA fluorescence-quenching (FQ) reporter molecule. Cleavage of the reporter molecule can provide a fluorescent readout indicating the presence of the target DNA in the sample. In some embodiments, the programmable nucleases disclosed herein can be combined, or multiplexed, with other programmable nucleases in a DETECTR assay. The principles of the DETECTR assay are described in Chen *et al.* (Science 2018 Apr 27;360(6387):436-439) and can be modified to facilitate the use of the programmable nucleases described herein. In some embodiments, the programmable nucleases disclosed herein can be used in a specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) assay. The principles of the SHERLOCK assay are described in Kellner *et al.* (Nat Protoc. 2019 Oct;14(10):2986-3012) and can be modified to facilitate the use of the programmable nucleases described herein. Thus some embodiments provide a method of detecting a target nucleic acid in a sample, the method comprising: contacting a sample comprising a target nucleic acid with (a) a

programmable CasΦ nuclease disclosed herein, (b) a guide RNA comprising a region that binds to the programmable CasΦ nuclease and an additional region that binds to the target nucleic acid, and (c) a detector nucleic acid that does not bind the guide RNA; cleaving the detector nucleic acid by the programmable CasΦ nuclease; and detecting the target nucleic acid by measuring a signal produced by the cleavage of the detector nucleic acid. In preferred embodiments, the detector nucleic acid is a single stranded DNA reporter.

[0172] The programmable nucleases of the present disclosure can show enhanced activity, as measured by enhanced cleavage of an ssDNA-FQ reporter, under certain conditions in the presence of the target DNA. For example, the programmable nucleases of the present disclosure can have variable levels of activity based on a buffer formulation, a pH level, temperature, or salt. Buffers consistent with the present disclosure include phosphate buffers, Tris buffers, and HEPES buffers. Programmable nucleases of the present disclosure can show optimal activity in phosphate buffers, Tris buffers, and HEPES buffers.

[0173] Programmable nucleases can also exhibit varying levels of nickase or double-stranded cleavage activity at different pH levels. For example, enhanced cleavage can be observed between pH 7 and pH 9. In some embodiments, programmable nuclease of the present disclosure exhibit enhanced cleavage at about pH 7, about pH 7.1, about pH 7.2, about pH 7.3, about pH 7.4, about pH 7.5, about pH 7.6, about pH 7.7, about pH 7.8, about pH 7.9, about pH 8, about pH 8.1, about pH 8.2, about pH 8.3, about pH 8.4, about pH 8.5, about pH 8.6, about pH 8.7, about pH 8.8, about pH 8.9, about pH 9, from pH 7 to 7.5, from pH 7.5 to 8, from pH 8 to 8.5, from pH 8.5 to 9, or from pH 7 to 8.5.

[0174] In some embodiments, the programmable nucleases of the present disclosure exhibit enhanced cleavage of ssDNA-FQ reporters DNA at a temperature of 25°C to 50°C in the presence of target DNA. For example, the programmable nucleases of the present disclosure can exhibit enhanced cleavage of an ssDNA-FQ reporter at about 25°C, about 26°C, about 27°C, about 28°C, about 29°C, about 30°C, about 31°C, about 32°C, about 33°C, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, about 40°C, about 41°C, about 42°C, about 43°C, about 44°C, about 45°C, about 46°C, about 47°C, about 48°C, about 49°C, about 50°C, from 30°C to 40°C, from 35°C to 45°C, or from 35°C to 40°C.

[0175] The programmable nucleases of the present disclosure may not be sensitive to salt concentrations in a sample in the presence of the target DNA. Advantageously, said programmable nucleases can be active and capable of cleaving ssDNA-FQ-reporter sequences under varying salt concentrations from 25 nM salt to 200 mM salt. Various salts are consistent with this property of the programmable nucleases disclosed herein, including NaCl or KCl. The programmable nucleases of the present disclosure can be active at salt concentrations of from 25 nM to 500 nM salt, from 500 nM to 1000 nM salt, from 1000 nM to 2000 nM salt, from 2000

nM to 3000 nM salt, from 3000 nM to 4000 nM salt, from 4000 nM to 5000 nM salt, from 5000 nM to 6000 nM salt, from 6000 nM to 7000 nM salt, from 7000 nM to 8000 nM salt, from 8000 nM to 9000 nM salt, from 9000 nM to 0.01 mM salt, from 0.01 mM to 0.05 mM salt, from 0.05 mM to 0.1 mM salt, from 0.1 mM to 10 mM salt, from 10 mM to 100 mM salt, or from 100 mM to 500 mM salt. Thus, the programmable nucleases of the present disclosure can exhibit cleavage activity independent of the salt concentration in a sample.

[0176] Programmable nucleases of the present disclosure can be capable of cleaving any ssDNA-FQ reporter, regardless of its sequence. The programmable nucleases provided herein can, thus, be capable of cleaving a universal ssDNA FQ reporter. In some embodiments, the programmable nucleases provided herein cleave homopolymer ssDNA-FQ reporter comprising 5 to 20 adenines, 5 to 20 thymines, 5 to 20 cytosines, or 5 to 20 guanines. Programmable nucleases of the present disclosure, thus, are capable of cleaving ssDNA-FQ reporters also cleaved by programmable nucleases, as disclosed elsewhere herein, allowing for facile multiplexing of multiple programmable nickases and programmable nucleases in a single assay having a single ssDNA-FQ reporter.

[0177] Programmable nucleases of the present disclosure can bind a wild type protospacer adjacent motif (PAM) or a mutant PAM in a target DNA. In some embodiments the programmable CasΦ nucleases of the present disclosure recognizes and bind a protospacer adjacent motif (PAM) of 5'-TBN-3', where B is one or more of C, G, or, T. For example, programmable CasΦ nucleases of the present disclosure may recognizes and bind a protospacer adjacent motif (PAM) of 5'-TTTN-3'. As another example, programmable CasΦ nucleases of the present disclosure may recognizes and bind a protospacer adjacent motif (PAM) of 5'-TTN-3.' In some embodiments, the PAM is 5'-TTTA-3', 5'-GTTK-3', 5'-VTTK-3', 5'-VTTS-3', 5'-TTTS-3' or 5'-VTTN-3', where K is G or T, V is A, C or G, and S is C or G. In some embodiments, the PAM is 5'-GTTB-3', wherein B is C, G, or, T.

[0178] In some embodiments of the present disclosure, the programmable CasΦ nucleases recognize and bind a PAM of 5'-NTTN-3'.

[0179] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 2, the programmable CasΦ nuclease or a variant recognizes a 5'-GTTK-3' PAM. In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 2, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTN-3' PAM.

[0180] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 4, the programmable CasΦ nuclease or a variant recognizes a 5'-VTTK-3' PAM. In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 4, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTN-3' PAM.

[0181] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 11, the programmable CasΦ nuclease or a variant recognizes a 5'-VTTS-3' PAM. In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 11, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTN-3' PAM.

[0182] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 12, the programmable CasΦ nuclease or a variant recognizes a 5'-TTTS-3' PAM. In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 12, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTN-3' PAM.

[0183] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 18, the programmable CasΦ nuclease or a variant recognizes a 5'-VTTN-3' PAM.

[0184] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 20, the programmable CasΦ nuclease or a variant recognizes a 5'-NTNN-3' PAM.

[0185] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 20, the programmable CasΦ nuclease or a variant recognizes a 5'-TTN-3' PAM.

[0186] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 26, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTG-3' PAM.

[0187] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 32, the programmable CasΦ nuclease or a variant recognizes a 5'-GTTB-3' PAM, wherein B is C, G, or N.

[0188] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 42, the programmable CasΦ nuclease or a variant recognizes a 5'-GTTN-3' PAM.

[0189] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 41, the programmable CasΦ nuclease or a variant recognizes a 5'-NTTN-3' PAM.

[0190] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 24, the programmable CasΦ nuclease or a variant recognizes a 5'-NTNN-3' PAM.

[0191] In some embodiments, when the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 25, the programmable CasΦ nuclease or a variant recognizes a 5'-NTNN-3' PAM.

[0192] The programmable nucleases and other reagents (e.g., a guide nucleic acid) can be formulated in a buffer disclosed herein. A wide variety of buffered solutions are compatible with the methods, compositions, reagents, enzymes, and kits disclosed herein. Buffers are compatible with different programmable nucleases described herein. Any of the methods, compositions, reagents, enzymes, or kits disclosed herein may comprise a buffer. These buffers may be compatible with the other reagents, samples, and support mediums as described herein for detection of an ailment, such as a disease, cancer, or genetic disorder, or genetic information, such as for phenotyping, genotyping, or determining ancestry. A buffer, as described herein, can enhance the cis- or trans-cleavage rates of any of the programmable nucleases described herein. The buffer can increase the discrimination of the programmable nucleases for the target nucleic acid. The methods as described herein can be performed in the buffer.

[0193] In some embodiments, a buffer may comprise one or more of a buffering agent, a salt, a crowding agent, or a detergent, or any combination thereof. A buffer may comprise a reducing agent. A buffer may comprise a competitor. Exemplary buffering agents include HEPES, TRIS, MES, ADA, PIPES, ACES, MOPSO, BIS-TRIS propane, BES, MOPS, TES, DISO, Trizma, TRICINE, GLY-GLY, HEPPS, BICINE, TAPS, A MPD, A MPSO, CHES, CAPSO, AMP, CAPS, phosphate, citrate, acetate, imidazole, or any combination thereof. A buffering agent may be compatible with a programmable nuclease. A buffer compatible with a programmable nuclease may comprise a buffering agent at a concentration of from 1 mM to 200 mM. A buffer compatible with a programmable nuclease may comprise a buffering agent at a concentration of from 10 mM to 30 mM. A buffer compatible with a programmable nuclease may comprise a buffering agent at a concentration of about 20 mM. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 2.5 to 3.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 3 to 4. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 3.5 to 4.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 4 to 5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 4.5 to 5.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 5 to 6. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 5.5 to 6.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 6 to 7. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 6.5 to 7.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 7 to 8. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 7.5 to 8.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 8 to 9. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 8.5 to 9.5. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 9 to 10. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 9.5 to 10.5.

[0194] A buffer may comprise a salt. Exemplary salts include NaCl, KCl, magnesium acetate, potassium acetate, $CaCl_2$ and $MgCl_2$. A buffer may comprise potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof. A buffer compatible with a programmable nuclease may comprise a salt at a concentration of from 5 mM to 100 mM. A buffer compatible with a programmable nuclease may comprise a salt at a concentration of from 5 mM to 10 mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt from 1 mM to 60 mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt from 1 mM to 10 mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt at about 105 mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt at about 55

mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt at about 7 mM. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt, wherein the salt comprises potassium acetate and magnesium acetate. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt, wherein the salt comprises sodium chloride and magnesium chloride. In some embodiments, a buffer compatible with a programmable nuclease comprises a salt, wherein the salt comprises potassium chloride and magnesium chloride.

[0195] A buffer may comprise a crowding agent. Exemplary crowding agents include glycerol and bovine serum albumin. A buffer may comprise glycerol. A crowding agent may reduce the volume of solvent available for other molecules in the solution, thereby increasing the effective concentrations of said molecules. A buffer compatible with a programmable nuclease may comprise a crowding agent at a concentration of from 0.01% (v/v) to 10% (v/v). A buffer compatible with a programmable nuclease may comprise a crowding agent at a concentration of from 0.5% (v/v) to 10% (v/v).

[0196] A buffer may comprise a detergent. Exemplary detergents include Tween, Triton-X, and IGEPAL. A buffer may comprise Tween, Triton-X, or any combination thereof. A buffer compatible with a programmable nuclease may comprise Triton-X. A buffer compatible with a programmable nuclease may comprise IGEPAL CA-630. In some embodiments, a buffer compatible with a programmable nuclease comprises a detergent at a concentration of 2% (v/v) or less. A buffer compatible with a programmable nuclease may comprise a detergent at a concentration of 2% (v/v) or less. A buffer compatible with a programmable nuclease may comprise a detergent at a concentration of from 0.00001% (v/v) to 0.01% (v/v). A buffer compatible with a programmable nuclease may comprise a detergent at a concentration of about 0.01% (v/v).

[0197] A buffer may comprise a reducing agent. Exemplary reducing agents comprise dithiothreitol (DTT), ß-mercaptoethanol (BME), or tris(2-carboxyethyl)phosphine (TCEP). A buffer compatible with a programmable nuclease may comprise DTT. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of from 0.01 mM to 100 mM. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of from 0.1 mM to 10 mM. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of from 0.5 mM to 2 mM. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of from 0.01 mM to 100 mM. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of from 0.1 mM to 10 mM. A buffer compatible with a programmable nuclease may comprise a reducing agent at a concentration of about 1 mM.

[0198] A buffer compatible with a programmable nuclease may comprise a competitor. Exemplary competitors compete with the target nucleic acid or the reporter nucleic acid for cleavage by the programmable nuclease. Exemplary competitors include heparin, and imidazole, and salmon sperm DNA. A buffer compatible with a programmable nuclease may comprise a competitor at a concentration of from 1 μg/mL to 100 μg/mL. A buffer compatible with a programmable nuclease may comprise a competitor at a concentration of from 40 μg/mL to 60 μg/mL.

[0199] In some embodiments, a programmable CasΦ nuclease is described as a "nickase" if the predominant cleavage product is a nicked nucleic acid when the target nucleic acid is a double-stranded nucleic acid. In some embodiments, a programmable CasΦ nuclease cleaves both strands of a double-stranded target nucleic acid. In some embodiments, the target nucleic acid is DNA. In some embodiments, the target nucleic acid is double-stranded DNA.

[0200] Where a programmable CasΦ nuclease disclosed herein cleaves both strands of a double-stranded target nucleic acid, the strand break may be a staggered cut with a 5' overhang. In some embodiments, the 5' overhang is an overhang of between 5 and 10 nucleotides. In some embodiments, the 5' overhang is an overhang of 5 or 6 nucleotides. In some embodiments, the 5' overhang is an overhang of 9 or 10 nucleotides.

[0201] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 20, the 5' overhang is a 9 or 10 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 20, the 5' overhang is a 9 or 10 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 20, the 5' overhang is a 9 or 10 nucleotide overhang.

[0202] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 22, the 5' overhang is a 9 or 10 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 22, the 5' overhang is a 10 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 22, the 5' overhang is a 10 nucleotide overhang.

[0203] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 28, the 5'

overhang is a 9 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 28, the 5' overhang is a 9 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 28, the 5' overhang is a 9 nucleotide overhang.

[0204] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 40, the 5' overhang is a 10 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 40, the 5' overhang is a 10 nucleotide overhang. In further embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 40, the 5' overhang is a 10 nucleotide overhang.

[0205] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 37, the 5' overhang is a 9 or 10 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 37, the 5' overhang is a 9 or 10 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 37, the 5' overhang is a 9 or 10 nucleotide overhang.

[0206] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 41, the 5' overhang is a 9 or 10 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 41, the 5' overhang is a 9 or 10 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 41, the 5' overhang is a 9 or 10 nucleotide overhang.

[0207] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 12, the 5' overhang is a 5 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 12, the 5' overhang is a 5 nucleotide overhang. In further preferred embodiments, where the

programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 12, the 5' overhang is a 5 nucleotide overhang.

[0208] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 24, the 5' overhang is a 6 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 24, the 5' overhang is a 6 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 24, the 5' overhang is a 6 nucleotide overhang.

[0209] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 25, the 5' overhang is a 6 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 25, the 5' overhang is a 6 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 25, the 5' overhang is a 6 nucleotide overhang.

[0210] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 32, the 5' overhang is a 6 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 32, the 5' overhang is a 6 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 32, the 5' overhang is a 6 nucleotide overhang.

[0211] In some embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with SEQ ID NO: 33, the 5' overhang is a 6 nucleotide overhang. In preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises at least 90% sequence identity with SEQ ID NO: 33, the 5' overhang is a 6 nucleotide overhang. In further preferred embodiments, where the programmable CasΦ nuclease or a variant thereof comprises the amino acid sequence of SEQ ID NO: 33, the 5' overhang is a 6 nucleotide overhang.

[0212] In some embodiments, a programmable CasΦ nuclease rapidly cleaves a strand of a double-stranded target nucleic acid. In some embodiments, the programmable CasΦ nuclease

cleaves the second strand of the target nucleic acid after it has cleaved the first strand of the target nucleic acid. The cleavage of target nucleic acid strands can be assessed in an *in vitro* cis-cleavage assay. To perform such as assay, the programmable CasΦ nuclease is complexed to its native crRNA, e.g. CasΦ.2 nuclease with the CasΦ.2 repeat, in buffer comprising 50mM potassium acetate, 20mM Tris-acetate, 10mM magnesium acetate, 100ug/ml BSA, and which is pH 7.9 at 25 °C. The complexing is carried out for 20 minutes at room temperature, e.g. 20-22 °C. The RNP is at a concentration of 200 nM. The target plasmid is a 2.2 kb super-coiled plasmid containing a target sequence, either 5'-TATTAAATACTCGTATTGCTGTTCGATTAT-3' (SEQ ID NO: 116) or 5'-CACAGCTTGTCTGTAAGCGGATGCCATATG-3' (SEQ ID NO: 117), which is immediately downstream of a 5'-GTTG-3' or 5'-TTTG-3' PAM. At time "0" 30 equal volumes of target plasmid, at 20 nM, and complexed RNP are mixed, so that the concentration of target plasmid is 10 nM and the concentration of complexed RNP is 100 nM. The incubation temperature is 37 °C. The reaction is quenched at desired time points, e.g. 1, 3, 6, 15, 30 and 60 minutes, with reaction quench comprising 1 mg/ml proteinase K, 0.08% SDS and 15 mM EDTA. The sample incubates for 30 minutes at 37 °C to deproteinize. The cleavage is quantified by agarose gel analysis.

[0213] In some embodiments, a programmable CasΦ nuclease creates at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90 or at least 95% of the maximum amount of nicked product within 1 minute, where the maximum amount of nicked product is the maximum amount detected within a 60 minute period from when the target plasmid is mixed with the programmable CasΦ nuclease. In preferred embodiments, at least 80% of the maximum amount of nicked product is created within 1 minute. In more preferred embodiments, at least 90% of the maximum amount of nicked product is created within 1 minute.

[0214] In some embodiments, a programmable CasΦ nuclease creates at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90 or at least 95% of the maximum amount of linearized product is created within 1 minute, where the maximum amount of linearized product is the maximum amount detected within a 60 minute period from when the target plasmid is mixed with the programmable CasΦ nuclease. In preferred embodiments, at least 80% of the maximum amount of linearized product is created within 1 minute. In more preferred embodiments, at least 90% of the maximum amount of linearized product is created within 1 minute.

[0215] In some embodiments, a programmable CasΦ nuclease uses a co-factor. In some embodiments, the co-factor allows the programmable CasΦ nuclease to perform a function. In some embodiments, the function is pre-crRNA processing and/or target nucleic acid cleavage. As discussed in Jiang F. and Doudna J.A. (Annu. Rev. Biophys. 2017. 46:505–29), Cas9 uses divalent metal ions as co-factors. The suitability of a divalent metal ion as a cofactor can easily

be assessed, such as by methods based on those described by Sundaresan *et al.* (Cell Rep. 2017 Dec 26; 21(13): 3728–3739). In some embodiments, the co-factor is a divalent metal ion. In some embodiments, the divalent metal ion is selected from Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Cu^{2+}. In a preferred embodiment, the divalent metal ion is Mg^{2+}. In some embodiments, a programmable CasΦ nuclease forms a complex with a divalent metal ion. In preferred embodiments, a programmable CasΦ nuclease forms a complex with Mg^{2+}.

[0216] In some aspects, the disclosure provides a composition comprising a programmable CasΦ nuclease disclosed herein and a cell, preferably wherein the cell is a eukaryotic cell. In some embodiments, a programmable CasΦ nuclease disclosed herein is in a cell, preferably wherein the cell is a eukaryotic cell.

[0217] In some aspects, the disclosure provides a composition comprising a nucleic acid encoding a programmable CasΦ nuclease disclosed herein and a cell, preferably wherein the cell is a eukaryotic cell. In some embodiments, a nucleic acid encoding a programmable CasΦ nuclease disclosed herein is in a cell, preferably wherein the cell is a eukaryotic cell.

Guide Nucleic Acids

[0218] The methods and compositions of the disclosure may comprise a guide nucleic acid. The guide nucleic acid can bind to a target nucleic acid (e.g., a single strand of a target nucleic acid) or portion thereof. For example, the guide nucleic acid can bind to a target nucleic acid such as nucleic acid from a virus or a bacterium or other agents responsible for a disease, or an amplicon thereof, as described herein. The guide nucleic acid can bind to a target nucleic acid such as a nucleic acid from a bacterium, a virus, a parasite, a protozoa, a fungus or other agents responsible for a disease, or an amplicon thereof, as described herein. The target nucleic acid can comprise a mutation, such as a single nucleotide polymorphism (SNP). A mutation can confer for example, resistance to a treatment, such as antibiotic treatment. A mutation can confer a gene malfunction or gene knockout. A mutation can confer a disease, contribution to a disease, or risk for a disease, such as a liver disease or disorder, eye disease or disorder, cystic fibrosis, or muscle disease or disorder. The guide nucleic acid can bind to a target nucleic acid such as a nucleic acid, preferably DNA, from a cancer gene or gene associated with a genetic disorder, or an amplicon thereof, as described herein. The guide nucleic acid comprises a segment of nucleic acids that are reverse complementary to the target nucleic acid. Often the guide nucleic acid binds specifically to the target nucleic acid. The target nucleic acid may be a reversed transcribed RNA, DNA, DNA amplicon, or synthetic nucleic acids. The target nucleic acid can be a single-stranded DNA or DNA amplicon of a nucleic acid of interest. A guide nucleic acid may be a non-naturally occurring guide nucleic acid. A non-naturally occurring guide nucleic acid may comprise an engineered sequence having a repeat and a spacer that hybridizes to a target nucleic acid sequence of interest. A non-naturally occurring guide nucleic acid may be recombinantly expressed or chemically synthesized.

[0219] A guide nucleic acid (e.g. gRNA) may hybridize to a target sequence of a target nucleic acid. The guide nucleic acid can bind to a programmable nuclease.

[0220] In some embodiments, a gRNA comprises a crRNA. In some embodiments, a gRNA of a CasΦ polypeptide or variants thereof does not comprise a tracrRNA. As described by Jiang F. and Doudna J.A. (*Annu. Rev. Biophys.* 2017. 46:505–29), Cas9 cleavage activity requires a tracrRNA. A tracrRNA is a polynucleotide that hybridizes with a crRNA to allow crRNA maturation such that the crRNA can bind to the Cas nuclease and locate the Cas nuclease to a target sequence. In some embodiments, a programmable CasΦ nuclease disclosed herein does not require a tracrRNA to locate and/or cleave a target nucleic acid. A crRNA may comprise a repeat region. Specifically, the crRNA of the guide nucleic acid may comprise a repeat region and a spacer region. The repeat region refers to the sequence of the crRNA that binds to the programmable nuclease. The spacer region refers to the sequence of the crRNA that hybridizes to a sequence of the target nucleic acid. In some embodiments, the repeat region may comprise mutations or truncations with respect to the repeat sequences in pre-crRNA. The repeat sequence of the crRNA may interact with a programmable nuclease, allowing for the guide nucleic acid and the programmable nuclease to form a complex. This complex may be referred to as a ribonucleoprotein (RNP) complex. The crRNA may comprise a spacer sequence. The spacer sequence may hybridize to a target sequence of the target nucleic acid, where the target sequence is a segment of a target nucleic acid. The spacer sequences may be reverse complementary to the target sequence. In some cases, the spacer sequence may be sufficiently reverse complementary to a target sequence to allow for hybridization, however, may not necessarily be 100% reverse complementary.

[0221] In some embodiments, a programmable nuclease may cleave a precursor RNA ("pre-crRNA") to produce (or "process") a guide RNA (gRNA), also referred to as a "mature guide RNA." A programmable nuclease that cleaves pre-crRNA to produce a mature guide RNA is said to have pre-crRNA processing activity.

[0222] Programmable nucleases disclosed herein may process the repeat sequence of a crRNA, where the repeat sequence is the region of the crRNA that binds to the programmable nuclease. For example, crRNA may be delivered to a mammalian cell, *e.g.* a HEK293T cell, wherein the crRNA includes a full length repeat region which is 36 nucleotides in length, along with a programmable nuclease. The programmable nuclease then cleaves the repeat region of the crRNA so that the mature crRNA comprises a shorter repeat region (*e.g.* 24 nucleotides in length). Accordingly, in some embodiments, programmable nucleases disclosed herein are capable of cleaving the repeat region of a crRNA. In preferred embodiments, programmable nucleases disclosed herein are capable of cleaving the repeat region of a crRNA in mammalian cells.

[0223] The guide nucleic acid can bind specifically to the target nucleic acid. A guide nucleic acid can comprise a sequence that is, at least in part, reverse complementary to the sequence of a target nucleic acid.

[0224] The guide nucleic acid may be a non-naturally occurring guide nucleic acid. A non-naturally occurring guide nucleic acid may comprise an engineered sequence having a repeat and a spacer that hybridizes to a target nucleic acid sequence of interest. A non-naturally occurring guide nucleic acid may be recombinantly expressed or chemically synthesized.

[0225] A guide nucleic acid can comprise RNA, DNA, or a combination thereof. The term "gRNA" refers to a guide nucleic acid comprising RNA. A gRNA may include nucleosides that are not ribonucleic. In some embodiments, all nucleosides in a gRNA are ribonucleic. In some embodiments, some of the nucleosides in a gRNA are not ribonucleic. In embodiments where nucleosides in a gRNA are not ribonucleic, non-ribonucleic nucleosides may be naturally-occurring or non-naturally-occurring nucleosides. In some embodiments, inter-nucleoside links are phosphodiester bonds. In some embodiments, the inter-nucleoside link between at least two nucleosides in a guide nucleic acid is not a phosphodiester bond. In some embodiments, the inter-nucleoside link between at least two nucleosides is a non-natural inter-nucleoside linkage. Non-natural inter-nucleoside linkages include phosphorous and non-phosphorous inter-nucleoside linkages. Phosphorous inter-nucleoside linkages include phosphorothioate linkages and thiophosphate linkages. An inter-nucleoside linkage may comprise a "C3 spacer". C3 spacers are known to the skilled person as comprising a chain of three carbon atoms.

[0226] Guide nucleic acids may be modified to improve genome editing efficiency, increase stability, reduce off-target effects, and/or increase the affinity of the guide nucleic acid for a CasΦ polypeptide disclosed herein. Modifications may include non-natural nucleotides and/or non-natural linkages. In addition or alternatively, one or more sugar moieties of the guide nucleic acid may be modified. Such sugar moiety modifications may include 2'-O-methyl (2'OMe,), 2'-O-methyoxy-ethyl and 2' fluoro. In some embodiments, editing efficiency, or genome editing efficiency, is determined by analyzing the frequency of indel mutations in a nucleic acid or gene knockout. In some embodiments, the use of a flow cytometer or next generation sequencing may be used to analyze cells for indel mutations or gene knockout. In other embodiments, off-target effects may be detected using a flow cytometer, next generation sequencing, or CIRCLE-seq.

[0227] In some preferred embodiments, first 3 nucleosides (or one of the first 3 nucleosides, or a combination of the first 3 nucleosides) from the 5' end of the repeat region comprise a 2'-O-methyl modification and the linkages between the 3 nucleosides at the 3' end of the spacer region comprise phosphorothioate linkages.

[0228] In some embodiments, the first nucleoside at the 5' end of the repeat region comprises a 2'-O-methyl modification. In some embodiments, the first two nucleosides at the 5' end of the repeat region comprise 2'-O-methyl modifications. In some embodiments, the first three nucleosides at the 5' end of the repeat region comprise 2'-O-methyl modifications. In some embodiments, the last nucleoside at the 3' end of the spacer region comprises a 2'-O-methyl modification. In some embodiments, the last two nucleosides at the 3' end of the spacer region comprise 2'-O-methyl modifications. In some embodiments, the last three nucleosides at the 3' end of the spacer region comprise 2'-O-methyl modifications.

[0229] In some embodiments, the first 3 nucleosides (or one of the first 3 nucleosides, or a combination of the first 3 nucleosides) from the 5' end of the repeat region and the 3 nucleosides at the 3' end of the spacer region comprise a 2'-O-methyl modification, and the linkages between the 3 nucleosides at the 3' end of the spacer region comprise phosphorothioate linkages.

[0230] In some embodiments, the first 3 nucleosides (or one of the first 3 nucleosides, or a combination of the first 3 nucleosides) from the 5' end of the repeat region and the 3 nucleosides at the 3' end of the spacer region comprise a 2' fluoro modification.

[0231] In some embodiments, the first nucleoside at the 5' end of the repeat region comprises a 2' fluoro modification. In some embodiments, the first two nucleosides at the 5' end of the repeat region comprise 2' fluoro modifications. In some embodiments, the first three nucleosides at the 5' end of the repeat region comprise 2' fluoro modifications. In some embodiments, the last nucleoside at the 3' end of the spacer region comprises a 2' fluoro modification. In some embodiments, the last two nucleosides at the 3' end of the spacer region comprise 2' fluoro modifications. In some embodiments, the last three nucleosides at the 3' end of the spacer region comprise 2' fluoro modifications. In preferred embodiments, the last three nucleosides at the 3' end of the spacer region comprise 2' fluoro modifications.

[0232] In preferred embodiments, the first two nucleosides at the 5' end of the repeat region comprise 2'-O-methyl modifications, the first two nucleosides at the 5' end of the repeat are linked by a phosphorothioate linkage, and the last three nucleosides at the 3' end of the spacer region comprise 2' fluoro modifications.

[0233] In some embodiments, the linkage between the two nucleosides at the 5' end of the repeat region comprises a 3C spacer and the linkage between the two nucleosides at the 3' end of the spacer region comprises a 3C spacer.

[0234] In some embodiments, the guide nucleic acid comprises ribonucleic nucleosides and deoxyribonucleic nucleosides. In some embodiments, the guide nucleic acid is a guide RNA wherein the first, eighth and nineth nucleosides from the 5' end of the spacer region and the four nucleosides at the 3' end of the spacer region are deoxyribonucleic nucleosides.

[0235] In some embodiments, the guide nucleic acid comprises a polyA tail. In some preferred embodiments, the guide nucleic acid comprises a polyA tail at the 3' end of the spacer region.

[0236] In some embodiments, a plurality of modified guides (e.g., a combination of modified guides disclosed herein) are complexed with one or more programmable nucleases (e.g., one or more programmable nucleases disclosed herein). In some examples, one or more of the plurality of modified guides comprise any of the nucleoside modifications described herein. In some examples, one or more of the plurality of the modified guides comprise any length of repeat or spacer region described herein. In some examples, one or more of the plurality of the modified guides comprise a repeat spacer length described herein, and a nucleoside modification described herein. In some embodiments, one or more of the plurality of modified guides comprise a repeat sequence from about 15 to about 20 nucleotides in length. In some embodiments, one or more of the plurality of modified guides comprise a spacer sequence or region from about 15 to about 20 nucleotides in length.

[0237] **TABLE 2** provides illustrative crRNA sequences for use with the compositions and methods of the disclosure. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99%, or 100% sequence identity to any one of SEQ ID NO: 48 – SEQ ID NO: 86, or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 49 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 51 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 52 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 54 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 57 or a reverse complement thereof.

TABLE 2. Illustrative crRNA sequences

CasΦ ortholog	crRNA repeat sequence (shown as DNA), 5'-to-3'	SEQ ID. NO.
CasΦ.01	GGAGAGATCTCAAACGATTGCTCGATTAGTCGAGAC	**48**
CasΦ.02	GTCGGAACGCTCAACGATTGCCCCTCACGAGGGGAC	**49**

CasΦ.04	ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC	**50**
CasΦ.07	GGATCCAATCCTTTTTGATTGCCCAATTCGTTGGGAC	**51**
CasΦ.10	GGATCTGAGGATCATTATTGCTCGTTACGACGAGAC	**52**
CasΦ.11	CCTGCGAAACCTTTTGATTGCTCAGTACGCTGAGAC	**53**
CasΦ.12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGAC	**54**
CasΦ.13	GTAGAAGACCTCGCTGATTGCTCGGTGCGCCGAGAC	**55**
CasΦ.17	ATGGCAACAGACTCTCATTGCGCGGTACGCCGCGAC	**56**
CasΦ.18	ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC	**57**
CasΦ.19	GTCGCTCTCTAACGCTTGCCCAGTACGCTGGGAC	**58**
CasΦ.20	GCTGGAAGACTCAATGATGGCTCCTTACGAGGAGAC	**59**
CasΦ.21	GGTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**60**
CasΦ.22	GGTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**61**
CasΦ.23	CTTGAAATCCTGTCAGATTGCTCCCTTCGGGGAGAC	**62**
CasΦ.24	GCTGGAAGACTCAATGATGGCTCCTTACGAGGAGAC	**63**
CasΦ.25	GCTGGAAGACTCAATGATGGCTCCTTACGAGGAGAC	**64**
CasΦ.26	CTAGGAACGCACGCAGATTGCTCGGTACGCCGAGAC	**65**
CasΦ.27	ATTGCAACGCCTAAAGATTGCTCGATACGTCGAGAC	**66**
CasΦ.28	GTTCGGCRAYCCTTTGATTGCTCAGTACGCTGAGAC	**67**
CasΦ.29	GTTGAACCTAGATCAGATGGCTCAGTACGCTGAGAC	**68**
CasΦ.30	CCCTCAACACGTCAGAAATGCCCGGCACGCCGGGAC	**69**
CasΦ.31	GTCGCAAGACTCGAATAATTGCCCCTCTATGGGGAC	**70**
CasΦ.32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGAC	**71**
CasΦ.33	CTCTCAATGGATAACGATTGCTCTCTACGGAGAGAC	**72**
CasΦ.34	GCTGGAAGACTCAATGATGGCTCCTTACGAGGAGAC	**73**
CasΦ.35	GTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**74**
CasΦ.36	GTCGCAAGACTCGAATAATTGCCCCTCTATGGGGAC	**75**
CasΦ.37	GTCGGAACGCTCAACGATTGCCCCTCACGAGGGGAC	**76**
CasΦ.38	GTTGAACCTAGATCAGATGGCTCAGTACGCTGAGAC	**77**
CasΦ.39	CTCTCAATGGATAACGATTGCTCTCTACGGAGAGAC	**78**

CasΦ.41	ACTGAAACCACCAACGATTGCGCTCCTCGGAGCGAC	**79**
CasΦ.42	ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC	**80**
CasΦ.43	GTTGAACCTAGATCAGATGGCTCAGTACGCTGAGAC	**81**
CasΦ.44	GTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**82**
CasΦ.45	GTTGAACCTAGATCAGATGGCTCAGTACGCTGAGAC	**83**
CasΦ.46	GTCGGAACGCTCAACGATTGCCCCTCACGAGGGGAC	**84**
CasΦ.47	GGTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**85**
CasΦ.48	GGTTGAACCCTCAACAGATTGCTCGGTAAGCCGAGAC	**86**

[0238] In some embodiments, the programmable nuclease disclosed herein is used in conjunction with a specific crRNA sequence. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 48 – SEQ ID NO: 86, or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 49 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 51 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 52 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 54 or a reverse complement thereof. In some embodiments, the crRNA sequence comprises at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to SEQ ID NO: 57 or a reverse complement thereof.

[0239] In some embodiments, the activity of a programmable CasΦ nuclease can be supported by a crRNA comprising any of the crRNA repeat sequences recited in **TABLE 2**. In some embodiments, the activity of a programmable CasΦ nuclease can be supported by a crRNA comprising a crRNA repeat sequence comprising at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity to any one of SEQ ID NO: 48 – SEQ ID NO: 86.

[0240] In some embodiments, the crRNA repeat sequence comprises a hairpin. In some embodiments, the hairpin is in the 3' portion of the crRNA repeat sequence. The hairpin

comprises a double-stranded stem portion and a single-stranded loop portion. In preferred embodiments, one stand of the stem portion comprises a CYC sequence and the other strand comprises a GRG sequence, wherein Y and R are complementary. In preferred embodiments, the crRNA repeat comprises a GAC sequence at the 3' end. In more preferred embodiments, the G of the GAC sequence is in the stem portion of the hairpin. In some embodiments, each strand of the stem portion comprises 3, 4, 5, 6, 7, 8, 9 or 10 nucleotides. In preferred embodiments, each strand of the stem portion comprises 3, 4 or 5 nucleotides. In some embodiments, the loop portion comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleotides. In preferred embodiments, the loop portion comprises 2, 3, 4, 5 or 6 nucleotides. In most preferred embodiments, the loop portion comprises 4 nucleotides. In some embodiments, the nucleotides are naturally occurring nucleotides. In some embodiments, the nucleotides are synthetic nucleotides.

[0241] In some cases, the guide nucleic acid is not naturally occurring and made by artificial combination of otherwise separate segments of sequence. Often, the artificial combination is performed by chemical synthesis, by genetic engineering techniques, or by the artificial manipulation of isolated segments of nucleic acids. In some cases, the segment of a guide nucleic acid that comprises a sequence that is reverse complementary to the target nucleic acid is 20 nucleotides in length. A guide nucleic acid can have at least 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides reverse complementary to a target nucleic acid. In some cases, the guide nucleic acid can be 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length. For example, a guide nucleic acid may be at least 10 bases. In some embodiments, a guide nucleic acid may be from 10 to 50 bases. In some embodiments, a guide nucleic acid may be at least 25 bases. In some cases, the guide nucleic acid has from exactly or about 12 nucleotides (nt) to about 80 nt, from about 12 nt to about 50 nt, from about 12 nt to about 45 nt, from about 12 nt to about 40 nt, from about 12 nt to about 35 nt, from about 12 nt to about 30 nt, from about 12 nt to about 25 nt, from about 12 nt to about 20 nt, from about 12 nt to about 19 nt, from about 19 nt to about 20 nt, from about 19 nt to about 25 nt, from about 19 nt to about 30 nt, from about 19 nt to about 35 nt, from about 19 nt to about 40 nt, from about 19 nt to about 45 nt, from about 19 nt to about 50 nt, from about 19 nt to about 60 nt, from about 20 nt to about 25 nt, from about 20 nt to about 30 nt, from about 20 nt to about 35 nt, from about 20 nt to about 40 nt, from about 20 nt to about 45 nt, from about 20 nt to about 50 nt, or from about 20 nt to about 60 nt reverse complementary to a target nucleic acid. In some cases, the guide nucleic acid has from about 10 nt to about 60 nt, from about 20 nt to about 50 nt, or from about 30 nt to about 40 nt reverse complementary to a target nucleic acid. It is understood that the sequence of a guide nucleic acid need not be 100% reverse complementary to that of its target nucleic acid to be specifically hybridizable, hybridizable, or bind specifically. The guide nucleic acid can have a sequence comprising at least one uracil in a region from nucleic acid residue 5 to 20 that is reverse complementary to a modification variable region in the target nucleic acid. The guide nucleic acid, in some cases, has a sequence comprising at least

one uracil in a region from nucleic acid residue 5 to 9, 10 to 14, or 15 to 20 that is reverse complementary to a modification variable region in the target nucleic acid. The guide nucleic acid can have a sequence comprising at least one uracil in a region from nucleic acid residue 5 to 20 that is reverse complementary to a methylation variable region in the target nucleic acid. The guide nucleic acid, in some cases, has a sequence comprising at least one uracil in a region from nucleic acid residue 5 to 9, 10 to 14, or 15 to 20 that is reverse complementary to a methylation variable region in the target nucleic acid. The guide nucleic acid can hybridize with a target nucleic acid.

[0242] In some instances, compositions comprise shorter versions of the guide nucleic acids disclosed herein. For instance, the guide nucleic acid sequence may consist of a portion of a guide nucleic acid disclosed herein. In some instances, shorter versions may provide enhanced activity relative to their longer versions. Examples of longer versions and shorter versions of guide RNA for CasΦ.12 are shown in Tables I, K, M, O, Q, S, U, and W, and Tables AB-AF, respectively, wherein the shorter versions are produced by removing sixteen nucleotides from the 5' end of the long version and three nucleotides from the 3' end of the long version. In some instances, the long version is a CasΦ.32 guide nucleic acid described in Tables J, L, N, P, R, T, V, X, and the short version is a guide nucleic acid without the sixteen nucleotides at the 5' end of the long version and without the three nucleotides at the 3' end of the long version.

[0243] The guide nucleic acid (e.g., a non-naturally occurring guide nucleic acid) can be selected from a group of guide nucleic acids that have been tiled against the nucleic acid sequence of a strain of an infection or genomic locus of interest. The guide nucleic acid can be selected from a group of guide nucleic acids that have been tiled against the nucleic acid sequence of a target nucleic acid, for example, a strain of HPV16 or HPV18. Often, guide nucleic acids that are tiled against the nucleic acid of a strain of an infection or genomic locus of interest can be pooled for use in a method described herein. Often, these guide nucleic acids are pooled for detecting a target nucleic acid in a single assay. The pooling of guide nucleic acids that are tiled against a single target nucleic acid can enhance the detection of the target nucleic using the methods described herein. The pooling of guide nucleic acids that are tiled against a single target nucleic acid can ensure broad coverage of the target nucleic acid within a single reaction using the methods described herein. The tiling, for example, is sequential along the target nucleic acid. Sometimes, the tiling is overlapping along the target nucleic acid. In some instances, the tiling comprises gaps between the tiled guide nucleic acids along the target nucleic acid. In some instances, the tiling of the guide nucleic acids is non-sequential. Often, a method for detecting a target nucleic acid comprises contacting a target nucleic acid to a pool of guide nucleic acids and a programmable nuclease or nickase as disclosed herein, wherein a guide nucleic acid sequence of the pool of guide nucleic acids has a sequence selected from a group of tiled guide nucleic acid that correspond to nucleic acid sequence of a target nucleic acid; and assaying for a signal

produce by cleavage of at least some nucleic acids of a reporter of a population of nucleic acids of a reporter. Pooling of guide nucleic acids can ensure broad spectrum identification, or broad coverage, of a target species within a single reaction. This can be particularly helpful in diseases or indications, like sepsis, that may be caused by multiple organisms.

[0244] In some embodiments, the spacer sequence is between 10 and 35 nucleotides in length, between 10 and 30 nucleotides in length, between 15 and 30 nucleotides in length, between 10 and 25 nucleotides in length, between 15 and 25 nucleotides in length, between 17 and 30 nucleotides in length, between 17 and 25 nucleotides in length, between 17 and 22 nucleotides in length, or between 17 and 20 nucleotides in length. In preferred embodiments, the spacer sequence between 17 and 25 nucleotides in length. In more preferred embodiments, the spacer sequence is between 17 and 20 nucleotides in length. In most preferred embodiments, the spacer sequence is 17 nucleotides in length.

[0245] In some embodiments, the repeat sequence is between 15 and 40 nucleotides in length, between 15 and 36 nucleotides in length, between 18 and 36 nucleotides in length, between 18 and 30 nucleotides in length, between 18 and 25 nucleotides in length, between 18 and 22 nucleotides in length, between 18 and 20 nucleotides in length. In preferred embodiments, the repeat sequence is between 20 and 22 nucleotides in length. In more preferred embodiments, the repeat sequence is 20 nucleotides in length.

[0246] The spacer region of guide nucleic acids for CasΦ polypeptides disclosed herein comprise a seed region. In some embodiments, the seed regions do not tolerate mismatches in the complentarity of a spacer and a target sequence within about 1 to about 20 nucleotides from the 5' end of a spacer sequence. The seed region starts from the 5' end of the spacer sequence and is a region in which mismatches in the complementarity between the spacer sequence and the target sequence are not tolerated when the guide nucleic acid is bound to a CasΦ polypeptide such that the guide nucleic acid does not hybridize to the target sequence to allow cleavage of the target nucleic acid by the CasΦ polypeptide. In some embodiments, the seed region comprises between 10 and 20 nucleosides, between 12 and 20 nucleosides, between 14 and 20 nucleosides, between 14 and 18 nucleosides, between 10 and 16 nucleosides, between 12 and 16 nucleosides, or between 14 and 16 nucleosides. In preferred embodiments, the seed region comprises 16 nucleotides.

[0247] A programmable nuclease of the present disclosure may be activated to exhibit cleavage activity (e.g., cis-cleavage of a target nucleic acid or trans-cleavage of a collateral nucleic acid) upon binding of a ribonucleoprotein (RNP) complex to a target nucleic acid, in which the spacer of the crRNA of the gRNA hybridizes to the target nucleic acid.

TABLE A: spacer sequences of gRNAs targeting human TRAC in T cells

Name	Spacer sequence (5' --> 3'), shown as DNA	Target	SEQ ID NO
R3040	TGGATATCTGTGGGACAAGA	TRAC	118
R3041	TCCCACAGATATCCAGAACC	TRAC	119
R3042	GAGTCTCTCAGCTGGTACAC	TRAC	120
R3043	AGAGTCTCTCAGCTGGTACA	TRAC	121
R3044	TCACTGGATTTAGAGTCTCT	TRAC	122
R3045	AGAATCAAAATCGGTGAATA	TRAC	123
R3046	GAGAATCAAAATCGGTGAAT	TRAC	124
R3047	ACCGATTTTGATTCTCAAAC	TRAC	125
R3048	TTTGAGAATCAAAATCGGTG	TRAC	126
R3049	GTTTGAGAATCAAAATCGGT	TRAC	127
R3050	TGATTCTCAAACAAATGTGT	TRAC	128
R3051	GATTCTCAAACAAATGTGTC	TRAC	129
R3052	ATTCTCAAACAAATGTGTCA	TRAC	130
R3053	TGACACATTTGTTTGAGAAT	TRAC	131
R3054	TCAAACAAATGTGTCACAAA	TRAC	132
R3055	GTGACACATTTGTTTGAGAA	TRAC	133
R3056	CTTTGTGACACATTTGTTTG	TRAC	134
R3057	TGATGTGTATATCACAGACA	TRAC	135
R3058	TCTGTGATATACACATCAGA	TRAC	136
R3059	GTCTGTGATATACACATCAG	TRAC	137
R3060	TGTCTGTGATATACACATCA	TRAC	138
R3061	AAGTCCATAGACCTCATGTC	TRAC	139
R3062	CTCTTGAAGTCCATAGACCT	TRAC	140
R3063	AAGAGCAACAGTGCTGTGGC	TRAC	141
R3064	CTCCAGGCCACAGCACTGTT	TRAC	142
R3065	TTGCTCCAGGCCACAGCACT	TRAC	143
R3066	GTTGCTCCAGGCCACAGCAC	TRAC	144
R3067	CACATGCAAAGTCAGATTTG	TRAC	145
R3068	GCACATGCAAAGTCAGATTT	TRAC	146
R3069	GCATGTGCAAACGCCTTCAA	TRAC	147
R3070	AAGGCGTTTGCACATGCAAA	TRAC	148
R3071	CATGTGCAAACGCCTTCAAC	TRAC	149
R3072	TTGAAGGCGTTTGCACATGC	TRAC	150
R3073	AACAACAGCATTATTCCAGA	TRAC	151
R3074	TGGAATAATGCTGTTGTTGA	TRAC	152
R3075	TTCCAGAAGACACCTTCTTC	TRAC	153

R3076	CAGAAGACACCTTCTTCCCC	TRAC	154
R3077	CCTGGGCTGGGGAAGAAGGT	TRAC	155
R3078	TTCCCCAGCCCAGGTAAGGG	TRAC	156
R3079	CCCAGCCCAGGTAAGGGCAG	TRAC	157
R3080	TAAAAGGAAAAACAGACATT	TRAC	158
R3081	CTAAAAGGAAAAACAGACAT	TRAC	159
R3082	TTCCTTTTAGAAAGTTCCTG	TRAC	160
R3083	TCCTTTTAGAAAGTTCCTGT	TRAC	161
R3084	CCTTTTAGAAAGTTCCTGTG	TRAC	162
R3085	CTTTTAGAAAGTTCCTGTGA	TRAC	163
R3086	TAGAAAGTTCCTGTGATGTC	TRAC	164
R3136	AGAAAGTTCCTGTGATGTCA	TRAC	165
R3137	GAAAGTTCCTGTGATGTCAA	TRAC	166
R3138	ACATCACAGGAACTTTCTAA	TRAC	167
R3139	CTGTGATGTCAAGCTGGTCG	TRAC	168
R3140	TCGACCAGCTTGACATCACA	TRAC	169
R3141	CTCGACCAGCTTGACATCAC	TRAC	170
R3142	TCTCGACCAGCTTGACATCA	TRAC	171
R3143	AAAGCTTTTCTCGACCAGCT	TRAC	172
R3144	CAAAGCTTTTCTCGACCAGC	TRAC	173
R3145	CCTGTTTCAAAGCTTTTCTC	TRAC	174
R3146	GAAACAGGTAAGACAGGGGT	TRAC	175
R3147	AAACAGGTAAGACAGGGGTC	TRAC	176

TABLE B: spacer sequences of gRNAs targeting human B2M in T cells

Name	Spacer Sequence (5' --> 3'), shown as DNA	Target	SEQ ID NO
R3087	AATATAAGTGGAGGCGTCGC	B2M	177
R3088	ATATAAGTGGAGGCGTCGCG	B2M	178
R3089	AGGAATGCCCGCCAGCGCGA	B2M	179
R3090	CTGAAGCTGACAGCATTCGG	B2M	180
R3091	GGGCCGAGATGTCTCGCTCC	B2M	181
R3092	GCTGTGCTCGCGCTACTCTC	B2M	182
R3093	CTGGCCTGGAGGCTATCCAG	B2M	183
R3094	TGGCCTGGAGGCTATCCAGC	B2M	184
R3095	ATGTGTCTTTTCCCGATATT	B2M	185
R3096	TCCCGATATTCCTCAGGTAC	B2M	186
R3097	CCCGATATTCCTCAGGTACT	B2M	187
R3098	CCGATATTCCTCAGGTACTC	B2M	188

R3099	GAGTACCTGAGGAATATCGG	B2M	189
R3100	GGAGTACCTGAGGAATATCG	B2M	190
R3101	CTCAGGTACTCCAAAGATTC	B2M	191
R3102	AGGTTTACTCACGTCATCCA	B2M	192
R3103	ACTCACGTCATCCAGCAGAG	B2M	193
R3104	CTCACGTCATCCAGCAGAGA	B2M	194
R3105	TCTGCTGGATGACGTGAGTA	B2M	195
R3106	CATTCTCTGCTGGATGACGT	B2M	196
R3107	CCATTCTCTGCTGGATGACG	B2M	197
R3108	ACTTTCCATTCTCTGCTGGA	B2M	198
R3109	GACTTTCCATTCTCTGCTGG	B2M	199
R3110	AGGAAATTTGACTTTCCATT	B2M	200
R3111	CCTGAATTGCTATGTGTCTG	B2M	201
R3112	CTGAATTGCTATGTGTCTGG	B2M	202
R3113	CTATGTGTCTGGGTTTCATC	B2M	203
R3114	AATGTCGGATGGATGAAACC	B2M	204
R3115	CATCCATCCGACATTGAAGT	B2M	205
R3116	ATCCATCCGACATTGAAGTT	B2M	206
R3117	AGTAAGTCAACTTCAATGTC	B2M	207
R3118	TTCAGTAAGTCAACTTCAAT	B2M	208
R3119	AAGTTGACTTACTGAAGAAT	B2M	209
R3120	ACTTACTGAAGAATGGAGAG	B2M	210
R3121	TCTCTCCATTCTTCAGTAAG	B2M	211
R3122	CTGAAGAATGGAGAGAGAAT	B2M	212
R3123	AATTCTCTCTCCATTCTTCA	B2M	213
R3124	CAATTCTCTCTCCATTCTTC	B2M	214
R3125	TCAATTCTCTCTCCATTCTT	B2M	215
R3126	TTCAATTCTCTCTCCATTCT	B2M	216
R3127	AAAAAGTGGAGCATTCAGAC	B2M	217
R3128	CTGAAAGACAAGTCTGAATG	B2M	218
R3129	AGACTTGTCTTTCAGCAAGG	B2M	219
R3130	TCTTTCAGCAAGGACTGGTC	B2M	220
R3131	CAGCAAGGACTGGTCTTTCT	B2M	221
R3132	AGCAAGGACTGGTCTTTCTA	B2M	222
R3133	CTATCTCTTGTACTACACTG	B2M	223
R3134	TATCTCTTGTACTACACTGA	B2M	224
R3135	AGTGTAGTACAAGAGATAGA	B2M	225
R3148	TACTACACTGAATTCACCCC	B2M	226
R3149	AGTGGGGGTGAATTCAGTGT	B2M	227

R3150	CAGTGGGGGTGAATTCAGTG	B2M	228
R3151	TCAGTGGGGGTGAATTCAGT	B2M	229
R3152	TTCAGTGGGGGTGAATTCAG	B2M	230
R3153	ACCCCCACTGAAAAAGATGA	B2M	231
R3154	ACACGGCAGGCATACTCATC	B2M	232
R3155	GGCTGTGACAAAGTCACATG	B2M	233
R3156	GTCACAGCCCAAGATAGTTA	B2M	234
R3157	TCACAGCCCAAGATAGTTAA	B2M	235
R3158	ACTATCTTGGGCTGTGACAA	B2M	236
R3159	CCCCACTTAACTATCTTGGG	B2M	237

TABLE C: spacer sequences of gRNAs that target human PD1 in T cells

Name	Spacer sequence (5' --> 3')	Target	SEQ ID NO
R2921	CCUUCCGCUCACCUCCGCCU	PD1	238
R2922	CCUUCCGCUCACCUCCGCCU	PD1	239
R2923	CGCUCACCUCCGCCUGAGCA	PD1	240
R2924	UCCACUGCUCAGGCGGAGGU	PD1	241
R2925	UAGCACCGCCCAGACGACUG	PD1	242
R2926	AGGCAUGCAGAUCCCACAGG	PD1	243
R2927	CACAGGCGCCCUGGCCAGUC	PD1	244
R2928	UCUGGGCGGUGCUACAACUG	PD1	245
R2929	GCAUGCCUGGAGCAGCCCCA	PD1	246
R2930	UAGCACCGCCCAGACGACUG	PD1	247
R2931	UGGCCGCCAGCCCAGUUGUA	PD1	248
R2932	CUUCCGCUCACCUCCGCCUG	PD1	249
R2933	CAGGGCCUGUCUGGGGAGUC	PD1	250
R2934	UCCCCAGCCCUGCUCGUGGU	PD1	251
R2935	GGUCACCACGAGCAGGGCUG	PD1	252
R2936	UCCCCUUCGGUCACCACGAG	PD1	253
R2937	GAGAAGCUGCAGGUGAAGGU	PD1	254
R2938	ACCUGCAGCUUCUCCAACAC	PD1	255
R2939	UCCAACACAUCGGAGAGCUU	PD1	256
R2940	GCACGAAGCUCUCCGAUGUG	PD1	257
R2941	AGCACGAAGCUCUCCGAUGU	PD1	258
R2942	GUGCUAAACUGGUACCGCAU	PD1	259
R2943	CUGGGGCUCAUGCGGUACCA	PD1	260
R2944	UCCGUCUGGUUGCUGGGGCU	PD1	261
R2945	CCCGAGGACCGCAGCCAGCC	PD1	262

R2946	UGUGACACGGAAGCGGCAGU	PD1	263
R2947	CGUGUCACACAACUGCCCAA	PD1	264
R2948	GGCAGUUGUGUGACACGGAA	PD1	265
R2949	CACAUGAGCGUGGUCAGGGC	PD1	266
R2950	CGCCGGGCCCUGACCACGCU	PD1	267
R2951	GGGGCCAGGGAGAUGGCCCC	PD1	268
R2952	AUCUGCGCCUUGGGGGCCAG	PD1	269
R2953	GAUCUGCGCCUUGGGGGCCA	PD1	270
R2954	CCAGACAGGCCCUGGAACCC	PD1	271
R2955	CCAGCCCUGCUCGUGGUGAC	PD1	272
R2956	UCUCUGGAAGGGCACAAAGG	PD1	273
R2957	GUGCCCUUCCAGAGAGAAGG	PD1	274
R2958	UGCCCUUCCAGAGAGAAGGG	PD1	275
R2959	UGCCCUUCUCUCUGGAAGGG	PD1	276
R2960	CAGAGAGAAGGGCAGAAGUG	PD1	277
R2961	GAACUGGCCGGCUGGCCUGG	PD1	278
R2962	GGAACUGGCCGGCUGGCCUG	PD1	279
R2963	CAAACCCUGGUGGUUGGUGU	PD1	280
R2964	GUGUCGUGGGCGGCCUGCUG	PD1	281
R2965	CCUCGUGCGGCCCGGGAGCA	PD1	282
R2966	UCCCUGCAGAGAAACACACU	PD1	283
R2967	CUCUGCAGGGACAAUAGGAG	PD1	284
R2968	UCUGCAGGGACAAUAGGAGC	PD1	285
R2969	CUCCUCAAAGAAGGAGGACC	PD1	286
R2970	UCCUCAAAGAAGGAGGACCC	PD1	287
R2971	UCUGUGGACUAUGGGGAGCU	PD1	288
R2972	UCUCGCCACUGGAAAUCCAG	PD1	289
R2973	CCAGUGGCGAGAGAAGACCC	PD1	290
R2974	CAGUGGCGAGAGAAGACCCC	PD1	291
R2975	CGCUAGGAAAGACAAUGGUG	PD1	292
R2976	UCUUUCCUAGCGGAAUGGGC	PD1	293
R2977	CCUAGCGGAAUGGGCACCUC	PD1	294
R2978	CUAGCGGAAUGGGCACCUCA	PD1	295
R2979	GCCCCUCUGACCGGCUUCCU	PD1	296
R2980	CUUGGCCACCAGUGUUCUGC	PD1	297
R2981	GCCACCAGUGUUCUGCAGAC	PD1	298
R2982	UGCAGACCCUCCACCAUGAG	PD1	299
R2983	UCCUGAGGAAAUGCGCUGAC	PD1	300
R2984	CCUCAGGAGAAGCAGGCAGG	PD1	301

R2985	CUCAGGAGAAGCAGGCAGGG	PD1	302
R2986	CAGGCCGUCCAGGGGCUGAG	PD1	303
R2987	AGACAUGAGUCCUGUGGUGG	PD1	304
R2988	AGGUCCUGCCAGCACAGAGC	PD1	305
R2989	AGGGAGCUGGACGCAGGCAG	PD1	306
R2990	AGCCCCGGGCCGCAGGCAGC	PD1	307
R2991	AGGCAGGAGGCUCCGGGGCG	PD1	308
R2992	GGGGCUGGUUGGAGAUGGCC	PD1	309
R2993	GAGAUGGCCUUGGAGCAGCC	PD1	310
R2994	GCUGCUCCAAGGCCAUCUCC	PD1	311
R2995	GAGCAGCCAAGGUGCCCCUG	PD1	312
R2996	GGGAUGCCACUGCCAGGGGC	PD1	313
R2997	CGGGAUGCCACUGCCAGGGG	PD1	314
R2998	GGCCCUGCGUCCAGGGCGUU	PD1	315
R2999	UCUGCUCCCUGCAGGCCUAG	PD1	316
R3000	UCUAGGCCUGCAGGGAGCAG	PD1	317
R3001	CCUGAAACUUCUCUAGGCCU	PD1	318
R3002	UGACCUUCCCUGAAACUUCU	PD1	319
R3003	CAGGGAAGGUCAGAAGAGCU	PD1	320
R3004	AGGGAAGGUCAGAAGAGCUC	PD1	321
R3005	CUGCCCUGCCCACCACAGCC	PD1	322
R3006	CCUGCCCUGCCCACCACAGC	PD1	323
R3007	ACACAUGCCCAGGCAGCACC	PD1	324
R3008	CACAUGCCCAGGCAGCACCU	PD1	325
R3009	CCUGCCCCACAAAGGGCCUG	PD1	326
R3010	GUGGGGCAGGGAAGCUGAGG	PD1	327
R3011	UGGGGCAGGGAAGCUGAGGC	PD1	328
R3012	CUGCCUCAGCUUCCCUGCCC	PD1	329
R3013	CAGGCCCAGCCAGCACUCUG	PD1	330
R3014	AGGCCCAGCCAGCACUCUGG	PD1	331
R3015	CACCCCAGCCCCUCACACCA	PD1	332
R3016	GGACCGUAGGAUGUCCCUCU	PD1	333

TABLE D: spacer sequences of gRNAs targeting human CIITA

Name	Spacer sequence (5' --> 3'), shown as DNA	Target	SEQ ID NO
R4503 C2TA_T1.1	CTACACAATGCGTTGCCTGG	CIITA	334
R4504 C2TA_T1.2	GGGCTCTGACAGGTAGGACC	CIITA	335

R4505 C2TA_T1.3	TGTAGGAATCCCAGCCAGGC	CIITA	336
R4506 C2TA_T1.8	CCTGGCTCCACGCCCTGCTG	CIITA	337
R4507 C2TA_T1.9	GGGAAGCTGAGGGCACGAGG	CIITA	338
R4508 C2TA_T2.1	ACAGCGATGCTGACCCCCTG	CIITA	339
R4509 C2TA_T2.2	TTAACAGCGATGCTGACCCC	CIITA	340
R4510 C2TA_T2.3	TATGACCAGATGGACCTGGC	CIITA	341
R4511 C2TA_T2.4	GGGCCCCTAGAAGGTGGCTA	CIITA	342
R4512 C2TA_T2.5	TAGGGGCCCCAACTCCATGG	CIITA	343
R4513 C2TA_T2.6	AGAAGCTCCAGGTAGCCACC	CIITA	344
R4514 C2TA_T2.7	TCCAGCCAGGTCCATCTGGT	CIITA	345
R4515 C2TA_T2.8	TTCTCCAGCCAGGTCCATCT	CIITA	346
R5200	AGCAGGCTGTTGTGTGACAT	CIITA	1934
R5201	CATGTCACACAACAGCCTGC	CIITA	1935
R5202	TGTGACATGGAAGGTGATGA	CIITA	1936
R5203	ATCACCTTCCATGTCACACA	CIITA	1937
R5204	GCATAAGCCTCCCTGGTCTC	CIITA	1938
R5205	CAGGACTCCCAGCTGGAGGG	CIITA	1939
R5206	CTCAGGCCCTCCAGCTGGGA	CIITA	1940
R5207	TGCTGGCATCTCCATACTCT	CIITA	1941
R5208	TGCCCAACTTCTGCTGGCAT	CIITA	1942
R5209	CTGCCCAACTTCTGCTGGCA	CIITA	1943
R5210	TCTGCCCAACTTCTGCTGGC	CIITA	1944
R5211	TGACTTTTCTGCCCAACTTC	CIITA	1945
R5212	CTGACTTTTCTGCCCAACTT	CIITA	1946
R5213	TCTGACTTTTCTGCCCAACT	CIITA	1947
R5214	CCAGAGGAGCTTCCGGCAGA	CIITA	1948
R5215	AGGTCTGCCGGAAGCTCCTC	CIITA	1949
R5216	CGGCAGACCTGAAGCACTGG	CIITA	1950
R5217	CAGTGCTTCAGGTCTGCCGG	CIITA	1951
R5218	AACAGCGCAGGCAGTGGCAG	CIITA	1952
R5219	AACCAGGAGCCAGCCTCCGG	CIITA	1953
R5220	TCCAGGCGCATCTGGCCGGA	CIITA	1954
R5221	CTCCAGGCGCATCTGGCCGG	CIITA	1955
R5222	TCTCCAGGCGCATCTGGCCG	CIITA	1956
R5223	CTCCAGTTCCTCGTTGAGCT	CIITA	1957
R5224	TCCAGTTCCTCGTTGAGCTG	CIITA	1958
R5225	AGGCAGCTCAACGAGGAACT	CIITA	1959
R5226	CTCGTTGAGCTGCCTGAATC	CIITA	1960
R5227	AGCTGCCTGAATCTCCCTGA	CIITA	1961
R5228	GTCCCCACCATCTCCACTCT	CIITA	1962
R5229	TCCCCACCATCTCCACTCTG	CIITA	1963
R5230	CCAGAGCCCATGGGGCAGAG	CIITA	1964

R5231	GCCAGAGCCCATGGGGCAGA	CIITA	1965
R5232	CAGCCTCAGAGATTTGCCAG	CIITA	1966
R5233	GGAGGCCGTGGACAGTGAAT	CIITA	1967
R5234	ACTGTCCACGGCCTCCCAAC	CIITA	1968
R5235	GCTCCATCAGCCACTGACCT	CIITA	1969
R5236	AGGCATGCTGGGCAGGTCAG	CIITA	1970
R5237	CTCGGGAGGTCAGGGCAGGT	CIITA	1971
R5238	GCTCGGGAGGTCAGGGCAGG	CIITA	1972
R5239	GAGACCTCTCCAGCTGCCGG	CIITA	1973
R5240	TTGGAGACCTCTCCAGCTGC	CIITA	1974
R5241	GAAGCTTGTTGGAGACCTCT	CIITA	1975
R5242	GGAAGCTTGTTGGAGACCTC	CIITA	1976
R5243	TGGAAGCTTGTTGGAGACCT	CIITA	1977
R5244	TACCGCTCACTGCAGGACAC	CIITA	1978
R5245	CTGCTGCTCCTCTCCAGCCT	CIITA	1979
R5246	CCGCTCCAGGCTCTTGCTGC	CIITA	1980
R5247	TGCCCAGTCCGGGGTGGCCA	CIITA	1981
R5248	GGCCAGCTGCCGTTCTGCCC	CIITA	1982
R5249	GCAGCCAACAGCACCTCAGC	CIITA	1983
R5250	GCTGCCAAGGAGCACCGGCG	CIITA	1984
R5251	CCCAGCACAGCAATCACTCG	CIITA	1985
R5252	GCCCAGCACAGCAATCACTC	CIITA	1986
R5253	CTGTGCTGGGCAAAGCTGGT	CIITA	1987
R5254	CCCTGACCAGCTTTGCCCAG	CIITA	1988
R5255	GGCTGGGGCAGTGAGCCGGG	CIITA	1989
R5256	TGGCCGGCTTCCCCAGTACG	CIITA	1990
R5257	CCCAGTACGACTTTGTCTTC	CIITA	1991
R5258	GTCTTCTCTGTCCCCTGCCA	CIITA	1992
R5259	TCTTCTCTGTCCCCTGCCAT	CIITA	1993
R5260	TCTGTCCCCTGCCATTGCTT	CIITA	1994
R5261	AAGCAATGGCAGGGGACAGA	CIITA	1995
R5262	CTTGAACCGTCCGGGGGATG	CIITA	1996
R5263	AACCGTCCGGGGGATGCCTA	CIITA	1997
R5264	TCCCTGGGCCCACAGCCACT	CIITA	1998
R5265	AAGATGTGGCTGAAAACCTC	CIITA	1999
R5266	TCAGCCACATCTTGAAGAGA	CIITA	2000
R5267	CAGCCACATCTTGAAGAGAC	CIITA	2001
R5268	AGCCACATCTTGAAGAGACC	CIITA	2002
R5269	AAGAGACCTGACCGCGTTCT	CIITA	2003
R5270	TGCTCATCCTAGACGGCTTC	CIITA	2004
R5271	CAGCTCCTCGAAGCCGTCTA	CIITA	2005
R5272	CGCTTCCAGCTCCTCGAAGC	CIITA	2006
R5273	GAGGAGCTGGAAGCGCAAGA	CIITA	2007

R5274	CTGCACAGCACGTGCGGACC	CIITA	2008
R5275	TGGAAAAGGCCGGCCAGCAG	CIITA	2009
R5276	TTCTGGAAAAGGCCGGCCAG	CIITA	2010
R5277	TCCAGAAGAAGCTGCTCCGA	CIITA	2011
R5278	CCAGAAGAAGCTGCTCCGAG	CIITA	2012
R5279	CAGAAGAAGCTGCTCCGAGG	CIITA	2013
R5280	CACCCTCCTCCTCACAGCCC	CIITA	2014
R5281	CTCAGGCTCTGGACCAGGCG	CIITA	2015
R5282	GAGCTGTCCGGCTTCTCCAT	CIITA	2016
R5283	AGCTGTCCGGCTTCTCCATG	CIITA	2017
R5284	TCCATGGAGCAGGCCCAGGC	CIITA	2018
R5285	GAGAGCTCAGGGATGACAGA	CIITA	2019
R5286	AGAGCTCAGGGATGACAGAG	CIITA	2020
R5287	GTGCTCTGTCATCCCTGAGC	CIITA	2021
R5288	TTCTCAGTCACAGCCACAGC	CIITA	2022
R5289	TCAGTCACAGCCACAGCCCT	CIITA	2023
R5290	GTGCCGGGCAGTGTGCCAGC	CIITA	2024
R5291	TGCCGGGCAGTGTGCCAGCT	CIITA	2025
R5292	GCGTCCTCCCCAAGCTCCAG	CIITA	2026
R5293	GGGAGGACGCCAAGCTGCCC	CIITA	2027
R5294	GCCAGCTCTGCCAGGGCCCC	CIITA	2028
R5295	ATGTCTGCGGCCCAGCTCCC	CIITA	2029
R5392	GATGTCTGCGGCCCAGCTCC	CIITA	2030
R5393	CCATCCGCAGACGTGAGGAC	CIITA	2031
R5394	GCCATCGCCCAGGTCCTCAC	CIITA	2032
R5395	GGCCATCGCCCAGGTCCTCA	CIITA	2033
R5396	GACTAAGCCTTTGGCCATCG	CIITA	2034
R5397	GTCCAACACCCACCGCGGGC	CIITA	2035
R5398	CAGGAGGAAGCTGGGGAAGG	CIITA	2036
R5399	CCCAGCTTCCTCCTGCAATG	CIITA	2037
R5400	CTCCTGCAATGCTTCCTGGG	CIITA	2038
R5401	CTGGGGGCCCTGTGGCTGGC	CIITA	2039
R5402	GCCACTCAGAGCCAGCCACA	CIITA	2040
R5403	CGCCACTCAGAGCCAGCCAC	CIITA	2041
R5404	ATTTCGCCACTCAGAGCCAG	CIITA	2042
R5405	TCCTTGATTTCGCCACTCAG	CIITA	2043
R5406	GGGTCAATGCTAGGTACTGC	CIITA	2044
R5407	CTTGGGGTCAATGCTAGGTA	CIITA	2045
R5408	TTCCTTGGGGTCAATGCTAG	CIITA	2046
R5409	ACCCCAAGGAAGAAGAGGCC	CIITA	2047
R5410	TCATAGGGCCTCTTCTTCCT	CIITA	2048
R5411	CTGGCTGGGCTGATCTTCCA	CIITA	2049
R5412	TGGCTGGGCTGATCTTCCAG	CIITA	2050

R5413	CAGCCTCCCGCCCGCTGCCT	CIITA	2051
R5414	CTGTCCACCGAGGCAGCCGC	CIITA	2052
R5415	TGCTTCCTGTCCACCGAGGC	CIITA	2053
R5416	AGGTACCTCGCAAGCACCTT	CIITA	2054
R5417	CGAGGTACCTGAAGCGGCTG	CIITA	2055
R5418	CAGCCTCCTCGGCCTCGTGG	CIITA	2056
R5419	GGCAGCACGTGGTACAGGAG	CIITA	2057
R5420	GCAGCACGTGGTACAGGAGC	CIITA	2058
R5421	TCTGGGCACCCGCCTCACGC	CIITA	2059
R5422	CTGGGCACCCGCCTCACGCC	CIITA	2060
R5423	TGGGCACCCGCCTCACGCCT	CIITA	2061
R5424	CCCAGTACATGTGCATCAGG	CIITA	2062
R5425	GCCCGCCGCCTCCAAGGCCT	CIITA	2063
R5426	GAGGCGGCGGGCCAAGACTT	CIITA	2064
R5427	TCCCTGGACCTCCGCAGCAC	CIITA	2065
R5428	GCCCCTCTGGATTGGGGAGC	CIITA	2066
R5429	CCCCTCTGGATTGGGGAGCC	CIITA	2067
R5430	GGGAGCCTCGTGGGACTCAG	CIITA	2068
R5431	GTCTCCCCATGCTGCTGCAG	CIITA	2069
R5432	TCCTCTGCTGCCTGAAGTAG	CIITA	2070
R5433	AGGCAGCAGAGGAGAAGTTC	CIITA	2071
R5434	AAAGGCTCGATGGTGAACTT	CIITA	2072
R5435	GAAAGGCTCGATGGTGAACT	CIITA	2073
R5436	ACCATCGAGCCTTTCAAAGC	CIITA	2074
R5437	GCTTTGAAAGGCTCGATGGT	CIITA	2075
R5438	AGGGACTTGGCTTTGAAAGG	CIITA	2076
R5439	CAAAGCCAAGTCCCTGAAGG	CIITA	2077
R5440	AAAGCCAAGTCCCTGAAGGA	CIITA	2078
R5441	CACATCCTTCAGGGACTTGG	CIITA	2079
R5442	CCAGGTCTTCCACATCCTTC	CIITA	2080
R5443	CCCAGGTCTTCCACATCCTT	CIITA	2081
R5444	CTCGGAAGACACAGCTGGGG	CIITA	2082
R5445	GGTCCCGAACAGCAGGGAGC	CIITA	2083
R5446	AGGTCCCGAACAGCAGGGAG	CIITA	2084
R5447	TTTAGGTCCCGAACAGCAGG	CIITA	2085
R5448	CTTTAGGTCCCGAACAGCAG	CIITA	2086
R5449	GGGACCTAAAGAAACTGGAG	CIITA	2087
R5450	GGGAAAGCCTGGGGGCCTGA	CIITA	2088
R5451	GGGGAAAGCCTGGGGGCCTG	CIITA	2089
R5452	CCCCAAACTGGTGCGGATCC	CIITA	2090
R5453	CCCAAACTGGTGCGGATCCT	CIITA	2091
R5454	TTCTCACTCAGCGCATCCAG	CIITA	2092
R5455	AGCTGGGGGAAGGTGGCTGA	CIITA	2093

R5456	CCCCAGCTGAAGTCCTTGGA	CIITA	2094
R5457	CAAGGACTTCAGCTGGGGGA	CIITA	2095
R5458	CCAAGGACTTCAGCTGGGGG	CIITA	2096
R5459	AGGGTTTCCAAGGACTTCAG	CIITA	2097
R5460	TAGGCACCCAGGTCAGTGAT	CIITA	2098
R5461	GTAGGCACCCAGGTCAGTGA	CIITA	2099
R5462	GCTCGCTGCATCCCTGCTCA	CIITA	2100
R5463	GCCTGAGCAGGGATGCAGCG	CIITA	2101
R5464	TACAATAACTGCATCTGCGA	CIITA	2102
R5465	GCTCGTGTGCTTCCGGACAT	CIITA	2103
R5466	CGGACATGGTGTCCCTCCGG	CIITA	2104
R5467	ACGGCTGCCGGGGCCCAGCA	CIITA	2105
R5468	GGAGGTGTCCTCATGTGGAG	CIITA	2106
R5469	CTGGACACTGAATGGGATGG	CIITA	2107
R5470	AGTGTCCAGGAACACCTGCA	CIITA	2108
R5471	CAGGTGTTCCTGGACACTGA	CIITA	2109
R5472	TTGCAGGTGTTCCTGGACAC	CIITA	2110
R5473	ACGGATCAGCCTGAGATGAT	CIITA	2111

TABLE E: spacer sequences of gRNAs targeting mouse PCSK9

Name	Spacer sequence (5' --> 3')	Target	SEQ ID NO
R4238	CCGCUGUUGCCGCCGCUGCU	PCSK9	347
R4239	CCGCCGCUGCUGCUGCUGUU	PCSK9	348
R4240	CUGCUACUGUGCCCCACCGG	PCSK9	349
R4241	AUAAUCUCCAUCCUCGUCCU	PCSK9	350
R4242	UGAAGAGCUGAUGCUCGCCC	PCSK9	351
R4243	GAGCAACGGCGGAAGGUGGC	PCSK9	352
R4244	CUGGCAGCCUCCAGGCCUCC	PCSK9	353
R4245	UGGUGCUGAUGGAGGAGACC	PCSK9	354
R4246	AAUCUGUAGCCUCUGGGUCU	PCSK9	355
R4247	UUCAAUCUGUAGCCUCUGGG	PCSK9	356
R4248	GUUCAAUCUGUAGCCUCUGG	PCSK9	357
R4249	AACAAACUGCCCACCGCCUG	PCSK9	358
R4250	AUGACAUAGCCCCGGCGGGC	PCSK9	359
R4251	UACAUAUCUUUUAUGACCUC	PCSK9	360
R4252	UAUGACCUCUUCCCUGGCUU	PCSK9	361
R4253	AUGACCUCUUCCCUGGCUUC	PCSK9	362
R4254	UGACCUCUUCCCUGGCUUCU	PCSK9	363
R4255	ACCAAGAAGCCAGGGAAGAG	PCSK9	364
R4256	CCUGGCUUCUUGGUGAAGAU	PCSK9	365

R4257	UUGGUGAAGAUGAGCAGUGA	PCSK9	366
R4258	GUGAAGAUGAGCAGUGACCU	PCSK9	367
R4259	CCCCAUGUGGAGUACAUUGA	PCSK9	368
R4260	CUCAAUGUACUCCACAUGGG	PCSK9	369
R4261	AGGAAGACUCCUUUGUCUUC	PCSK9	370
R4262	GUCUUCGCCCAGAGCAUCCC	PCSK9	371
R4263	UCUUCGCCCAGAGCAUCCCA	PCSK9	372
R4264	GCCCAGAGCAUCCCAUGGAA	PCSK9	373
R4265	CAUGGGAUGCUCUGGGCGAA	PCSK9	374
R4266	GCUCCAGGUUCCAUGGGAUG	PCSK9	375
R4267	UCCCAGCAUGGCACCAGACA	PCSK9	376
R4268	CUCUGUCUGGUGCCAUGCUG	PCSK9	377
R4269	GAUACCAGCAUCCAGGGUGC	PCSK9	378
R4270	AGGGCAGGGUCACCAUCACC	PCSK9	379
R4271	AAGUCGGUGAUGGUGACCCU	PCSK9	380
R4272	AACAGCGUGCCGGAGGAGGA	PCSK9	381
R4273	GCCACACCAGCAUCCCGGCC	PCSK9	382
R4274	AGCACACGCAGGCUGUGCAG	PCSK9	383
R4275	ACAGUUGAGCACACGCAGGC	PCSK9	384
R4276	CCUUGACAGUUGAGCACACG	PCSK9	385
R4277	GCUGACUCUUCCGAAUAAAC	PCSK9	386
R4278	AUUCGGAAGAGUCAGCUAAU	PCSK9	387
R4279	UUCGGAAGAGUCAGCUAAUC	PCSK9	388
R4280	GGAAGAGUCAGCUAAUCCAG	PCSK9	389
R4281	UGCUGCCCCUGGCCGGUGGG	PCSK9	390
R4282	AGGAUGCGGCUAUACCCACC	PCSK9	391
R4283	CCAGCUGCUGCAACCAGCAC	PCSK9	392
R4284	CAGCAGCUGGGAACUUCCGG	PCSK9	393
R4285	CGGGACGACGCCUGCCUCUA	PCSK9	394
R4286	GUGGCCCCGACUGUGAUGAC	PCSK9	395
R4287	CCUUGGGGACUUUGGGGACU	PCSK9	396
R4288	GUCCCCAAAGUCCCCAAGGU	PCSK9	397
R4289	GGGACUUUGGGGACUAAUUU	PCSK9	398
R4290	GGGGACUAAUUUUGGACGCU	PCSK9	399
R4291	GGGACUAAUUUUGGACGCUG	PCSK9	400
R4292	UGGACGCUGUGUGGAUCUCU	PCSK9	401
R4293	GGACGCUGUGUGGAUCUCUU	PCSK9	402
R4294	GACGCUGUGUGGAUCUCUUU	PCSK9	403
R4295	CCGGGGGCAAAGAGAUCCAC	PCSK9	404

R4296	GCCCCCGGGAAGGACAUCAU	PCSK9	405
R4297	CCCCCGGGAAGGACAUCAUC	PCSK9	406
R4298	AUGUCACAGAGUGGGACCUC	PCSK9	407
R4299	UGGCUCGGAUGCUGAGCCGG	PCSK9	408
R4300	CCCUGGCCGAGCUGCGGCAG	PCSK9	409
R4301	GUAGAGAAGUGGAUCAGCCU	PCSK9	410
R4302	GGUAGAGAAGUGGAUCAGCC	PCSK9	411
R4303	UCUACCAAAGACGUCAUCAA	PCSK9	412
R4304	AUGACGUCUUUGGUAGAGAA	PCSK9	413
R4305	CCUGAGGACCAGCAGGUGCU	PCSK9	414
R4306	GGGGUCAGCACCUGCUGGUC	PCSK9	415
R4307	GAGUGGGCCCCGAGUGUGCC	PCSK9	416
R4308	UGGGGCACAGCGGGCUGUAG	PCSK9	417
R4309	UCCAGGAGCGGGAGGCGUCG	PCSK9	418
R4310	CAGACCUGCUGGCCUCCUAU	PCSK9	419
R4311	AGGGCCUUGCAGACCUGCUG	PCSK9	420
R4312	GGGGGUGAGGGUGUCUAUGC	PCSK9	421
R4313	GGGGUGAGGGUGUCUAUGCC	PCSK9	422
R4314	GCACGGGGAACCAGGCAGCA	PCSK9	423
R4315	CCCGUGCCAACUGCAGCAUC	PCSK9	424
R4316	UGGAUGCUGCAGUUGGCACG	PCSK9	425
R4317	UGGUGGCAGUGGACAUGGGU	PCSK9	426
R4318	CACUUCCCAAUGGAAGCUGC	PCSK9	427
R4319	CAUUGGGAAGUGGAAGACCU	PCSK9	428
R4320	GGAAGUGGAAGACCUUAGUG	PCSK9	429
R4321	GUGUCCGGAGGCAGCCUGCG	PCSK9	430
R4322	GCCACCAGGCGGCCAGUGUC	PCSK9	431
R4323	CUGCUGCCAUGCCCCAGGGC	PCSK9	432
R4324	CAGCCCUGGGGCAUGGCAGC	PCSK9	433
R4325	CAUUCCAGCCCUGGGGCAUG	PCSK9	434
R4326	GCAUUCCAGCCCUGGGGCAU	PCSK9	435
R4327	UGCAUUCCAGCCCUGGGGCA	PCSK9	436
R4328	AUUUUGCAUUCCAGCCCUGG	PCSK9	437
R4329	CAUCCAGUCAGGGUCCAUCC	PCSK9	438
R4330	UCCACGCUGUAGGCUCCCAG	PCSK9	439
R4331	CCACACACAGGUUGUCCACG	PCSK9	440
R4332	UCCACUGGUCCUGUCUGCUC	PCSK9	441
R4333	CUGAAGGCCGGCUCCGGCAG	PCSK9	442

TABLE F: spacer sequences of gRNAs targets Bak1 in CHO cells

Name	Spacer sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2452 Bak1_CasPhi_1	GAAGCTATGTTTTCCATCTC	443
R2453 Bak1_CasPhi_2	GCAGGGGCAGCCGCCCCCTG	444
R2454 Bak1_CasPhi_3	CTCCTAGAACCCAACAGGTA	445
R2455 Bak1_CasPhi_4	GAAAGACCTCCTCTGTGTCC	446
R2456 Bak1_CasPhi_5	TCCATCTCGGGGTTGGCAGG	447
R2457 Bak1_CasPhi_6	TTCCTGATGGTGGAGATGGA	448
R2849_Bak1_nsd_sg1	CTGACTCCCAGCTCTGACCC	449
R2850_Bak1_nsd_sg2	TGGGGTCAGAGCTGGGAGTC	450
R2851_Bak1_nsd_sg3	GAAAGACCTCCTCTGTGTCC	451
R2852_Bak1_nsd_sg4	CGAAGCTATGTTTTCCATCT	452
R2853_Bak1_nsd_sg5	GAAGCTATGTTTTCCATCTC	453
R2854_Bak1_nsd_sg6	TCCATCTCCACCATCAGGAA	454
R2855_Bak1_nsd_sg7	CCATCTCCACCATCAGGAAC	455
R2856_Bak1_nsd_sg8	CTGATGGTGGAGATGGAAAA	456
R2857_Bak1_nsd_sg9	CATCTCCACCATCAGGAACA	457
R2858_Bak1_nsd_sg10	TTCCTGATGGTGGAGATGGA	458
R2859_Bak1_nsd_sg11	GCAGGGGCAGCCGCCCCCTG	459
R2860_Bak1_nsd_sg12	TCCATCTCGGGGTTGGCAGG	460
R2861_Bak1_nsd_sg13	TAGGAGCAAATTGTCCATCT	461
R2862_Bak1_nsd_sg14	GGTTCTAGGAGCAAATTGTC	462
R2863_Bak1_nsd_sg15	GCTCCTAGAACCCAACAGGT	463
R2864_Bak1_nsd_sg16	CTCCTAGAACCCAACAGGTA	464
R3977 Bak1_exon1_sg1	TCCAGACGCCATCTTTCAGG	465
R3978 Bak1_exon1_sg2	TGGTAAGAGTCCTCCTGCCC	466
R3979 Bak1_exon3_sg1	TTACAGCATCTTGGGTCAGG	467
R3980 Bak1_exon3_sg2	GGTCAGGTGGGCCGGCAGCT	468
R3981 Bak1_exon3_sg3	CTATCATTGGAGATGACATT	469
R3982 Bak1_exon3_sg4	GAGATGACATTAACCGGAGA	470
R3983 Bak1_exon3_sg5	TGGAACTCTGTGTCGTATCT	471
R3984 Bak1_exon3_sg6	CAGAATTTACTGGAGCAGCT	472
R3985 Bak1_exon3_sg7	ACTGGAGCAGCTGCAGCCCA	473
R3986 Bak1_exon3_sg8	CCAGCTGTGGGCTGCAGCTG	474
R3987 Bak1_exon3_sg9	GTAGGCATTCCCAGCTGTGG	475
R3988 Bak1_exon3_sg10	GTGAAGAGTTCGTAGGCATT	476
R3989 Bak1_exon3_sg11	ACCAAGATTGCCTCCAGGTA	477
R3990 Bak1_exon3_sg12	CCTCCAGGTACCCACCACCA	478

TABLE G: spacer sequences of gRNAs targeting Bax in CHO cells

Name	Spacer sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2458 Bax_CasPhi_1	CTAATGTGGATACTAACTCC	479
R2459 Bax_CasPhi_2	TTCCGTGTGGCAGCTGACAT	480
R2460 Bax_CasPhi_3	CTGATGGCAACTTCAACTGG	481
R2461 Bax_CasPhi_4	TACTTTGCTAGCAAACTGGT	482
R2462 Bax_CasPhi_5	AGCACCAGTTTGCTAGCAAA	483
R2463 Bax_CasPhi_6	AACTGGGGCCGGGTTGTTGC	484
R2865_Bax_nsd_sg1	TTCTCTTTCCTGTAGGATGA	485
R2866_Bax_nsd_sg2	TCTTTCCTGTAGGATGATTG	486
R2867_Bax_nsd_sg3	CCTGTAGGATGATTGCTAAT	487
R2868_Bax_nsd_sg4	CTGTAGGATGATTGCTAATG	488
R2869_Bax_nsd_sg5	CTAATGTGGATACTAACTCC	489
R2870_Bax_nsd_sg6	TTCCGTGTGGCAGCTGACAT	490
R2871_Bax_nsd_sg7	CGTGTGGCAGCTGACATGTT	491
R2872_Bax_nsd_sg8	CCATCAGCAAACATGTCAGC	492
R2873_Bax_nsd_sg9	AAGTTGCCATCAGCAAACAT	493
R2874_Bax_nsd_sg10	GCTGATGGCAACTTCAACTG	494
R2875_Bax_nsd_sg11	CTGATGGCAACTTCAACTGG	495
R2876_Bax_nsd_sg12	AACTGGGGCCGGGTTGTTGC	496
R2877_Bax_nsd_sg13	TTGCCCTTTTCTACTTTGCT	497
R2878_Bax_nsd_sg14	CCCTTTTCTACTTTGCTAGC	498
R2879_Bax_nsd_sg15	CTAGCAAAGTAGAAAAGGGC	499
R2880_Bax_nsd_sg16	GCTAGCAAAGTAGAAAAGGG	500
R2881_Bax_nsd_sg17	TCTACTTTGCTAGCAAACTG	501
R2882_Bax_nsd_sg18	CTACTTTGCTAGCAAACTGG	502
R2883_Bax_nsd_sg19	TACTTTGCTAGCAAACTGGT	503
R2884_Bax_nsd_sg20	GCTAGCAAACTGGTGCTCAA	504
R2885_Bax_nsd_sg21	CTAGCAAACTGGTGCTCAAG	505
R2886_Bax_nsd_sg22	AGCACCAGTTTGCTAGCAAA	506

TABLE H: spacer sequences of gRNAs targeting Fut8 in CHO cells

Name	Spacer sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2464 Fut8_CasPhi_1	CCACTTTGTCAGTGCGTCTG	507
R2465 Fut8_casPhi_2	CTCAATGGGATGGAAGGCTG	508
R2466 Fut8_CasPhi_3	AGGAATACATGGTACACGTT	509

R2467 Fut8_CasPhi_4	AAGAACATTTTCAGCTTCTC	510
R2468 Fut8_CasPhi_5	ATCCACTTTCATTCTGCGTT	511
R2469 Fut8_CasPhi_6	TTTGTTAAAGGAGGCAAAGA	512
R2887_Fut8_nsd_sg1	TCCCCAGAGTCCATGTCAGA	513
R2888_Fut8_nsd_sg2	TCAGTGCGTCTGACATGGAC	514
R2889_Fut8_nsd_sg3	GTCAGTGCGTCTGACATGGA	515
R2890_Fut8_nsd_sg4	CCACTTTGTCAGTGCGTCTG	516
R2891_Fut8_nsd_sg5	TGTTCCCACTTTGTCAGTGC	517
R2892_Fut8_nsd_sg6	CTCAATGGGATGGAAGGCTG	518
R2893_Fut8_nsd_sg7	CATCCCATTGAGGAATACAT	519
R2894_Fut8_nsd_sg8	AGGAATACATGGTACACGTT	520
R2895_Fut8_nsd_sg9	AACGTGTACCATGTATTCCT	521
R2896_Fut8_nsd_sg10	TTCAACGTGTACCATGTATT	522
R2897_Fut8_nsd_sg11	AAGAACATTTTCAGCTTCTC	523
R2898_Fut8_nsd_sg12	GAGAAGCTGAAAATGTTCTT	524
R2899_Fut8_nsd_sg13	TCAGCTTCTCGAACGCAGAA	525
R2900_Fut8_nsd_sg14	CAGCTTCTCGAACGCAGAAT	526
R2901_Fut8_nsd_sg15	TGCGTTCGAGAAGCTGAAAA	527
R2902_Fut8_nsd_sg16	AGCTTCTCGAACGCAGAATG	528
R2903_Fut8_nsd_sg17	ATTCTGCGTTCGAGAAGCTG	529
R2904_Fut8_nsd_sg18	CATTCTGCGTTCGAGAAGCT	530
R2905_Fut8_nsd_sg19	TCGAACGCAGAATGAAAGTG	531
R2906_Fut8_nsd_sg20	ATCCACTTTCATTCTGCGTT	532
R2907_Fut8_nsd_sg21	TATCCACTTTCATTCTGCGT	533
R2908_Fut8_nsd_sg22	TTATCCACTTTCATTCTGCG	534
R2909_Fut8_nsd_sg23	TTTATCCACTTTCATTCTGC	535
R2910_Fut8_nsd_sg24	TTTTATCCACTTTCATTCTG	536
R2911_Fut8_nsd_sg25	AACAAAGAAGGGTCATCAGT	537
R2912_Fut8_nsd_sg26	CCTCCTTTAACAAAGAAGGG	538
R2913_Fut8_nsd_sg27	GCCTCCTTTAACAAAGAAGG	539
R2914_Fut8_nsd_sg28	TTTGTTAAAGGAGGCAAAGA	540
R2915_Fut8_nsd_sg29	GTTAAAGGAGGCAAAGACAA	541
R2916_Fut8_nsd_sg30	TTAAAGGAGGCAAAGACAAA	542
R2917_Fut8_nsd_sg31	TCTTTGCCTCCTTTAACAAA	543
R2918_Fut8_nsd_sg32	GTCTTTGCCTCCTTTAACAA	544
R2919_Fut8_nsd_sg33	GTCTAACTTACTTTGTCTTT	545
R2920_Fut8_nsd_sg34	TTGGTCTAACTTACTTTGTC	546

TABLE I: CasΦ.12 gRNAs targeting human TRAC in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3040_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGATATCTGTGGGACAAGA	547
R3041_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCCACAGATATCCAGAACC	548
R3042_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAGTCTCTCAGCTGGTACAC	549
R3043_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGAGTCTCTCAGCTGGTACA	550
R3044_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCACTGGATTTAGAGTCTCT	551
R3045_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGAATCAAAATCGGTGAATA	552
R3046_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAGAATCAAAATCGGTGAAT	553
R3047_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACCGATTTTGATTCTCAAAC	554
R3048_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTTGAGAATCAAAATCGGTG	555
R3049_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTTTGAGAATCAAAATCGGT	556
R3050_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGATTCTCAAACAAATGTGT	557
R3051_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGATTCTCAAACAAATGTGTC	558
R3052_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATTCTCAAACAAATGTGTCA	559
R3053_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGACACATTTGTTTGAGAAT	560
R3054_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCAAACAAATGTGTCACAAA	561
R3055_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTGACACATTTGTTTGAGAA	562
R3056_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTTTGTGACACATTTGTTTG	563
R3057_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGATGTGTATATCACAGACA	564
R3058_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCTGTGATATACACATCAGA	565
R3059_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTCTGTGATATACACATCAG	566

R3060_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGTCTGTGATATACACATCA	567
R3061_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAGTCCATAGACCTCATGTC	568
R3062_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCTTGAAGTCCATAGACCT	569
R3063_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAGAGCAACAGTGCTGTGGC	570
R3064_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCCAGGCCACAGCACTGTT	571
R3065_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTGCTCCAGGCCACAGCACT	572
R3066_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTTGCTCCAGGCCACAGCAC	573
R3067_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCACATGCAAAGTCAGATTTG	574
R3068_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCACATGCAAAGTCAGATTT	575
R3069_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCATGTGCAAACGCCTTCAA	576
R3070_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAGGCGTTTGCACATGCAAA	577
R3071_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCATGTGCAAACGCCTTCAAC	578
R3072_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTGAAGGCGTTTGCACATGC	579
R3073_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAACAACAGCATTATTCCAGA	580
R3074_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGAATAATGCTGTTGTTGA	581
R3075_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCAGAAGACACCTTCTTC	582
R3076_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAGAAGACACCTTCTTCCCC	583
R3077_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCTGGGCTGGGGAAGAAGGT	584
R3078_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCCCAGCCCAGGTAAGGG	585
R3079_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCCAGCCCAGGTAAGGGCAG	586
R3080_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTAAAAGGAAAAACAGACATT	587
R3081_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTAAAAGGAAAAACAGACAT	588

R3082_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCTTTTAGAAAGTTCCTG	589
R3083_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCTTTTAGAAAGTTCCTGT	590
R3084_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCTTTTAGAAAGTTCCTGTG	591
R3085_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTTTTAGAAAGTTCCTGTGA	592
R3086_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTAGAAAGTTCCTGTGATGTC	593
R3136_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGAAAGTTCCTGTGATGTCA	594
R3137_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAAGTTCCTGTGATGTCAA	595
R3138_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACATCACAGGAACTTTCTAA	596
R3139_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGTGATGTCAAGCTGGTCG	597
R3140_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCGACCAGCTTGACATCACA	598
R3141_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCGACCAGCTTGACATCAC	599
R3142_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCTCGACCAGCTTGACATCA	600
R3143_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAAGCTTTTCTCGACCAGCT	601
R3144_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAAAGCTTTTCTCGACCAGC	602
R3145_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCTGTTTCAAAGCTTTTCTC	603
R3146_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAACAGGTAAGACAGGGGT	604
R3147_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAACAGGTAAGACAGGGGTC	605

TABLE J: CasΦ.32 gRNAs targeting human TRAC in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3040_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTGGATATCTGTGGGACAAGA	606
R3041_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCCCACAGATATCCAGAACC	607
R3042_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGAGTCTCTCAGCTGGTACAC	608

R3043_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAGAGTCTCTCAGCTGGTACA	609
R3044_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTCACTGGATTTAGAGTCTCT	610
R3045_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAGAATCAAAATCGGTGAATA	611
R3046_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGAGAATCAAAATCGGTGAAT	612
R3047_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CACCGATTTTGATTCTCAAAC	613
R3048_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTTGAGAATCAAAATCGGTG	614
R3049_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGTTTGAGAATCAAAATCGGT	615
R3050_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTGATTCTCAAACAAATGTGT	616
R3051_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGATTCTCAAACAAATGTGTC	617
R3052_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CATTCTCAAACAAATGTGTCA	618
R3053_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTGACACATTTGTTTGAGAAT	619
R3054_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTCAAACAAATGTGTCACAAA	620
R3055_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGTGACACATTTGTTTGAGAA	621
R3056_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCTTTGTGACACATTTGTTTG	622
R3057_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTGATGTGTATATCACAGACA	623
R3058_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTCTGTGATATACACATCAGA	624
R3059_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGTCTGTGATATACACATCAG	625
R3060_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTGTCTGTGATATACACATCA	626
R3061_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAAGTCCATAGACCTCATGTC	627
R3062_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCTCTTGAAGTCCATAGACCT	628
R3063_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAAGAGCAACAGTGCTGTGGC	629
R3064_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCTCCAGGCCACAGCACTGTT	630

R3065_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTGCTCCAGGCCACAGCACT	631
R3066_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGTTGCTCCAGGCCACAGCAC	632
R3067_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCACATGCAAAGTCAGATTTG	633
R3068_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGCACATGCAAAGTCAGATTT	634
R3069_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CGCATGTGCAAACGCCTTCAA	635
R3070_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAAGGCGTTTGCACATGCAAA	636
R3071_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCATGTGCAAACGCCTTCAAC	637
R3072_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTGAAGGCGTTTGCACATGC	638
R3073_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CAACAACAGCATTATTCCAGA	639
R3074_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTGGAATAATGCTGTTGTTGA	640
R3075_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTCCAGAAGACACCTTCTTC	641
R3076_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCAGAAGACACCTTCTTCCCC	642
R3077_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCCTGGGCTGGGGAAGAAGGT	643
R3078_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTCCCCAGCCCAGGTAAGGG	644
R3079_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCCCAGCCCAGGTAAGGGCAG	645
R3080_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTAAAAGGAAAAACAGACATT	646
R3081_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCTAAAAGGAAAAACAGACAT	647
R3082_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTTCCTTTTAGAAAGTTCCTG	648
R3083_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTCCTTTTAGAAAGTTCCTGT	649
R3084_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCCTTTTAGAAAGTTCCTGTG	650
R3085_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CCTTTTAGAAAGTTCCTGTGA	651
R3086_Cas Phi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGA CTAGAAAGTTCCTGTGATGTC	652

R3136_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGAAAGTTCCTGTGATGTCA	653
R3137_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGAAAGTTCCTGTGATGTCAA	654
R3138_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACATCACAGGAACTTTCTAA	655
R3139_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGTGATGTCAAGCTGGTCG	656
R3140_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCGACCAGCTTGACATCACA	657
R3141_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTCGACCAGCTTGACATCAC	658
R3142_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCTCGACCAGCTTGACATCA	659
R3143_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAAAGCTTTTCTCGACCAGCT	660
R3144_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCAAAGCTTTTCTCGACCAGC	661
R3145_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCTGTTTCAAAGCTTTTCTC	662
R3146_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGAAACAGGTAAGACAGGGGT	663
R3147_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAAACAGGTAAGACAGGGGTC	664

TABLE K: CasΦ.12 gRNAs targeting human B2M in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3087_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAATATAAGTGGAGGCGTCGC	665
R3088_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATATAAGTGGAGGCGTCGCG	666
R3089_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGGAATGCCCGCCAGCGCGA	667
R3090_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGAAGCTGACAGCATTCGG	668
R3091_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGGCCGAGATGTCTCGCTCC	669
R3092_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCTGTGCTCGCGCTACTCTC	670
R3093_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGGCCTGGAGGCTATCCAG	671
R3094_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGCCTGGAGGCTATCCAGC	672

R3095_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATGTGTCTTTTCCCGATATT	673
R3096_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCCGATATTCCTCAGGTAC	674
R3097_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCCGATATTCCTCAGGTACT	675
R3098_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCGATATTCCTCAGGTACTC	676
R3099_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAGTACCTGAGGAATATCGG	677
R3100_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGAGTACCTGAGGAATATCG	678
R3101_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCAGGTACTCCAAAGATTC	679
R3102_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGGTTTACTCACGTCATCCA	680
R3103_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACTCACGTCATCCAGCAGAG	681
R3104_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCACGTCATCCAGCAGAGA	682
R3105_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCTGCTGGATGACGTGAGTA	683
R3106_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCATTCTCTGCTGGATGACGT	684
R3107_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCATTCTCTGCTGGATGACG	685
R3108_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACTTTCCATTCTCTGCTGGA	686
R3109_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGACTTTCCATTCTCTGCTGG	687
R3110_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGGAAATTTGACTTTCCATT	688
R3111_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCTGAATTGCTATGTGTCTG	689
R3112_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGAATTGCTATGTGTCTGG	690
R3113_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTATGTGTCTGGGTTTCATC	691
R3114_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAATGTCGGATGGATGAAACC	692
R3115_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCATCCATCCGACATTGAAGT	693
R3116_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATCCATCCGACATTGAAGTT	694

R3117_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGTAAGTCAACTTCAATGTC	695
R3118_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCAGTAAGTCAACTTCAAT	696
R3119_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAGTTGACTTACTGAAGAAT	697
R3120_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACTTACTGAAGAATGGAGAG	698
R3121_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCTCTCCATTCTTCAGTAAG	699
R3122_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGAAGAATGGAGAGAGAAT	700
R3123_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAATTCTCTCTCCATTCTTCA	701
R3124_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAATTCTCTCTCCATTCTTC	702
R3125_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCAATTCTCTCTCCATTCTT	703
R3126_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCAATTCTCTCTCCATTCT	704
R3127_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAAAAGTGGAGCATTCAGAC	705
R3128_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGAAAGACAAGTCTGAATG	706
R3129_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGACTTGTCTTTCAGCAAGG	707
R3130_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCTTTCAGCAAGGACTGGTC	708
R3131_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAGCAAGGACTGGTCTTTCT	709
R3132_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGCAAGGACTGGTCTTTCTA	710
R3133_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTATCTCTTGTACTACACTG	711
R3134_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTATCTCTTGTACTACACTGA	712
R3135_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGTGTAGTACAAGAGATAGA	713
R3148_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTACTACACTGAATTCACCCC	714
R3149_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGTGGGGGTGAATTCAGTGT	715
R3150_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAGTGGGGGTGAATTCAGTG	716

R3151_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCAGTGGGGGTGAATTCAGT	717
R3152_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCAGTGGGGGTGAATTCAG	718
R3153_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACCCCCACTGAAAAAGATGA	719
R3154_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACACGGCAGGCATACTCATC	720
R3155_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGCTGTGACAAAGTCACATG	721
R3156_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTCACAGCCCAAGATAGTTA	722
R3157_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCACAGCCCAAGATAGTTAA	723
R3158_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACTATCTTGGGCTGTGACAA	724
R3159_CasPhi12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCCCACTTAACTATCTTGGG	725

TABLE L: CasΦ.32 gRNAs targeting human B2M in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3087_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAATATAAGTGGAGGCGTCGC	726
R3088_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACATATAAGTGGAGGCGTCGCG	727
R3089_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGGAATGCCCGCCAGCGCGA	728
R3090_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGAAGCTGACAGCATTCGG	729
R3091_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGGGCCGAGATGTCTCGCTCC	730
R3092_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGCTGTGCTCGCGCTACTCTC	731
R3093_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGGCCTGGAGGCTATCCAG	732
R3094_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTGGCCTGGAGGCTATCCAGC	733
R3095_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACATGTGTCTTTTCCCGATATT	734
R3096_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCCCGATATTCCTCAGGTAC	735
R3097_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCCGATATTCCTCAGGTACT	736

R3098_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCGATATTCCTCAGGTACTC	737
R3099_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGAGTACCTGAGGAATATCGG	738
R3100_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGGAGTACCTGAGGAATATCG	739
R3101_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTCAGGTACTCCAAAGATTC	740
R3102_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGGTTTACTCACGTCATCCA	741
R3103_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACTCACGTCATCCAGCAGAG	742
R3104_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTCACGTCATCCAGCAGAGA	743
R3105_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCTGCTGGATGACGTGAGTA	744
R3106_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCATTCTCTGCTGGATGACGT	745
R3107_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCATTCTCTGCTGGATGACG	746
R3108_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACTTTCCATTCTCTGCTGGA	747
R3109_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGACTTTCCATTCTCTGCTGG	748
R3110_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGGAAATTTGACTTTCCATT	749
R3111_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCTGAATTGCTATGTGTCTG	750
R3112_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGAATTGCTATGTGTCTGG	751
R3113_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTATGTGTCTGGGTTTCATC	752
R3114_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAATGTCGGATGGATGAAACC	753
R3115_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCATCCATCCGACATTGAAGT	754
R3116_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACATCCATCCGACATTGAAGTT	755
R3117_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGTAAGTCAACTTCAATGTC	756
R3118_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTTCAGTAAGTCAACTTCAAT	757
R3119_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAAGTTGACTTACTGAAGAAT	758

R3120_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACTTACTGAAGAATGGAGAG	759
R3121_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCTCTCCATTCTTCAGTAAG	760
R3122_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGAAGAATGGAGAGAGAAT	761
R3123_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAATTCTCTCTCCATTCTTCA	762
R3124_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCAATTCTCTCTCCATTCTTC	763
R3125_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCAATTCTCTCTCCATTCTT	764
R3126_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTTCAATTCTCTCTCCATTCT	765
R3127_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAAAAAGTGGAGCATTCAGAC	766
R3128_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTGAAAGACAAGTCTGAATG	767
R3129_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGACTTGTCTTTCAGCAAGG	768
R3130_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCTTTCAGCAAGGACTGGTC	769
R3131_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCAGCAAGGACTGGTCTTTCT	770
R3132_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGCAAGGACTGGTCTTTCTA	771
R3133_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCTATCTCTTGTACTACACTG	772
R3134_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTATCTCTTGTACTACACTGA	773
R3135_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGTGTAGTACAAGAGATAGA	774
R3148_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTACTACACTGAATTCACCCC	775
R3149_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACAGTGGGGGTGAATTCAGTGT	776
R3150_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCAGTGGGGGTGAATTCAGTG	777
R3151_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCAGTGGGGGTGAATTCAGT	778
R3152_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTTCAGTGGGGGTGAATTCAG	779
R3153_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACCCCCACTGAAAAAGATGA	780

R3154_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACACGGCAGGCATACTCATC	781
R3155_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGGCTGTGACAAAGTCACATG	782
R3156_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACGTCACAGCCCAAGATAGTTA	783
R3157_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACTCACAGCCCAAGATAGTTAA	784
R3158_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACACTATCTTGGGCTGTGACAA	785
R3159_CasPhi32	GCTGGGGACCGATCCTGATTGCTCGCTGCGGCGAGACCCCCACTTAACTATCTTGGG	786

TABLE M: CasΦ.12 gRNAs targeting human PD1 in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3')	SEQ ID NO
R2921_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUUCCGCUCACCUCCGCCU	787
R2922_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUUCCGCUCACCUCCGCCU	788
R2923_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGCUCACCUCCGCCUGAGCA	789
R2924_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCACUGCUCAGGCGGAGGU	790
R2925_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUAGCACCGCCCAGACGACUG	791
R2926_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCAUGCAGAUCCCACAGG	792
R2927_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACAGGCGCCCUGGCCAGUC	793
R2928_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGGGCGGUGCUACAACUG	794
R2929_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCAUGCCUGGAGCAGCCCCA	795
R2930_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUAGCACCGCCCAGACGACUG	796
R2931_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGCCGCCAGCCCAGUUGUA	797
R2932_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUUCCGCUCACCUCCGCCUG	798
R2933_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGGCCUGUCUGGGGAGUC	799
R2934_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCCAGCCCUGCUCGUGGU	800

R2935_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGUCACCACGAGCAGGGCUG	801
R2936_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCCUUCGGUCACCACGAG	802
R2937_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGAAGCUGCAGGUGAAGGU	803
R2938_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACCUGCAGCUUCUCCAACAC	804
R2939_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAACACAUCGGAGAGCUU	805
R2940_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCACGAAGCUCUCCGAUGUG	806
R2941_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCACGAAGCUCUCCGAUGU	807
R2942_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGCUAAACUGGUACCGCAU	808
R2943_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGGGGCUCAUGCGGUACCA	809
R2944_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCGUCUGGUUGCUGGGGCU	810
R2945_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCGAGGACCGCAGCCAGCC	811
R2946_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGUGACACGGAAGCGGCAGU	812
R2947_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGUGUCACACAACUGCCCAA	813
R2948_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCAGUUGUGUGACACGGAA	814
R2949_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACAUGAGCGUGGUCAGGGC	815
R2950_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGCCGGGCCCUGACCACGCU	816
R2951_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGGCCAGGGAGAUGGCCCC	817
R2952_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUCUGCGCCUUGGGGGCCAG	818
R2953_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAUCUGCGCCUUGGGGGCCA	819
R2954_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGACAGGCCCUGGAACCC	820
R2955_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGCCCUGCUCGUGGUGAC	821
R2956_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUCUGGAAGGGCACAAAGG	822

R2957_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGCCCUUCCAGAGAGAAGG	823
R2958_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCCCUUCCAGAGAGAAGGG	824
R2959_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCCCUUCUCUCUGGAAGGG	825
R2960_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGAGAGAAGGGCAGAAGUG	826
R2961_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAACUGGCCGGCUGGCCUGG	827
R2962_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAACUGGCCGGCUGGCCUG	828
R2963_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAAACCCUGGUGGUUGGUGU	829
R2964_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGUCGUGGGCGGCCUGCUG	830
R2965_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUCGUGCGGCCCGGGAGCA	831
R2966_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCUGCAGAGAAACACACU	832
R2967_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCUGCAGGGACAAUAGGAG	833
R2968_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGCAGGGACAAUAGGAGC	834
R2969_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCCUCAAAGAAGGAGGACC	835
R2970_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCUCAAAGAAGGAGGACCC	836
R2971_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGUGGACUAUGGGGAGCU	837
R2972_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUCGCCACUGGAAAUCCAG	838
R2973_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGUGGCGAGAGAAGACCC	839
R2974_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGUGGCGAGAGAAGACCCC	840
R2975_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGCUAGGAAAGACAAUGGUG	841
R2976_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUUUCCUAGCGGAAUGGGC	842
R2977_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUAGCGGAAUGGGCACCUC	843
R2978_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUAGCGGAAUGGGCACCUCA	844

R2979_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCCCUCUGACCGGCUUCCU	845
R2980_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUUGGCCACCAGUGUUCUGC	846
R2981_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCACCAGUGUUCUGCAGAC	847
R2982_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCAGACCCUCCACCAUGAG	848
R2983_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCUGAGGAAAUGCGCUGAC	849
R2984_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUCAGGAGAAGCAGGCAGG	850
R2985_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCAGGAGAAGCAGGCAGGG	851
R2986_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGCCGUCCAGGGGCUGAG	852
R2987_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGACAUGAGUCCUGUGGUGG	853
R2988_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGUCCUGCCAGCACAGAGC	854
R2989_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGAGCUGGACGCAGGCAG	855
R2990_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCCCCGGGCCGCAGGCAGC	856
R2991_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCAGGAGGCUCCGGGGCG	857
R2992_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGGCUGGUUGGAGAUGGCC	858
R2993_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGAUGGCCUUGGAGCAGCC	859
R2994_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUGCUCCAAGGCCAUCUCC	860
R2995_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGCAGCCAAGGUGCCCCUG	861
R2996_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGAUGCCACUGCCAGGGGC	862
R2997_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGGGAUGCCACUGCCAGGGG	863
R2998_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCCCUGCGUCCAGGGCGUU	864
R2999_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGCUCCCUGCAGGCCUAG	865
R3000_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUAGGCCUGCAGGGAGCAG	866

R3001_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUGAAACUUCUCUAGGCCU	867
R3002_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGACCUUCCCUGAAACUUCU	868
R3003_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGGAAGGUCAGAAGAGCU	869
R3004_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGAAGGUCAGAAGAGCUC	870
R3005_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCCCUGCCCACCACAGCC	871
R3006_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUGCCCUGCCCACCACAGC	872
R3007_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACACAUGCCCAGGCAGCACC	873
R3008_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACAUGCCCAGGCAGCACCU	874
R3009_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUGCCCCACAAAGGGCCUG	875
R3010_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGGGGCAGGGAAGCUGAGG	876
R3011_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGGGCAGGGAAGCUGAGGC	877
R3012_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCCUCAGCUUCCCUGCCC	878
R3013_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGCCCAGCCAGCACUCUG	879
R3014_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCCCAGCCAGCACUCUGG	880
R3015_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACCCCAGCCCCUCACACCA	881
R3016_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGACCGUAGGAUGUCCCUCU	882

TABLE N: CasΦ.32 gRNAs targeting human PD1 in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3')	SEQ ID NO
R2921_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUUCCGCUCACCUCCGCCU	883
R2922_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUUCCGCUCACCUCCGCCU	884
R2923_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCGCUCACCUCCGCCUGAGCA	885
R2924_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCACUGCUCAGGCGGAGGU	886

R2925_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUAGCACCGCCCAGACGACUG	887
R2926_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGCAUGCAGAUCCCACAGG	888
R2927_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCACAGGCGCCCUGGCCAGUC	889
R2928_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUGGGCGGUGCUACAACUG	890
R2929_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGCAUGCCUGGAGCAGCCCCA	891
R2930_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUAGCACCGCCCAGACGACUG	892
R2931_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGGCCGCCAGCCCAGUUGUA	893
R2932_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUUCCGCUCACCUCCGCCUG	894
R2933_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGGGCCUGUCUGGGGAGUC	895
R2934_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCCCAGCCCUGCUCGUGGU	896
R2935_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGUCACCACGAGCAGGGCUG	897
R2936_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCCCUUCGGUCACCACGAG	898
R2937_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGAGAAGCUGCAGGUGAAGGU	899
R2938_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACACCUGCAGCUUCUCCAACAC	900
R2939_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCAACACAUCGGAGAGCUU	901
R2940_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGCACGAAGCUCUCCGAUGUG	902
R2941_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGCACGAAGCUCUCCGAUGU	903
R2942_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGUGCUAAACUGGUACCGCAU	904
R2943_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUGGGGCUCAUGCGGUACCA	905
R2944_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCGUCUGGUUGCUGGGGCU	906
R2945_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCCGAGGACCGCAGCCAGCC	907
R2946_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGUGACACGGAAGCGGCAGU	908

R2947_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCGUGUCACACAACUGCCCAA	909
R2948_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGCAGUUGUGUGACACGGAA	910
R2949_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCACAUGAGCGUGGUCAGGGC	911
R2950_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCGCCGGGCCCUGACCACGCU	912
R2951_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGGCCAGGGAGAUGGCCCC	913
R2952_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAUCUGCGCCUUGGGGGCCAG	914
R2953_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGAUCUGCGCCUUGGGGGCCA	915
R2954_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCAGACAGGCCCUGGAACCC	916
R2955_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCAGCCCUGCUCGUGGUGAC	917
R2956_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUCUGGAAGGGCACAAAGG	918
R2957_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGUGCCCUUCCAGAGAGAAGG	919
R2958_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGCCCUUCCAGAGAGAAGGG	920
R2959_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGCCCUUCUCUCUGGAAGGG	921
R2960_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGAGAGAAGGGCAGAAGUG	922
R2961_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGAACUGGCCGGCUGGCCUGG	923
R2962_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGAACUGGCCGGCUGGCCUG	924
R2963_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAAACCCUGGUGGUUGGUGU	925
R2964_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGUGUCGUGGGCGGCCUGCUG	926
R2965_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUCGUGCGGCCCGGGAGCA	927
R2966_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCCUGCAGAGAAACACACU	928
R2967_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUCUGCAGGGACAAUAGGAG	929
R2968_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUGCAGGGACAAUAGGAGC	930

R2969_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUCCUCAAAGAAGGAGGACC	931
R2970_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCUCAAAGAAGGAGGACCC	932
R2971_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUGUGGACUAUGGGGAGCU	933
R2972_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUCGCCACUGGAAAUCCAG	934
R2973_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCAGUGGCGAGAGAAGACCC	935
R2974_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGUGGCGAGAGAAGACCCC	936
R2975_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCGCUAGGAAAGACAAUGGUG	937
R2976_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUUUCCUAGCGGAAUGGGC	938
R2977_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUAGCGGAAUGGGCACCUC	939
R2978_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUAGCGGAAUGGGCACCUCA	940
R2979_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGCCCCUCUGACCGGCUUCCU	941
R2980_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUUGGCCACCAGUGUUCUGC	942
R2981_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGCCACCAGUGUUCUGCAGAC	943
R2982_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGCAGACCCUCCACCAUGAG	944
R2983_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCUGAGGAAAUGCGCUGAC	945
R2984_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUCAGGAGAAGCAGGCAGG	946
R2985_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUCAGGAGAAGCAGGCAGGG	947
R2986_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGGCCGUCCAGGGGCUGAG	948
R2987_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGACAUGAGUCCUGUGGUGG	949
R2988_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGUCCUGCCAGCACAGAGC	950
R2989_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGGAGCUGGACGCAGGCAG	951
R2990_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGCCCCGGGCCGCAGGCAGC	952

R2991_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGCAGGAGGCUCCGGGGCG	953
R2992_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGGCUGGUUGGAGAUGGCC	954
R2993_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGAGAUGGCCUUGGAGCAGCC	955
R2994_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGCUGCUCCAAGGCCAUCUCC	956
R2995_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGAGCAGCCAAGGUGCCCCUG	957
R2996_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGAUGCCACUGCCAGGGGC	958
R2997_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCGGGAUGCCACUGCCAGGGG	959
R2998_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGCCCUGCGUCCAGGGCGUU	960
R2999_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUGCUCCCUGCAGGCCUAG	961
R3000_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCUAGGCCUGCAGGGAGCAG	962
R3001_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUGAAACUUCUCUAGGCCU	963
R3002_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGACCUUCCCUGAAACUUCU	964
R3003_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGGGAAGGUCAGAAGAGCU	965
R3004_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGGAAGGUCAGAAGAGCUC	966
R3005_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUGCCCUGCCCACCACAGCC	967
R3006_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUGCCCUGCCCACCACAGC	968
R3007_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACACACAUGCCCAGGCAGCACC	969
R3008_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCACAUGCCCAGGCAGCACCU	970
R3009_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUGCCCCACAAAGGGCCUG	971
R3010_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGUGGGGCAGGGAAGCUGAGG	972
R3011_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGGGGCAGGGAAGCUGAGGC	973
R3012_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUGCCUCAGCUUCCCUGCCC	974

R3013_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCAGGCCCAGCCAGCACUCUG	975
R3014_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGGCCCAGCCAGCACUCUGG	976
R3015_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCACCCCAGCCCCUCACACCA	977
R3016_CasPhi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGACCGUAGGAUGUCCCUCU	978

TABLE O: CasΦ.12 gRNAs targeting human CIITA

Name	Repeat+spacer sequence RNA Sequence (5' --> 3')	SEQ ID NO
R4503_CasPhi12_C2TA_T1.1	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUACACAAUGCGUUGCCUGG	979
R4504_CasPhi12_C2TA_T1.2	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGCUCUGACAGGUAGGACC	980
R4505_CasPhi12_C2TA_T1.3	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGUAGGAAUCCCAGCCAGGC	981
R4506_CasPhi12_C2TA_T1.8	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUGGCUCCACGCCCUGCUG	982
R4507_CasPhi12_C2TA_T1.9	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGAAGCUGAGGGCACGAGG	983
R4508_CasPhi12_C2TA_T2.1	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACAGCGAUGCUGACCCCCUG	984
R4509_CasPhi12_C2TA_T2.2	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUAACAGCGAUGCUGACCCC	985
R4510_CasPhi12_C2TA_T2.3	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUAUGACCAGAUGGACCUGGC	986
R4511_CasPhi12_C2TA_T2.4	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGCCCCUAGAAGGUGGCUA	987
R4512_CasPhi12_C2TA_T2.5	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUAGGGGCCCCAACUCCAUGG	988
R4513_CasPhi12_C2TA_T2.6	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGAAGCUCCAGGUAGCCACC	989
R4514_CasPhi12_C2TA_T2.7	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAGCCAGGUCCAUCUGGU	990
R4515_CasPhi12_C2TA_T2.8	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCUCCAGCCAGGUCCAUCU	991
R5200_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCAGGCUGUUGUGUGACAU	2112
R5201_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAUGUCACACAACAGCCUGC	2113
R5202_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGUGACAUGGAAGGUGAUGA	2114

R5203_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUCACCUUCCAUGUCACACA	2115
R5204_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCAUAAGCCUCCCUGGUCUC	2116
R5205_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGACUCCCAGCUGGAGGG	2117
R5206_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCAGGCCCUCCAGCUGGGA	2118
R5207_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCUGGCAUCUCCAUACUCU	2119
R5208_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCCCAACUUCUGCUGGCAU	2120
R5209_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCCCAACUUCUGCUGGCA	2121
R5210_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGCCCAACUUCUGCUGGC	2122
R5211_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGACUUUUCUGCCCAACUUC	2123
R5212_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGACUUUUCUGCCCAACUU	2124
R5213_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGACUUUUCUGCCCAACU	2125
R5214_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGAGGAGCUUCCGGCAGA	2126
R5215_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGUCUGCCGGAAGCUCCUC	2127
R5216_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGGCAGACCUGAAGCACUGG	2128
R5217_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGUGCUUCAGGUCUGCCGG	2129
R5218_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAACAGCGCAGGCAGUGGCAG	2130
R5219_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAACCAGGAGCCAGCCUCCGG	2131
R5220_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAGGCGCAUCUGGCCGGA	2132
R5221_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCCAGGCGCAUCUGGCCGG	2133
R5222_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUCCAGGCGCAUCUGGCCG	2134
R5223_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCCAGUUCCUCGUUGAGCU	2135
R5224_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAGUUCCUCGUUGAGCUG	2136
R5225_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCAGCUCAACGAGGAACU	2137
R5226_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCGUUGAGCUGCCUGAAUC	2138
R5227_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCUGCCUGAAUCUCCCUGA	2139

R5228_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUCCCCACCAUCUCCACUCU	2140
R5229_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCCACCAUCUCCACUCUG	2141
R5230_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGAGCCCAUGGGGCAGAG	2142
R5231_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCAGAGCCCAUGGGGCAGA	2143
R5232_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCCUCAGAGAUUUGCCAG	2144
R5233_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAGGCCGUGGACAGUGAAU	2145
R5234_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACUGUCCACGGCCUCCCAAC	2146
R5235_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUCCAUCAGCCACUGACCU	2147
R5236_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCAUGCUGGGCAGGUCAG	2148
R5237_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCGGGAGGUCAGGGCAGGU	2149
R5238_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUCGGGAGGUCAGGGCAGG	2150
R5239_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGACCUCUCCAGCUGCCGG	2151
R5240_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUGGAGACCUCUCCAGCUGC	2152
R5241_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAAGCUUGUUGGAGACCUCU	2153
R5242_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAAGCUUGUUGGAGACCUC	2154
R5243_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGAAGCUUGUUGGAGACCU	2155
R5244_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUACCGCUCACUGCAGGACAC	2156
R5245_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCUGCUCCUCUCCAGCCU	2157
R5246_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCGCUCCAGGCUCUUGCUGC	2158
R5247_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCCCAGUCCGGGGUGGCCA	2159
R5248_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCCAGCUGCCGUUCUGCCC	2160
R5249_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCAGCCAACAGCACCUCAGC	2161
R5250_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUGCCAAGGAGCACCGGCG	2162
R5251_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAGCACAGCAAUCACUCG	2163
R5252_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCCAGCACAGCAAUCACUC	2164

R5253_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGUGCUGGGCAAAGCUGGU	2165
R5254_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCUGACCAGCUUUGCCCAG	2166
R5255_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCUGGGGCAGUGAGCCGGG	2167
R5256_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGCCGGCUUCCCCAGUACG	2168
R5257_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAGUACGACUUUGUCUUC	2169
R5258_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUCUUCUCUGUCCCCUGCCA	2170
R5259_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUUCUCUGUCCCCUGCCAU	2171
R5260_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGUCCCCUGCCAUUGCUU	2172
R5261_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAGCAAUGGCAGGGGACAGA	2173
R5262_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUUGAACCGUCCGGGGGAUG	2174
R5263_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAACCGUCCGGGGGAUGCCUA	2175
R5264_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCUGGGCCCACAGCCACU	2176
R5265_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAGAUGUGGCUGAAAACCUC	2177
R5266_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCAGCCACAUCUUGAAGAGA	2178
R5267_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCCACAUCUUGAAGAGAC	2179
R5268_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCCACAUCUUGAAGAGACC	2180
R5269_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAGAGACCUGACCGCGUUCU	2181
R5270_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCUCAUCCUAGACGGCUUC	2182
R5271_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCUCCUCGAAGCCGUCUA	2183
R5272_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGCUUCCAGCUCCUCGAAGC	2184
R5273_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGGAGCUGGAAGCGCAAGA	2185
R5274_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCACAGCACGUGCGGACC	2186
R5275_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGAAAAGGCCGGCCAGCAG	2187
R5276_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCUGGAAAAGGCCGGCCAG	2188
R5277_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAGAAGAAGCUGCUCCGA	2189

R5278_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGAAGAAGCUGCUCCGAG	2190
R5279_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGAAGAAGCUGCUCCGAGG	2191
R5280_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACCCUCCUCCUCACAGCCC	2192
R5281_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCAGGCUCUGGACCAGGCG	2193
R5282_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGCUGUCCGGCUUCUCCAU	2194
R5283_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCUGUCCGGCUUCUCCAUG	2195
R5284_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAUGGAGCAGGCCCAGGC	2196
R5285_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGAGCUCAGGGAUGACAGA	2197
R5286_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGAGCUCAGGGAUGACAGAG	2198
R5287_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGCUCUGUCAUCCCUGAGC	2199
R5288_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCUCAGUCACAGCCACAGC	2200
R5289_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCAGUCACAGCCACAGCCCU	2201
R5290_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGCCGGGCAGUGUGCCAGC	2202
R5291_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCCGGGCAGUGUGCCAGCU	2203
R5292_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCGUCCUCCCCAAGCUCCAG	2204
R5293_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGAGGACGCCAAGCUGCCC	2205
R5294_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCAGCUCUGCCAGGGCCCC	2206
R5295_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUGUCUGCGGCCCAGCUCCC	2207
R5392_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAUGUCUGCGGCCCAGCUCC	2208
R5393_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAUCCGCAGACGUGAGGAC	2209
R5394_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCAUCGCCCAGGUCCUCAC	2210
R5395_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCCAUCGCCCAGGUCCUCA	2211
R5396_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGACUAAGCCUUUGGCCAUCG	2212
R5397_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUCCAACACCCACCGCGGGC	2213
R5398_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGAGGAAGCUGGGGAAGG	2214

R5399_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAGCUUCCUCCUGCAAUG	2215
R5400_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCCUGCAAUGCUUCCUGGG	2216
R5401_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGGGGGCCCUGUGGCUGGC	2217
R5402_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCACUCAGAGCCAGCCACA	2218
R5403_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGCCACUCAGAGCCAGCCAC	2219
R5404_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUUUCGCCACUCAGAGCCAG	2220
R5405_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCUUGAUUUCGCCACUCAG	2221
R5406_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGUCAAUGCUAGGUACUGC	2222
R5407_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUUGGGGUCAAUGCUAGGUA	2223
R5408_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCCUUGGGGUCAAUGCUAG	2224
R5409_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACCCCAAGGAAGAAGAGGCC	2225
R5410_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCAUAGGGCCUCUUCUUCCU	2226
R5411_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGGCUGGGCUGAUCUUCCA	2227
R5412_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGCUGGGCUGAUCUUCCAG	2228
R5413_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCCUCCCGCCCGCUGCCU	2229
R5414_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGUCCACCGAGGCAGCCGC	2230
R5415_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCUUCCUGUCCACCGAGGC	2231
R5416_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGUACCUCGCAAGCACCUU	2232
R5417_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGAGGUACCUGAAGCGGCUG	2233
R5418_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCCUCCUCGGCCUCGUGG	2234
R5419_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGCAGCACGUGGUACAGGAG	2235
R5420_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCAGCACGUGGUACAGGAGC	2236
R5421_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCUGGGCACCCGCCUCACGC	2237
R5422_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGGGCACCCGCCUCACGCC	2238
R5423_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGGCACCCGCCUCACGCCU	2239

R5424_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAGUACAUGUGCAUCAGG	2240
R5425_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCCGCCGCCUCCAAGGCCU	2241
R5426_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGGCGGCGGGCCAAGACUU	2242
R5427_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCUGGACCUCCGCAGCAC	2243
R5428_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCCCUCUGGAUUGGGGAGC	2244
R5429_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCCUCUGGAUUGGGGAGCC	2245
R5430_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGAGCCUCGUGGGACUCAG	2246
R5431_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUCUCCCCAUGCUGCUGCAG	2247
R5432_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCUCUGCUGCCUGAAGUAG	2248
R5433_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGCAGCAGAGGAGAAGUUC	2249
R5434_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAAGGCUCGAUGGUGAACUU	2250
R5435_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAAAGGCUCGAUGGUGAACU	2251
R5436_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACCAUCGAGCCUUUCAAAGC	2252
R5437_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUUUGAAAGGCUCGAUGGU	2253
R5438_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGACUUGGCUUUGAAAGG	2254
R5439_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAAAGCCAAGUCCCUGAAGG	2255
R5440_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAAGCCAAGUCCCUGAAGGA	2256
R5441_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACAUCCUUCAGGGACUUGG	2257
R5442_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGGUCUUCCACAUCCUUC	2258
R5443_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAGGUCUUCCACAUCCUU	2259
R5444_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCGGAAGACACAGCUGGGG	2260
R5445_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGUCCCGAACAGCAGGGAGC	2261
R5446_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGUCCCGAACAGCAGGGAG	2262
R5447_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUUAGGUCCCGAACAGCAGG	2263
R5448_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUUUAGGUCCCGAACAGCAG	2264

R5449_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGACCUAAAGAAACUGGAG	2265
R5450_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGAAAGCCUGGGGGCCUGA	2266
R5451_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGGAAAGCCUGGGGGCCUG	2267
R5452_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCCAAACUGGUGCGGAUCC	2268
R5453_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCAAACUGGUGCGGAUCCU	2269
R5454_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCUCACUCAGCGCAUCCAG	2270
R5455_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCUGGGGGAAGGUGGCUGA	2271
R5456_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCCAGCUGAAGUCCUUGGA	2272
R5457_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAAGGACUUCAGCUGGGGGA	2273
R5458_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAAGGACUUCAGCUGGGGG	2274
R5459_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGUUUCCAAGGACUUCAG	2275
R5460_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUAGGCACCCAGGUCAGUGAU	2276
R5461_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUAGGCACCCAGGUCAGUGA	2277
R5462_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUCGCUGCAUCCCUGCUCA	2278
R5463_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCUGAGCAGGGAUGCAGCG	2279
R5464_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUACAAUAACUGCAUCUGCGA	2280
R5465_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUCGUGUGCUUCCGGACAU	2281
R5466_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGGACAUGGUGUCCCUCCGG	2282
R5467_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACGGCUGCCGGGGCCCAGCA	2283
R5468_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAGGUGUCCUCAUGUGGAG	2284
R5469_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGGACACUGAAUGGGAUGG	2285
R5470_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGUGUCCAGGAACACCUGCA	2286
R5471_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGGUGUUCCUGGACACUGA	2287
R5472_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUGCAGGUGUUCCUGGACAC	2288
R5473_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACGGAUCAGCCUGAGAUGAU	2289

TABLE P: CasΦ.32 gRNAs targeting human CIITA

Name	Repeat+spacer sequence RNA Sequence (5' --> 3')	SEQ ID NO
R4503_CasPhi32_C2TA_T1.1	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCUACACAAUGCGUUGCCUGG	992
R4504_CasPhi32_C2TA_T1.2	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGCUCUGACAGGUAGGACC	993
R4505_CasPhi32_C2TA_T1.3	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUGUAGGAAUCCCAGCCAGGC	994
R4506_CasPhi32_C2TA_T1.8	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACCCUGGCUCCACGCCCUGCUG	995
R4507_CasPhi32_C2TA_T1.9	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGAAGCUGAGGGCACGAGG	996
R4508_CasPhi32_C2TA_T2.1	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACACAGCGAUGCUGACCCCCUG	997
R4509_CasPhi32_C2TA_T2.2	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUUAACAGCGAUGCUGACCCC	998
R4510_CasPhi32_C2TA_T2.3	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUAUGACCAGAUGGACCUGGC	999
R4511_CasPhi32_C2TA_T2.4	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACGGGCCCCUAGAAGGUGGCUA	1000
R4512_CasPhi32_C2TA_T2.5	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUAGGGGCCCCAACUCCAUGG	1001
R4513_CasPhi32_C2TA_T2.6	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACAGAAGCUCCAGGUAGCCACC	1002
R4514_CasPhi32_C2TA_T2.7	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUCCAGCCAGGUCCAUCUGGU	1003
R4515_CasPhi32_C2TA_T2.8	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAGACUUCUCCAGCCAGGUCCAUCU	1004

TABLE Q: CasΦ.12 gRNAs targeting mouse PCSK9

Name	Repeat+spacer sequence RNA Sequence (5' --> 3')	SEQ ID NO
R4238_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCGCUGUUGCCGCCGCUGCU	1005
R4239_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCGCCGCUGCUGCUGCUGUU	1006
R4240_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCUACUGUGCCCCACCGG	1007
R4241_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUAAUCUCCAUCCUCGUCCU	1008
R4242_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGAAGAGCUGAUGCUCGCCC	1009

R4243_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGAGCAACGGCGGAAGGUGGC	1010
R4244_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCUGGCAGCCUCCAGGCCUCC	1011
R4245_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUGGUGCUGAUGGAGGAGACC	1012
R4246_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAAUCUGUAGCCUCUGGGUCU	1013
R4247_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUUCAAUCUGUAGCCUCUGGG	1014
R4248_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGUUCAAUCUGUAGCCUCUGG	1015
R4249_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAACAAACUGCCCACCGCCUG	1016
R4250_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAUGACAUAGCCCCGGCGGGC	1017
R4251_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUACAUAUCUUUUAUGACCUC	1018
R4252_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUAUGACCUCUUCCCUGGCUU	1019
R4253_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAUGACCUCUUCCCUGGCUUC	1020
R4254_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUGACCUCUUCCCUGGCUUCU	1021
R4255_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACACCAAGAAGCCAGGGAAGAG	1022
R4256_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCUGGCUUCUUGGUGAAGAU	1023
R4257_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUUGGUGAAGAUGAGCAGUGA	1024
R4258_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGUGAAGAUGAGCAGUGACCU	1025
R4259_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCCCAUGUGGAGUACAUUGA	1026
R4260_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCUCAAUGUACUCCACAUGGG	1027
R4261_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAGGAAGACUCCUUUGUCUUC	1028
R4262_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGUCUUCGCCCAGAGCAUCCC	1029
R4263_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUCUUCGCCCAGAGCAUCCCA	1030
R4264_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGCCCAGAGCAUCCCAUGGAA	1031

R4265_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAUGGGAUGCUCUGGGCGAA	1032
R4266_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUCCAGGUUCCAUGGGAUG	1033
R4267_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCCAGCAUGGCACCAGACA	1034
R4268_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUCUGUCUGGUGCCAUGCUG	1035
R4269_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAUACCAGCAUCCAGGGUGC	1036
R4270_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGCAGGGUCACCAUCACC	1037
R4271_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAAGUCGGUGAUGGUGACCCU	1038
R4272_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAACAGCGUGCCGGAGGAGGA	1039
R4273_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCACACCAGCAUCCCGGCC	1040
R4274_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGCACACGCAGGCUGUGCAG	1041
R4275_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACACAGUUGAGCACACGCAGGC	1042
R4276_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCUUGACAGUUGAGCACACG	1043
R4277_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCUGACUCUUCCGAAUAAAC	1044
R4278_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUUCGGAAGAGUCAGCUAAU	1045
R4279_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUUCGGAAGAGUCAGCUAAUC	1046
R4280_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAAGAGUCAGCUAAUCCAG	1047
R4281_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCUGCCCCUGGCCGGUGGG	1048
R4282_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAUGCGGCUAUACCCACC	1049
R4283_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCAGCUGCUGCAACCAGCAC	1050
R4284_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCAGCUGGGAACUUCCGG	1051
R4285_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCGGGACGACGCCUGCCUCUA	1052
R4286_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGGCCCCGACUGUGAUGAC	1053

R4287_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCUUGGGGACUUUGGGGACU	1054
R4288_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGUCCCCAAAGUCCCCAAGGU	1055
R4289_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGGACUUUGGGGACUAAUUU	1056
R4290_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGGGACUAAUUUUGGACGCU	1057
R4291_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGGACUAAUUUUGGACGCUG	1058
R4292_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUGGACGCUGUGUGGAUCUCU	1059
R4293_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGACGCUGUGUGGAUCUCUU	1060
R4294_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGACGCUGUGUGGAUCUCUUU	1061
R4295_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCGGGGGCAAAGAGAUCCAC	1062
R4296_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGCCCCCGGGAAGGACAUCAU	1063
R4297_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCCCCGGGAAGGACAUCAUC	1064
R4298_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAUGUCACAGAGUGGGACCUC	1065
R4299_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUGGCUCGGAUGCUGAGCCGG	1066
R4300_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCCUGGCCGAGCUGCGGCAG	1067
R4301_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGUAGAGAAGUGGAUCAGCCU	1068
R4302_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGUAGAGAAGUGGAUCAGCC	1069
R4303_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUCUACCAAAGACGUCAUCAA	1070
R4304_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAUGACGUCUUUGGUAGAGAA	1071
R4305_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCUGAGGACCAGCAGGUGCU	1072
R4306_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGGGGUCAGCACCUGCUGGUC	1073
R4307_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACGAGUGGGCCCCGAGUGUGCC	1074
R4308_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUGGGGCACAGCGGGCUGUAG	1075

R4309_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCAGGAGCGGGAGGCGUCG	1076
R4310_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGACCUGCUGGCCUCCUAU	1077
R4311_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGGCCUUGCAGACCUGCUG	1078
R4312_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGGGUGAGGGUGUCUAUGC	1079
R4313_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGGUGAGGGUGUCUAUGCC	1080
R4314_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCACGGGGAACCAGGCAGCA	1081
R4315_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCCCGUGCCAACUGCAGCAUC	1082
R4316_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGAUGCUGCAGUUGGCACG	1083
R4317_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGGUGGCAGUGGACAUGGGU	1084
R4318_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCACUUCCCAAUGGAAGCUGC	1085
R4319_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAUUGGGAAGUGGAAGACCU	1086
R4320_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGAAGUGGAAGACCUUAGUG	1087
R4321_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGUGUCCGGAGGCAGCCUGCG	1088
R4322_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCCACCAGGCGGCCAGUGUC	1089
R4323_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCUGCUGCCAUGCCCCAGGGC	1090
R4324_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAGCCCUGGGGCAUGGCAGC	1091
R4325_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAUUCCAGCCCUGGGGCAUG	1092
R4326_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGCAUUCCAGCCCUGGGGCAU	1093
R4327_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUGCAUUCCAGCCCUGGGGCA	1094
R4328_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAUUUUGCAUUCCAGCCCUGG	1095
R4329_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACCAUCCAGUCAGGGUCCAUCC	1096
R4330_CasPhi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACUCCACGCUGUAGGCUCCCAG	1097

R4331_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCCACACACAGGUUGUCCACG	1098
R4332_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACUCCACUGGUCCUGUCUGCUC	1099
R4333_Cas Phi12	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACCUGAAGGCCGGCUCCGGCAG	1100

TABLE R: CasΦ.32 gRNAs targeting mouse PCSK9

Name	Repeat+spacer sequence RNA Sequence (5' --> 3')	SEQ ID NO
R4238_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCGCUGUUGCCGCCGCUGCU	1101
R4239_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCGCCGCUGCUGCUGCUGUU	1102
R4240_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUGCUACUGUGCCCCACCGG	1103
R4241_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUAAUCUCCAUCCUCGUCCU	1104
R4242_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGAAGAGCUGAUGCUCGCCC	1105
R4243_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGAGCAACGGCGGAAGGUGGC	1106
R4244_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUGGCAGCCUCCAGGCCUCC	1107
R4245_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGUGCUGAUGGAGGAGACC	1108
R4246_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAAUCUGUAGCCUCUGGGUCU	1109
R4247_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUUCAAUCUGUAGCCUCUGGG	1110
R4248_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUUCAAUCUGUAGCCUCUGG	1111
R4249_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAACAAACUGCCCACCGCCUG	1112
R4250_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUGACAUAGCCCCGGCGGGC	1113
R4251_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUACAUAUCUUUUAUGACCUC	1114
R4252_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUAUGACCUCUUCCCUGGCUU	1115
R4253_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUGACCUCUUCCCUGGCUUC	1116
R4254_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGACCUCUUCCCUGGCUUCU	1117

R4255_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACACCAAGAAGCCAGGGAAGAG	1118
R4256_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCUGGCUUCUUGGUGAAGAU	1119
R4257_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUUGGUGAAGAUGAGCAGUGA	1120
R4258_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUGAAGAUGAGCAGUGACCU	1121
R4259_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCCCAUGUGGAGUACAUUGA	1122
R4260_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUCAAUGUACUCCACAUGGG	1123
R4261_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAGGAAGACUCCUUUGUCUUC	1124
R4262_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUCUUCGCCCAGAGCAUCCC	1125
R4263_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCUUCGCCCAGAGCAUCCCA	1126
R4264_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCCCAGAGCAUCCCAUGGAA	1127
R4265_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAUGGGAUGCUCUGGGCGAA	1128
R4266_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCUCCAGGUUCCAUGGGAUG	1129
R4267_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCCCAGCAUGGCACCAGACA	1130
R4268_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUCUGUCUGGUGCCAUGCUG	1131
R4269_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGAUACCAGCAUCCAGGGUGC	1132
R4270_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAGGGCAGGGUCACCAUCACC	1133
R4271_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAAGUCGGUGAUGGUGACCCU	1134
R4272_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAACAGCGUGCCGGAGGAGGA	1135
R4273_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCCACACCAGCAUCCCGGCC	1136
R4274_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAGCACACGCAGGCUGUGCAG	1137
R4275_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACACAGUUGAGCACACGCAGGC	1138
R4276_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCUUGACAGUUGAGCACACG	1139

R4277_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCUGACUCUUCCGAAUAAAC	1140
R4278_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUUCGGAAGAGUCAGCUAAU	1141
R4279_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUUCGGAAGAGUCAGCUAAUC	1142
R4280_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGAAGAGUCAGCUAAUCCAG	1143
R4281_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGCUGCCCCUGGCCGGUGGG	1144
R4282_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAGGAUGCGGCUAUACCCACC	1145
R4283_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCAGCUGCUGCAACCAGCAC	1146
R4284_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAGCAGCUGGGAACUUCCGG	1147
R4285_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCGGGACGACGCCUGCCUCUA	1148
R4286_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUGGCCCCGACUGUGAUGAC	1149
R4287_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCUUGGGGACUUUGGGGACU	1150
R4288_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUCCCCAAAGUCCCCAAGGU	1151
R4289_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGACUUUGGGGACUAAUUU	1152
R4290_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGGACUAAUUUUGGACGCU	1153
R4291_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGACUAAUUUUGGACGCUG	1154
R4292_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGACGCUGUGUGGAUCUCU	1155
R4293_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGACGCUGUGUGGAUCUCUU	1156
R4294_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGACGCUGUGUGGAUCUCUUU	1157
R4295_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCGGGGGCAAAGAGAUCCAC	1158
R4296_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCCCCCGGGAAGGACAUCAU	1159
R4297_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCCCCGGGAAGGACAUCAUC	1160
R4298_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUGUCACAGAGUGGGACCUC	1161

R4299_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGCUCGGAUGCUGAGCCGG	1162
R4300_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCCUGGCCGAGCUGCGGCAG	1163
R4301_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUAGAGAAGUGGAUCAGCCU	1164
R4302_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGUAGAGAAGUGGAUCAGCC	1165
R4303_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCUACCAAAGACGUCAUCAA	1166
R4304_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUGACGUCUUUGGUAGAGAA	1167
R4305_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCUGAGGACCAGCAGGUGCU	1168
R4306_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGGUCAGCACCUGCUGGUC	1169
R4307_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGAGUGGGCCCCGAGUGUGCC	1170
R4308_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGGGCACAGCGGGCUGUAG	1171
R4309_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCCAGGAGCGGGAGGCGUCG	1172
R4310_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAGACCUGCUGGCCUCCUAU	1173
R4311_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAGGGCCUUGCAGACCUGCUG	1174
R4312_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGGGUGAGGGUGUCUAUGC	1175
R4313_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGGGUGAGGGUGUCUAUGCC	1176
R4314_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCACGGGGAACCAGGCAGCA	1177
R4315_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCCGUGCCAACUGCAGCAUC	1178
R4316_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGAUGCUGCAGUUGGCACG	1179
R4317_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGGUGGCAGUGGACAUGGGU	1180
R4318_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCACUUCCCAAUGGAAGCUGC	1181
R4319_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAUUGGGAAGUGGAAGACCU	1182
R4320_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGGAAGUGGAAGACCUUAGUG	1183

R4321_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGUGUCCGGAGGCAGCCUGCG	1184
R4322_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCCACCAGGCGGCCAGUGUC	1185
R4323_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUGCUGCCAUGCCCCAGGGC	1186
R4324_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAGCCCUGGGGCAUGGCAGC	1187
R4325_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAUUCCAGCCCUGGGGCAUG	1188
R4326_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACGCAUUCCAGCCCUGGGGCAU	1189
R4327_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUGCAUUCCAGCCCUGGGGCA	1190
R4328_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACAUUUUGCAUUCCAGCCCUGG	1191
R4329_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCAUCCAGUCAGGGUCCAUCC	1192
R4330_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCCACGCUGUAGGCUCCCAG	1193
R4331_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCCACACACAGGUUGUCCACG	1194
R4332_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACUCCACUGGUCCUGUCUGCUC	1195
R4333_Cas Phi32	GCUGGGGACCGAUCCUGAUUGCUCGCUGCGGCGAG ACCUGAAGGCCGGCUCCGGCAG	1196

TABLE S: CasΦ.12 gRNAs targeting Bak1 in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA**	**SEQ ID NO**
R2452 Bak1_CasPhi12_1	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGAAGCTATGTTTTCCATCTC	1197
R2453 Bak1_CasPhi12_2	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGCAGGGGCAGCCGCCCCCTG	1198
R2454 Bak1_CasPhi12_3	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTCCTAGAACCCAACAGGTA	1199
R2455 Bak1_CasPhi12_4	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGAAAGACCTCCTCTGTGTCC	1200
R2456 Bak1_CasPhi12_5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCCATCTCGGGGTTGGCAGG	1201
R2457 Bak1_CasPhi12_6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTCCTGATGGTGGAGATGGA	1202

R2849_Bak1_CasPhi12_nsd_sg1	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGACTCCCAGCTCTGACCC	1203
R2850_Bak1_CasPhi12_nsd_sg2	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGGGTCAGAGCTGGGAGTC	1204
R2851_Bak1_CasPhi12_nsd_sg3	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAAGACCTCCTCTGTGTCC	1205
R2852_Bak1_CasPhi12_nsd_sg4	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCGAAGCTATGTTTTCCATCT	1206
R2853_Bak1_CasPhi12_nsd_sg5	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAGCTATGTTTTCCATCTC	1207
R2854_Bak1_CasPhi12_nsd_sg6	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCATCTCCACCATCAGGAA	1208
R2855_Bak1_CasPhi12_nsd_sg7	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCATCTCCACCATCAGGAAC	1209
R2856_Bak1_CasPhi12_nsd_sg8	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGATGGTGGAGATGGAAAA	1210
R2857_Bak1_CasPhi12_nsd_sg9	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCATCTCCACCATCAGGAACA	1211
R2858_Bak1_CasPhi12_nsd_sg10	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCTGATGGTGGAGATGGA	1212
R2859_Bak1_CasPhi12_nsd_sg11	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCAGGGGCAGCCGCCCCCTG	1213
R2860_Bak1_CasPhi12_nsd_sg12	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCATCTCGGGGTTGGCAGG	1214
R2861_Bak1_CasPhi12_nsd_sg13	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTAGGAGCAAATTGTCCATCT	1215
R2862_Bak1_CasPhi12_nsd_sg14	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGTTCTAGGAGCAAATTGTC	1216
R2863_Bak1_CasPhi12_nsd_sg15	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCTCCTAGAACCCAACAGGT	1217
R2864_Bak1_CasPhi12_nsd_sg16	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCCTAGAACCCAACAGGTA	1218
R3977 Bak1_CasPhi12_exon1_sg1	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCAGACGCCATCTTTCAGG	1219
R3978 Bak1_CasPhi12_exon1_sg2	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGTAAGAGTCCTCCTGCCC	1220
R3979 Bak1_CasPhi12_exon3_sg1	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTACAGCATCTTGGGTCAGG	1221
R3980 Bak1_CasPhi12_exon3_sg2	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGTCAGGTGGGCCGGCAGCT	1222
R3981 Bak1_CasPhi12_exon3_sg3	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTATCATTGGAGATGACATT	1223
R3982 Bak1_CasPhi12_exon3_sg4	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAGATGACATTAACCGGAGA	1224

R3983 Bak1_ CasPhi12_exon3_sg5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTGGAACTCTGTGTCGTATCT	1225
R3984 Bak1_ CasPhi12_exon3_sg6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCAGAATTTACTGGAGCAGCT	1226
R3985 Bak1_ CasPhi12_exon3_sg7	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACACTGGAGCAGCTGCAGCCCA	1227
R3986 Bak1_ CasPhi12_exon3_sg8	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCAGCTGTGGGCTGCAGCTG	1228
R3987 Bak1_ CasPhi12_exon3_sg9	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTAGGCATTCCCAGCTGTGG	1229
R3988 Bak1_ CasPhi12_exon3_sg10	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTGAAGAGTTCGTAGGCATT	1230
R3989 Bak1_ CasPhi12_exon3_sg11	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACACCAAGATTGCCTCCAGGTA	1231
R3990 Bak1_ CasPhi12_exon3_sg12	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCTCCAGGTACCCACCACCA	1232

TABLE T: CasΦ.32 gRNAs targeting Bak1 in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA**	**SEQ ID NO**
R2452 Bak1_CasPhi32_1	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGAAGCTATGTTTTCCATCTC	1233
R2453 Bak1_CasPhi32_2	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGCAGGGGCAGCCGCCCCCTG	1234
R2454 Bak1_CasPhi32_3	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCTCCTAGAACCCAACAGGTA	1235
R2455 Bak1_CasPhi32_4	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGAAAGACCTCCTCTGTGTCC	1236
R2456 Bak1_CasPhi32_5	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTCCATCTCGGGGTTGGCAGG	1237
R2457 Bak1_CasPhi32_6	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTTCCTGATGGTGGAGATGGA	1238
R2849_Bak1_CasPhi32_nsd_sg1	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCTGACTCCCAGCTCTGACCC	1239
R2850_Bak1_CasPhi32_nsd_sg2	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTGGGGTCAGAGCTGGGAGTC	1240
R2851_Bak1_ CasPhi32_nsd_sg3	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGAAAGACCTCCTCTGTGTCC	1241
R2852_Bak1 CasPhi32__nsd_sg4	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCGAAGCTATGTTTTCCATCT	1242

R2853_Bak1_ CasPhi32_nsd_sg5	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGAAGCTATGTTTTCCATCTC	1243
R2854_Bak1_ CasPhi32_nsd_sg6	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTCCATCTCCACCATCAGGAA	1244
R2855_Bak1_ CasPhi32_nsd_sg7	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCCATCTCCACCATCAGGAAC	1245
R2856_Bak1_ CasPhi32_nsd_sg8	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCTGATGGTGGAGATGGAAAA	1246
R2857_Bak1_ CasPhi32_nsd_sg9	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCATCTCCACCATCAGGAACA	1247
R2858_Bak1_ CasPhi32_nsd_sg10	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTTCCTGATGGTGGAGATGGA	1248
R2859_Bak1_ CasPhi32_nsd_sg11	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGCAGGGGCAGCCGCCCCCTG	1249
R2860_Bak1_ CasPhi32_nsd_sg12	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTCCATCTCGGGGTTGGCAGG	1250
R2861_Bak1_ CasPhi32_nsd_sg13	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTAGGAGCAAATTGTCCATCT	1251
R2862_Bak1_ CasPhi32_nsd_sg14	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGGTTCTAGGAGCAAATTGTC	1252
R2863_Bak1_ CasPhi32_nsd_sg15	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGCTCCTAGAACCCAACAGGT	1253
R2864_Bak1_ CasPhi32_nsd_sg16	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCTCCTAGAACCCAACAGGTA	1254
R3977 Bak1_ CasPhi32_exon1_sg1	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTCCAGACGCCATCTTTCAGG	1255
R3978 Bak1_ CasPhi32_exon1_sg2	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTGGTAAGAGTCCTCCTGCCC	1256
R3979 Bak1_ CasPhi32_exon3_sg1	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTTACAGCATCTTGGGTCAGG	1257
R3980 Bak1_ CasPhi32_exon3_sg2	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGGTCAGGTGGGCCGGCAGCT	1258
R3981 Bak1_ CasPhi32_exon3_sg3	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCTATCATTGGAGATGACATT	1259
R3982 Bak1_ CasPhi32_exon3_sg4	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGAGATGACATTAACCGGAGA	1260
R3983 Bak1_ CasPhi32_exon3_sg5	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACTGGAACTCTGTGTCGTATCT	1261
R3984 Bak1_ CasPhi32_exon3_sg6	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCAGAATTTACTGGAGCAGCT	1262
R3985 Bak1_ CasPhi32_exon3_sg7	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACACTGGAGCAGCTGCAGCCCA	1263
R3986 Bak1_ CasPhi32_exon3_sg8	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCCAGCTGTGGGCTGCAGCTG	1264

R3987 Bak1_ CasPhi32_exon3_sg9	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGTAGGCATTCCCAGCTGTGG	1265
R3988 Bak1_ CasPhi32_exon3_sg10	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACGTGAAGAGTTCGTAGGCATT	1266
R3989 Bak1_ CasPhi32_exon3_sg11	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACACCAAGATTGCCTCCAGGTA	1267
R3990 Bak1_ CasPhi32_exon3_sg12	GCTGGGGACCGATCCTGATTGCTCGCTGCGG CGAGACCCTCCAGGTACCCACCACCA	1268

TABLE U: CasΦ.12 gRNAs targeting Bax in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA**	**SEQ ID NO**
R2458 Bax_CasPhi12_1	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTAATGTGGATACTAACTCC	1269
R2459 Bax_CasPhi12_2	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTCCGTGTGGCAGCTGACAT	1270
R2460 Bax_CasPhi12_3	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTGATGGCAACTTCAACTGG	1271
R2461 Bax_CasPhi12_4	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTACTTTGCTAGCAAACTGGT	1272
R2462 Bax_CasPhi12_5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAGCACCAGTTTGCTAGCAAA	1273
R2463 Bax_CasPhi12_6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAACTGGGGCCGGGTTGTTGC	1274
R2865_Bax_CasPhi12_nsd_sg1	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTCTCTTTCCTGTAGGATGA	1275
R2866_Bax_CasPhi12_nsd_sg2	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCTTTCCTGTAGGATGATTG	1276
R2867_Bax_ CasPhi12_nsd_sg3	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCTGTAGGATGATTGCTAAT	1277
R2868_Bax_ CasPhi12_nsd_sg4	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTGTAGGATGATTGCTAATG	1278
R2869_Bax_ CasPhi12_nsd_sg5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTAATGTGGATACTAACTCC	1279
R2870_Bax_ CasPhi12_nsd_sg6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTCCGTGTGGCAGCTGACAT	1280
R2871_Bax_ CasPhi12_nsd_sg7	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCGTGTGGCAGCTGACATGTT	1281
R2872_Bax_ CasPhi12_nsd_sg8	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCATCAGCAAACATGTCAGC	1282

R2873_Bax_ CasPhi12_nsd_sg9	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAAGTTGCCATCAGCAAACAT	1283
R2874_Bax_ CasPhi12_nsd_sg10	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGCTGATGGCAACTTCAACTG	1284
R2875_Bax_ CasPhi12_nsd_sg11	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTGATGGCAACTTCAACTGG	1285
R2876_Bax_ CasPhi12_nsd_sg12	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAACTGGGGCCGGGTTGTTGC	1286
R2877_Bax_ CasPhi12_nsd_sg13	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTGCCCTTTTCTACTTTGCT	1287
R2878_Bax_ CasPhi12_nsd_sg14	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCCTTTTCTACTTTGCTAGC	1288
R2879_Bax_ CasPhi12_nsd_sg15	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTAGCAAAGTAGAAAAGGGC	1289
R2880_Bax_ CasPhi12_nsd_sg16	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGCTAGCAAAGTAGAAAAGGG	1290
R2881_Bax_ CasPhi12_nsd_sg17	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCTACTTTGCTAGCAAACTG	1291
R2882_Bax_ CasPhi12_nsd_sg18	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTACTTTGCTAGCAAACTGG	1292
R2883_Bax_ CasPhi12_nsd_sg19	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTACTTTGCTAGCAAACTGGT	1293
R2884_Bax_ CasPhi12_nsd_sg20	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGCTAGCAAACTGGTGCTCAA	1294
R2885_Bax_ CasPhi12_nsd_sg21	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTAGCAAACTGGTGCTCAAG	1295
R2886_Bax_ CasPhi12_nsd_sg22	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAGCACCAGTTTGCTAGCAAA	1296

TABLE V: CasΦ.32 gRNAs targeting Bax in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA**	**SEQ ID NO**
R2458 Bax_CasPhi32_1	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTAATGTGGATACTAACTCC	1297
R2459 Bax_CasPhi32_2	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTCCGTGTGGCAGCTGACAT	1298
R2460 Bax_CasPhi32_3	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTGATGGCAACTTCAACTGG	1299
R2461 Bax_CasPhi32_4	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTACTTTGCTAGCAAACTGGT	1300
R2462 Bax_CasPhi32_5	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAGCACCAGTTTGCTAGCAAA	1301

R2463 Bax_CasPhi32_6	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAACTGGGGCCGGGTTGTTGC	1302
R2865_Bax_CasPhi32_nsd_sg1	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTCTCTTTCCTGTAGGATGA	1303
R2866_Bax_CasPhi32_nsd_sg2	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCTTTCCTGTAGGATGATTG	1304
R2867_Bax_CasPhi32_nsd_sg3	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCTGTAGGATGATTGCTAAT	1305
R2868_Bax_CasPhi32_nsd_sg4	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTGTAGGATGATTGCTAATG	1306
R2869_Bax_CasPhi32_nsd_sg5	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTAATGTGGATACTAACTCC	1307
R2870_Bax_CasPhi32_nsd_sg6	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTCCGTGTGGCAGCTGACAT	1308
R2871_Bax_CasPhi32_nsd_sg7	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCGTGTGGCAGCTGACATGTT	1309
R2872_Bax_CasPhi32_nsd_sg8	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCATCAGCAAACATGTCAGC	1310
R2873_Bax_CasPhi32_nsd_sg9	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAAGTTGCCATCAGCAAACAT	1311
R2874_Bax_CasPhi32_nsd_sg10	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGCTGATGGCAACTTCAACTG	1312
R2875_Bax_CasPhi32_nsd_sg11	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTGATGGCAACTTCAACTGG	1313
R2876_Bax_CasPhi32_nsd_sg12	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAACTGGGGCCGGGTTGTTGC	1314
R2877_Bax_CasPhi32_nsd_sg13	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTGCCCTTTTCTACTTTGCT	1315
R2878_Bax_CasPhi32_nsd_sg14	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCCTTTTCTACTTTGCTAGC	1316
R2879_Bax_CasPhi32_nsd_sg15	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTAGCAAAGTAGAAAAGGGC	1317
R2880_Bax_CasPhi32_nsd_sg16	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGCTAGCAAAGTAGAAAAGGG	1318
R2881_Bax_CasPhi32_nsd_sg17	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCTACTTTGCTAGCAAACTG	1319
R2882_Bax_CasPhi32_nsd_sg18	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTACTTTGCTAGCAAACTGG	1320
R2883_Bax_CasPhi32_nsd_sg19	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTACTTTGCTAGCAAACTGGT	1321
R2884_Bax_CasPhi32_nsd_sg20	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGCTAGCAAACTGGTGCTCAA	1322
R2885_Bax_CasPhi32_nsd_sg21	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTAGCAAACTGGTGCTCAAG	1323

R2886_Bax_ CasPhi32_nsd_sg22	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAGCACCAGTTTGCTAGCAAA	1324

TABLE W: CasΦ.12 gRNAs targeting Fut8 in CHO cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2464 Fut8_CasPhi12_1	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCACTTTGTCAGTGCGTCTG	1325
R2465 Fut8_CasPhi12_2	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTCAATGGGATGGAAGGCTG	1326
R2466 Fut8_CasPhi12_3	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAGGAATACATGGTACACGTT	1327
R2467 Fut8_CasPhi12_4	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAAGAACATTTTCAGCTTCTC	1328
R2468 Fut8_CasPhi12_5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACATCCACTTTCATTCTGCGTT	1329
R2469 Fut8_CasPhi12_6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTTGTTAAAGGAGGCAAAGA	1330
R2887_Fut8_CasPhi12_nsd_sg1	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCCCCAGAGTCCATGTCAGA	1331
R2888_Fut8_ CasPhi12_nsd_sg2	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCAGTGCGTCTGACATGGAC	1332
R2889_Fut8_CasPhi12_nsd_sg3	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTCAGTGCGTCTGACATGGA	1333
R2890_Fut8_ CasPhi12_nsd_sg4	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCACTTTGTCAGTGCGTCTG	1334
R2891_Fut8_ CasPhi12_nsd_sg5	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTGTTCCCACTTTGTCAGTGC	1335
R2892_Fut8_ CasPhi12_nsd_sg6	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCTCAATGGGATGGAAGGCTG	1336
R2893_Fut8_ CasPhi12_nsd_sg7	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCATCCCATTGAGGAATACAT	1337
R2894_Fut8_ CasPhi12_nsd_sg8	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAGGAATACATGGTACACGTT	1338
R2895_Fut8_ CasPhi12_nsd_sg9	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAACGTGTACCATGTATTCCT	1339
R2896_Fut8_ CasPhi12_nsd_sg10	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTCAACGTGTACCATGTATT	1340
R2897_Fut8_ CasPhi12_nsd_sg11	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAAGAACATTTTCAGCTTCTC	1341
R2898_Fut8_ CasPhi12_nsd_sg12	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGAGAAGCTGAAAATGTTCTT	1342

R2899_Fut8_ CasPhi12_nsd_sg13	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCAGCTTCTCGAACGCAGAA	1343
R2900_Fut8_ CasPhi12_nsd_sg14	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCAGCTTCTCGAACGCAGAAT	1344
R2901_Fut8_ CasPhi12_nsd_sg15	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTGCGTTCGAGAAGCTGAAAA	1345
R2902_Fut8_ CasPhi12_nsd_sg16	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAGCTTCTCGAACGCAGAATG	1346
R2903_Fut8_ CasPhi12_nsd_sg17	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACATTCTGCGTTCGAGAAGCTG	1347
R2904_Fut8_ CasPhi12_nsd_sg18	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCATTCTGCGTTCGAGAAGCT	1348
R2905_Fut8_ CasPhi12_nsd_sg19	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCGAACGCAGAATGAAAGTG	1349
R2906_Fut8_ CasPhi12_nsd_sg20	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACATCCACTTTCATTCTGCGTT	1350
R2907_Fut8_ CasPhi12_nsd_sg21	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTATCCACTTTCATTCTGCGT	1351
R2908_Fut8_ CasPhi12_nsd_sg22	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTATCCACTTTCATTCTGCG	1352
R2909_Fut8_ CasPhi12_nsd_sg23	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTTATCCACTTTCATTCTGC	1353
R2910_Fut8_ CasPhi12_nsd_sg24	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTTTATCCACTTTCATTCTG	1354
R2911_Fut8_ CasPhi12_nsd_sg25	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACAACAAAGAAGGGTCATCAGT	1355
R2912_Fut8_ CasPhi12_nsd_sg26	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACCCTCCTTTAACAAAGAAGGG	1356
R2913_Fut8_ CasPhi12_nsd_sg27	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGCCTCCTTTAACAAAGAAGG	1357
R2914_Fut8_ CasPhi12_nsd_sg28	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTTGTTAAAGGAGGCAAAGA	1358
R2915_Fut8_ CasPhi12_nsd_sg29	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTTAAAGGAGGCAAAGACAA	1359
R2916_Fut8_ CasPhi12_nsd_sg30	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTAAAGGAGGCAAAGACAAA	1360
R2917_Fut8_ CasPhi12_nsd_sg31	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTCTTTGCCTCCTTTAACAAA	1361
R2918_Fut8_ CasPhi12_nsd_sg32	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTCTTTGCCTCCTTTAACAA	1362
R2919_Fut8_ CasPhi12_nsd_sg33	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACGTCTAACTTACTTTGTCTTT	1363
R2920_Fut8_ CasPhi12_nsd_sg34	CTTTCAAGACTAATAGATTGCTCCTTACGAG GAGACTTGGTCTAACTTACTTTGTC	1364

TABLE X: CasΦ.32 gRNAs targeting Fut8 in CHO cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2464 Fut8_CasPhi32_1	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCACTTTGTCAGTGCGTCTG	1365
R2465 Fut8_CasPhi32_2	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTCAATGGGATGGAAGGCTG	1366
R2466 Fut8_CasPhi32_3	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAGGAATACATGGTACACGTT	1367
R2467 Fut8_CasPhi32_4	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAAGAACATTTTCAGCTTCTC	1368
R2468 Fut8_CasPhi32_5	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACATCCACTTTCATTCTGCGTT	1369
R2469 Fut8_CasPhi32_6	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTTGTTAAAGGAGGCAAAGA	1370
R2887_Fut8_CasPhi32_nsd_sg1	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCCCCAGAGTCCATGTCAGA	1371
R2888_Fut8_ CasPhi32_nsd_sg2	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCAGTGCGTCTGACATGGAC	1372
R2889_Fut8_ CasPhi32_nsd_sg3	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGTCAGTGCGTCTGACATGGA	1373
R2890_Fut8_ CasPhi32_nsd_sg4	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCACTTTGTCAGTGCGTCTG	1374
R2891_Fut8_ CasPhi32_nsd_sg5	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTGTTCCCACTTTGTCAGTGC	1375
R2892_Fut8_ CasPhi32_nsd_sg6	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCTCAATGGGATGGAAGGCTG	1376
R2893_Fut8_ CasPhi32_nsd_sg7	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCATCCCATTGAGGAATACAT	1377
R2894_Fut8_ CasPhi32_nsd_sg8	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAGGAATACATGGTACACGTT	1378
R2895_Fut8_ CasPhi32_nsd_sg9	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAACGTGTACCATGTATTCCT	1379
R2896_Fut8_ CasPhi32_nsd_sg10	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTCAACGTGTACCATGTATT	1380
R2897_Fut8_ CasPhi32_nsd_sg11	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAAGAACATTTTCAGCTTCTC	1381
R2898_Fut8_ CasPhi32_nsd_sg12	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGAGAAGCTGAAAATGTTCTT	1382
R2899_Fut8_ CasPhi32_nsd_sg13	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCAGCTTCTCGAACGCAGAA	1383

R2900_Fut8_ CasPhi32_nsd_sg14	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCAGCTTCTCGAACGCAGAAT	1384
R2901_Fut8_ CasPhi32_nsd_sg15	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTGCGTTCGAGAAGCTGAAAA	1385
R2902_Fut8_ CasPhi32_nsd_sg16	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAGCTTCTCGAACGCAGAATG	1386
R2903_Fut8_ CasPhi32_nsd_sg17	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACATTCTGCGTTCGAGAAGCTG	1387
R2904_Fut8_ CasPhi32_nsd_sg18	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCATTCTGCGTTCGAGAAGCT	1388
R2905_Fut8_ CasPhi32_nsd_sg19	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCGAACGCAGAATGAAAGTG	1389
R2906_Fut8_ CasPhi32_ CasPhi32_nsd_sg20	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACATCCACTTTCATTCTGCGTT	1390
R2907_Fut8_ CasPhi32_nsd_sg21	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTATCCACTTTCATTCTGCGT	1391
R2908_Fut8_ CasPhi32_nsd_sg22	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTATCCACTTTCATTCTGCG	1392
R2909_Fut8_ CasPhi32_nsd_sg23	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTTATCCACTTTCATTCTGC	1393
R2910_Fut8_ CasPhi32_nsd_sg24	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTTTATCCACTTTCATTCTG	1394
R2911_Fut8_ CasPhi32_nsd_sg25	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACAACAAAGAAGGGTCATCAGT	1395
R2912_Fut8_ CasPhi32_nsd_sg26	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACCCTCCTTTAACAAAGAAGGG	1396
R2913_Fut8_ CasPhi32_nsd_sg27	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGCCTCCTTTAACAAAGAAGG	1397
R2914_Fut8_ CasPhi32_nsd_sg28	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTTGTTAAAGGAGGCAAAGA	1398
R2915_Fut8_ CasPhi32_nsd_sg29	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGTTAAAGGAGGCAAAGACAA	1399
R2916_Fut8_ CasPhi32_nsd_sg30	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTAAAGGAGGCAAAGACAAA	1400
R2917_Fut8_ CasPhi32_nsd_sg31	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTCTTTGCCTCCTTTAACAAA	1401
R2918_Fut8_ CasPhi32_nsd_sg32	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGTCTTTGCCTCCTTTAACAA	1402
R2919_Fut8_ CasPhi32_nsd_sg33	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACGTCTAACTTACTTTGTCTTT	1403
R2920_Fut8_ CasPhi32_nsd_sg34	GCTGGGGACCGATCCTGATTGCTCGCTGCG GCGAGACTTGGTCTAACTTACTTTGTC	1404

TABLE Y: CasΦ.12 gRNAs targeting human TRAC in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3040_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGGATATCTGTGGGACA	1533
R3041_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCCCACAGATATCCAGA	1534
R3042_CasPhi12_S	ATTGCTCCTTACGAGGAGACGAGTCTCTCAGCTGGTA	1535
R3043_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGAGTCTCTCAGCTGGT	1536
R3044_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCACTGGATTTAGAGTC	1537
R3045_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGAATCAAAATCGGTGA	1538
R3046_CasPhi12_S	ATTGCTCCTTACGAGGAGACGAGAATCAAAATCGGTG	1539
R3047_CasPhi12_S	ATTGCTCCTTACGAGGAGACACCGATTTTGATTCTCA	1540
R3048_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTTGAGAATCAAAATCG	1541
R3049_CasPhi12_S	ATTGCTCCTTACGAGGAGACGTTTGAGAATCAAAATC	1542
R3050_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGATTCTCAAACAAATG	1543
R3051_CasPhi12_S	ATTGCTCCTTACGAGGAGACGATTCTCAAACAAATGT	1544
R3052_CasPhi12_S	ATTGCTCCTTACGAGGAGACATTCTCAAACAAATGTG	1545
R3053_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGACACATTTGTTTGAG	1546
R3054_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCAAACAAATGTGTCAC	1547
R3055_CasPhi12_S	ATTGCTCCTTACGAGGAGACGTGACACATTTGTTTGA	1548
R3056_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTTTGTGACACATTTGT	1549
R3057_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGATGTGTATATCACAG	1550
R3058_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCTGTGATATACACATC	1551
R3059_CasPhi12_S	ATTGCTCCTTACGAGGAGACGTCTGTGATATACACAT	1552
R3060_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGTCTGTGATATACACA	1553
R3061_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAGTCCATAGACCTCAT	1554

R3062_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTCTTGAAGTC CATAGA	1555
R3063_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAGAGCAACAG TGCTGT	1556
R3064_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTCCAGGCCAC AGCACT	1557
R3065_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTGCTCCAGGC CACAGC	1558
R3066_CasPhi12_S	ATTGCTCCTTACGAGGAGACGTTGCTCCAGG CCACAG	1559
R3067_CasPhi12_S	ATTGCTCCTTACGAGGAGACCACATGCAAAG TCAGAT	1560
R3068_CasPhi12_S	ATTGCTCCTTACGAGGAGACGCACATGCAAA GTCAGA	1561
R3069_CasPhi12_S	ATTGCTCCTTACGAGGAGACGCATGTGCAAA CGCCTT	1562
R3070_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAGGCGTTTGC ACATGC	1563
R3071_CasPhi12_S	ATTGCTCCTTACGAGGAGACCATGTGCAAAC GCCTTC	1564
R3072_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTGAAGGCGTT TGCACA	1565
R3073_CasPhi12_S	ATTGCTCCTTACGAGGAGACAACAACAGCAT TATTCC	1566
R3074_CasPhi12_S	ATTGCTCCTTACGAGGAGACTGGAATAATGC TGTTGT	1567
R3075_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCCAGAAGAC ACCTTC	1568
R3076_CasPhi12_S	ATTGCTCCTTACGAGGAGACCAGAAGACACC TTCTTC	1569
R3077_CasPhi12_S	ATTGCTCCTTACGAGGAGACCCTGGGCTGGG GAAGAA	1570
R3078_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCCCCAGCCC AGGTAA	1571
R3079_CasPhi12_S	ATTGCTCCTTACGAGGAGACCCCAGCCCAGG TAAGGG	1572
R3080_CasPhi12_S	ATTGCTCCTTACGAGGAGACTAAAAGGAAAA ACAGAC	1573
R3081_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTAAAAGGAAA AACAGA	1574
R3082_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCCTTTTAGAA AGTTC	1575
R3083_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCCTTTTAGAA AGTTCC	1576
R3084_CasPhi12_S	ATTGCTCCTTACGAGGAGACCCTTTTAGAAA GTTCCT	1577
R3085_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTTTTAGAAAG TTCCTG	1578
R3086_CasPhi12_S	ATTGCTCCTTACGAGGAGACTAGAAAGTTCC TGTGAT	1579

R3136_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGAAAGTTCCTGTGATG	1580
R3137_CasPhi12_S	ATTGCTCCTTACGAGGAGACGAAAGTTCCTGTGATGT	1581
R3138_CasPhi12_S	ATTGCTCCTTACGAGGAGACACATCACAGGAACTTTC	1582
R3139_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTGTGATGTCAAGCTGG	1583
R3140_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCGACCAGCTTGACATC	1584
R3141_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTCGACCAGCTTGACAT	1585
R3142_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCTCGACCAGCTTGACA	1586
R3143_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAAGCTTTTCTCGACCA	1587
R3144_CasPhi12_S	ATTGCTCCTTACGAGGAGACCAAAGCTTTTCTCGACC	1588
R3145_CasPhi12_S	ATTGCTCCTTACGAGGAGACCCTGTTTCAAAGCTTTT	1589
R3146_CasPhi12_S	ATTGCTCCTTACGAGGAGACGAAACAGGTAAGACAGG	1590
R3147_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAACAGGTAAGACAGGG	1591

TABLE Z: CasΦ.12 gRNAs targeting human B2M in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R3115_CasPhi12_S	ATTGCTCCTTACGAGGAGACCATCCATCCGACATTGA	1592
R3116_CasPhi12_S	ATTGCTCCTTACGAGGAGACATCCATCCGACATTGAA	1593
R3117_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGTAAGTCAACTTCAAT	1594
R3118_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCAGTAAGTCAACTTC	1595
R3119_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAGTTGACTTACTGAAG	1596
R3120_CasPhi12_S	ATTGCTCCTTACGAGGAGACACTTACTGAAGAATGGA	1597
R3121_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCTCTCCATTCTTCAGT	1598
R3122_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTGAAGAATGGAGAGAG	1599
R3123_CasPhi12_S	ATTGCTCCTTACGAGGAGACAATTCTCTCTCCATTCT	1600
R3124_CasPhi12_S	ATTGCTCCTTACGAGGAGACCAATTCTCTCTCCATTC	1601

R3125_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCAATTCTCTCTCCATT	1602
R3126_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCAATTCTCTCTCCAT	1603
R3127_CasPhi12_S	ATTGCTCCTTACGAGGAGACAAAAAGTGGAGCATTCA	1604
R3128_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTGAAAGACAAGTCTGA	1605
R3129_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGACTTGTCTTTCAGCA	1606
R3130_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCTTTCAGCAAGGACTG	1607
R3131_CasPhi12_S	ATTGCTCCTTACGAGGAGACCAGCAAGGACTGGTCTT	1608
R3132_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGCAAGGACTGGTCTTT	1609
R3133_CasPhi12_S	ATTGCTCCTTACGAGGAGACCTATCTCTTGTACTACA	1610
R3134_CasPhi12_S	ATTGCTCCTTACGAGGAGACTATCTCTTGTACTACAC	1611
R3135_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGTGTAGTACAAGAGAT	1612
R3148_CasPhi12_S	ATTGCTCCTTACGAGGAGACTACTACACTGAATTCAC	1613
R3149_CasPhi12_S	ATTGCTCCTTACGAGGAGACAGTGGGGGTGAATTCAG	1614
R3150_CasPhi12_S	ATTGCTCCTTACGAGGAGACCAGTGGGGGTGAATTCA	1615
R3151_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCAGTGGGGGTGAATTC	1616
R3152_CasPhi12_S	ATTGCTCCTTACGAGGAGACTTCAGTGGGGGTGAATT	1617
R3153_CasPhi12_S	ATTGCTCCTTACGAGGAGACACCCCCACTGAAAAAGA	1618
R3154_CasPhi12_S	ATTGCTCCTTACGAGGAGACACACGGCAGGCATACTC	1619
R3155_CasPhi12_S	ATTGCTCCTTACGAGGAGACGGCTGTGACAAAGTCAC	1620
R3156_CasPhi12_S	ATTGCTCCTTACGAGGAGACGTCACAGCCCAAGATAG	1621
R3157_CasPhi12_S	ATTGCTCCTTACGAGGAGACTCACAGCCCAAGATAGT	1622
R3158_CasPhi12_S	ATTGCTCCTTACGAGGAGACACTATCTTGGGCTGTGA	1623
R3159_CasPhi12_S	ATTGCTCCTTACGAGGAGACCCCCACTTAACTATCTT	1624

TABLE AA: CasΦ.12 gRNAs targeting human PD1 in T cells

Name	Repeat+spacer RNA Sequence (5' --> 3')	SEQ ID NO
R2921_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUUCCGC UCACCUCCG	1625
R2922_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUUCCGC UCACCUCCG	1626
R2923_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGCUCACC UCCGCCUGA	1627
R2924_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCACUGC UCAGGCGGA	1628
R2925_CasPhi12_S	AUUGCUCCUUACGAGGAGACUAGCACCG CCCAGACGA	1629
R2926_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCAUGC AGAUCCCAC	1630
R2927_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACAGGCG CCCUGGCCA	1631
R2928_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGGGCG GUGCUACAA	1632
R2929_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCAUGCCU GGAGCAGCC	1633
R2930_CasPhi12_S	AUUGCUCCUUACGAGGAGACUAGCACCG CCCAGACGA	1634
R2931_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGCCGCC AGCCCAGUU	1635
R2932_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUUCCGCU CACCUCCGC	1636
R2933_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGGCCU GUCUGGGGA	1637
R2934_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCCAGC CCUGCUCGU	1638
R2935_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGUCACCA CGAGCAGGG	1639
R2936_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCCUUC GGUCACCAC	1640
R2937_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGAAGCU GCAGGUGAA	1641
R2938_CasPhi12_S	AUUGCUCCUUACGAGGAGACACCUGCAG CUUCUCCAA	1642
R2939_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAACAC AUCGGAGAG	1643
R2940_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCACGAAG CUCUCCGAU	1644
R2941_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCACGAA GCUCUCCGA	1645
R2942_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGCUAAA CUGGUACCG	1646
R2943_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGGGCU CAUGCGGUA	1647

R2944_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCGUCUG GUUGCUGGG	1648
R2945_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCGAGGA CCGCAGCCA	1649
R2946_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGUGACAC GGAAGCGGC	1650
R2947_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGUGUCAC ACAACUGCC	1651
R2948_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCAGUUG UGUGACACG	1652
R2949_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACAUGAG CGUGGUCAG	1653
R2950_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGCCGGGC CCUGACCAC	1654
R2951_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGCCAG GGAGAUGGC	1655
R2952_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUCUGCGC CUUGGGGGC	1656
R2953_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAUCUGCG CCUUGGGGG	1657
R2954_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGACAG GCCCUGGAA	1658
R2955_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGCCCU GCUCGUGGU	1659
R2956_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUCUGGA AGGGCACAA	1660
R2957_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGCCCUU CCAGAGAGA	1661
R2958_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCCCUUC CAGAGAGAA	1662
R2959_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCCCUUC UCUCUGGAA	1663
R2960_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGAGAGA AGGGCAGAA	1664
R2961_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAACUGGC CGGCUGGCC	1665
R2962_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAACUGG CCGGCUGGC	1666
R2963_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAAACCCU GGUGGUUGG	1667
R2964_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGUCGUG GGCGGCCUG	1668
R2965_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUCGUGC GGCCCGGGA	1669
R2966_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCUGCA GAGAAACAC	1670
R2967_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCUGCAG GGACAAUAG	1671
R2968_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGCAGG GACAAUAGG	1672

R2969_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCCUCAAAGAAGGAGG	1673
R2970_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCUCAAAGAAGGAGGA	1674
R2971_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGUGGACUAUGGGGA	1675
R2972_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUCGCCACUGGAAAUC	1676
R2973_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGUGGCGAGAGAAGA	1677
R2974_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGUGGCGAGAGAAGAC	1678
R2975_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGCUAGGAAAGACAAUG	1679
R2976_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUUUCCUAGCGGAAUG	1680
R2977_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUAGCGGAAUGGGCAC	1681
R2978_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUAGCGGAAUGGGCACC	1682
R2979_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCCUCUGACCGGCUU	1683
R2980_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUUGGCCACCAGUGUUC	1684
R2981_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCACCAGUGUUCUGCA	1685
R2982_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCAGACCCUCCACCAU	1686
R2983_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCUGAGGAAAUGCGCU	1687
R2984_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUCAGGAGAAGCAGGC	1688
R2985_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCAGGAGAAGCAGGCA	1689
R2986_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGCCGUCCAGGGGCU	1690
R2987_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGACAUGAGUCCUGUGG	1691
R2988_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGUCCUGCCAGCACAG	1692
R2989_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGAGCUGGACGCAGG	1693
R2990_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCCCCGGGCCGCAGGC	1694
R2991_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCAGGAGGCUCCGGG	1695
R2992_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGCUGGUUGGAGAUG	1696
R2993_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGAUGGCCUUGGAGCA	1697

R2994_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUGCUCC AAGGCCAUC	1698
R2995_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGCAGCC AAGGUGCCC	1699
R2996_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGAUGCC ACUGCCAGG	1700
R2997_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGGGAUGC CACUGCCAG	1701
R2998_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCCCUGC GUCCAGGGC	1702
R2999_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGCUCC CUGCAGGCC	1703
R3000_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUAGGCC UGCAGGGAG	1704
R3001_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUGAAAC UUCUCUAGG	1705
R3002_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGACCUUC CCUGAAACU	1706
R3003_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGGAAG GUCAGAAGA	1707
R3004_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGAAGG UCAGAAGAG	1708
R3005_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCCCUG CCCACCACA	1709
R3006_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUGCCCU GCCCACCAC	1710
R3007_CasPhi12_S	AUUGCUCCUUACGAGGAGACACACAUGC CCAGGCAGC	1711
R3008_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACAUGCC CAGGCAGCA	1712
R3009_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUGCCCC ACAAAGGGC	1713
R3010_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGGGGCA GGGAAGCUG	1714
R3011_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGGGCAG GGAAGCUGA	1715
R3012_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCCUCA GCUUCCCUG	1716
R3013_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGCCCA GCCAGCACU	1717
R3014_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCCCAG CCAGCACUC	1718
R3015_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACCCCAG CCCCUCACA	1719
R3016_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGACCGUA GGAUGUCCC	1720

TABLE AB: shortened CasΦ.12 gRNAs targeting human CIITA

Name	Repeat+spacer RNA Sequence (5' --> 3')	SEQ ID NO
R4503_CasPhi12_C2TA_T1.1_S	AUUGCUCCUUACGAGGAGACCUACACAAUGCGUUGCC	1721
R4504_CasPhi12_C2TA_T1.2_S	AUUGCUCCUUACGAGGAGACGGGCUCUGACAGGUAGG	1722
R4505_CasPhi12_C2TA_T1.3_S	AUUGCUCCUUACGAGGAGACUGUAGGAAUCCCAGCCA	1723
R4506_CasPhi12_C2TA_T1.8_S	AUUGCUCCUUACGAGGAGACCCUGGCUCCACGCCCUG	1724
R4507_CasPhi12_C2TA_T1.9_S	AUUGCUCCUUACGAGGAGACGGGAAGCUGAGGGCACG	1725
R4508_CasPhi12_C2TA_T2.1_S	AUUGCUCCUUACGAGGAGACACAGCGAUGCUGACCCC	1726
R4509_CasPhi12_C2TA_T2.2_S	AUUGCUCCUUACGAGGAGACUUAACAGCGAUGCUGAC	1727
R4510_CasPhi12_C2TA_T2.3_S	AUUGCUCCUUACGAGGAGACUAUGACCAGAUGGACCU	1728
R4511_CasPhi12_C2TA_T2.4_S	AUUGCUCCUUACGAGGAGACGGGCCCCUAGAAGGUGG	1729
R4512_CasPhi12_C2TA_T2.5_S	AUUGCUCCUUACGAGGAGACUAGGGGCCCCAACUCCA	1730
R4513_CasPhi12_C2TA_T2.6_S	AUUGCUCCUUACGAGGAGACAGAAGCUCCAGGUAGCC	1731
R4514_CasPhi12_C2TA_T2.7_S	AUUGCUCCUUACGAGGAGACUCCAGCCAGGUCCAUCU	1732
R4515_CasPhi12_C2TA_T2.8_S	AUUGCUCCUUACGAGGAGACUUCUCCAGCCAGGUCCA	1733
R5200_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCAGGCUGUUGUGUGA	2290
R5201_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAUGUCACACAACAGCC	2291
R5202_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGUGACAUGGAAGGUGA	2292
R5203_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUCACCUUCCAUGUCAC	2293
R5204_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCAUAAGCCUCCCUGGU	2294
R5205_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGACUCCCAGCUGGA	2295
R5206_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCAGGCCCUCCAGCUG	2296
R5207_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCUGGCAUCUCCAUAC	2297
R5208_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCCCAACUUCUGCUGG	2298
R5209_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCCCAACUUCUGCUG	2299

R5210_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGCCCA ACUUCUGCU	2300
R5211_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGACUUUU CUGCCCAAC	2301
R5212_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGACUUU UCUGCCCAA	2302
R5213_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGACUU UUCUGCCCA	2303
R5214_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGAGGA GCUUCCGGC	2304
R5215_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGUCUGC CGGAAGCUC	2305
R5216_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGGCAGAC CUGAAGCAC	2306
R5217_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGUGCUU CAGGUCUGC	2307
R5218_CasPhi12_S	AUUGCUCCUUACGAGGAGACAACAGCGC AGGCAGUGG	2308
R5219_CasPhi12_S	AUUGCUCCUUACGAGGAGACAACCAGGA GCCAGCCUC	2309
R5220_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAGGCG CAUCUGGCC	2310
R5221_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCCAGGC GCAUCUGGC	2311
R5222_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUCCAGG CGCAUCUGG	2312
R5223_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCCAGUU CCUCGUUGA	2313
R5224_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAGUUC CUCGUUGAG	2314
R5225_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCAGCU CAACGAGGA	2315
R5226_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCGUUGA GCUGCCUGA	2316
R5227_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCUGCCU GAAUCUCCC	2317
R5228_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUCCCCAC CAUCUCCAC	2318
R5229_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCCACC AUCUCCACU	2319
R5230_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGAGCC CAUGGGGCA	2320
R5231_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCAGAGC CCAUGGGGC	2321
R5232_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCCUCA GAGAUUUGC	2322
R5233_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAGGCCG UGGACAGUG	2323
R5234_CasPhi12_S	AUUGCUCCUUACGAGGAGACACUGUCCA CGGCCUCCC	2324

R5235_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUCCAUCAGCCACUGA	2325
R5236_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCAUGCUGGGCAGGU	2326
R5237_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCGGGAGGUCAGGGCA	2327
R5238_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUCGGGAGGUCAGGGC	2328
R5239_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGACCUCUCCAGCUGC	2329
R5240_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUGGAGACCUCUCCAGC	2330
R5241_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAAGCUUGUUGGAGACC	2331
R5242_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAAGCUUGUUGGAGAC	2332
R5243_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGAAGCUUGUUGGAGA	2333
R5244_CasPhi12_S	AUUGCUCCUUACGAGGAGACUACCGCUCACUGCAGGA	2334
R5245_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCUGCUCCUCUCCAG	2335
R5246_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCGCUCCAGGCUCUUGC	2336
R5247_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCCCAGUCCGGGGUGG	2337
R5248_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCCAGCUGCCGUUCUG	2338
R5249_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCAGCCAACAGCACCUC	2339
R5250_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUGCCAAGGAGCACCG	2340
R5251_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAGCACAGCAAUCAC	2341
R5252_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCAGCACAGCAAUCA	2342
R5253_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGUGCUGGGCAAAGCU	2343
R5254_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCUGACCAGCUUUGCC	2344
R5255_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCUGGGGCAGUGAGCC	2345
R5256_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGCCGGCUUCCCCAGU	2346
R5257_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAGUACGACUUUGUC	2347
R5258_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUCUUCUCUGUCCCCUG	2348
R5259_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUUCUCUGUCCCCUGC	2349

R5260_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUGUCCC CUGCCAUUG	2350
R5261_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAGCAAUG GCAGGGGAC	2351
R5262_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUUGAACC GUCCGGGGG	2352
R5263_CasPhi12_S	AUUGCUCCUUACGAGGAGACAACCGUCC GGGGGAUGC	2353
R5264_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCUGGG CCCACAGCC	2354
R5265_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAGAUGUG GCUGAAAAC	2355
R5266_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCAGCCAC AUCUUGAAG	2356
R5267_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCCACA UCUUGAAGA	2357
R5268_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCCACAU CUUGAAGAG	2358
R5269_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAGAGACC UGACCGCGU	2359
R5270_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCUCAUC CUAGACGGC	2360
R5271_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCUCCU CGAAGCCGU	2361
R5272_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGCUUCCA GCUCCUCGA	2362
R5273_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGGAGCU GGAAGCGCA	2363
R5274_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCACAG CACGUGCGG	2364
R5275_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGAAAAG GCCGGCCAG	2365
R5276_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCUGGAA AAGGCCGGC	2366
R5277_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAGAAG AAGCUGCUC	2367
R5278_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGAAGA AGCUGCUCC	2368
R5279_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGAAGAA GCUGCUCCG	2369
R5280_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACCCUCC UCCUCACAG	2370
R5281_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCAGGCU CUGGACCAG	2371
R5282_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGCUGUC CGGCUUCUC	2372
R5283_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCUGUCC GGCUUCUCC	2373
R5284_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAUGGA GCAGGCCCA	2374

R5285_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGAGCUCAGGGAUGAC	2375
R5286_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGAGCUCAGGGAUGACA	2376
R5287_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGCUCUGUCAUCCCUG	2377
R5288_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCUCAGUCACAGCCAC	2378
R5289_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCAGUCACAGCCACAGC	2379
R5290_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGCCGGGCAGUGUGCC	2380
R5291_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCCGGGCAGUGUGCCA	2381
R5292_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCGUCCUCCCCAAGCUC	2382
R5293_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGAGGACGCCAAGCUG	2383
R5294_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCAGCUCUGCCAGGGC	2384
R5295_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUGUCUGCGGCCCAGCU	2385
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R5393_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAUCCGCAGACGUGAG	2387
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R5395_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCCAUCGCCCAGGUCC	2389
R5396_CasPhi12_S	AUUGCUCCUUACGAGGAGACGACUAAGCCUUUGGCCA	2390
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R5399_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAGCUUCCUCCUGCA	2393
R5400_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCCUGCAAUGCUUCCU	2394
R5401_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGGGGCCCUGUGGCU	2395
R5402_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCACUCAGAGCCAGCC	2396
R5403_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGCCACUCAGAGCCAGC	2397
R5404_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUUUCGCCACUCAGAGC	2398
R5405_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCUUGAUUUCGCCACU	2399

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R5407_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUUGGGGUCAAUGCUAG	2401
R5408_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCCUUGGGGUCAAUGC	2402
R5409_CasPhi12_S	AUUGCUCCUUACGAGGAGACACCCCAAGGAAGAAGAG	2403
R5410_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCAUAGGGCCUCUUCUU	2404
R5411_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGCUGGGCUGAUCUU	2405
R5412_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGCUGGGCUGAUCUUC	2406
R5413_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCCUCCCGCCCGCUG	2407
R5414_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGUCCACCGAGGCAGC	2408
R5415_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCUUCCUGUCCACCGA	2409
R5416_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGUACCUCGCAAGCAC	2410
R5417_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGAGGUACCUGAAGCGG	2411
R5418_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCCUCCUCGGCCUCG	2412
R5419_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGCAGCACGUGGUACAG	2413
R5420_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCAGCACGUGGUACAGG	2414
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R5422_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGGCACCCGCCUCAC	2416
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R5424_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAGUACAUGUGCAUC	2418
R5425_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCGCCGCCUCCAAGG	2419
R5426_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGGCGGCGGGCCAAGA	2420
R5427_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCUGGACCUCCGCAG	2421
R5428_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCCUCUGGAUUGGGG	2422
R5429_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCCUCUGGAUUGGGGA	2423
R5430_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGAGCCUCGUGGGACU	2424

R5431_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUCUCCCC AUGCUGCUG	2425
R5432_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCUCUGC UGCCUGAAG	2426
R5433_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGCAGCA GAGGAGAAG	2427
R5434_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAAGGCUC GAUGGUGAA	2428
R5435_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAAAGGCU CGAUGGUGA	2429
R5436_CasPhi12_S	AUUGCUCCUUACGAGGAGACACCAUCGA GCCUUUCAA	2430
R5437_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUUUGAA AGGCUCGAU	2431
R5438_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGACUU GGCUUUGAA	2432
R5439_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAAAGCCA AGUCCCUGA	2433
R5440_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAAGCCAA GUCCCUGAA	2434
R5441_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACAUCCU UCAGGGACU	2435
R5442_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGGUCU UCCACAUCC	2436
R5443_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAGGUC UUCCACAUC	2437
R5444_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCGGAAG ACACAGCUG	2438
R5445_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGUCCCGA ACAGCAGGG	2439
R5446_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGUCCCG AACAGCAGG	2440
R5447_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUUAGGUC CCGAACAGC	2441
R5448_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUUUAGGU CCCGAACAG	2442
R5449_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGACCUA AAGAAACUG	2443
R5450_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGAAAGC CUGGGGGCC	2444
R5451_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGAAAG CCUGGGGGC	2445
R5452_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCCAAAC UGGUGCGGA	2446
R5453_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCAAACU GGUGCGGAU	2447
R5454_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCUCACU CAGCGCAUC	2448
R5455_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCUGGGG GAAGGUGGC	2449

R5456_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCCAGCU GAAGUCCUU	2450
R5457_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAAGGACU UCAGCUGGG	2451
R5458_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAAGGAC UUCAGCUGG	2452
R5459_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGUUUC CAAGGACUU	2453
R5460_CasPhi12_S	AUUGCUCCUUACGAGGAGACUAGGCACC CAGGUCAGU	2454
R5461_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUAGGCAC CCAGGUCAG	2455
R5462_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUCGCUG CAUCCCUGC	2456
R5463_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCUGAGC AGGGAUGCA	2457
R5464_CasPhi12_S	AUUGCUCCUUACGAGGAGACUACAAUAA CUGCAUCUG	2458
R5465_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUCGUGU GCUUCCGGA	2459
R5466_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGGACAUG GUGUCCCUC	2460
R5467_CasPhi12_S	AUUGCUCCUUACGAGGAGACACGGCUGC CGGGGCCCA	2461
R5468_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAGGUGU CCUCAUGUG	2462
R5469_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGACAC UGAAUGGGA	2463
R5470_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGUGUCCA GGAACACCU	2464
R5471_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGGUGUU CCUGGACAC	2465
R5472_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUGCAGGU GUUCCUGGA	2466
R5473_CasPhi12_S	AUUGCUCCUUACGAGGAGACACGGAUCA GCCUGAGAU	2467

TABLE AC: CasΦ.12 gRNAs targeting mouse PCSK9

Name	**Repeat+spacer RNA Sequence (5' --> 3')**	**SEQ ID NO**
R4238_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCGCUGUUGCCG CCGCU	1734
R4239_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCGCCGCUGCUG CUGCU	1735
R4240_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCUACUGUGC CCCAC	1736
R4241_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUAAUCUCCAUC CUCGU	1737

R4242_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGAAGAGCUGAUGCUCG	1738
R4243_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGCAACGGCGGAAGGU	1739
R4244_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGGCAGCCUCCAGGCC	1740
R4245_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGUGCUGAUGGAGGAG	1741
R4246_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAUCUGUAGCCUCUGGG	1742
R4247_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCAAUCUGUAGCCUCU	1743
R4248_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUUCAAUCUGUAGCCUC	1744
R4249_CasPhi12_S	AUUGCUCCUUACGAGGAGACAACAAACUGCCCACCGC	1745
R4250_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUGACAUAGCCCCGGCG	1746
R4251_CasPhi12_S	AUUGCUCCUUACGAGGAGACUACAUAUCUUUUAUGAC	1747
R4252_CasPhi12_S	AUUGCUCCUUACGAGGAGACUAUGACCUCUUCCCUGG	1748
R4253_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUGACCUCUUCCCUGGC	1749
R4254_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGACCUCUUCCCUGGCU	1750
R4255_CasPhi12_S	AUUGCUCCUUACGAGGAGACACCAAGAAGCCAGGGAA	1751
R4256_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUGGCUUCUUGGUGAA	1752
R4257_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUGGUGAAGAUGAGCAG	1753
R4258_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGAAGAUGAGCAGUGA	1754
R4259_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCCAUGUGGAGUACAU	1755
R4260_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCAAUGUACUCCACAU	1756
R4261_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGAAGACUCCUUUGUC	1757
R4262_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUCUUCGCCCAGAGCAU	1758
R4263_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUUCGCCCAGAGCAUC	1759
R4264_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCAGAGCAUCCCAUG	1760
R4265_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAUGGGAUGCUCUGGGC	1761
R4266_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUCCAGGUUCCAUGGG	1762

R4267_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCCAGCAUGGCACCAG	1763
R4268_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUCUGUCUGGUGCCAUG	1764
R4269_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAUACCAGCAUCCAGGG	1765
R4270_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGCAGGGUCACCAUC	1766
R4271_CasPhi12_S	AUUGCUCCUUACGAGGAGACAAGUCGGUGAUGGUGAC	1767
R4272_CasPhi12_S	AUUGCUCCUUACGAGGAGACAACAGCGUGCCGGAGGA	1768
R4273_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCACACCAGCAUCCCG	1769
R4274_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGCACACGCAGGCUGUG	1770
R4275_CasPhi12_S	AUUGCUCCUUACGAGGAGACACAGUUGAGCACACGCA	1771
R4276_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUUGACAGUUGAGCAC	1772
R4277_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCUGACUCUUCCGAAUA	1773
R4278_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUUCGGAAGAGUCAGCU	1774
R4279_CasPhi12_S	AUUGCUCCUUACGAGGAGACUUCGGAAGAGUCAGCUA	1775
R4280_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAAGAGUCAGCUAAUC	1776
R4281_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCUGCCCCUGGCCGGU	1777
R4282_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGAUGCGGCUAUACCC	1778
R4283_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCAGCUGCUGCAACCAG	1779
R4284_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCAGCUGGGAACUUC	1780
R4285_CasPhi12_S	AUUGCUCCUUACGAGGAGACCGGGACGACGCCUGCCU	1781
R4286_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGGCCCCGACUGUGAU	1782
R4287_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUUGGGGACUUUGGGG	1783
R4288_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUCCCCAAAGUCCCCAA	1784
R4289_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGACUUUGGGGACUAA	1785
R4290_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGACUAAUUUUGGAC	1786
R4291_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGACUAAUUUUGGACG	1787

R4292_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGACGCUGUGUGGAUC	1788
R4293_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGACGCUGUGUGGAUCU	1789
R4294_CasPhi12_S	AUUGCUCCUUACGAGGAGACGACGCUGUGUGGAUCUC	1790
R4295_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCGGGGGCAAAGAGAUC	1791
R4296_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCCCCGGGAAGGACAU	1792
R4297_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCCCGGGAAGGACAUC	1793
R4298_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUGUCACAGAGUGGGAC	1794
R4299_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGCUCGGAUGCUGAGC	1795
R4300_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCUGGCCGAGCUGCGG	1796
R4301_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUAGAGAAGUGGAUCAG	1797
R4302_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGUAGAGAAGUGGAUCA	1798
R4303_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCUACCAAAGACGUCAU	1799
R4304_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUGACGUCUUUGGUAGA	1800
R4305_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCUGAGGACCAGCAGGU	1801
R4306_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGUCAGCACCUGCUG	1802
R4307_CasPhi12_S	AUUGCUCCUUACGAGGAGACGAGUGGGCCCCGAGUGU	1803
R4308_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGGGCACAGCGGGCUG	1804
R4309_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCAGGAGCGGGAGGCG	1805
R4310_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGACCUGCUGGCCUCC	1806
R4311_CasPhi12_S	AUUGCUCCUUACGAGGAGACAGGGCCUUGCAGACCUG	1807
R4312_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGGUGAGGGUGUCUA	1808
R4313_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGGGUGAGGGUGUCUAU	1809
R4314_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCACGGGGAACCAGGCA	1810
R4315_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCCGUGCCAACUGCAGC	1811
R4316_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGAUGCUGCAGUUGGC	1812

R4317_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGGUGGCAGUGGACAUG	1813
R4318_CasPhi12_S	AUUGCUCCUUACGAGGAGACCACUUCCCAAUGGAAGC	1814
R4319_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAUUGGGAAGUGGAAGA	1815
R4320_CasPhi12_S	AUUGCUCCUUACGAGGAGACGGAAGUGGAAGACCUUA	1816
R4321_CasPhi12_S	AUUGCUCCUUACGAGGAGACGUGUCCGGAGGCAGCCU	1817
R4322_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCCACCAGGCGGCCAGU	1818
R4323_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGCUGCCAUGCCCCAG	1819
R4324_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAGCCCUGGGGCAUGGC	1820
R4325_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAUUCCAGCCCUGGGGC	1821
R4326_CasPhi12_S	AUUGCUCCUUACGAGGAGACGCAUUCCAGCCCUGGGG	1822
R4327_CasPhi12_S	AUUGCUCCUUACGAGGAGACUGCAUUCCAGCCCUGGG	1823
R4328_CasPhi12_S	AUUGCUCCUUACGAGGAGACAUUUUGCAUUCCAGCCC	1824
R4329_CasPhi12_S	AUUGCUCCUUACGAGGAGACCAUCCAGUCAGGGUCCA	1825
R4330_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCACGCUGUAGGCUCC	1826
R4331_CasPhi12_S	AUUGCUCCUUACGAGGAGACCCACACACAGGUUGUCC	1827
R4332_CasPhi12_S	AUUGCUCCUUACGAGGAGACUCCACUGGUCCUGUCUG	1828
R4333_CasPhi12_S	AUUGCUCCUUACGAGGAGACCUGAAGGCCGGCUCCGG	1829

TABLE AD: CasΦ.12 gRNAs targeting Bak1 in CHO cells

Name	Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA	SEQ ID NO
R2452 Bak1_CasPhi12_1_S	ATTGCTCCTTACGAGGAGACGAAGCTATGTTTTCCAT	1830
R2453 Bak1_CasPhi12_2_S	ATTGCTCCTTACGAGGAGACGCAGGGGCAGCCGCCCC	1831
R2454 Bak1_CasPhi12_3_S	ATTGCTCCTTACGAGGAGACCTCCTAGAACCCAACAG	1832
R2455 Bak1_CasPhi12_4_S	ATTGCTCCTTACGAGGAGACGAAAGACCTCCTCTGTG	1833
R2456 Bak1_CasPhi12_5_S	ATTGCTCCTTACGAGGAGACTCCATCTCGGGGTTGGC	1834

R2457 Bak1_CasPhi12_6_S	ATTGCTCCTTACGAGGAGACTTCCTGATGGTGGAGAT	1835
R2849_Bak1_CasPhi12_nsd_sg1_S	ATTGCTCCTTACGAGGAGACCTGACTCCCAGCTCTGA	1836
R2850_Bak1_CasPhi12_nsd_sg2_S	ATTGCTCCTTACGAGGAGACTGGGGTCAGAGCTGGGA	1837
R2851_Bak1_CasPhi12_nsd_sg3_S	ATTGCTCCTTACGAGGAGACGAAAGACCTCCTCTGTG	1838
R2852_Bak1_CasPhi12_nsd_sg4_S	ATTGCTCCTTACGAGGAGACCGAAGCTATGTTTTCCA	1839
R2853_Bak1_CasPhi12_nsd_sg5_S	ATTGCTCCTTACGAGGAGACGAAGCTATGTTTTCCAT	1840
R2854_Bak1_CasPhi12_nsd_sg6_S	ATTGCTCCTTACGAGGAGACTCCATCTCCACCATCAG	1841
R2855_Bak1_CasPhi12_nsd_sg7_S	ATTGCTCCTTACGAGGAGACCCATCTCCACCATCAGG	1842
R2856_Bak1_CasPhi12_nsd_sg8_S	ATTGCTCCTTACGAGGAGACCTGATGGTGGAGATGGA	1843
R2857_Bak1_CasPhi12_nsd_sg9_S	ATTGCTCCTTACGAGGAGACCATCTCCACCATCAGGA	1844
R2858_Bak1_CasPhi12_nsd_sg10_S	ATTGCTCCTTACGAGGAGACTTCCTGATGGTGGAGAT	1845
R2859_Bak1_CasPhi12_nsd_sg11_S	ATTGCTCCTTACGAGGAGACGCAGGGGCAGCCGCCCC	1846
R2860_Bak1_CasPhi12_nsd_sg12_S	ATTGCTCCTTACGAGGAGACTCCATCTCGGGGTTGGC	1847
R2861_Bak1_CasPhi12_nsd_sg13_S	ATTGCTCCTTACGAGGAGACTAGGAGCAAATTGTCCA	1848
R2862_Bak1_CasPhi12_nsd_sg14_S	ATTGCTCCTTACGAGGAGACGGTTCTAGGAGCAAATT	1849
R2863_Bak1_CasPhi12_nsd_sg15_S	ATTGCTCCTTACGAGGAGACGCTCCTAGAACCCAACA	1850
R2864_Bak1_CasPhi12_nsd_sg16_S	ATTGCTCCTTACGAGGAGACCTCCTAGAACCCAACAG	1851
R3977 Bak1_CasPhi12_exon1_sg1_S	ATTGCTCCTTACGAGGAGACTCCAGACGCCATCTTTC	1852
R3978 Bak1_CasPhi12_exon1_sg2_S	ATTGCTCCTTACGAGGAGACTGGTAAGAGTCCTCCTG	1853
R3979 Bak1_CasPhi12_exon3_sg1_S	ATTGCTCCTTACGAGGAGACTTACAGCATCTTGGGTC	1854
R3980 Bak1_CasPhi12_exon3_sg2_S	ATTGCTCCTTACGAGGAGACGGTCAGGTGGGCCGGCA	1855
R3981 Bak1_CasPhi12_exon3_sg3_S	ATTGCTCCTTACGAGGAGACCTATCATTGGAGATGAC	1856

R3982 Bak1_ CasPhi12_exon3_sg4_ S	ATTGCTCCTTACGAGGAGACGAGATGACATT AACCGG	1857
R3983 Bak1_ CasPhi12_exon3_sg5_ S	ATTGCTCCTTACGAGGAGACTGGAACTCTGT GTCGTA	1858
R3984 Bak1_ CasPhi12_exon3_sg6_ S	ATTGCTCCTTACGAGGAGACCAGAATTTACT GGAGCA	1859
R3985 Bak1_ CasPhi12_exon3_sg7_ S	ATTGCTCCTTACGAGGAGACACTGGAGCAGC TGCAGC	1860
R3986 Bak1_ CasPhi12_exon3_sg8_ S	ATTGCTCCTTACGAGGAGACCCAGCTGTGGG CTGCAG	1861
R3987 Bak1_ CasPhi12_exon3_sg9_ S	ATTGCTCCTTACGAGGAGACGTAGGCATTCC CAGCTG	1862
R3988 Bak1_ CasPhi12_exon3_sg10 _S	ATTGCTCCTTACGAGGAGACGTGAAGAGTTC GTAGGC	1863
R3989 Bak1_ CasPhi12_exon3_sg11 _S	ATTGCTCCTTACGAGGAGACACCAAGATTGC CTCCAG	1864
R3990 Bak1_ CasPhi12_exon3_sg12 _S	ATTGCTCCTTACGAGGAGACCCTCCAGGTAC CCACCA	1865

TABLE AE: CasΦ.12 gRNAs targeting Bax in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA)**	**SEQ ID NO**
R2458 Bax_CasPhi12_1_S	ATTGCTCCTTACGAGGAGACCTAATGTGGAT ACTAAC	1866
R2459 Bax_CasPhi12_2_S	ATTGCTCCTTACGAGGAGACTTCCGTGTGGC AGCTGA	1867
R2460 Bax_CasPhi12_3_S	ATTGCTCCTTACGAGGAGACCTGATGGCAAC TTCAAC	1868
R2461 Bax_CasPhi12_4_S	ATTGCTCCTTACGAGGAGACTACTTTGCTAGC AAACT	1869
R2462 Bax_CasPhi12_5_S	ATTGCTCCTTACGAGGAGACAGCACCAGTTT GCTAGC	1870
R2463 Bax_CasPhi12_6_S	ATTGCTCCTTACGAGGAGACAACTGGGGCCG GGTTGT	1871
R2865_Bax_CasPhi12 _nsd_sg1_S	ATTGCTCCTTACGAGGAGACTTCTCTTTCCTG TAGGA	1872
R2866_Bax_CasPhi12 _nsd_sg2_S	ATTGCTCCTTACGAGGAGACTCTTTCCTGTAG GATGA	1873
R2867_Bax_ CasPhi12_nsd_sg3_S	ATTGCTCCTTACGAGGAGACCCTGTAGGATG ATTGCT	1874

R2868_Bax_ CasPhi12_nsd_sg4_S	ATTGCTCCTTACGAGGAGACCTGTAGGATGA TTGCTA	1875
R2869_Bax_ CasPhi12_nsd_sg5_S	ATTGCTCCTTACGAGGAGACCTAATGTGGAT ACTAAC	1876
R2870_Bax_ CasPhi12_nsd_sg6_S	ATTGCTCCTTACGAGGAGACTTCCGTGTGGC AGCTGA	1877
R2871_Bax_ CasPhi12_nsd_sg7_S	ATTGCTCCTTACGAGGAGACCGTGTGGCAGC TGACAT	1878
R2872_Bax_ CasPhi12_nsd_sg8_S	ATTGCTCCTTACGAGGAGACCCATCAGCAAA CATGTC	1879
R2873_Bax_ CasPhi12_nsd_sg9_S	ATTGCTCCTTACGAGGAGACAAGTTGCCATC AGCAAA	1880
R2874_Bax_ CasPhi12_nsd_sg10_S	ATTGCTCCTTACGAGGAGACGCTGATGGCAA CTTCAA	1881
R2875_Bax_ CasPhi12_nsd_sg11_S	ATTGCTCCTTACGAGGAGACCTGATGGCAAC TTCAAC	1882
R2876_Bax_ CasPhi12_nsd_sg12_S	ATTGCTCCTTACGAGGAGACAACTGGGGCCG GGTTGT	1883
R2877_Bax_ CasPhi12_nsd_sg13_S	ATTGCTCCTTACGAGGAGACTTGCCCTTTTCT ACTTT	1884
R2878_Bax_ CasPhi12_nsd_sg14_S	ATTGCTCCTTACGAGGAGACCCCTTTTCTACT TTGCT	1885
R2879_Bax_ CasPhi12_nsd_sg15_S	ATTGCTCCTTACGAGGAGACCTAGCAAAGTA GAAAAG	1886
R2880_Bax_ CasPhi12_nsd_sg16_S	ATTGCTCCTTACGAGGAGACGCTAGCAAAGT AGAAAA	1887
R2881_Bax_ CasPhi12_nsd_sg17_S	ATTGCTCCTTACGAGGAGACTCTACTTTGCTA GCAAA	1888
R2882_Bax_ CasPhi12_nsd_sg18_S	ATTGCTCCTTACGAGGAGACCTACTTTGCTAG CAAAC	1889
R2883_Bax_ CasPhi12_nsd_sg19_S	ATTGCTCCTTACGAGGAGACTACTTTGCTAGC AAACT	1890
R2884_Bax_ CasPhi12_nsd_sg20_S	ATTGCTCCTTACGAGGAGACGCTAGCAAACT GGTGCT	1891
R2885_Bax_ CasPhi12_nsd_sg21_S	ATTGCTCCTTACGAGGAGACCTAGCAAACTG GTGCTC	1892
R2886_Bax_ CasPhi12_nsd_sg22_S	ATTGCTCCTTACGAGGAGACAGCACCAGTTT GCTAGC	1893

TABLE AF: CasΦ.12 gRNAs targeting Fut8 in CHO cells

Name	**Repeat+spacer RNA Sequence (5' --> 3'), shown as DNA)**	**SEQ ID NO**
R2464 Fut8_CasPhi12_1_S	ATTGCTCCTTACGAGGAGACCCACTTTGTCA GTGCGT	1894
R2465 Fut8_CasPhi12_2_S	ATTGCTCCTTACGAGGAGACCTCAATGGGAT GGAAGG	1895
R2466 Fut8_CasPhi12_3_S	ATTGCTCCTTACGAGGAGACAGGAATACATG GTACAC	1896

R2467 Fut8_CasPhi12_4_S	ATTGCTCCTTACGAGGAGACAAGAACATTTTCAGCTT	1897
R2468 Fut8_CasPhi12_5_S	ATTGCTCCTTACGAGGAGACATCCACTTTCATTCTGC	1898
R2469 Fut8_CasPhi12_6_S	ATTGCTCCTTACGAGGAGACTTTGTTAAAGGAGGCAA	1899
R2887_Fut8_CasPhi12_nsd_sg1_S	ATTGCTCCTTACGAGGAGACTCCCCAGAGTCCATGTC	1900
R2888_Fut8_CasPhi12_nsd_sg2_S	ATTGCTCCTTACGAGGAGACTCAGTGCGTCTGACATG	1901
R2889_Fut8_CasPhi12_nsd_sg3_S	ATTGCTCCTTACGAGGAGACGTCAGTGCGTCTGACAT	1902
R2890_Fut8_CasPhi12_nsd_sg4_S	ATTGCTCCTTACGAGGAGACCCACTTTGTCAGTGCGT	1903
R2891_Fut8_CasPhi12_nsd_sg5_S	ATTGCTCCTTACGAGGAGACTGTTCCCACTTTGTCAG	1904
R2892_Fut8_CasPhi12_nsd_sg6_S	ATTGCTCCTTACGAGGAGACCTCAATGGGATGGAAGG	1905
R2893_Fut8_CasPhi12_nsd_sg7_S	ATTGCTCCTTACGAGGAGACCATCCCATTGAGGAATA	1906
R2894_Fut8_CasPhi12_nsd_sg8_S	ATTGCTCCTTACGAGGAGACAGGAATACATGGTACAC	1907
R2895_Fut8_CasPhi12_nsd_sg9_S	ATTGCTCCTTACGAGGAGACAACGTGTACCATGTATT	1908
R2896_Fut8_CasPhi12_nsd_sg10_S	ATTGCTCCTTACGAGGAGACTTCAACGTGTACCATGT	1909
R2897_Fut8_CasPhi12_nsd_sg11_S	ATTGCTCCTTACGAGGAGACAAGAACATTTTCAGCTT	1910
R2898_Fut8_CasPhi12_nsd_sg12_S	ATTGCTCCTTACGAGGAGACGAGAAGCTGAAAATGTT	1911
R2899_Fut8_CasPhi12_nsd_sg13_S	ATTGCTCCTTACGAGGAGACTCAGCTTCTCGAACGCA	1912
R2900_Fut8_CasPhi12_nsd_sg14_S	ATTGCTCCTTACGAGGAGACCAGCTTCTCGAACGCAG	1913
R2901_Fut8_CasPhi12_nsd_sg15_S	ATTGCTCCTTACGAGGAGACTGCGTTCGAGAAGCTGA	1914
R2902_Fut8_CasPhi12_nsd_sg16_S	ATTGCTCCTTACGAGGAGACAGCTTCTCGAACGCAGA	1915
R2903_Fut8_CasPhi12_nsd_sg17_S	ATTGCTCCTTACGAGGAGACATTCTGCGTTCGAGAAG	1916
R2904_Fut8_CasPhi12_nsd_sg18_S	ATTGCTCCTTACGAGGAGACCATTCTGCGTTCGAGAA	1917
R2905_Fut8_CasPhi12_nsd_sg19_S	ATTGCTCCTTACGAGGAGACTCGAACGCAGAATGAAA	1918
R2906_Fut8_CasPhi12_nsd_sg20_S	ATTGCTCCTTACGAGGAGACATCCACTTTCATTCTGC	1919
R2907_Fut8_CasPhi12_nsd_sg21_S	ATTGCTCCTTACGAGGAGACTATCCACTTTCATTCTG	1920
R2908_Fut8_CasPhi12_nsd_sg22_S	ATTGCTCCTTACGAGGAGACTTATCCACTTTCATTCT	1921

R2909_Fut8_CasPhi12_nsd_sg23_S	ATTGCTCCTTACGAGGAGACTTTATCCACTTTCATTC	1922
R2910_Fut8_CasPhi12_nsd_sg24_S	ATTGCTCCTTACGAGGAGACTTTTATCCACTTTCATT	1923
R2911_Fut8_CasPhi12_nsd_sg25_S	ATTGCTCCTTACGAGGAGACAACAAAGAAGGGTCATC	1924
R2912_Fut8_CasPhi12_nsd_sg26_S	ATTGCTCCTTACGAGGAGACCCTCCTTTAACAAAGAA	1925
R2913_Fut8_CasPhi12_nsd_sg27_S	ATTGCTCCTTACGAGGAGACGCCTCCTTTAACAAAGA	1926
R2914_Fut8_CasPhi12_nsd_sg28_S	ATTGCTCCTTACGAGGAGACTTTGTTAAAGGAGGCAA	1927
R2915_Fut8_CasPhi12_nsd_sg29_S	ATTGCTCCTTACGAGGAGACGTTAAAGGAGGCAAAGA	1928
R2916_Fut8_CasPhi12_nsd_sg30_S	ATTGCTCCTTACGAGGAGACTTAAAGGAGGCAAAGAC	1929
R2917_Fut8_CasPhi12_nsd_sg31_S	ATTGCTCCTTACGAGGAGACTCTTTGCCTCCTTTAAC	1930
R2918_Fut8_CasPhi12_nsd_sg32_S	ATTGCTCCTTACGAGGAGACGTCTTTGCCTCCTTTAA	1931
R2919_Fut8_CasPhi12_nsd_sg33_S	ATTGCTCCTTACGAGGAGACGTCTAACTTACTTTGTC	1932
R2920_Fut8_CasPhi12_nsd_sg34_S	ATTGCTCCTTACGAGGAGACTTGGTCTAACTTACTTT	1933

TABLE AG: CasΦ.12 gRNAs targeting Fut8

Name	**Repeat length**	**Spacer length**	**Repeat sequence** (5' --> 3')	**Spacer sequence** (5' --> 3')	**crRNA sequence** (5' --> 3')
R3582	36	30	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAUGGUACACGUUGAAGAACAUU (SEQ ID NO: 1482)	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUUGAAGAACAUU (SEQ ID NO:1499)
R3583	36	29	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAUGGUACACGUUGAAGAACAU (SEQ ID NO: 1483)	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUUGAAGAACAU (SEQ ID NO:1500)
R3584	36	28	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC	AGGAAUACAUGGUACACGUUGAAGAACA	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA

			(SEQ ID NO: 2469)	(SEQ ID NO: 1484)	CGUUGAAGAACA (SEQ ID NO:1501)
R3585	36	27	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GAAGAAC (SEQ ID NO: 1485)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAAC (SEQ ID NO:1502)
R3586	36	26	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GAAGAA (SEQ ID NO: 1486)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAA (SEQ ID NO:1503)
R3587	36	25	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GAAGA (SEQ ID NO: 1487)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGA (SEQ ID NO:1504)
R3588	36	24	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GAAG (SEQ ID NO: 1488)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAG (SEQ ID NO:1505)
R3589	36	23	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GAA (SEQ ID NO: 1489)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAA (SEQ ID NO:1506)
R3590	36	22	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU GA (SEQ ID NO: 1490)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGA (SEQ ID NO:1507)
R3591	36	21	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC	AGGAAUACAU GGUACACGUU	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA

			(SEQ ID NO: 2469)	G (SEQ ID NO: 1491)	CGUUG (SEQ ID NO:1508)
R3592	36	20	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1492)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUU (SEQ ID NO:1509)
R3593	36	19	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACGU (SEQ ID NO: 1493)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGU (SEQ ID NO:1510)
R3594	36	18	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACACG (SEQ ID NO: 1494)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CG (SEQ ID NO:1511)
R3595	36	17	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACAC (SEQ ID NO: 1495)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA C (SEQ ID NO:1512)
R3596	36	16	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUACA (SEQ ID NO: 1496)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA (SEQ ID NO:1513)
R3597	36	15	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 2469)	AGGAAUACAU GGUAC (SEQ ID NO: 1497)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUAC (SEQ ID NO:1514)
R3598	35	20	UUUCAAGAC UAAUAGAUU GCUCCUUAC GAGGAGAC	AGGAAUACAU GGUACACGUU	UUUCAAGACUAAU AGAUUGCUCCUUA CGAGGAGACAGGA AUACAUGGUACAC

			(SEQ ID NO: 1466)	(SEQ ID NO: 1498)	GUU (SEQ ID NO:1515)
R3599	34	20	UUCAAGACU AAUAGAUUG CUCCUUACG AGGAGAC (SEQ ID NO: 1467)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	UUCAAGACUAAUA GAUUGCUCCUUAC GAGGAGACAGGAA UACAUGGUACACG UU (SEQ ID NO:1516)
R3600	33	20	UCAAGACUA AUAGAUUGC UCCUUACGA GGAGAC (SEQ ID NO: 1468)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	UCAAGACUAAUAG AUUGCUCCUUACG AGGAGACAGGAAU ACAUGGUACACGU U (SEQ ID NO:1517)
R3601	32	20	CAAGACUAA UAGAUUGCU CCUUACGAG GAGAC (SEQ ID NO: 1469)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	CAAGACUAAUAGA UUGCUCCUUACGA GGAGACAGGAAUA CAUGGUACACGUU (SEQ ID NO:1518)
R3602	31	20	AAGACUAAU AGAUUGCUC CUUACGAGG AGAC (SEQ ID NO: 1470)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	AAGACUAAUAGAU UGCUCCUUACGAG GAGACAGGAAUAC AUGGUACACGUU (SEQ ID NO:1519)
R3603	30	20	AGACUAAUA GAUUGCUCC UUACGAGGA GAC (SEQ ID NO: 1471)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	AGACUAAUAGAUU GCUCCUUACGAGG AGACAGGAAUACA UGGUACACGUU (SEQ ID NO:1520)
R3604	29	20	GACUAAUAG AUUGCUCCU UACGAGGAG AC (SEQ ID NO: 1472)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	GACUAAUAGAUUG CUCCUUACGAGGA GACAGGAAUACAU GGUACACGUU (SEQ ID NO:1521)
R3605	28	20	ACUAAUAGA UUGCUCCUU ACGAGGAGA C (SEQ ID NO: 1473)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	ACUAAUAGAUUGC UCCUUACGAGGAG ACAGGAAUACAUG GUACACGUU (SEQ ID NO:1522)
R3606	27	20	CUAAUAGAU UGCUCCUUA CGAGGAGAC	AGGAAUACAU GGUACACGUU	CUAAUAGAUUGCU CCUUACGAGGAGA CAGGAAUACAUGG

			(SEQ ID NO: 1474)	(SEQ ID NO: 1498)	UACACGUU (SEQ ID NO:1523)
R3607	26	20	UAAUAGAUU GCUCCUUAC GAGGAGAC (SEQ ID NO: 1475)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	UAAUAGAUUGCUC CUUACGAGGAGAC AGGAAUACAUGGU ACACGUU (SEQ ID NO:1524)
R3608	25	20	AAUAGAUUG CUCCUUACG AGGAGAC (SEQ ID NO: 1476)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AAUAGAUUGCUCC UUACGAGGAGACA GGAAUACAUGGUA CACGUU (SEQ ID NO:1525)
R3609	24	20	AUAGAUUGC UCCUUACGA GGAGAC (SEQ ID NO: 1477)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AUAGAUUGCUCCU UACGAGGAGACAG GAAUACAUGGUAC ACGUU (SEQ ID NO:1526)
R3610	23	20	UAGAUUGCU CCUUACGAG GAGAC (SEQ ID NO: 1478)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUU (SEQ ID NO:1527)
R3611	22	20	AGAUUGCUC CUUACGAGG AGAC (SEQ ID NO: 1479)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AGAUUGCUCCUUA CGAGGAGACAGGA AUACAUGGUACAC GUU (SEQ ID NO:1528)
R3612	21	20	GAUUGCUCC UUACGAGGA GAC (SEQ ID NO: 1480)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	GAUUGCUCCUUAC GAGGAGACAGGAA UACAUGGUACACG UU (SEQ ID NO:1529)
R3613	20	20	AUUGCUCCU UACGAGGAG AC (SEQ ID NO: 1481)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU	AUUGCUCCUUACG AGGAGACAGGAAU ACAUGGUACACGU U (SEQ ID NO:1530)

				(SEQ ID NO: 2487)	

TABLE AH: CasΦ.12 gRNAs targeting B2M and TRAC

Name	**Target**	**Modification**	**Repeat sequence** (5' --> 3')	**Spacer sequence** (5' --> 3')	**crRNA sequence** (5' --> 3')
R3150 20-20	B2M Exon 2	Unmodified, 2'OMe at last 3' base (1me) 2'OMe at last two 3' bases (2me) 2'OMe at last three 3' bases (3me)	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCAG UG (SEQ ID NO: 1434)	AUUGCUCCUUAC GAGGAGACCAG UGGGGGUGAAU UCAGUG (SEQ ID NO: 1435)
R3042 20-20	TRAC Exon 1	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	GAGUCUCUC AGCUGGUAC AC (SEQ ID NO: 1436)	AUUGCUCCUUAC GAGGAGACGAG UCUCUCAGCUGG UACAC (SEQ ID NO: 1437)
R3150 20-17	B2M Exon 2	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCA (SEQ ID NO: 1438)	AUUGCUCCUUAC GAGGAGACCAG UGGGGGUGAAU UCA (SEQ ID NO: 1439)
R3042 20-17	TRAC Exon 1	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCA (SEQ ID NO: 1440)	AUUGCUCCUUAC GAGGAGACGAG UCUCUCAGCUGG UA (SEQ ID NO: 1441)

[0248] In some embodiments, the guide nucleic acid comprises a spacer sequence that is the same as or differs by no more than 5 nucleotides from a spacer sequence from Tables A to H by no more than 4 nucleotides from a spacer sequence from Tables A to H, by no more than 3 nucleotides from a spacer sequence from Tables A to H, no more than 2 nucleotides from a spacer sequence from Tables A to H, or no more than 1 nucleotide from a spacer sequence from

Tables A to H. A difference may be addition, deletion or substitution and where there are multiple differences, the differences may be addition, deletion and/or substitution.

[0249] In some embodiments, the guide nucleic acid comprisies a sequence that is the same as or differs by no more than 5 nucleotides from a sequence from Tables I to AH by no more than 4 nucleotides from a sequence from Tables I to AH, by no more than 3 nucleotides from a sequence from Tables I to X, no more than 2 nucleotides from a sequence from Table I to AH, or no more than 1 nucleotide from a sequence from Tables I to AH. A difference may be addition, deletion or substitution and where there are multiple differences, the differences may be addition, deletion and/or substitution.

[0250] In some embodiments, the guide nucleic acid comprises a sequence that is at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, at least 41, at least 42, at least 43, at least 44, at least 45, at least 46, at least 47, at least 48, at least 49, at least 50, at least 51, at least 52, at least 53, at least 54, at least 55, at least 56 or at least 57 contiguous nucleobases of a sequence from Tables I to X, AG and AH (SEQ ID NO: 547-1404, 1433-1441, 1466-1530 or 2112-2289).

[0251] In some embodiments, the guide nucleic acid comprises a sequence that is 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 or 57 contiguous nucleobases of a sequence from Tables I to X, AG and AH (SEQ ID NO: 547-1404, 1433-1441, 1466-1530 or 2112-2289).

[0252] In some embodiments, the guide nucleic acid comprises a sequence that is at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36 or at least 37 contiguous nucleobases of a sequence from Tables Y to AF (SEQ ID NO: 1533-1933 or 2290-2467).

[0253] In some embodiments, the guide nucleic acid comprises a sequence that is 30, 31, 32, 33, 34, 35, 36 or 37 contiguous nucleobases of a sequence from Tables Y to AF (SEQ ID NO: 1533-1933 or 2290-2467).

[0254] In some embodiments, the guide nucleic acid comprises a repeat sequence from Table 2 and a spacer sequence from Tables A to H

[0255] In the sequences provided in Tables A-AH, the base T is interchangeable with U when a guide nucleic either is or comprises ribonucleic or deoxyribonucleic nucleosides.

Coding sequences and expression vectors

[0256] In some aspects, the present disclosure provides a nucleic acid encoding a programmable CasΦ nuclease disclosed herein. In some embodiments, the nucleic acid is a vector, preferably the vector is an expression vector. Suitable expression vectors are easily identifiable for the cell type of interest. For example, an expression vector comprises a suitable promoter for

transcription in the cell type of interest. An expression vector can also include other elements to support transcription, such as a Woodchuck Hepatitis Virus (WHP) Posttranscriptional regulatory Element (WPRE).

[0257] In some embodiments, a nucleic acid encoding a programmable CasΦ nuclease *(e.g.* within an expression vector) comprises elements suitable for expression in a eukaryotic cell. In some embodiments, the nucleic acid comprises a promoter suitable for transcription in a eukaryotic cell *e.g.* containing a TATA box and/or a TFIIB recognition element. The nucleic acid *(e.g.* within an expression vector) will typically include a promoter suitable for transcription in a eukaryotic cell upstream of the sequence encoding the programmable CasΦ nuclease, and may include a transcription terminator downstream of the sequence encoding the programmable CasΦ nuclease. The nucleic acid *(e.g.* within an expression vector) may also include enhancer(s) upstream and/or downstream of the sequence encoding the programmable CasΦ nuclease. A promoter may be an inducible promoter. The nucleic acid may also comprise a guide RNA. Suitable promoters are well known in the art and include the CMV promoter, EF1a promoter, intron-less EF1a short promoter, SV40 promoter, human or mouse PGK1 promoter, Ubc (ubiquitin C) promoter and mouse or human U6 promoter. Suitable mammalian promoters include the EF1a promoter, intron-less EF1a short promoter, and human U6 promoter.

[0258] In some embodiments, the vector is a viral vector. In some embodiments, the vector is a retroviral vector or a lentiviral vector. In preferred embodiments, the vector is an adeno-associated viral (AAV) vector. Several serotypes are available for AAV vectors that can be used in the compositions and methods disclosed herein, including AAV1, AAV2, AAV5, AAV6, AAV8, AAV9 and AAV DJ. In more preferred emodiments, the AAV vector is an AAV DJ vector.

[0259] A vector may be integrated into a host cell genome.

[0260] In some embodiments, a vector comprises a nucleic acid encoding a programmable CasΦ nuclease. In some embodiments, a vector comprises a nucleic acid encoding a guide nucleic acid. In some embodiments, a vector comprises a donor polynucleotide. In some embodiments, a nucleic acid encoding a programmable CasΦ nuclease, a nucleic acid encoding a guide nucleic acid and a donor polynucleotide are comprised by separate vectors. In some embodiments, a vector comprises a nucleic acid encoding a programmable CasΦ nuclease and a nucleic acid encoding a guide nucleic acid.

[0261] It is well known in the field that the large size of Cas9 nucleases makes Cas9 impractical for several applications. For example, packaging vectors into viral particles becomes more difficult as the size of the vector increases. It is therefore difficult to include other components in a viral vector that includes a nucleic acid encoding a Cas9 nuclease. Accordingly, one of the advantages of the programmable CasΦ nucleases disclosed herein arises from the smaller size of

the programmable CasΦ nucleases which allows vectors comprising a nucleic acid encoding a programmable CasΦ nuclease to be easily packaged into viral particles when the vector also includes nucleic acids encoding other components, such a nucleic acid encoding a guide nucleic acid and/or donor polynucleotide. In preferred embodiments, a vector encodes a nucleic acid encoding a programmable CasΦ nuclease and a nucleic acid encoding a guide nucleic acid. In preferred embodiments, a vector encodes a nucleic acid encoding a programmable CasΦ nuclease, a nucleic acid encoding a guide nucleic acid and a donor polynucleotide. In some preferred embodiments, a vector comprises up to 1 kb donor polynucleotide, a promoter for expression of a guide nucleic acid, a nucleic acid encoding the nucleic acid, a mammalian promoter for expression of a programmable CasΦ nuclease, a nucleic acid encoding the programmable CasΦ nuclease, and a polyA signal. In alternative preferred embodiments, the donor polynucleotide is included in a nucleic acid encoding a tag, such as a fluorescent protein. In further preferred embodiments, the programmable CasΦ nuclease encoded by the vector is fuzed or linked to two nuclear localization signals.

[0262] In some embodiments, the expression vector comprises elements suitable for expression in a prokaryotic cell. In some embodiments, the expression vector comprises a promoter suitable for transcription in a prokaryotic cell *e.g.* comprising a Shine Dalgarno sequence.

[0263] In some embodiments, a CasΦ nuclease, a guide nucleic acid, or a nucleic acid encoding any combination thereof, may be inserted into a host cell by manner of electroporation, nucleofection, chemical methods, transfection, transduction, transformation, or microinjection. In some embodiments, a CasΦ nuclease, a guide nucleic acid, or a nucleic acid encoding any combination thereof, may be introduced into a cell by squeezing the cell to deform it, thereby disrupting the cell membrane and allowing the CasΦ nuclease, the guide nucleic acid, or the nucleic acid encoding any combination thereof, to pass into the cell.

[0264] In some embodiments, an Amaxa 4D nucleofector may be used to carry out nucleofection. In some embodiments, the chemical method or transfection comprises lipofectamine.

[0265] Lipid nanoparticle (LNP) delivery is one of the most clinically advanced non-viral delivery systems for gene therapy. LNPs have many properties that make them ideal candidates for delivery of nucleic acids, including ease of manufacture, low cytotoxicity and immunogenicity, high efficiency of nucleic acid encapsulation and cell transfection, multidosing capabilities and flexibility of design (Kulkarni *et al.,* (2018) *Nucleic Acid Therapeutics*). In some embodiments, LNP is used to deliver a nucleic acid encoding a programmable CasΦ nuclease described herein. In some embodiments, LNP is used to deliver a nucleic acid encoding a guide nucleic acid. In some embodiments, LNP is used to deliver a nucleic acid encoding encoding a programmable CasΦ nuclease and a guide nucleic acid. In some embodiments, the LNP has an

amine group to phosphate (N/P) ratio of between 2 and 10, between 3 and 10, or between 5 and 9. In preferred embodiments, the LNP has a N/P ratio of between 5 and 9. In more preferred embodiments, the LNP has a N/P ratio of 5. In some embodiments, the LNP additional components, *e.g.*, nucleic acids, proteins, peptides, small molecules, sugars, lipids.

[0266] In more preferred embodiments, the LNP has a N/P ratio of 4 to 5. In preferred embodiments, the LNP comprises a nucleic acid encoding a programmable CasΦ nuclease, and the LNP has an N/P ratio of 4 to 5.

Target Nucleic Acid and Sample

[0267] A wide array of samples is compatible with the compositions and methods disclosed herein. The samples, as described herein, may be used in the methods of nicking a target nucleic acid disclosed herein. The samples, as described herein, may be used in the DETECTR assay methods disclosed herein. The samples, as described herein, are compatible with any of the programmable nucleases disclosed herein and use of said programmable nuclease in a method of detecting a target nucleic acid. The samples, as described herein, are compatible with any of the compositions comprising a programmable nuclease and a buffer. Described herein are samples that contain deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or both, which can be modified or detected using a programmable nuclease of the present disclosure. As described herein, programmable nucleases are activated upon binding to a target nucleic acid of interest in a sample upon hybridization of a guide nucleic acid to the target nucleic acid. Subsequently, the activated programmable nucleases exhibit sequence-independent cleavage of a nucleic acid in a reporter. The reporter additionally includes a detectable moiety, which is released upon sequence-independent cleavage of the nucleic acid in the reporter. The detectable moiety emits a detectable signal, which can be measured by various methods (e.g., spectrophotometry, fluorescence measurements, electrochemical measurements).

[0268] Various sample types comprising a target nucleic acid of interest are consistent with the present disclosure. These samples can comprise a target nucleic acid sequence for detection. In some embodiments, the detection of the target nucleic indicates an ailment, such as a disease, cancer, or genetic disorder, or genetic information, such as for phenotyping, genotyping, or determining ancestry and are compatible with the reagents and support mediums as described herein. Generally, a sample from an individual or an animal or an environmental sample can be obtained to test for presence of a disease, cancer, genetic disorder, or any mutation of interest. A biological sample from the individual may be blood, serum, plasma, saliva, urine, mucosal sample, peritoneal sample, cerebrospinal fluid, gastric secretions, nasal secretions, sputum, pharyngeal exudates, urethral or vaginal secretions, an exudate, an effusion, or tissue. A tissue sample may be dissociated or liquified prior to application to detection system of the present disclosure. A sample from an environment may be from soil, air, or water. In some instances, the

environmental sample is taken as a swab from a surface of interest or taken directly from the surface of interest. In some instances, the raw sample is applied to the detection system. In some instances, the sample is diluted with a buffer or a fluid or concentrated prior to application to the detection system or be applied neat to the detection system. Sometimes, the sample is contained in no more 20 µl. The sample, in some cases, is contained in no more than 1, 5, 10, 15, 20, 25, 30, 35 40, 45, 50, 55, 60, 65, 70, 75, 80, 90, 100, 200, 300, 400, 500 µl, or any of value from 1 µl to 500 µl, preferably from 10 µL to 200 µL, or more preferably from 50 µL to 100 µL. Sometimes, the sample is contained in more than 500 µl.

[0269] In some embodiments, the target nucleic acid is single-stranded DNA. The methods, reagents, enzymes, and kits disclosed herein may enable the direct detection of a DNA encoding a sequence of interest, in particular a single-stranded DNA encoding a sequence of interest, without transcribing the DNA into RNA, for example, by using an RNA polymerase. The compositions and methods disclosed herein may enable the detection of target nucleic acid that is an amplified nucleic acid of a nucleic acid of interest. In some embodiments, the target nucleic acid is a cDNA, genomic DNA, an amplicon of genomic DNA or a DNA amplicon of an RNA. A nucleic acid can encode a sequence from a genomic locus. In some cases, the target nucleic acid that binds to the guide nucleic acid is from 5 to 100, 5 to 90, 5 to 80, 5 to 70, 5 to 60, 5 to 50, 5 to 40, 5 to 30, 5 to 25, 5 to 20, 5 to 15, or 5 to 10 nucleotides in length. The nucleic acid can be from 10 to 90, from 20 to 80, from 30 to 70, or from 40 to 60 nucleotides in length. A nucleic acid can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 60, 70, 80, 90, or 100 nucleotides in length. The target nucleic acid can encode a sequence reverse complementary to a guide nucleic acid sequence.

[0270] In some instances, the sample is taken from single-cell eukaryotic organisms; a plant or a plant cell; an algal cell; a fungal cell; an animal cell, tissue, or organ; a cell, tissue, or organ from an invertebrate animal; a cell, tissue, fluid, or organ from a vertebrate animal such as fish, amphibian, reptile, bird, and mammal; a cell, tissue, fluid, or organ from a mammal such as a human, a non-human primate, an ungulate, a feline, a bovine, an ovine, and a caprine. In some instances, the sample is taken from nematodes, protozoans, helminths, or malarial parasites. In some cases, the sample comprises nucleic acids from a cell lysate from a eukaryotic cell, a mammalian cell, a human cell, a prokaryotic cell, or a plant cell. In some cases, the sample comprises nucleic acids expressed from a cell.

[0271] The sample described herein may comprise at least one target nucleic acid. The target nucleic acid comprises a segment that is reverse complementary to a segment of a guide nucleic acid. Often, the sample comprises the segment of the target nucleic acid and at least one nucleic acid comprising at least 50% sequence identity to a segment of the target nucleic acid. Sometimes, the at least one nucleic acid comprises a segment comprising at least 60%, 70%,

75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the segment of the target nucleic acid. Often, a sample comprises the segment of the target nucleic acid and at least one nucleic acid a segment comprising less than 100% sequence identity to the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. Sometimes, a sample comprises the segment of the target nucleic acid and at least one nucleic acid a segment comprising less than 100% sequence identity to the target nucleic acid but no less than 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the segment of the target nucleic acid. For example, the segment of the target nucleic acid comprises a mutation as compared to at least one nucleic acid comprising a segment comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. Sometimes, the segment of the target nucleic acid comprises a mutation as compared to at least one nucleic acid comprising a segment comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 60%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the segment of the target nucleic acid. Often, the segment of the target nucleic acid comprises a mutation as compared to at least one nucleic acid comprising a segment comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. The mutation can be a mutation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides. Often, the mutation is a single nucleotide mutation. The single nucleotide mutation can be a single nucleotide polymorphism (SNP), which is a single base pair variation in a DNA sequence present in less than 1% of a population. Sometimes, the target nucleic acid comprises a single nucleotide mutation, wherein the single nucleotide mutation comprises the wild type variant of the SNP. The single nucleotide mutation or SNP can be associated with a phenotype of the sample or a phenotype of the organism from which the sample was taken. The SNP, in some cases, is associated with altered phenotype from wild type phenotype. Often, the segment of the target nucleic acid sequence comprises a deletion as compared to at least one nucleic acid comprising a segment comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. The mutation can be a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more nucleotides. The mutation can be a deletion of about 5, about 10, about 15, about 20, about 25, about 30, about 35, about 40, about 45, about 50, about 55, about 60, about 65, about 70, about 75, about 80, about 85, about 90, about 95, about 100, about 200, about 300, about 400, about 500, about 600, about 700, about 800, about 900, or about 1000 nucleotides. The mutation can be a deletion of from 1 to 5, from 5 to 10, from 10 to 15, from 15 to 20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, from 45 to 50, from 50 to 55, from 55 to 60, from 60 to 65, from 65 to 70, from 70 to 75, from 75 to 80, from 80 to 85, from 85 to 90, from 90 to 95, from 95 to 100, from 100 to 200, from 200

to 300, from 300 to 400, from 400 to 500, from 500 to 600, from 600 to 700, from 700 to 800, from 800 to 900, from 900 to 1000, from 1 to 50, from 1 to 100, from 25 to 50, from 25 to 100, from 50 to 100, from 100 to 500, from 100 to 1000, or from 500 to 1000 nucleotides. The segment of the target nucleic acid that the guide nucleic acid of the methods describe herein binds to comprises the mutation, such as the SNP or the deletion. The mutation can be a single nucleotide mutation or a SNP. The SNP can be a synonymous substitution or a nonsynonymous substitution. The nonsynonymous substitution can be a missense substitution or a nonsense point mutation. The synonymous substitution can be a silent substitution. The mutation can be a deletion of one or more nucleotides. Often, the single nucleotide mutation, SNP, or deletion is associated with a disease such as cancer or a genetic disorder. The mutation, such as a single nucleotide mutation, a SNP, or a deletion, can be encoded in the sequence of a target nucleic acid from the germline of an organism or can be encoded in a target nucleic acid from a diseased cell, such as a cancer cell.

[0272] The sample used for disease testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The sample used for disease testing may comprise at least nucleic acid of interest that is amplified to produce a target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The nucleic acid of interest can comprise DNA, RNA, or a combination thereof.

[0273] The target nucleic acid (e.g., a target DNA) may be a portion of a nucleic acid from a virus or a bacterium or other agents responsible for a disease in the sample. The target nucleic acid may be a portion of a nucleic acid from a gene expressed in a cancer or genetic disorder in the sample. In some cases, the sequence is a segment of a target nucleic acid sequence. A segment of a target nucleic acid sequence can be from a genomic locus, a transcribed mRNA, or a reverse transcribed cDNA. A segment of a target nucleic acid sequence can be from 5 to 100, 5 to 90, 5 to 80, 5 to 70, 5 to 60, 5 to 50, 5 to 40, 5 to 30, 5 to 25, 5 to 20, 5 to 15, or 5 to 10 nucleotides in length. A segment of a target nucleic acid sequence can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 60, 70, 80, 90, or 100 nucleotides in length. The sequence of the target nucleic acid segment can be reverse complementary to a segment of a guide nucleic acid sequence. The target nucleic acid may comprise a genetic variation (e.g., a single nucleotide polymorphism), with respect to a standard sample, associated with a disease phenotype or disease predisposition. The target nucleic acid may be an amplicon of a portion of an RNA, may be a DNA, or may be a DNA amplicon from any organism in the sample.

[0274] In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a virus or a bacterium or other agents responsible for a disease in the sample. In some embodiments, the target nucleic acid comprises DNA that is reverse transcribed from RNA using a reverse transcriptase prior to detection by a programmable nuclease using the

compositions, systems, and methods disclosed herein. The target nucleic acid, in some cases, is a portion of a nucleic acid from a sexually transmitted infection or a contagious disease, in the sample. In some cases, the target nucleic acid is a portion of a nucleic acid from a genomic locus, or any DNA amplicon, such as a reverse transcribed mRNA or a cDNA from a gene locus, a transcribed mRNA, or a reverse transcribed cDNA from a gene locus in at least one of: human immunodeficiency virus (HIV), human papillomavirus (HPV), chlamydia, gonorrhea, syphilis, trichomoniasis, sexually transmitted infection, malaria, Dengue fever, Ebola, chikungunya, and leishmaniasis. Pathogens include viruses, fungi, helminths, protozoa, malarial parasites, *Plasmodium* parasites, *Toxoplasma* parasites, and *Schistosoma* parasites. Helminths include roundworms, heartworms, and phytophagous nematodes, flukes, Acanthocephala, and tapeworms. Protozoan infections include infections from *Giardia spp., Trichomonas spp.*, African trypanosomiasis, amoebic dysentery, babesiosis, balantidial dysentery, Chaga's disease, coccidiosis, malaria and toxoplasmosis. Examples of pathogens such as parasitic/protozoan pathogens include, but are not limited to: *Plasmodium falciparum*, *P. vivax, Trypanosoma cruzi* and *Toxoplasma gondii*. Fungal pathogens include, but are not limited to *Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Chlamydia trachomatis, and Candida albicans*. Pathogenic viruses include but are not limited to coronavirus; immunodeficiency virus (e.g., HIV); influenza virus; dengue; West Nile virus; herpes virus; yellow fever virus; Hepatitis Virus C; Hepatitis Virus A; Hepatitis Virus B; papillomavirus; and the like. Pathogens include, e.g., HIV virus, *Mycobacterium tuberculosis, Streptococcus agalactiae, methicillin-resistant Staphylococcus aureus, Legionella pneumophila, Streptococcus pyogenes, Escherichia coli, Neisseria gonorrhoeae, Neisseria meningitidis, Pneumococcus, Cryptococcus neoformans, Histoplasma capsulatum, Hemophilus influenzae B, Treponema pallidum*, Lyme disease spirochetes, *Pseudomonas aeruginosa, Mycobacterium leprae, Brucella abortus*, rabies virus, influenza virus, cytomegalovirus, herpes simplex virus I, herpes simplex virus II, human serum parvo-like virus, respiratory syncytial virus (RSV), *M. genitalium*, *T. vaginalis*, varicella-zoster virus, hepatitis B virus, hepatitis C virus, measles virus, adenovirus, human T-cell leukemia viruses, Epstein-Barr virus, murine leukemia virus, mumps virus, vesicular stomatitis virus, Sindbis virus, lymphocytic choriomeningitis virus, wart virus, blue tongue virus, Sendai virus, feline leukemia virus, Reovirus, polio virus, simian virus 40, mouse mammary tumor virus, dengue virus, rubella virus, West Nile virus, *Plasmodium falciparum, Plasmodium vivax, Toxoplasma gondii, Trypanosoma rangeli, Trypanosoma cruzi, Trypanosoma rhodesiense, Trypanosoma brucei, Schistosoma mansoni, Schistosoma japonicum, Babesia bovis, Eimeria tenella, Onchocerca volvulus, Leishmania tropica, Mycobacterium tuberculosis, Trichinella spiralis, Theileria parva, Taenia hydatigena, Taenia ovis, Taenia saginata, Echinococcus granulosus, Mesocestoides corti, Mycoplasma arthritidis, M. hyorhinis, M. orale, M. arginini, Acholeplasma laidlawii, M. salivarium* and *M. pneumoniae*. In some cases, the target sequence is a portion of a nucleic acid from a genomic locus, a transcribed

mRNA, or a reverse transcribed cDNA from a gene locus of bacterium or other agents responsible for a disease in the sample comprising a mutation that confers resistance to a treatment, such as a single nucleotide mutation that confers resistance to antibiotic treatment. In some cases, the mutation that confers resistance to a treatment is a deletion.

[0275] Compositions and methods of the disclosure can be used for cell line engineering (e.g., engineering a cell from a cell line for bioproduction). For example, compositions and methods of the disclosure can be used to express a desired protein from a cell line. In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a cell line. In some embodiments, the target nucleic acid sequence comprises a genomic nucleic acid sequence of a cell line. In some embodiments, the cell line is a Chinese hamster ovary cell line (CHO), human embryonic kidney cell line (HEK), cell lines derived from cancer cells, cell lines derived from lymphocytes, and the like. Non-limiting examples of cell lines includes: C8161, CCRF-CEM, MOLT, mIMCD-3, NHDF, HeLa-S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEKn, HEKa, MiaPaCell, Panc1, PC-3, TF1, CTLL-2, CIR, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu1, SW480, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, TIB55, Jurkat, J45.01, LRMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, 132-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, A2780ADR, A2780cis, A172, A20, A253, A431, A-549, ALC, AsPC-1, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293, BxPC3, C3H-10T1/2, C6/36, Cal-27, Capan-1, CHO, CHO-7, CHO-IR, CHO-K1, CHO-K2, CHO-S, CHO-T, CHO Dhfr −/−, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV-434, CML T1, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HAP1, HB54, HB55, HCA2, HEK-293, HeLa, Hepa1-6, Hep3B, Hepa1c1c7, HL-60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KYO1, LNCap, Ma-Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK II, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NALM-1, Neuro2A, NK92, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-5F, RMA/RMAS, Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-1, and YAR. Non-limiting examples of other cells that can be used with the disclosure include immune cells, such as CART, T-cells, B-cells, NK cells (including iNK cells), granulocytes, basophils, eosinophils, neutrophils, mast cells, monocytes, macrophages, dendritic cells, antigen-presenting cells (APC), or adaptive cells. Non-limiting examples of cells that can be used with this disclosure also include plant cells, such as parenchyma, sclerenchyma, collenchyma, xylem, phloem, germline (*e.g.*, pollen). Cells may be from lycophytes, ferns, gymnosperms, angiosperms, bryophytes, charophytes, chloropytes, rhodophytes, or glaucophytes. Cells may be obtained from non-human animals, including, but

not limited to, rats, dogs, rabbits, cats, and monkeys. Non-limiting examples of cells that can be used with this disclosure also include stem cells, such as human stem cells, animal stem cells, stem cells that are not derived from human embryonic stem cells, embryonic stem cells, mesenchymal stem cells, pluripotent stem cells, induced pluripotent stem cells (iPS), somatic stem cells, adult stem cells, hematopoietic stem cells, tissue-specific stem cells. Non-limiting examples of cells that can be used with this disclosure also include neuronal cells from various organs of an animal, *e.g.*, brain, heart, lung, liver, pancreas, and muscle. In preferred embodiments, the cells that can be used with the disclosure are T cells, such as CAR-T (CART) cells.

[0276] CHO cells are an epithelial cell line which is particularly useful in biological and medical research. In particular, CHO cells are frequently used for the industrial production of recombinant therapeutics. In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a CHO cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a CHO cell. In some embodiments, a method disclosed herein comprises modifying or editing a CHO cell. In some embodiments, a modified CHO cell is provided wherein the CHO cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a CHO cell is provided wherein the CHO cell comprises a CasΦ polypeptide disclosed herein.

[0277] T cells are important therapeutic targets. In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a T cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a T cell. In some embodiments, a method disclosed herein comprises modifying or editing a T cell. In some embodiments, a method disclosed herein comprises modifying a PDCD1 gene of a T cell. In some embodiments, a method disclosed herein comprises modifying a TRAC gene of a T cell. In some emobdiments, a method disclosed herein comprises modifying a B2M gene of a T cell. In some embodiments, a method disclosed herein comprises modifying a PDCD1 gene of a T cell, a TRAC gene of a T cell, a B2M gene of a T cell or a combination thereof. In some embodiments, a method disclosed herein comprises modifying a PDCD1 gene, a TRAC gene, and a B2M gene of a T cell. In some embodiments, a modified T cell is provided wherein the T cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a T cell is provided wherein the T cell comprises a CasΦ polypeptide disclosed herein.

[0278] T cells, also known as T lymphocytes, are easily identifiable by the surface expression of the T- cell receptor (TCR). In some embodiments, the T cells include one or more subsets of T cells, such as CD4+ cells, CD8+ cells, and sub-populations thereof. In some embodiments, a T cell is a CD4+ cell. In some embodiments, a T cell is a CD8+ T cells. In some embodiments, a population of T cells comprises CD4+ T cells and CD8+ T cells. In some embodiments, T cells comprise TCR-T, Tscm, or iT cells.

[0279] Sub-populations of CD4+ and CD8+ T cells include naive T cells, effector T cells, memory T cells, immature T cells, mature T cells, helper T cells, cytotoxic T cells, regulatory T cells, alpha/beta T cells, and delta/gamma T cells. Sub-types of memory T cells include stem cell memory T cells, central memory T cells, effector memory T cells, and terminally differentiated effector memory T cells. Sub-types of helper T cells, include T helper 1 cells, T helper 2 cells, T helper 3 cells, T helper 17 cells, T helper 9 cells, T helper 22 cells, and follicular helper T cells. In some embodiments, the cell is a regulatory T cell (Treg).

[0280] CART cells are T cells that have been genetically engineered to express unique chimeric antigen receptors (CARs) targeting specific antigens. CART cells are important targets for immunotherapy. In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a CART cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a CART cell. In some embodiments, a method disclosed herein comprises modifying or editing a CART cell. In some embodiments, a modified CART cell is provided wherein the CART cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a CART cell is provided wherein the CART cell comprises a CasΦ polypeptide disclosed herein.

[0281] Modified stem cells and methods of modifying stem cells are also provided. In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a stem cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a stem cell. In some embodiments, a method disclosed herein comprises modifying or editing a stem cell. In some embodiments, a modified stem cell is provided wherein a stem cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a stem cell is provided wherein the stem cell comprises a CasΦ polypeptide disclosed herein.In some embodiments, a modified stem cell is obtained or is obtainable by a method disclosed herein. In some embodiments, a modified stem cell is provided wherein the CART cell is modified by a CasΦ polypeptide disclosed herein.

[0282] Induced pluripotent stem cells (iPSCs) are pluripotent stem cells that are generated from somatic cells. They can propagate indefinitely and give rise to any cell type in the body. These features make iPSCs a powerful tool for researching human disease and provide a promising prospect for cell therapies for a range of medical conditions. iPSCs can be generated in a patient-specific manner and used in autologous transplant, thereby overcoming complications of rejection by the host immune system (Moradi *et al.* (2019), *Stem Cell Research & Therapy*).

[0283] In some embodiments, a CasΦ polypeptide disclosed herein is expressed in an induced pluripotent stem cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in an induced pluripotent stem cell. In some embodiments, a method disclosed herein comprises modifying or editing an induced pluripotent stem cell. In

some embodiments, a modified induced pluripotent stem cell is provided wherein an induced pluripotent stem cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, an induced pluripotent stem cell is provided wherein the induced pluripotent stem cell comprises a CasΦ polypeptide disclosed herein. In some embodiments, a modified induced pluripotent cell is obtained or is obtainable by a method disclosed herein.

[0284] Hematopoietic stem cells (HSCs) are identifiable by the marker CD34. HSCs are stem cells that differentiate to give rise blood cells, such as T and B lymphocytes, erythrocytes, monocytes and macrophages. HSCs are important cells for future stem cell therapies as they have the potential to be used to treat genetic blood cell diseases (Morgan *et al.* (2017), *Cell Stem Cell*).

[0285] In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a hematopoietic stem cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a hematopoietic stem cell. In some embodiments, a method disclosed herein comprises modifying or editing a hematopoietic stem cell. In some embodiments, a modified hematopoietic stem cell is provided wherein a hematopoietic stem cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a hematopoietic stem cell is provided wherein the hematopoietic stem cell comprises a CasΦ polypeptide disclosed herein. In some embodiments, a modified hematopoietic stem cell is obtained or is obtainable by a method disclosed herein.

[0286] Compositions and methods of the disclosure can be used for agricultural engineering. For example, compositions and methods of the disclosure can be used to confer desired traits on a plant. A plant can be engineered for the desired physiological and agronomic characteristic using the present disclosure. In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a plant. In some embodiments, the target nucleic acid sequence comprises a genomic nucleic acid sequence of a plant cell. In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of an organelle of a plant cell. In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a chloroplast of a plant cell.

[0287] The plant can be a monocotyledonous plant. The plant can be a dicotyledonous plant. Non-limiting examples of orders of dicotyledonous plants include Magniolales, Illiciales, Laurales, Piperales, Aristochiales, Nymphaeales, Ranunculales, Papeverales, Sarraceniaceae, Trochodendrales, Hamamelidales, Eucomiales, Leitneriales, Myricales, Fagales, Casuarinales, Caryophyllales, Batales, Polygonales, Plumbaginales, Dilleniales, Theales, Malvales, Urticales, Lecythidales, Violales, Salicales, Capparales, Ericales, Diapensales, Ebenales, Primulales, Rosales, Fabales, Podostemales, Haloragales, Myrtales, Cornales, Proteales, San tales, Rafflesiales, Celastrales, Euphorbiales, Rhamnales, Sapindales, Juglandales, Geraniales,

Polygalales, Umbellales, Gentianales, Polemoniales, Lamiales, Plantaginales, Scrophulariales, Campanulales, Rubiales, Dipsacales, and Asterales.

[0288] Non-limiting examples of orders of monocotyledonous plants include Alismatales, Hydrocharitales, Najadales, Triuridales, Commelinales, Eriocaulales, Restionales, Poales, Juncales, Cyperales, Typhales, Bromeliales, Zingiberales, Arecales, Cyclanthales, Pandanales, Arales, Lilliales, and Orchid ales. A plant can belong to the order, for example, Gymnospermae, Pinales, Ginkgoales, Cycadales, Araucariales, Cupressales and Gnetales.

[0289] Non-limiting examples of plants include plant crops, fruits, vegetables, grains, soy bean, corn, maize, wheat, seeds, tomatoes, rice, cassava, sugarcane, pumpkin, hay, potatoes, cotton, cannabis, tobacco, flowering plants, conifers, gymnosperms, ferns, clubmosses, hornworts, liverworts, mosses, wheat, maize, rice, millet, barley, tomato, apple, pear, strawberry, orange, acacia, carrot, potato, sugar beets, yam, lettuce, spinach, sunflower, rape seed, Arabidopsis, alfalfa, amaranth, apple, apricot, artichoke, ash tree, asparagus, avocado, banana, barley, beans, beet, birch, beech, blackberry, blueberry, broccoli, Brussel's sprouts, cabbage, canola, cantaloupe, carrot, cassava, cauliflower, cedar, a cereal, celery, chestnut, cherry, Chinese cabbage, citrus, clementine, clover, coffee, corn, cotton, cowpea, cucumber, cypress, eggplant, elm, endive, eucalyptus, fennel, figs, fir, geranium, grape, grapefruit, groundnuts, ground cherry, gum hemlock, hickory, kale, kiwifruit, kohlrabi, larch, lettuce, leek, lemon, lime, locust, pine, maidenhair, maize, mango, maple, melon, millet, mushroom, mustard, nuts, oak, oats, oil palm, okra, onion, orange, an ornamental plant or flower or tree, papaya, palm, parsley, parsnip, pea, peach, peanut, pear, peat, pepper, persimmon, pigeon pea, pine, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, safflower, sallow, soybean, spinach, spruce, squash, strawberry, sugar beet, sugarcane, sunflower, sweet potato, sweet corn, tangerine, tea, tobacco, tomato, trees, triticale, turf grasses, turnips, vine, walnut, watercress, watermelon, wheat, yams, yew, and zucchini. A plant can include algae.

[0290] In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a virus, a bacterium, or other pathogen responsible for a disease in a plant (e.g., a crop). Methods and compositions of the disclosure can be used to treat or detect a disease in a plant. For example, the methods of the disclosure can be used to target a viral nucleic acid sequence in a plant. A programmable nuclease of the disclosure (e.g., CasΦ) can cleave the viral nucleic acid. In some embodiments, the target nucleic acid sequence comprises a nucleic acid sequence of a virus or a bacterium or other agents (e.g., any pathogen) responsible for a disease in the plant (e.g., a crop). In some embodiments, the target nucleic acid comprises DNA that is reverse transcribed from RNA using a reverse transcriptase prior to detection by a programmable nuclease using the compositions, systems, and methods disclosed herein. The target nucleic acid, in some cases, is a portion of a nucleic acid from a virus or a bacterium or other agents

responsible for a disease in the plant (e.g., a crop). In some cases, the target nucleic acid is a portion of a nucleic acid from a genomic locus, or any DNA amplicon, such as a reverse transcribed mRNA or a cDNA from a gene locus, a transcribed mRNA, or a reverse transcribed cDNA from a gene locus in at a virus or a bacterium or other agents (e.g., any pathogen) responsible for a disease in the plant (e.g., a crop). A virus infecting the plant can be an RNA virus. A virus infecting the plant can be a DNA virus. Non-limiting examples of viruses that can be targeted with the disclosure include Tobacco mosaic virus (TMV), Tomato spotted wilt virus (TSWV), Cucumber mosaic virus (CMV), Potato virus Y (PVY), Cauliflower mosaic virus (CaMV) (RT virus), Plum pox virus (PPV), Brome mosaic virus (BMV) and Potato virus X (PVX).

[0291] The sample used for cancer testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The target nucleic acid, in some cases, comprises a portion of a gene comprising a mutation associated with cancer, a gene whose overexpression is associated with cancer, a tumor suppressor gene, an oncogene, a checkpoint inhibitor gene, a gene associated with cellular growth, a gene associated with cellular metabolism, or a gene associated with cell cycle. Sometimes, the target nucleic acid encodes a cancer biomarker, such as a prostate cancer biomarker or non-small cell lung cancer. In some cases, the assay can be used to detect "hotspots" in target nucleic acids that can be predictive of lung cancer. In some cases, the target nucleic acid comprises a portion of a nucleic acid that is associated with a blood fever. In some cases, the target nucleic acid is a portion of a nucleic acid from a genomic locus, any DNA amplicon of, a reverse transcribed mRNA, or a cDNA from a locus of at least one of: ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CHEK2, CTNNA1, DICER1, DIS3L2, EGFR, EPCAM, FH, FLCN, GATA2, GPC3, GREM1, HOXB13, HRAS, KIT, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL4, RET, RUNX1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERC, TERT, TMEM127, TP53, TSC1, TSC2, VHL, WRN, and WT1. Any region of the aforementioned gene loci can be probed for a mutation or deletion using the compositions and methods disclosed herein. For example, in the EGFR gene locus, the compositions and methods for detection disclosed herein can be used to detect a single nucleotide polymorphism or a deletion. The SNP or deletion can occur in a non-coding region or a coding region. The SNP or deletion can occur in an Exon, such as Exon19. A SNP, deletion, or other mutation may mediate gene knockout.

[0292] The sample used for genetic disorder testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. In some embodiments, the

genetic disorder is hemophilia, sickle cell anemia, β-thalassemia, Duchene muscular dystrophy, severe combined immunodeficiency, Huntington's disease, or cystic fibrosis. The target nucleic acid, in some cases, is from a gene with a mutation associated with a genetic disorder, from a gene whose overexpression is associated with a genetic disorder, from a gene associated with abnormal cellular growth resulting in a genetic disorder, or from a gene associated with abnormal cellular metabolism resulting in a genetic disorder. In some cases, the target nucleic acid is a nucleic acid from a genomic locus, a transcribed mRNA, or a reverse transcribed mRNA, a DNA amplicon of or a cDNA from a locus of at least one of: CFTR, FMR1, SMN1, ABCB11, ABCC8, ABCD1, ACAD9, ACADM, ACADVL, ACAT1, ACOX1, ACSF3, ADA, ADAMTS2, ADGRG1, AGA, AGL, AGPS, AGXT, AIRE, ALDH3A2, ALDOB, ALG6, ALMS1, ALPL, AMT, AQP2, ARG1, ARSA, ARSB, ASL, ASNS, ASPA, ASS1, ATM, ATP6V1B1, ATP7A, ATP7B, ATRX, BBS1, BBS10, BBS12, BBS2, BCKDHA, BCKDHB, BCS1L, BLM, BSND, CAPN3, CBS, CDH23, CEP290, CERKL, CHM, CHRNE, CIITA, CLN3, CLN5, CLN6, CLN8, CLRN1, CNGB3, COL27A1, COL4A3, COL4A4, COL4A5, COL7A1, CPS1, CPT1A, CPT2, CRB1, CTNS, CTSK, CYBA, CYBB, CYP11B1, CYP11B2, CYP17A1, CYP19A1, CYP27A1, DBT, DCLRE1C, DHCR7, DHDDS, DLD, DMD, DNAH5, DNAI1, DNAI2, DYSF, EDA, EIF2B5, EMD, ERCC6, ERCC8, ESCO2, ETFA, ETFDH, ETHE1, EVC, EVC2, EYS, F9, FAH, FAM161A, FANCA, FANCC, FANCG, FH, FKRP, FKTN, G6PC, GAA, GALC, GALK1, GALT, GAMT, GBA, GBE1, GCDH, GFM1, GJB1, GJB2, GLA, GLB1, GLDC, GLE1, GNE, GNPTAB, GNPTG, GNS, GRHPR, HADHA, HAX1, HBA1,, HBA2, HBB, HEXA, HEXB, HGSNAT, HLCS, HMGCL, HOGA1, HPS1, HPS3, HSD17B4, HSD3B2, HYAL1, HYLS1, IDS, IDUA, IKBKAP, IL2RG, IVD, KCNJ11, LAMA2, LAMA3, LAMB3, LAMC2, LCA5, LDLR, LDLRAP1, LHX3, LIFR, LIPA, LOXHD1, LPL, LRPPRC, MAN2B1, MCOLN1, MED17, MESP2, MFSD8, MKS1, MLC1, MMAA, MMAB, MMACHC, MMADHC, MPI, MPL, MPV17, MTHFR, MTM1, MTRR, MTTP, MUT, MYO7A, NAGLU, NAGS, NBN, NDRG1, NDUFAF5, NDUFS6, NEB, NPC1, NPC2, NPHS1, NPHS2, NR2E3, NTRK1, OAT, OPA3, OTC, PAH, PC, PCCA, PCCB, PCDH15, PDHA1, PDHB, PEX1, PEX10, PEX12, PEX2, PEX6, PEX7, PFKM, PHGDH, PKHD1, PMM2, POMGNT1, PPT1, PROP1, PRPS1, PSAP, PTS, PUS1, PYGM, RAB23, RAG2, RAPSN, RARS2, RDH12, RMRP, RPE65, RPGRIP1L, RS1, RTEL1, SACS, SAMHD1, SEPSECS, SGCA, SGCB, SGCG, SGSH, SLC12A3, SLC12A6, SLC17A5, SLC22A5, SLC25A13, SLC25A15, SLC26A2, SLC26A4, SLC35A3, SLC37A4, SLC39A4, SLC4A11, SLC6A8, SLC7A7, SMARCAL1, SMPD1, STAR, SUMF1, TAT, TCIRG1, TECPR2, TFR2, TGM1, TH, TMEM216, TPP1, TRMU, TSFM, TTPA, TYMP, USH1C, USH2A, VPS13A, VPS13B, VPS45, VRK1, VSX2, WNT10A, XPA, XPC, and ZFYVE26.

[0293] The sample used for phenotyping testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The target nucleic acid, in some cases, is a nucleic acid encoding a sequence associated with a phenotypic trait.

[0294] The sample used for genotyping testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The target nucleic acid, in some cases, is a nucleic acid encoding a sequence associated with a genotype of interest.

[0295] The sample used for ancestral testing may comprise at least one target nucleic acid that can bind to a guide nucleic acid of the reagents described herein. The target nucleic acid, in some cases, is a nucleic acid encoding a sequence associated with a geographic region of origin or ethnic group.

[0296] The sample can be used for identifying a disease status. For example, a sample is any sample described herein, and is obtained from a subject for use in identifying a disease status of a subject. The disease can be a cancer or genetic disorder. Sometimes, a method comprises obtaining a serum sample from a subject; and identifying a disease status of the subject. Often, the disease status is prostate disease status, but the status of any disease can be assessed.

[0297] In some instances, the target nucleic acid is a single stranded nucleic acid. Alternatively, or in combination, the target nucleic acid is a double stranded nucleic acid and is prepared into single stranded nucleic acids before or upon contacting the reagents. The target nucleic acid may be a reverse transcribed RNA, DNA, DNA amplicon, synthetic nucleic acids, or nucleic acids found in biological or environmental samples. The target nucleic acids include but are not limited to mRNA, rRNA, tRNA, non-coding RNA, long non-coding RNA, and microRNA (miRNA). In some cases, the target nucleic acid is single-stranded DNA (ssDNA) or mRNA. In some cases, the target nucleic acid is from a virus, a parasite, or a bacterium described herein. In some cases, the target nucleic acid is transcribed from a gene as described herein and then reverse transcribed into a DNA amplicon. In some cases, miRNA is extracted using a mirVANA kit. In some cases, RNA may be treated with shrimp alkaline phosphatase to remove phosphates from the 5' and 3' ends of an RNA for analysis. RNA analysis may further comprise the use of a thermocycler, SR Adaptors for Illumina, ligation enzymes, reverse transcriptase, and suitable primers for polymerase chain reaction.

[0298] A number of target nucleic acids are consistent with the methods and compositions disclosed herein. Some methods described herein can detect a target nucleic acid present in the sample in various concentrations or amounts as a target nucleic acid population. In some cases, the sample has at least 2 target nucleic acids. In some cases, the sample has at least 3, 5, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, or 10000 target nucleic acids. In some cases, the sample as from 1 to 10,000, from 100 to 8000, from 400 to 6000, from 500 to 5000, from 1000 to 4000, or from 2000 to 3000 target nucleic acids. In some cases, the method detects target nucleic acid present at least at one copy per 10 non-target nucleic acids, 10^2 non-target nucleic acids, 10^3 non-target nucleic acids, 10^4 non-target nucleic acids, 10^5 non-target nucleic acids, 10^6 non-target nucleic acids, 10^7 non-

target nucleic acids, 10^8 non-target nucleic acids, 10^9 non-target nucleic acids, or 10^{10} non-target nucleic acids. Often, the target nucleic acid can be from 0.05% to 20% of total nucleic acids in the sample. Sometimes, the target nucleic acid is from 0.1% to 10% of the total nucleic acids in the sample. The target nucleic acid, in some cases, is from 0.1% to 5% of the total nucleic acids in the sample. The target nucleic acid can also be from 0.1% to 1% of the total nucleic acids in the sample. The target nucleic acid can be DNA or RNA. The target nucleic acid can be any amount less than 100% of the total nucleic acids in the sample. The target nucleic acid can be 100% of the total nucleic acids in the sample.

[0299] In some embodiments, the sample comprises a target nucleic acid at a concentration of less than 1 nM, less than 2 nM, less than 3 nM, less than 4 nM, less than 5 nM, less than 6 nM, less than 7 nM, less than 8 nM, less than 9 nM, less than 10 nM, less than 20 nM, less than 30 nM, less than 40 nM, less than 50 nM, less than 60 nM, less than 70 nM, less than 80 nM, less than 90 nM, less than 100 nM, less than 200 nM, less than 300 nM, less than 400 nM, less than 500 nM, less than 600 nM, less than 700 nM, less than 800 nM, less than 900 nM, less than 1 μM, less than 2 μM, less than 3 μM, less than 4 μM, less than 5 μM, less than 6 μM, less than 7 μM, less than 8 μM, less than 9 μM, less than 10 μM, less than 100 μM, or less than 1 mM. In some embodiments, the sample comprises a target nucleic acid sequence at a concentration of from 1 nM to 2 nM, from 2 nM to 3 nM, from 3 nM to 4 nM, from 4 nM to 5 nM, from 5 nM to 6 nM, from 6 nM to 7 nM, from 7 nM to 8 nM, from 8 nM to 9 nM, from 9 nM to 10 nM, from 10 nM to 20 nM, from 20 nM to 30 nM, from 30 nM to 40 nM, from 40 nM to 50 nM, from 50 nM to 60 nM, from 60 nM to 70 nM, from 70 nM to 80 nM, from 80 nM to 90 nM, from 90 nM to 100 nM, from 100 nM to 200 nM, from 200 nM to 300 nM, from 300 nM to 400 nM, from 400 nM to 500 nM, from 500 nM to 600 nM, from 600 nM to 700 nM, from 700 nM to 800 nM, from 800 nM to 900 nM, from 900 nM to 1 μM, from 1 μM to 2 μM, from 2 μM to 3 μM, from 3 μM to 4 μM, from 4 μM to 5 μM, from 5 μM to 6 μM, from 6 μM to 7 μM, from 7 μM to 8 μM, from 8 μM to 9 μM, from 9 μM to 10 μM, from 10 μM to 100 μM, from 100 μM to 1 mM, from 1 nM to 10 nM, from 1 nM to 100 nM, from 1 nM to 1 μM, from 1 nM to 10 μM, from 1 nM to 100 μM, from 1 nM to 1 mM, from 10 nM to 100 nM, from 10 nM to 1 μM, from 10 nM to 10 μM, from 10 nM to 100 μM, from 10 nM to 1 mM, from 100 nM to 1 μM, from 100 nM to 10 μM, from 100 nM to 100 μM, from 100 nM to 1 mM, from 1 μM to 10 μM, from 1 μM to 100 μM, from 1 μM to 1 mM, from 10 μM to 100 μM, from 10 μM to 1 mM, or from 100 μM to 1 mM. In some embodiments, the sample comprises a target nucleic acid at a concentration of from 20 nM to 200 μM, from 50 nM to 100 μM, from 200 nM to 50 μM, from 500 nM to 20 μM, or from 2 μM to 10 μM. In some embodiments, the target nucleic acid is not present in the sample.

[0300] In some embodiments, the sample comprises fewer than 10 copies, fewer than 100 copies, fewer than 1000 copies, fewer than 10,000 copies, fewer than 100,000 copies, or fewer

than 1,000,000 copies of a target nucleic acid sequence. In some embodiments, the sample comprises from 10 copies to 100 copies, from 100 copies to 1000 copies, from 1000 copies to 10,000 copies, from 10,000 copies to 100,000 copies, from 100,000 copies to 1,000,000 copies, from 10 copies to 1000 copies, from 10 copies to 10,000 copies, from 10 copies to 100,000 copies, from 10 copies to 1,000,000 copies, from 100 copies to 10,000 copies, from 100 copies to 100,000 copies, from 100 copies to 1,000,000 copies, from 1,000 copies to 100,000 copies, or from 1,000 copies to 1,000,000 copies of a target nucleic acid sequence. In some embodiments, the sample comprises from 10 copies to 500,000 copies, from 200 copies to 200,000 copies, from 500 copies to 100,000 copies, from 1000 copies to 50,000 copies, from 2000 copies to 20,000 copies, from 3000 copies to 10,000 copies, or from 4000 copies to 8000 copies. In some embodiments, the target nucleic acid is not present in the sample.

[0301] A number of target nucleic acid populations are consistent with the methods and compositions disclosed herein. Some methods described herein can detect two or more target nucleic acid populations present in the sample in various concentrations or amounts. In some cases, the sample has at least 2 target nucleic acid populations. In some cases, the sample has at least 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, or 50 target nucleic acid populations. In some cases, the sample has from 3 to 50, from 5 to 40, or from 10 to 25 target nucleic acid populations. In some cases, the method detects target nucleic acid populations that are present at least at one copy per 10^1 non-target nucleic acids, 10^2 non-target nucleic acids, 10^3 non-target nucleic acids, 10^4 non-target nucleic acids, 10^5 non-target nucleic acids, 10^6 non-target nucleic acids, 10^7 non-target nucleic acids, 10^8 non-target nucleic acids, 10^9 non-target nucleic acids, or 10^{10} non-target nucleic acids. The target nucleic acid populations can be present at different concentrations or amounts in the sample.

[0302] In some embodiments, the target nucleic acid as disclosed herein can activate the programmable nuclease to initiate sequence-independent cleavage of a nucleic acid-based reporter (e.g., a reporter comprising a DNA sequence, a reporter comprising an RNA sequence, or a reporter comprising DNA and RNA). For example, a programmable nuclease of the present disclosure is activated by a target DNA to cleave reporters having an RNA (also referred to herein as an "RNA reporter"). Alternatively, a programmable nuclease of the present disclosure is activated by a target RNA to cleave reporters having an RNA. Alternatively, a programmable nuclease of the present disclosure is activated by a target DNA to cleave reporters having a DNA (also referred to herein as a "DNA reporter"). The RNA reporter can comprise a single-stranded RNA labelled with a detection moiety or can be any RNA reporter as disclosed herein. The DNA reporter can comprise a single-stranded DNA labelled with a detection moiety or can be any DNA reporter as disclosed herein.

[0303] In some embodiments, the target nucleic acid as described in the methods herein does not initially comprise a PAM sequence. However, any target nucleic acid of interest may be

generated using the methods described herein to comprise a PAM sequence, and thus be a PAM target nucleic acid. A PAM target nucleic acid, as used herein, refers to a target nucleic acid that has been amplified to insert a PAM sequence that is recognized by a CRISPR/Cas system.

[0304] In some embodiments, the target nucleic acid is in a cell. In some embodiments, the cell is a single-cell eukaryotic organism; a plant cell an algal cell; a fungal cell; an animal cell; a cell from an invertebrate animal; a cell from a vertebrate animal such as fish, amphibian, reptile, bird, and mammal; or a cell from a mammal such as a human, a non-human primate, an ungulate, a feline, a bovine, an ovine, and a caprine. In preferred embodiments, the cell is a eukaryotic cell. In preferred embodiments, the cell is a mammalian cell, a human cell, or a plant cell.

[0305] Any of the above disclosed samples are consistent with the methods, compositions, reagents, enzymes, and kits disclosed herein and can be used as a companion diagnostic with any of the diseases disclosed herein, or can be used in reagent kits, point-of-care diagnostics, or over-the-counter diagnostics.

Methods of Modifying or Editing a Target Nucleic Acid Sequence

[0306] The disclosure provides compositions and methods for modifying or editing a target nucleic acid sequence. In some embodiments, the target nucleic acid sequence is associated with (e.g., causes, at least in part) a disease or disorder described herein, including a liver disease or disorder, an eye disease or disorder, cystic fibrosis, or a muscle disease or disorder. In some examples, the target nucleic acid comprises at least a portion of any one of the following genes: DNMT1, HPRT1, RPL32P3, CCR5, FANCF, GRIN2B, EMX1, AAVS1, ALKBH5, CLTA, CDK11, CTNNB1, AXIN1, LRP6, TBK1, BAP1,TLE3, PPM1A, BCL2L2, SUFU, RICTOR, VPS35, TOP1, SIRT1, PTEN, MMD, PAQR8, H2AX, POU5F1, OCT4, SYS1, ARFRP1, TSPAN14, EMC2, EMC3, SEL1L, DERL2, UBE2G2, UBE2J1, HRD1, PCSK9, BAK1 and CFTR. In some embodiments, the target nucleic acid comprises at least a portion of a PCSK9 gene. In some embodiments, the PCSK9 gene comprises a mutation associated with a liver disease or disorder. In some embodiments, the target nucleic acid comprises at least a portion of a BAK1 gene. In some embodiments, the BAK1 gene comprises a mutation associated with an eye disease or disorder. In some embodiments, the target nucleic acid comprises at least a portion of a CFTR gene. In some embodiments, the CFTR gene comprises a mutation associated with cystic fibrosis. In some embodiments, the CFTR gene comprises a delta F508 mutation. Compositions and methods of the disclosure can be used for introducing a site-specific cleavage in a target nucleic acid sequence. The site-specific cleavage can be a double-strand cleavage. The site-specific cleavage can be a single-strand cleavage (e.g. nicking). The modification can result in introducing a mutation (e.g., point mutations, deletions) in a target nucleic acid. The modification can result in removing a disease-causing mutation in a nucleic acid sequence. Methods of the disclosure can be targeted to any locus in a genome of a cell. They can generate point mutations, deletions, null mutations, or tissue-specific mutations in a target nucleic acid

sequence. A complex comprising a programmable nuclease and guide nucleic acid of the disclosure can be used to generate gene knock-out, gene knock-in, gene editing, gene tagging, or a combination thereof. In some embodiments, the activity of a nuclease, such as a cleavage product, may be analyzed using gel electrophoresis or nucleic acid sequencing.

[0307] The methods described herein (e.g., methods of introducing a nick or a double-stranded break into a target nucleic acid) may be used to edit or modify a target nucleic acid. Methods of modifying a target nucleic acid may use the compositions comprising a programmable nuclease and a gRNA as described herein. Modifying a target nucleic acid may comprise one or more of cleaving the target nucleic acid, deleting one or more nucleotides of the target nucleic acid, inserting one or more nucleotides into the target nucleic acid, mutating one or more nucleotides of the target nucleic acid, or modifying (e.g., methylating, demethylating, deaminating, or oxidizing) of one or more nucleotides of the target nucleic acid.

[0308] In some embodiments, modifying a target nucleic acid comprises genome editing. Genome editing may comprise modifying a genome, chromosome, plasmid, or other genetic material of a cell or organism. In some embodiments the genome, chromosome, plasmid, or other genetic material of the cell or organism is modified *in vivo.* In some embodiments the genome, chromosome, plasmid, or other genetic material of the cell or organism is modified in a cell. In some embodiments the genome, chromosome, plasmid, or other genetic material of the cell or organism is modified *in vitro*. For example, a plasmid may be modified *in vitro* using a composition described herein and introduced into a cell or organism. In some embodiments, modifying a target nucleic acid may comprise deleting a sequence from a target nucleic acid. For example, a mutated sequence or a sequence associated with a disease may be removed from a target nucleic acid. In some embodiments, modifying a target nucleic acid may comprise replacing a sequence in a target nucleic acid with a second sequence. For example, a mutated sequence or a sequence associated with a disease may be replaced with a second sequence lacking the mutation or that is not associated with the disease. In some embodiments, modifying a target nucleic acid may comprise introducing a sequence into a target nucleic acid. For example, a beneficial sequence or a sequence that may reduce or eliminate a disease may inserted into the target nucleic acid.

[0309] In some embodiments, the present disclosure provides methods and compositions for editing a target nucleic acid sequence comprising a programmable nuclease capable of introducing a double-strand break in a double stranded DNA (dsDNA) target sequence. The programmable nuclease can be coupled to a guide nucleic acid that targets a particular region of interest in the dsDNA. A double-strand break can be repaired and rejoined by non-homologous end joining (NHEJ) or homology directed repair (HDR). Thus, a programmable nuclease capable of introducing a double-strand break as disclosed herein can be useful in a genome editing method, for example, used for therapeutic applications to treat a disease or disorder, or for

agricultural applications. Such diseases or disorders that can be treated by the methods and compositions described herein include a liver disease or disorder, an eye disease or disorder, cystic fibrosis, or a muscle disease or disorder. CasΦ programmable nuclease disclosed herein can be used for genome editing purposes to generate double strand breaks in order to excise a region of DNA and subsequently introduce a region of DNA (e.g., donor DNA) into the excised region.

[0310] In some embodiments, the present disclosure provides methods and compositions for modifying or editing a target nucleic acid sequence comprising two or more programmable nickases. For example, modifying a target nucleic acid may comprise introducing a two or more single-stranded breaks in the target nucleic acid. In some embodiments, a break may be introduced by contacting a target nucleic acid with a programmable nickase and a guide nucleic acid. The guide nucleic acid may bind to the programmable nickase and hybridize to a region of the target nucleic acid, thereby recruiting the programmable nickase to the region of the target nucleic acid. Binding of the programmable nickase to the guide nucleic acid and the region of the target nucleic acid may activate the programmable nickase, and the programmable nickase may introduce a break (e.g., a single stranded break) in the region of the target nucleic acid. In some embodiments, modifying a target nucleic acid may comprise introducing a first break in a first region of the target nucleic acid and a second break in a second region of the target nucleic acid. For example, modifying a target nucleic acid may comprise contacting a target nucleic acid with a first guide nucleic acid that binds to a first programmable nickase and hybridizes to a first region of the target nucleic acid and a second guide nucleic acid that binds to a second programmable nickase and hybridizes to a second region of the target nucleic acid. The first programmable nickase may introduce a first break in a first strand at the first region of the target nucleic acid, and the second programmable nickase may introduce a second break in a second strand at the second region of the target nucleic acid. In some embodiments, a segment of the target nucleic acid between the first break and the second break may be removed, thereby modifying the target nucleic acid. In some embodiments, a segment of the target nucleic acid between the first break and the second break may be replaced (e.g., with an insert sequence), thereby modifying the target nucleic acid.

[0311] The methods of the disclosure can use HDR or NHEJ. Following cleavage of a targeted genomic sequence, one of two alternative DNA repair mechanisms can restore chromosomal integrity: non-homologous end joining (NHEJ) which can generate insertions and/or deletions of a few base-pairs of DNA at the cut site. Alternatively, the cell can employ homology-directed repair (HDR), which can correct the lesion via an additional DNA template (e.g., donor) that spans the cut site. In some instances, the methods of the disclosure use microhomology-mediated end-joining (MMEJ).

[0312] Methods and compositions of the disclosure can be used to insert a donor polynucleotide into a target nucleic acid sequence. A donor polynucleotide can comprise a segment of nucleic acid to be integrated at a target genomic locus. The donor polynucleotide can comprise one or more polynucleotides of interest. The donor polynucleotide can comprise one or more expression cassettes. The expression cassette can comprise a donor polynucleotide of interest, a polynucleotide encoding a selection marker and/or a reporter gene, and regulatory components that influence expression.

[0313] The donor polynucleotide can comprise a genomic nucleic acid. The genomic nucleic acid can be derived from an animal, a mouse, a human, a non-human, a rodent, a non-human, a rat, a hamster, a rabbit, a pig, a bovine, a deer, a sheep, a goat, a chicken, a cat, a dog, a ferret, a primate (e.g., marmoset, rhesus monkey), domesticated mammal or an agricultural mammal, an avian, a bacterium, a archaeon, a virus, or any other organism of interest or a combination thereof. The donor polynucleotide may be synthetic.

[0314] Donor polynucleotides of any suitable size can be integrated into a genome. In some embodiments, the donor polynucleotide integrated into a genome is less than 3, about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 or more than 500 kilobases (kb) in length. In some embodiments, the donor polynucleotide integrated into a genome is at least about 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 or more than 500 kb in length. In some embodiments, the donor polynucleotide integrated into a genome is up to about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 or more than 500 kb in length.

[0315] The donor polynucleotide can be flanked by site-specific recombination target sequences (e.g., 5' and 3' homology arms) on a targeting vector. The length of a homology arm may be from about 50 to about 1000 bp. The length of a homology arm may be from about 400 to about 1000 bp. A homology arm can be of any length that is sufficient to promote a homologous recombination event with a corresponding target site, including for example, from about 400 bp to about 500 bp, from about 500 bp to about 600 bp, from about 600 bp to about 700 bp, from about 700 bp to about 800 bp, from about 800 bp to about 900 bp, or from about 900 bp to about 1000 bp. In preferred embodiments, the length of a homology arm may be from about 200 to about 300 bp. The sum total of 5' and 3' homology arms can be about 0.5 kb, 1 kb, 1.5 kb, 2 kb, 3 kb, 4 kb, 5kb, 6kb, 7kb, 8kb, 9kb, about 0.5 kb to about 1 kb, about 1 kb to about 1.5 kb, about 1.5 kb to about 2 kb, about 2 kb to about 3 kb, about 3 kb to about 4 kb, about 4 kb to about 5kb, about 5kb to about 6 kb, about 6 kb to about 7 kb, about 8 kb to about 9 kb, or is at least 10 kb.

[0316] In some embodiments, the donor polynucleotide comprises one or more phosphorothioate bonds between nucleobases. In some embodiments, one or more of the first five 5' nucleobases of the donor polynucleotide are linked by phosphorothioate bonds. In some embodiments, one or more of the five nucleobases at the 3' end of the donor polynucleotide are linked by phosphorothioate bonds. In some embodiments, one or more of the first three 5' nucleobases of the donor polynucleotide are linked by phosphorothioate bonds. In some embodiments, one or more of the three nucleobases at the 3' end of the donor polynucleotide are linked by phosphorothioate bonds. In preferred embodiments, the two nucleobases at 5' end of the donor polynucleotide are linked by a phosphorothioate bond. In some embodiments, the two nucleobases at the 3' end of the donor polynucleotide are linked by a phosphorothioate bond. In more preferred embodiments, the two nucleobases at 5' end of the donor polynucleotide are linked by a phosphorothioate bond and the two nucleobases at the 3' end of the donor polynucleotide are linked by a phosphorothioate bond.

[0317] Examples of site-specific recombinases that can be used include, but are not limited to, Cre, Flp, and Dre recombinases. The site-specific recombinase can be introduced into the cell by any means, including by introducing the recombinase polypeptide into the cell or by introducing a polynucleotide encoding the site- specific recombinase into the host cell. The polynucleotide encoding the site-specific recombinase can be located within the insert polynucleotide or within a separate polynucleotide. The site-specific recombinase can be operably linked to a promoter active in the cell including, for example, an inducible promoter, a promoter that is endogenous to the cell, a promoter that is heterologous to the cell, a cell-specific promoter, a tissue-specific promoter, or a developmental stage- specific promoter. Site-specific recombination target sequences which can flank the insert polynucleotide or any polynucleotide of interest in the insert polynucleotide can include, but are not limited to, loxP, lox511, 1oχ2272, 1oχ66, lox71 , loxM2, lox5171, FRT, FRT11, FRT71, attp, att, FRT, rox, and a combination thereof.

[0318] The target nucleic acid may comprise one or more of a genome, a chromosome, a plasmid, a gene, a promoter, an untranslated region, an open reading frame, an intron, an exon, or an operator. The target nucleic acid may comprise a segment of one or more of a genome, a chromosome, a plasmid, a gene, a promoter, an untranslated region, an open reading frame, an intron, an exon, or an operator. In some embodiments, the target nucleic acid may be part of a cell or an organism. In some embodiments, the target nucleic acid may be a cell-free genetic component.

[0319] In some embodiments, gene modifying or gene editing is achieved by fusing a programmable nuclease such as a CasΦ protein to a heterologous sequence. The heterologous sequence can be a suitable fusion partner, e.g., a polypeptide that provides recombinase activity by acting on the target nucleic acid sequence. In some embodiments, the fusion protein

comprises a programmable nuclease such as a CasΦ protein fused to a heterologous sequence by a linker.

[0320] The heterologous sequence or fusion partner can be a site specific recombinase. The site specific recombinase can have recombinase activity. Examples of site-specific recombinases that can be used include, but are not limited to, Cre, Hin, Tre, and FLP recombinases. The heterologous sequence or fusion partner can be a recombinase catalytic domain. The recombinase catalytic domains can be from, for example, a tyrosine recombinase, a serine recombinase, a Gin recombinase, a Hin recombinase, a β recombinase, a Sin recombinase, a Tn3 recombinase, a γδ recombinase, a Cre recombinase, a FLP recombinase, or a phC31 integrase.

[0321] The heterologous sequence or fusion partner can be fused to the C-terminus, N-terminus, or an internal portion (e.g., a portion other than the N- or C-terminus) of the programmable nuclease, for example a dead CasΦ polypeptide.

[0322] The heterologous sequence or fusion partner can be fused to the programmable nuclease by a linker. A linker can be a peptide linker or a non-peptide linker. In some embodiments, the linker is an XTEN linker. In some embodiments, the linker comprises one or more repeats a tri-peptide GGS. In some embodiments, the linker is from 1 to 100 amino acids in length. In some embodiments, the linker is more 100 amino acids in length. In some embodiments, the linker is from 10 to 27 amino acids in length. A non-peptide linker can be a polyethylene glycol (PEG), polypropylene glycol (PPG), co-poly(ethylene/propylene) glycol, polyoxyethylene (POE), polyurethane, polyphosphazene, polysaccharides, dextran, polyvinyl alcohol, polyvinylpyrrolidones, polyvinyl ethyl ether, polyacryl amide, polyacrylate, polycyanoacrylates, lipid polymers, chitins, hyaluronic acid, heparin, or an alkyl linker.

[0323] In some embodiments, the CasΦ protein can comprise an enzymatically inactive and/or "dead" (abbreviated by "d") programmable nuclease in combination (e.g., fusion) with a polypeptide comprising recombinase activity. Although a programmable CasΦ nuclease normally has nuclease activity, in some embodiments, a programmable CasΦ nuclease does not have nuclease activity.

[0324] A programmable nuclease can comprise a modified form of a wild type counterpart. The modified form of the wild type counterpart can comprise an amino acid change (e.g., deletion, insertion, or substitution) that reduces the nucleic acid-cleaving activity of the programmable nuclease. For example, a nuclease domain (e.g., RuvC domain) of a CasΦ polypeptide can be deleted or mutated so that it is no longer functional or comprises reduced nuclease activity. The modified form of the programmable nuclease can have less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nucleic acid-cleaving activity of the wild-type counterpart. The modified form of a programmable nuclease can have no substantial nucleic acid-cleaving

activity. When a programmable nuclease is a modified form that has no substantial nucleic acid-cleaving activity, it can be referred to as enzymatically inactive and/or dead. A dead CasΦ polypeptide (e.g., dCasΦ) can bind to a target nucleic acid sequence but may not cleave the target nucleic acid sequence. A dCasΦ polypeptide can associate with a guide nucleic acid to activate or repress transcription of a target nucleic acid sequence.

[0325] In some embodiments, a programmable nuclease is a dead CasΦ polypeptide. A dead CasΦ polypeptide can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 85% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 90% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 95% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 98% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107.

[0326] A deadCasΦ (also referred to herein as "dCasΦ") polypeptide can form a ribonucleoprotein complex with a guide nucleic acid. The guide nucleic acid can comprise a crRNA sequence comprising at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99%, or 100% sequence identity to any one of SEQ ID NO: 48 – SEQ ID NO: 86, or a reverse complement thereof.

[0327] Enzymatically inactive can refer to a polypeptide that can bind to a nucleic acid sequence in a polynucleotide in a sequence-specific manner, but may not cleave a target polynucleotide. An enzymatically inactive site-directed polypeptide can comprise an enzymatically inactive domain (e.g. a programmable nuclease domain). Enzymatically inactive can refer to no activity. Enzymatically inactive can refer to substantially no activity. Enzymatically inactive can refer to essentially no activity. Enzymatically inactive can refer to an activity less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% activity compared to a wild-type exemplary activity (e.g., nucleic acid cleaving activity, wild-type CasΦ activity).

[0328] In further embodiments, methods of modifying cells are provided. In some embodiments, a method of modifying a cell comprising a target nucleic acid wherein the method comprises introducing a programmable CasΦ nuclease or variant thereof disclosed herein to the cell, wherein the programmable CasΦ nuclease or variant cleaves or modifies the target nucleic acid.

[0329] Modified cells obtained or obtainable by the methods described herein are provided. In some embodiments, a modified cell is obtained or is obtained by a method of modifying a cell disclosed herein.

[0330] In some embodiments, a CasΦ polypeptide disclosed herein is expressed in a cell. In some embodiments, a CasΦ polypeptide disclosed herein complexed with a guide nucleic is expressed in a cell. In some embodiments, a method disclosed herein comprises modifying or editing a cell. In some embodiments, a modified cell is provided wherein a cell is modified by a CasΦ polypeptide disclosed herein. In some embodiments, a cell is provided wherein the cell comprises a CasΦ polypeptide disclosed herein.

Methods of Nicking of a Target Nucleic Acid

[0331] Disclosed herein are methods of introducing a break into a target nucleic acid. In some embodiments, the break may be a single stranded break (e.g., a nick). The programmable nickases disclosed herein and a gRNA disclosed herein may be used to introduce a single-stranded break into a target nucleic acid, for example a single stranded break in a double-stranded DNA.

[0332] A method of introducing a break into a target nucleic acid may comprise contacting the target nucleic acid with a first guide nucleic acid (e.g., a guide nucleic acid comprising a region that binds to a first programmable nickase) and a second guide nucleic acid (e.g., a guide nucleic acid comprising a region that binds to a second programmable nickase). The first guide nucleic acid may comprise an additional region that binds to the target nucleic acid, and the second guide nucleic acid may comprise an additional region that binds to the target nucleic acid. The additional region of the first guide nucleic acid and the additional region of the second guide nucleic acid may bind opposing strands of the target nucleic acid.

[0333] In some embodiments, a programmable nickase of the disclosure can cleave a non-target strand of a double-stranded target nucleic acid (e.g., DNA). In some embodiments, the programmable nickase may not cleave the target strand of the double-stranded target nucleic acid (e.g., DNA). The strand of a double-stranded target nucleic acid that is complementary to and hybridizes with the guide nucleic acid can be called the target strand. The strand of the double-stranded target DNA that is complementary to the target strand, and therefore is not complementary to the guide nucleic acid can be called non-target strand.

[0334] The temperature at which a ribonucleoprotein (RNP) complex comprising a programmable nuclease and a guide nucleic acid is formed (i.e. the RNP complexing temperature) can affect the nickase activity of the programmable nuclease. For example, an RNP complex formed at room temperature can have a greater nickase activity than an RNP complex formed at 37°C. In some cases, the RNP complex can be formed at room temperature, for example, from about 20°C to 22°C. In some cases, the RNP complex can be formed at, for

example, about 15°C, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C.

[0335] In some embodiments, a programmable nuclease may exhibit at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, or at least about 50-fold greater nicking activity when complexed with a guide RNA at room temperature as compared to when complexed at 37°C.

[0336] The crRNA repeat sequence of a guide nucleic acid can affect the nickase activity of a programmable nuclease. For example, a programmable nuclease can comprise enhanced or greater nickase activity when complexed with guide nucleic acids comprising certain crRNA repeat sequences. For example, a programmable nuclease can comprise greater nickase activity when complexed with a guide RNA comprising a crRNA repeat sequence of CasΦ.18 as shown in **TABLE 2**. In another example, a programmable nuclease can comprise greater nickase activity when complexed with a guide RNA comprising a crRNA repeat sequence of CasΦ.7 as shown in **TABLE 2**. In some embodiments, a programmable nuclease may exhibit at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, or at least about 50-fold greater nicking activity when complexed with a guide RNA comprising a specific crRNA repeat sequence as compared to when in a complex with a guide RNA comprising another crRNA repeat sequence.

[0337] The programmable nucleases disclosed herein may exhibit cis-cleavage activity or target cleavage activity. Target cleavage activity may refer to the cleavage of a target nucleic acid by the programmable nuclease. In some cases, the cis-cleavage activity results in double-stranded breaks in the target nucleic acids. In some cases, the cis-cleavage activity results in single-stranded breaks in the target nucleic acids. In some cases, the cis-cleavage activity produces a

mixture of double- and single-stranded breaks in the target nucleic acids. In further cases, the rates of cis-cleavage double- and single-strand break formation may be dependent on the sequence of the guide nucleic acid. In some cases, the ratio of cis-cleavage double- and single-strand break formation may be dependent on the sequence of the guide nucleic acid. In some cases, the ratio or rate of cis-cleavage double- and single-strand break formation may be dependent on the repeat sequence of the crRNA of the guide nucleic acid. In some cases, the ratio or rate of cis-cleavage double- and single-strand break formation may be dependent on the temperature at which the ribonucleoprotein complex comprising the programmable nuclease and the guide nucleic acid are complexed.

[0338] A programmable nuclease for use in modifying a target nucleic acid may have greater nicking activity as compared to double stranded cleavage activity. In some embodiments, a programmable nuclease may exhibit at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, or at least about 50-fold greater nicking activity as compared to double stranded cleavage activity.

[0339] In other cases, a programmable nuclease for use in modifying a target nucleic acid may have greater double stranded cleavage activity as compared to nicking activity. In some embodiments, a programmable nuclease may exhibit at least about 1.1-fold, at least about 1.2-fold, at least about 1.3-fold, at least about 1.4-fold, at least about 1.5-fold, at least about 1.6-fold, at least about 1.7-fold, at least about 1.8-fold, at least about 1.9-fold, at least about 2-fold, at least about 2.1-fold, at least about 2.2-fold, at least about 2.3-fold, at least about 2.4-fold, at least about 2.5-fold, at least about 2.6-fold, at least about 2.7-fold, at least about 2.8-fold, at least about 2.9-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 7-fold, at least about 8-fold, at least about 9-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 30-fold, at least about 40-fold, or at least about 50-fold greater double stranded cleavage activity as compared to nicking activity.

[0340] In some embodiments, the nicking activity and double stranded cleavage activity of a programmable nuclease depend on the conditions and species present in the sample containing the programmable nuclease. In some cases, the nicking activity and double stranded cleavage activity of the programmable nuclease are responsive to the sequence of the crRNA present in

the guide nucleic acid. In some cases, the ratio of nicking activity and double stranded cleavage activity can be modulated by changing the sequence of the crRNA present. In some cases, the nicking activity and double stranded cleavage activity of the programmable nuclease respond differently to changes in temperature (e.g., RNP complexing temperature), pH, osmolarity, buffer, target nucleic acid concentration, ionic strength, and inhibitor concentration. In some embodiments, the ratio of nicking activity to cleavage activity by a programmable nuclease can be actively controlled by adjusting sample conditions and crRNA sequences.

Methods of Regulating Gene Expression

[0341] In some embodiments, the disclosure provided methods and compositions for regulating gene expression. The methods and compositions can comprise use of an enzymatically inactive and/or "dead" (abbreviated by "d") programmable nuclease in combination (e.g., fusion) with a polypeptide comprising transcriptional regulation activity. Although a programmable CasΦ nuclease normally has nuclease activity, in some embodiments, a programmable CasΦ nuclease does not have nuclease activity.

[0342] A programmable nuclease can comprise a modified form of a wild type counterpart. The modified form of the wild type counterpart can comprise an amino acid change (e.g., deletion, insertion, or substitution) that reduces the nucleic acid-cleaving activity of the programmable nuclease. For example, a nuclease domain (e.g., RuvC domain) of a CasΦ polypeptide can be deleted or mutated so that it is no longer functional or comprises reduced nuclease activity. The modified form of the programmable nuclease can have less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5%, or less than 1% of the nucleic acid-cleaving activity of the wild-type counterpart. The modified form of a programmable nuclease can have no substantial nucleic acid-cleaving activity. When a programmable nuclease is a modified form that has no substantial nucleic acid-cleaving activity, it can be referred to as enzymatically inactive and/or dead. A dead CasΦ polypeptide (e.g., dCasΦ) can bind to a target nucleic acid sequence but may not cleave the target nucleic acid sequence. A dCasΦ polypeptide can associate with a guide nucleic acid to activate or repress transcription of a target nucleic acid sequence.

[0343] In some embodiments, the disclosure provides a method of selectively modulating transcription of a gene in a cell. The method can comprise introducing into a cell a (i) fusion polypeptide comprising a dCasΦ polypeptide and a polypeptide comprising transcriptional regulation activity, or a nucleic acid comprising a nucleotide sequence encoding the fusion polypeptide, wherein the dCasΦ polypeptide is enzymatically inactive or exhibits reduced nucleic acid cleavage activity; and ii) a guide nucleic acid, or a nucleic acid comprising a nucleotide sequence encoding the guide nucleic acid.

[0344] In some embodiments, a programmable nuclease is a dead CasΦ polypeptide. A dead CasΦ polypeptide can comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, at least 99%, or 100% sequence identity with any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 85% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 90% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 95% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107. In some embodiments, a programmable nuclease is a dead CasΦ polypeptide comprising at least 98% sequence identity to any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO. 105, and SEQ ID NO 107.

[0345] A deadCasΦ (also referred to herein as "dCasΦ") polypeptide can form a ribonucleoprotein complex with a guide nucleic acid. The guide nucleic acid can comprise a crRNA sequence comprising at least 70%, at least 80%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99%, or 100% sequence identity to any one of SEQ ID NO: 48 – SEQ ID NO: 86, or a reverse complement thereof.

[0346] Enzymatically inactive can refer to a polypeptide that can bind to a nucleic acid sequence in a polynucleotide in a sequence-specific manner, but may not cleave a target polynucleotide. An enzymatically inactive site-directed polypeptide can comprise an enzymatically inactive domain (e.g. a programmable nuclease domain). Enzymatically inactive can refer to no activity. Enzymatically inactive can refer to substantially no activity. Enzymatically inactive can refer to essentially no activity. Enzymatically inactive can refer to an activity less than 1%, less than 2%, less than 3%, less than 4%, less than 5%, less than 6%, less than 7%, less than 8%, less than 9%, or less than 10% activity compared to a wild-type exemplary activity (e.g., nucleic acid cleaving activity, wild-type CasΦ activity).

[0347] Transcription regulation can be achieved by fusing a programmable nuclease such as a dead CasΦ protein to a heterologous sequence. The heterologous sequence can be a suitable fusion partner, e.g., a polypeptide that provides an activity that increases, decreases, or otherwise regulates transcription by acting on the target nucleic acid sequence or on a polypeptide (e.g., a histone or other DNA-binding protein) associated with the target nucleic acid sequence. Non-limiting examples of suitable fusion partners include a polypeptide that provides for transcription activation activity, transcription repression activity, nuclease activity, transcription release factor activity, histone modification activity, histone acetyltransferase activity, nucleic acid association activity, DNA methylase activity, direct or indirect DNA demethylase activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity,

phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deaminase activity, deadenylation activity, SUMOylating activity, deSUMOylating activity, ribosylation activity, deribosylation activity, myristoylation activity, or demyristoylation activity.

[0348] Illustrative modifications performed by a fusion polypeptide can comprise methylation, demethylation, acetylation, deacetylation , ubiquitination, deubiquitination, deamination, alkylation, depurination, oxidation, pyrimidine dimer formation, transposition, recombination, chain elongation, ligation, glycosylation. Phosphorylation, dephosphorylation, adenylation, deadenylation, SUMOylation, deSUMOylation, ribosylation, deribosylation, myristoylation, remodeling, cleavage, oxidoreduction, hydrolation, or isomerization.

[0349] The heterologous sequence or fusion partner can be fused to the C-terminus, N-terminus, or an internal portion (e.g., a portion other than the N- or C-terminus) of the programmable nuclease, for example a dead CasΦ polypeptide. Non-limiting examples of fusion partners include transcription activators, transcription repressors, histone lysine methyltransferases (KMT), Histone Lysine Demethylates, Histone lysine acetyltransferases (KAT), Histone lysine deacetylase, DNA methylases (adenosine or cytosine modification), deaminases, CTCF, periphery recruitment elements (e.g., Lamin A, Lamin B), and protein docking elements (e.g., FKBP/FRB).

[0350] Non-limiting examples of transcription activators include GAL4, VP16, VP64, and p65 subdomain (NFkappaB).

[0351] Non-limiting examples of transcription repressors include Kruippel associated box (KRAB or SKD), the Mad mSIN3 interaction domain (SID), and the ERF repressor domain (ERD).

[0352] Non-limiting examples of histone lysine methyltransferases (KMT) include members from KMT1 family (e.g., SUV39H1, SUV39H2, G9A, ESET/SETDB1, Clr4, Su(var)3-9), KMT2 family members (e.g., hSET1A, hSET1 B, MLL 1 to 5, ASH1, and homologs (Trx, Trr, Ash1)), KMT3 family (SYMD2, NSD1), KMT4 (DOT1L and homologs), KMT5 family (Pr-SET7/8, SUV4-20H1, and homologs), KMT6 (EZH2), and KMT8 (e.g., RIZ1).

[0353] Non-limiting examples of Histone Lysine Demethylates (KDM) include members from KDM1 family (LSD1/BHC110, Splsd1/Swm1/Saf11 0, Su(var)3-3), KDM3 family (JHDM2a/b), KDM4 family (JMJD2A/JHDM3A, JMJD2B, JMJD2C/GASC1, JMJD2D, and homologs (Rph1)), KDM5 family (JARID1A/RBP2, JARID1 B/PLU-1,JARIDIC/SMCX, JARID1D/SMCY, and homologs (Lid, Jhn2, Jmj2)), and KDM6 family (e.g., UTX, JMJD3).

[0354] Non-limiting examples of KAT include members of KAT2 family (hGCN5, PCAF, and homologs (dGCN5/PCAF, Gcn5), KAT3 family (CBP, p300, and homologs (dCBP/NEJ)), KAT4, KAT5, KAT6, KAT7, KAT8, and KAT13.

[0355] In some embodiments, the disclosure provides methods for increasing transcription of a target nucleic acid sequence. The transcription of a target nucleic acid sequence can increase by at least about 1.1 fold, at least about 1.2 fold, at least about 1.3 fold, at least about 1.4 fold, at least about 1.5 fold, at least about 1.6 fold, at least about 1.7 fold, at least about 1.8 fold, at least about 1.9 fold, at least about 2 fold, at least about 2.5 fold, at least about 3 fold, at least about 3.5 fold, at least about 4 fold, at least about 4.5 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 12 fold, at least about 15 fold, at least about 20-fold, at least about 50-fold, at least about 70-fold, or at least about 100-fold compared to the level of transcription of the target nucleic acid sequence in the absence of a fusion polypeptide comprising a enzymatically inactive or enzymatically reduced programmable nuclease (e.g., dead CasΦ protein).

[0356] In some embodiments, the disclosure provides methods for decreasing transcription of a target nucleic acid sequence. The transcription of a target nucleic acid sequence can decrease by at least about 1.1 fold, at least about 1.2 fold, at least about 1.3 fold, at least about 1.4 fold, at least about 1.5 fold, at least about 1.6 fold, at least about 1.7 fold, at least about 1.8 fold, at least about 1.9 fold, at least about 2 fold, at least about 2.5 fold, at least about 3 fold, at least about 3.5 fold, at least about 4 fold, at least about 4.5 fold, at least about 5 fold, at least about 6 fold, at least about 7 fold, at least about 8 fold, at least about 9 fold, at least about 10 fold, at least about 12 fold, at least about 15 fold, at least about 20-fold, at least about 50-fold, at least about 70-fold, or at least about 100-fold compared to the level of transcription of the target nucleic acid sequence in the absence of a fusion polypeptide comprising a enzymatically inactive or enzymatically reduced programmable nuclease (e.g., dead Cas 12j protein).

Method of Treating a Disorder

[0357] The compositions and methods described herein may be used to treat, prevent, or inhibit an ailment in a subject. The ailments may include diseases, cancers, genetic disorders, neoplasias, and infections. In some cases, the disease or disorder for treatment is a liver disease or disorder, an eye disease or disorder, cystic fibrosis, or a muscle disease or disorder. In some cases, the ailments are associated with one or more genetic sequences, including but not limited to 11-hydroxylase deficiency; 17,20-desmolase deficiency; 17-hydroxylase deficiency; 3-hydroxyisobutyrate aciduria; 3-hydroxysteroid dehydrogenase deficiency; 46,XY gonadal dysgenesis; AAA syndrome; ABCA3 deficiency; ABCC8-associated hyperinsulinism; aceruloplasminemia; achondrogenesis type 2; acral peeling skin syndrome; acrodermatitis enteropathica; adrenocortical micronodular hyperplasia; adrenoleukodystrophies; adrenomyeloneuropathies; Aicardi-Goutieres syndrome; Alagille disease; Alpers syndrome; alpha-mannosidosis; Alstrom syndrome; Alzheimer disease; amelogenesis imperfecta; amish type microcephaly; amyotrophic lateral sclerosis (ALS); anauxetic dysplasia; androgen insensitivity syndrome; Antley-Bixler syndrome; APECED, Apert syndrome, aplasia of lacrimal

and salivary glands, argininemia, arrhythmogenic right ventricular dysplasia, Arts syndrome, ARVD2, arylsulfatase deficiency type metachromatic leokodystrophy, ataxia telangiectasia, autoimmune lymphoproliferative syndrome; autoimmune polyglandular syndrome type 1; autosomal dominant anhidrotic ectodermal dysplasia; autosomal dominant polycystic kidney disease; autosomal recessive microtia; autosomal recessive renal glucosuria; autosomal visceral heterotaxy; Bardet-Biedl syndrome; Bartter syndrome; basal cell nevus syndrome; Batten disease; benign recurrent intrahepatic cholestasis; beta-mannosidosis; Bethlem myopathy; Blackfan-Diamond anemia; blepharophimosis; Byler disease; C syndrome; CADASIL; carbamyl phosphate synthetase deficiency; cardiofaciocutaneous syndrome; Carney triad; carnitine palmitoyltransferase deficiencies; cartilage-hair hypoplasia; cblC type of combined methylmalonic aciduria; CD18 deficiency; CD3Z-associated primary T-cell immunodeficiency; CD40L deficiency; CDAGS syndrome; CDG1A; CDG1B; CDG1M; CDG2C; CEDNIK syndrome; central core disease; centronuclear myopathy; cerebral capillary malformation; cerebrooculofacioskeletal syndrome type 4; cerebrooculogacioskeletal syndrome; cerebrotendinous xanthomatosis; CHARGE association; cherubism; CHILD syndrome; chronic granulomatous disease; chronic recurrent multifocal osteomyelitis; citrin deficiency; classic hemochromatosis; CNPPB syndrome; cobalamin C disease; Cockayne syndrome; coenzyme Q10 deficiency; Coffin-Lowry syndrome; Cohen syndrome; combined deficiency of coagulation factors V; common variable immune deficiency; complete androgen insentivity; cone rod dystrophies; conformational diseases; congenital bile adid synthesis defect type 1; congenital bile adid synthesis defect type 2; congenital defect in bile acid synthesis type; congenital erythropoietic porphyria; congenital generalized osteosclerosis; Cornelia de Lange syndrome; Cousin syndrome; Cowden disease; COX deficiency; Crigler-Najjar disease; Crigler-Najjar syndrome type 1; Crisponi syndrome; Currarino syndrome; Curth-Macklin type ichthyosis hystrix; cutis laxa; cystic fibrosis; cystinosis; d-2-hydroxyglutaric aciduria; DDP syndrome; Dejerine-Sottas disease; Denys-Drash syndrome; desmin cardiomyopathy; desmin myopathy; DGUOK-associated mitochondrial DNA depletion; disorders of glutamate metabolism; distal spinal muscular atrophy type 5; DNA repair diseases; dominant optic atrophy; Doyne honeycomb retinal dystrophy; Duchenne muscular dystrophy; dyskeratosis congenita; Ehlers-Danlos syndrome type 4; Ehlers-Danlos syndromes; Elejalde disease; Ellis-van Creveld disease; Emery-Dreifuss muscular dystrophies; encephalomyopathic mtDNA depletion syndrome; enzymatic diseases; EPCAM-associated congenital tufting enteropathy; epidermolysis bullosa with pyloric atresia; exercise-induced hypoglycemia; facioscapulohumeral muscular dystrophy; Faisalabad histiocytosis; familial atypical mycobacteriosis; familial capillary malformation-arteriovenous; familial esophageal achalasia; familial glomuvenous malformation; familial hemophagocytic lymphohistiocytosis; familial mediterranean fever; familial megacalyces; familial schwannomatosisl; familial spina bifida; familial splenic asplenia/hypoplasia; familial thrombotic thrombocytopenic purpura; Fanconi disease; Feingold syndrome; FENIB;

fibrodysplasia ossificans progressiva; FKTN; Francois-Neetens fleck corneal dystrophy; Frasier syndrome; Friedreich ataxia; FTDP-17; fucosidosis; G6PD deficiency; galactosialidosis; Galloway syndrome; Gardner syndrome; Gaucher disease; Gitelman syndrome; GLUT1 deficiency; glycogen storage disease type 1b; glycogen storage disease type 2; glycogen storage disease type 3; glycogen storage disease type 4; glycogen storage disease type 9a; glycogen storage diseases; GM1-gangliosidosis; Greenberg syndrome; Greig cephalopolysyndactyly syndrome; hair genetic diseases; HANAC syndrome; harlequin type ichtyosis congenita; HDR syndrome; hemochromatosis type 3; hemochromatosis type 4; hemophilia A; hereditary angioedema type 3; hereditary angioedemas; hereditary hemorrhagic telangiectasia; hereditary hypofibrinogenemia; hereditary intraosseous vascular malformation; hereditary leiomyomatosis and renal cell cancer; hereditary neuralgic amyotrophy; hereditary sensory and autonomic neuropathy type; Hermansky-Pudlak disease; HHH syndrome; HHT2; hidrotic ectodermal dysplasia type 1; hidrotic ectodermal dysplasias; HNF4A-associated hyperinsulinism; HNPCC; human immunodeficiency with microcephaly; Huntington disease; hyper-IgD syndrome; hyperinsulinism-hyperammonemia syndrome; hypertrophy of the retinal pigment epithelium; hypochondrogenesis; hypohidrotic ectodermal dysplasia; ICF syndrome; idiopathic congenital intestinal pseudo-obstruction; immunodeficiency with hyper-IgM type 1; immunodeficiency with hyper-IgM type 3; immunodeficiency with hyper-IgM type 4; immunodeficiency with hyper-IgM type 5; inborm errors of thyroid metabolism; infantile visceral myopathy; infantile X-linked spinal muscular atrophy; intrahepatic cholestasis of pregnancy; IPEX syndrome; IRAK4 deficiency; isolated congenital asplenia; Jeune syndrome Imag; Johanson-Blizzard syndrome; Joubert syndrome; JP-HHT syndrome; juvenile hemochromatosis; juvenile hyalin fibromatosis; juvenile nephronophthisis; Kabuki mask syndrome; Kallmann syndromes; Kartagener syndrome; KCNJ11-associated hyperinsulinism; Kearns-Sayre syndrome; Kostmann disease; Kozlowski type of spondylometaphyseal dysplasia; Krabbe disease; LADD syndrome; late infantile-onset neuronal ceroid lipofuscinosis; LCK deficiency; LDHCP syndrome; Legius syndrome; Leigh syndrome; lethal congenital contracture syndrome 2; lethal congenital contracture syndromes; lethal contractural syndrome type 3; lethal neonatal CPT deficiency type 2; lethal osteosclerotic bone dysplasia; LIG4 syndrome; lissencephaly type 1 Imag; lissencephaly type 3; Loeys-Dietz syndrome; low phospholipid-associated cholelithiasis; lysinuric protein intolerance; Maffucci syndrome; Majeed syndrome; mannose-binding protein deficiency; Marfan disease; Marshall syndrome; MASA syndrome; MCAD deficiency; McCune-Albright syndrome; MCKD2; Meckel syndrome; Meesmann corneal dystrophy; megacystis-microcolon-intestinal hypoperistalsis; megaloblastic anemia type 1; MEHMO; MELAS; Melnick-Needles syndrome; MEN2s; Menkes disease; metachromatic leukodystrophies; methylmalonic acidurias; methylvalonic aciduria; microcoria-congenital nephrosis syndrome; microvillous atrophy; mitochondrial neurogastrointestinal encephalomyopathy; monilethrix; monosomy X; mosaic trisomy 9 syndrome; Mowat-Wilson syndrome; mucolipidosis type 2; mucolipidosis type Ma;

mucolipidosis type IV; mucopolysaccharidoses; mucopolysaccharidosis type 3A; mucopolysaccharidosis type 3C; mucopolysaccharidosis type 4B; multiminicore disease; multiple acyl-CoA dehydrogenation deficiency; multiple cutaneous and mucosal venous malformations; multiple endocrine neoplasia type 1; multiple sulfatase deficiency; NAIC; nail-patella syndrome; nemaline myopathies; neonatal diabetes mellitus; neonatal surfactant deficiency; nephronophtisis; Netherton disease; neurofibromatoses; neurofibromatosis type 1; Niemann-Pick disease type A; Niemann-Pick disease type B; Niemann-Pick disease type C; NKX2E; Noonan syndrome; North American Indian childhood cirrhosis; NROB1 duplication-associated DSD; ocular genetic diseases; oculo-auricular syndrome; OLEDAID; oligomeganephronia; oligomeganephronic renal hypolasia; Ollier disease; Opitz-Kaveggia syndrome; orofaciodigital syndrome type 1; orofaciodigital syndrome type 2; osseous Paget disease; otopalatodigital syndrome type 2; OXPHOS diseases; palmoplantar hyperkeratosis; panlobar nephroblastomatosis; Parkes-Weber syndrome; Parkinson disease; partial deletion of 21q22.2-q22.3; Pearson syndrome; Pelizaeus-Merzbacher disease; Pendred syndrome; pentalogy of Cantrell; peroxisomal acyl-CoA-oxidase deficiency; Peutz-Jeghers syndrome; Pfeiffer syndrome; Pierson syndrome; pigmented nodular adrenocortical disease; pipecolic acidemia; Pitt-Hopkins syndrome; plasmalogens deficiency; pleuropulmonary blastoma and cystic nephroma; polycystic lipomembranous osteodysplasia; porphyrias; premature ovarian failure; primary erythermalgia; primary hemochromatoses; primary hyperoxaluria; progressive familial intrahepatic cholestasis; propionic acidemia; pyruvate decarboxylase deficiency; RAPADILINO syndrome; renal cystinosis; rhabdoid tumor predisposition syndrome; Rieger syndrome; ring chromosome 4; Roberts syndrome; Robinow-Sorauf syndrome; Rothmund-Thomson syndrome; SCID; Saethre-Chotzen syndrome; Sandhoff disease; SC phocomelia syndrome; SCAS; Schinzel phocomelia syndrome; short rib-polydactyly syndrome type 1; short rib-polydactyly syndrome type 4; short-rib polydactyly syndrome type 2; short-rib polydactyly syndrome type 3; Shwachman disease; Shwachman-Diamond disease; sickle cell anemia; Silver-Russell syndrome; Simpson-Golabi-Behmel syndrome; Smith-Lemli-Opitz syndrome; SPG7-associated hereditary spastic paraplegia; spherocytosis; split-hand/foot malformation with long bone deficiencies; spondylocostal dysostosis; sporadic visceral myopathy with inclusion bodies; storage diseases; STRA6-associated syndrome; Tay-Sachs disease; thanatophoric dysplasia; thyroid metabolism diseases; Tourette syndrome; transthyretin-associated amyloidosis; trisomy 13; trisomy 22; trisomy 2p syndrome; tuberous sclerosis; tufting enteropathy; urea cycle diseases; Van Den Ende-Gupta syndrome; Van der Woude syndrome; variegated mosaic aneuploidy syndrome; VLCAD deficiency; von Hippel-Lindau disease; Waardenburg syndrome; WAGR syndrome; Walker-Warburg syndrome; Werner syndrome; Wilson disease; Wolcott-Rallison syndrome; Wolfram syndrome; X-linked agammaglobulinemia; X-linked chronic idiopathic intestinal pseudo-obstruction; X-linked cleft palate with ankyloglossia; X-linked dominant chondrodysplasia punctata; X-linked ectodermal dysplasia; X-linked Emery-Dreifuss muscular

dystrophy; X-linked lissencephaly; X-linked lymphoproliferative disease; X-linked visceral heterotaxy; xanthinuria type 1; xanthinuria type 2; xeroderma pigmentosum; XPV; and Zellweger disease. In some embodiments, the ailment is Duchenne muscular dystrophy. In some embodiments, the ailment is myotonic dystrophy Type 1 (DM1). In some embodiments, the ailment is blindness or an inherited disease affecting the back of the eye. In some embodiments, the ailment is deafness. In some embodiments, the ailment is progeria. In some embodiments, the ailment is multiple sclerosis. In some embodiments, the ailment is cancer. In some embodiments, the ailment is a lysosomal storage disease, *e.g.*, Hunter syndrome, Hurler syndrome. In some embodiments, the ailment is hypercholesterolemia. In some embodiments, the ailment is Stargardt macular dystrophy. In some embodiments, the ailment is In preferred embodiments, the ailment is cystic fibrosis.

[0358] In some embodiments, treating, preventing, or inhibiting an ailment in a subject may comprise contacting a target nucleic acid associated with a particular ailment to a programmable nuclease (e.g., a CasΦ programmable nuclease). In some aspects, the methods of treating, preventing, or inhibiting an ailment may involve removing, modifying, replacing, transposing, or affecting the regulation of a genomic sequence of a patient in need thereof. In some embodiments, the methods of treating, preventing, or inhibiting an ailment may involve modulating gene expression. In some embodiments, the methods of treating, preventing, or inhibiting an ailment may comprise targeting a nucleic acid sequence associated with a pathogen, such as a virus or bacteria, to a programmable nuclease of the present disclosure.

[0359] The compositions and methods described herein may be used to treat, prevent, diagnose, or identify a cancer in a subject. In some aspects, the methods may target cells or tissues. In some embodiments, the methods may be applied to subjects, such as humans. As used herein, the term “cancer” refers to a physiological condition that may be characterized by abnormal or unregulated cell growth or activity. In some cases, cancer may involve the spread of the cells exhibiting abnormal or unregulated growth or activity between various tissues in a subject. In some aspects, cancer may be a genetic condition. Examples of cancers include, but are not limited to Acute Lymphoblastic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Anal Cancer, Astrocytomas, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Cancer, Breast Cancer, Bronchial Cancer, Burkitt Lymphoma, Carcinoma, Cardiac Tumors, Cervical Cancer, Chordoma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Chronic Myeloproliferative Neoplasms, Colon Cancer, Colorectal Cancer, Craniopharyngioma, Cutaneous T-cell lymphoma, Ductal Carcinoma, Embryonal Tumors, Endometrial Cancer, Ependymoma, Esophageal Cancer, Esthesioneuroblastoma, Ewing Sarcoma, Extracranial Germ Cell Tumors, Extragonadal Germ Cell Tumors, Fallopian Tube Cancer, Fibrous Histiocytoma, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Cancer, Gastrointestinal Carcinoid Cancer, Gastrointestinal Stromal Tumors, Gestational Trophoblastic Disease, Hairy Cell Leukemia, Head

and Neck Cancer, Heart Tumors, Hepatocellular Cancer, Histiocytosis, Hodgkin Lymphoma, Hypopharyngeal Cancer, Intraocular Melanoma, Islet Cell Tumors, Kaposi Sarcoma, Kidney cancer, Langerhans Cell Histiocytosis, Laryngeal Cancer, Leukemia, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoma, Malignant Fibrous Histiocytoma, Melanoma, Merkel Cell Carcinoma, Mesothelioma, Metastatic Squamous Neck Cancer, Midline Tract Carcinoma, Mouth Cancer, Multiple Endocrine Neoplasia Syndromes, Multiple Myeloma, Mycosis Fungoides, Myelodysplastic Syndromes, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Neoplasms, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Oral Cancer, Osteosarcoma, Ovarian Cancer, Pancreatic Cancer, Pancreatic Neuroendocrine Tumors, Papillomatosis, Paraganglioma, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm, Pleuropulmonary Blastoma, Primary Central Nervous System (CNS) Lymphoma, Primary Peritoneal Cancer, Prostate Cancer, Rectal Cancer, Recurrent Cancer, Renal Cell Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sézary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer with Occult Primary, Stomach Cancer, T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Tracheobronchial Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Ureter Cancer, Renal Pelvis Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Vascular Tumors, Vulvar Cancer, and Wilms Tumor.

[0360] In some cases, a cancer is associated with one or more particular biomarkers. A biomarker is a chemical species or profile that may serve as an indicator of a cellular or organismal state (e.g., the presence or absence of a disease). Non-limiting examples of biomarkers include biomolecules, nucleic acid sequences, proteins, metabolites, nucleic acids, protein modifications. A biomarker may refer to one species or to a plurality of species, such as a cell surface profile.

[0361] The methods of the present disclosure (e.g., methods of modifying a target nucleic acid) may comprise targeting a biomarker or a nucleic acid associated with a biomarker with a programmable nuclease of the disclosure (e.g., a CasΦ). In some cases, the biomarker is a gene associated with a cancer. Non-limiting examples of genes associated with cancers include, ABL, AF4/HRX, AKT-2, ALK, ALK/NPM, AML1, AML1/MTG8, APC, ATM, AXIN2, AXL, BAP1, BARD1, BCL-2, BCL-3, BCL- 6, BCR/ABL, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, c-MYC, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN1C, CDKN2A, CEBPA, CHEK2, CTNNA1, DBL, DEK/CAN, DICER1, DIS3L2, E2A/PBX1, EGFR, ENL/HRX, EPCAM, ERG/TLS, ERBB, ERBB-2, ETS-1, EWS/FLI-1, FH, FLCN, FMS, FOS, FPS, GATA2, GLI, GPGSP, GREM1, HER2/neu, HOX11, HOXB13, HST, IL-3, INT-2, JUN, KIT, KS3, K-SAM,

LBC, LCK, LMO1, LMO2, L-MYC, LYL-1, LYT-10, LYT-10/Cα1, MAS, MAX, MDM-2, MEN1, MET, MITF, MLH1, MLL, MOS, MSH1, MSH2, MSH3, MSH6, MTG8/AML1, MUTYH, MYB, MYH11/CBFB, NBN, NEU, NF1, NF2, N-MYC, NTHL1, OST, PALB2, PAX-5, PBX1/E2A, PDGFRA, PHOX2B, PIM-1, PMS2, POLD1, POLE, POT1, PRAD-1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RAF, RAR/PML, RAS-H, RAS-K, RAS-N, RB1, RECQL4, REL/NRG, RET, RHOM1, RHOM2, ROS, RUNX1, SDHA, SDHAF, SDHB, SDHC, SDHD, SET/CAN, SIS, SKI, SMAD4, SMARCA4, SMARCB1, SMARCE1, SRC, STK11, SUFU, TAL1, TAL2, TAN-1, TIAM1, TERC, TERT, TMEM127, TP53, TSC1, TSC2, TRK, VHL, WRN, and WT1. In some cases, a gene biomarker for cancer will carry one or more mutations. In some cases, a gene biomarker for a cancer will be upregulated or downregulated relative to a patient or sample that does not have the cancer.

[0362] The compositions and methods described herein may be suitable for autologous or allogeneic treatment, as well as *ex vivo* cell-based treatments.

[0363] The compositions and methods described herein may be used to treat, prevent, diagnose, or identify an infection in a subject. In some embodiments, the subject is an animal (e.g., a mammal, such as a human). In some embodiments, the subject is a plant (e.g., a crop).

[0364] In some aspects, the disclosure provides the programmable CasΦ nucleases and compositions described herein for use in a method of treatment. In some embodiments, the disclosure provides the CasΦ programmable nucleases and compositions described herein for use in a method of treating an ailment recited above.

[0365] In some aspects, the disclosure provides the programmable CasΦ nucleases and compositions described herein for use as a medicament.

Methods of Detecting a Target Nucleic Acid

[0366] The present disclosure provides methods and compositions, which enable target nucleic acid detection by programmable nuclease platforms, such as the DNA Endonuclease Targeted CRISPR TransReporter (DETECTR) platform. In some embodiments, the target nucleic acid is a DNA. In some embodiments, the target nucleic acid is a RNA.

[0367] A number of reagents are consistent with the compositions and methods disclosed herein. The reagents described herein may be used for nicking target nucleic acids and for detection of target nucleic acids. The reagents disclosed herein can include programmable nucleases, guide nucleic acids, target nucleic acids, and buffers. As described herein, target nucleic acid comprising DNA or RNA may be modified or detected (e.g., the target nucleic acid hybridizes to the guide nucleic) using a programmable nuclease (e.g., a CasΦ as disclosed herein) and other reagents disclosed herein. As described herein, target nucleic acids comprising DNA may be an amplicon of a nucleic acid of interest and the amplicon can be detected using a programmable

nuclease (e.g., a CasΦ as disclosed herein) and other reagents disclosed herein. Additionally, detection of multiple target nucleic acids is possible using two or more programmable nickases or a programmable nickase with a non-nickase programmable nuclease complexed to guide nucleic acids that target the multiple target nucleic acids, wherein the programmable nucleases exhibit different sequence-independent cleavage of the nucleic acid of a reporter (e.g., cleavage of an RNA reporter by a first programmable nuclease and cleavage of a DNA reporter by a second programmable nuclease).

[0368] In some embodiments, target nucleic acid from a sample is amplified before assaying for cleavage of reporters. Target DNA can be amplified by PCR or isothermal amplification techniques. DNA amplification methods that are compatible with the DETECTR technology can be used for programmable nucleases disclosed herein. For example, ssDNA can be amplified. Amplification of ssDNA instead of dsDNA can enable PAM-independent detection of nucleic acids by proteins with PAM requirements for dsDNA-activated trans-cleavage.

[0369] Certain programmable nucleases (e.g., a CasΦ as disclosed herein) of the disclosure can exhibit indiscriminate trans-cleavage of ssDNA, enabling their use for detection of DNA in samples. In some embodiments, target ssDNA are generated from many nucleic acid templates (RNA, ss/dsDNA) in order to achieve cleavage of the FQ reporter in the DETECTR platform. Certain programmable nucleases can be activated by ssDNA, upon which they can exhibit trans-cleavage of ssDNA and can, thereby, be used to cleave ssDNA FQ reporter molecules in the DETECTR system. These programmable nucleases can target ssDNA present in the sample, or generated and/or amplified from any number of nucleic acid templates (RNA, ssDNA, or dsDNA).

[0370] The compositions, kits and methods disclosed herein may be implemented in methods of assaying for a target nucleic acid. In some embodiments, a method of assaying for a target nucleic acid in a sample, comprises: contacting the sample to a complex comprising a guide nucleic acid comprising a segment that is reverse complementary to a segment of the target nucleic acid and a programmable nuclease (e.g., a CasΦ as disclosed herein) of the disclosure that exhibits sequence independent cleavage upon forming a complex comprising the segment of the guide nucleic acid binding to the segment of the target nucleic acid, wherein the sample comprises at least one nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid; and assaying for cleavage of at least one reporter nucleic acids of a population of reporter nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein absence of the cleavage indicates an absence of the target nucleic acid in the sample.

[0371] The target nucleic acid can be from 0.05% to 20% of total nucleic acids in the sample. Sometimes, the target nucleic acid is from 0.1% to 10% of the total nucleic acids in the sample.

The target nucleic acid, in some cases, is from 0.1% to 5% of the total nucleic acids in the sample. Often, a sample comprises the segment of the target nucleic acid and at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. For example, the segment of the target nucleic acid comprises a mutation as compared to at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid. Often, the segment of the target nucleic acid comprises a single nucleotide mutation as compared to at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid but no less than 50% sequence identity to the segment of the target nucleic acid.

[0372] The concentrations of the various reagents in the programmable nuclease DETECTR reaction mix can vary depending on the particular scale of the reaction. For example, the final concentration of the programmable nuclease can vary from 1 pM to 1 nM, from 1 pM to 10 pM, from 10 pM to 100 pM, from 100 pM to 1 nM, from 1 nM to 10 nM, from 10 nM to 20 nM, from 20 nM to 30 nM, from 30 nM to 40 nM, from 40 nM to 50 nM, from 50 nM to 60 nM, from 60 nM to 70 nM, from 70 nM to 80 nM, from 80 nM to 90 nM, from 90 nM to 100 nM, from 100 nM to 200 nM, from 200 nM to 300 nM, from 300 nM to 400 nM, from 400 nM to 500 nM, from 500 nM to 600 nM, from 600 nM to 700 nM, from 700 nM to 800 nM, from 800 nM to 900 nM, from 900 nM to 1000 nM. The final concentration of the sgRNA complementary to the target nucleic acid can be from 1 pM to 1 nM, from 1 pM to 10 pM, from 10 pM to 100 pM, from 100 pM to 1 nM, from 1 nM to 10 nM, from 10 nM to 20 nM, from 20 nM to 30 nM, from 30 nM to 40 nM, from 40 nM to 50 nM, from 50 nM to 60 nM, from 60 nM to 70 nM, from 70 nM to 80 nM, from 80 nM to 90 nM, from 90 nM to 100 nM, from 100 nM to 200 nM, from 200 nM to 300 nM, from 300 nM to 400 nM, from 400 nM to 500 nM, from 500 nM to 600 nM, from 600 nM to 700 nM, from 700 nM to 800 nM, from 800 nM to 900 nM, from 900 nM to 1000 nM. The concentration of the ssDNA-FQ reporter can be from 1 pM to 1 nM, from 1 pM to 10 pM, from 10 pM to 100 pM, from 100 pM to 1 nM, from 1 nM to 10 nM, from 10 nM to 20 nM, from 20 nM to 30 nM, from 30 nM to 40 nM, from 40 nM to 50 nM, from 50 nM to 60 nM, from 60 nM to 70 nM, from 70 nM to 80 nM, from 80 nM to 90 nM, from 90 nM to 100 nM, from 100 nM to 200 nM, from 200 nM to 300 nM, from 300 nM to 400 nM, from 400 nM to 500 nM, from 500 nM to 600 nM, from 600 nM to 700 nM, from 700 nM to 800 nM, from 800 nM to 900 nM, from 900 nM to 1000 nM.

[0373] An example of a DETECTR reaction comprises, consists, or consists essentially of a final concentration of 100nM CasΦ polypeptide or variant thereof, 125nM sgRNA, and 50 nM ssDNA-FQ reporter in a total reaction volume of 20 μL. Reactions are incubated in a fluorescence plate reader (Tecan Infinite Pro 200 M Plex) for 2 hours at 37°C with fluorescence

measurements taken every 30 seconds (e.g., λex: 485 nm; λem: 535 nm). The fluorescence wavelength detected can vary depending on the reporter molecule.

[0374] Described herein are reagents comprising a single stranded reporter nucleic acid comprising a detection moiety, wherein the reporter nucleic acid (e.g., the ssDNA-FQ reporter described above) is capable of being cleaved by the programmable nuclease, upon generation and amplification of ssDNA from a nucleic acid template using the methods disclosed herein, thereby generating a first detectable signal.

[0375] The methods disclosed herein, thus, include generation and amplification of ssDNA from a target nucleic acid template (e.g., cDNA, ssDNA, or dsDNA) of interest in a sample, incubation of the ssDNA with an ssDNA activated programmable nuclease leading to indiscriminate, PAM-independent cleavage of reporter nucleic acids (also referred to as ssDNA-FQ reporters) to generate a detectable signal, and quantification of the detectable signal to detect a target nucleic acid sequence of interest.

Reporters

[0376] Described herein are reagents comprising a reporter. The reporter can comprise a single stranded nucleic acid and a detection moiety (e.g., a labeled single stranded DNA reporter), wherein the nucleic acid is capable of being cleaved by the activated programmable nuclease (e.g., a CasΦ as disclosed herein), releasing the detection moiety, and, generating a detectable signal. As used herein, "reporter" is used interchangeably with "reporter nucleic acid" or "reporter molecule". The programmable nucleases disclosed herein, activated upon hybridization of a guide RNA to a target nucleic acid, can cleave the reporter. Cleaving the "reporter" may be referred to herein as cleaving the "reporter nucleic acid," the "reporter molecule," or the "nucleic acid of the reporter."

[0377] A major advantage of the compositions and methods disclosed herein can be the design of excess reporters to total nucleic acids in an unamplified or an amplified sample, not including the nucleic acid of the reporter. Total nucleic acids can include the target nucleic acids and non-target nucleic acids, not including the nucleic acid of the reporter. The non-target nucleic acids can be from the original sample, either lysed or unlysed. The non-target nucleic acids can also be byproducts of amplification. Thus, the non-target nucleic acids can include both non-target nucleic acids from the original sample, lysed or unlysed, and from an amplified sample. The presence of a large amount of non-target nucleic acids, an activated programmable nuclease (e.g., a CasΦ as disclosed herein) may be inhibited in its ability to bind and cleave the reporter sequences. This is because the activated programmable nuclease collaterally cleaves any nucleic acids. If total nucleic acids are in present in large amounts, they may outcompete reporters for the programmable nucleases. The compositions and methods disclosed herein are designed to have an excess of reporter to total nucleic acids, such that the detectable signals from DETECTR

reactions are particularly superior. In some embodiments, the reporter can be present in at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 11 fold, at least 12 fold, at least 13 fold, at least 14 fold, at least 15 fold, at least 16 fold, at least 17 fold, at least 18 fold, at least 19 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, from 1.5 fold to 100 fold, from 2 fold to 10 fold, from 10 fold to 20 fold, from 20 fold to 30 fold, from 30 fold to 40 fold, from 40 fold to 50 fold, from 50 fold to 60 fold, from 60 fold to 70 fold, from 70 fold to 80 fold, from 80 fold to 90 fold, from 90 fold to 100 fold, from 1.5 fold to 10 fold, from 1.5 fold to 20 fold, from 10 fold to 40 fold, from 20 fold to 60 fold, or from 10 fold to 80 fold excess of total nucleic acids.

[0378] Another significant advantage of the compositions and methods disclosed herein can be the design of an excess volume comprising the guide nucleic acid, the programmable nuclease (e.g., a CasΦ as disclosed herein), and the reporter, which contacts a smaller volume comprising the sample with the target nucleic acid of interest. The smaller volume comprising the sample can be unlysed sample, lysed sample, or lysed sample which has undergone any combination of reverse transcription, amplification, and in vitro transcription. The presence of various reagents in a crude, non-lysed sample, a lysed sample, or a lysed and amplified sample, such as buffer, magnesium sulfate, salts, the pH, a reducing agent, primers, dNTPs, NTPs, cellular lysates, non-target nucleic acids, primers, or other components, can inhibit the ability of the programmable nuclease to become activated or to find and cleave the nucleic acid of the reporter. This may be due to nucleic acids that are not the reporter outcompeting the nucleic acid of the reporter, for the programmable nuclease. Alternatively, various reagents in the sample may simply inhibit the activity of the programmable nuclease. Thus, the compositions and methods provided herein for contacting an excess volume comprising the guide nucleic acid, the programmable nuclease, and the reporter to a smaller volume comprising the sample with the target nucleic acid of interest provides for superior detection of the target nucleic acid by ensuring that the programmable nuclease is able to find and cleaves the nucleic acid of the reporter. In some embodiments, the volume comprising the guide nucleic acid, the programmable nuclease, and the reporter (can be referred to as "a second volume") is 4-fold greater than a volume comprising the sample (can be referred to as "a first volume"). In some embodiments, the volume comprising the guide nucleic acid, the programmable nuclease, and the reporter (can be referred to as "a second volume") is at least 1.5 fold, at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 11 fold, at least 12 fold, at least 13 fold, at least 14 fold, at least 15 fold, at least 16 fold, at least 17 fold, at least 18 fold, at least 19 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 60 fold, at least 70 fold, at least 80 fold, at least 90 fold, at least 100 fold, from 1.5 fold to 100 fold, from 2 fold to 10 fold, from 10 fold to 20 fold, from 20 fold to 30 fold, from 30 fold to 40 fold, from 40 fold to 50 fold, from 50 fold to 60 fold, from 60 fold to 70 fold, from 70 fold to 80 fold, from 80 fold to

90 fold, from 90 fold to 100 fold, from 1.5 fold to 10 fold, from 1.5 fold to 20 fold, from 10 fold to 40 fold, from 20 fold to 60 fold, or from 10 fold to 80 fold greater than a volume comprising the sample (can be referred to as "a first volume"). In some embodiments, the volume comprising the sample is at least 0.5 μL, at least 1 μL, at least at least 1 μL, at least 2 μL, at least 3 μL, at least 4 μL, at least 5 μL, at least 6 μL, at least 7 μL, at least 8 μL, at least 9 μL, at least 10 μL, at least 11 μL, at least 12 μL, at least 13 μL, at least 14 μL, at least 15 μL, at least 16 μL, at least 17 μL, at least 18 μL, at least 19 μL, at least 20 μL, at least 25 μL, at least 30 μL, at least 35 μL, at least 40 μL, at least 45 μL, at least 50 μL, at least 55 μL, at least 60 μL, at least 65 μL, at least 70 μL, at least 75 μL, at least 80 μL, at least 85 μL, at least 90 μL, at least 95 μL, at least 100 μL, from 0.5 μL to 5 μL μL, from 5 μL to 10 μL, from 10 μL to 15 μL, from 15 μL to 20 μL, from 20 μL to 25 μL, from 25 μL to 30 μL, from 30 μL to 35 μL, from 35 μL to 40 μL, from 40 μL to 45 μL, from 45 μL to 50 μL, from 10 μL to 20 μL, from 5 μL to 20 μL, from 1 μL to 40 μL, from 2 μL to 10 μL, or from 1 μL to 10 μL. In some embodiments, the volume comprising the programmable nuclease, the guide nucleic acid, and the reporter is at least 10 μL, at least 11 μL, at least 12 μL, at least 13 μL, at least 14 μL, at least 15 μL, at least 16 μL, at least 17 μL, at least 18 μL, at least 19 μL, at least 20 μL, at least 21 μL, at least 22 μL, at least 23 μL, at least 24 μL, at least 25 μL, at least 26 μL, at least 27 μL, at least 28 μL, at least 29 μL, at least 30 μL, at least 40 μL, at least 50 μL, at least 60 μL, at least 70 μL, at least 80 μL, at least 90 μL, at least 100 μL, at least 150 μL, at least 200 μL, at least 250 μL, at least 300 μL, at least 350 μL, at least 400 μL, at least 450 μL, at least 500 μL, from 10 μL to 15 μL μL, from 15 μL to 20 μL, from 20 μL to 25 μL, from 25 μL to 30 μL, from 30 μL to 35 μL, from 35 μL to 40 μL, from 40 μL to 45 μL, from 45 μL to 50 μL, from 50 μL to 55 μL, from 55 μL to 60 μL, from 60 μL to 65 μL, from 65 μL to 70 μL, from 70 μL to 75 μL, from 75 μL to 80 μL, from 80 μL to 85 μL, from 85 μL to 90 μL, from 90 μL to 95 μL, from 95 μL to 100 μL, from 100 μL to 150 μL, from 150 μL to 200 μL, from 200 μL to 250 μL, from 250 μL to 300 μL, from 300 μL to 350 μL, from 350 μL to 400 μL, from 400 μL to 450 μL, from 450 μL to 500 μL, from 10 μL to 20 μL, from 10 μL to 30 μL, from 25 μL to 35 μL, from 10 μL to 40 μL, from 20 μL to 50 μL, from 18 μL to 28 μL, or from 17 μL to 22 μL.

[0379] In some cases, the reporter nucleic acid is a single-stranded nucleic acid sequence comprising deoxyribonucleotides. In other cases, the reporter nucleic acid is a single-stranded nucleic acid sequence comprising ribonucleotides. The nucleic acid of a reporter can be a single-stranded nucleic acid sequence comprising at least one deoxyribonucleotide and at least one ribonucleotide. In some cases, the nucleic acid of a reporter is a single-stranded nucleic acid comprising at least one ribonucleotide residue at an internal position that functions as a cleavage site. In some cases, the nucleic acid of a reporter comprises at least 2, 3, 4, 5, 6, 7, 8, 9, or 10 ribonucleotide residues at an internal position. In some cases, the nucleic acid of a reporter comprises from 2 to 10, from 3 to 9, from 4 to 8, or from 5 to 7 ribonucleotide residues at an internal position. Sometimes the ribonucleotide residues are continuous. Alternatively, the

ribonucleotide residues are interspersed in between non-ribonucleotide residues. In some cases, the nucleic acid of a reporter has only ribonucleotide residues. In some cases, the nucleic acid of a reporter has only deoxyribonucleotide residues. In some cases, the nucleic acid comprises nucleotides resistant to cleavage by the programmable nuclease described herein. In some cases, the nucleic acid of a reporter comprises synthetic nucleotides. In some cases, the nucleic acid of a reporter comprises at least one ribonucleotide residue and at least one non-ribonucleotide residue. In some cases, the nucleic acid of a reporter is 5-20, 5-15, 5-10, 7-20, 7-15, or 7-10 nucleotides in length. In some cases, the nucleic acid of a reporter is from 3 to 20, from 4 to 10, from 5 to 10, or from 5 to 8 nucleotides in length. In some cases, the nucleic acid of a reporter comprises at least one uracil ribonucleotide. In some cases, the nucleic acid of a reporter comprises at least two uracil ribonucleotides. Sometimes the nucleic acid of a reporter has only uracil ribonucleotides. In some cases, the nucleic acid of a reporter comprises at least one adenine ribonucleotide. In some cases, the nucleic acid of a reporter comprises at least two adenine ribonucleotides. In some cases, the nucleic acid of a reporter has only adenine ribonucleotides. In some cases, the nucleic acid of a reporter comprises at least one cytosine ribonucleotide. In some cases, the nucleic acid of a reporter comprises at least two cytosine ribonucleotides. In some cases, the nucleic acid of a reporter comprises at least one guanine ribonucleotide. In some cases, the nucleic acid of a reporter comprises at least two guanine ribonucleotides. A nucleic acid of a reporter can comprise only unmodified ribonucleotides, only unmodified deoxyribonucleotides, or a combination thereof. In some cases, the nucleic acid of a reporter is from 5 to12 nucleotides in length. In some cases, the reporter nucleic acid is at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, or at least 30 nucleotides in length. In some cases, the reporter nucleic acid is 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length.

[0380] The single stranded nucleic acid of a reporter comprises a detection moiety capable of generating a first detectable signal. Sometimes the reporter nucleic acid comprises a protein capable of generating a signal. A signal can be a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric signal. In some cases, a detection moiety is on one side of the cleavage site. Optionally, a quenching moiety is on the other side of the cleavage site. Sometimes the quenching moiety is a fluorescence quenching moiety. In some cases, the quenching moiety is 5' to the cleavage site and the detection moiety is 3' to the cleavage site. In some cases, the detection moiety is 5' to the cleavage site and the quenching moiety is 3' to the cleavage site. Sometimes the quenching moiety is at the 5' terminus of the nucleic acid of a reporter. Sometimes the detection moiety is at the 3' terminus of the nucleic acid of a reporter. In some cases, the detection moiety is at the 5' terminus of the nucleic acid of

a reporter. In some cases, the quenching moiety is at the 3' terminus of the nucleic acid of a reporter. In some cases, the single-stranded nucleic acid of a reporter is at least one population of the single-stranded nucleic acid capable of generating a first detectable signal. In some cases, the single-stranded nucleic acid of a reporter is a population of the single stranded nucleic acid capable of generating a first detectable signal. Optionally, there is more than one population of single-stranded nucleic acid of a reporter. In some cases, there are 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, or greater than 50, or any number spanned by the range of this list of different populations of single-stranded nucleic acids of a reporter capable of generating a detectable signal. In some cases, there are from 2 to 50, from 3 to 40, from 4 to 30, from 5 to 20, or from 6 to 10 different populations of single-stranded nucleic acids of a reporter capable of generating a detectable signal.

TABLE 3 – Examples of Single Stranded Nucleic Acids in a Reporter

5' Detection Moiety*	Sequence (SEQ ID NO)	3' Quencher*
/56-FAM/	TTATTATT (SEQ ID NO: 95)	/3IABkFQ/
/56-FAM/	TTATTATT (SEQ ID NO: 95)	/3IABkFQ/
/5IRD700/	TTATTATT (SEQ ID NO: 95)	/3IRQC1N/
/5TYE665/	TTATTATT (SEQ ID NO: 95)	/3IAbRQSp/
/5Alex594N/	TTATTATT (SEQ ID NO: 95)	/3IAbRQSp/
/5ATTO633N/	TTATTATT (SEQ ID NO: 95)	/3IAbRQSp/
/56-FAM/	TTTTTT (SEQ ID NO: 96)	/3IABkFQ/
/56-FAM/	TTTTTTTT (SEQ ID NO: 97)	/3IABkFQ/
/56-FAM/	TTTTTTTTTT (SEQ ID NO: 98)	/3IABkFQ/
/56-FAM/	TTTTTTTTTTTT (SEQ ID NO: 99)	/3IABkFQ/
/56-FAM/	TTTTTTTTTTTTTT (SEQ ID NO: 100)	/3IABkFQ/
/56-FAM/	AAAAAA (SEQ ID NO: 101)	/3IABkFQ/
/56-FAM/	CCCCCC (SEQ ID NO: 102)	/3IABkFQ/
/56-FAM/	GGGGGG (SEQ ID NO: 103)	/3IABkFQ/
/56-FAM/	TTATTATT (SEQ ID NO: 104)	/3IABkFQ/

*This Table refers to the detection moiety and quencher moiety as their tradenames and their source is identified. However, alternatives, generics, or non-tradename moieties with similar function from other sources can also be used.

/56-FAM/: 5′ 6-Fluorescein (Integrated DNA Technologies)
/3IABkFQ/: 3′ Iowa Black FQ (Integrated DNA Technologies)
/5IRD700/: 5′ IRDye 700 (Integrated DNA Technologies)
/5TYE665/: 5′ TYE 665 (Integrated DNA Technologies)
/5Alex594N/: 5′ Alexa Fluor 594 (NHS Ester) (Integrated DNA Technologies)
/5ATTO633N/: 5′ ATTO TM 633 (NHS Ester) (Integrated DNA Technologies)

/3IRQC1N/: 3′ IRDye QC-1 Quencher (Li-Cor)
/3IAbRQSp/: 3′ Iowa Black RQ (Integrated DNA Technologies)

[0381] A detection moiety can be an infrared fluorophore. A detection moiety can be a fluorophore that emits fluorescence in the range of from 500 nm and 720 nm. A detection moiety can be a fluorophore that emits fluorescence in the range of from 500 nm and 720 nm. In some cases, the detection moiety emits fluorescence at a wavelength of 700 nm or higher. In other cases, the detection moiety emits fluorescence at about 660 nm or about 670 nm. In some cases, the detection moiety emits fluorescence in the range of from 500 to 520, 500 to 540, 500 to 590, 590 to 600, 600 to 610, 610 to 620, 620 to 630, 630 to 640, 640 to 650, 650 to 660, 660 to 670, 670 to 680, 690 to 690, 690 to 700, 700 to 710, 710 to 720, or 720 to 730 nm. In some cases, the detection moiety emits fluorescence in the range from 450 nm to 750 nm, from 500 nm to 650 nm, or from 550 to 650 nm. A detection moiety can be a fluorophore that emits a detectable fluorescence signal in the same range as 6-Fluorescein, IRDye 700, TYE 665, Alex Fluor, or ATTO TM 633 (NHS Ester). A detection moiety can be fluorescein amidite, 6-Fluorescein, IRDye 700, TYE 665, Alex Fluor 594, or ATTO TM 633 (NHS Ester). A detection moiety can be a fluorophore that emits a fluorescence in the same range as 6-Fluorescein (Integrated DNA Technologies), IRDye 700 (Integrated DNA Technologies), TYE 665 (Integrated DNA Technologies), Alex Fluor 594 (Integrated DNA Technologies), or ATTO TM 633 (NHS Ester) (Integrated DNA Technologies). A detection moiety can be fluorescein amidite, 6-Fluorescein (Integrated DNA Technologies), IRDye 700 (Integrated DNA Technologies), TYE 665 (Integrated DNA Technologies), Alex Fluor 594 (Integrated DNA Technologies), or ATTO TM 633 (NHS Ester) (Integrated DNA Technologies). Any of the detection moieties described herein can be from any commercially available source, can be an alternative with a similar function, a generic, or a non-tradename of the detection moieties listed.

[0382] A detection moiety can be chosen for use based on the type of sample to be tested. For example, a detection moiety that is an infrared fluorophore is used with a urine sample. As another example, SEQ ID NO: 87 with a fluorophore that emits a fluorescence around 520 nm is used for testing in non-urine samples, and SEQ ID NO: 94 with a fluorophore that emits a fluorescence around 700 nm is used for testing in urine samples.

[0383] A quenching moiety can be chosen based on its ability to quench the detection moiety. A quenching moiety can be a non-fluorescent fluorescence quencher. A quenching moiety can quench a detection moiety that emits fluorescence in the range of from 500 nm and 720 nm. A quenching moiety can quench a detection moiety that emits fluorescence in the range of from 500 nm and 720 nm. In some cases, the quenching moiety quenches a detection moiety that emits fluorescence at a wavelength of 700 nm or higher. In other cases, the quenching moiety quenches a detection moiety that emits fluorescence at about 660 nm or about 670 nm. In some cases, the

quenching moiety quenches a detection moiety that emits fluorescence in the range of from 500 to 520, 500 to 540, 500 to 590, 590 to 600, 600 to 610, 610 to 620, 620 to 630, 630 to 640, 640 to 650, 650 to 660, 660 to 670, 670 to 680, 690 to 690, 690 to 700, 700 to 710, 710 to 720, or 720 to 730 nm. In some cases, the quenching moiety quenches a detection moiety that emits fluorescence in the range from 450 nm to 750 nm, from 500 nm to 650 nm, or from 550 to 650 nm. A quenching moiety can quench fluorescein amidite, 6-Fluorescein, IRDye 700, TYE 665, Alex Fluor 594, or ATTO TM 633 (NHS Ester). A quenching moiety can be Iowa Black RQ, Iowa Black FQ or IRDye QC-1 Quencher. A quenching moiety can quench fluorescein amidite, 6-Fluorescein (Integrated DNA Technologies), IRDye 700 (Integrated DNA Technologies), TYE 665 (Integrated DNA Technologies), Alex Fluor 594 (Integrated DNA Technologies), or ATTO TM 633 (NHS Ester) (Integrated DNA Technologies). A quenching moiety can be Iowa Black RQ (Integrated DNA Technologies), Iowa Black FQ (Integrated DNA Technologies) or IRDye QC-1 Quencher (LiCor). Any of the quenching moieties described herein can be from any commercially available source, can be an alternative with a similar function, a generic, or a non-tradename of the quenching moieties listed.

[0384] The generation of the detectable signal from the release of the detection moiety indicates that cleavage by the programmable nucleases has occurred and that the sample contains the target nucleic acid. In some cases, the detection moiety comprises a fluorescent dye. Sometimes the detection moiety comprises a fluorescence resonance energy transfer (FRET) pair. In some cases, the detection moiety comprises an infrared (IR) dye. In some cases, the detection moiety comprises an ultraviolet (UV) dye. Alternatively or in combination, the detection moiety comprises a polypeptide. Sometimes the detection moiety comprises a biotin. Sometimes the detection moiety comprises at least one of avidin or streptavidin. In some instances, the detection moiety comprises a polysaccharide, a polymer, or a nanoparticle. In some instances, the detection moiety comprises a gold nanoparticle or a latex nanoparticle.

[0385] A detection moiety can be any moiety capable of generating a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric signal. A nucleic acid of a reporter, sometimes, is protein-nucleic acid that is capable of generating a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric signal upon cleavage of the nucleic acid. Often a calorimetric signal is heat produced after cleavage of the nucleic acids of a reporter. Sometimes, a calorimetric signal is heat absorbed after cleavage of the nucleic acids of a reporter. A potentiometric signal, for example, is electrical potential produced after cleavage of the nucleic acids of a reporter. An amperometric signal can be movement of electrons produced after the cleavage of nucleic acid of a reporter. Often, the signal is an optical signal, such as a colorimetric signal or a fluorescence signal. An optical signal is, for example, a light output produced after the cleavage of the nucleic acids of a reporter. Sometimes, an optical signal is a change in light absorbance between before

and after the cleavage of nucleic acids of a reporter. Often, a piezo-electric signal is a change in mass between before and after the cleavage of the nucleic acid of a reporter.

[0386] The detectable signal can be a colorimetric signal or a signal visible by eye. In some instances, the detectable signal can be fluorescent, electrical, chemical, electrochemical, or magnetic. In some cases, the first detection signal can be generated by binding of the detection moiety to the capture molecule in the detection region, where the first detection signal indicates that the sample contained the target nucleic acid. Sometimes the system can be capable of detecting more than one type of target nucleic acid, wherein the system comprises more than one type of guide nucleic acid and more than one type of reporter nucleic acid. In some cases, the detectable signal can be generated directly by the cleavage event. Alternatively or in combination, the detectable signal can be generated indirectly by the signal event. Sometimes the detectable signal is not a fluorescent signal. In some instances, the detectable signal can be a colorimetric or color-based signal. In some cases, the detected target nucleic acid can be identified based on its spatial location on the detection region of the support medium. In some cases, the second detectable signal can be generated in a spatially distinct location than the first generated signal.

[0387] Often, the protein-nucleic acid is an enzyme-nucleic acid. The enzyme may be sterically hindered when present as in the enzyme-nucleic acid, but then functional upon cleavage from the nucleic acid. Often, the enzyme is an enzyme that produces a reaction with a substrate. An enzyme can be invertase. Often, the substrate of invertase is sucrose. A DNS reagent produces a colorimetric change when invertase converts sucrose to glucose. In some cases, it is preferred that the nucleic acid (e.g., DNA) and invertase are conjugated using a heterobifunctional linker via sulfo-SMCC chemistry. Sometimes the protein-nucleic acid is a substrate-nucleic acid. Often the substrate is a substrate that produces a reaction with an enzyme.

[0388] A protein-nucleic acid may be attached to a solid support. The solid support, for example, is a surface. A surface can be an electrode. Sometimes the solid support is a bead. Often the bead is a magnetic bead. Upon cleavage, the protein is liberated from the solid and interacts with other mixtures. For example, the protein is an enzyme, and upon cleavage of the nucleic acid of the enzyme-nucleic acid, the enzyme flows through a chamber into a mixture comprising the substrate. When the enzyme meets the enzyme substrate, a reaction occurs, such as a colorimetric reaction, which is then detected. As another example, the protein is an enzyme substrate, and upon cleavage of the nucleic acid of the enzyme substrate-nucleic acid, the enzyme flows through a chamber into a mixture comprising the enzyme. When the enzyme substrate meets the enzyme, a reaction occurs, such as a calorimetric reaction, which is then detected.

[0389] Often, the signal is a colorimetric signal or a signal visible by eye. In some instances, the signal is fluorescent, electrical, chemical, electrochemical, or magnetic. A signal can be a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric signal. In some cases, the detectable signal is a colorimetric signal or a signal visible by eye. In some instances, the detectable signal is fluorescent, electrical, chemical, electrochemical, or magnetic. In some cases, the first detection signal is generated by binding of the detection moiety to the capture molecule in the detection region, where the first detection signal indicates that the sample contained the target nucleic acid. Sometimes the system is capable of detecting more than one type of target nucleic acid, wherein the system comprises more than one type of guide nucleic acid and more than one type of nucleic acid of a reporter. In some cases, the detectable signal is generated directly by the cleavage event. Alternatively or in combination, the detectable signal is generated indirectly by the signal event. Sometimes the detectable signal is not a fluorescent signal. In some instances, the detectable signal is a colorimetric or color-based signal. In some cases, the detected target nucleic acid is identified based on its spatial location on the detection region of the support medium. In some cases, the second detectable signal is generated in a spatially distinct location than the first generated signal.

[0390] In some cases, the threshold of detection, for a subject method of detecting a single stranded target nucleic acid in a sample, is less than or equal to 10 nM. The term "threshold of detection" is used herein to describe the minimal amount of target nucleic acid that must be present in a sample in order for detection to occur. For example, when a threshold of detection is 10 nM, then a signal can be detected when a target nucleic acid is present in the sample at a concentration of 10 nM or more. In some cases, the threshold of detection is less than or equal to 5 nM, 1 nM, 0.5 nM, 0.1 nM, 0.05 nM, 0.01 nM, 0.005 nM, 0.001 nM, 0.0005 nM, 0.0001 nM, 0.00005 nM, 0.00001 nM, 10 pM, 1 pM, 500 fM, 250 fM, 100 fM, 50 fM, 10 fM, 5 fM, 1 fM, 500 attomole (aM), 100 aM, 50 aM, 10 aM, or 1 aM. In some cases, the threshold of detection is in a range of from 1 aM to 1 nM, 1 aM to 500 pM, 1 aM to 200 pM, 1 aM to 100 pM, 1 aM to 10 pM, 1 aM to 1 pM, 1 aM to 500 fM, 1 aM to 100 fM, 1 aM to 1 fM, 1 aM to 500 aM, 1 aM to 100 aM, 1 aM to 50 aM, 1 aM to 10 aM, 10 aM to 1 nM, 10 aM to 500 pM, 10 aM to 200 pM, 10 aM to 100 pM, 10 aM to 10 pM, 10 aM to 1 pM, 10 aM to 500 fM, 10 aM to 100 fM, 10 aM to 1 fM, 10 aM to 500 aM, 10 aM to 100 aM, 10 aM to 50 aM, 100 aM to 1 nM, 100 aM to 500 pM, 100 aM to 200 pM, 100 aM to 100 pM, 100 aM to 10 pM, 100 aM to 1 pM, 100 aM to 500 fM, 100 aM to 100 fM, 100 aM to 1 fM, 100 aM to 500 aM, 500 aM to 1 nM, 500 aM to 500 pM, 500 aM to 200 pM, 500 aM to 100 pM, 500 aM to 10 pM, 500 aM to 1 pM, 500 aM to 500 fM, 500 aM to 100 fM, 500 aM to 1 fM, 1 fM to 1 nM, 1 fM to 500 pM, 1 fM to 200 pM, 1 fM to 100 pM, 1 fM to 10 pM, 1 fM to 1 pM, 10 fM to 1 nM, 10 fM to 500 pM, 10 fM to 200 pM, 10 fM to 100 pM, 10 fM to 10 pM, 10 fM to 1 pM, 500 fM to 1 nM, 500 fM to 500 pM, 500 fM to 200 pM, 500 fM to 100 pM, 500 fM to 10 pM, 500 fM to 1 pM, 800 fM to 1 nM, 800 fM to 500

pM, 800 fM to 200 pM, 800 fM to 100 pM, 800 fM to 10 pM, 800 fM to 1 pM, 1 pM to 1 nM, 1 pM to 500 pM, 1 pM to 200 pM, 1 pM to 100 pM, or 1 pM to 10 pM. In some cases, the threshold of detection in a range of from 800 fM to 100 pM, 1 pM to 10 pM, 10 fM to 500 fM, 10 fM to 50 fM, 50 fM to 100 fM, 100 fM to 250 fM, or 250 fM to 500 fM. In some cases the threshold of detection is in a range of from 2 aM to 100 pM, from 20 aM to 50 pM, from 50 aM to 20 pM, from 200 aM to 5 pM, or from 500 aM to 2 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid is detected in a sample is in a range of from 1 aM to 1 nM, 10 aM to 1 nM, 100 aM to 1 nM, 500 aM to 1 nM, 1 fM to 1 nM, 1 fM to 500 pM, 1 fM to 200 pM, 1 fM to 100 pM, 1 fM to 10 pM, 1 fM to 1 pM, 10 fM to 1 nM, 10 fM to 500 pM, 10 fM to 200 pM, 10 fM to 100 pM, 10 fM to 10 pM, 10 fM to 1 pM, 500 fM to 1 nM, 500 fM to 500 pM, 500 fM to 200 pM, 500 fM to 100 pM, 500 fM to 10 pM, 500 fM to 1 pM, 800 fM to 1 nM, 800 fM to 500 pM, 800 fM to 200 pM, 800 fM to 100 pM, 800 fM to 10 pM, 800 fM to 1 pM, 1 pM to 1 nM, 1 pM to 500 pM, from 1 pM to 200 pM, 1 pM to 100 pM, or 1 pM to 10 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid is detected in a sample is in a range of from 2 aM to 100 pM, from 20 aM to 50 pM, from 50 aM to 20 pM, from 200 aM to 5 pM, or from 500 aM to 2 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid can be detected in a sample is in a range of from 1 aM to 100 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid can be detected in a sample is in a range of from 1 fM to 100 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid can be detected in a sample is in a range of from 10 fM to 100 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid can be detected in a sample is in a range of from 800 fM to 100 pM. In some cases, the minimum concentration at which a single stranded target nucleic acid can be detected in a sample is in a range of from 1 pM to 10 pM. In some cases, the devices, systems, fluidic devices, kits, and methods described herein detect a target single-stranded nucleic acid in a sample comprising a plurality of nucleic acids such as a plurality of non-target nucleic acids, where the target single-stranded nucleic acid is present at a concentration as low as 1 aM, 10 aM, 100 aM, 500 aM, 1 fM, 10 fM, 500 fM, 800 fM, 1 pM, 10 pM, 100 pM, or 1 pM.

[0391] In some embodiments, the target nucleic acid is present in the cleavage reaction at a concentration of about 10 nM, about 20 nM, about 30 nM, about 40 nM, about 50 nM, about 60 nM, about 70 nM, about 80 nM, about 90 nM, about 100 nM, about 200 nM, about 300 nM, about 400 nM, about 500 nM, about 600 nM, about 700 nM, about 800 nM, about 900 nM, about 1 μM, about 10 μM, or about 100 μM. In some embodiments, the target nucleic acid is present in the cleavage reaction at a concentration of from 10 nM to 20 nM, from 20 nM to 30 nM, from 30 nM to 40 nM, from 40 nM to 50 nM, from 50 nM to 60 nM, from 60 nM to 70 nM, from 70 nM to 80 nM, from 80 nM to 90 nM, from 90 nM to 100 nM, from 100 nM to 200 nM, from 200 nM to 300 nM, from 300 nM to 400 nM, from 400 nM to 500 nM, from 500 nM to 600 nM, from

600 nM to 700 nM, from 700 nM to 800 nM, from 800 nM to 900 nM, from 900 nM to 1 μM, from 1 μM to 10 μM, from 10 μM to 100 μM, from 10 nM to 100 nM, from 10 nM to 1 μM, from 10 nM to 10 μM, from 10 nM to 100 μM, from 100 nM to 1 μM, from 100 nM to 10 μM, from 100 nM to 100 μM, or from 1 μM to 100 μM. In some embodiments, the target nucleic acid is present in the cleavage reaction at a concentration of from 20 nM to 50 μM, from 50 nM to 20 μM, or from 200 nM to 5 μM.

[0392] In some cases, the methods, compositions, reagents, enzymes, and kits described herein may be used to detect a target single-stranded nucleic acid in a sample where the sample is contacted with the reagents for a predetermined length of time sufficient for the trans-cleavage to occur or cleavage reaction to reach completion. In some cases, the devices, systems, fluidic devices, kits, and methods described herein detect a target single-stranded nucleic acid in a sample where the sample is contacted with the reagents for no greater than 60 minutes. Sometimes the sample is contacted with the reagents for no greater than 120 minutes, 110 minutes, 100 minutes, 90 minutes, 80 minutes, 70 minutes, 60 minutes, 55 minutes, 50 minutes, 45 minutes, 40 minutes, 35 minutes, 30 minutes, 25 minutes, 20 minutes, 15 minutes, 10 minutes, 5 minutes, 4 minutes, 3 minutes, 2 minutes, or 1 minute. Sometimes the sample is contacted with the reagents for at least 120 minutes, 110 minutes, 100 minutes, 90 minutes, 80 minutes, 70 minutes, 60 minutes, 55 minutes, 50 minutes, 45 minutes, 40 minutes, 35 minutes, 30 minutes, 25 minutes, 20 minutes, 15 minutes, 10 minutes, or 5 minutes. In some cases, the sample is contacted with the reagents for from 5 minutes to 120 minutes, from 5 minutes to 100 minutes, from 10 minutes to 90 minutes, from 15 minutes to 45 minutes, or from 20 minutes to 35 minutes. In some cases, the devices, systems, fluidic devices, kits, and methods described herein can detect a target nucleic acid in a sample in less than 10 hours, less than 9 hours, less than 8 hours, less than 7 hours, less than 6 hours, less than 5 hours, less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, less than 50 minutes, less than 45 minutes, less than 40 minutes, less than 35 minutes, less than 30 minutes, less than 25 minutes, less than 20 minutes, less than 15 minutes, less than 10 minutes, less than 9 minutes, less than 8 minutes, less than 7 minutes, less than 6 minutes, or less than 5 minutes. In some cases, the devices, systems, fluidic devices, kits, and methods described herein can detect a target nucleic acid in a sample in from 5 minutes to 10 hours, from 10 minutes to 8 hours, from 15 minutes to 6 hours, from 20 minutes to 5 hours, from 30 minutes to 2 hours, or from 45 minutes to 1 hour.

[0393] When a guide nucleic acid binds to a target nucleic acid, the programmable nuclease's trans-cleavage activity can be initiated, and nucleic acids of a reporter can be cleaved, resulting in the detection of fluorescence. The guide nucleic acid may be a non-naturally occurring guide nucleic acid. A non-naturally occurring guide nucleic acid may comprise an engineered sequence having a repeat and a spacer that hybridizes to a target nucleic acid sequence of interest. A non-naturally occurring guide nucleic acid may be recombinantly expressed or chemically

synthesized. Nucleic acid reporters can comprise a detection moiety, wherein the nucleic acid reporter can be cleaved by the activated programmable nuclease, thereby generating a signal. Some methods as described herein can a method of assaying for a target nucleic acid in a sample comprises contacting the sample to a complex comprising a guide nucleic acid comprising a segment that is reverse complementary to a segment of the target nucleic acid and a programmable nuclease that exhibits sequence independent cleavage upon forming a complex comprising the segment of the guide nucleic acid binding to the segment of the target nucleic acid; and assaying for a signal indicating cleavage of at least some protein-nucleic acids of a population of protein-nucleic acids, wherein the signal indicates a presence of the target nucleic acid in the sample and wherein absence of the signal indicates an absence of the target nucleic acid in the sample. The cleaving of the nucleic acid of a reporter using the programmable nuclease may cleave with an efficiency of 50% as measured by a change in a signal that is calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric, as non-limiting examples. Some methods as described herein can be a method of detecting a target nucleic acid in a sample comprising contacting the sample comprising the target nucleic acid with a guide nucleic acid targeting a target nucleic acid segment, a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target nucleic acid segment, a single stranded nucleic acid of a reporter comprising a detection moiety, wherein the nucleic acid of a reporter is capable of being cleaved by the activated programmable nuclease, thereby generating a first detectable signal, cleaving the single stranded nucleic acid of a reporter using the programmable nuclease that cleaves as measured by a change in color, and measuring the first detectable signal on the support medium. The cleaving of the single stranded nucleic acid of a reporter using the programmable nuclease may cleave with an efficiency of 50% as measured by a change in color. In some cases, the cleavage efficiency is at least 40%, 50%, 60%, 70%, 80%, 90%, or 95% as measured by a change in color. The change in color may be a detectable colorimetric signal or a signal visible by eye. The change in color may be measured as a first detectable signal. The first detectable signal can be detectable within 5 minutes of contacting the sample comprising the target nucleic acid with a guide nucleic acid targeting a target nucleic acid segment, a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target nucleic acid segment, and a single stranded nucleic acid of a reporter comprising a detection moiety, wherein the nucleic acid of a reporter is capable of being cleaved by the activated nuclease. The first detectable signal can be detectable within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, or 120 minutes of contacting the sample. In some embodiments, the first detectable signal can be detectable within from 1 to 120, from 5 to 100, from 10 to 90, from 15 to 80, from 20 to 60, or from 30 to 45 minutes of contacting the sample.

[0394] In some cases, the methods, reagents, enzymes, and kits described herein detect a target single-stranded nucleic acid with a programmable nuclease and a single-stranded nucleic acid of

a reporter in a sample where the sample is contacted with the reagents for a predetermined length of time sufficient for trans-cleavage of the single stranded nucleic acid of a reporter.

Some methods as described herein can be a method of detecting a target nucleic acid in a sample comprising contacting the sample comprising the target nucleic acid with a guide nucleic acid targeting a target sequence, a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence, a single stranded reporter nucleic acid comprising a detection moiety, wherein the reporter nucleic acid is capable of being cleaved by the activated nuclease, thereby generating a first detectable signal, cleaving the single stranded reporter nucleic acid using the programmable nuclease that cleaves as measured by a change in color, and measuring the first detectable signal on the support medium. The cleaving of the single stranded reporter nucleic acid using the programmable nuclease may cleave with an efficiency of 50% as measured by a change in color. In some cases, the cleavage efficiency is at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% as measured by a change in color. The change in color may be a detectable colorimetric signal or a signal visible by eye. The change in color may be measured as a first detectable signal. The first detectable signal can be detectable within 5 minutes of contacting the sample comprising the target nucleic acid with a guide nucleic acid targeting a target sequence, a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence, and a single stranded reporter nucleic acid comprising a detection moiety, wherein the reporter nucleic acid is capable of being cleaved by the activated nuclease. The first detectable signal can be detectable within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100, 110, or 120 minutes of contacting the sample.

Multiplexing Programmable Nucleases and Programmable Nickases

[0395] Described herein are compositions comprising a programmable nuclease (e.g., a CasΦ as disclosed herein) capable of being activated when complexed with the guide nucleic acid and the target nucleic acid molecule. Furthermore, these reagents can be used with different types of programmable nuclease, e.g., for multiplexing programmable nucleases. In some embodiments, the programmable nucleases can exist in RNP complexes that target multiple genes simultaneously. In some embodiments, a programmable nickase may be multiplexed with an additional programmable nuclease. For example, a programmable nickase may be multiplexed with an additional programmable nuclease for modification or detection of a target nucleic acid. In some embodiments, a first programmable nickase may be multiplexed with a second programmable nickase. In some embodiments, the programmable nickase may be a CasΦ programmable nickase.

[0396] In some embodiments, a CasΦ polypeptide disclosed herein may be multiplexed with multiple guide nucleic acids in the same sample, wherein the guide nucleic acids may comprise different sequences.

[0397] In some embodiments, an additional programmable nuclease used in multiplexing is any suitable programmable nuclease. Sometimes, the programmable nuclease is any Cas protein (also referred to as a Cas nuclease herein). In some cases, the programmable nuclease is Cas13. In some embodiments, the Cas13 is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. In some cases, the programmable nuclease can be Mad7 or Mad2. In some cases, the programmable nuclease is a Cas12 protein. Sometimes the Cas12 is Cas12a, Cas12b, Cas12c, Cas12d, Cas12e, Cas12g, Cas12h, or Cas12i. In some cases, the programmable nuclease is another CasΦ protein. In some cases, the programmable nuclease is Csm1, Cas9, C2c4, C2c8, C2c5, C2c10, C2c9, or CasZ. Sometimes, the Csm1 can be also called smCms1, miCms1, obCms1, or suCms1. Sometimes CasZ can be also called Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. Sometimes, the programmable nuclease can be a type V CRISPR-Cas system. In some cases, the programmable nuclease can be a type VI CRISPR-Cas system. Sometimes the programmable nuclease can be a type III CRISPR-Cas system.

[0398] In some cases, an additional programmable nuclease used in multiplexing can be from, for example, *Leptotrichia shahii (Lsh), Listeria seeligeri (Lse*), *Leptotrichia buccalis (Lbu), Leptotrichia wadeu (Lwa), Rhodobacter capsulatus (Rca), Herbinix hemicellulosilytica (Hhe), Paludibacter propionicigenes (Ppr), Lachnospiraceae bacterium (Lba), Eubacterium rectale (Ere), Listeria newyorkensis (Lny), Clostridium aminophilum (Cam), Prevotella sp. (Psm), Capnocytophaga canimorsus (Cca, Lachnospiraceae bacterium (Lba), Bergeyella zoohelcum (Bzo), Prevotella intermedia (Pin), Prevotella buccae (Pbu), Alistipes sp. (Asp), Riemerella anatipestifer (Ran), Prevotella aurantiaca (Pau), Prevotella saccharolytica (Psa), Prevotella intermedia (Pin2), Capnocytophaga canimorsus (Cca), Porphyromonas gulae (Pgu), Prevotella sp. (Psp), Porphyromonas gingivalis (Pig), Prevotella intermedia (Pin3), Enterococcus italicus (Ei), Lactobacillus salivarius (Ls),* or *Thermus thermophilus (Tt).* In some cases, an additional programmable nuclease used in multiplexing can be from, for example, a phage such as a bacteriophage also called a megaphage. The nucleases may come from a particular bacteriophage clade called Biggiephage. Any combination of programmable nucleases can be used in multiplexing. In some embodiments, multiplexing of programmable nucleases takes place in one reaction volume. In other embodiments, multiplexing of programmable nucleases takes place in separate reaction volumes in a single device.

Amplification of a Target Nucleic Acid

[0399] Disclosed herein are methods of amplifying a target nucleic acid for detection using any of the methods, reagents, kits or devices described herein. The compositions for amplification of target nucleic acids and methods of use thereof, as described herein, are compatible with the DETECTR assay methods disclosed herein. The compositions for amplification of target nucleic acids and methods of use thereof, as described herein, are compatible with any of the programmable nucleases disclosed herein and use of said programmable nuclease in a method of

detecting a target nucleic acid. A target nucleic acid can be an amplified nucleic acid of interest. The nucleic acid of interest may be any nucleic acid disclosed herein or from any sample as disclosed herein. This amplification can be thermal amplification (e.g., using PCR) or isothermal amplification. This nucleic acid amplification of the sample can improve at least one of sensitivity, specificity, or accuracy of the detection the target nucleic acid. The reagents for nucleic acid amplification can comprise a recombinase, an oligonucleotide primer, a single-stranded DNA binding (SSB) protein, and a polymerase. The nucleic acid amplification can be transcription mediated amplification (TMA). Nucleic acid amplification can be helicase dependent amplification (HDA) or circular helicase dependent amplification (cHDA). In additional cases, nucleic acid amplification is strand displacement amplification (SDA). The nucleic acid amplification can be recombinase polymerase amplification (RPA). The nucleic acid amplification can be at least one of loop mediated amplification (LAMP) or the exponential amplification reaction (EXPAR). Nucleic acid amplification is, in some cases, by rolling circle amplification (RCA), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), single primer isothermal amplification (SPIA), multiple displacement amplification (MDA), nucleic acid sequence based amplification (NASBA), hinge-initiated primer-dependent amplification of nucleic acids (HIP), nicking enzyme amplification reaction (NEAR), or improved multiple displacement amplification (IMDA). The nucleic acid amplification can be performed for no greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or 60 minutes. Sometimes, the nucleic acid amplification reaction is performed at a temperature of around 20-45°C. The nucleic acid amplification reaction can be performed at a temperature no greater than 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 45°C. The nucleic acid amplification reaction can be performed at a temperature of at least 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, or 45°C.

[0400] The compositions for amplification of target nucleic acids and methods of use thereof, as described herein, are compatible with any of the compositions comprising a programmable nuclease and a buffer, which has been developed to improve the function of the programmable nuclease and use of said compositions in a method of detecting a target nucleic acid. The compositions for amplification of target nucleic acids and methods of use thereof, as described herein, are compatible with any of the methods disclosed herein including methods of assaying for at least one base difference (e.g., assaying for a SNP or a base mutation) in a target nucleic acid sequence, methods of assaying for a target nucleic acid that lacks a PAM by amplifying the target nucleic acid sequence to introduce a PAM, and compositions used in introducing a PAM via amplification into the target nucleic acid sequence. In some cases, amplification of the target nucleic acid may increase the sensitivity of a detection reaction. In some cases, amplification of the target nucleic acid may increase the specificity of a detection reaction. Amplification of the target nucleic acid may increase the concentration of the target nucleic acid in the sample relative to the concentration of nucleic acids that do not correspond to the target nucleic acid. In some

embodiments, amplification of the target nucleic acid may be used to modify the sequence of the target nucleic acid. For example, amplification may be used to insert a PAM sequence into a target nucleic acid that lacks a PAM sequence. In some cases, amplification may be used to increase the homogeneity of a target nucleic acid sequence. For example, amplification may be used to remove a nucleic acid variation that is not of interest in the target nucleic acid sequence.

[0401] An amplified target nucleic acid may be present in a DETECTR reaction in an amount relative to an amount of a programmable nuclease. In some embodiments, the amplified target nucleic acid is present in at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the programmable nuclease. In some embodiments, the amplified target nucleic acid is present in no more than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the programmable nuclease. In some embodiments, the amplified target nucleic acid is present in from 1-fold to 2-fold, from 1-fold to 3-fold, from 1-fold to 4-fold, from 1-fold to 5-fold, from 1-fold to 10-fold, from 1-fold to 25-fold, from 1-fold to 50-fold, from 1-fold to 100-fold, from 1-fold to 500-fold, from 1-fold to 1000-fold, from 1-fold to 10,000-fold, from 1-fold to 100,000-fold, from 5-fold to 10-fold, from 5-fold to 25-fold, from 5-fold to 50-fold, from 5-fold to 100-fold, from 5-fold to 500-fold, from 5-fold to 1000-fold, from 5-fold to 10,000-fold, from 5-fold to 100,000-fold, from 10-fold to 25-fold, from 10-fold to 50-fold, from 10-fold to 100-fold, from 10-fold to 500-fold, from 10-fold to 1000-fold, from 10-fold to 10,000-fold, from 10-fold to 100,000-fold, from 100-fold to 500-fold, from 100-fold to 1000-fold, from 100-fold to 10,000-fold, from 100-fold to 100,000-fold, from 1000-fold to 10,000-fold, from 1000-fold to 100,000-fold, or from 10,000-fold to 100,000-fold molar excess relative to the amount of the programmable nuclease. In some embodiments, the programmable nuclease is present in at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the programmable nuclease is present in no more than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the programmable nuclease is present in from 1-fold to 2-fold, from 1-fold to 3-fold, from 1-fold to 4-fold, from 1-fold to 5-fold, from 1-fold to 10-fold, from 1-fold to 25-fold, from 1-fold to 50-fold, from 1-fold to 100-fold, from 1-fold to 500-fold, from 1-fold to 1000-fold, from 1-fold to 10,000-fold, from 1-fold to 100,000-fold, from 5-fold to 10-fold, from 5-fold to 25-fold, from 5-fold to 50-fold, from 5-fold to 100-fold, from 5-fold to 500-fold, from 5-fold to 1000-fold, from 5-fold to 10,000-fold, from 5-fold to 100,000-fold, from 10-fold to 25-fold, from 10-fold to 50-fold, from 10-fold to 100-fold, from 10-fold to 500-fold, from 10-fold to 1000-fold, from 10-fold to 10,000-fold, from 10-fold to 100,000-fold, from 100-fold to 500-fold, from 100-fold to 1000-fold, from 100-fold to 10,000-fold, from 100-fold to 100,000-fold, from 1000-fold to 10,000-

fold, from 1000-fold to 100,000-fold, or from 10,000-fold to 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the target nucleic acid is not present in the sample.

[0402] An amplified target nucleic acid may be present in a DETECTR reaction in an amount relative to an amount of a guide nucleic acid. In some embodiments, the amplified target nucleic acid is present in at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the guide nucleic acid. In some embodiments, the amplified target nucleic acid is present in no more than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the guide nucleic acid. In some embodiments, the amplified target nucleic acid is present in from 1-fold to 2-fold, from 1-fold to 3-fold, from 1-fold to 4-fold, from 1-fold to 5-fold, from 1-fold to 10-fold, from 1-fold to 25-fold, from 1-fold to 50-fold, from 1-fold to 100-fold, from 1-fold to 500-fold, from 1-fold to 1000-fold, from 1-fold to 10,000-fold, from 1-fold to 100,000-fold, from 5-fold to 10-fold, from 5-fold to 25-fold, from 5-fold to 50-fold, from 5-fold to 100-fold, from 5-fold to 500-fold, from 5-fold to 1000-fold, from 5-fold to 10,000-fold, from 5-fold to 100,000-fold, from 10-fold to 25-fold, from 10-fold to 50-fold, from 10-fold to 100-fold, from 10-fold to 500-fold, from 10-fold to 1000-fold, from 10-fold to 10,000-fold, from 10-fold to 100,000-fold, from 100-fold to 500-fold, from 100-fold to 1000-fold, from 100-fold to 10,000-fold, from 100-fold to 100,000-fold, from 1000-fold to 10,000-fold, from 1000-fold to 100,000-fold, or from 10,000-fold to 100,000-fold molar excess relative to the amount of the guide nucleic acid. In some embodiments, the guide nucleic acid is present in at least 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the guide nucleic acid is present in no more than 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, 1000-fold, 10,000-fold, or 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the guide nucleic acid is present in from 1-fold to 2-fold, from 1-fold to 3-fold, from 1-fold to 4-fold, from 1-fold to 5-fold, from 1-fold to 10-fold, from 1-fold to 25-fold, from 1-fold to 50-fold, from 1-fold to 100-fold, from 1-fold to 500-fold, from 1-fold to 1000-fold, from 1-fold to 10,000-fold, from 1-fold to 100,000-fold, from 5-fold to 10-fold, from 5-fold to 25-fold, from 5-fold to 50-fold, from 5-fold to 100-fold, from 5-fold to 500-fold, from 5-fold to 1000-fold, from 5-fold to 10,000-fold, from 5-fold to 100,000-fold, from 10-fold to 25-fold, from 10-fold to 50-fold, from 10-fold to 100-fold, from 10-fold to 500-fold, from 10-fold to 1000-fold, from 10-fold to 10,000-fold, from 10-fold to 100,000-fold, from 100-fold to 500-fold, from 100-fold to 1000-fold, from 100-fold to 10,000-fold, from 100-fold to 100,000-fold, from 1000-fold to 10,000-fold, from 1000-fold to 100,000-fold, or from 10,000-fold to 100,000-fold molar excess relative to the amount of the target nucleic acid. In some embodiments, the target nucleic acid is not present in the sample.

Kits

[0403] Disclosed herein are kits for use to detect, modify, edit, or regulate a target nucleic acid sequence as disclosed herein using the methods as discuss above. In some embodiments, the kit comprises the programmable nuclease system, reagents, and the support medium. The reagents and programmable nuclease system can be provided in a reagent chamber or on the support medium. Alternatively, the reagent and programmable nuclease system can be placed into the reagent chamber or the support medium by the individual using the kit. Optionally, the kit further comprises a buffer and a dropper. The reagent chamber can be a test well or container. The opening of the reagent chamber can be large enough to accommodate the support medium. The buffer can be provided in a dropper bottle for ease of dispensing. The dropper can be disposable and transfer a fixed volume. The dropper can be used to place a sample into the reagent chamber or on the support medium.

[0404] The kit or system for detection of a target nucleic acid described herein further comprises reagents for nucleic acid amplification of target nucleic acids in the sample. Isothermal nucleic acid amplification allows the use of the kit or system in remote regions or low resource settings without specialized equipment for amplification. Often, the reagents for nucleic acid amplification comprise a recombinase, an oligonucleotide primer, a single-stranded DNA binding (SSB) protein, and a polymerase. Sometimes, nucleic acid amplification of the sample improves at least one of sensitivity, specificity, or accuracy of the assay in detecting the target nucleic acid. In some cases, the nucleic acid amplification is performed in a nucleic acid amplification region on the support medium. Alternatively, or in combination, the nucleic acid amplification is performed in a reagent chamber, and the resulting sample is applied to the support medium. Sometimes, the nucleic acid amplification is isothermal nucleic acid amplification. In some cases, the nucleic acid amplification is transcription mediated amplification (TMA). Nucleic acid amplification is helicase dependent amplification (HDA) or circular helicase dependent amplification (cHDA) in other cases. In additional cases, nucleic acid amplification is strand displacement amplification (SDA). In some cases, nucleic acid amplification is by recombinase polymerase amplification (RPA). In some cases, nucleic acid amplification is by at least one of loop mediated amplification (LAMP) or the exponential amplification reaction (EXPAR). Nucleic acid amplification is, in some cases, by rolling circle amplification (RCA), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), single primer isothermal amplification (SPIA), multiple displacement amplification (MDA), nucleic acid sequence based amplification (NASBA), hinge-initiated primer-dependent amplification of nucleic acids (HIP), nicking enzyme amplification reaction (NEAR), or improved multiple displacement amplification (IMDA). Often, the nucleic acid amplification is performed for no greater than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, or 60 minutes, or any value from 1 to 60 minutes. Sometimes, the nucleic acid

amplification is performed for from 1 to 60, from 5 to 55, from 10 to 50, from 15 to 45, from 20 to 40, or from 25 to 35 minutes. Sometimes, the nucleic acid amplification reaction is performed at a temperature of around 20-45°C. In some cases, the nucleic acid amplification reaction is performed at a temperature no greater than 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, 45°C, or any value from 20 °C to 45 °C. In some cases, the nucleic acid amplification reaction is performed at a temperature of at least 20°C, 25°C, 30°C, 35°C, 37°C, 40°C, or 45°C, or any value from 20 °C to 45 °C. In some cases, the nucleic acid amplification reaction is performed at a temperature of from 20°C to 45°C, from 25°C to 40°C, from 30°C to 40°C, or from 35°C to 40°C.

[0405] In some embodiments, a kit for detecting a target nucleic acid comprising a support medium; a guide nucleic acid targeting a target sequence; a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence; and a reporter nucleic acid comprising a detection moiety, wherein the reporter nucleic acid is capable of being cleaved by the activated nuclease, thereby generating a first detectable signal. Often, the kit further comprises primers for amplifying a target nucleic acid of interest to produce a PAM target nucleic acid.

[0406] In some embodiments, a kit for detecting a target nucleic acid comprising a PCR plate; a guide nucleic acid targeting a target sequence; a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence; and a single stranded reporter nucleic acid comprising a detection moiety, wherein the reporter nucleic acid is capable of being cleaved by the activated nuclease, thereby generating a first detectable signal. The wells of the PCR plate can be pre-aliquoted with the guide nucleic acid targeting a target sequence, a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence, and at least one population of a single stranded reporter nucleic acid comprising a detection moiety. A user can thus add the biological sample of interest to a well of the pre-aliquoted PCR plate and measure for the detectable signal with a fluorescent light reader or a visible light reader.

[0407] In some embodiments, a kit for modifying a target nucleic acid comprising a support medium; a guide nucleic acid targeting a target sequence; and a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence.

[0408] In some embodiments, a kit for modifying a target nucleic acid comprising a PCR plate; a guide nucleic acid targeting a target sequence; and a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence. The wells of the PCR plate can be pre-aliquoted with the guide nucleic acid targeting a target sequence, and a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence. A user can thus add the biological sample of interest to a well of the pre-aliquoted PCR plate.

[0409] In some instances, such kits may include a package, carrier, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in a method described herein.

[0410] Suitable containers include, for example, test wells, bottles, vials, and test tubes. In one embodiment, the containers are formed from a variety of materials such as glass, plastic, or polymers.

[0411] The kit or systems described herein contain packaging materials. Examples of packaging materials include, but are not limited to, pouches, blister packs, bottles, tubes, bags, containers, bottles, and any packaging material suitable for intended mode of use.

[0412] A kit typically includes labels listing contents and/or instructions for use, and package inserts with instructions for use. A set of instructions will also typically be included. In one embodiment, a label is on or associated with the container. In some instances, a label is on a container when letters, numbers or other characters forming the label are attached, molded or etched into the container itself; a label is associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. In one embodiment, a label is used to indicate that the contents are to be used for a specific therapeutic application. The label also indicates directions for use of the contents, such as in the methods described herein.

[0413] After packaging the formed product and wrapping or boxing to maintain a sterile barrier, the product may be terminally sterilized by heat sterilization, gas sterilization, gamma irradiation, or by electron beam sterilization. Alternatively, the product may be prepared and packaged by aseptic processing.

[0414] Unless otherwise defined, all technical terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Any reference to “or” herein is intended to encompass “and/or” unless otherwise stated.

[0415] As used herein, the term “comprising” and its grammatical equivalents specifies the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0416] Unless specifically stated or obvious from context, as used herein, the term “about” in reference to a number or range of numbers is understood to mean the stated number and numbers +/- 10% thereof, or 10% below the lower listed limit and 10% above the higher listed limit for the values listed for a range.

[0417] As used herein the terms "individual," "subject," and "patient" are used interchangeably and include any member of the animal kingdom, including humans.

[0418] Methods of the disclosure can be performed in a subject. Compositions of the disclosure can be administered to a subject. A subject can be a human. A subject can be a mammal (e.g., rat, mouse, cow, dog, pig, sheep, horse). A subject can be a vertebrate or an invertebrate. A subject can be a laboratory animal. A subject can be a patient. A subject can be suffering from a disease. A subject can display symptoms of a disease. A subject may not display symptoms of a disease, but still have a disease. A subject can be under medical care of a caregiver (e.g., the subject is hospitalized and is treated by a physician). A subject can be a plant or a crop.

[0419] Methods of the disclosure can be performed in a cell. A cell can be *in vitro*. A cell can be *in vivo*. A cell can be *ex vivo*. A cell can be an isolated cell. A cell can be a cell inside of an organism. A cell can be an organism. A cell can be a cell in a cell culture. A cell can be one of a collection of cells. A cell can be a mammalian cell or derived from a mammalian cell. A cell can be a rodent cell or derived from a rodent cell. A cell can be a human cell or derived from a human cell. A cell can be a prokaryotic cell or derived from a prokaryotic cell. A cell can be a bacterial cell or can be derived from a bacterial cell. A cell can be an archaeal cell or derived from an archaeal cell. A cell can be a eukaryotic cell or derived from a eukaryotic cell. A cell can be a pluripotent stem cell. A cell can be a plant cell or derived from a plant cell. A cell can be an animal cell or derived from an animal cell. A cell can be an invertebrate cell or derived from an invertebrate cell. A cell can be a vertebrate cell or derived from a vertebrate cell. A cell can be a microbe cell or derived from a microbe cell. A cell can be a fungi cell or derived from a fungi cell. A cell can be from a specific organ or tissue.

[0420] Methods of the disclosure can be performed in a eukaryotic cell or cell line. In some embodiments, the eukaryotic cell is a Chinese hamster ovary (CHO) cell. In some embodiments, the eukaryotic cell is a Human embryonic kidney 293 cells (also referred to as HEK or HEK 293) cell. In some embodiments, the eukaryotic cell is a K562 cell.

[0421] Non-limiting examples of cell lines that can be used with the disclosure include C8161, CCRF-CEM, MOLT, mIMCD-3, NHDF, HeLa-S3, Huh1, Huh4, Huh7, HUVEC, HASMC, HEKn, HEKa, MiaPaCell, Panc1, PC-3, TF1, CTLL-2, CIR, Rat6, CV1, RPTE, A10, T24, J82, A375, ARH-77, Calu1, SW480, SW620, SKOV3, SK-UT, CaCo2, P388D1, SEM-K2, WEHI-231, HB56, TIB55, Jurkat, J45.01, LRMB, Bcl-1, BC-3, IC21, DLD2, Raw264.7, NRK, NRK-52E, MRC5, MEF, Hep G2, HeLa B, HeLa T4, COS, COS-1, COS-6, COS-M6A, BS-C-1 monkey kidney epithelial, BALB/3T3 mouse embryo fibroblast, 3T3 Swiss, 3T3-L1, 132-d5 human fetal fibroblasts; 10.1 mouse fibroblasts, 293-T, 3T3, 721, 9L, A2780, A2780ADR, A2780cis, A172, A20, A253, A431, A-549, ALC, B16, B35, BCP-1 cells, BEAS-2B, bEnd.3, BHK-21, BR 293, BxPC3, C3H-10T1/2, C6/36, Cal-27, CHO, CHO-7, CHO-IR, CHO-K1,

CHO-K2, CHO-T, CHO Dhfr −/−, COR-L23, COR-L23/CPR, COR-L23/5010, COR-L23/R23, COS-7, COV-434, CML T1, CMT, CT26, D17, DH82, DU145, DuCaP, EL4, EM2, EM3, EMT6/AR1, EMT6/AR10.0, FM3, H1299, H69, HB54, HB55, HCA2, HEK-293, HeLa, Hepa1-6, Hepa1c1c7, HL-60, HMEC, HT-29, Jurkat, JY cells, K562 cells, Ku812, KCL22, KG1, KYO1, LNCap, Ma-Mel 1-48, MC-38, MCF-7, MCF-10A, MDA-MB-231, MDA-MB-468, MDA-MB-435, MDCK II, MDCK II, MOR/0.2R, MONO-MAC 6, MTD-1A, MyEnd, NCI-H69/CPR, NCI-H69/LX10, NCI-H69/LX20, NCI-H69/LX4, NIH-3T3, NALM-1, NW-145, OPCN/OPCT cell lines, Peer, PNT-1A/PNT 2, RenCa, RIN-5F, RMA/RMAS, Saos-2 cells, Sf-9, SkBr3, T2, T-47D, T84, THP1 cell line, U373, U87, U937, VCaP, Vero cells, WM39, WT-49, X63, YAC-1, and YAR. Non-limiting examples of other cells that can be used with the disclosure include immune cells, such as CART, T-cells, B-cells, NK cells, granulocytes, basophils, eosinophils, neutrophils, mast cells, monocytes, macrophages, dendritic cells, antigen-presenting cells (APC), or adaptive cells. Non-limiting examples of cells that can be used with this disclosure also include plant cells, such as Parenchyma, sclerenchyma, collenchyma, xylem, phloem, germline (e.g., pollen). Cells from lycophytes, ferns, gymnosperms, angiosperms, bryophytes, charophytes, chloropytes, rhodophytes, or glaucophytes. Non-limiting examples of cells that can be used with this disclosure also include stem cells, such as human stem cells, animal stem cells, stem cells that are not derived from human embryonic stem cells, embryonic stem cells, mesenchymal stem cells, pluripotent stem cells, induced pluripotent stem cells (iPS), somatic stem cells, adult stem cells, hematopoietic stem cells, tissue-specific stem cells.

[0422] Methods described herein may be used to create populations of cells comprising at least one of the cells described herein. In some cases, a population of cells comprises a non-naturally occurring compositions described herein.

[0423] Compositions of the disclosure include populations of cells, or any progeny thereof, comprising other compositions described herein or that have been modified by the methods described herein.

[0424] Methods described herein may include producing a protein from a cell or a population of cells described herein. In some cases, the method comprises producing a protein, and industrial protein, or a protein at large scale using a cell provided for herein that has been modified by any of the methods described herein. In some cases, a rodent cell or CHO cell is modified by a nuclease or cas enzyme described herein and is later used, expanded, or cultured for protein production. In some cases, a derivative or progeny of a modified CHO cell, as describered herein, is used, expanded, or cultured for protein production. A method of protein production may further comprise a donor template, additional guide RNA, a buffer, a protease inhibitor, a nuclease inhibitor, or a detergent.

EXAMPLES

[0425] The following examples are included to further describe some aspects of the present disclosure and should not be used to limit the scope of the invention.

EXAMPLE 1

Human Codon Optimized CasΦ polypeptide

[0426] Human codon-optimized nucleotide sequences of illustrative CasΦ polypeptides were prepared. **TABLE 4** provides human codon optimized nucleotide sequences of illustrative CasΦ polypeptides that are suitable for use with the methods and compositions of the disclosure.

TABLE 4. Human codon optimized nucleotide sequences

Name	Endogenous Amino Acid Sequence	Human Codon Optimized Nucleotide Sequence
CasΦ.2	MPKPAVESEFSKVLK KHFPGERFRSSYMKR GGKILAAQGEEAVVA YLQGKSEEEPPNFQPP AKCHVVTKSRDFAE WPIMKASEAIQRYIYA LSTTERAACKPGKSSE SHAAWFAATGVSNH GYSHVQGLNLIFDHT LGRYDGVLKKVQLR NEKARARLESINASR ADEGLPEIKAEEEEVA TNETGHLLQPPGINPS FYVYQTISPQAYRPRD EIVLPPEYAGYVRDPN APIPLGVVRNRCDIQK GCPGYIPEWQREAGT AISPKTGKAVTVPGLS PKKNKRMRRYWRSE KEKAQDALLVTVRIG TDWVVIDVRGLLRNA RWRTIAPKDISLNALL DLFTGDPVIDVRRNIV TFTYTLDACGTYARK WTLKGKQTKATLDK LTATQTVALVAIDLG QTNPISAGISRVTQEN GALQCEPLDRFTLPD DLLKDISAYRIAWDR NEEELRARSVEALPE AQQAEVRALDGVSKE TARTQLCADFGLDPK RLPWDKMSSNTTFISE	ATGCCTAAGCCTGCCGTGGAAAGCGAGTTCAG CAAGGTGCTGAAGAAGCACTTCCCCGGCGAGC GGTTCAGATCCAGCTACATGAAGAGAGGCGGC AAGATCCTGGCCGCTCAAGGCGAAGAAGCCGT GGTCGCATATCTGCAGGGCAAGAGCGAGGAA GAACCTCCTAACTTCCAGCCTCCTGCCAAGTG CCACGTGGTCACCAAGAGCAGAGATTTCGCCG AGTGGCCCATCATGAAGGCCTCTGAAGCCATC CAGCGGTACATCTACGCCCTGAGCACAACAGA AAGAGCCGCCTGCAAGCCTGGCAAGAGCAGC GAATCTCACGCCGCTTGGTTTGCCGCTACCGG CGTGTCCAATCACGGCTACTCTCATGTGCAGG GCCTGAACCTGATCTTCGATCACACCCTGGGC AGATACGACGGCGTGCTGAAAAAGGTGCAGC TGCGGAACGAGAAGGCCAGAGCCAGACTGGA ATCCATCAACGCCAGCAGAGCCGATGAGGGCC TGCCTGAGATTAAGGCCGAAGAGGAAGAGGT GGCCACAAACGAAACCGGCCATCTGCTGCAGC CACCTGGCATCAACCCTAGCTTCTACGTGTAC CAGACAATCAGCCCTCAGGCCTACAGACCCAG GGACGAGATTGTGCTGCCTCCTGAGTATGCCG GCTACGTGCGGGATCCCAACGCTCCTATTCCT CTGGGCGTCGTGCGGAACAGATGCGACATCCA GAAAGGCTGCCCCGGCTACATTCCCGAGTGGC AGAGAGAAGCCGGCACCGCCATTTCTCCAAAG ACAGGCAAAGCCGTGACCGTGCCTGGCCTGTC TCCTAAGAAAAACAAGCGGATGCGGCGGTACT GGCGGAGCGAGAAAGAAAAAGCCCAGGACGC CCTGCTGGTCACAGTGCGGATTGGCACAGATT GGGTCGTGATCGATGTGCGCGGCCTGCTGAGA AATGCCAGATGGCGGACAATCGCCCCTAAGGA CATCAGCCTGAACGCACTGCTGGACCTGTTCA CCGGCGATCCTGTGATTGACGTGCGGCGGAAC

	ALLSNSVSRDQVFFTP APKKGAKKKAPVEV MRKDRTWARAYKPR LSVEAQKLKNEALW ALKRTSPEYLKLSRR KEELCRRSINYVIEKT RRRTQCQIVIPVIEDL NVRFFHGSGKRLPGW DNFFTAKKENRWFIQ GLHKAFSDLRTHRSF YVFEVRPERTSITCPK CGHCEVGNRDGEAFQ CLSCGKTCNADLDVA THNLTQVALTGKTMP KREEPRDAQGTAPAR KTKKASKSKAPPAER EDQTPAQEPSQTS (SEQ ID NO: 2)	ATCGTGACCTTCACCTACACACTGGACGCCTG CGGCACCTACGCCAGAAAGTGGACACTGAAG GGCAAGCAGACCAAGGCCACTCTGGACAAGC TGACCGCCACACAGACAGTGGCCCTGGTGGCT ATTGATCTGGGCCAGACAAACCCTATCAGCGC CGGCATCAGCAGAGTGACCCAAGAAAATGGC GCCCTGCAGTGCGAGCCCCTGGACAGATTCAC ACTGCCCGACGACCTGCTGAAGGACATCTCCG CCTATAGAATCGCCTGGGACCGCAATGAAGAG GAACTGAGAGCCAGAAGCGTGGAAGCCCTGC CTGAAGCACAGCAGGCTGAAGTGCGAGCACT GGACGGGGTGTCCAAAGAGACAGCCAGAACT CAGCTGTGCGCCGACTTTGGACTGGACCCCAA AAGACTGCCCTGGGACAAGATGAGCAGCAAC ACCACCTTCATCAGCGAGGCCCTGCTGAGCAA TAGCGTGTCCAGAGATCAGGTGTTCTTCACCC CTGCTCCAAAGAAGGGCGCCAAGAAGAAAGC CCCTGTCGAAGTGATGCGGAAGGACCGGACAT GGGCCAGAGCTTACAAGCCCAGACTGTCCGTG GAAGCTCAGAAGCTGAAGAACGAAGCCCTGT GGGCCCTGAAGAGAACAAGCCCCGAGTACCT GAAGCTGAGCCGGCGGAAAGAAGAACTCTGC CGGCGGAGCATCAACTACGTGATCGAGAAAA CCCGGCGGAGAACCCAGTGCCAGATCGTGATT CCTGTGATCGAGGACCTGAACGTGCGGTTCTT TCACGGCAGCGGCAAGAGACTGCCCGGCTGG GATAATTTCTTCACCGCCAAAAAAGAAAACCG GTGGTTCATCCAGGGCCTGCACAAGGCCTTCA GCGACCTGAGAACCCACCGGTCCTTTTACGTG TTCGAAGTGCGGCCCGAGCGGACCAGCATCAC CTGTCCTAAATGCGGCCACTGCGAAGTGGGCA ACAGAGATGGCGAGGCCTTCCAGTGTCTGAGC TGTGGCAAGACCTGCAACGCCGACCTGGATGT GGCCACTCACAATCTGACACAGGTGGCCCTGA CCGGCAAGACCATGCCTAAGAGAGAGGAACC TAGGGACGCCCAGGGTACAGCCCCTGCCAGAA AGACAAAGAAAGCCAGCAAGAGCAAGGCCCC TCCTGCCGAGAGAGAAGATCAGACCCCAGCTC AAGAGCCCAGCCAGACATCT (SEQ ID NO: 1405)
CasΦ.4	MEKEITELTKIRREFP NKKFSSTDMKKAGKL LKAEGPDAVRDFLNS CQEIIGDFKPPVKTNI VSISRPFEEWPVSMVG RAIQEYYFSLTKEELE SVHPGTSSEDHKSFFN ITGLSNYNYTSVQGL NLIFKNAKAIYDGTLV KANNKNKKLEKKFN EINHKRSLEGLPIITPD	ATGGAAAAAGAGATCACCGAGCTGACCAAGA TCCGCAGAGAGTTCCCCAACAAGAAGTTCAGC AGCACCGACATGAAGAAGGCCGGCAAGCTGC TGAAGGCCGAAGGACCTGATGCCGTGCGGGA CTTCCTGAACAGCTGCCAAGAGATCATCGGCG ACTTCAAGCCTCCAGTCAAGACCAACATCGTG TCCATCAGCAGACCCTTCGAGGAATGGCCCGT GTCCATGGTTGGACGGGCCATCCAAGAGTACT ACTTCAGCCTGACCAAAGAGGAACTGGAAAG CGTTCACCCCGGCACCAGCAGCGAGGACCACA AGAGCTTTTTCAACATCACCGGCCTGAGCAAC

	FEEPFDENGHLNNPPG INRNIYGYQGCAAKV FVPSKHKMVSLPKEY EGYNRDPNLSLAGFR NRLEIPEGEPGHVPWF QRMDIPEGQIGHVNKI QRFNFVHGKNSGKVK FSDKTGRVKRYHHSK YKDATKPYKFLEESK KVSALDSILAIITIGDD WVVFDIRGLYRNVFY RELAQKGLTAVQLLD LFTGDPVIDPKKGVV TFSYKEGVVPVFSQKI VPRFKSRDTLEKLTSQ GPVALLSVDLGQNEP VAARVCSLKNINDKIT LDNSCRISFLDDYKK QIKDYRDSLDELEIKI RLEAINSLETNQQVEI RDLDVFSADRAKANT VDMFDIDPNLISWDS MSDARVSTQISDLYL KNGGDESRVYFEINN KRIKRSDYNISQLVRP KLSDSTRKNLNDSIW KLKRTSEEYLKLSKR KLELSRAVVNYTIRQS KLLSGINDIVIILEDLD VKKKFNGRGIRDIGW DNFFSSRKENRWFIPA FHKAFSELSSNRGLCV IEVNPAWTSATCPDC GFCSKENRDGINFTCR KCGVSYHADIDVATL NIARVAVLGKPMSGP ADRERLGDTKKPRVA RSRKTMKRKDISNST VEAMVTA (SEQ ID NO: 4)	TACAACTACACCAGCGTGCAGGGCCTGAACCT GATCTTCAAGAACGCCAAGGCCATCTACGACG GCACCCTGGTCAAGGCCAACAACAAGAACAA GAAGCTCGAGAAGAAGTTTAACGAGATCAAC CACAAGCGGAGCCTGGAAGGCCTGCCTATCAT CACCCCTGATTTCGAGGAACCCTTCGACGAGA ACGGCCACCTGAACAACCCTCCAGGCATCAAC CGGAACATCTACGGCTATCAGGGCTGCGCCGC CAAGGTGTTCGTGCCTTCTAAGCACAAGATGG TGTCCCTGCCTAAAGAGTACGAGGGCTACAAC AGGGACCCCAACCTGTCTCTGGCCGGCTTCAG AAACAGACTGGAAATCCCTGAGGGCGAGCCT GGCCATGTGCCATGGTTCCAGAGAATGGATAT CCCCGAGGGCCAGATCGGACACGTGAACAAG ATCCAGCGGTTCAACTTCGTGCACGGCAAGAA CAGCGGCAAAGTGAAGTTCTCCGACAAGACCG GCAGAGTGAAGAGATACCACCACAGCAAGTA CAAGGACGCTACCAAGCCTTACAAGTTCCTGG AAGAGTCCAAGAAGGTGTCAGCCCTGGACAG CATCCTGGCCATCATCACAATCGGCGACGACT GGGTCGTGTTCGACATCAGAGGCCTGTACCGG AACGTGTTCTACAGAGAGCTGGCCCAGAAAGG CCTGACAGCTGTGCAACTGCTGGACCTGTTTA CCGGCGATCCCGTGATCGACCCCAAGAAAGGC GTGGTCACCTTCAGCTACAAAGAGGGCGTCGT CCCCGTCTTTAGCCAGAAAATCGTGCCCCGGT TCAAGAGCCGGGACACCCTGGAAAAGCTGAC CTCTCAGGGACCTGTGGCTCTGCTGTCTGTGG ACCTGGGACAGAATGAACCTGTGGCCGCCAGA GTGTGCAGCCTGAAGAACATCAACGACAAGAT CACCCTGGACAACTCTTGCCGGATCAGCTTCC TGGACGACTACAAGAAGCAGATCAAGGACTA CAGAGACAGCCTGGACGAGCTGGAAATCAAG ATCCGGCTGGAAGCCATCAACTCCCTCGAGAC AAACCAGCAGGTCGAGATCAGAGATCTGGAC GTGTTCAGCGCCGACCGGGCCAAAGCCAATAC CGTGGACATGTTTGACATCGACCCTAACCTGA TCAGCTGGGACTCCATGAGCGACGCCAGAGTC AGCACCCAGATCAGCGACCTGTACCTGAAGAA TGGCGGCGACGAGAGCCGGGTGTACTTTGAGA TTAACAACAAACGGATTAAGCGGAGCGACTAC AACATCAGCCAGCTCGTGCGGCCCAAGCTGAG CGATAGCACCAGAAAGAACCTGAACGACAGC ATCTGGAAGCTGAAGCGGACCAGCGAGGAAT ACCTGAAGCTGAGCAAGCGGAAGCTGGAACT GAGCAGAGCCGTCGTGAATTACACCATCCGGC AGAGCAAACTGCTGAGCGGCATCAATGACATC GTGATCATTCTCGAGGACCTGGACGTGAAGAA GAAATTCAACGGCAGAGGCATCCGCGATATCG GCTGGGACAACTTCTTCAGCTCCCGGAAAGAA AACCGGTGGTTCATCCCCGCCTTCCACAAGGC

		CTTTAGCGAGCTGAGCAGCAACAGGGGCCTGT GCGTGATCGAAGTGAATCCTGCCTGGACCAGC GCCACCTGTCCTGATTGTGGCTTCTGCAGCAA AGAAAACAGAGATGGCATCAACTTCACGTGCC GGAAGTGCGGCGTGTCCTACCACGCCGATATT GACGTGGCCACACTGAATATTGCCAGAGTGGC CGTGCTGGGCAAGCCTATGTCTGGACCTGCCG ACAGAGAGAGACTGGGCGACACCAAGAAACC TAGAGTGGCCCGCAGCAGAAAGACCATGAAG CGGAAGGACATCAGCAACAGCACCGTCGAGG CCATGGTTACAGCT (SEQ ID NO: 1406)
CasΦ.11	MSNTAVSTREHMSNK TTPPSPLSLLLRAHFP GLKFESQDYKIAGKK LRDGGPEAVISYLTG KGQAKLKDVKPPAK AFVIAQSRPFIEWDLV RVSRQIQEKIFGIPATK GRPKQDGLSETAFNE AVASLEVDGKSKLNE ETRAAFYEVLGLDAP SLHAQAQNALIKSAIS IREGVLKKVENRNEK NLSKTKRRKEAGEEA TFVEEKAHDERGYLI HPPGVNQTIPGYQAV VIKSCPSDFIGLPSGCL AKESAEALTDYLPHD RMTIPKGQPGYVPEW QHPLLNRRKNRRRRD WYSASLNKPKATCSK RSGTPNRKNSRTDQIQ SGRFKGAIPVLMRFQ DEWVIIDIRGLLRNAR YRKLLKEKSTIPDLLS LFTGDPSIDMRQGVC TFIYKAGQACSAKMV KTKNAPEILSELTKSG PVVLVSIDLGQTNPIA AKVSRVTQLSDGQLS HETLLRELLSNDSSDG KEIARYRVASDRLRD KLANLAVERLSPEHK SEILRAKNDTPALCKA RVCAALGLNPEMIAW DKMTPYTEFLATAYL EKGGDRKVATLKPKN RPEMLRRDIKFKGTE GVRIEVSPEAAEAYRE AQWDLQRTSPEYLRL	ATGAGCAACACCGCCGTGTCCACCAGAGAACA CATGTCCAACAAGACAACCCCTCCATCTCCTC TGAGCCTGCTGCTGAGAGCCCACTTTCCTGGC CTGAAGTTCGAGAGCCAGGACTACAAGATCGC CGGCAAGAAACTGAGAGATGGCGGACCTGAG GCCGTGATCAGCTACCTGACTGGAAAAGGCCA GGCCAAGCTGAAGGACGTGAAGCCTCCTGCCA AGGCCTTTGTGATCGCCCAGAGCAGACCCTTC ATCGAGTGGGACCTCGTCAGAGTGTCCCGGCA GATCCAAGAGAAGATCTTTGGCATCCCCGCCA CCAAGGGCAGACCTAAGCAAGATGGCCTGAG CGAGACAGCCTTCAACGAAGCCGTGGCCAGCC TGGAAGTGGACGGCAAGAGCAAGCTGAACGA GGAAACCAGAGCCGCCTTCTACGAGGTGCTGG GACTTGATGCCCCAAGCCTGCATGCTCAGGCC CAGAATGCCCTGATCAAGAGCGCCATCAGCAT CAGAGAAGGCGTGCTGAAGAAGGTGGAAAAC CGGAACGAGAAGAACCTGAGCAAGACCAAGC GGCGGAAAGAGGCTGGCGAAGAGGCCACCTT TGTGGAAGAGAAGGCCCACGACGAGCGGGGC TATCTGATTCATCCTCCTGGCGTGAACCAGAC AATCCCCGGCTATCAGGCCGTGGTCATCAAGA GCTGCCCCAGCGATTTCATCGGCCTGCCTAGT GGCTGTCTGGCCAAAGAGTCTGCCGAGGCTCT GACCGATTACCTGCCTCACGACCGGATGACTA TCCCCAAGGGACAGCCTGGCTATGTGCCCGAA TGGCAGCACCCTCTGCTGAACAGAAGAAAGA ACCGGCGCAGAAGAGACTGGTACAGCGCCAG CCTGAACAAGCCCAAGGCCACCTGTAGCAAGA GATCCGGCACACCCAACCGGAAGAACAGCAG AACCGACCAGATCCAGAGCGGCAGATTCAAG GGCGCCATTCCTGTGCTGATGCGGTTCCAGGA TGAGTGGGTCATCATCGACATCCGGGGCCTGC TGAGAAACGCCCGGTATCGGAAGCTGCTGAAA GAGAAGTCCACCATTCCTGACCTGCTGAGCCT GTTCACCGGCGATCCCAGCATCGATATGAGAC AGGGCGTGTGCACCTTCATCTACAAGGCCGGC CAGGCCTGTAGCGCCAAGATGGTCAAGACAA AGAACGCCCCTGAGATCCTGTCCGAGCTGACC

	STWKQELTKRILNQL RHKAAKSSQCEVVV MAFEDLNIKMMHGN GKWADGGWDAFFIK KRENRWFMQAFHKS LTELGAHKGVPTIEVT PHRTSITCTKCGHCDK ANRDGERFACQKCGF VAHADLEIATDNIERV ALTGKPMPKPESERS GDAKKSVGARKAAF KPEEDAEAAE (SEQ ID NO: 2468)	AAGTCTGGACCTGTGGTGCTGGTGTCCATCGA CCTGGGCCAGACAAATCCTATCGCCGCCAAGG TGTCCAGAGTGACCCAGCTGTCTGATGGCCAG CTGAGCCACGAGACACTGCTGAGGGAACTGCT GAGCAACGATAGCAGCGACGGCAAAGAGATC GCCCGGTACAGAGTGGCCAGCGACAGACTGA GAGACAAGCTGGCCAATCTGGCCGTGGAAAG ACTGAGCCCTGAGCACAAGAGCGAGATCCTGA GAGCCAAGAACGACACCCCTGCTCTGTGCAAG GCCAGAGTGTGTGCTGCCCTGGGACTGAACCC TGAAATGATCGCCTGGGACAAGATGACCCCTT ACACCGAGTTTCTGGCCACCGCCTACCTGGAA AAAGGCGGCGACAGAAAAGTGGCCACACTGA AGCCCAAGAACAGACCCGAGATGCTGCGGCG GGACATCAAGTTCAAGGGAACCGAGGGCGTC AGAATCGAGGTGTCACCTGAAGCCGCCGAGGC CTATAGAGAAGCCCAGTGGGATCTGCAGAGG ACAAGCCCCGAGTACCTGAGACTGTCCACCTG GAAGCAAGAGCTGACAAAGAGAATCCTGAAC CAGCTGCGGCACAAGGCCGCCAAAAGCAGCC AGTGTGAAGTGGTGGTCATGGCCTTCGAGGAC CTGAACATCAAGATGATGCACGGCAACGGCA AGTGGGCCGATGGTGGATGGGATGCCTTCTTC ATCAAGAAACGCGAGAACCGGTGGTTCATGCA GGCCTTCCACAAGAGCCTGACAGAGCTGGGAG CACACAAGGGCGTGCCAACCATCGAAGTGACC CCTCACAGAACCAGCATCACCTGTACCAAGTG CGGCCACTGCGACAAGGCCAACAGAGATGGG GAGAGATTCGCCTGCCAGAAATGCGGCTTTGT GGCCCACGCCGATCTGGAAATCGCCACCGACA ACATCGAGAGAGTGGCCCTGACAGGCAAGCC CATGCCTAAGCCTGAGAGCGAGAGAAGCGGC GACGCCAAGAAATCTGTGGGAGCCAGAAAGG CCGCCTTCAAGCCTGAGGAAGATGCCGAAGCT GCCGAG (SEQ ID NO: 1407)
CasΦ.12	MIKPTVSQFLTPGFKL IRNHSRTAGLKLKNE GEEACKKFVRENEIPK DECPNFQGGPAIANII AKSREFTEWEIYQSSL AIQEVIFTLPKDKLPEP ILKEEWRAQWLSEHG LDTVPYKEAAGLNLII KNAVNTYKGVQVKV DNKNKNNLAKINRKN EIAKLNGEQEISFEEIK AFDDKGYLLQKPSPN KSIYCYQSVSPKPFITS KYHNVNLPEEYIGYY RKSNEPIVSPYQFDRL	ATGATCAAGCCTACCGTCAGCCAGTTTCTGAC CCCTGGCTTCAAGCTGATCCGGAACCACTCTA GAACAGCCGGCCTGAAGCTGAAGAACGAGGG CGAAGAGGCCTGCAAGAAATTCGTGCGCGAG AACGAGATCCCCAAGGACGAGTGCCCCAACTT TCAAGGCGGACCCGCCATTGCCAACATCATTG CCAAGAGCCGCGAGTTCACCGAGTGGGAGATC TACCAGTCTAGCCTGGCCATCCAAGAAGTGAT CTTCACCCTGCCTAAGGACAAGCTGCCCGAGC CTATCCTGAAAGAGGAATGGCGAGCCCAGTGG CTGTCTGAGCACGGACTGGATACCGTGCCTTA CAAAGAAGCCGCCGGACTGAACCTGATCATCA AGAACGCCGTGAACACCTACAAGGGCGTGCA AGTGAAGGTGGACAACAAGAACAAAAACAAC CTGGCCAAGATCAACCGGAAGAATGAGATCG

	RIPIGEPGYVPKWQYT FLSKKENKRRKLSKRI KNVSPILGIICIKKDW CVFDMRGLLRTNHW KKYHKPTDSINDLFD YFTGDPVIDTKANVV RFRYKMENGIVNYKP VREKKGKELLENICD QNGSCKLATVDVGQ NNPVAIGLFELKKVN GELTKTLISRHPTPIDF CNKITAYRERYDKLE SSIKLDAIKQLTSEQKI EVDNYNNNFTPQNTK QIVCSKLNINPNDLPW DKMISGTHFISEKAQV SNKSEIYFTSTDKGKT KDVMKSDYKWFQDY KPKLSKEVRDALSDIE WRLRRESLEFNKLSK SREQDARQLANWISS MCDVIGIENLVKKNN FFGGSGKREPGWDNF YKPKKENRWWINAIH KALTELSQNKGKRVI LLPAMRTSITCPKCKY CDSKNRNGEKFNCLK CGIELNADIDVATENL ATVAITAQSMPKPTC ERSGDAKKPVRARKA KAPEFHDKLAPSYTV VLREAV (SEQ ID NO: 12)	CCAAGCTGAACGGCGAGCAAGAGATCAGCTTC GAGGAAATCAAGGCCTTCGACGACAAGGGCT ACCTGCTGCAGAAGCCCTCTCCAAACAAGAGC ATCTACTGCTACCAGAGCGTGTCCCCTAAGCC TTTCATCACCAGCAAGTACCACAACGTGAACC TGCCTGAAGAGTACATCGGCTACTACCGGAAG TCCAACGAGCCCATCGTGTCCCCATACCAGTT CGACAGACTGCGGATCCCTATCGGCGAGCCTG GCTATGTGCCTAAGTGGCAGTACACCTTCCTG AGCAAGAAAGAGAACAAGCGGCGGAAGCTGA GCAAGCGGATCAAGAATGTGTCCCCAATCCTG GGCATCATCTGCATCAAGAAAGATTGGTGCGT GTTCGACATGCGGGGCCTGCTGAGAACAAACC ACTGGAAGAAGTATCACAAGCCCACCGACAG CATCAACGACCTGTTCGACTACTTCACCGGCG ATCCCGTGATCGACACCAAGGCCAATGTCGTG CGGTTCCGGTACAAGATGGAAAACGGCATCGT GAACTACAAGCCCGTGCGGGAAAAGAAGGGC AAAGAGCTGCTGGAAAACATCTGCGACCAGA ACGGCAGCTGCAAGCTGGCCACAGTGGATGTG GGCCAGAACAACCCTGTGGCCATCGGCCTGTT CGAGCTGAAAAAAGTGAACGGGGAGCTGACC AAGACACTGATCAGCAGACACCCCACACCTAT CGATTTCTGCAACAAGATCACCGCCTACCGCG AGAGATACGACAAGCTGGAAAGCAGCATCAA GCTGGACGCCATCAAGCAGCTGACCAGCGAGC AGAAAATCGAAGTGGACAACTACAACAACAA CTTCACGCCCCAGAACACCAAGCAGATCGTGT GCAGCAAGCTGAATATCAACCCCAACGATCTG CCCTGGGACAAGATGATCAGCGGCACCCACTT CATCAGCGAGAAGGCCCAGGTGTCCAACAAG AGCGAGATCTACTTTACCAGCACCGATAAGGG CAAGACCAAGGACGTGATGAAGTCCGACTAC AAGTGGTTCCAGGACTATAAGCCCAAGCTGTC CAAAGAAGTGCGGGACGCCCTGAGCGATATTG AGTGGCGGCTGAGAAGAGAGAGCCTGGAATT CAACAAGCTCAGCAAGAGCAGAGAGCAGGAC GCCAGACAGCTGGCCAATTGGATCAGCAGCAT GTGCGACGTGATCGGCATCGAGAACCTGGTCA AGAAGAACAACTTCTTCGGCGGCAGCGGCAA GAGAGAACCCGGCTGGGACAACTTCTACAAGC CGAAGAAAGAAAACCGGTGGTGGATCAACGC CATCCACAAGGCCCTGACAGAGCTGTCCCAGA ACAAGGGAAAGAGAGTGATCCTGCTGCCTGCC ATGCGGACCAGCATCACCTGTCCTAAGTGCAA GTACTGCGACAGCAAGAACCGCAACGGCGAG AAGTTCAATTGCCTGAAGTGTGGCATTGAGCT GAACGCCGACATCGACGTGGCCACCGAAAATC TGGCTACCGTGGCCATCACAGCCCAGAGCATG CCTAAGCCAACCTGCGAGAGAAGCGGCGACG CCAAGAAACCTGTGCGGGCCAGAAAAGCCAA

		GGCTCCCGAGTTCCACGATAAGCTGGCCCCTA GCTACACCGTGGTGCTGAGAGAAGCTGTG (SEQ ID NO: 1408)
CasΦ.17	MYSLEMADLKSEPSL LAKLLRDRFPGKYWL PKYWKLAEKKRLTG GEEAACEYMADKQL DSPPPNFRPPARCVIL AKSRPFEDWPVHRVA SKAQSFVIGLSEQGFA ALRAAPPSTADARRD WLRSHGASEDDLMA LEAQLLETIMGNAISL HGGVLKKIDNANVK AAKRLSGRNEARLNK GLQELPPEQEGSAYG ADGLLVNPPGLNLNI YCRKSCCPKPVKNTA RFVGHYPGYLRDSDSI LISGTMDRLTIIEGMP GHIPAWQREQGLVKP GGRRRRLSGSESNMR QKVDPSTGPRRSTRS GTVNRSNQRTGRNGD PLLVEIRMKEDWVLL DARGLLRNLRWRESK RGLSCDHEDLSLSGLL ALFSGDPVIDPVRNEV VFLYGEGIIPVRSTKP VGTRQSKKLLERQAS MGPLTLISCDLGQTNL IAGRASAISLTHGSLG VRSSVRIELDPEIIKSF ERLRKDADRLETEILT AAKETLSDEQRGEVN SHEKDSPQTAKASLC RELGLHPPSLPWGQM GPSTTFIADMLISHGR DDDAFLSHGEFPTLE KRKKFDKRFCLESRP LLSSETRKALNESLW EVKRTSSEYARLSQR KKEMARRAVNFVVEI SRRKTGLSNVIVNIED LNVRIFHGGGKQAPG WDGFFRPKSENRWFI QAIHKAFSDLAAHHG IPVIESDPQRTSMTCPE CGHCDSKNRNGVRFL CKGCGASMDADFDA	ATGTACAGCCTGGAAATGGCCGACCTGAAGTC CGAGCCTTCTCTGCTGGCTAAGCTGCTGAGAG ACAGATTCCCCGGCAAGTACTGGCTGCCTAAG TACTGGAAGCTGGCCGAGAAGAAGAGACTGA CAGGCGGAGAAGAAGCCGCCTGCGAGTACAT GGCTGACAAGCAGCTGGATAGCCCTCCACCTA ACTTCCGGCCTCCAGCCAGATGTGTGATCCTG GCCAAGAGCAGACCCTTCGAGGATTGGCCAGT GCACAGAGTGGCCAGCAAGGCCCAGTCTTTTG TGATCGGCCTGAGCGAGCAGGGCTTCGCTGCT CTTAGAGCTGCCCCTCCTAGCACAGCCGACGC CAGAAGAGATTGGCTGAGAAGCCATGGCGCC AGCGAGGATGATCTGATGGCTCTGGAAGCCCA GCTGCTGGAAACCATCATGGGCAACGCCATTT CTCTGCACGGCGGCGTGCTGAAGAAGATCGAC AACGCCAACGTGAAGGCCGCCAAGAGACTGT CCGGAAGAAACGAGGCCAGACTGAACAAGGG CCTGCAAGAGCTGCCTCCTGAGCAAGAGGGAT CTGCCTATGGCGCCGATGGCCTGCTGGTTAAT CCTCCTGGCCTGAACCTGAACATCTACTGCAG AAAGAGCTGCTGCCCCAAGCCTGTGAAGAACA CCGCCAGATTCGTGGGACACTACCCCGGCTAC CTGAGAGACTCCGACAGCATCCTGATCAGCGG CACCATGGACCGGCTGACAATCATCGAGGGAA TGCCCGGACACATCCCCGCCTGGCAACGAGAA CAGGGACTTGTGAAACCTGGCGGCAGAAGGC GGAGACTGTCTGGCAGCGAGAGCAACATGAG ACAGAAGGTGGACCCCAGCACAGGCCCCAGA AGAAGCACAAGATCCGGCACCGTGAACAGAA GCAACCAGCGGACAGGCAGAAACGGCGATCC TCTGCTGGTGGAAATCCGGATGAAGGAAGATT GGGTCCTGCTGGACGCCAGAGGCCTGCTGAGA AATCTGAGATGGCGCGAGTCCAAGAGAGGCCT GAGCTGCGATCACGAGGATCTGAGCCTGTCTG GACTGCTGGCCCTGTTTTCTGGCGACCCCGTG ATCGATCCTGTGCGGAATGAGGTGGTGTTCCT GTACGGCGAGGGCATCATTCCAGTGCGGAGCA CAAAGCCTGTGGGCACCAGACAGAGCAAGAA ACTGCTGGAACGGCAGGCCAGCATGGGCCCTC TGACACTGATCTCTTGTGACCTGGGCCAGACC AACCTGATTGCCGGCAGAGCCTCTGCTATCAG CCTGACACATGGATCTCTGGGCGTCAGATCCA GCGTGCGGATTGAGCTGGACCCCGAGATCATC AAGAGCTTCGAGCGGCTGAGAAAGGACGCCG ACAGACTGGAAACCGAGATCCTGACCGCCGCC AAAGAAACCCTGAGCGACGAACAGAGGGGCG AAGTGAACAGCCACGAGAAGGATAGCCCACA

	ACRNLERVALTGKPM PKPSTSCERLLSATTG KVCSDHSLSHDAIEK AS (SEQ ID NO: 17)	GACAGCCAAGGCCAGCCTGTGTAGAGAGCTG GGACTGCACCCTCCATCTCTGCCTTGGGGACA GATGGGCCCTAGCACCACCTTTATCGCCGACA TGCTGATCTCCCACGGCAGGGACGATGATGCC TTTCTGAGCCACGGCGAGTTCCCCACACTGGA AAAGCGGAAGAAGTTCGATAAGCGGTTCTGCC TGGAAAGCAGACCCCTGCTGAGCAGCGAGAC AAGAAAGGCCCTGAACGAGTCCCTGTGGGAA GTGAAGAGAACCAGCAGCGAGTACGCCCGGC TGAGCCAGAGAAAGAAAGAGATGGCTAGACG GGCCGTGAACTTCGTGGTCGAGATCTCCAGAA GAAAGACCGGCCTGTCCAACGTGATCGTGAAC ATCGAGGACCTGAACGTGCGGATCTTTCACGG CGGAGGAAAACAGGCTCCTGGCTGGGATGGCT TCTTCAGACCCAAGTCCGAGAACCGGTGGTTC ATCCAGGCCATCCACAAGGCCTTCAGCGATCT GGCCGCTCACCACGGAATCCCTGTGATCGAGA GCGACCCTCAGCGGACCAGCATGACCTGTCCT GAGTGTGGCCACTGCGACAGCAAGAACCGGA ATGGCGTTCGGTTCCTGTGCAAAGGCTGTGGC GCCTCCATGGACGCCGATTTTGATGCCGCCTG CCGGAACCTGGAAAGAGTGGCTCTGACAGGC AAGCCCATGCCTAAGCCTAGCACCTCCTGTGA AAGACTGCTGAGCGCCACCACCGGCAAAGTGT GCTCTGATCACTCCCTGTCTCACGACGCCATCG AGAAGGCTTCTTAA (SEQ ID NO: 1409)
CasΦ.18	MEKEITELTKIRREFP NKKFSSTDMKKAGKL LKAEGPDAVRDFLNS CQEIIGDFKPPVKTNI VSISRPFEEWPVSMVG RAIQEYYFSLTKEELE SVHPGTSSEDHKSFFN ITGLSNYNYTSVQGL NLIFKNAKAIYDGTLV KANNKNKKLEKKFN EINHKRSLEGLPIITPD FEEPFDENGHLNNPPG INRNIYGYQGCAAKV FVPSKHKMVSLPKEY EGYNRDPNLSLAGFR NRLEIPEGEPGHVPWF QRMDIPEGQIGHVNKI QRFNFVHGKNSGKVK FSDKTGRVKRYHHSK YKDATKPYKFLEESK KVSALDSILAIITIGDD WVVFDIRGLYRNVFY RELAQKGLTAVQLLD LFTGDPVIDPKKGVV	ATGGAAAAAGAGATCACCGAGCTGACCAAGA TCCGCAGAGAGTTCCCCAACAAGAAGTTCAGC AGCACCGACATGAAGAAGGCCGGCAAGCTGC TGAAGGCCGAAGGACCTGATGCCGTGCGGGA CTTCCTGAACAGCTGCCAAGAGATCATCGGCG ACTTCAAGCCTCCAGTCAAGACCAACATCGTG TCCATCAGCAGACCCTTCGAGGAATGGCCCGT GTCCATGGTTGGACGGGCCATCCAAGAGTACT ACTTCAGCCTGACCAAAGAGGAACTGGAAAG CGTTCACCCCGGCACCAGCAGCGAGGACCACA AGAGCTTTTTCAACATCACCGGCCTGAGCAAC TACAACTACACCAGCGTGCAGGGCCTGAACCT GATCTTCAAGAACGCCAAGGCCATCTACGACG GCACCCTGGTCAAGGCCAACAACAAGAACAA GAAGCTCGAGAAGAAGTTTAACGAGATCAAC CACAAGCGGAGCCTGGAAGGCCTGCCTATCAT CACCCCTGATTTCGAGGAACCCTTCGACGAGA ACGGCCACCTGAACAACCCTCCAGGCATCAAC CGGAACATCTACGGCTATCAGGGCTGCGCCGC CAAGGTGTTCGTGCCTTCTAAGCACAAGATGG TGTCCCTGCCTAAAGAGTACGAGGGCTACAAC AGGGACCCCAACCTGTCTCTGGCCGGCTTCAG AAACAGACTGGAAATCCCTGAGGGCGAGCCT GGCCATGTGCCATGGTTCCAGAGAATGGATAT

	TFSYKEGVVPVFSQKI VPRFKSRDTLEKLTSQ GPVALLSVDLGQNEP VAARVCSLKNINDKIT LDNSCRISFLDDYKK QIKDYRDSLDELEIKI RLEAINSLETNQQVEI RDLDVFSADRAKANT VDMFDIDPNLISWDS MSDARVSTQISDLYL KNGGDESRVYFEINN KRIKRSDYNISQLVRP KLSDSTRKNLNDSIW KLKRTSEEYLKLSKR KLELSRAVVNYTIRQS KLLSGINDIVIILEDLD VKKKFNGRGIRDIGW DNFFSSRKENRWFIPA FHKTFSELSSNRGLCV IEVNPAWTSATCPDC GFCSKENRDGINFTCR KCGVSYHADIDVATL NIARVAVLGKPMSGP ADRERLGDTKKPRVA RSRKTMKRKDISNST VEAMVTA (SEQ ID NO: 18)	CCCCGAGGGCCAGATCGGACACGTGAACAAG ATCCAGCGGTTCAACTTCGTGCACGGCAAGAA CAGCGGCAAAGTGAAGTTCTCCGACAAGACCG GCAGAGTGAAGAGATACCACCACAGCAAGTA CAAGGACGCTACCAAGCCTTACAAGTTCCTGG AAGAGTCCAAGAAGGTGTCAGCCCTGGACAG CATCCTGGCCATCATCACAATCGGCGACGACT GGGTCGTGTTCGACATCAGAGGCCTGTACCGG AACGTGTTCTACAGAGAGCTGGCCCAGAAAGG CCTGACAGCTGTGCAACTGCTGGACCTGTTTA CCGGCGATCCCGTGATCGACCCCAAGAAAGGC GTGGTCACCTTCAGCTACAAAGAGGGCGTCGT CCCCGTCTTTAGCCAGAAAATCGTGCCCCGGT TCAAGAGCCGGGACACCCTGGAAAAGCTGAC CTCTCAGGGACCTGTGGCTCTGCTGTCTGTGG ACCTGGGACAGAATGAACCTGTGGCCGCCAGA GTGTGCAGCCTGAAGAACATCAACGACAAGAT CACCCTGGACAACTCTTGCCGGATCAGCTTCC TGGACGACTACAAGAAGCAGATCAAGGACTA CAGAGACAGCCTGGACGAGCTGGAAATCAAG ATCCGGCTGGAAGCCATCAACTCCCTCGAGAC AAACCAGCAGGTCGAGATCAGAGATCTGGAC GTGTTCAGCGCCGACCGGGCCAAAGCCAATAC CGTGGACATGTTTGACATCGACCCTAACCTGA TCAGCTGGGACTCCATGAGCGACGCCAGAGTC AGCACCCAGATCAGCGACCTGTACCTGAAGAA TGGCGGCGACGAGAGCCGGGTGTACTTTGAGA TTAACAACAAACGGATTAAGCGGAGCGACTAC AACATCAGCCAGCTCGTGCGGCCCAAGCTGAG CGATAGCACCAGAAAGAACCTGAACGACAGC ATCTGGAAGCTGAAGCGGACCAGCGAGGAAT ACCTGAAGCTGAGCAAGCGGAAGCTGGAACT GAGCAGAGCCGTCGTGAATTACACCATCCGGC AGAGCAAACTGCTGAGCGGCATCAATGACATC GTGATCATTCTCGAGGACCTGGACGTGAAGAA GAAATTCAACGGCAGAGGCATCCGCGATATCG GCTGGGACAACTTCTTCAGCTCCCGGAAAGAA AACCGGTGGTTCATCCCCGCCTTCCACAAGAC CTTTAGCGAGCTGAGCAGCAACAGGGGCCTGT GCGTGATCGAAGTGAATCCTGCCTGGACCAGC GCCACCTGTCCTGATTGTGGCTTCTGCAGCAA AGAAAACAGAGATGGCATCAACTTCACGTGCC GGAAGTGCGGCGTGTCCTACCACGCCGATATT GACGTGGCCACACTGAATATTGCCAGAGTGGC CGTGCTGGGCAAGCCTATGTCTGGACCTGCCG ACAGAGAGAGACTGGGCGACACCAAGAAACC TAGAGTGGCCCGCAGCAGAAAGACCATGAAG CGGAAGGACATCAGCAACAGCACCGTCGAGG CCATGGTTACAGCTTAA (SEQ ID NO: 1410)

EXAMPLE 2

Illustrative CasΦ Guide RNA sequences

[0427] Guide RNA sequences for complexing with the CasΦ polypeptides of the disclosure were prepared. **TABLE 5** provides illustrative guide RNA sequences to target the target nucleic acid sequence TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 1411). A guide nucleic acid of the disclosure can comprise the sequence of any of the guide RNAs provided in Table 5 or a portion thereof.

TABLE 5. Illustrative CasΦ guide RNA sequences

Name	RNA Type	Repeat length	Spacer length	RNA sequence (5' --> 3'), shown as DNA BOLD = spacer
CasΦ.2	crRNA	36	30	GTCGGAACGCTCAACGATTGCCCCTCACGAGGGGAC (SEQ ID NO: 49)
CasΦ.7	crRNA	36	30	GGATCCAATCCTTTTTGATTGCCCAATTCGTTGGGAC (SEQ ID NO: 51)
CasΦ.10	crRNA	36	30	GGATCTGAGGATCATTATTGCTCGTTACGACGAGAC (SEQ ID NO: 52)
CasΦ.18	crRNA	36	30	ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC (SEQ ID NO: 57)

EXAMPLE 3

CasΦ acts as a programmable nickase

[0428] The present example shows that a CasΦ polypeptide can comprise programmable nickase activity. **FIG. 1** shows data from an experiment to analyze nicking ability of CasΦ ortholog proteins. For this experiment, five different CasΦ polypeptides: designated CasΦ.2, CasΦ.11, CasΦ.17, CasΦ.18, and CasΦ.12 in **FIG. 1**, were analyzed. Amino acid sequences of the proteins used in the experiment are shown in **TABLE 4**.

[0429] All reactions were carried out using guide RNA comprising a crRNA sequence comprising the CasΦ.18 repeat sequence (ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC (SEQ ID NO: 57)). Complexing of the CasΦ polypeptide with a guide RNA to form the ribonucleoprotein (RNP) complex was carried out at room temperature for 20 minutes. The RNP complex was incubated with the target DNA at 37°C for 60 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C). The target nucleic acid

used for the reactions was a super-coiled plasmid DNA comprising the target sequence TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 116), which was immediately downstream of a TTTN PAM sequence. The plasmid DNA sequence is provided below with the target sequence in bold:

gtgtagataactacgatacgggagggcttaccatctggccccagtgctgcaatgataccgcgagacccacgctcaccggc
tccagatttatcagcaataaaccagccagccggaagggccgagcgcagaagtggtcctgcaactttatccgcctccatcca
gtctattaattgttgccgggaagctagagtaagtagttcgccagttaatagtttgcgcaacgttgttgccattgctacaggcatc
gtggtgtcacgctcgtcgtttggtatggcttcattcagctccggttcccaacgatcaaggcgagttacatgatcccccatgttg
tgcaaaaaagcggttagctccttcggtcctccgatcgttgtcagaagtaagttggccgcagtgttatcactcatggttatggc
agcactgcataattctcttactgtcatgccatccgtaagatgcttttctgtgactggtgagtactcaaccaagtcattctgagaat
agtgtatgcggcgaccgagttgctcttgcccggcgtcaatacgggataataccgcgccacatagcagaactttaaaagtgc
tcatcattggaaaacgttcttcggggcgaaaactctcaaggatcttaccgctgttgagatccagttcgatgtaacccactcgt
gcacccaactgatcttcagcatcttttactttcaccagcgtttctgggtgagcaaaaacaggaaggcaaaatgccgcaaaaa
agggaataagggcgacacggaaatgttgaatactcatactcttccttttcaatattattgaagcatttatcagggttattgtctc
atgagcggatacatatttgaatgtatttagaaaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctga
cgtctaagaaaccattattatcatgacattaacctataaaaataggcgtatcacgaggccctttcgtctcgcgcgtttcggtgat
gacggtgaaaacctctgacacatgcagctcccggagacggtcacagcttgtctgccatggacatgttta**TATTAAA**
TACTCGTATTGCTGTTCGATTATgaccgaattccctgtcgtgccagctgcattaatgaatcggcca
acgcgcggggagaggcggtttgcgtattgggcgctcttccgcttcctcgctcactgactcgctgcgctcggtcgttcggct
gcggcgagcggtatcagctcactcaaaggcggtaatacggttatccacagaatcaggggataacgcaggaaagaacatg
tgagcaaaaggccagcaaaaggccaggaaccgtaaaaaggccgcgttgctggcgtttttccataggctccgccccctg
acgagcatcacaaaaatcgacgctcaagtcagaggtggcgaaacccgacaggactataaagataccaggcgtttccccc
tggaagctccctcgtgcgctctcctgttccgaccctgccgcttaccggatacctgtccgcctttctcccttcgggaagcgtgg
cgctttctcatagctcacgctgtaggtatctcagttcggtgtaggtcgttcgctccaagctgggctgtgtgcacgaacccccc
gttcagcccgaccgctgcgccttatccggtaactatcgtcttgagtccaacccggtaagacacgacttatcgccactggcag
cagccactggtaacaggattagcagagcgaggtatgtaggcggtgctacagagttcttgaagtggtggcctaactacggct
acactagaagaacagtatttggtatctgcgctctgctgaagccagttaccttcggaaaaagagttggtagctcttgatccggc
aaacaaaccaccgctggtagcggtggtttttttgtttgcaagcagcagattacgcgcagaaaaaaaggatctcaagaagat
cctttgatcttttctacggggtctgacgctcagtggaacgaaaactcacgttaagggattttggtcatgagattatcaaaaagg
atcttcacctagatccttttaaattaaaaatgaagttttaaatcaatctaaagtatatatgagtaaacttggtctgacagttaccaat
gcttaatcagtgaggcacctatctcagcgatctgtctatttcgttcatccatagttgcctgactccccgtc (SEQ ID NO: 1412)

[0430] As shown in **FIG 1**, CasΦ.17 and CasΦ.18 produced only nicked product (i.e. single strand breaks; "nicked") by 60 minutes. By way of comparison, CasΦ.12 generated almost entirely linearized product demonstrating double-stranded breaks, while CasΦ.2 and CasΦ.11 generated some linearized product (i.e. double strand breaks) but primarily produced nicked intermediate. This data demonstrates that CasΦ orthologs can comprise programmable nickase activity.

EXAMPLE 4

Effect of crRNA repeat sequence and RNP complexing temperature on CasΦ nickase activity

[0431] The present example shows that the crRNA repeat sequence and RNP complexing temperature can affect nickase activity of CasΦ. **FIG. 2A and FIG. 2B** illustrate results of a *cis*-

cleavage experiment showing the percentage of input plasmid DNA that was nicked after 60 minutes of reaction at 37°C by CasΦ RNP complex assembled at room temperature (**FIG. 2A**) or at 37°C (**FIG. 2B**). **FIG. 2C** illustrates alignment of CasΦ.2, CasΦ.7, CasΦ.10, and CasΦ.18 repeat sequences showing conserved (highlighted in black) and diverged nucleotides.

[0432] For this study, each of three CasΦ polypeptides (CasΦ.11, CasΦ.17 and CasΦ.18 in FIG. 2A and 2B) was tested for their ability to nick input plasmid DNA when complexed with one of four crRNAs comprising the repeat sequences of CasΦ.2, CasΦ.7, CasΦ.10 and CasΦ.18 (abbreviated j2, j7, j10 and j18, respectively in **FIG. 2A** and **FIG. 2B**). Amino acid sequences of the proteins used in the experiment are shown in **TABLE 4**. Guide RNA sequences corresponding to j2, j7, j10 and j18 are provided in **TABLE 5**. The input plasmid was a super-coiled plasmid (sequence shown in EXAMPLE 3) comprising the target sequence TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 108) immediately downstream of a TTTN PAM. The incubation reaction to form the RNP complex was performed either at room temperature or at 37°C for 60 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C). The RNP complex was incubated with the input plasmid for 60 minutes at 37°C. The reaction was quenched with 1 mg/ml proteinase K, 0.08% SDS, and 15 mM EDTA. The data illustrated in FIG. 2A and FIG. 2B comes from a single replicate of the *in vitro cis*-cleavage experiment.

[0433] As shown in **FIG. 2A**, when the CasΦ polypeptides were assembled into RNP complexes with the guide nucleic acids at room temperature, crRNAs comprising repeat sequences from any of the proteins supported nickase activity by CasΦ.11, CasΦ.17 and CasΦ.18, with the exception of the CasΦ.17/CasΦ.2-repeat pairing. As shown in **FIG. 2B**, when the CasΦ polypeptides were assembled into RNP complexes with the guide nucleic acids at 37°C, as opposed to at room temperature, the activity of each protein was completely abolished when complexed with crRNAs comprising a repeat sequence from CasΦ.2 or CasΦ.10.

[0434] This example showed that the nickase activity of CasΦ can be affected by the crRNA repeat sequence. The data also showed that the nickase activity of CasΦ can be affected by the RNP complexing temperature.

[0435] **FIG. 2D** provides further examples of the nickase activity of CasΦ affected by the RNP complexing temperature. Nickase activity was assessed as described above for CasΦ.2, CasΦ.4, CasΦ.6, CasΦ.9, CasΦ.10, CasΦ.12 and CasΦ.13. Amino acid sequences of the proteins used in the experiment are shown in **TABLE 1**.

[0436] The effect of complexing temperature on the double strand cutting activity of CasΦ polypeptides was also assessed as described above. As shown in **FIG. 2D**, generally the double strand cutting activity of CasΦ polypeptides, particularly CasΦ.2, CasΦ.4 and CasΦ.12, is not affected by the RNP complexing temperature. Although some systems with less efficient double strand cutting activity, such as CasΦ.10, CasΦ.11 and CasΦ.13 in this example, are sensitive to RNP complexing temperature.

EXAMPLE 5

CasΦ nickase cleaves non-target strand

[0437] The present example shows that CasΦ nickase cleaves the non-target DNA strand. Results of the study are shown in **FIG. 3**. For this study, four different CasΦ polypeptides (CasΦ.12, CasΦ.2, CasΦ.11, and CasΦ.18 as shown in FIG. 1) were analyzed using a cis-cleavage assay. Amino acid sequences of the proteins used in the experiment are shown in **TABLE 4**. The CasΦ polypeptides were complexed with guide RNA to form RNP complexes All reactions were carried out using guide RNA comprising a crRNA sequence comprising the CasΦ.18 repeat sequence (ACCAAAACGACTATTGATTGCCCAGTACGCTGGGAC (SEQ ID NO: 57)). Complexing of the CasΦ polypeptides with guide RNA to form the ribonucleoprotein (RNP) complex was carried out at room temperature for 20 minutes. The RNP complex was incubated with the target DNA at 37°C for 60 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C. The target nucleic acid used for the reactions was a super-coiled plasmid DNA (sequence shown in EXAMPLE 3) comprising the target sequence TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 116), which was immediately downstream of a TTTN PAM sequence. The reaction was quenched with 1 mg/ml proteinase K, 0.08% SDS, and 15 mM EDTA. The resulting cleaved DNA from the reaction was Sanger sequenced using forward and reverse primers. The forward primer provided the sequence of the target strand (TS), while the reverse primer provided the sequence of the non-target strand (NTS). If a strand had been cleaved by the CasΦ polypeptide, the sequencing signal would drop off from the cleavage site in the sequencing data. **FIG. 3** illustrates results of the Sanger sequencing.

[0438] **FIG. 3, panel A**, shows a control reaction where no CasΦ polypeptide was added. As a result, the target DNA was uncut and resulted in complete sequencing of both target and non-target strands. **FIG. 3, panel B**, illustrates the cleavage pattern for CasΦ.12, which comprises double-stranded DNA cleavage activity. The sequencing signal dropped off on both the target and the non-target strands (as shown by arrows), demonstrating cleavage of both strands of the

target DNA. **FIG. 3, panel C**, illustrates the cleavage pattern for CasΦ.2, which predominantly nicks DNA (as illustrated in **FIG. 1**). The data showed that the sequencing signal dropped off on only the non-target strand (bottom arrow) demonstrating cleavage of the non-target strand. **FIG. 3, panel D**, illustrates the cleavage pattern for CasΦ.11, which comprises strong nickase activity (as illustrated in **FIG. 1**). The data showed that the sequencing signal dropped off on only the non-target strand (bottom arrow) demonstrating cleavage of the non-target strand. **FIG. 3, panel E**, illustrates the cleavage pattern for CasΦ.18, which comprises strong nickase activity (as illustrated in **FIG. 1**). The data showed that the sequencing signal dropped off on only the non-target strand (bottom arrow) demonstrating cleavage of the non-target strand. Thus, this example shows that CasΦ polypeptides comprising nickase activity cleave the non-target strand of a target DNA.

EXAMPLE 6
Editing a Target Nucleic Acid

[0439] This example describes genetic modification of a target nucleic acid with a programmable CasΦ nuclease (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO: 105 or SEQ ID NO: 107) of the present disclosure. The programmable CasΦ nuclease is administered with a guide nucleic acid capable of hybridizing to a segment of a target nucleic acid sequence of interests in a ribonucleoprotein complex or as separate nucleic acids encoding for each component. Subjects administered said composition are humans or non-human mammals. Upon binding of the guide nucleic acid to the segment of the target nucleic acid, the programmable CasΦ nuclease nicks or induces a double stranded break in the target. The target undergoes NHEJ or HDR. A donor nucleic acid may be co-administered. The donor nucleic acid may be to replace or repair a mutated segment of the target nucleic acid. The subject may have a disease. Upon genetic modification of the target nucleic acid, the disease or a symptom of the disease may be alleviated, or the disease may be cured.

EXAMPLE 7
Editing a Plant or Crop Target Nucleic Acid

[0440] This example describes genetic modification of a plant or crop target nucleic acid with a programmable CasΦ nuclease (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ I DNO: 105 or SEQ ID NO: 107) of the present disclosure. The programmable CasΦ nuclease is administered with a guide nucleic acid capable of hybridizing to a segment of a target nucleic acid sequence of interests in a ribonucleoprotein complex or as separate nucleic acids encoding for each component. Subjects administered said composition are plant or crop cells. Upon binding of the guide nucleic acid to the segment of the target nucleic acid, the programmable

CasΦ nuclease nicks or induces a double stranded break in the target. The target undergoes NHEJ or HDR. A donor nucleic acid may be co-administered. The donor nucleic acid may be to replace or repair a mutated segment of the target nucleic acid. The result is an engineered plant or crop cell.

EXAMPLE 8

Genetic Modification of a Target Nucleic Acid

[0441] This example describes genetic modification of a target nucleic acid with a dead programmable CasΦ nuclease (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO: 105 or SEQ ID NO: 107 with a mutation rendering it catalytically inactive) of the present disclosure. The programmable CasΦ nuclease is further linked to a transcriptional regulator. The programmable CasΦ nuclease, the transcriptional regulator, and the guide nucleic acid capable of hybridizing to a segment of a target nucleic acid sequence of interests are administered as a ribonucleoprotein complex or as separate nucleic acids encoding for each component. Subjects administered said composition are humans or non-human mammals. Upon binding of the guide nucleic acid to the segment of the target nucleic acid, the dead programmable CasΦ nuclease upregulates or downregulates transcription. The subject may have a disease. Upon genetic modification of the target nucleic acid, the disease or a symptom of the disease may be alleviated, or the disease may be cured.

EXAMPLE 9

Genetic Modification of a Plant of Crop Target Nucleic Acid

[0442] This example describes genetic modification of a plant or crop target nucleic acid with a dead programmable CasΦ nuclease (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO: 105 or SEQ ID NO: 107 with a mutation rendering it catalytically inactive) of the present disclosure. The programmable CasΦ nuclease is further linked to a transcriptional regulator. The programmable CasΦ nuclease, the transcriptional regulator, and the guide nucleic acid capable of hybridizing to a segment of a target nucleic acid sequence of interests are administered as a ribonucleoprotein complex or as separate nucleic acids encoding for each component. Subjects administered said composition are humans or non-human mammals. Upon binding of the guide nucleic acid to the segment of the target nucleic acid, the dead programmable CasΦ nuclease upregulates or downregulates transcription. The result is an engineered plant or crop cell.

EXAMPLE 10

Detection of a Target Nucleic Acid

[0443] This example describes detection of a target nucleic acid with a programmable CasΦ nuclease (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 47, SEQ ID NO: 105 or SEQ ID NO: 107) of the present disclosure. The programmable CasΦ nuclease, the guide nucleic acid capable of hybridizing to a segment of a target nucleic acid sequence of interests, and a labeled ssDNA reporter are contacted to a sample. In the presence of the target nucleic acid in the sample, the guide nucleic acid binds to its target, thereby activating the programmable CasΦ nuclease to cleave the labeled ssDNA reporter and releasing a detectable label. The detectable label emits a detectable signal that is, optionally, quantified. In the absence of the target nucleic acid in the sample, the guide nucleic acid does not bind to its target, the labeled ssDNA reporter is not cleaved, and low or no signal is detected.

EXAMPLE 11

Preference for nicking or double strand cleavage of target DNA is a property of CasΦ enzymes, independent of crRNA repeat or target sequences

[0444] This example describes how the preference of a CasΦ polypeptide to cleave a single or both strands of a double-strand target DNA is independent of the crRNA repeat or target sequence. For this study, each of twelve CasΦ polypeptide (CasΦ.1, CasΦ.2, CasΦ.3, CasΦ.4, CasΦ.6, CasΦ.9, CasΦ.10, CasΦ.11, CasΦ.12, CasΦ.13, CasΦ.17 and CasΦ.18) was complexed with one of the crRNAs comprising the repeat sequences of CasΦ.1, CasΦ.2, CasΦ.4, CasΦ.7, CasΦ.10, CasΦ.11, CasΦ.12, CasΦ.13, CasΦ.17 and CasΦ.18. Amino acid sequences of the proteins used in the experiment are shown in **TABLE 1** and crRNA sequences are provided in **TABLE 2**. The input plasmid was one of two super-coiled plasmids containing a target sequence (TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 108) or CACAGCTTGTCTGTAAGCGGATGCCATATG (SEQ ID NO: 109)) immediately downstream of a TTTN PAM. The incubation reaction to form the RNP complex was performed at room temperature for 20 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C). The RNP complex was incubated with the input plasmid for 60 minutes at 37°C. The reaction was quenched with 1 mg/ml proteinase K, 0.08% SDS, and 15 mM EDTA.

[0445] As shown in **FIG. 4A**, CasΦ polypeptides have a preference for nicking or linearizing (i.e. cleaving both strands) a double strand plasmid DNA target and this preference is not affected by the crRNA repeat or target DNA sequence.

[0446] Raw data used to generate a subset of the heatmap in FIG. 4A is shown in **FIG. 4B**. These data show that CasΦ.12 is predominantly a linearizer of plasmid DNA, i.e. CasΦ.12 predominantly cleaves both strands of a double strand target DNA. Whereas CasΦ.18 is predominantly a nickase and predominantly cleaves one strand of a double strand target DNA.

[0447] This example showed that the preference of a CasΦ polypeptide to cleave a single or both strands of a double-strand target DNA is independent of the crRNA repeat or target sequence.

EXAMPLE 12

Structural conservation across the CasΦ repeats

[0448] This example describes the conservation of structure across the CasΦ repeats. In particular, **FIG. 5A** shows the structure of the crRNA repeats for CasΦ.1, CasΦ.2, CasΦ.7, CasΦ.11, CasΦ.12, CasΦ.13, CasΦ.18, and CasΦ.32. crRNA sequences are provided in **TABLE 2**. There is high sequence and structure conservation in the 3' half of the CasΦ repeats. The LocARNA alignment tool was used to confirm the consensus structure of CasΦ repeats, which is shown in **FIG.5B**. The consensus was determined on the basis of the following crRNA repeats: CasΦ.1, CasΦ.2, CasΦ.4, CasΦ.7, CasΦ.10, CasΦ.11, CasΦ.12, CasΦ.13, Cas12Φ.17, CasΦ.18, CasΦ.19, CasΦ.21, CasΦ.22, CasΦ.23, CasΦ.24, CasΦ.25, CasΦ.26, CasΦ.27, CasΦ.28, CasΦ.29, CasΦ.30, CasΦ.31, CasΦ.32, CasΦ.33, CasΦ.35, CasΦ.41. The sequence of these repeats is provided in **TABLE 5**. As shown in FIG.5B, CasΦ repeats have a highly conserved 3' hairpin which includes a double stranded stem portion and a single-stranded loop portion. One strand of the stem includes the sequence CYC and the other strand includes the sequence GRG, where Y and R are complementary. The loop portion typically comprises four nucleotides. The 3' end of CasΦ repeats comprise the sequence GAC and the G of this sequence is in the stem of the hairpin.

[0449] This example shows the conserved structure of CasΦ crRNA repeats.

EXAMPLE 13

CasΦ PAM preferences on linear targets

[0450] The present example shows the PAM preferences for CasΦ polypeptides on linear double stranded DNA targets. For this study, five different CasΦ polypeptides (CasΦ.2, CasΦ.4, CasΦ.11, CasΦ.12 and CasΦ.18) were analyzed using a cis-cleavage assay. Amino acid sequences of the proteins used are shown in **TABLE 1**. The CasΦ polypeptides were complexed their native crRNAs (i.e. the corresponding CasΦ.2, CasΦ.4, CasΦ.11, CasΦ.12 and CasΦ.18 repeats) to form RNP complexes at room temperature for 20 minutes. The RNP complex was incubated with target DNA at 37°C for 60 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C). The target DNA was a 1.1 kb PCR-amplified DNA product. Stating with a TTTA PAM, each position was varied one by one to the other 3 nucleotides for a total of 12 variants in addition to

the parental TTTA PAM. Linear fragments were used to disfavor cleavage for greater sensitivity of PAM preference determination. **FIG.6A** illustrates the absolute levels of double strand cleavage (or nicking for CasΦ.18). **FIG.6B** illustrates the data from FIG.6A after normalization to the parental TTTA PAM as 100%. **FIG.6C** provides a summary of the optimal PAM preferences from the data in FIG.6A and FIG.6B. CasΦ.2 recognizes a GTTK PAM, where K is G or T. CasΦ.4 recognizes a VTTK PAM, where V is A, C or G and K is G or T. CasΦ.11 recognizes a VTTS PAM, where V is A, C or G and S is C or G. CasΦ.12 recognizes a TTTS PAM, where S is C or G. CasΦ.18 recognizes a VTTN PAM, where V is A, C or G and N is A, C, G or T.

[0451] This example shows the optimized PAM preferences for some of the CasΦ polypeptides.

EXAMPLE 14

CasΦ polypeptides rapidly nick supercoiled DNA

[0452] The present example shows that CasΦ polypeptides rapidly nick supercoiled DNA but vary in their ability to deliver the second strand cleavage. For this study, five different CasΦ polypeptides (CasΦ.2, CasΦ.4, CasΦ.11, CasΦ.12 and CasΦ.18) were analyzed using a cis-cleavage assay. Amino acid sequences of the proteins used are shown in **TABLE 1**. The CasΦ polypeptides were complexed with their native crRNA to form 200nM RNP complexes at room temperature in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25 °C) for 20 minutes in a volume of 30 µl. The target plasmid was one of two 2.2 kb super-coiled plasmids containing a target sequence (TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 108) or CACAGCTTGTCTGTAAGCGGATGCCATATG (SEQ ID NO: 109), the guide RNAs targeted the underlined sequence) immediately downstream of a GTTG or TTTG PAM. At time "0" 30 µl of 20 nM target plasmid was mixed with RNP for a total volume of 60 µl. The incubation temperature was 37 °C. At 1, 3, 6, 15, 30 and 60 minutes, 9 µl portions of the reaction were withdrawn and stopped with reaction quench (1 mg/ml proteinase K, 0.08% SDS and 15 mM EDTA) and allowed to deproteinize for 30 minutes at 37 °C before agarose gel analysis. The cleavage was quantified as nicked or linear. **FIG.7** shows the rapid nicking of supercoiled target DNA by CasΦ polypeptides. The decrease in nicked products over time is due to the formation of linear product as the CasΦ polypeptides cleaves the second strand of the target DNA. CasΦ.12 rapidly cleaves both strands of supercoiled DNA.

[0453] This example shows that CasΦ polypeptides rapidly nick supercoiled DNA.

EXAMPLE 15

CasΦ polypeptides prefers full length repeats and spacers form 16-20 nucleotide

[0454] The present example shows that CasΦ polypeptides prefer full-length repeats and spacers from 16 to 20 nucleotides. For this study, each of five CasΦ polypeptides (CasΦ.2, CasΦ.4,

CasΦ.11, CasΦ.12 and CasΦ.18in FIG. 8A and 8B) was tested for their ability to cleave input plasmid DNA when complexed with one of either of the crRNAs comprising the repeat sequences of CasΦ.2 or CasΦ.18 (abbreviated j2 and j18, respectively in **FIG. 8A** and **FIG. 8B**). Amino acid sequences of the proteins used in the experiment are shown in **TABLE 1**. Guide RNA sequences corresponding to j2 and j18 are provided in **TABLE 2**. The CasΦ polypeptides were complexed to the crRNA in NEB CutSmart Buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25 °C) for 20 minutes at room temperature. The ability of the CasΦ polypeptides to cleave a 2.2kb plasmid containing a target sequence was assessed (FUT8_1: ACGCGTTTTAGAAGAGCAGCTTGTTAAGGCCAAAGAACAGATTGA (SEQ ID NO: 1413) and DNMT_1: AAAGATTTGTCCTTGGAGAACGGTGCTCATGCTTACAACCGGGA (SEQ ID NO: 1414), the PAM is underlined). Spacers targeting these target sequences were shortened from the 3' end. The cleavage incubation was at 37 °C and the reaction was quenched after 10 minutes with 1 mg/ml proteinase K, 0.08% SDS and 15 mM EDTA. To assess the effect of shortening the crRNA repeats, the repeats were shortened from the 5' end.

[0455] As shown in **FIG.8A**, cRNA repeats with a length of 19 to 37 nucleotides supported cleavage activity of CasΦ polypeptides.

[0456] As shown in **FIG.8B**, cleavage activity was observed over the range of spacer lengths tested (16 to 35 nucleotides). The optimal spacer length to support the cleavage activity of CasΦ polypeptides in this *in vitro* system is 16 to 20 nucleotides.

[0457] This example shows that CasΦ polypeptides prefer crRNA repeat lengths of 19 to 37 nucleotides and spacer lengths of 16 to 20 nucleotides *in vitro*.

EXAMPLE 16

CasΦ.12 spacer length optimization in HEK293T cells

[0458] The present example shows the use of CasΦ.12 as a gene editing tool in HEK293T cells and the effect of changing the length of the spacer. As illustrated in the schematic in **FIG.9A**, a stable HEK293T cell line that expresses AcGFP was established. A plasmid expressing the crRNA under the control of the U6 promoter and CasΦ.12 under the control of the EF1a promoter was transfected into the AcGFP-expressing HEK293T cell line. The CasΦ.12 was expressed as FLAGtag-SV40NLS-Cas12j.12-NLS-T2A-PuroR. GFP expression was assessed by flow cytometry at days 5, 7 and 10. The 30 nucleotide spacer sequence is 5'-TTGCCCAGGATGTTGCCATCCTCCTTGAAA-3' (SEQ ID NO: 1415). To assess the effect of different spacer length, the spacer was shortened from its 3' end. As shown in **FIG.9B**, a spacer length of 15 to 30 nucleotides supported CasΦ.12 cleavage activity in HEK293T cells, but with less cleavage detected with the 15 and 16 nucleotide spacers. There is a preference for CasΦ.12

to have a spacer length of 17 to 22 nucleotides, but cleavage activity is still supported with the longer spacers tested.

EXAMPLE 17

CasΦ nucleases are a novel class of protein

[0459] This example illustrates that the CasΦ nucleases identified herein are a novel class of Cas proteins. SEQ ID NOs: 1 to 47 and SEQ ID NO. 105 were searched in the InterPro database, but were not identified as belonging to a class of protein. As an example, the results for SEQ ID NO: 2 are shown in **FIG.10A**. As a positive control, the Cpf1 sequence from *Acidaminococcus sp.* (strain BV3L6) was also searched and was identified as a CRISPR-associated endonuclease Cas12a family member, as shown in **FIG.10B**.

EXAMPLE 18

DNA Cleavage by CasΦ.19- CasΦ.48

[0460] This example illustrates the DNA cleavage activity of CasΦ.19 to CasΦ.45. Amino acid sequences of the proteins used in the experiment are shown in **TABLE 1**. The CasΦ polypeptides were complexed with their native crRNA (or the crRNA of the CasΦ polypeptide with the closest match based on amino acid sequence identity) to form 100nM RNP complexes at room temperature in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25 °C) for 20 minutes in a volume of 30 µl. crRNA sequences are provided in **TABLE 2**. The target plasmid was a 2.1 kb plasmid containing the target sequence **TATTAAATACTCGTATTGCT**GTTCGATTAT (SEQ ID NO: 108). The cleavage incubation was performed at 37 °C and the reaction was quenched after 60 minutes. Cleavage products where then analyzed by gel electrophoresis, as shown in **FIG.13A**. This analysis identifies CasΦ.20, CasΦ.22, CasΦ.24, CasΦ.25, CasΦ.28, CasΦ.31, CasΦ.32, CasΦ.37, CasΦ.43 and CasΦ.45 as enzymes that predominantly linearize plasmid DNA, i.e. they predominantly cleave both strands of a double strand target DNA. Whereas DNA cleavage by CasΦ.21 results in mixed nicked and linear product, indicating that CasΦ.21 functions as a nickase as well as a linearizer of plasmid DNA with a preference for nickase activity under the conditions of the present study. Mixed nicked and linearized cleavage products were also identified following cleavage by CasΦ.26, CasΦ.29, CasΦ.33, CasΦ.34, CasΦ.38 and CasΦ.44. 'SC' represents 'super-coiled' un-cut target plasmid.

[0461] This example shows robust DNA cleavage by CasΦ polypeptides.

[0462] The inventors went on to demonstrate the robust generation of indels following targeting by CasΦ.12, CasΦ.20, CasΦ.21, CasΦ.22, CasΦ.25, CasΦ.28, CasΦ.31, CasΦ.32, CasΦ.33, CasΦ.34, CasΦ.37, CasΦ.43, and CasΦ.45. A stable HEK293T cell line that expresses AcGFP was established. HEK293T-AcGFP cells were transfected with crRNA and CasΦ expression plasmids using lipofectamine on day 0. Target sequences are provided in **TABLE 6**. Cells were

harvested by trypsinization on day 3 for TIDE analysis. The target locus was amplified by PCR and the amplified product was then sequenced using Sanger sequencing. The TIDE analysis provides the frequency of indel mutations (https://tide.nki.nl/#about). As shown in **FIG.13B**, targeting CasΦ.12, CasΦ.20, CasΦ.21, CasΦ.22, CasΦ.25, CasΦ.28, CasΦ.31, CasΦ.32, CasΦ.33, CasΦ.34, CasΦ.37, CasΦ.43, and CasΦ.45 to AcGFP led to the robust generation of indel mutations. **FIG.13C** provides an alternative representation of the data shown in **FIG.13B** for CasΦ.12, CasΦ.28, CasΦ.31, CasΦ.32 and CasΦ.33. These data further demonstrate the genome editing ability of CasΦ.20, CasΦ.21, CasΦ.22, CasΦ.25, CasΦ.28, CasΦ.31, CasΦ.32, CasΦ.33, CasΦ.34, CasΦ.37, CasΦ.43, and CasΦ.45.

TABLE 6

	Target Sequence	PAM eGFP	PAM acGFP	SEQ ID NO
KT_eGFP	TTAAGGCCAAAGAACAGATT	CTTG	CTTG	1416
OT_eGFP	CGTGATGGTCTCGATTGAGT	None	None	1417
T1_eGFP	AAGAAGTCGTGCTGCTTCAT	CTTG	CTTG	1418
T2_eGFP	ATCTGCACCACCGGCAAGCT	GTTC	GTTC	1419
T3_eGFP	TGGCGGATCTTGAAGTTCAC	GTTG	GTTG	1420
T4_eGFP	CCGTAGGTGGCATCGCCCTC	GTTC	CTTC	1421
T5_eGFP	ACGTCGCCGTCCAGCTCGAC	GTTT	None	1422
T6_eGFP	AAGAAGATGGTGCGCTCCTG	CTTG	CTCG	1423

EXAMPLE 19

PAM requirement for CasΦ determined by *in vitro* enrichment

[0463] This example illustrates the NTTN PAM requirement for CasΦ.2, CasΦ.4, CasΦ.11 and CasΦ.12. An *in vitro* enrichment (IVE) analysis was performed. The CasΦ polypeptides were complexed with crRNA to form 500 nM RNP complexes at room temperature in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25 ^{o}C) for 30 minutes in a volume of 25 µl. crRNA sequences are provided in **TABLE 2**. The cleavage incubation was performed at 37 ^{o}C and the reaction was quenched after 30 minutes. The substrate for the cleavage incubation was a pooled plasmid library which includes different PAM sequences. After quenching, the cleavage reactions were cleaned using Beckman SPRi beads. The samples were sequenced to identify which PAM sequences enabled target cleavage by the CasΦ polypeptides. As shown in **FIG. 14A**, this analysis revealed an NTTN PAM requirement for CasΦ.2, CasΦ.4, CasΦ.11 and CasΦ.12.

[0464] The inventors went on to assess the PAM requirement of CasΦ.20, CasΦ.26, CasΦ.32, CasΦ.38 and CasΦ.45. An IVE analysis was performed using the protocol described above for

CasΦ.2, CasΦ.4, CasΦ.11 and CasΦ.12. As shown in **FIG.14B**, Sanger sequencing revealed a NTNN PAM requirement for CasΦ.20, a NTTG PAM requirement for CasΦ.26, a GTTN PAM requirement for CasΦ.32 and CasΦ.38, and a NTTN PAM requirement for CasΦ.45.

[0465] The inventors also determined a single-base PAM requirement for CasΦ.20, CasΦ.24 and CasΦ.25. Amino acid sequences of the proteins used are shown in **TABLE 1**. The CasΦ polypeptides were complexed with their native crRNAs to form RNP complexes at room temperature for 20 minutes. crRNA sequences are provided in **TABLE 2**. The RNP complexes were incubated with target DNA at 37°C for 60 minutes in NEB CutSmart buffer (50mM Potassium Acetate, 20mM Tris-Acetate, 10mM Magnesium Acetate, 100ug/ml BSA, pH 7.9 at 25°C). The RNPs were then used in cleavage reactions with plasmid DNA comprising a target sequence and a PAM. Stating with a TTTg PAM, the PAM was mutated to each of the sequences shown in **FIG.14C** to assess the PAM requirement. The products of the cleavage reactions were analyzed by gel electrophoresis, as seen in **FIG.14C**. **FIG.14D** provides the quantification of the gels shown in **FIG.14C**. Together, the data in **FIG.14C** and **FIG.14D** demonstrate a NTNN PAM for DNA cleavage by CasΦ.20, CasΦ.24 and CasΦ.25.

[0466] This example demonstrates PAM sequences that enable CasΦ polypeptides to be targeted to a target sequence.

EXAMPLE 20

CasΦ-mediated genome editing in HEK293T cells

[0467] This example illustrates the ability of CasΦ polypeptides to mediate genome editing in HEK293T cells, a cell line which is widely used in biological research. In this study, a CasΦ.12 plasmid, including both CasΦ polypeptide sequence and gRNA sequence, sometimes called an all-in-one, was delivered via lipofection. Spacers targeted exon 4 of the *Fut8* gene. The spacer sequences are provided in **TABLE 7**. Cells were transfected on day 0 and harvested for analysis on day 5. As shown in **FIG.15**, the target locus was modified following delivery of CasΦ.12 and gRNA 2. Cas9 was delivered to HEK293T cells to provide a positive control and no modification was detected when a non-targeting (NT) gRNA was used. The presence of indels was confirmed by next generation sequence analysis. The sample targeted by CasΦ.12 and gRNA 2 is shown in **FIG.15**. The next generation sequence analysis revealed a diverse pattern of indels. The most frequent mutations were deletion mutations of 4 to 18 base pairs. The frequency of mutations was quantified and is illustrated as "% modified", which is defined as the % of modification in the DNA sequence when aligned to unedited cells. Modifications can be deletions, insertions and substitutions.

[0468] This example demonstrates the use of CasΦ.12 as a robust genome editing tool.

TABLE 7

Name	Target	Spacer sequence (5' --> 3') [SEQ ID NO]
Fut8_1	CasPhi target	GAAGAGCAGCTTGTTAAGGC (SEQ ID NO: 1424)
Fut8_2	CasPhi target	GCCTTAACAAGCTGCTCTTC (SEQ ID NO: 1425)
Fut8_3	Cas9 target (control)	ATTGATCAGGGGCCAgctat (SEQ ID NO: 1426)
Fut8_4	Cas9 target (control)	Acgcgtactcttcctatagc (SEQ ID NO: 1427)
NT	Non target	CGTGATGGTCTCGATTGAGT (SEQ ID NO: 1428)

EXAMPLE 21

CasΦ-mediated genome editing in CHO cells

[0469] This example illustrates the ability of CasΦ polypeptides to mediate genome editing in CHO cells, an epithelial cell line which is frequently used in biological and medical research. To test the function of CasΦ.12 in CHO cells, 40 pmol CasΦ.12 was complexed to its native crRNA (2.5:1 crRNA:CasΦ). To prepare a mastermix of CasΦ.12 RNP, 3 µl crRNA (at 100 nM) was added to 1.6 µl CasΦ.12 (at 75 µM). Spacer sequences are provided in **Table 8**. The RNP complexes were incubated at 37°C for 30 minutes. CHO cells were resuspended at 1.2 x10^6 cells/ml in SF solution (Lonza). 40 µl of the cell suspension was added to the RNP complexes and 20 µl of the resultant suspension was then transferred to individual tubes for nucleofection. Lonza setting FF-137 was used to nucleofect the CHO cells. Cells were then harvested for analysis on day 5. As shown in **FIG.16A**, CasΦ.12 induced the generation of indels in each of the endogenous genes tested (*Bak1, Bax* and *Fut8*). The ability of CasΦ.12 to induce indel mutations in each of these genes is further shown in **FIG.16F** for *Bak1*, **FIG.16G** for *Bax* and **FIG.16H** for *Fut8*. Spacer sequences for **FIG.16F**, **FIG.16G** and **FIG.16H** are provided in Tables F, G, and H, respectively. The data shown in **FIG.16F-H** were produced with 200,000 CHO cells per transfection, RNP complexed with 250 pmol of CasΦ.12, and full-length unmodified guide RNA in molar excess relative to CasΦ.12, using the same Lonza reagents described for producing data presented in **FIGS.16A-E**.

TABLE 8

Name	Spacer sequence (5' --> 3')	Repeat+Spacer sequence (5' --> 3'), shown as DNA
Bak1_1	GAAGCTATGTTTTCCATCTC (SEQ ID NO: 443)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAGCTATGTTTTCCATCTC (SEQ ID NO: 1197)
Bak1_2	GCAGGGGCAGCCGCCCCCTG (SEQ ID NO: 444)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGCAGGGGCAGCCGCCCCCTG (SEQ ID NO: 1198)

Bak1_3	CTCCTAGAACCCAACAGGTA (SEQ ID NO: 445)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCCTAGAACCCAACAGGTA (SEQ ID NO: 1199)
Bak1_4	GAAAGACCTCCTCTGTGTCC (SEQ ID NO: 446)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAAAGACCTCCTCTGTGTCC (SEQ ID NO: 1200)
Bak1_5	TCCATCTCGGGGTTGGCAGG (SEQ ID NO: 447)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCATCTCGGGGTTGGCAGG (SEQ ID NO: 1201)
Bak1_6	TTCCTGATGGTGGAGATGGA (SEQ ID NO: 448)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCTGATGGTGGAGATGGA (SEQ ID NO: 1202)
Bax_1	CTAATGTGGATACTAACTCC (SEQ ID NO: 479)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTAATGTGGATACTAACTCC (SEQ ID NO: 1269)
Bax_2	TTCCGTGTGGCAGCTGACAT (SEQ ID NO: 480)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTCCGTGTGGCAGCTGACAT (SEQ ID NO: 1270)
Bax_3	CTGATGGCAACTTCAACTGG (SEQ ID NO: 481)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGATGGCAACTTCAACTGG (SEQ ID NO: 1271)
Bax_4	TACTTTGCTAGCAAACTGGT (SEQ ID NO: 482)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTACTTTGCTAGCAAACTGGT (SEQ ID NO: 1272)
Bax_5	AGCACCAGTTTGCTAGCAAA (SEQ ID NO: 483)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGCACCAGTTTGCTAGCAAA (SEQ ID NO: 1273)
Bax_6	AACTGGGGCCGGGTTGTTGC (SEQ ID NO: 484)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAACTGGGGCCGGGTTGTTGC (SEQ ID NO: 1274)
Fut8_1	CCACTTTGTCAGTGCGTCTG (SEQ ID NO: 507)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCACTTTGTCAGTGCGTCTG (SEQ ID NO: 1325)
Fut8_2	CTCAATGGGATGGAAGGCTG (SEQ ID NO: 508)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTCAATGGGATGGAAGGCTG (SEQ ID NO: 1326)
Fut8_3	AGGAATACATGGTACACGTT (SEQ ID NO: 509)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAGGAATACATGGTACACGTT (SEQ ID NO: 1327)
Fut8_4	AAGAACATTTTCAGCTTCTC (SEQ ID NO: 510)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACAAGAACATTTTCAGCTTCTC (SEQ ID NO: 1328)
Fut8_5	ATCCACTTTCATTCTGCGTT (SEQ ID NO: 511)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATCCACTTTCATTCTGCGTT (SEQ ID NO: 1329)

Fut8_6	TTTGTTAAAGGAGGCA AAGA (SEQ ID NO: 512)	CTTTCAAGACTAATAGATTGCTCCTTACGA GGAGACTTTGTTAAAGGAGGCAAAGA (SEQ ID NO: 1330)

[0470] The inventors went on to demonstrate the ability of CasΦ.12 to mediate gene editing via the homology directed repair pathway. The inventors tested DNA donor oligos with 25 bp, 50 bp or 90 bp homology arms (HA), as shown in **FIG.16B**. The donor oligos were delivered to CHO cells with or without CasΦ.12 and crRNA. As seen in **FIG.16C**, indels were not detected in the absence of CasΦ.12. Whereas, indels were detected in the presence of CasΦ.12 and confirmed by sequencing the endogenous targeted locus (**FIG.16D**). The sequencing analysis also showed the successful incorporation of a DNA donor oligo into the endogenous targeted locus (**FIG.16E**).

[0471] The inventors further demonstrated the ability of CasΦ.12 to mediate gene editing of *Bax* and *Fut8* genes via the homology directed repair pathway. In this additional study, DNA donor oligos with 20 bp, 25 bp, 30 bp or 40 bp 90 bp HA were used, shown in **FIG.16I**. These DNA donor oligos were either unmodified or modified with phosphorothioate (PS) bonds between the first 5', and the last two 3' bases. As shown in **FIG.16J**, CasΦ.12 mediated successful incorporation of a DNA donor oligo into the endogenous targeted locus. Finally, the inventors further optimized CasΦ.12-mediated genome editing of *Fut8* using AAV6 delivery of the DNA donor. In this study, CHO cells were transfected with *Fut8*-targeting RNP (500 pmol) using Lonza nucleofection protocols. AAV6 donors at different MOIs were added to cells immediately after transfection. The frequency of indels and HDR was analyzed by NGS. As shown in **FIG.16K** and **FIG.16L,** CasΦ.12 induced the generation of indels and HDR.

[0472] These data further demonstrate the utility of CasΦ polypeptides as a genome editing tool.

EXAMPLE 22

CasΦ-mediated genome editing in K562 cells

[0473] This example illustrates the ability of CasΦ polypeptides to mediate genome editing in K562 cells, a myelogenous leukemia cell line which is particularly useful for biological and medical research by virtue of its amenability for nucleofection by electroporation. In this study, K562 cells were nucleofected with Cas9 or CasΦ.12. To nucleofect the cells, 150,000 cells in SF solution (SF Cell Line 96 Amaxa) were added to the amount of plasmid (expressing the gRNA targeting the Fut8 gene and either Cas9 or CasΦ.12) indicated in **FIG.17**. Amaxa program 96-FF-120 was used to nucleofect the cells. The cells were harvested two days after nucleofection and the frequency of indel mutations was determined. As shown in **FIG.17**, as the amount of CasΦ.12 plasmid increased, the amount of indels detected in the endogenous *Fut8* gene also increased.

EXAMPLE 23

CasΦ-mediated genome editing in primary cells

[0474] This example illustrates the ability of CasΦ polypeptides to mediate genome editing in primary cells, such as T cells. In this study, CasΦ.12 was delivered to human T cells. CasΦ.12 was complexed to its native crRNA comprising the spacer sequence 5'-GGGCCGAGAUGUCUCGCUCC-3' (SEQ ID NO: 1429). Complexes were formed in a 3:1 ratio of crRNA:protein. For nucleofection, 50 pmol RNP was mixed with 320,000 cells per well and the Amaxa EH115 program was used. Immediately after nucleofection, 80 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 15 minutes before transfer to the culture plate. Genomic DNA was extracted from cells on day 3 and day 5. Flow cytometry analysis was performed on day 5. As shown in **FIG. 18A**, when CasΦ.12 was delivered with a gRNA targeting the endogenous beta-2 microglobulin (B2M) gene, a distinct population of B2M-negative cells was detected by flow cytometry analysis demonstrating the CasΦ.12-mediated knockout of the endogenous B2M gene. In the absence of the B2M-targeting gRNA, the population of B2M-negative cells was not observed by flow cytometry. Indels were confirmed by next generation sequencing analysis, as shown **FIG.18C**, and quantified, as shown in **FIG.18B**.

[0475] The inventors went on to use CasΦ.12 to target the T-cell receptor alpha-constant (TRAC) gene. Knockout of the *TRAC* gene prevents expression of the T cell receptor. Accordingly, TRAC knockout T cells are beneficial for T cell therapies (e.g. CAR-T cell therapies) because TRAC knockout T cells have a longer half-life *in vivo* as the T cells have less potential to attack the recipient's normal cells. In this study, CasΦ.12 and gRNA targeting the *TRAC* gene (CasPhi1 or CasPhi7) were delivered to T cells. As shown in **FIG.18D**, the delivery of the CasΦ.12 and the gRNA resulted in a population of TRAC-negative cells, which were detected by flow cytometry. The inventors went on to confirm the presence of indel mutations by sequencing the target locus. As shown in **FIG.18E**, the sequence analysis revealed insertion, deletion and substitution mutations at the endogenous targeted locus. The frequency of indel mutations was quantified, as shown in **FIG.18F**.

[0476] These data demonstrate the utility of CasΦ polypeptides as a robust genome editing tool in primary human cells.

EXAMPLE 24

Separable DNA strand cleavage reactions of CasΦ nucleases

[0477] This example further illustrates the mechanism of DNA strand cleavage by CasΦ polypeptides. In this study, CasΦ.4, CasΦ.12 and CasΦ.18 were complexed with their native crRNA. RNP complexes were formed by a 20 minute incubation at room temperature. The target plasmid was a 2.1 kb plasmid containing the target sequence

TATTAAATACTCGTATTGCTGTTCGATTAT (SEQ ID NO: 108). The cleavage reaction was carried out at 37 °C and had a duration of 30 minutes. The cleavage products were then analyzed by gel electrophoresis. As shown in **FIG.19**, CasΦ polypeptides nick supercoiled (sc) DNA by cleaving the non-target DNA strand. Some CasΦ polypeptides, such as CasΦ.4 and CasΦ.12, then go on to cleave the second (target) strand to generate a linear product from a plasmid target. Whereas some CasΦ polypeptides, such as CasΦ.18, function as nickases and do not go on to cleave the second strand. CasΦ cleavage activity is dependent on metal cations, such as Mg^{2+}. Varying the concentration of Mg^{2+} allows the cleavage of the first strand and then second strand by CasΦ.4 and CasΦ.12 to be visualized. As the concentration of Mg^{2+} increases, the amount of linearized product detected increases indicating that the second strand has been cleaved in the CasΦ.4 and CasΦ.12 reactions.

EXAMPLE 25

Detection of a target nucleic acid by CasΦ polypeptides

[0478] This example illustrates the use of CasΦ.4 and CasΦ.18 in a nucleic acid detection assay by virtue of trans cleavage activity of ssDNA. In this study, 100 nM RNP was prepared and used in a detection assay. In the detection assay, the target dsDNA was at a concentration of 10 nM and the ssDNA reporter molecule was at a concentration of 100nM. The target dsDNA included 5 target sequences, which were targeted by a pool of 5 gRNAs) with 7 base pairs flanking the 20 nucleotide target sequences on both 5' and 3' sides, as shown in **FIG.20**. The detection assay was carried out at 37 °C. The buffer conditions provided in **TABLE 9** were tested in the detection assay. All buffers were supplemented with 0.1 mg/ml BSA and 1 mM TCEP. As seen in **FIG.20**, when a gRNA (complexed to a CasΦ polypeptide) hybridizes to a target nucleic acid, the CasΦ's trans cleavage activity is activated such that a labeled ssDNA reporter is degraded. The degradation of the ssDNA reporter is detected as fluorescence thus allowing CasΦ polypeptides to be used in assays to achieve fast and high-fidelity detection of target nucleic acid molecules in a sample. As shown in **FIG.20**, high pH (e.g. 8-9) and high Mg^{2+} concentration (e.g. 12-15 mM) provided preferred conditions for the detection assay.

TABLE 9

buffer ID #	pH	1X NaCl (mM)	1X $MgCl_2$ (mM)
1	9	150	15
2	9	150	3
3	7.5	0	3
4	9	0	3
5	9	0	15
6	7.5	150	3
7	7.5	150	15

8	8	37.5	3
9	8.5	150	12
10	7.5	0	15
11	8.5	0	6
12	9	150	3
13	9	0	3
14	9	150	15
15	8	150	6
16	7.5	150	15
17	8	112.5	15
18	9	0	15
19	7.5	150	3
20	8.5	112.5	3
21	8.5	37.5	12
22	7.5	0	3
23	8.5	112.5	6
24	7.5	37.5	6
25	8	0	12
26	7.5	112.5	6
27	8.5	37.5	15
28	9	37.5	6
29	9	112.5	12
30	7.5	37.5	12
31	7.5	0	15
32	7.5	112.5	12

[0479] These data demonstrate the utility of CasΦ polypeptides in nucleic acid detection assays.

EXAMPLE 26

High efficiency of CasΦ polypeptide-mediated genome editing in primary cells

[0480] The present example shows that CasΦ.12 mediates high genome editing efficiency that is comparable the editing efficiency mediated by Cas9. Results of the study are shown in **FIG.21.** In this study, CasΦ.12 mRNA (SEQ ID NO: 107) with a gRNA (CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC**GGGCCGAGAUGUCUCGCUCC** (SEQ ID NO: 1430)); spacer sequence is bold and underlined) or Cas9 mRNA with a gRNA (GGCCGAGATGTCTCGCTCCG (SEQ ID NO: 1431)) was delivered to T cells. gRNAs used in this study targeted the B2M gene. For nucleofection, T cells were resuspended in BTXpress electroporation medium (5 x 10^5 cells per well) and mixed with CasΦ.12 or Cas9 mRNA and

500 pmol gRNA. Cells were collected on day 2 for extraction of genomic DNA, and the frequency of indel mutations was determined. As shown in **FIG.21A,** when 20 μg of CasΦ.12 mRNA was delivered with gRNA to T cells, high genome editing efficiency was achieved, and this was at a similar level to of genome editing achieved using Cas9. Cells were also collected on Day 2 for flow cytometry to determine the frequency of B2M knockout. As shown in **FIG.21B** and quantified in **FIG.21A,** a similar percentage of B2M-negative cells were detected after delivery of CasΦ.12 or Cas9 mRNA. Accordingly, this example demonstrates high efficiency of CasΦ polypeptide-mediated genome efficiency in primary cells.

EXAMPLE 27

CasΦ polypeptide-mediated genome editing in CHO cells

[0481] This present example describes the identification of optimized gRNAs for CasΦ.12-mediated genome editing in CHO cells. In this study, CasΦ.12 polypeptides (SEQ ID NO: 107) were complexed with a gRNA shown in **TABLE 10.** CHO cells were resuspended in SF solution and Lonza setting FF-137 was used to nucleofect the cells (200,000 cells per well) with 250 pmol RNP. Genomic DNA was extracted and the presence of indels was confirmed by next generation sequence analysis. **FIG.22A** shows the frequency of indel mutations induced by CasΦ.12 polypeptides complexed with a 2'fluoro modified gRNA. As shown in **FIG.22B,** gRNAs with ~20% or greater editing efficiency were identified.

TABLE 10

Name	**Spacer sequence (5' --> 3')**	**RNA sequence (5' --> 3'), shown as DNA**
R2849_Bak1_nsd_sg1	CTGACTCCCAGCTCTGACCC (SEQ ID NO:449)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTGACTCCCAGCTCTGACCC (SEQ ID NO: 1203)
R2855_Bak1_nsd_sg7	CCATCTCCACCATCAGGAAC (SEQ ID NO:455)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCATCTCCACCATCAGGAAC (SEQ ID NO: 1209)
R3977 Bak1_exon1_sg1	TCCAGACGCCATCTTTCAGG (SEQ ID NO:465)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTCCAGACGCCATCTTTCAGG (SEQ ID NO: 1219)
R3978 Bak1_exon1_sg2	TGGTAAGAGTCCTCCTGCCC (SEQ ID NO:466)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGTAAGAGTCCTCCTGCCC (SEQ ID NO: 1220)

R3979 Bak1_exon3_sg1	TTACAGCATCTTGGGTCAGG (SEQ ID NO:467)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTTACAGCATCTTGGGTCAGG (SEQ ID NO: 1221)
R3980 Bak1_exon3_sg2	GGTCAGGTGGGCCGGCAGCT (SEQ ID NO:468)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGGTCAGGTGGGCCGGCAGCT (SEQ ID NO: 1222)
R3981 Bak1_exon3_sg3	CTATCATTGGAGATGACATT (SEQ ID NO:469)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCTATCATTGGAGATGACATT (SEQ ID NO: 1223)
R3982 Bak1_exon3_sg4	GAGATGACATTAACCGGAGA (SEQ ID NO:470)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGAGATGACATTAACCGGAGA (SEQ ID NO: 1224)
R3983 Bak1_exon3_sg5	TGGAACTCTGTGTCGTATCT (SEQ ID NO:471)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACTGGAACTCTGTGTCGTATCT (SEQ ID NO: 1225)
R3984 Bak1_exon3_sg6	CAGAATTTACTGGAGCAGCT (SEQ ID NO:472)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCAGAATTTACTGGAGCAGCT (SEQ ID NO: 1226)
R3985 Bak1_exon3_sg7	ACTGGAGCAGCTGCAGCCCA (SEQ ID NO:473)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACACTGGAGCAGCTGCAGCCCA (SEQ ID NO: 1227)
R3986 Bak1_exon3_sg8	CCAGCTGTGGGCTGCAGCTG (SEQ ID NO:474)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACCCAGCTGTGGGCTGCAGCTG (SEQ ID NO: 1228)
R3987 Bak1_exon3_sg9	GTAGGCATTCCCAGCTGTGG (SEQ ID NO:475)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTAGGCATTCCCAGCTGTGG (SEQ ID NO: 1229)
R3988 Bak1_exon3_sg10	GTGAAGAGTTCGTAGGCATT (SEQ ID NO:476)	CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACGTGAAGAGTTCGTAGGCATT (SEQ ID NO: 1230)

R3989 Bak1_exon3_sg11	ACCAAGATTGCCTCCAG GTA (SEQ ID NO:477)	CTTTCAAGACTAATAGATTGCTCC TTACGAGGAGACACCAAGATTGC CTCCAGGTA (SEQ ID NO: 1231)
R3990 Bak1_exon3_sg12	CCTCCAGGTACCCACCA CCA (SEQ ID NO:478)	CTTTCAAGACTAATAGATTGCTCC TTACGAGGAGACCCTCCAGGTAC CCACCACCA (SEQ ID NO: 1232)

EXAMPLE 28

Minimal off-target effects of CasΦ polypeptides

[0482] This example illustrates the off-target profiles of CasΦ.12 and Cas9. A major challenge in the translation of CRISPR/Cas9 technology into the clinic has been overcoming off-target effects. Off-target effects arise where a gRNA tolerates mismatches in complementarity of the gRNA and target sequence, and so the gRNA hybridizes to a sequence that is not the target sequence. Off-target effects are a source of major concern as it is important to avoid the production in unnecessary mutations that could be detrimental. In this study, CIRCLE-seq was performed to detect off-target sites (Tsai *et al.* 2017 *Nature Methods*). Sequencing was performed on genomic DNA extracted from CHO cells that had been transfected with CasΦ.12 polypeptide (SEQ ID NO: 107) and a gRNA targeting Fut8, CasΦ.12 polypeptide and a gRNA targeting BAX or Cas9 polypeptide and a gRNA targeting BAX. As shown in **FIG.23A**, Casϕ.12 targeting Fut8 induced minimal off-target mutations. **FIG.23D** shows the off-target mutations induced by Cas9 editing of Fut8. Similarly, Casϕ.12 targeting BAX induced minimal off-target mutations, as shown in **FIG.23B.** Cas9 targeting BAX induced a higher percentage of off-targets mutations, as shown in **FIG.23C,** compared to Casϕ.12. Cas9 targeting Bak1 also induced a higher percentage of off-targets mutations, as shown in **FIG.23E,** compared to Casϕ.12, as shown in **FIG.23F.**

[0483] In a further study, GUIDE-Seq was performed to detect off-target sites (Tsai *et al.* 2015 *Nature Biotechnology*). Sequencing was performed on genomic DNA extracted from HEK293 cells following delivery of either CasΦ.12 polypeptide or Cas9 polypeptide and a gRNA targeting human Fut8. As shown in **FIG.23G**, no off target mutations were detected in the CasΦ.12 polypeptide sample. Whereas, several off-target mutations were detected in Cas9 polypeptide sample, as shown in **FIG.23H**. Accordingly, this example demonstrates that CasΦ polypeptides have fewer off-target effects than Cas9.

EXAMPLE 29

CasΦ polypeptide-mediated genome editing via homology directed repair (HDR)

[0484] The present example illustrates the ability of that CasΦ.12 to mediate HDR. In this study, CasΦ.12 polypeptide (SEQ ID NO: 107) was complexed with a gRNA (CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGAGUCUCUCAGCUGGUAC AC (SEQ ID NO: 1432)) targeting the TRAC gene and delivered to T cells. RNP complexes were formed by a 10 minute incubation at room temperature. T cells were resuspended at 5 x 10^5 cells/20 µL in electroporation solution (Lonza). T cells were nucleofected using the Amaxa P3 kit and Amaxa 4D Nucleofector with pulse code EH115. Immediately after nucleofection, 80 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 10 minutes before transfer to the culture plate. Cells were harvested and genomic DNA was extracted. The frequency of indel mutations HDR was determined and shown in **FIG.24A**. The frequency of indel mutations and HDR was combined to determine the frequency of modification. Flow cytometry was also performed to determine the frequency of TRAC knockout, as assessed by the loss of CD3 at the cell surface. **FIG.24A** shows CasΦ.12-mediated gene editing via the HDR pathway. **FIG.24B** shows a schematic of the donor oligonucleotide. Thus, this example demonstrates the use of CasΦ polypeptides as robust genome editing tools.

EXAMPLE 30

Multiplex genome editing with CasΦ polypeptides

[0485] This example illustrates the ability of CasΦ RNP complexes to target multiple genes simultaneously. In this study, gRNAs targeting B2M or TRAC were incubated with CasΦ.12 polypeptides (SEQ ID NO: 107) for 10 minutes at room temperature to form RNP complexes. RNP complexes were formed with a variety of gRNAs with different modifications (unmodified, 2'-O-methyl on the last 3' nucleotide of the crRNA (1me), 2'-O-methyl on the last two 3' nucleotides of the crRNA (2me) and 2'-O-methyl on the last three 3' nucleotides of the crRNA(3me)) and with different repeat and spacer sequences (20-20, which corresponds to 20 nucleotide repeat and 20 nucleotide spacer, and 20-17, which corresponds to 20 nucleotide repeat and 17 nucleotide spacer), as shown in **TABLE 11**. B2M targeting RNPs, TRAC targeting RNPs or B2M targeting RNPs and TRAC targeting RNPs were added to T cells. T cells were resuspended at 5 x 10^5 cells/20 µL in Nucleofection P3 solution and an Amaxa 4D 96-well electroporation system with pulse code EH115 was used to nucleofect the cells. Immediately after nucleofection, 85 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 10 minutes before transfer to the culture plate. On Day 3, genomic DNA was extracted. On Day 5, cells were harvested for flow cytometry. Quantification of the percentage of B2M-negative and CD3-negative cells is shown in **FIG. 25A** for gRNAs with a repeat length of 20 nucleotides and a spacer length of 20 nucleotides, and in **FIG. 25B** for gRNAs with a repeat length of 20 nucleotides and a spacer length of 17 nucleotides.

Corresponding flow cytometry panels can be seen in **FIG.25C** for gRNAs of different repeat and spacer lengths and with different modifications.

[0486] In a further study, RNP complexes were formed using CasΦ.12 and modified gRNAs (unmodified, 1me, 2me, 3me, 2'-fluoro on the last 3' nucleotide of the crRNA (1F), 2'-fluoro on the last two 3' nucleotides of the crRNA (2F) and 2'-fluoro on the last three 3' nucleotides of the crRNA (3F)) with different lengths of spacer sequences (20-20 and 20-17 as above) that target TRAC. T cells were nucleofected with RNP complexes (125 pmol) using the P3 primary cell nucleofection kit and an Amaxa 4D 96-well electroporation system with pulse code EH115. As shown in **FIG.25D**, ~90% editing efficiency was achieved using CasΦ.12 and modified gRNAs. **FIG.25E** shows a flow cytometry plot illustrating ~90% TRAC knockout in T cells after delivery of CasΦ.12 and modified gRNAs. This data further demonstrates the ability of CasΦ to mediate high efficiency genome editing.

TABLE 11

Name	**Target**	**Modification**	**Repeat sequence** (5' --> 3')	**Spacer sequence** (5' --> 3')	**crRNA sequence** (5' --> 3')
R3150 20-20	B2M Exon 2	Unmodified, 2'OMe at last 3' base (1me) 2'OMe at last two 3' bases (2me) 2'OMe at last three 3' bases (3me)	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCAG UG (SEQ ID NO: 1434)	AUUGCUCCUUAC GAGGAGACCAG UGGGGGUGAAU UCAGUG (SEQ ID NO: 1435)
R3042 20-20	TRAC Exon 1	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	GAGUCUCUC AGCUGGUAC AC (SEQ ID NO: 1436)	AUUGCUCCUUAC GAGGAGACGAG UCUCUCAGCUGG UACAC (SEQ ID NO: 1437)
R3150 20-17	B2M Exon 2	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCA (SEQ ID NO: 1438)	AUUGCUCCUUAC GAGGAGACCAG UGGGGGUGAAU UCA (SEQ ID NO: 1439)

R3042 20-17	TRAC Exon 1	Unmodified, 1me 2me 3me	AUUGCUC CUUACGA GGAGAC (SEQ ID NO: 1433)	CAGUGGGGG UGAAUUCA (SEQ ID NO: 1440)	AUUGCUCCUUAC GAGGAGACGAG UCUCUCAGCUGG UA (SEQ ID NO: 1441)

EXAMPLE 31

CasΦ polypeptides have an extended seed region

[0487] The present example shows that CasΦ.12 has an extended seed region compared to Cas9 and does not tolerate mismatches in the complementarity of the spacer and target sequences within the first 1-16 nucleotides from the 5' of the spacer sequence. In this study, CasΦ.12 (SEQ ID NO: 107) was complexed with a gRNA targeting TRAC gene and delivered to T cells. Spacer sequences contained a single mismatch at the position indicated in **FIG.26A** or a mismatch at each of the two positions indicated in **FIG.26B**. Mismatches were generated by substituting a purine for a purine (i.e. A to G and vice versa) and a pyrimidine for a pyrimidine (*i.e.* U to C and *vice versa*). RNP complexes were formed by a 10 minute incubation at room temperature. T cells were resuspended at 5 x 10^5 cells/20 µL in electroporation solution (Lonza). Amaxa P3 kit and Amaxa 4D Nucleofector was used to nucleofect the T cells. Immediately after nucleofection, 80 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 10 minutes before transfer to the culture plate. Cells were harvested for extraction of genomic DNA to determine the frequency of indel mutations and for flow cytometry to determine the percentage of CD3 knockout cells. As shown in **FIG.26A**, no indel mutations or CD3 knockout were detected when there was a single mismatch in the complementarity of the spacer and target sequences at positions 1-16 from the 5' end of the spacer sequence. Similarly, no indels or CD3 knockout cells were detected when there was a double mismatch in the complementarity of the spacer and target sequences at positions 1-16 from the 5' end of the spacer sequence as shown in **FIG.26B.** The data shown in **FIG.26A** and **FIG.26B** demonstrate that CasΦ polypeptides do not tolerate mismatches in complementarity between the spacer sequence and target sequence in the 5' 16 positions of the spacer. This region in which mismatches are not tolerated is known as the "seed region". Thus the seed region of CasΦ.12 is the first 16 bases from the 5' end of the spacer. In contrast, the seed region of Cas9 is much shorter and is reported to be only 5 nucleotides long (Wu *et al., Quant Biol.* 2014 Jun; 2(2): 59-70). Shorter seed regions result in increased likelihood of off-target effects because the likelihood of mismatches between the spacer and target occurring outside the seed region is increased. Accordingly, longer seed regions result in a reduced likelihood of off-target effects. The long seed region of CasΦ.12 is therefore advantageous over the short seed region of Cas9 and contributes to the reduced off-target effects of CasΦ.12. **FIG. 26C** and **FIG. 26D** provide schematics of the gRNAs with mismatches.

EXAMPLE 32

Use of modified guide RNAs with CasΦ polypeptides

[0488] This example illustrates the ability of CasΦ.12 to mediate genome editing in CHO cells with modified gRNAs. In this study, RNP complexes were formed using CasΦ.12 polypeptide (SEQ ID NO: 107) and a modified gRNA shown in **TABLE 12**. For nucleofection, 200 pmol RNP was mixed with 200,000 cells per well. CHO cells were resuspended in SF solution and Lonza setting FF-137 was used to nucleofect the cells. Genomic DNA was extracted 48 hours after transfection and the frequency of indel mutations was determined. As shown in **FIG.27A**, several modified gRNAs with editing efficiency of ~10% were identified. In a further study, additional modified gRNAs were tested. As shown in **FIG.27B**, modified gRNAs with editing efficiency of up to 40-50% were identified.

[0489] gRNAs with phosphorothioate (PS) backbone modifications, 2'-fluoro (2'-F) and 2'-O-Methyl (2'OMe) sugar modifications are known to increase metabolic stability and binding affinity to RNA, and replacing RNA nucleotides with DNA generates gRNAs with highly efficient gene-editing activity compared to the natural crRNA (Rahdar *et al,* 2015, PNA; McMahon *et al.* 2017, Molecular Therapy Vol. 26 No 5).

TABLE 12

SEQ ID NO.	Name (FIG. 27A)	Modification	Position	Full modified guide (repeat and spacer)	Name (FIG.27A,B)
1442	R2466_Mo1	2'-O-Methyl (2'OMe), 3' phosphorothioate (PS) bonds	2'OMe at 3 first (5') and last (3') bases, 3' PS bonds between first 3 (5') and last 2 (3') bases	mC*mU*mU*UCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACmG*mU*mU*	Synthego_Mod
1443	R2466_Mo2	2'OMe, 3', 25 nucleotide repeat	2'OMe at 3 first (5') and last (3') bases, 3' PS bonds between first 3 (5') and last 2 (3') bases	mA*mA*mU*AGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACmG*mU*mU	

1444	R2466_Mo3	2'-O-methoxy-ethyl bases	2'-O-Methoxy-ethyl bases at 2 first (5') and last (3') bases, 3' PS bonds between first 2 (5') and last 2 (3') bases	**/52MOErA*/i2MOErA*/**UAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACG**/i2MOErT/32MOErT**	
1445	R2466_Mo4	2'-Fluoro (2'-F)	First (5') and last (3') base	/52FC/UUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGU/32FU/	
1446	R2466_Mo5	2'-F, 25 nucleotide repeat	First (5') and last (3') base	/52FA/AUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGU/32FU/	1F, 45F (25nt R)
1447	R2466_Mo6	2'-F, PS, 2'OMe	First (5') base 2'OMe, PS between first two(5') bases, last 4 (3') bases 2'-F	**mC***U*UUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA/i2FC/i2FG/i2FU/32FU/	1, 2 OMe-PS, 54, 55, 56 'F
1448	R2466_Mo7	2'-F, PS, 2'OMe, 25 nucleotide repeat	First (5') base 2'OMe, PS between first two(5') bases, last 4 (3')bases 2'-F	mA*A*UAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA/i2FC/i2FG/i2FU/32FU	1, 2 OMe-PS, 54, 55, 56 'F (25nt R)
1449	R2466_Mo8	2'-F	Last 4 (3') bases 2'-F	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA/i2FC/i2FG/i2FU/32FU	54, 55, 56 2'F
1450	R2466_Mo9	2'-F, 25 nucleotide repeat	Last 4 (3') bases 2'-F	AAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA/i2FC/i2FG/i2FU/32FU	54, 55, 56 2'F (25 nt R)

1451	R2466_Mo10	C3 Spacer, 21 nucleotide spacer	First (5') and last (3') base	**C**UUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU**G**	
1452	R2466_Mo11	C3 Spacer, 21 nucleotide spacer, 25 nucleotide spacer	First (5') and last (3') base	**A**AUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU**G**	
1453	R2466_Mo12	DNA bases + 2'OMe, PS	2'OMe at 3 first(5') bases, last 4(3') bases DNA	mC*mU*mU*UCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA**CGTT**	1, 2, 3 Ome-PS 54, 55, 56 DNA
1454	R2466_Mo13	DNA nucleoside	Last (3') 4 nucleoside	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACA**CGTT**	
1455	R2466_Mo14	DNA nucleosides	Nucleoside 1 of spacer and last (3') 4 nucleosides	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC**A**GGAAUACAUGGUACA**CGTT**	1, 54, 55, 56 DNA
1456	R2466_Mo15	DNA nucleosides	Nucleoside 8 of spacer and last (3') 4 nucleosides	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUA**C**AUGGUACA**CGTT**	
1457	R2466_Mo16	DNA nucleosides	Nucleoside 9 of spacer and last (3') 4 nucleosides	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUAC**A**UGGUACA**CGTT**	
1458	R2466_Mo17	DNA nucleosides	Nucleoside 1 and 8 of spacer and	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG	1, 8, 54, 55, 56 DNA

			last (3') 4 nucleosides	AC**A**GGAAUA**C**AUGGUAC A**CGTT**	
1459	R2466_Mo18	DNA nucleosides	Nucleoside 1 and 9 of spacer and last (3') 4 nucleosides	CUUUCAAGACUAAUAGA UUGCUCCUUACGAGGAG AC**A**GGAAUAC**A**UGGUAC A**CGTT**	
1460	R2466_Mo19	DNA nucleosides	Nucleoside 1, 8 and 9 of spacer and last (3') 4 nucleosides	CUUUCAAGACUAAUAGA UUGCUCCUUACGAGGAG AC**A**GGAAUA**CA**UGGUAC A**CGTT**	1, 8, 9, 54, 55, 56 DNA
1461	R2466_Mo20	DNA bases, 25 nucleotide repeat	Nucleoside 1, 8 and 9 of spacer and last (3') 4 nucleosides	AAUAGAUUGCUCCUUAC GAGGAGAC**A**GGAAUA**CA** UGGUACA**CGTT**	
1462	R2466_Mo21	Poly-A-tail, 25 nucleotide repeat		AAUAGAUUGCUCCUUAC GAGGAGACAGGAAUACA UGGUACACGUUAAAAAA A	
1463	R2466_Mo22	DNA bases, 2'OMe, PS	2'OMe and PS at first 3(5') bases, DNA bases at 1,8 and 9 of spacer, PS at last 4 (3') bases	mC*mU*mU*UCAAGACUA AUAGAUUGCUCCUUACG AGGAGAC**A**GGAAUA**CA**U GGUACA**CGTT**	1,2,3 OMe, 1, 8, 9, 54, 55, 56 DNA
1464	R2466_Mo23	Unmodified, 25 nucleotide repeat		AAUAGAUUGCUCCUUAC GAGGAGACAGGAAUACA UGGUACACGUU	
1465	R2466 (Unmodified)	Unmodified	Unmodified	CUUUCAAGACUAAUAGA UUGCUCCUUACGAGGAG ACAGGAAUACAUGGUAC ACGUU	

EXAMPLE 33

Optimization of guide RNA repeat and spacer length in CHO cells

[0490] This example describes the optimization of repeat and spacer lengths of gRNAs for genome editing in CHO cells. In this study, RNP complexes were formed by incubating CasΦ.12 polypeptides (SEQ ID NO: 107) with a gRNA targeting Fut8 gene shown in **TABLE 13**. The gRNAs had different repeat lengths (20 to 36 nucleotides) or spacer lengths (15 to 30 nucleotides). Genomic DNA was extracted and the frequency of indel mutations was determined. For nucleofection, 250 pmol RNP was mixed with 200,000 cells per well. After 2 days, cells were collected and genomic DNA was extracted to determine the frequency of indel mutations. **FIG.28A** shows the generation of indels by CasΦ.12 with gRNAs containing repeat sequences of different lengths. **FIG.28B** the shows the generation of indels by CasΦ.12 with gRNAs containing spacer sequences of different lengths. The optimal gRNA for CasΦ.12-mediated genome editing in CHO cells was found to have a 20-nucleotide repeat length and a 17-nucleotide spacer length.

TABLE 13

Name	**Repeat length**	**Spacer length**	**Repeat sequence** (5' --> 3')	**Spacer sequence** (5' --> 3')	**crRNA sequence** (5' --> 3')
R3582	36	30	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAGAACAUU (SEQ ID NO: 1482)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAACAU U (SEQ ID NO:1499)
R3583	36	29	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAGAACAU (SEQ ID NO: 1483)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAACAU (SEQ ID NO:1500)
R3584	36	28	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAGAACA (SEQ ID NO: 1484)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAACA (SEQ ID NO:1501)
R3585	36	27	CUUUCAAGA CUAAUAGAU UGCUCCUUA	AGGAAUACAU GGUACACGUU GAAGAAC	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG

			CGAGGAGAC (SEQ ID NO: 54)	(SEQ ID NO: 1485)	AAUACAUGGUACA CGUUGAAGAAC (SEQ ID NO:1502)
R3586	36	26	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAGAA (SEQ ID NO: 1486)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGAA (SEQ ID NO:1503)
R3587	36	25	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAGA (SEQ ID NO: 1487)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAGA (SEQ ID NO:1504)
R3588	36	24	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAAG (SEQ ID NO: 1488)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAAG (SEQ ID NO:1505)
R3589	36	23	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GAA (SEQ ID NO: 1489)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGAA (SEQ ID NO:1506)
R3590	36	22	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU GA (SEQ ID NO: 1490)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUGA (SEQ ID NO:1507)
R3591	36	21	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGUU G (SEQ ID NO: 1491)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUUG (SEQ ID NO:1508)
R3592	36	20	CUUUCAAGA CUAAUAGAU UGCUCCUUA	AGGAAUACAU GGUACACGUU	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG

			CGAGGAGAC (SEQ ID NO: 54)	(SEQ ID NO: 1492)	AAUACAUGGUACA CGUU (SEQ ID NO:1509)
R3593	36	19	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACGU (SEQ ID NO: 1493)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGU (SEQ ID NO:1510)
R3594	36	18	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACACG (SEQ ID NO: 1494)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CG (SEQ ID NO:1511)
R3595	36	17	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACAC (SEQ ID NO: 1495)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA C (SEQ ID NO:1512)
R3596	36	16	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUACA (SEQ ID NO: 1496)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA (SEQ ID NO:1513)
R3597	36	15	CUUUCAAGA CUAAUAGAU UGCUCCUUA CGAGGAGAC (SEQ ID NO: 54)	AGGAAUACAU GGUAC (SEQ ID NO: 1497)	CUUUCAAGACUAA UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUAC (SEQ ID NO:1514)
R3598	35	20	UUUCAAGAC UAAUAGAUU GCUCCUUAC GAGGAGAC (SEQ ID NO: 1466)	AGGAAUACAU GGUACACGUU (SEQ ID NO: 1498)	UUUCAAGACUAAU AGAUUGCUCCUUA CGAGGAGACAGGA AUACAUGGUACAC GUU (SEQ ID NO:1515)
R3599	34	20	UUCAAGACU AAUAGAUUG CUCCUUACG	AGGAAUACAU GGUACACGUU	UUCAAGACUAAUA GAUUGCUCCUUAC GAGGAGACAGGAA

			AGGAGAC (SEQ ID NO: 1467)	(SEQ ID NO: 1498)	UACAUGGUACACGUU (SEQ ID NO:1516)
R3600	33	20	UCAAGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1468)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	UCAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1517)
R3601	32	20	CAAGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1469)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	CAAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1518)
R3602	31	20	AAGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1470)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	AAGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1519)
R3603	30	20	AGACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1471)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	AGACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1520)
R3604	29	20	GACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1472)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	GACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1521)
R3605	28	20	ACUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1473)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	ACUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1522)
R3606	27	20	CUAAUAGAUUGCUCCUUACGAGGAGAC (SEQ ID NO: 1474)	AGGAAUACAUGGUACACGUU (SEQ ID NO: 1498)	CUAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGUACACGUU (SEQ ID NO:1523)
R3607	26	20	UAAUAGAUUGCUCCUUACGAGGAGAC	AGGAAUACAUGGUACACGUU	UAAUAGAUUGCUCCUUACGAGGAGACAGGAAUACAUGGU

			(SEQ ID NO: 1475)	(SEQ ID NO: 1498)	ACACGUU (SEQ ID NO:1524)
R3608	25	20	AAUAGAUUG CUCCUUACG AGGAGAC (SEQ ID NO: 1476)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AAUAGAUUGCUCC UUACGAGGAGACA GGAAUACAUGGUA CACGUU (SEQ ID NO:1525)
R3609	24	20	AUAGAUUGC UCCUUACGA GGAGAC (SEQ ID NO: 1477)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AUAGAUUGCUCCU UACGAGGAGACAG GAAUACAUGGUAC ACGUU (SEQ ID NO:1526)
R3610	23	20	UAGAUUGCU CCUUACGAG GAGAC (SEQ ID NO: 1478)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	UAGAUUGCUCCUU ACGAGGAGACAGG AAUACAUGGUACA CGUU (SEQ ID NO:1527)
R3611	22	20	AGAUUGCUC CUUACGAGG AGAC (SEQ ID NO: 1479)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AGAUUGCUCCUUA CGAGGAGACAGGA AUACAUGGUACAC GUU (SEQ ID NO:1528)
R3612	21	20	GAUUGCUCC UUACGAGGA GAC (SEQ ID NO: 1480)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	GAUUGCUCCUUAC GAGGAGACAGGAA UACAUGGUACACG UU (SEQ ID NO:1529)
R3613	20	20	AUUGCUCCU UACGAGGAG AC (SEQ ID NO: 1481)	AGGAAUACAU GGUACACGUU AGGAAUACAU GGUACACGUU (SEQ ID NO: 2487)	AUUGCUCCUUACG AGGAGACAGGAAU ACAUGGUACACGU U (SEQ ID NO:1530)

EXAMPLE 34

Identification of optimal guide RNAs for CasΦ polypeptide-mediated genome editing in primary cells

[0491] The present example shows identification of the best performing gRNAs that target TRAC, B2M and programmed cell death protein 1 (PD1) in T cells. In this study, CasΦ.12 polypeptides (SEQ ID NO: 107) were incubated with different gRNAs (shown in **Table 14**) at room temperature for 10 minutes to form RNP complexes. T cells were resuspended at 5 x 10^5 cells/20 µL in electroporation solution (Lonza) and an Amaxa 4D Nucleofector with pulse code EH115 was used to nucleofect the cells Immediately after nucleofection, 80 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 10 minutes before transfer to the culture plate. After 48 hours, DNA was extracted from half of the cells and PCR was performed to detect the frequency of indels. The rest of the cells were cultured until Day 5, and were then collected for flow cytometry to detect the frequency of TRAC or B2M knockout. **FIG.29A** and **FIG.29B** show exemplary gRNAs for targeting TRAC. **FIG.29B** and **FIG.29C** show exemplary gRNAs for targeting B2M. **FIG.29E** shows exemplary gRNAs for targeting PD1. Additionally, this example demonstrates that a guide RNAs targeting a non-coding region can mediate gene knockout. For example, R3007, R2995, R2992 and R3014 target non-coding regions of the PD1 gene. The screening for gRNAs targeting TRAC is shown in **FIG.29F** and for gRNAs targeting B2M is shown in **FIG.29H**. Flow cytometry plots of exemplary gRNAs targeting TRAC are shown in **FIG.29G** and of exemplary gRNAs targeting B2M in **FIG.29I.**

TABLE 14

Name	**Target**	**Spacer sequence** (5' --> 3')
R3041	TRAC	UCCCACAGAUAUCCAGAACC (SEQ ID NO: 2470)
R3042	TRAC	GAGUCUCUCAGCUGGUACAC (SEQ ID NO: 1436)
R3043	TRAC	AGAGUCUCUCAGCUGGUACA (SEQ ID NO: 2471)
R3061	TRAC	AAGUCCAUAGACCUCAUGUC (SEQ ID NO: 2472)
R3063	TRAC	AAGAGCAACAGUGCUGUGGC (SEQ ID NO: 2473)
R3066	TRAC	GUUGCUCCAGGCCACAGCAC (SEQ ID NO: 2474)
R3068	TRAC	GCACAUGCAAAGUCAGAUUU (SEQ ID NO: 2475)
R3069	TRAC	GCAUGUGCAAACGCCUUCAA (SEQ ID NO: 2476)
R3081	TRAC	CUAAAAGGAAAAACAGACAU (SEQ ID NO: 2477)
R3141	TRAC	CUCGACCAGCUUGACAUCAC (SEQ ID NO: 2478)
R3088	B2M	AUAUAAGUGGAGGCGUCGCG (SEQ ID NO: 2479)
R3091	B2M	GGGCCGAGAUGUCUCGCUCC (SEQ ID NO: 1429)
R3094	B2M	UGGCCUGGAGGCUAUCCAGC (SEQ ID NO: 2480)
R3119	B2M	AAGUUGACUUACUGAAGAAU (SEQ ID NO: 2481)

R3132	B2M	AGCAAGGACUGGUCUUUCUA (SEQ ID NO: 2482)
R3149	B2M	AGUGGGGGUGAAUUCAGUGU (SEQ ID NO: 2483)
R3150	B2M	CAGUGGGGGUGAAUUCAGUG (SEQ ID NO: 1434)
R3155	B2M	GGCUGUGACAAAGUCACAUG (SEQ ID NO: 2484)
R3156	B2M	GUCACAGCCCAAGAUAGUUA (SEQ ID NO: 2485)
R3157	B2M	UCACAGCCCAAGAUAGUUAA (SEQ ID NO: 2486)
R2946	PD1	UGUGACACGGAAGCGGCAGU (SEQ ID NO: 263)
R2992	PD1	GGGGCUGGUUGGAGAUGGCC (SEQ ID NO: 309)
R2995	PD1	GAGCAGCCAAGGUGCCCCUG (SEQ ID NO: 312)
R3007	PD1	ACACAUGCCCAGGCAGCACC (SEQ ID NO: 324)
R3014	PD1	AGGCCCAGCCAGCACUCUGG (SEQ ID NO: 331)

EXAMPLE 35

RNP and mRNA delivery of CasΦ polypeptides

[0492] This example illustrates that CasΦ.12 can be delivered to primary cells as mRNA or as an RNP complex. In one study, RNP complexes were formed using CasΦ.12 protein (0, 100, 200 or 400 pmol) (SEQ ID NO: 107) and gRNAs (0, 400 or 800 pmol) targeting B2M or TRAC. RNP complexes were added to T cells. T cells were nucleofected using the Amaxa P3 kit and Amaxa 4D 96-well electroporation system with pulse code EH115. Cells were harvested for flow cytometry to determine the percentage of B2M or TRAC knockout cells, and genomic DNA was extracted to detect the frequency of indel mutations. As shown in **FIG. 30A**, a distinct population of B2M-negative cells was detected in T cells transfected with CasΦ.12 RNP complex targeting B2M. A distinct population of TRAC-negative cells was detected in in T cells transfected with CasΦ.12 RNP complex targeting TRAC, and shown in **FIG. 30B.** Quantification of the percentage of B2M knockout cells is shown in **FIG.30C** and quantification of the percentage of TRAC knockout cells is shown in **FIG. 30D.** A high frequency of indel mutations was also seen after delivery of RNP complexes. As shown in **FIG. 30E**, ~55% indel mutations was detected when RNP complexes targeting B2M were formed using 400 pmol protein and 800 pmol guide RNA. A similar frequency of indel mutations was detected when RNP complexes targeting TRAC were formed using the same conditions, as illustrated in **FIG. 30F.**

[0493] In a second study, CasΦ.12 mRNA was delivered to T cells with a gRNA targeting the B2M gene. For nucleofection, T cells were resuspended in BTXpress electroporation medium (5 x 10^5 cells per well) and mixed with CasΦ.12 mRNA and 500 pmol gRNA. Cells were collected on Day 2 for extraction of genomic DNA, and the frequency of indel mutations was determined. As shown in **FIG.30G,** delivery of CasΦ.12 mRNA and gRNA resulted in a high frequency of indel mutations. This was at a comparable level to genome editing with delivery of Cas9 mRNA. Further data from this study are shown in **FIG.30I** and **FIG.30J. FIG.30I** shows the frequency of indel mutations and functional knockout, as assessed by flow cytometry, of the B2M gene induced by either CasΦ.12 or Cas9 targeting the same site. **FIG.30J** shows the distribution of the

size of indel mutations induced by CasΦ.12 or Cas9 determined by NGS analysis. CasΦ.12 predominantly induced larger deletion mutations whereas Cas9 induced mostly small 1bp InDels. This data further confirms the ability of CasΦ.12 to mediate genome editing at the B2M locus.

EXAMPLE 36

gRNA processing by CasΦ polypeptides in mammalian cells

[0494] This example illustrates the ability of CasΦ polypeptides to process gRNA in mammalian cells. In this study, HEK293T cells were transfected with crRNA and expression plasmids encoding CasΦ.12 (SEQ ID NO: 107) using lipofectamine on day 0. The crRNA had the repeat sequence (the region that binds to CasΦ.12) CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGAC (SEQ ID NO: 54). To determine the nature of the crRNAs expressed in the HEK293T cells, the microRNA species in the HEK293T cells were analyzed by next generation sequencing. After 2 days, miRNA was extracted using the mirVANA kit. RNA was treated with recombinant Shrimp Alkaline Phosphatase (rSAP) to remove all the phosphates from the 5' and 3' ends of the RNA. PNK phosphorylation was then performed to add phosphate back to the 5' ends in preparation for adaptor ligation to the RNA. RNA was then mixed with 3' SR Adaptor for Illumina, followed by 3' ligation enzyme mix and incubated for 1 hour at 25°C in a thermal cycler. The reverse transcription primer was then hybridized to prevent adaptor-dimer formation. The SR RT primer hybridizes to the excess of 3' SR Adaptor (that remains free after the 3' ligation reaction) and transforms the single stranded DNA adaptor into a double-stranded DNA molecule. Double-stranded DNAs are not substrates for ligation mediated by T4 RNA Ligase 1 and therefore do not ligate to the 5′ SR. The RNA-ligation mixture from the previous step was mixed with SR RT primer for Illumina and placed in a thermocycler for the following program: 5 minutes at 75°C, 15 minutes at 37°C, 15 minutes at 25°C, hold at 4°C. The RNA-ligation mixture was then incubated with 5' SR adaptor for 1 hour at 25°C in a thermal cycler. Finally, RNA was reverse transcribed using ProtoScript II Reverse Transcriptase and amplified for PCR. The sample was then analyzed by next generation sequencing.

[0495] As shown in **FIG.31** the major crRNA molecule detected by sequence analysis was 24 nucleotides long (ATAGATTGCTCCTTACGAGGAGAC (SEQ ID NO: 1531) which is 12 nucleotides shorter than the full length repeat sequence (CTTTCAAGACTAATAGATTGCTCCTTACGAGGAGAC (SED ID NO: 54)) that was delivered to the HEK293T cells. This demonstrates how CasΦ.12 can process the repeat region of its crRNA in mammalian cells.

EXAMPLE 37

CasΦ polypeptide cleavage generates 5' overhangs

[0496] This example illustrates different CasΦ polypeptide-induced cleavage patterns. In this study, CasΦ polypeptides (CasΦ.12, CasΦ.45, CasΦ.43, CasΦ.39. CasΦ.37, CasΦ.33, CasΦ.32, CasΦ.30, CasΦ.28, CasΦ.25, CasΦ.24, CasΦ.22, CasΦ.20, CasΦ.18) were complexed with a crRNA to form RNPs. The RNPs were then used in cleavage reactions with plasmid DNA comprising a target sequence and a PAM (GTTG). The cleavage reaction was carried out at 37 °C and had a duration of 15 minutes. The cleavage products were then analyzed by gel electrophoresis. As shown in **FIG.32A**, the majority of CasΦ polypeptides generated a linear product from a plasmid target, whilst some CasΦ polypeptides introduced nicks into the plasmid DNA.

[0497] **FIG32B** shows a schematic of the cut sites on the target and non-target strand of a double-stranded target nucleic acid. The nature of the cleavage patterns resulting from the location of the cut sites on the target and non-target strands was investigated by sequence analysis, as shown in **FIG.32C** and represented in **FIG.32D**. These data show that the cleavage pattern following CasΦ polypeptide mediated cleavage of target nucleic acid is a staggered cut comprising 5' overhangs. **FIG.32E** shows a table of cut sites and overhangs of the different CasΦ polypeptides. The "#bp overlap" corresponds to the length of the 5' overhang for each CasΦ polypeptide. For comparison, Cpf1 introduces a staggered double-stranded DNA break with a 4- or 5-nucleotide 5' overhang (Zetsche *et. al* 2015 Cell).

EXAMPLE 38

Multiplex genome editing with CasΦ polypeptides

[0498] This example illustrates the ability of CasΦ RNP complexes to knockout multiple genes simultaneously. In this study, gRNAs targeting B2M, TRAC and PDCD1 (provided in **Table 15**) were incubated with CasΦ.12 (SEQ ID NO: 12) for 10 minutes at room temperature to form B2M, TRAC, and PDC1 targeting RNPs, respectively. The B2M targeting RNPs, TRAC targeting RNPs, PDCD1 targeting RNPs and combinations thereof were added to T cells. T cells were resuspended at 5 x 10^5 cells/20 µL in Nucleofection P3 solution and an Amaxa 4D 96-well electroporation system with pulse code EH115 was used to nucleofect the cells. Immediately after nucleofection, 85 µl pre-warmed culture medium was added to each well. The cells were then left in the cuvette plate for 10 minutes before transfer to the culture plate. On Day 3, genomic DNA was extracted and sent for NGS sequencing and the % indel was measured with a positive % indel being indicative of % knockout. On Day 5, cells were harvested for flow cytometry and the % knockout was measured with fluorescently labeled antibodies to TRAC and B2M (antibody to PDCD1 unavailable). % indel results are presented in **Table 16** and flow cytometry data presented in **Table 17**. Corresponding flow cytometry panels are shown in **FIG.33**.

TABLE 15

Description	SEQ ID	Sequence
B2M gRNA (R3132)	1532	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAG ACAGCAAGGACUGGUCUUUCUA
TRAC gRNA (R3042)	1432	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGA CGAGUCUCUCAGCUGGUACAC
PDCD1 gRNA (R2925)	791	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGA CUAGCACCGCCCAGACGACUG

TABLE 16

Description	**RNP Guide ID(s)**	**Amplicon**	**% INDEL**
TRAC single KO	R3042	TRAC	77.6%
B2M single KO	R3132	B2M	85.5%
PDCD1 single KO	R2925	PDCD1	44.6%
TRAC, B2M double KO	R3132 & R3042	TRAC	58.8%
TRAC, B2M double KO	R3132 & R3042	B2M	61.2%
TRAC, B2M, PDCD1 triple KO	R3132, R3042, R2925	TRAC	59.2%
TRAC, B2M, PDCD1 triple KO	R3132, R3042, R2925	B2M	69.4%
TRAC, B2M, PDCD1 triple KO	R3132, R3042, R2925	PDCD1	42.1%

TABLE 17

gRNA	B2M+ CD3-	B2M+, CD3+	B2M- , CD3+	B2M-,CD3-
TRAC	94	5.91	0.00418	0.1
B2M	0.051	8.65	90.7	0.59
TRAC + B2M	4.2	4.89	4.01	86.9
TRAC + B2M + PDCD1	4.74	14.1	4.33	76.8

EXAMPLE 39

Genome editing with CasΦ polypeptides mediates efficient editing of PCSK9 in mouse hepatoma cells

[0499] The present example shows that CasΦ.12 RNP complexes are highly effective at mediating editing the PCSK9 gene. In this study, 95 CasΦ gRNAs targeting PCSK9 (sequences shown in Tables E and Q), were incubated with CasΦ.12 (SEQ ID NO: 12) to form RNP complexes. Positive control RNP complexes were also formed using Cas9 and a gRNA. Hepa1-6 mouse hepatoma cells (100,000 cells) were resuspended in SF solution (Lonza) and nucleofected with CasΦ RNPs (250 pmoles) or the control Cas9 RNPs (60 pmoles) using program CM-137 or CM-148 (Amaxa nucleofector). Cells were collected after 48 hours, genomic DNA was extracted and the frequency of indel mutations was determined using NGS. **FIG.34** shows that CasΦ.12 is a highly effective genome editing tool, with an indel frequency of up to 48% induced by CasΦ.12 RNP complexes. Whereas, the maximum indel frequency induced by Cas9 was only about 22%.

EXAMPLE 40

Adeno-associated virus encoding CasΦ.12 facilitates genome editing

[0500] This example shows that a CasΦ.12 plasmid, including both CasΦ polypeptide sequence and gRNA sequence, sometimes called an all-in-one, can be used to facilitate genome editing. In this study, the crRNAs (sequences shown in Tables E and Q) from the initial RNP screen were chosen and truncations of these crRNAs were generated with repeat lengths of 36, 25, 20, or 19 nucleotides in combination with spacer lengths of 20, 17, or 16 nucleotides. Each crRNA was then cloned into an AAV vector consisting of U6 promoter to drive crRNA expression, intron-less EF1alpha short (EFS) promoter driving CasΦ expression, PolyA signal, and 1 kb stuffer sequence genomic. Hepa1-6 mouse hepatoma cells were nucleofected with 10 μg of each AAV plasmid. After 72 hours, genomic DNA was extracted and the frequency of indel mutations was determined using NGS. **FIG.35A** shows a plasmid map of the adeno-associated virus (AAV) encoding the CasΦ polypeptide sequence and gRNA sequence. **FIG.35D** shows the frequency of CasΦ.12 induced indel mutations in Hepa1-6 cells transduced with 10 μg of each AAV plasmid. gRNAs containing repeat sequences of 19, 20, 25 or 36 nucleotides and spacer sequences of 16, 17 or 20 nucleotides were used in this study. In the graph legend, repeat and spacer lengths are indicated as the number of nucleotides in the repeat followed by the number of nucleotides in the spacer, eg 20-17 has a repeat length of 20 nucleotides and a spacer length of 17 nucleotides. The

frequency of indel mutations is comparable to that of Cas9. **FIG.35E** and **FIG. 35F** show the frequency of CasΦ.12 induced indel mutations with different gRNA containing repeat and spacer sequences of different lengths (indicated as in **FIG.35F** with repeat length followed by spacer length). This study demonstrates that the all-in-one vector method of CasΦ.12 mediated genome editing is robust across different gRNA sequences and with gRNAs of different repeat and spacer lengths.

[0501] AAV vectors are a leading platform for delivery of gene therapy for treatment of human disease (Wang *et al.*, (2019) *Nature Reviews Drug Discovery*). One of the limitations of viral vector delivery of CRISPR/Cas9 is the size of Cas9. AAVs are roughly 20 nm, allowing for 4.5 kb genomic material to be packaged within it. This makes packaging Cas9 and a gRNA (~4.2 kB) with any additional elements such as multiple gRNAs or a donor polynucleotide for HDR challenging (Lino *et al.*, (2018), *Drug Delivery*). Whereas CasΦ is much smaller, allowing all of the components of the CRISPR system to be packaged in one viral vector.

EXAMPLE 41

Optimization of lipid nanoparticle delivery of CasΦ

[0502] This example describes the optimization of lipid nanoparticle (LNP) delivery of CasΦ mRNA and gRNA. In this study, the encapsulation efficiency of LNPs was optimized by testing different amine group to phosphate group ratio (N/P) of LNPs containing CasΦ mRNA and gRNA. An LNP kit from Precision Nanosystems (GenVoy-ILM™) was used to generate LNPs with different N/P ratios. LNPs were then dropped into HEK293T cells. Genomic DNA was extracted and the frequency of indel mutations was determined using NGS. The gRNA used in this study was R2470 with 2' O-methyl on the first three 5' and last three 3' nucleotides and phosphorotioate bonds in between the first three 5' nucleotides and in between the last two 3' nucleotides. The sequence of R2470 from 5' to 3' is 42256-779_601_SL. The mRNA was generated using T7 messenger mRNA IVT kit. As shown in **FIG.36**, indel mutations were detected following the use of a range of N/P ratios.

[0503] LNPs are one of the most clinically advanced non-viral delivery systems for gene therapy. LNPs have many properties that make them ideal candidates for delivery of nucleic acids, including ease of manufacture, low cytotoxicity and immunogenicity, high effiency of nucleic acid encapsulation and cell transfection, multidosing capabilities and flexibility of design (Kulkarni *et al.*, (2018) *Nucleic Acid Therapeutics*).

EXAMPLE 42

Genome editing in hematopoietic stem cells with CasΦ polypeptides

[0504] This example demonstrates CasΦ-mediated genome editing of $CD34^+$ hematopoietic stem cells (HSCs). HSCs are stem cells that differentiate to give rise blood cells, such as T and B lymphocytes, erythrocytes, monocytes and macrophages. HSCs are important cells for future stem cell therapies as they have the potential to be used to treat genetic blood cell diseases (Morgan *et al.* (2017), *Cell Stem Cell*).

[0505] In this study human $CD34^+$ cells were grown in XVIVO10 media (+ 5% FBS, +1X CC110) for three days. On the third day, the cells were nucleofected using the Lonza P3 kit with either RNP containing CasΦ.12 polypeptides complexed with B2M-targeting guide R3132 (42256-779_601_SL), or a mixture of CasΦ.12 mRNA with B2M-targeting guide. Cells were collected after 3 days, genomic DNA was purified and the frequency of indel mutations at the B2M locus was analyzed by NGS. As shown in **FIG.37**, CasΦ.12 is an effective tool for genome editing when CasΦ.12 is delivered to cells as CasΦ.12 RNP complexes or CasΦ.12 mRNA.

[0506] This example illustrates the utility of CasΦ polypetides as genome editing tools in stem cells, such as HSCs.

EXAMPLE 43

Genome editing in induced pluripotent stem cells with CasΦ polypeptides

[0507] This example demonstrates CasΦ-mediated genome editing of induced pluripotent stem cells (iPSCs). iPSCs are pluripotent stem cells that are generated from somatic cells. They can propagate indefinitely and give rise to any cell type in the body. These features make iPSCs a powerful tool for researching human disease and provide a promising prospect for cell therapies for a range of medical conditions. iPSCs can be generated in a patient-specific manner and used in autologous transplant, thereby overcoming complications of rejection by the host immune system (Moradi *et al.* (2019), *Stem Cell Research & Therapy*).

[0508] In this study, high quality WTC-11 iPSCs were harvested as single cells using Accutase treatment for 5 minutes. RNP complexes were formed using CasΦ.12 polypeptides and gRNAs targeting either the B2M locus or targeting a CIITA locus (sequences shown in **Table 19**). RNP complexes were formed using 2:1 gRNA:CasΦ.12 RNP (1000 pmol gRNA + 500 pmol Cas12Φ.12) and incubating at room temperature for approximately 15 minutes. WTC-11 iPSCs (200,000 cells) were resuspended in 20 uL of P3 nucleofection solution per reaction and 40 uL of cell suspension was added to each RNP tube. Half of the volume of each RNP/cell suspension

mixture was added to the Lonza 96 well shuttle and nucleofection was performed using the program CD118. To recover the transfected cells, 80 µL of warm StemFlex media supplemented with 2 µM of Thiazovivin was added to the wells of the shuttle. The entire volume of the shuttle well was transferred to a 96 well plate previously coated with 0.337 mg/mL Matrigel containing 100 µL of 2 µM of Thiazovivin. Cells were allowed to recover for 24 hours in 37°C incubator with humidity control. Cells were confluent 48 hours post-transfection, and single-cell passaged using Accutase. Genomic DNA was extracted using KingFisher Tissue and DNA kit. NGS library preparation was performed using in house protocols and the frequency of indel mutations was quantified using Crispresso. As shown in **FIG.38**, effective genome editing at the B2M and CIITA loci was achieved with CasΦ.12 RNP complexes in iPSCs.

[0509] This example demonstrates the utility of CasΦ as genome editing tools in iPSCs.

TABLE 19

Name	Target	Sequence	SEQ ID NO
R3132	B2M	AUUGCUCCUUACGAGGAGACAGCAAGGACUGGUCUUU	2488
R4504_CasPhi12_S	CIITA	AUUGCUCCUUACGAGGAGACGGGCUCUGACAGGUAGG	1722
R5406_CasPhi12	CIITA	CUUUCAAGACUAAUAGAUUGCUCCUUACGAGGAGACGGGUCAAUGCUAGGUACUGC	2222

EXAMPLE 44

Genome editing with CasΦ polypeptides mediates efficient editing of CIITA locus

[0510] This example demonstrates CasΦ-mediated genome editing of the CIITA locus. In this study, RNP complexes were formed using CasΦ polypeptides and gRNAs targeting CIITA (sequences shown in Tables D and O). K562 cells were nucleofected with RNP complexes (250 pmol) using Lonza nucleofection protocols. Cells were harvested after 48 hours, genomic DNA was isolated and the frequency of indel mutations was evaluated using NGS analysis (MiSeq, Illumina). As shown in **FIG. 39**, effective genome editing of the CIITA locus was achieved using CasΦ RNP complexes.

[0511] While preferred embodiments of the present invention have been shown and described herein, it will be apparent to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A composition comprising:

a) a programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and

b) a guide nucleic acid or a nucleic acid encoding said guide nucleic acid, wherein said guide nucleic acid comprises a region comprising a nucleotide sequence that is complementary to a target nucleic acid sequence and an additional region, wherein said region and said additional region are heterologous to each other.

2. The composition of claim 1, wherein the additional region of the guide nucleic acid comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

3. The composition of claim 1, wherein the guide nucleic acid comprises a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

4. The composition of claim 1, wherein the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

5. The composition of claim 1, wherein the programmable CasΦ nuclease comprises nickase activity.

6. The composition of claim 1, wherein the programmable CasΦ nuclease comprises double-strand cleavage activity.

7. The composition of claim 1, wherein the programmable CasΦ nuclease comprises at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

8. The composition of claim 1, wherein the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

9. The composition of claim 1, wherein the programmable CasΦ nuclease comprises at least 98% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

10. The composition of claim 1, wherein the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

11. The composition of claim 1, wherein the guide nucleic acid does not comprise a tracrRNA.

12. The composition of claim 1, wherein the programmable CasΦ nuclease comprises greater nickase activity when complexed with the guide nucleic acid at a temperature from about 20°C to about 25°C, as compared with complex formation at a temperature of about 37°C.

13. The composition of claim 1, wherein the additional region comprises at least 98% sequence identity to SEQ ID NO: 57.

14. The composition of claim 13, wherein the programmable CasΦ nuclease comprises greater nickase activity when complexed with the guide nucleic acid comprising a sequence comprising at least 98% sequence identity to SEQ ID NO: 57, as compared to when complexed with a guide nucleic acid comprising SEQ ID NO: 49.

15. The composition of claim 1, wherein the programmable CasΦ nuclease exhibits greater nicking activity as compared to double stranded cleavage activity.

16. The composition of claim 1, wherein the programmable CasΦ nuclease exhibits greater double stranded cleavage activity as compared to nicking activity.

17. The composition of any one of claims 1-16, wherein the programmable CasΦ nuclease comprises a single active site in a RuvC domain that is capable of catalyzing pre-crRNA processing and nicking or cleaving of nucleic acids.

18. The composition of any one of claims 1-17, wherein the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TBN-3', wherein B is one or more of C, G, or T.

19. The composition of claim 18, wherein the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5’-TTTN-3’.

20. A method of modifying a target nucleic acid sequence, the method comprising:

contacting a target nucleic acid sequence with a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and a guide nucleic acid,

wherein the programmable CasΦ nuclease cleaves the target nucleic acid sequence, thereby modifying the target nucleic acid sequence.

21. The method of claim 20, wherein the programmable CasΦ nuclease introduces a double-stranded break in the target nucleic acid sequence.

22. The method of claim 20, wherein the programmable CasΦ nuclease comprises double-strand cleavage activity.

23. The method of claim 20, wherein the programmable CasΦ nuclease cleaves a single-strand of the target nucleic acid sequence.

24. The method of claim 20, wherein the programmable CasΦ nuclease comprises nickase activity.

25. The method of claim 20, wherein the programmable CasΦ nuclease exhibits greater nicking activity as compared to double stranded cleavage activity.

26. The method of claim 20, wherein the programmable CasΦ nuclease exhibits greater double stranded cleavage activity as compared to nicking activity.

27. The method of claim 20, wherein the target nucleic acid is DNA.

28. The method of claim 20, wherein the target nucleic acid is double-stranded DNA.

29. The method of claim 20, wherein the programmable CasΦ nuclease cleaves a non-target strand of the double-stranded DNA, wherein the non-target strand is non-complementary to the guide nucleic acid.

30. The method of claim 20, wherein the programmable CasΦ nuclease does not cleave a target strand of the double-stranded DNA, wherein the target strand is complementary to the guide nucleic acid.

31. The method of claim 20, wherein the programmable CasΦ nuclease comprises at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

32. The method of claim 20, wherein the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

33. The method of claim 20, wherein the programmable CasΦ nuclease comprises at least 98% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105 and SEQ ID NO. 107.

34. The method of claim 20, wherein the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

35. The method of claim 20, wherein the guide nucleic acid comprises a sequence comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

36. The method of claim 20, wherein the guide nucleic acid comprises a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

37. The method of claim 20, wherein the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

38. The method of claim 20, wherein the guide nucleic acid does not comprise a tracrRNA.

39. The method of claim 20, wherein the target nucleic acid sequence comprises a mutated sequence or a sequence associated with a disease.

40. The method of claim 39, wherein the mutated sequence is removed after the programmable CasΦ nuclease cleaves the target nucleic acid sequence.

41. The method of claim 20, wherein the target nucleic acid sequence is in a human cell.

42. The method of claim 20, wherein the method is performed in vivo.

43. The method of claim 20, wherein the method is performed ex vivo.

44. The method of claim 20, further comprising inserting a donor polynucleotide into the target nucleic acid sequence at the site of cleavage.

45. A method of introducing a break in a target nucleic acid, the method comprising: contacting the target nucleic acid with:

(a) a first guide nucleic acid comprising a region that binds to a first programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107; and

(b) a second guide nucleic acid comprising a region that binds to a second programmable nickase comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107,

wherein the first guide nucleic acid comprises a first additional region that binds to the target nucleic acid and wherein the second guide nucleic acid comprises a second additional region that binds to the target nucleic acid and wherein the first additional region of the first guide nucleic acid and the second additional region of the second guide nucleic acid bind opposing strands of the target nucleic acid.

46. The method of claim 45, wherein the first programmable nickase, the second programmable nickase, or both comprise at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, SEQ ID NO. 107.

47. The method of claim 45, wherein the first programmable nickase, the second programmable nickase, or both comprise at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, SEQ ID NO. 107.

48. The method of claim 45, wherein the first programmable nickase, the second programmable nickase, or both comprise a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

49. The method of claim 45, wherein the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

50. The method of claim 45, wherein the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence comprising at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

51. The method of claim 45, wherein the first guide nucleic acid, the second guide nucleic acid, or both comprise a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

52. The method of claim 45, wherein the first programmable nickase and the second programmable nickase exhibit greater nicking activity as compared to double stranded cleavage activity.

53. The method of claim 45, wherein the first programmable nickase and the second programmable nickase nick the target nucleic acid at two different sites.

54. The method of claim 45, wherein the target nucleic acid comprises double stranded DNA.

55. The method of claim 53, wherein the two different sites are on opposing strands of the double stranded DNA.

56. The method of claim 45, wherein the target nucleic acid comprises a mutated sequence or a sequence is associated with a disease.

57. The method of claim 56, wherein the mutated sequence is removed after the first programmable nickase and the second programmable nickase nick the target nucleic acid.

58. The method of claim 45, wherein the target nucleic acid is in a cell.

59. The method of claim 45, wherein the method is performed in vivo.

60. The method of claim 45, wherein the method is performed ex vivo.

61. The method of any one of claims 45-60, wherein the first programmable nickase and the second programmable nickase are the same.

62. The method of any one of claims 45-60, wherein the first programmable nickase and the second programmable nickase are different.

63. A method of detecting a target nucleic acid in a sample, the method comprising

contacting a sample comprising a target nucleic acid with

(a) a programmable CasΦ nuclease comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 105;

(b) a guide RNA comprising a region that binds to the programmable CasΦ nuclease and an additional region that binds to the target nucleic acid; and

(c) a labeled single stranded DNA reporter that does not bind the guide RNA;

cleaving the labeled single stranded DNA reporter by the programmable CasΦ nuclease to release a detectable label; and

detecting the target nucleic acid by measuring a signal from the detectable label.

64. The method of claim 63, wherein the target nucleic acid is single stranded DNA.

65. The method of claim 63, wherein the target nucleic acid is double stranded DNA.

66. The method of claim 63, wherein the target nucleic acid is a viral nucleic acid.

67. The method of claim 63, wherein the target nucleic acid is bacterial nucleic acid.

68. The method of claim 63, wherein the target nucleic acid is from a human cell.

69. The method of claim 63, wherein the target nucleic acid is a fetal nucleic acid.

70. The method of claim 63, wherein the sample is derived from a subject's saliva, blood, serum, plasma, urine, aspirate, or biopsy sample.

71. The method of claim 63, wherein the programmable CasΦ nuclease comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, SEQ ID NO. 107.

72. The method of claim 63, wherein the programmable CasΦ nuclease comprises a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

73. The method of claim 63, wherein the guide RNA comprises at least about 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

74. The method of claim 63, wherein the guide RNA comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

75. The method of claim 63, wherein the sample comprises a phosphate buffer, a Tris buffer, or a HEPES buffer.

76. The method of claim 63, wherein the sample comprises a pH of 7 to 9.

77. The method of claim 63, wherein the sample comprises a pH of 7.5 to 8.

78. The method of claim 63, wherein the sample comprises a salt concentration of 25 nM to 200 mM.

79. The method of claim 63, wherein the single stranded DNA reporter comprises an ssDNA-fluorescence quenching DNA reporter.

80. The method of claim 63, wherein the ssDNA- fluorescence quenching DNA reporter is a universal ssDNA- fluorescence quenching DNA reporter.

81. The method of claim 63, wherein the programmable CasΦ nuclease exhibits PAM-independent cleaving.

82. A method of modulating transcription of a gene in a cell, the method comprising: introducing into a cell comprising a target nucleic acid sequence:

(i) a fusion polypeptide or a nucleic acid encoding the fusion polypeptide, wherein the fusion polypeptide comprises:

(a) a dCasΦ polypeptide comprising at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, wherein the dCasΦ polypeptide is enzymatically inactive; and

(b) a polypeptide comprising transcriptional regulation activity; and

(ii) a guide nucleic acid, or a nucleic acid comprising a nucleotide sequence encoding the guide nucleic acid, wherein the guide nucleic acid comprises a region that binds to the dCasΦ polypeptide and an additional region that binds to the target nucleic acid;

wherein transcription of the gene is modulated through the fusion polypeptide acting on the target nucleic acid sequence.

83. The method of claim 82, wherein the dCasΦ polypeptide comprises at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107.

84. The method of claim 82, wherein the guide nucleic acid comprises at least about 95% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

85. The method of claim 82, wherein the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

86. The method of claim 82, wherein the guide nucleic acid comprises a sequence selected from the group consisting of SEQ ID NOs: 48 to 86.

87. The method of claim 82, wherein the polypeptide comprising transcriptional regulation activity polypeptide comprises transcription activation activity.

88. The method of claim 82, wherein the polypeptide comprising transcriptional regulation activity polypeptide comprises transcription repressor activity.

89. The method of claim 82, wherein the polypeptide comprising transcriptional regulation activity polypeptide comprises an activity selected from the group consisting of transcription activation activity, transcription repression activity, nuclease activity, transcription release factor activity, histone modification activity, histone acetyltransferase activity, nucleic acid association activity, DNA methylase activity, direct or indirect DNA demethylase activity, methyltransferase activity, demethylase activity, acetyltransferase activity, deacetylase activity, kinase activity, phosphatase activity, ubiquitin ligase activity, deubiquitinating activity, adenylation activity, deadenylation activity, deaminase activity, SUMOylating activity, deSUMOylating activity,

ribosylation activity, deribosylation activity, myristoylation activity, and demyristoylation activity.

90. A composition comprising:

a) a Cas nuclease or nucleic acid encoding said Cas nuclease, and

b) a guide nucleic acid or a nucleic acid encoding said guide nucleic acid, wherein said guide nucleic acid comprises a region comprising a nucleotide sequence that is complementary to a target nucleic acid sequence and an additional region, wherein said region and said additional region are heterologous to each other;

wherein the Cas nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid.

91. The composition of claim 90, wherein the same active site in the RuvC domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid.

92. The composition of claims 90 or 91, wherein the Cas nuclease is the programmable CasΦ nuclease of any one of claims 1-18.

93. The composition of any one of claims 90-92, wherein the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TBN-3', wherein B is one or more of C, G, or, T.

94. The composition of claim 93, wherein the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTTN-3', optionally wherein the PAM is 5'-TTTN-3'.

95. The composition of claim 93, wherein the PAM is 5'-GTTK-3', 5'-VTTK-3', 5'-VTTS-3', 5'-TTTS-3' or 5'-VTTN-3', where K is G or T, V is A, C or G, and S is C or G.

96. The composition of any one of claims 90-94, wherein the composition is used in a method of any one of claims 20-89.

97. The use of a programmable CasΦ nuclease to modify a target nucleic acid sequence according to the method of claims 20 to 44.

98. The use of a first programmable nickase and a second programmable nickase to introduce a break in a target nucleic acid according to the method of claims 45 to 62.

99. The use of a programmable CasΦ nuclease to detect a target nucleic acid in a sample according to the method of claims 63 to 81.

100. The use of a dCasΦ polypeptide to modulate transcription of a gene in a cell according to the method of claims 82 to 89.

101. The composition of any one of claims 1-19 or 45-100, wherein the region is a spacer region and the additional region is a repeat region.

102. The method, composition, or use of any one of claims 1-19 or 45-100, wherein the region is a repeat region and the additional region is a spacer region.

103. The method, composition, or use of claim 101 or 102, wherein the repeat region comprises a GAC sequence, optionally wherein the GAC sequence is at the 3' end of the repeat region.

104. The method, composition, or use of claims 101-103, wherein the repeat region comprises a hairpin, optionally wherein the hairpin is in the 3' portion of the repeat region.

105. The method, composition, or use of claim 104, wherein the hairpin comprises a double-stranded stem portion and a single-stranded loop portion.

106. The method, composition, or use of claim 105, wherein a strand of the stem portion comprises a CYC sequence and the other strand of the stem portion comprises a GRG sequence, wherein Y and R are complementary.

107. The method, composition, or use of claim 106, wherein the G of the GAC sequence is in the stem portion of the hairpin.

108. The method, composition, or use of any one of claims 105-107, wherein each strand of the stem portion comprises 3, 4 or 5 nucleotides.

109. The method, composition, or use of any one of claims 105-108, wherein the loop portion comprises between 2 and 8 nucleotides, optionally wherein the loop portion comprises 4 nucleotides.

110. The composition of claim 1, wherein the guide nucleic acid comprises at least 98% sequence identity to SEQ ID NO: 54.

111. The method, composition, or use according to any one of claims 101-110, wherein the repeat region is between 15 and 50 nucleotides in length, preferably, wherein the repeat region is between 19 and 37 nucleotides in length.

112. The method, composition, or use according to any one of claims 101-111, wherein the spacer region is between 15 and 50 nucleotides in length, between 15 and 40 nucleotides in length, or between 15 and 35 nucleotides in length, preferably wherein the spacer region is between 16 and 30 nucleotides in length.

113. The method, composition, or use according to claim 112, wherein the spacer region is between 16 and 20 nucleotides in length.

114. The composition according to any one of claims 1-19, 90-95, 101-113, wherein the programmable CasΦ nuclease forms a complex with a divalent metal ion, preferably wherein the divalent metal ion is Mg^{2+}.

115. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

116. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

117. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, or SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516;

b) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

c) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

d) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

118. The programmable CasΦ nuclease or a nucleic acid of claims 115-117, wherein the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid.

119. The programmable CasΦ nuclease or a nucleic acid of claims 115-118, wherein the programmable CasΦ nuclease is fused or linked to one or more NLS.

120. The programmable CasΦ nuclease or a nucleic acid of claims 115-119, wherein:

a) the one or more NLS are fused or linked to the N-terminus of the programmable CasΦ nuclease;

b) the one or more NLS are fused or linked to the C-terminus of the programmable CasΦ nuclease; or

c) the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable CasΦ nuclease.

121. A composition comprising the programmable CasΦ nuclease or a nucleic acid of claims 115-120 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

122. A composition comprising the programmable CasΦ nuclease or a nucleic acid of claims 115-120 and a cell, preferably wherein the cell is a eukaryotic cell.

123. A composition comprising the programmable CasΦ nuclease or a nucleic acid of claims 115-120 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

124. A eukaryotic cell comprising the programmable CasΦ nuclease or a nucleic acid of claims 115-120.

125. The eukaryotic cell of claim 124, wherein the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

126. A vector comprising the nucleic acid of claims 115-120.

127. The vector of claim 126, wherein the vector is a viral vector.

128. The composition of claim 18, wherein the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTN-3'.

129. The composition of any one of claims 1-17, wherein the programmable CasΦ nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTB-3', wherein B is C, G, or T.

130. The composition of claim 93, wherein the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-TTN-3', optionally wherein the PAM is 5'-TTN-3'.

131. The composition of any one of claims 90-94, wherein the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTK-3', 5'-VTTK-3', 5'-VTTS-3', 5'-TTTS-3' or 5'-VTTN-3', where K is G or T, V is A, C or G, and S is C or G.

132. The composition of any one of claims 90-94, wherein the Cas nuclease recognizes a protospacer adjacent motif (PAM) of 5'-GTTB-3', wherein B is C, G, or T.

133. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

134. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

135. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

136. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

137. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

138. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

139. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

140. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

141. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

142. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

143. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

144. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

145. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

146. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

147. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

148. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

149. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

150. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

151. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to a sequence selected from the group consisting of SEQ ID NOs: 1 to 47, SEQ ID NO. 105, and SEQ ID NO. 107, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

152. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises a RuvC-like domain which matches PFAM family PF07282 and does not match PFAM family PF18516, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

153. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable CasΦ nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable CasΦ nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable CasΦ nuclease does not require a tracrRNA to cleave the target nucleic acid.

154. The programmable CasΦ nuclease or a nucleic acid of any of claims 133-153, wherein the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid.

155. The programmable CasΦ nuclease or a nucleic acid of any of claims 133-154, wherein the programmable CasΦ nuclease is fused or linked to one or more NLS.

156. The programmable CasΦ nuclease or a nucleic acid of any of claims 133-155, wherein:

a) the one or more NLS are fused or linked to the N-terminus of the programmable CasΦ nuclease;

b) the one or more NLS are fused or linked to the C-terminus of the programmable CasΦ nuclease; or

c) the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable CasΦ nuclease.

157. A composition comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 133-156 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

158. The composition of claim 157, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

159. The composition of claim 158, wherein the seed region comprises 16 nucleosides.

160. A composition comprising the programmable CasΦ nuclease or a nucleic acid of claims 133-156 and a cell, preferably wherein the cell is a eukaryotic cell.

161. A composition comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 133-156 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

162. The composition of claim 161, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

163. The composition of claim 162, wherein the seed region comprises 16 nucleosides.

164. A eukaryotic cell comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 133-156.

165. The eukaryotic cell of claim 164, wherein the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

166. The eukaryotic cell of claim 165, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

167. The eukaryotic cell of claim 166, wherein the seed region comprises 16 nucleosides.

168. A vector comprising the nucleic acid of any of claims 133-156.

169. The vector of claim 168, wherein the vector is a viral vector.

170. A guide nucleic acid, or a nucleic acid encoding said guide nucleic acid, comprising a sequence that is the same as or differs by no more than 5, 4, 3, 2, or 1 nucleotides from:

a) a sequence from Tables A to AH; or

b) a sequence comprising a repeat sequence from Table 2 and a spacer sequence from Tables A to H.

171. The guide nucleic acid of claim 170 comprising:

a) a sequence from Tables A to AH; or

b) a sequence comprising a repeat sequence from Table 2 and a spacer sequence from Tables A to H.

172. The guide nucleic acid of claim 170 or claim 171, wherein the guide nucleic acid comprises RNA and/or DNA.

173. The guide nucleic acid of claim 172, wherein the guide nucleic acid is a guide RNA.

174. A complex comprising the guide nucleic acid of any of claims 171 to 173 and a programmable CasΦ nuclease.

175. A eukaryotic cell comprising the guide nucleic acid of any of claims 165 to 167.

176. The eukaryotic cell of claim 175 further comprising a programmable CasΦ nuclease.

177. A vector encoding the guide nucleic acid of any of claims 170 to 173.

178. The vector of claim 177, wherein the vector is a viral vector.

179. A method of introducing a first modification in a first gene and a second modification in a second gene, the method comprising contacting a cell with a CasΦ nuclease; a first guide RNA that is at least partially complementary to an equal length portion of the first gene; and a second guide RNA that is at least partially complementary to an equal length portion of the second gene.

180. The method of claim 179, wherein the CasΦ nuclease is a CasΦ12 nuclease.

181. The method of claim 180, wherein the CasΦ12 nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 12.

182. The method of any one of claims 179-181, wherein the first and/or second modification comprises an insertion of a nucleotide, a deletion of a nucleotide or a combination thereof.

183. The method of any one of claims 179-181, wherein the first and/or second modification comprises an epigenetic modification.

184. The method of any one of claims 179-183, wherein the first and/or second mutation results in a reduction in the expression of the first gene and/or second gene, respectively.

185. The method of any one of claims 179-184, wherein the reduction in the expression is at least about a 10% reduction, at least about a 20% reduction, at least about a 30% reduction, at least about a 40% reduction, at least about a 50% reduction, at least about a 60% reduction, at least about a 70% reduction, at least about an 80% reduction, or at least about a 90% reduction.

186. The method of any one of claims 179-185, comprising contacting the cell with three different guide RNAs targeting three different genes.

187. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 12.

188. The programmable CasΦ nuclease or a nucleic acid of claim 187, wherein said programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 12.

189. The programmable CasΦ nuclease or a nucleic acid of claim 187, wherein said programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 12.

190. The programmable CasΦ nuclease or a nucleic acid of claim 187, wherein said programmable CasΦ nuclease comprises at least 98% sequence identity to SEQ ID NO: 12.

191. The programmable CasΦ nuclease or a nucleic acid of claim 187, wherein said programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 12.

192. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 18.

193. The programmable CasΦ nuclease or a nucleic acid of claim 192, wherein said programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 18.

194. The programmable CasΦ nuclease or a nucleic acid of claim 192, wherein said programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 18.

195. The programmable CasΦ nuclease or a nucleic acid of claim 192, wherein said programmable CasΦ nuclease comprises at least 98% sequence identity to SEQ ID NO: 18.

196. The programmable CasΦ nuclease or a nucleic acid of claim 192, wherein said programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 18.

197. A programmable CasΦ nuclease or a nucleic acid encoding said programmable CasΦ nuclease, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 32.

198. The programmable CasΦ nuclease or a nucleic acid of claim 197, wherein said programmable CasΦ nuclease comprises at least 85% sequence identity to SEQ ID NO: 32.

199. The programmable CasΦ nuclease or a nucleic acid of claim 197, wherein said programmable CasΦ nuclease comprises at least 90% sequence identity to SEQ ID NO: 32.

200. The programmable CasΦ nuclease or a nucleic acid of claim 197, wherein said programmable CasΦ nuclease comprises at least 95% sequence identity to SEQ ID NO: 32.

201. The programmable CasΦ nuclease or a nucleic acid of claim 197, wherein said programmable CasΦ nuclease comprises at least 98% sequence identity to SEQ ID NO: 32.

202. The programmable CasΦ nuclease or a nucleic acid of claim 197, wherein said programmable CasΦ nuclease comprises or consists of an amino acid sequence of SEQ ID NO: 32.

203. The programmable CasΦ nuclease or a nucleic acid of any one of claims 187 to 202, wherein the programmable CasΦ nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

204. The programmable CasΦ nuclease or a nucleic acid of claim 203, wherein a complex comprising the programmable CasΦ nuclease and the guide RNA binds to the target sequence.

205. The programmable CasΦ nuclease or a nucleic acid of any one of claims 187 to 204, wherein the programmable CasΦ nuclease does not require a tracrRNA to cleave a target nucleic acid.

206. The programmable CasΦ nuclease or a nucleic acid of any one of claims 187 to 205, wherein the programmable CasΦ nuclease wherein the programmable CasΦ nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving a target nucleic acid.

207. A composition comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 187-206 and a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

208. The composition of claim 207, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

209. The composition of claim 209, wherein the seed region comprises 16 nucleosides.

210. A composition comprising the programmable CasΦ nuclease or a nucleic acid of claims 187-206 and a cell, preferably wherein the cell is a eukaryotic cell.

211. A composition comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 187-206 and a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

212. The composition of claim 211, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

213. The composition of claim 212, wherein the seed region comprises 16 nucleosides.

214. A eukaryotic cell comprising the programmable CasΦ nuclease or a nucleic acid of any of claims 187-206.

215. The eukaryotic cell of claim 214, wherein the cell further comprises a guide nucleic acid comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

216. The eukaryotic cell of claim 215, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

217. The eukaryotic cell of claim 217, wherein the seed region comprises 16 nucleosides.

218. A vector comprising the nucleic acid of any of claims 187-206.

219. The vector of claim 218, wherein the vector is a viral vector.

220. The vector of claim 168 or claim 218, wherein the vector further comprises a nucleic acid encoding a guide nucleic acid, wherein the guide nucleic acid comprises a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable CasΦ nuclease.

221. The vector of claim 220, wherein the guide nucleic acid is a guide RNA.

222. The vector of any one of claims 168, 219-221, wherein the further comprises a donor polynucleotide.

223. The composition of claim 207 or claim 211 or the eukaryotic cell of claim 215, wherein the guide nucleic acid is a guide RNA.

224. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the programmable nuclease comprises a RuvC domain, wherein the RuvC domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

225. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

226. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

227. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid; and

d) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

228. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease cleaves both strands of the target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang; and

e) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

229. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

e) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

230. A programmable nuclease or a nucleic acid encoding said programmable nuclease, wherein said programmable nuclease is a Type V CRISPR/Cas enzyme nuclease and comprises between 400 and 900 amino acids, and wherein

a) the programmable nuclease is capable of binding to a guide RNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome

and a second region that binds to the programmable nuclease, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides;

b) a complex comprising the programmable nuclease and the guide RNA binds to the target sequence;

c) the RuvC-like domain is capable of processing a pre-crRNA and cleaving the target nucleic acid;

d) the programmable nuclease cleaves both strands of a target nucleic acid comprising the target sequence, wherein the strand break is a staggered cut with a 5' overhang;

e) the programmable nuclease is capable of cleaving the second region of the guide RNA in mammalian cells; and

f) the programmable nuclease does not require a tracrRNA to cleave the target nucleic acid.

231. The programmable nuclease or a nucleic acid of any of claims 224-230, wherein the same active site in the RuvC domain or RuvC-like domain catalyzes the processing of the pre-crRNA and the cleaving of the target nucleic acid.

232. The programmable nuclease or a nucleic acid of any of claims 224-231, wherein the programmable nuclease is fused or linked to one or more NLS.

233. The programmable nuclease or a nucleic acid of claims 232, wherein:

a) the one or more NLS are fused or linked to the N-terminus of the programmable nuclease;

b) the one or more NLS are fused or linked to the C-terminus of the programmable nuclease; or

c) the one or more NLS are fused or linked to the N-terminus and the C-terminus of the programmable nuclease.

234. A composition comprising the programmable nuclease or a nucleic acid of any of claims 224-233 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease.

235. The composition of claim 234, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

236. The composition of claim 235, wherein the seed region comprises 16 nucleosides.

237. A composition comprising the programmable nuclease or a nucleic acid of claims 224-233 and a cell, preferably wherein the cell is a eukaryotic cell.

238. A composition comprising the programmable nuclease or a nucleic acid of any of claims 224-233 and a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease and a cell, preferably wherein the cell is a eukaryotic cell.

239. The composition of claim 238, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

240. The composition of claim 239, wherein the seed region comprises 16 nucleosides.

241. A eukaryotic cell comprising the programmable nuclease or a nucleic acid of any of claims 224-233.

242. The eukaryotic cell of claim 241, wherein the cell further comprises a gRNA comprising a first region that is complementary to a target nucleic acid sequence in a eukaryotic genome and a second region that binds to the programmable nuclease.

243. The eukaryotic cell of claim 242, wherein the first region comprises a seed region comprising between 10 and 16 nucleosides.

244. The eukaryotic cell of claim 243, wherein the seed region comprises 16 nucleosides.

245. A vector comprising the nucleic acid of any of claims 224-233.

246. The vector of claim 245, wherein the vector is a viral vector.

247. A complex comprising a first programmable CasΦ nuclease and a second programmable CasΦ nuclease.

248. The complex of claim 224, wherein the first programmable CasΦ nuclease and the second programmable CasΦ nuclease are the same programmable CasΦ nuclease.

249. A dimer comprising a first programmable CasΦ nuclease and a second programmable CasΦ nuclease.

250. A homodimer comprising a first programmable CasΦ nuclease and a second programmable CasΦ nuclease.

251. A method of modifying a cell comprising a target nucleic acid, comprising introducing the composition of any one of claims 1-19, 90-95, 157-159, 207-209, 234-236 to the cell,

wherein the programmable CasΦ nuclease, programmable nuclease or the cas nuclease cleaves the target nucleic acid, thereby modifying the cell.

252. A method of modifying a cell comprising a target nucleic acid, comprising introducing to the cell (i) the programmable CasΦ nuclease or programmable nuclease of any one of claims 115-120, 133-156, 187-206, or 224-233 and (ii) a guide nucleic acid, wherein the programmable CasΦ nuclease or programmable Cas nuclease cleaves the target nucleic acid, thereby modifying the cell.

253. The method of claim 252, wherein the guide nucleic acid is a guide RNA.

254. The method of any one of claims 251-253, wherein the method further comprises introducing a donor polynucleotide to the cell.

255. The method of claim 254, wherein the method comprises inserting the donor polynucleotide into the target nucleic acid at the site of cleavage.

256. The method of any one of claims 251-255, wherein the cell is a eukaryotic cell, preferably a human cell.

257. The method of claim 256, wherein the cell is a T cell.

258. The method of claim 257, wherein the T cell is a CAR-T cell.

259. The method of claim 256, wherein the cell is a stem cell.

260. The method of claim 259, wherein the cell is a hematopoietic stem cell.

261. The method of claim 259, wherein the stem cell is a pluripotent stem cell, preferably an induced pluripotent stem cell.

262. A modified cell obtained or obtainable by the method of any one of claims 251-261.

263. A modified human cell obtained or obtainable by the method of claim 41.

264. A modified cell obtained or obtainable by the method of claim 58.

265. The modified cell of claim 264, wherein the cell is a eukaryotic cell, preferably a human cell.

266. The modified cell of any one of claims 263-265, wherein the cell is a T cell.

267. The modified cell of claim 266, wherein the T cell is a CAR-T cell.

268. The modified cell of any one of claims 263-265, wherein the cell is a stem cell.

269. The modified cell of claim 268, wherein the cell is a hematopoietic stem cell.

270. The modified cell of claim 268, wherein the cell is a pluripotent stem cell, preferably an induced pluripotent stem cell.

271. The use of a CasΦ nuclease to introduce a first modification in a first gene and a second modification in a gene according to the method of any one of claims 179 to 186.

272. The use of a programmable CasΦ nuclease, programmable nuclease or a cas nuclease to modify a cell according to the method of any one of claims 251 to 261.

273. The method of claim 251 or claim 252, wherein the introducing comprises lipid nanoparticle delivery of nucleic acid encoding the programmable CasΦ nuclease, programmable nuclease or cas nuclease and the guide nucleic acid.

274. The method of claim 273, wherein the nucleic acid further comprises a donor polynucleotide.

275. The method of claim 273 or claim 274, wherein the nucleic acid is a viral vector.

276. The method of claim 275, wherein the viral vector is an AAV vector.

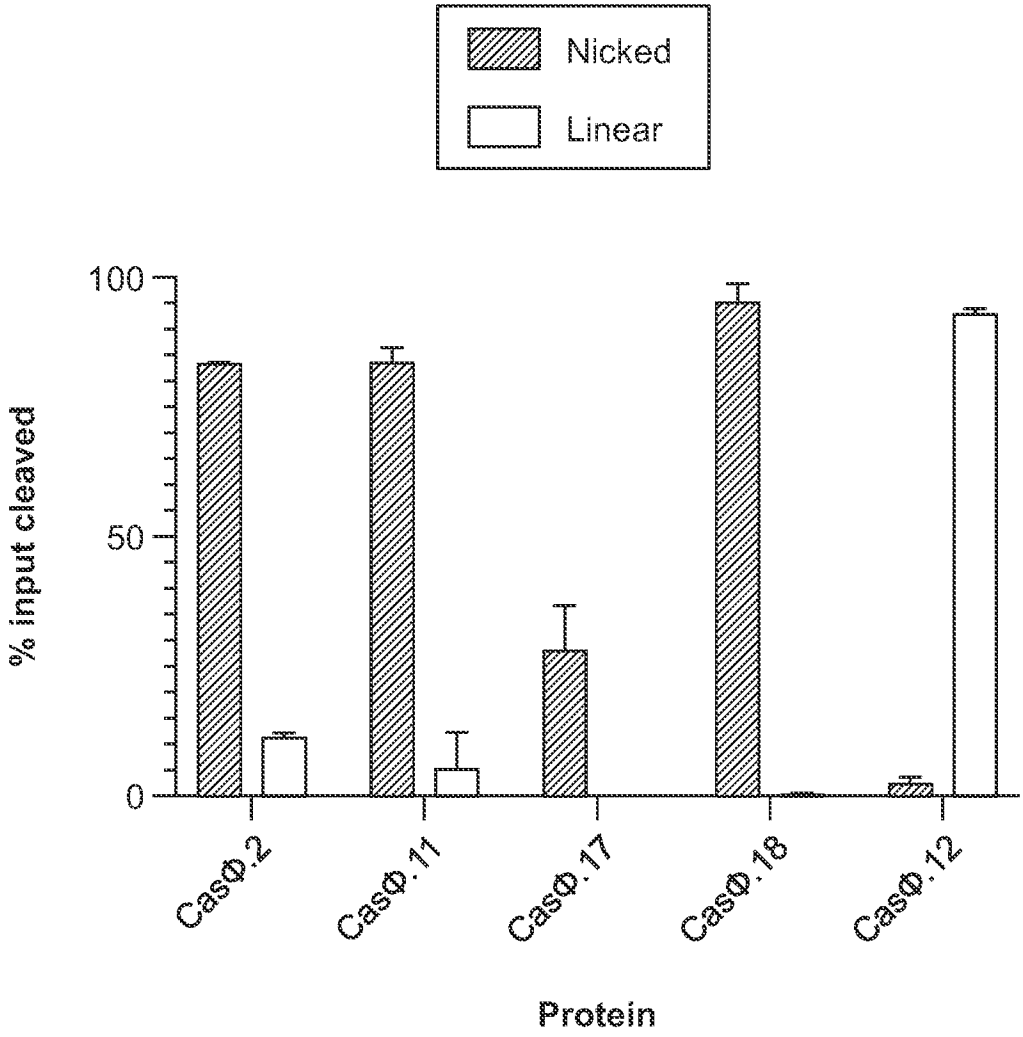

Nicked
Linear
100
50
0
% input cleaved
CasΦ.2
CasΦ.11
CasΦ.17
CasΦ.18
CasΦ.12
Protein

FIG. 1

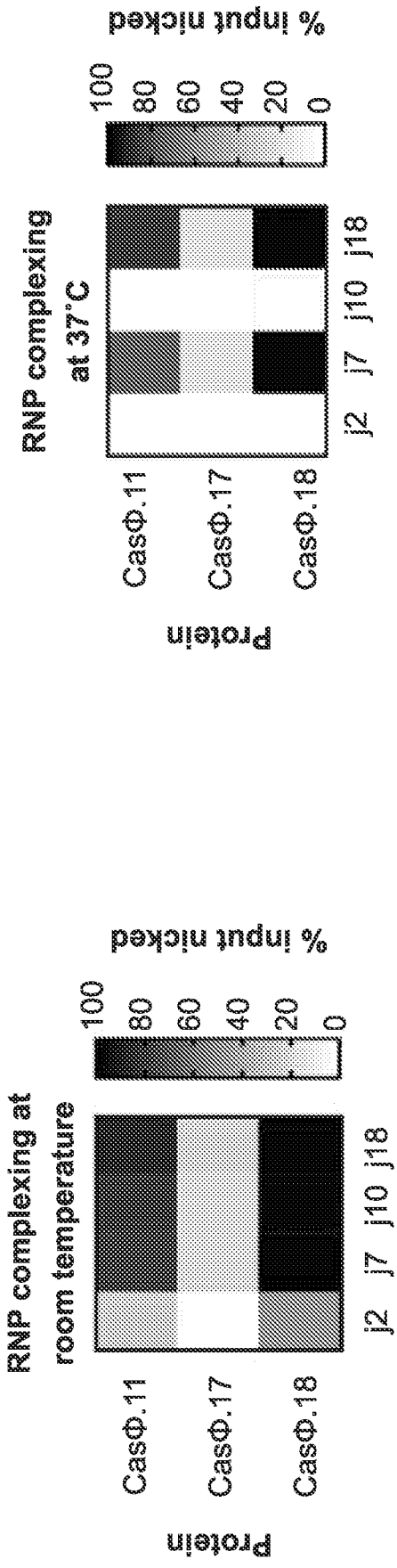


FIG. 2A

FIG. 2B

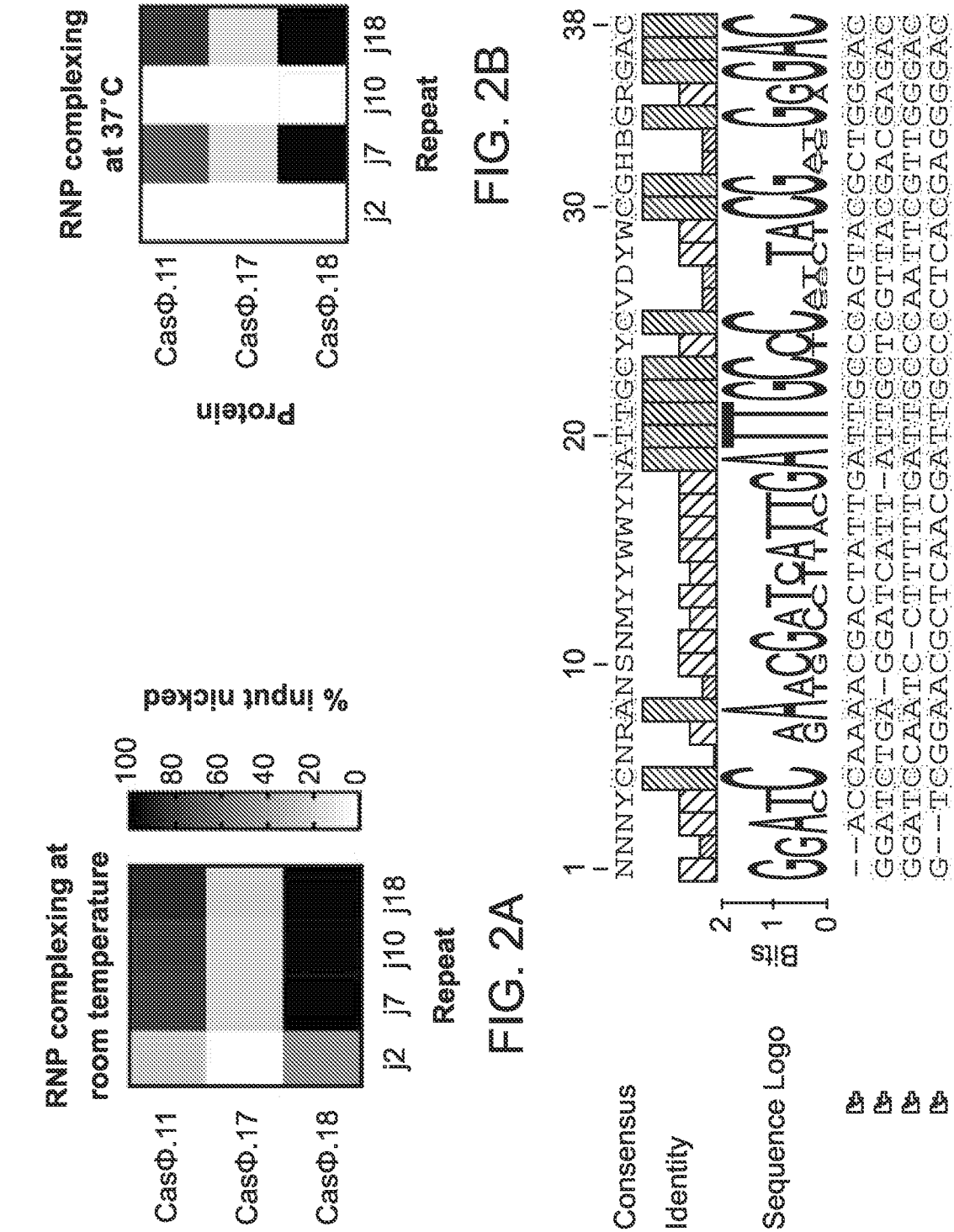


FIG. 2C

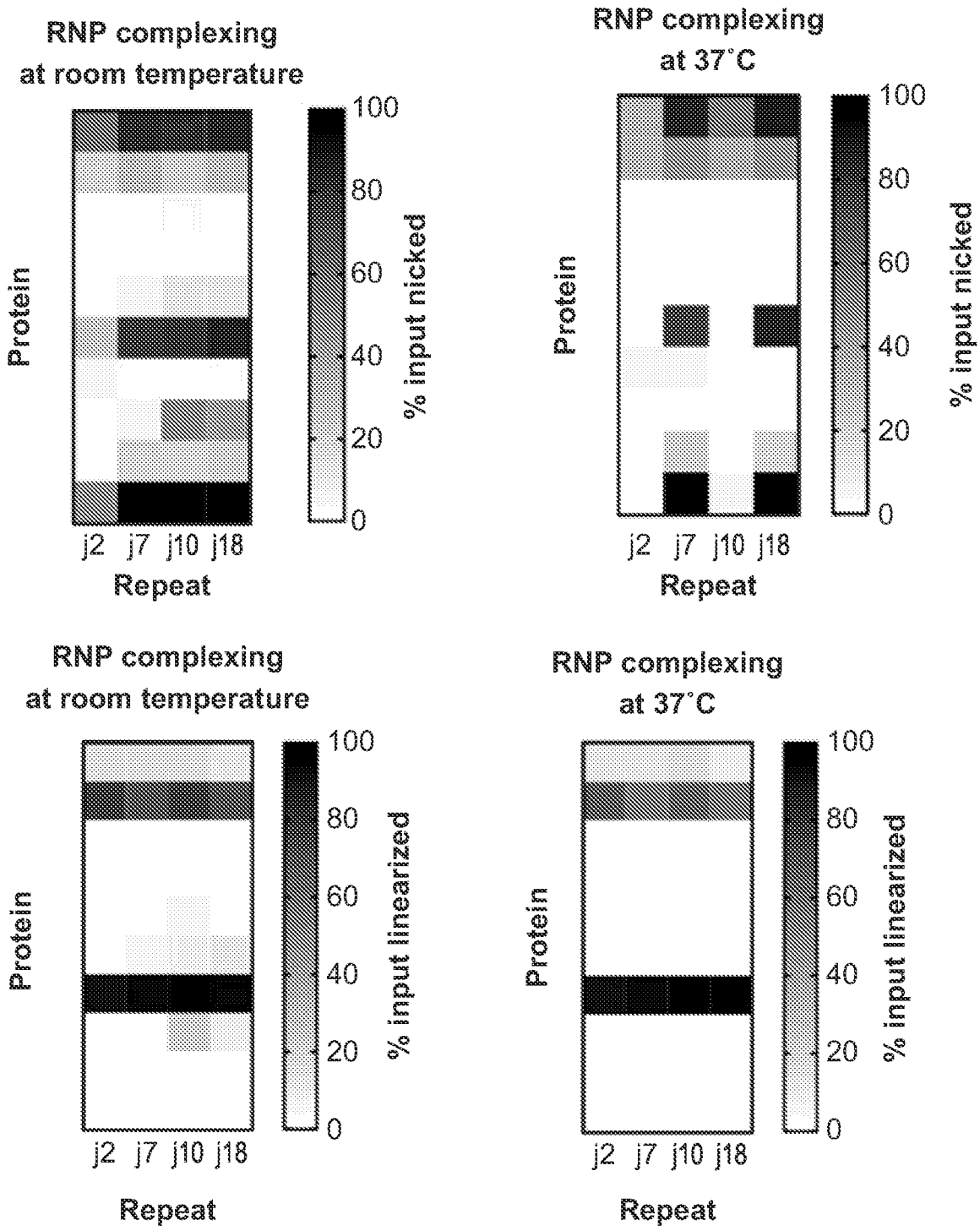

RNP complexing
at room temperature
Protein
j2 j7 j10 j18
Repeat
100
80
60
40
20
0
% input nicked
RNP complexing
at 37˚C
Protein
j2 j7 j10 j18
Repeat
100
80
60
40
20
0
% input nicked
RNP complexing
at room temperature
Protein
j2 j7 j10 j18
Repeat
100
80
60
40
20
0
% input linearized
RNP complexing
at 37˚C
Protein
j2 j7 j10 j18
Repeat
100
80
60
40
20
0
% input linearized

FIG. 2D

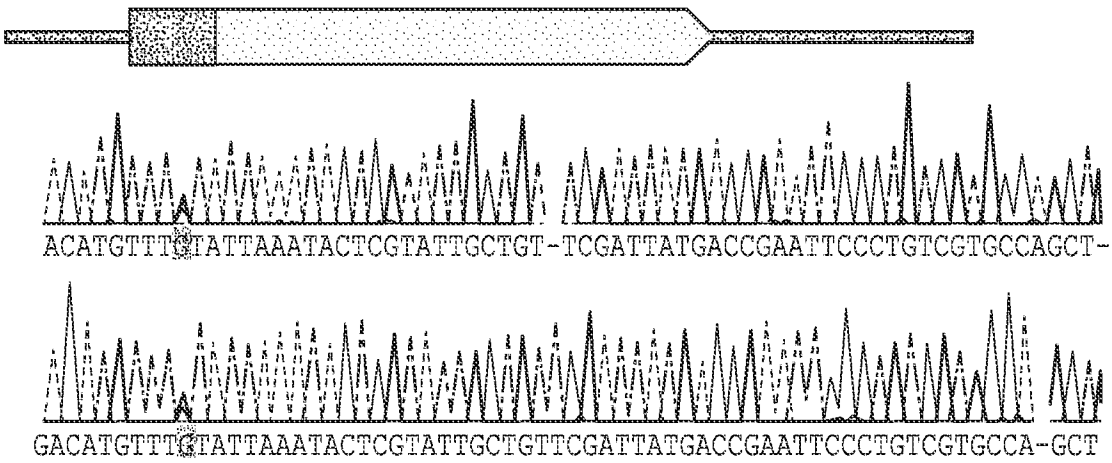


FIG. 3A

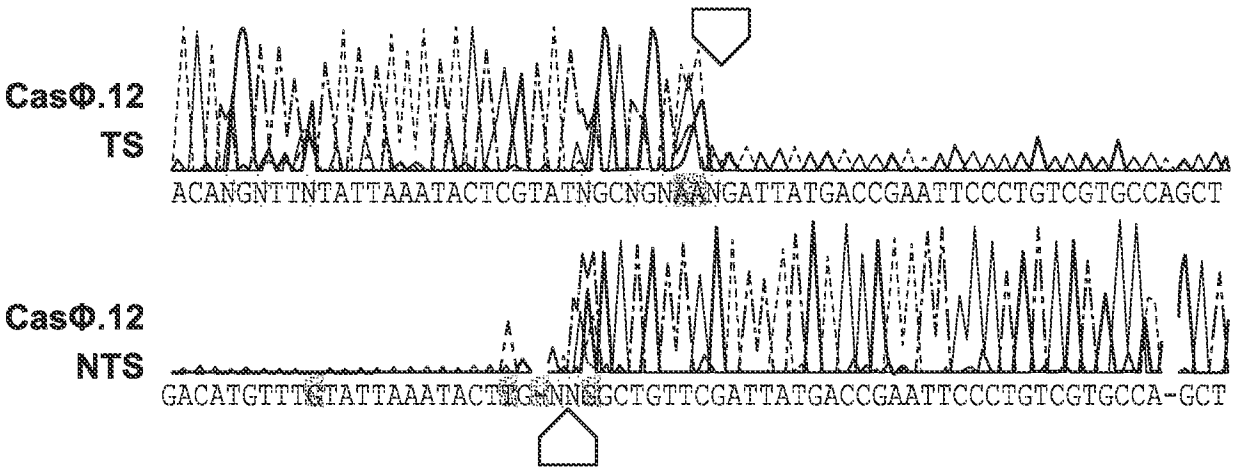


FIG. 3B

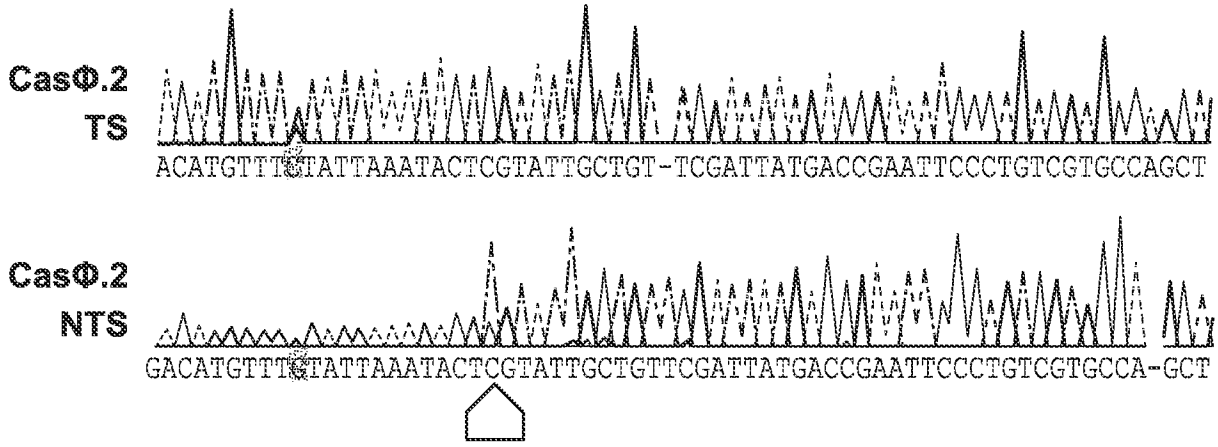


FIG. 3C

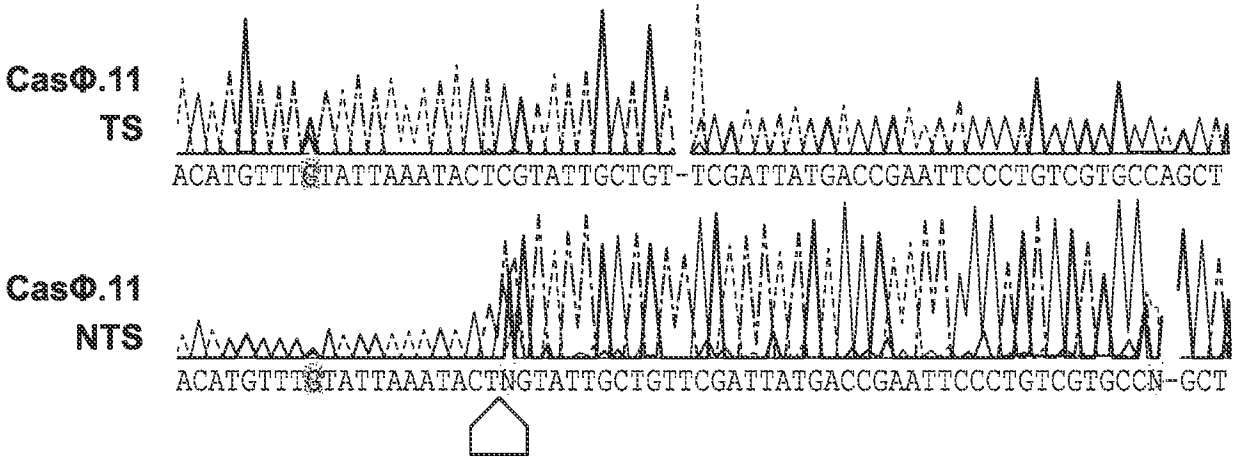

CasΦ.11
TS
ACATGTTTGTATTAAATACTCGTATTGCTGT-TCGATTATGACCGAATTCCCTGTCGTGCCAGCT
CasΦ.11
NTS
ACATGTTTGTATTAAATACTNGTATTGCTGTTCGATTATGACCGAATTCCCTGTCGTGCCN-GCT

FIG. 3D

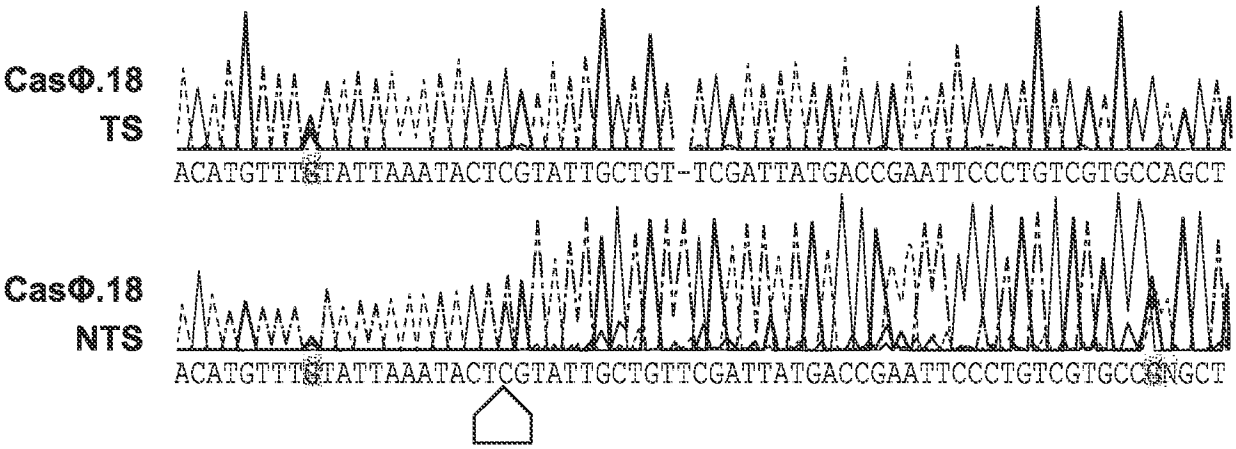

CasΦ.18
TS
ACATGTTTGTATTAAATACTCGTATTGCTGT-TCGATTATGACCGAATTCCCTGTCGTGCCAGCT
CasΦ.18
NTS
ACATGTTTGTATTAAATACTCGTATTGCTGTTCGATTATGACCGAATTCCCTGTCGTGCCNGCT

FIG. 3E

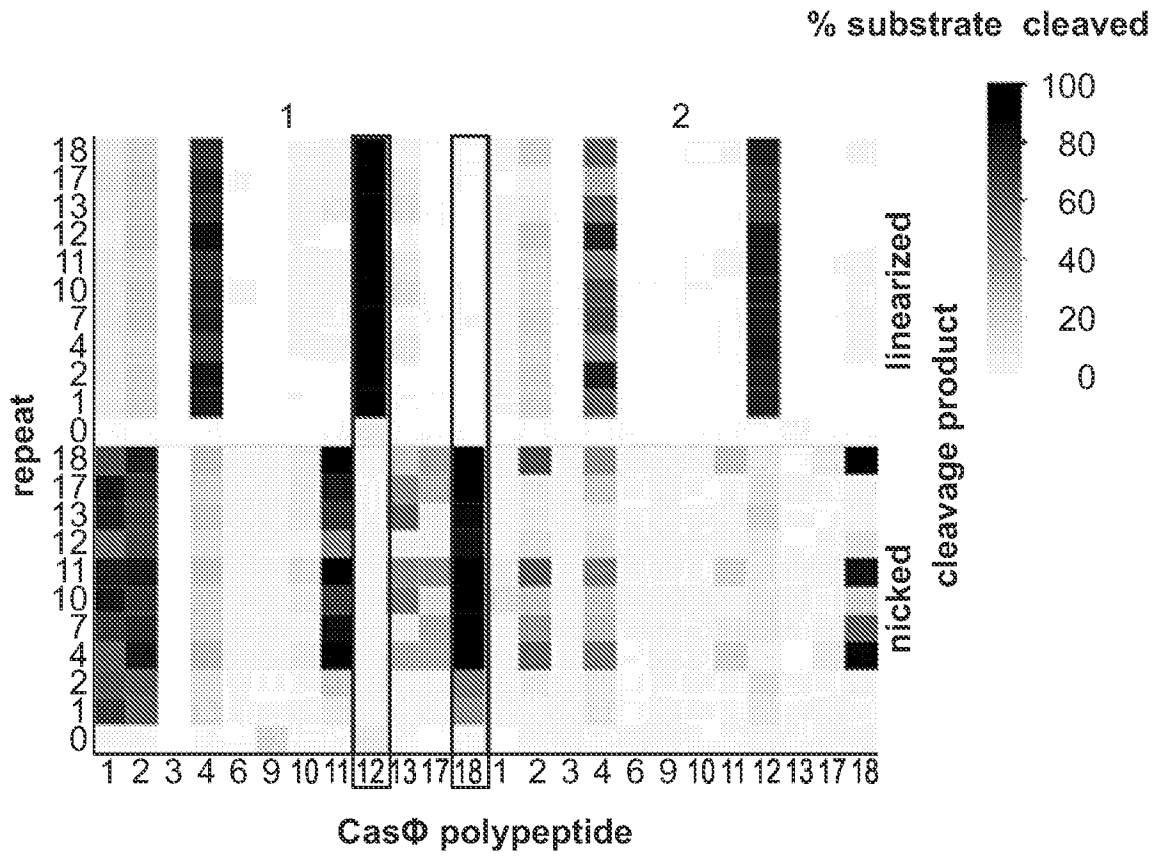

% substrate cleaved
100
80
60
40
20
0
1
2
repeat
linearized
nicked
cleavage product
1 2 3 4 6 9 10 11 12 13 17 18 1 2 3 4 6 9 10 11 12 13 17 18
CasΦ polypeptide

FIG. 4A

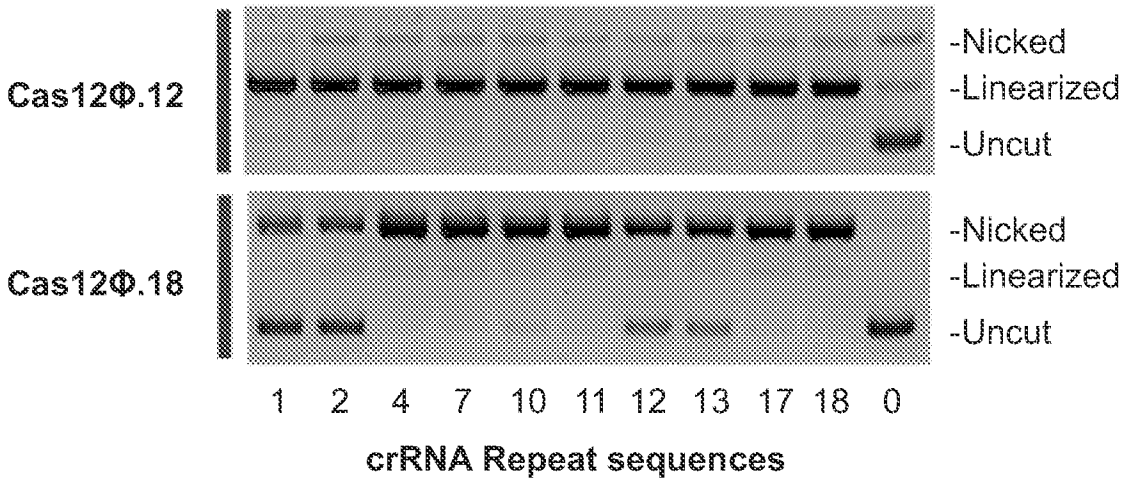

Cas12Φ.12
-Nicked
-Linearized
-Uncut
Cas12Φ.18
-Nicked
-Linearized
-Uncut
1 2 4 7 10 11 12 13 17 18 0
crRNA Repeat sequences

FIG. 4B

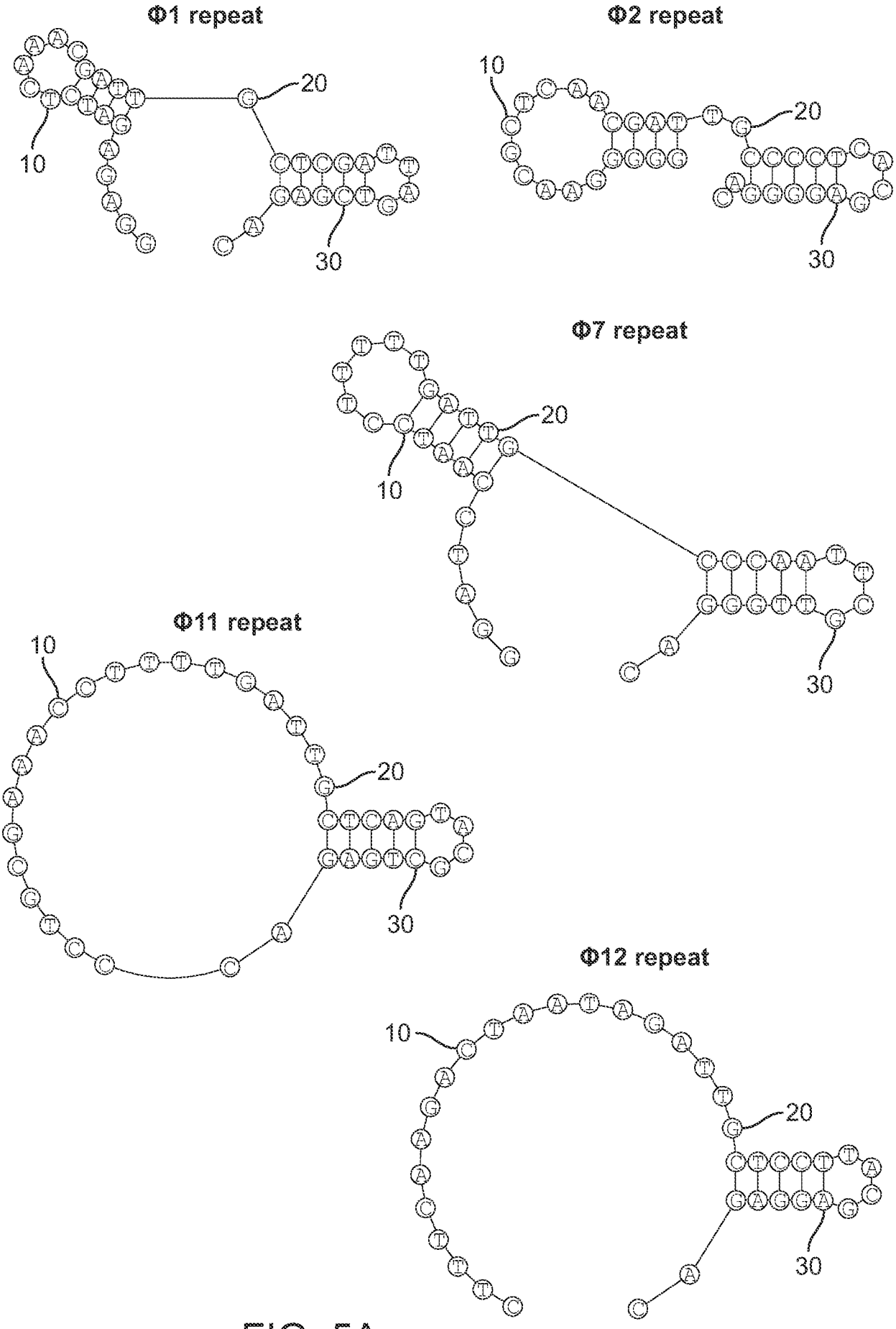

Φ1 repeat
10
20
30
Φ2 repeat
10
20
30
Φ7 repeat
10
20
30
Φ11 repeat
10
20
30
Φ12 repeat
10
20
30

FIG. 5A

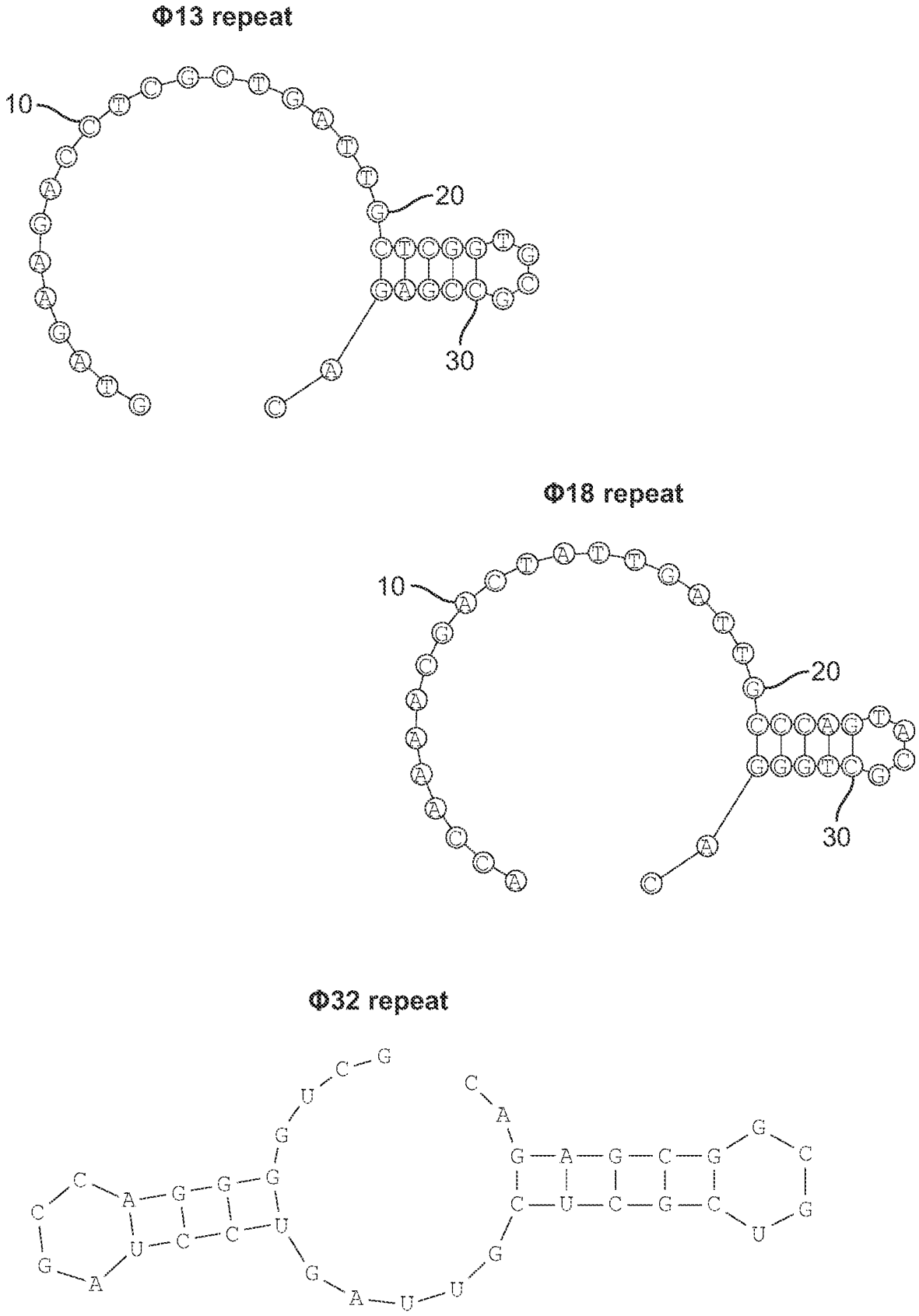


FIG. 5A (Cont.)

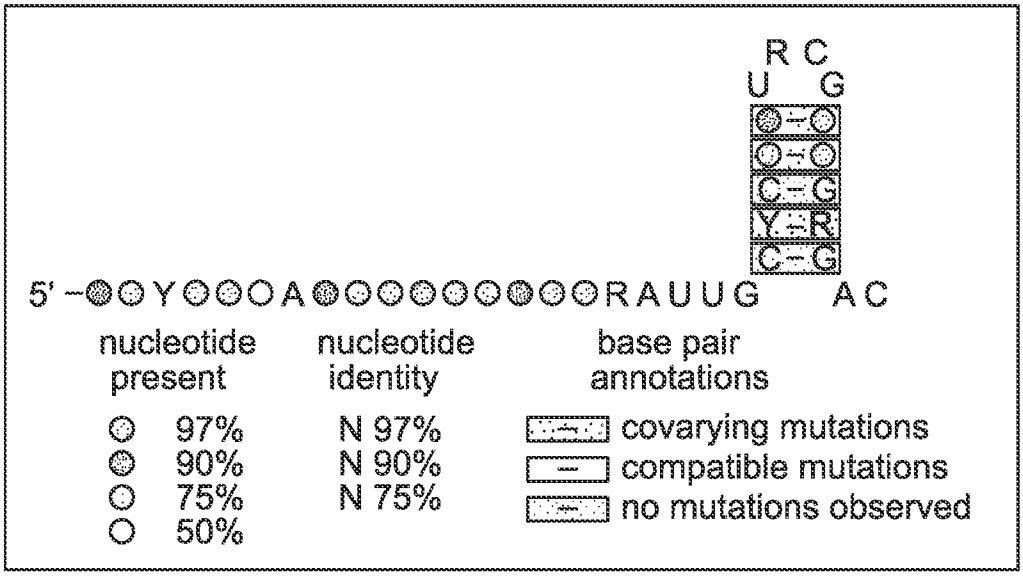

R C
U G
C–G
Y–R
C–G
5' –Y A R A U U G A C
nucleotide present
97%
90%
75%
50%
nucleotide identity
N 97%
N 90%
N 75%
base pair annotations
covarying mutations
compatible mutations
no mutations observed

FIG. 5B

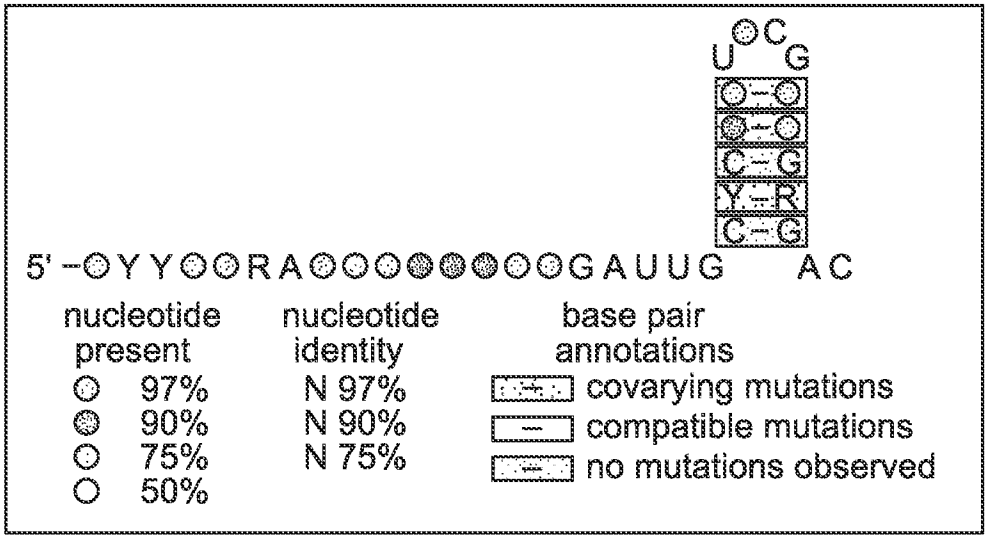

C
U G
C–G
Y–R
C–G
5' –Y Y R A G A U U G A C
nucleotide present
97%
90%
75%
50%
nucleotide identity
N 97%
N 90%
N 75%
base pair annotations
covarying mutations
compatible mutations
no mutations observed

FIG. 5C

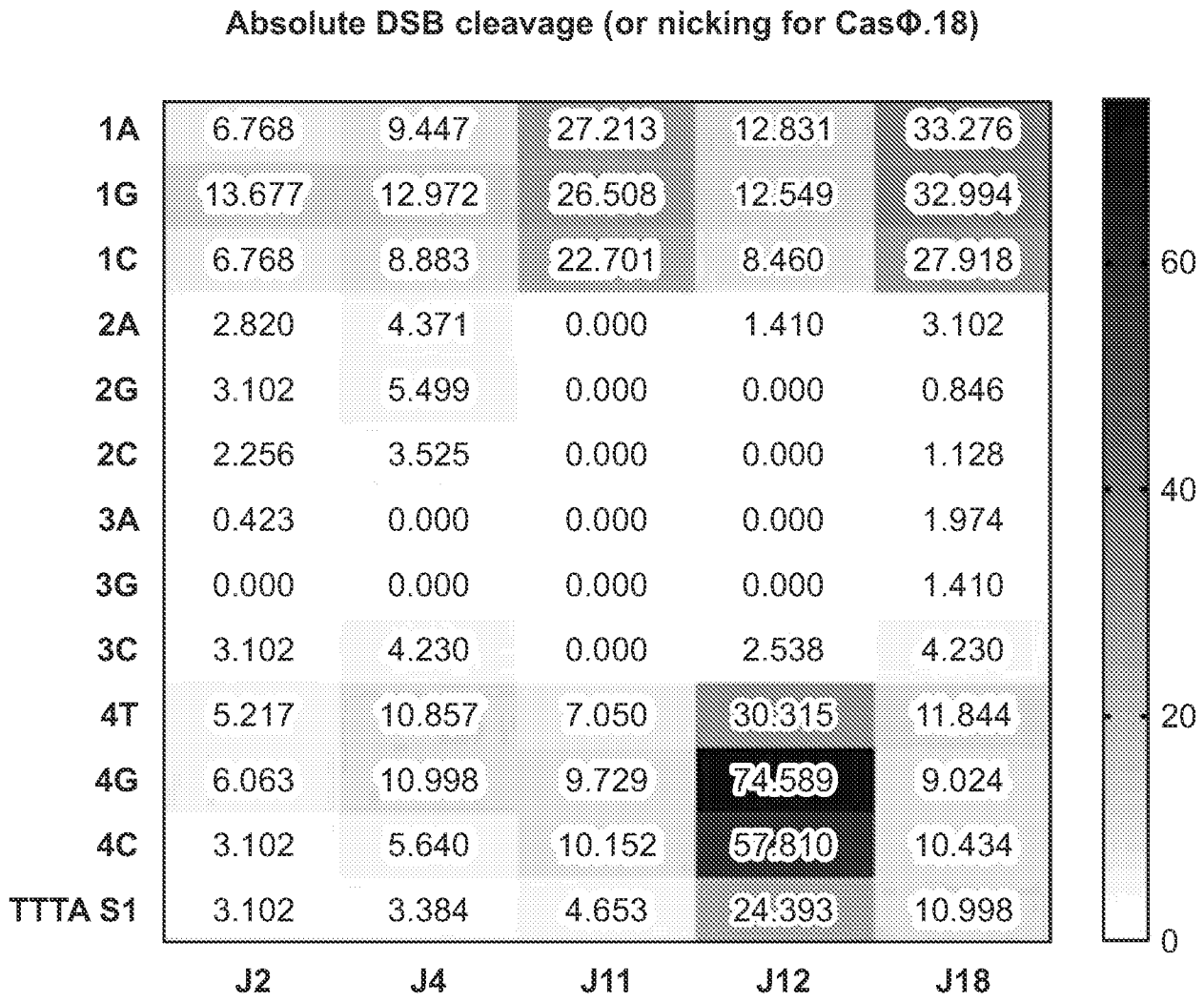

Absolute DSB cleavage (or nicking for CasΦ.18)
1A 6.768 9.447 27.213 12.831 33.276
1G 13.677 12.972 26.508 12.549 32.994
1C 6.768 8.883 22.701 8.460 27.918
2A 2.820 4.371 0.000 1.410 3.102
2G 3.102 5.499 0.000 0.000 0.846
2C 2.256 3.525 0.000 0.000 1.128
3A 0.423 0.000 0.000 0.000 1.974
3G 0.000 0.000 0.000 0.000 1.410
3C 3.102 4.230 0.000 2.538 4.230
4T 5.217 10.857 7.050 30.315 11.844
4G 6.063 10.998 9.729 74.589 9.024
4C 3.102 5.640 10.152 57.810 10.434
TTTA S1 3.102 3.384 4.653 24.393 10.998
J2 J4 J11 J12 J18
60
40
20
0

FIG. 6A

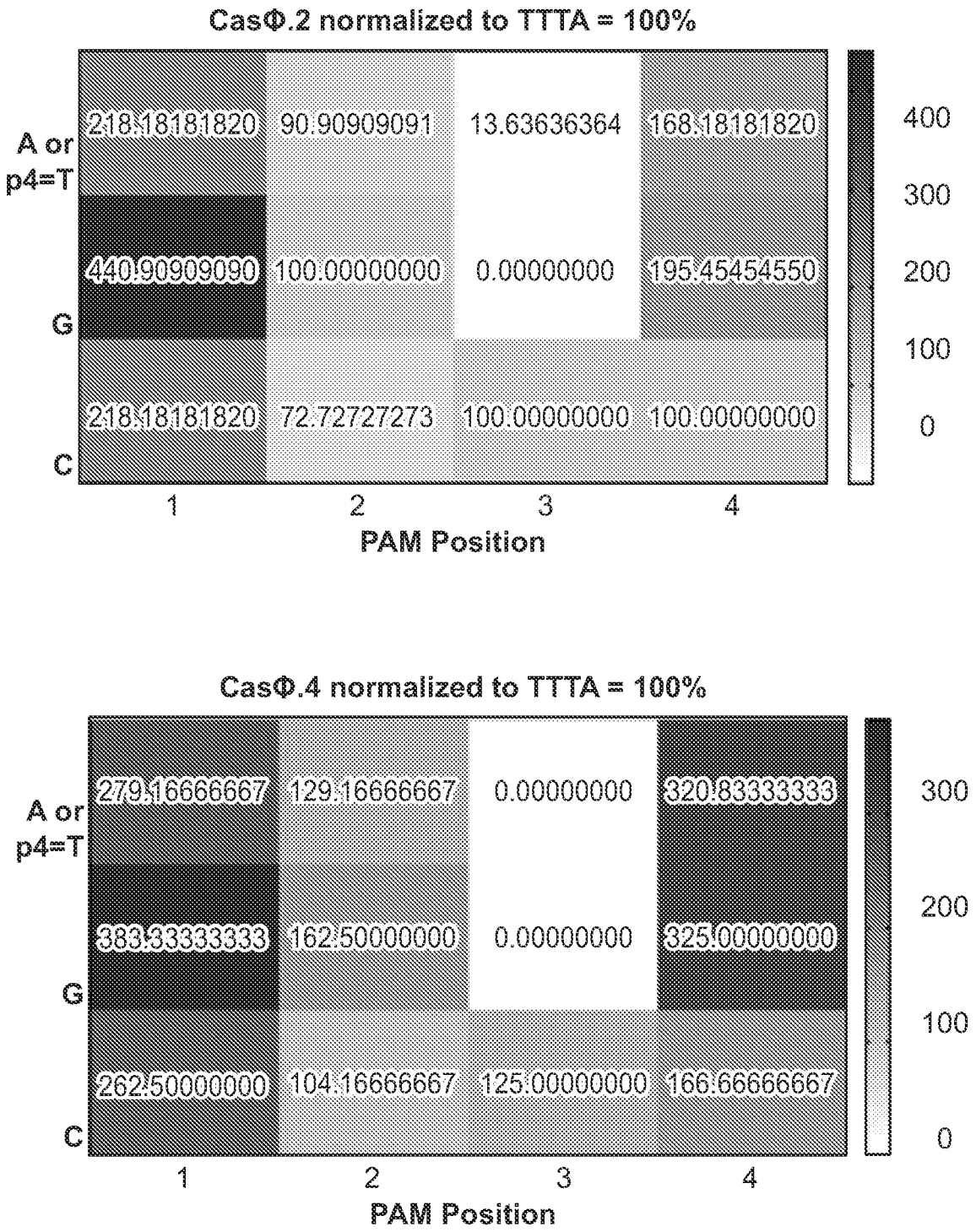

CasΦ.2 normalized to TTTA = 100%
A or p4=T
G
C
218.18181820
90.90909091
13.63636364
168.18181820
440.90909090
100.00000000
0.00000000
195.45454550
218.18181820
72.72727273
100.00000000
100.00000000
1
2
3
4
PAM Position
400
300
200
100
0
CasΦ.4 normalized to TTTA = 100%
A or p4=T
G
C
279.16666667
129.16666667
0.00000000
320.83333333
383.33333333
162.50000000
0.00000000
325.00000000
262.50000000
104.16666667
125.00000000
166.66666667
1
2
3
4
PAM Position
300
200
100
0

FIG. 6B

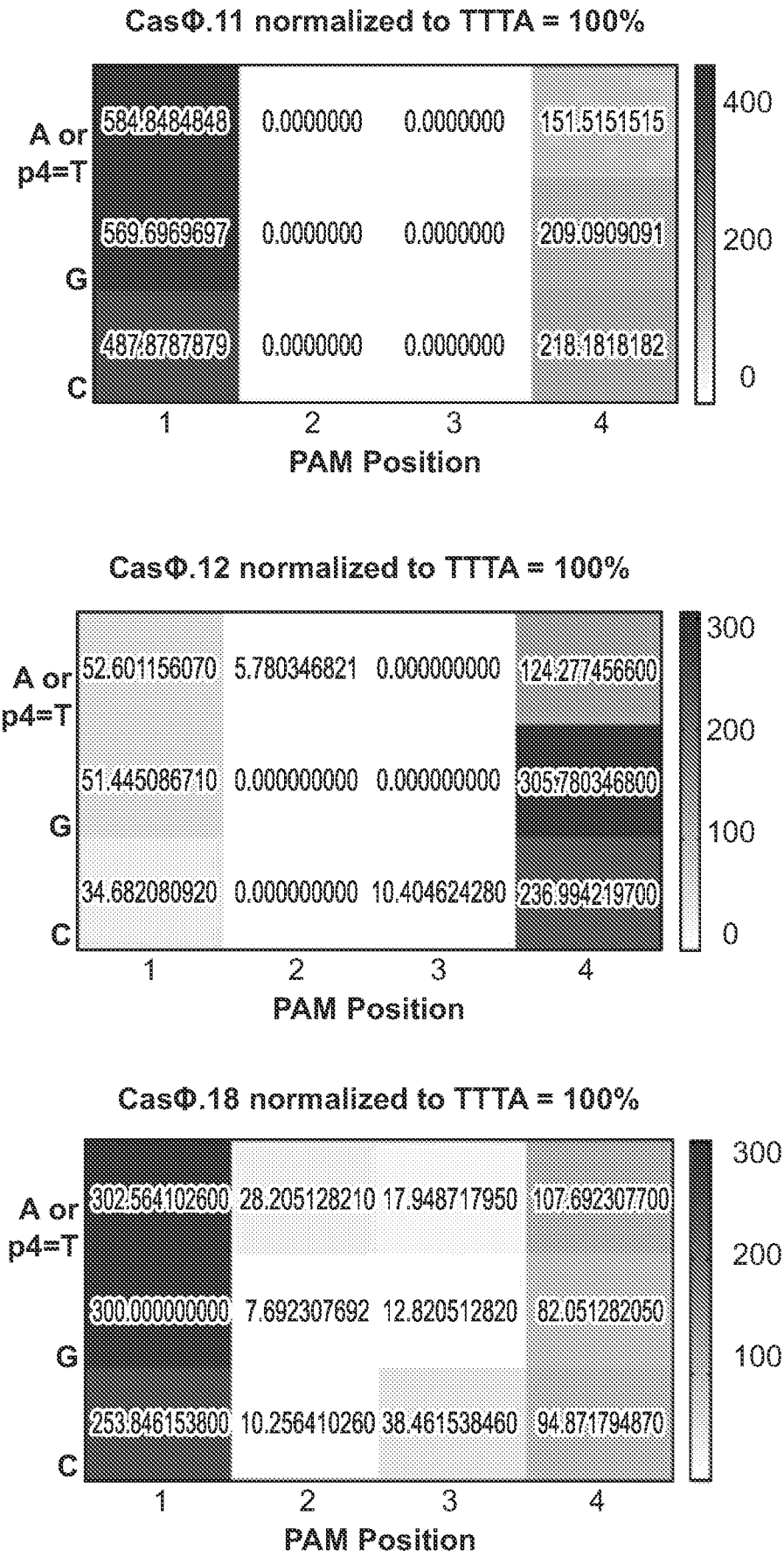

CasΦ.11 normalized to TTTA = 100%
A or p4=T
G
C
584.8484848
0.0000000
0.0000000
151.5151515
569.6969697
0.0000000
0.0000000
209.0909091
487.8787879
0.0000000
0.0000000
218.1818182
400
200
0
1
2
3
4
PAM Position
CasΦ.12 normalized to TTTA = 100%
A or p4=T
G
C
52.601156070
5.780346821
0.000000000
124.277456600
51.445086710
0.000000000
0.000000000
305.780346800
34.682080920
0.000000000
10.404624280
236.994219700
300
200
100
0
1
2
3
4
PAM Position
CasΦ.18 normalized to TTTA = 100%
A or p4=T
G
C
302.564102600
28.205128210
17.948717950
107.692307700
300.000000000
7.692307692
12.820512820
82.051282050
253.846153800
10.256410260
38.461538460
94.871794870
300
200
100
1
2
3
4
PAM Position

FIG. 6B (Cont.)

Protein	Optimal PAM
CasΦ_2	GTTK
CasΦ_4	VTTK
CasΦ_11	VTTS
CasΦ_12	TTTS
CasΦ_18	VTTN

K = G,T
S = C,G
V = A,C,G
N = A,C,G,T

FIG. 6C

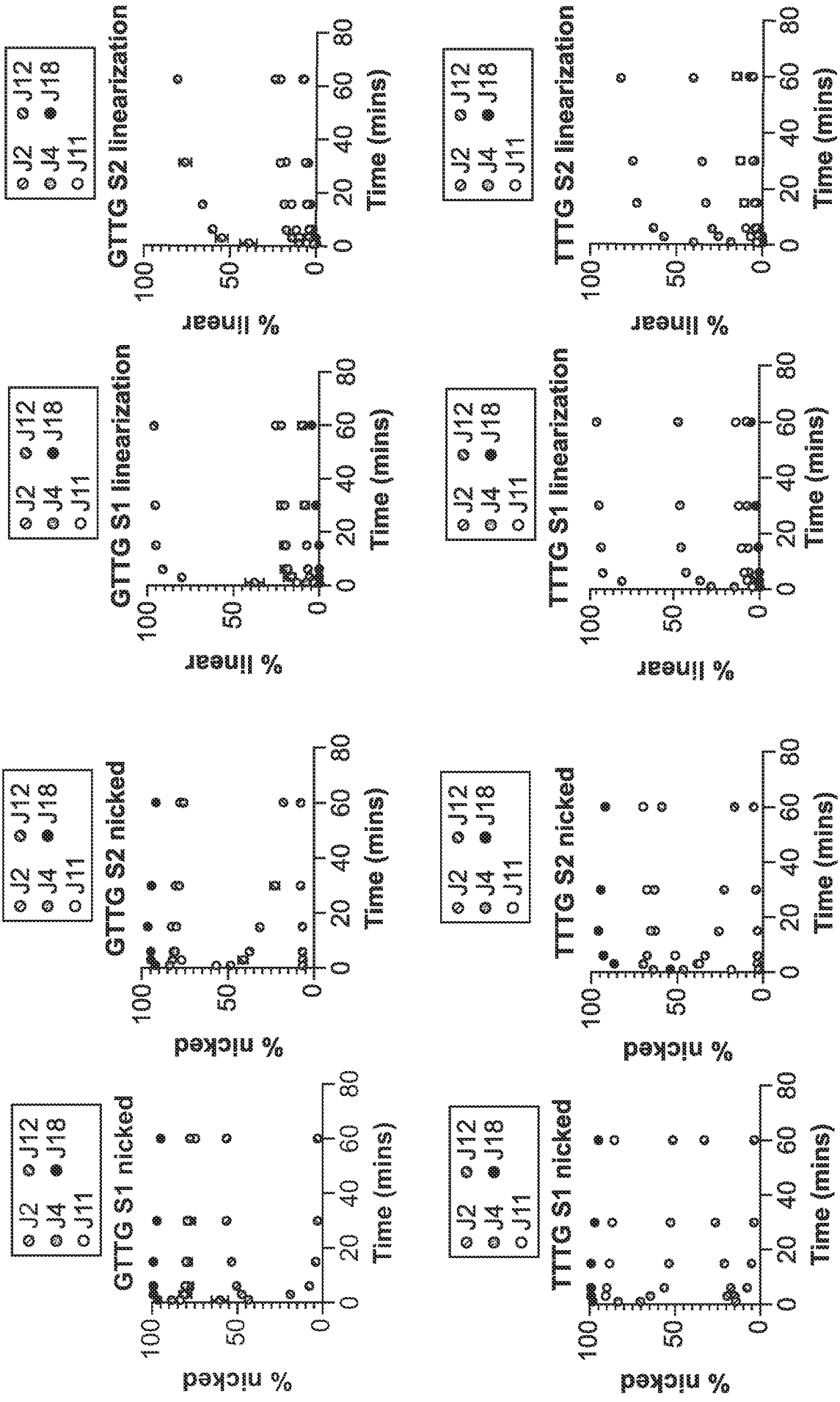

GTTG S1 nicked
TTTG S1 nicked
GTTG S2 nicked
TTTG S2 nicked
GTTG S1 linearization
TTTG S1 linearization
GTTG S2 linearization
TTTG S2 linearization
% nicked
% linear
Time (mins)
J2
J4
J11
J12
J18

FIG. 7

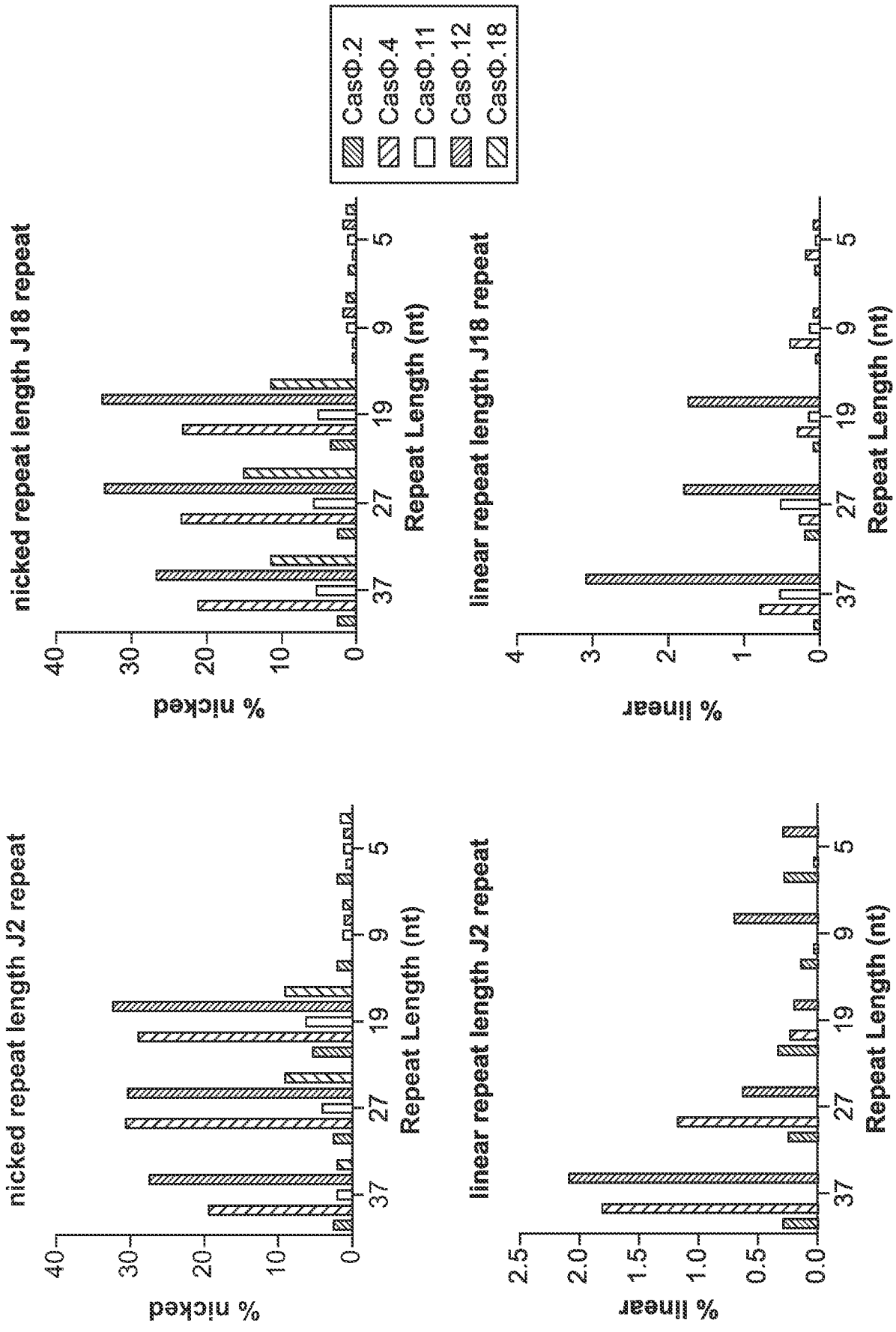

nicked repeat length J18 repeat
% nicked
Repeat Length (nt)
linear repeat length J18 repeat
% linear
Repeat Length (nt)
nicked repeat length J2 repeat
% nicked
Repeat Length (nt)
linear repeat length J2 repeat
% linear
Repeat Length (nt)
CasΦ.2
CasΦ.4
CasΦ.11
CasΦ.12
CasΦ.18

FIG. 8A

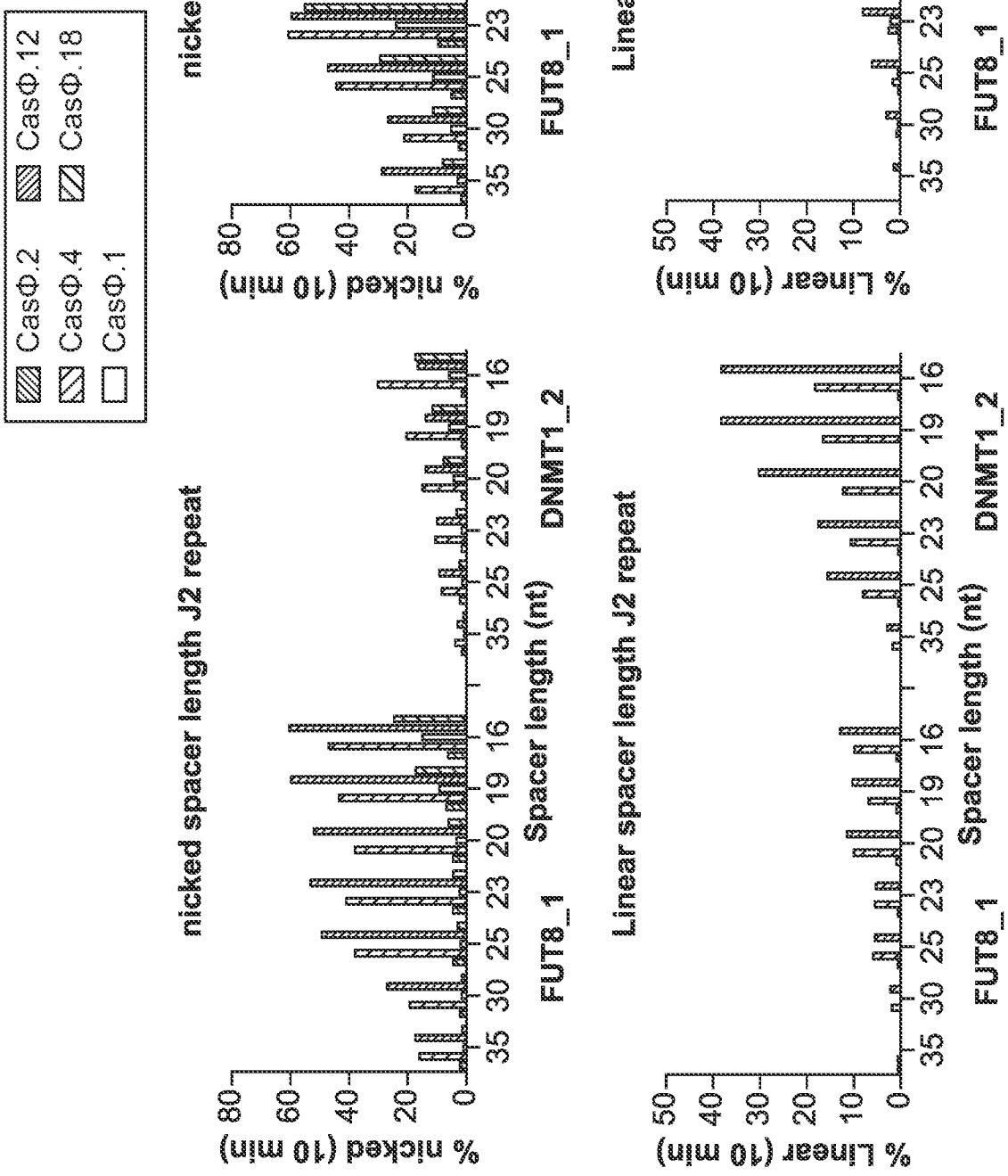

CasΦ.2
CasΦ.4
CasΦ.1
CasΦ.12
CasΦ.18
nicked spacer length J18 repeat
% nicked (10 min)
Spacer length (nt)
FUT8_1
DNMT1_2
Linear spacer length J18 repeat
% Linear (10 min)
nicked spacer length J2 repeat
Linear spacer length J2 repeat

FIG. 8B

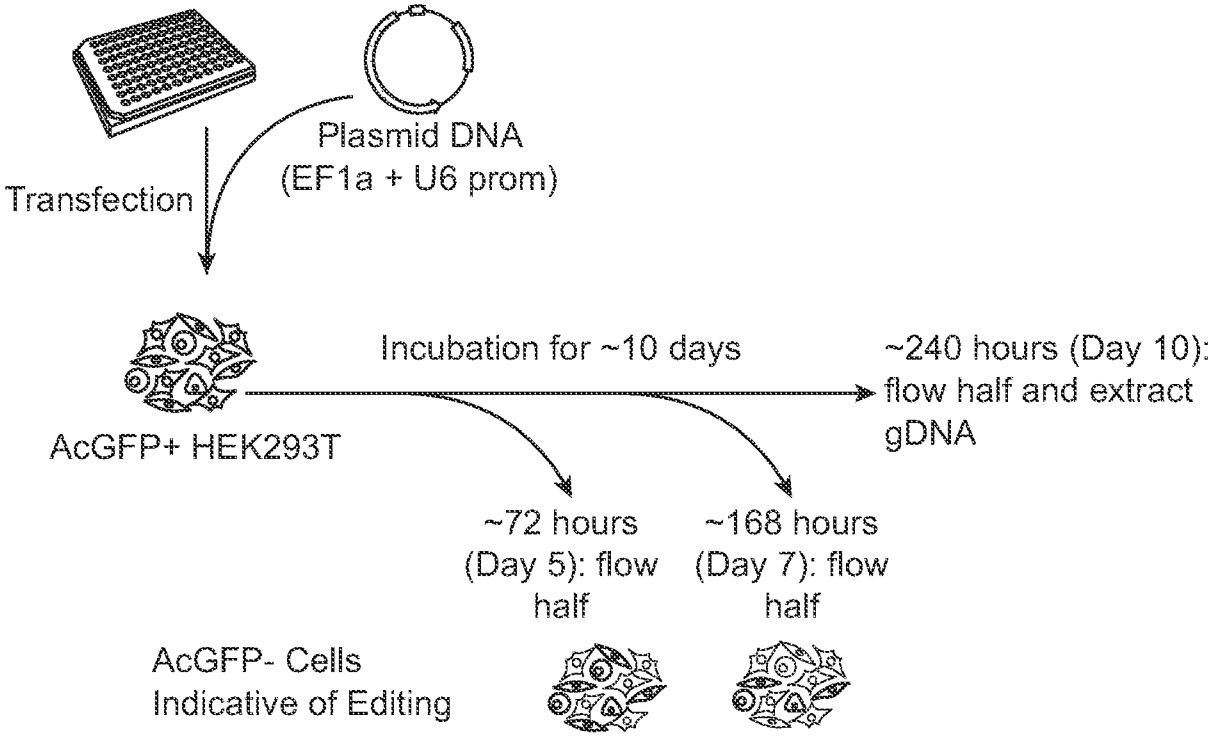

Plasmid DNA
(EF1a + U6 prom)
Transfection
Incubation for ~10 days
~240 hours (Day 10): flow half and extract gDNA
AcGFP+ HEK293T
~72 hours (Day 5): flow half
~168 hours (Day 7): flow half
AcGFP- Cells Indicative of Editing

FIG. 9A

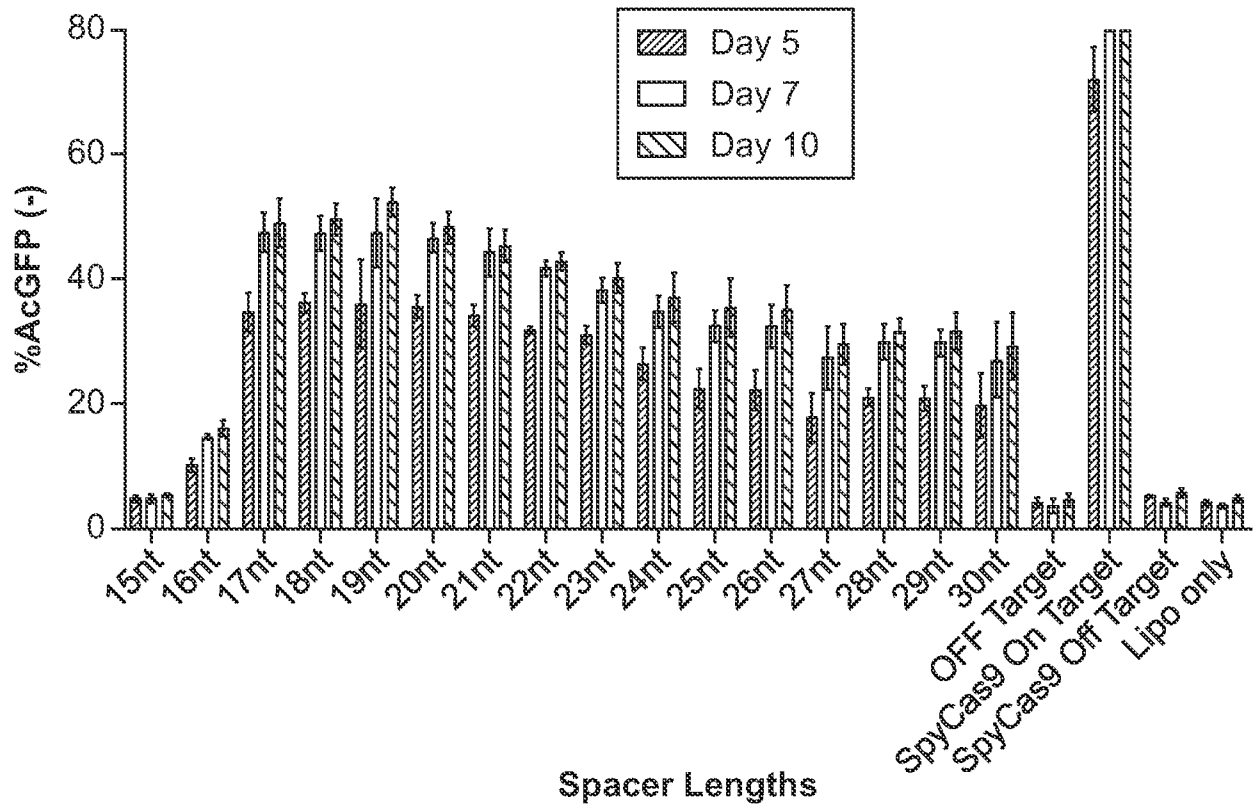

Day 5
Day 7
Day 10
%AcGFP (-)
0
20
40
60
80
15nt
16nt
17nt
18nt
19nt
20nt
21nt
22nt
23nt
24nt
25nt
26nt
27nt
28nt
29nt
30nt
OFF Target
SpyCas9 On Target
SpyCas9 Off Target
Lipo only
Spacer Lengths

FIG. 9B

Overview
Entries 1
Sequence
InterProScan Search Result
Title
SEQ ID NO 2
Job ID
iprscan5-R20200609-201723-0087-81535480-p2m
Length
757 amino acids
Action
Status
finished
Protein family membership
None predicted
Entry matches to this protein
Colour By: Accession
Collapse All
Display
Export
1
100
200
300
400
500
600
700
757
Domain
Predictions
IPR010095
PF07282
mobidb-lite (1)
mobidb-lite (2)
Coil (3)

FIG. 10A

Overview
Entries 4
Sequence
InterProScan Search Result
Title
Asp sp.Cpf1
Job ID
iprscan5-R20200609-200020-0653-62825152-p2m
Length
1307 amino acids
Action
Status
finished
Protein family membership
CRISPR -associated endonuclease Cas12a (IPR027620)
Entry matches to this protein
Colour By: Accession
Collapse All
Display
Export
1
100
200
300
400
500
600
700
800
900
1,000
1,100
1,200
1,300
1307
500
1000
Family
Domain
IPR027620
TIGR04330
IPR040852
PF18516
IPR040882
PF18510
IPR040787
PF18501

FIG. 10B

```
HMMER3/f [3.1b2 I February 2015]
NAME  OrfB_Zn_ribbon
ACC   PF07282.12
DESC  Putative transposase DNA-binding domain
LENG  69
ALPH  amino
RF    no
MM    no
CONS  yes
CS    no
MAP   yes
DATE  Wed Apr 22 09:44:42 2020
NSEQ  54
EFFN  4.670288
CKSUM 986868954
GA    26.30 26.30 ;
TC    26.30 26.30 ;
NC    26.20 26.20 ;
BM    hmmbuild HMM.ann SEED.ann
SM    hmmsearch -z 47079205 -E 1000 --cpu 4 HMM pfamseq
STATS LOCAL MSV       -  9.1774  0.71875
STATS LOCAL VITERBI   -  9.2725  0.71875
STATS LOCAL FORWARD   -  4.2633  0.71875
HMM     A     C     D     E     F     G     H     I     K     L     M     N     P     Q     R     S     T     V     W     Y
        m->m  m->i  m->d  i->m  i->i  d->m  d->d
COMPO 2.51685 3.05041 3.00924 2.67502 3.46385 2.89097 3.66927 2.99268 2.57262 2.69875 3.96709 2.90797 3.64145 3.14274 2.76568 2.59594 2.86953 2.76494 4.51641 3.37755
      2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73139 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
      0.01739 5.26646 4.40710 0.61958 0.77255 0.00000       *
    1 2.59421 5.43676 5.62081 5.23623 1.66218 5.11093 2.59667 3.95914 5.04271 2.77635 4.58762 4.93760 5.42808 4.98845 5.66714 4.45252 1.40339 3.80774 1.74892 1.98727  1 f - - .
      2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
      0.00760 5.27666 6.00102 0.61958 0.77255 0.46390 0.99169
```

FIG. 11

```
2  2.34855 5.49449 2.91304 2.70751 3.75662  2.26017  3.45327 4.30986 2.18998 3.79564 4.53759 2.80120 4.17641 3.07853 2.13565 2.22500 2.94085 3.88018 5.93272 3.28328 2  r  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 3.58471 3.61503
   0.00753 5.26646 6.01081 0.61958 0.77255  0.48576  0.95510
3  2.75943 5.51157 3.13164 2.06597 4.85478  3.47646  3.96709 3.94817 1.68329 3.44021 4.17473 3.09392 4.17331 2.56410 2.25438 2.31506 2.77362 3.90121 5.94535 3.66482 3  k  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26646 6.01081 0.61958 0.77255  0.48576  0.95510
4  3.59275 4.91710 5.56423 4.95986 1.38444  4.82609  5.15870 2.32706 4.76708 1.19220 2.98351 4.99047 5.10526 4.82667 4.75819 4.15582 3.04756 2.11200 5.51674 4.40627 4  1  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26646 6.01081 0.61958 0.77255  0.48576  0.95510
5  2.90988 5.17770 3.44270 2.51257 4.40485  3.57719  3.35914 2.52800 2.15119 2.44260 3.42963 3.39890 4.24650 2.24405 2.46620 3.06248 2.67322 3.08132 4.49416 3.93115 5  k  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26846 6.01081 0.61958 0.77255  0.48576  0.95510
6  2.56424 5.50936 3.02009 1.95369 3.95812  2.88052  3.37131 3.93380 2.44202 3.47691 3.52559 2.51325 4.17357 2.41510 2.34207 2.86908 2.64094 3.89850 5.94373 3. 95501 6  e  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26846 6.01081 0.61958 0.77255  0.48576  0.95510
7  2.62483 4.05956 4.07864 3.08563 2.52078  4.06126  4.32936 2.75794 2.61177 2.68453 2.87971 3.86588 4.43761 2.34347 2.52116 3.00013 3.27465 2.72128 3.34240 2.55429 7  q  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26846 6.01081 0.61958 0.77255  0.48576  0.95510
8  4.29426 5.47911 6.34784 5.80324 3.98232  5.80272  6.22401 1.30628 5.69818 0.82107 2.81794 5.98170 5.84917 5.61019 5.66793 5.2 1738 4.51437 1.93965 6.22312 5.24562 8  1  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26846 6.01081 0.61958 0.77255  0.48576  0.95510
9  2.50444 5.50403 3.07553 1.37663 4.84391  3.78163  3.96914 3.45620 1.99739 3.80703 3.80611 3.25112 4.17498 2.60522 2.77943 2.52974 3.08509 3.66851 5.93977 4.53212 9  e  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.26846 6.01081 0.61958 0.77255  0.48576  0.95510
10 2.87793 4.06620 2.90943 2.49665 3.93498  3. 51811 4.19987 3.34443 2.96365 3.00211 4.14293 2.70611 4.28056 3.13278 3.18796 3.10686 3.25311 3.36787 3.94746 1.31311 10 y  -  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354  2.40513  3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255  0.48576  0.95510
```

FIG. 11 (Cont. 1)

11 3.03566 5.15446 3.49110 2.86608 3.72582 3.88327 4.08615 3.49085 0.93271 3.15424 4.23096 3.43604 4.27076 2.83864 3.33395 3.09329 3.01605 2.92857 5.66822 3.86192 11 k - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 1.58177 3.61503
0.00753 5.28846 6.01061 0.61958 0.77255 0.48576 0.95510
12 0.96876 2.54237 4.23999 3.68508 3.94950 2.99042 4.48372 3.10057 3.61660 2.69845 3.93373 3.98228 4.47968 3.59764 3.90996 2.69134 2.96146 3.07050 5.41821 4.28701 12 a - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
13 2.67743 5.49487 3.14846 1.93627 4.83094 3.78296 3.65042 3.83839 1.67719 2.80020 4.06118 3.13067 3.77061 2.41958 2.72879 2.86667 2.86801 3.16101 5.93297 4.05753 13 k - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.04584 5.28846 3.22496 0.61958 0.77255 0.48576 0.95510
14 2.86278 5.35695 2.90563 1.53478 4.20177 3.78266 3.97501 3.86335 2.32864 2.68823 3.50044 3.11021 4.17494 2.99757 2.37949 2.85297 3.22130 3.52761 2.92577 4.46498 14 e - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00782 5.25045 5.97279 0.61958 0.77255 0.61711 0.77544
15 2.33025 4.45495 3.33409 2.65448 2.40540 3.88293 3.66494 3.59562 2.55167 2.79029 4.10009 3.31088 4.26946 3.17022 2.43803 3.09831 3.23512 3.31970 4.40518 1.87947 15 y - - -
2.68619 4.42226 2.77521 2.73124 3.46355 2.40514 3.72496 3.29355 2.67742 2.69356 4.24691 2.93348 2.73741 3.18147 2.89802 2.37888 2.77521 2.98519 4.58478 3.61460
0.03070 3.58681 5.97279 0.39588 1.11806 0.61711 0.77544
16 3.12789 5.67204 3.28025 2.76211 5.43893 0.50455 4.66675 4.90924 3.55977 4.45907 5.29100 3.32482 4.60767 3.84731 3.47259 3.13331 3.83520 4.42112 6.63363 5.26243 17 G - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00782 5.25045 5.97279 0.61958 0.77255 0.49813 0.93565
17 3.04534 4.54622 4.42423 3.83968 3.65300 3.50653 4.42847 1.41051 2.74209 2.39094 2.88391 3.55119 4.50358 3.96408 2.79792 3.17534 3.27722 1.82821 5.15504 3.95935 18 i - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 1.58477 3.61503
0.00766 5.27168 5.99403 0.61958 0.77255 0.54651 0.86504
18 2.87230 5.49318 2.95364 2.01695 4.15343 3.77359 3.63936 3.98773 1.83813 2.69207 4.53549 3.10614 2.43480 2.59305 3.03928 2.58919 2.89656 3.37303 5.92863 4.52249 19 k - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00766 5.27168 5.99403 0.61958 0.77255 0.44957 1.01585
19 3.08100 4.46612 5.03010 4.41867 3.15857 4.26415 4.59956 2.33477 4.21152 2.24257 3.14608 4.41157 4.62581 4.34855 4.23064 3.20686 2.54647 0.97597 5.09892 3.08142 20 v - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
20 3.01638 5.18607 3.09263 1.89649 3.70297 3.85496 3.63569 2.06106 2.49467 3.03278 4.25931 3.39411 4.24427 2.85666 2.99621 2.96229 2.79668 2.10878 5.69487 4.36689 21 e - - -
2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.93347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510

FIG. 11 (Cont. 2)

```
21 2.60118 5.02242 3.57194 1.97818 3.43219 3.58669 3.76722 3.21471 2.31941 2.28885 4.10853 3.49998 4.29233 3.00840 3.26572 3.12231 2.66717 2.61617 5.56225 2.39107 22 e - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
22 2.40155 4.56403 4.45463 3.87490 3.67957 3.04389 4.45950 2.02204 3.75918 2.76645 3.68584 4.10794 3.85862 3.36458 3.47571 3.43235 3.29836 1.07273 5.18328 3.98821 23 v - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67711 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
23 3.00896 4.55311 1.80936 2.59496 4.84566 3.28857 3.97221 4.32798 2.58483 3.60946 4.54901 1.77200 3.42849 3.07061 3.03600 2.09979 3.03612 3.42158 5.94275 4.53496 24 n - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
24 2.79798 5.49014 3.29740 1.75718 4.81952 3.81551 4.60629 3.93265 2.52531 3.78760 4.54305 3.29583 1.36840 3.11231 2.68778 2.78443 3.11669 3.87399 5.93574 4.54520 25 p - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
25 1.50542 3.77739 3.66760 2.61957 3.17147 3.93972 3.48483 3.47900 3.08109 3.15863 4.02470 3.13230 4.32402 3.40878 2.66468 2.63877 3.25959 3.22436 3.28971 2.51084 26 a - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
26 3.04676 4.97415 3.21007 2.96503 2.66902 3.13549 3.58691 3.52993 3.07516 3.01463 4.06559 2.48718 4.33257 3.40449 3.29236 3.17106 3.27821 3.27105 4.17777 1.29748 27 y - - -
   2.68599 4.42235 2.77530 2.73133 3.46364 2.40523 3.72505 3.29364 2.67751 2.69365 4.24700 2.90357 2.73658 3.18156 2.89811 2.37897 2.77491 2.98528 4.58487 3.61513
   0.21638 1.64963 6.01081 0.10645 2.29266 0.48576 0.95510
27 3.14716 5.18103 4.48841 4.31633 5.57769 3.84275 5.40780 5.05927 4.45805 4.72093 5.50469 3.32330 4.65244 4.69761 4.72766 1.45784 0.59423 4.32898 6.89303 5.71885 29 T - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
28 3.25075 5.17378 4.88271 4.88931 5.93563 2.79144 5.87421 5.52685 5.14089 5.18695 5.94740 4.65466 4.78410 5.26462 5.25851 0.23285 3.83615 4.59695 7.21569 6.16123 30 S - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
29 3.04439 5.37069 3.37716 2.81463 4.65566 3.84369 4.02732 2.94092 1.46956 3.64527 4.43061 3.34409 4.23343 1.77045 2.92252 2.65564 2.77039 2.76399 5.83746 4.47927 31 k - - -
   2.68618 4.42225 2.77519 2.73123 3.46351 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
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FIG. 11 (Cont. 3)

```
30 3.00747 5.43830 2.70974 2.10734 4.75135 3.79417 3.93487 2.90708 2.37661 2.92723 4.14062 2.97921 4.18683 2.85784 2.75581 2.61609 1.83458 3.68093 5.89064 3.75773 32 t - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
31 3.26535 0.25198 5.58899 5.57276 5.70674 3.96294 6.04137 5.21916 5.39983 5.89409 5.84078 4.88032 4.83145 5.55459 5.36441 2.62458 3.85544 4.45501 7 .06727 5.97763 33 C - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
32 2.43519 5.32884 3.42185 2.90211 4.74015 3.28703 2.96586 4.18538 2.91810 3.47001 4.52811 3.06155 1.62149 3.26836 3.38980 1.49946 3.31401 3.79284 5.94081 4.58182 34 s - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
33 2.61078 3.35079 3.23449 2.19394 4.56376 3.29554 3.71861 2.91187 2.51018 3.33982 3.88749 2.44731 4.21602 3.04792 2.48477 2.78455 2.96230 2.25992 5.78464 3.71618 35 e - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
34 3.65788 0.47624 3.98655 2.82069 4.74288 4.26728 2.70257 4.43727 3.68875 4.98661 5.01017 4.17099 4.87343 4.19297 3.95531 3.79192 4.60847 4.12089 6.12585 4.77667 36 C - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
35 2.97605 5.18783 3.14635 3.17765 4.47189 0.69383 4.33865 3.91092 3.18962 3.26416 4.41360 3.64580 4.43597 3.55200 3.28738 3.27290 3.45476 3.66671 4.46388 4.50728 37 g - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
36 2.39341 3.97392 3.38026 2.35103 3.54015 3.29371 2.36669 3.95173 2.60637 3.53630 4.33637 3.34999 4.22289 2.73394 2.87111 2.16482 2.97759 3.69069 4.50967 2.63248 38 s - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
37 2.87624 5.20494 3.42470 2.56669 4.43970 3.85052 4.05489 2.35000 1.91063 3.17554 3.95459 3.10561 3.79808 3.07869 2.15555 3.05411 2.49982 2.35762 5.70983 4.02721 39 k - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
38 2.78686 4.33285 2.90613 2.84412 4.48071 2.60065 3.07373 3.14623 2.37215 2.31764 3.49084 2.28640 4.23115 3.01692 3.14262 2.58401 3.24558 2.56091 5.73498 4.39476 40 n - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
39 3.00535 5.51120 2.85275 1.97441 4.85425 3.06905 3.96713 3.51572 1.80752 3.81562 4.55239 3.00845 3.54511 2.91052 2.57707 2.33719 2.81395 3.07210 5.94509 3.31924 41 k - - -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
```

FIG. 11 (Cont. 4)

40 2.79461 5.51679 2.78555 1.87270 4.86227 3.48679 3.54080 4.38853 2.15763 3.48334 4.55735 3.09403 3.02458 2.60123 2.14734 2.74315 2.65797 3.58974 5.94922 3.41566 42 e - - -
2.68624 4.42289 2.77494 2.73103 3.46418 2.40540 3.72559 3.29418 2.67710 2.69236 4.24754 2.90337 2.73746 3.18182 2.69763 2.37951 2.77506 2.98538 4.58541 3.61362
1.32716 0.90106 1.11281 1.04708 0.43226 0.48576 0.95510
41 2.14549 5.29641 2.74530 2.54532 4.13981 2.92965 3.80413 4.09470 2.45590 3.26892 4.34959 2.68262 2.97204 2.90711 2.34597 2.19643 2.69569 3.67678 5.74126 4.34240 51 a - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.01120 4.89367 5.61602 0.61958 0.77255 0.60842 0.78573
42 2.78895 5.02703 2.85851 2.84197 3.76011 3.41921 3.99330 3.38549 2.73637 1.66688 4.10605 3.03596 2.88030 2.97473 2.48037 2.76102 3.16184 2.58848 5.54967 4.23825 52 1 - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00905 5.10509 5.82744 0.61958 0.77255 0.26919 1.44393
43 2.41703 5.51166 2.81678 2.29243 4.85492 3.37853 3.22639 3.76865 2.37006 3.34870 4.55279 2.69047 3.15111 2.72796 2.99009 1.99472 2.69710 3.01190 5.94543 4.53557 53 s - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
44 3.00576 5.49550 2.74803 2.56141 4.83184 2.23449 3.73329 3.52233 2.24774 3.57063 4.53046 3.09923 3.00131 2.35765 2.58756 2.54292 2.76968 2.91429 4.36571 3.95073 54 g - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
45 2.59304 5.53215 2.87161 2.44987 4.87671 2.70363 3.98031 4.36160 2.31962 3.63621 4.57396 3.09533 3.82619 2.91333 1.25026 2.76559 3.25519 3.92272 5.96382 4.55391 55 r - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
46 2.91730 5.34660 3.16251 2.20229 4.62597 2.88148 4.00970 4.06808 2.49801 2.26903 3.79794 3.00554 4.20569 2.99135 2.44311 2.91068 2.09646 2.56280 4.46325 4.45336 56 t - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
47 3.07404 4.45967 4.96404 4.35568 1.54143 3.20252 3.19334 2.89354 4.15600 2.10912 2.80888 4.37040 4.59854 4.29864 4.18851 3.19956 3.30692 2.30735 2.67545 2.56780 57 f - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
48 2.70371 5.32180 3.04046 2.49323 3.44716 3.81985 3.50849 3.16594 2.05956 3.31159 4.38279 3.13993 4.21111 2.89553 2.31619 3.01684 2.59689 2.21734 5.80159 3.03769 58 k - - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.69601 2.37887 2.77519 2.98518 4.58477 3.61503
0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510

FIG. 11 (Cont. 5)

```
49 3.43228 0.35397 4.93218 4.57677 5.23780 4.12022 5.24832 4.76158 3.85479 4.43261 5.32121 3.41861 4.85781 4.56634 2.85610 3.63431 3.91437 4.27343 6.51772 5.33189 59  C  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37881 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
50 2.90235 5.49119 2.79978 2.21367 3.11628 3.52721 3.97181 3.81893 2.49862 3.79195 4.53464 3.10548 1.92793 2.66275 2.82236 2.53353 2.34154 3.43865 5.93025 4.52517 60  p  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37881 2.77519 2.98518 4.58477 3.61503
     0.15876 5.28846 1.95371 0.61958 0.77255 0.48576 0.95510
51 2.42137 5.44418 2.64787 2.45597 4.78837 3.34231 3.11703 4.27312 2.21583 3.74906 4.48510 2.01541 3.52159 2.99339 2.42819 2.38051 3.06531 2.99604 5.87737 4.46693 61  n  -  -
     2.68611 4.42234 2.77439 2.73097 3.46363 2.40521 3.72503 3.29363 2.67749 2.69364 4.24698 2.90355 2.73748 3.18155 2.89809 2.37895 2.77528 2.98527 4.58486 3.61512
     0.10781 2.30853 5.86081 0.67459 0.71265 0.29096 1.37652
52 3.12714 0.51171 5.26215 4.93130 4.73210 2.84875 5.40988 3.95106 4.74065 2.51364 4.72705 4.61813 4.69769 4.94392 4.81333 3.40061 2.94398 3.59675 6.23451 5.08178 64  C  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37881 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510
53 3.02864 5.22708 3.24416 2.87007 4.46645 1.15780 3.81090 3.55248 2.51169 2.79176 4.29803 3.08949 4.24766 3.05520 3.01257 3.66284 3.25918 3.17603 3.48227 4.39417 65  g  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37881 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510
54 2.40365 4.66049 4.11497 2.73912 1.99022 3.19575 3.02364 2.97871 3.04860 2.32962 3.39617 3.23425 4.44610 3.75342 3.77696 3.14743 2.78217 2.37042 4.63637 2.84025 66  f  -  -
     2.68610 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510
55 2.87226 5.40375 3.12694 2.10029 3.48700 3.45001 3.05224 3.40157 2.17907 3.68954 4.45651 3.28896 4.19366 2.89854 2.77197 2.58416 2.36149 2.31846 5.86446 3.24460 67  e  -  -
     2.68622 4.42229 2.77524 2.73099 3.46388 2.40511 3.72499 3.29358 2.67745 2.69359 4.24694 2.90351 2.73744 3.18116 2.89805 2.37891 2.77524 2.98591 4.58481 3.61507
     0.09213 2.45854 6.01081 0.20065 1.70393 0.48576 0.95510
56 1.92398 3.61677 3.15008 3.27007 2.69745 3.56706 2.73192 2.56530 3.56523 2.17657 3.26149 3.32916 4.46930 3.56678 3.83643 2.98321 3.27866 2.88023 3.49247 2.87097 68  a  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90341 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
57 3.26479 4.36049 1.25687 2.78658 5.09728 3.88340 1.83438 4.59558 2.99414 4.07950 4.84599 2.02585 4.35503 2.80468 3.50667 3.20760 3.51018 4.16444 6.21720 4.78378 70  d  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
58 1.23853 5.29374 4.56249 4.01115 5.45569 4.01738 4.88734 4.86441 3.31439 4.43320 5.25952 4.21073 4.69469 4.07868 0.79443 2.96054 3.75959 4.31075 6.5461B 5.38597 71  r  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
59 4.23844 6.90487 0.26040 3.07112 6.19796 4.19894 4.96642 5.94050 4.20174 5.34626 6.33855 3.68062 4.92678 3.11787 4.94287 4.04612 4.58985 5.43768 7.36664 5.78034 72  D  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37881 2.77519 2.98518 4.58477 3.61503
     0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
```

FIG. 11 (Cont. 6)

```
60 2.93945 4.40654 4.71529 3.83327 3.17614 2.99810 3.66840 1.94060 3.36839 1.99214 3.60335 4.24473 4.55597 3.70054 3.13856 3.48570 2.67283 1.70881 3.61192 3.09309 73  v  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
61 3.05529 4.93693 3.35440 3.14649 4.10597 3.96319 3.70433 3.19792 3.12971 2.93067 4.03701 1.20424 4.35471 3.45639 3.53504 3.28081 3.02427 1.76674 5.50259 4.23456 74  n  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
62 0.66653 5.04829 4.74593 4.48579 5.56793 1.44837 5.45202 5.04659 4.49890 4.69952 5.47180 4.41412 4.63532 4.74114 3.79579 2.41637 3.64520 4.29564 6.88210 5.73877 75  A  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
63 1.22811 2.79256 4.63608 4.06360 3.74414 4.14349 4.57733 2.83369 3.93924 2.44450 3.75090 4.22387 4.56892 4.15266 4.10711 1.80257 3.14338 2.37335 5.25148 4.06859 76  S  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510
64 2.84218 4.47424 4.78141 3.36613 2.49959 3.88625 4.51887 1.81894 2.92767 1.97340 3.28741 4.27841 4.56507 4.19153 2.62686 3.49894 3.29019 2.26288 4.42160 2.44681 77  i  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510                                                                                                                  78  N  -  -
65 3.24098 5.22877 4.05555 3.93311 5.51602 3.89082 5.20175 4.95733 4.18540 4.64613 5.46412 0.36554 4.66730 4.45710 4.51557 2.97042 3.17609 3.54434 6.82127 5.57035
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61956 0.77255 0.48576 0.95510
66 4.36648 5.53779 6.41524 5.87613 3.34469 5.89675 6.29833 0.74710 5.77945 1.12347 3.70298 6.07426 5.90719 5.65972 5.73964 5.32369 4.58410 2.54442 6.24393 5.26425 79  i  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
67 1.77383 4.51595 4.56619 3.98635 3.13870 3.46254 4.47329 2.41413 3.02892 1.45875 3.22243 3.68276 4.53513 4.06735 2.94148 3.26910 3.28669 2.68668 4.07643 3.56501 80  l  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00753 5.28846 6.01081 0.61958 0.77255 0.48576 0.95510
68 2.74054 5.50211 2.90007 2.48939 4.84120 3.42564 3.28407 4.32288 1.56965 2.60197 4.54436 2.74584 4.17522 2.83528 2.23727 2.97101 2.96556 3.33612 4.17207 4.53114 81  k  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.09234 5.28846 2.48704 0.61958 0.77255 0.48576 0.95510
69 2.79150 5.19360 3.44199 2.87423 4.43324 3.84913 4.03522 3.52601 1.74699 3.12499 3.84765 3.19641 4.23545 3.18909 1.35441 3.65949 3.24778 3.17337 5.68846 3.24148 82  r  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.00552 5.20165      * 0.61958 0.77255 0.00000      *
```

FIG. 11 (Cont. 7)

```
HMMER3/f [3.1b2 | February 2015]
NAME  RuvC_1
ACC   PF18516.2
DESC  RuvC nuclease domain
LENG  244
ALPH  amino
RF    yes
MM    no
CONS  yes
CS    no
MAP   yes
DATE  Wed Apr 22 16:45:27 2020
NSEQ  10
EFFN  0.893555
CKSUM 1712232851
GA    26.20 26.20 ;
TC    33.30 108.10 ;
NC    26.10 24.00 ;
BM    hmmbuild --hand&MM,mm SEED.mm
SM    hmmsearch -Z 47079205 -E 1000 --cpu 4 HMM pfamseq
STATS LOCAL MSV       -10.8985  0.70314
STATS LOCAL VITERBI   -11.7760  0.70314
STATS LOCAL FORWARD    -5.3232  0.70314
HMM     A        C        D        E        F        G        H        I        K        L        M        N        P        Q        R        S        T        V        W        Y
        m->M     m->I     m->D     i->M     i->I     d->M     d->D
COMPO  2.61196  4.58549  2.88726  2.63091  3.35172  2.81203  3.77793  2.82099  2.55752  2.46803  3.74028  2.89868  3.43302  3.00266  2.94492  2.65321  2.88515  2.70318  4.61036  3.36995
       2.68616  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
       0.02416  4.13012  4.85247  0.61956  0.77255  0.00000        *
     1 1.17964  4.20743  3.69450  3.30076  4.08077  3.10800  4.25604  3.28756  3.26624  3.13101  4.02115  3.44534  3.81345  3.56234  3.56465  2.08158  2.78226  2.42975  5.49377  4.28849     1  a  x  -  -
       2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
       0.02418  4.13012  4.85247  0.61956  0.77255  0.48576  0.95510
```

FIG. 12

```
2  2.61338 4.76339 3.03582 2.35283 3.99657 3.44570 3.64687 3.06553 2.43076 3.01712 3.84155 2.75850 3.83658 2.63371 2.52070 2.66041 2.24062 2.89411 5.27120 3.95254  2  t  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
3  2.21665 4.53168 3.10038 2.69084 4.18810 1.89003 3.88295 3.60371 2.74016 3.23467 4.05719 3.10740 3.80465 2.70371 3.15670 2.32621 2.32509 3.19738 5.40153 4.19015  3  g  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
4  2.63243 4.84749 3.01544 2.45510 4.10295 2.89526 3.62801 3.18693 2.11366 2.67736 3.91821 2.62724 3.83413 2.60153 2.56835 2.65895 2.85984 3.19003 5.33136 4.00347  4  k  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
5  2.60791 4.94309 2.54097 2.36145 3.66130 3.10574 3.60815 3.67356 2.25315 3.22894 4.01276 2.66013 3.34049 2.73349 2.66324 2.36293 2.52666 3.30509 5.42647 4.05725  5  k  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
6  2.76032 4.88657 2.91332 2.52137 3.84125 3.47085 3.69464 3.55282 2.29226 3.14619 4.00632 1.77890 3.91621 2.90792 2.87288 2.79227 3.00550 3.24722 5.19429 2.64657  6  n  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
7  3.19121 4.48415 4.95588 4.40495 3.36883 4.54836 4.96244 1.32087 4.29273 1.56042 2.76714 4.60788 4.78057 4.44476 4.42269 3.90171 3.42735 1.56612 5.35179 4.23466  7  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
8  2.96076 4.95199 2.88656 2.80222 4.35872 3.37616 4.16275 4.13369 3.09325 3.75289 4.71266 0.77153 4.04408 3.44256 3.45107 3.02220 3.35515 3.72910 5.63500 4.29369  8  n  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
9  2.75063 5.21636 2.07567 2.28073 4.53723 3.36420 3.66861 4.01175 2.09749 3.50944 4.29078 2.83645 3.83621 1.99368 2.91573 2.49471 2.99387 3.60052 5.65768 4.24801  9  q  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
10 2.62726 4.67268 2.62391 2.43068 4.13184 3.43386 3.62174 3.55265 2.03494 2.91672 2.80808 2.95116 3.82510 2.61063 2.63268 2.64659 2.85568 3.21664 5.35306 4.01524 10  k  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
11 3.01701 4.49722 4.47638 4.15497 3.75279 3.98410 4.91687 2.07467 4.02703 2.39951 3.68566 4.30155 4.53576 4.37166 4.21377 3.50986 3.37571 0.75204 5.57832 4.32290 11  v  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
12 2.60646 4.66709 3.08808 2.27534 3.87230 3.46447 3.67557 2.58561 2.37654 2.73189 3.74953 2.64358 3.85413 2.66244 2.92895 2.68481 2.83980 2.71323 5.19305 3.89239 12  e  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 1)

```
13 2.50357 5.08649 2.46544 1.89999 4.40363 3.38370 3.59669 3.86929 2.19236 3.07858 4.13908 2.86032 3.79644 2.54470 2.65409 2.61378 2.87675 3.46262 5.53041 4.13682 13  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
14 2.60806 4.80200 2.59460 2.30492 3.20749 3.08742 3.30732 3.45328 2.30745 3.05826 3.87468 2.95672 3.82280 2.79332 2.90946 2.64218 2.84029 3.13273 5.29579 2.91353 14  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
15 2.88773 4.28327 4.47199 3.89826 2.83426 4.08526 4.42622 1.72819 3.77903 1.56781 3.14992 4.09997 4.40345 3.97076 3.93773 3.39682 2.52549 1.89635 4.98667 3.82286 15  l  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
16 2.46257 4.86245 3.10163 2.51832 4.12621 3.69816 3.63581 3.11830 1.97674 2.82660 3.93402 3.01768 3.86992 2.42967 2.25603 2.71382 2.90267 3.20791 5.32888 4.02513 16  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
17 2.49846 5.11276 2.10873 2.29316 4.42856 3.36386 3.62120 3.89701 2.25509 3.41104 4.18231 2.64044 3.80599 2.50669 2.90980 2.31664 2.71745 3.49182 5.57442 4.17104 17  d  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
18 2.55290 5.23598 2.10259 2.11174 4.56386 3.32556 3.70425 4.04437 2.56985 3.55806 4.35044 2.02836 3.83982 2.83963 3.09704 2.12842 3.03401 3.63106 5.72659 4.29469 18  n  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
19 2.63785 5.09756 2.46416 2.18844 4.42488 3.38520 3.58740 3.89667 2.10114 3.39083 4.14725 2.85930 3.11120 2.51326 2.46748 2.60533 2.69856 3.47951 5.53543 4.13996 19  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.12378 4.13012 2.29911 0.61958 0.77255 0.48576 0.95510
20 2.86792 5.39617 1.44492 1.99820 4.69413 2.79410 3.72513 4.19107 2.36047 3.69138 4.51116 2.72897 3.83830 2.87590 3.20907 2.77723 3.13843 3.77226 5.85525 4.38629 20  d  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02668 4.03302 4.75536 0.61958 0.77255 0.42595 1.05886
21 2.50268 4.16392 4.35277 3.76917 2.85330 3.91816 4.24798 1.93946 3.63961 1.82764 2.58761 3.94449 4.25926 3.83283 3.78510 3.22101 2.73956 1.87772 4.83890 3.66642 21  l  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
22 2.33717 4.98033 2.84018 2.42871 4.27056 3.41781 2.25664 3.71667 2.21927 3.27872 4.09175 2.34917 3.85973 2.81176 2.80422 2.71502 2.95322 3.36636 5.46155 4.11349 22  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 2)

```
23 3.21001 4.48351 5.00710 4.49017 3.56745 4.63166 5.15013 1.14391 4.38335 1.86764 3.35586 4.70186 4.88300 4.60444 4.55348 4.01183 3.46101 1.33368 5.57477 4.40062 23  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
24 3.23702 4.59462 4.68672 4.31863 3.56122 4.33356 5.00626 0.80564 4.15201 2.10269 3.46483 4.52917 4.78715 4.47860 4.32027 3.86073 3.53449 1.66845 5.49992 4.25552 24  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.12378 4.13012 2.29911 0.61958 0.77255 0.48576 0.95510
25 2.87641 4.63319 3.58285 3.50401 4.63619 0.51940 4.61199 4.27928 3.78636 3.91250 4.89143 3.74753 4.02934 4.01863 3.92297 3.05336 3.37016 3.77662 5.68838 4.74640 25  G  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02668 4.03302 4.75536 0.61958 0.77255 0.42595 1.05886
26 3.21894 4.49916 4.99254 4.47815 3.54954 4.62760 5.14426 1.05243 4.36339 1.86864 3.34032 4.69575 4.88155 4.58909 4.53596 4.01003 3.47232 1.46147 5.56778 4.39303 26  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
27 2.84130 5.13234 1.16743 2.33119 4.61251 3.27696 3.89116 4.01102 2.87173 3.65288 4.52675 2.87820 3.89895 3.07109 3.40373 2.81917 2.40137 3.61690 5.86277 4.43976 27  d  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
28 3.31989 5.12537 3.83907 3.30568 4.54668 3.74796 4.04471 4.11322 2.38651 3.58243 4.58251 3.64214 4.24405 3.25804 0.64748 3.39580 3.57210 3.82161 5.52863 4.42539 28  R  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
29 2.60320 4.63832 2.86675 2.74901 4.52763 1.13376 4.12695 4.07134 3.07711 3.69178 4.54267 2.26698 3.87241 3.36129 3.46898 2.70318 3.04064 3.55403 5.79194 4.49135 29  g  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
30 2.85837 4.98436 2.81209 1.30813 4.11124 3.47919 3.82687 3.42409 2.62220 2.42891 4.06096 3.05946 3.97245 3.01400 3.02578 2.88814 3.11607 3.18269 5.46792 4.11535 30  e  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
31 3.18938 5.25166 3.64930 2.94657 4.73459 3.77275 3.65658 4.07151 1.63813 3.49742 4.38409 3.31980 4.13835 2.82434 1.08956 3.18835 3.24551 3.76276 5.51611 4.37690 31  r  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 3)

```
32 2.83245 5.28996 2.08737 2.24643 4.51612 3.33543 2.36769 4.07934 2.60076 3.59061 4.40089 1.80556 3.86560 2.87374 3.11681 2.76903 3.09281 3.67605 5.71360 4.26117 32  n  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
33 3.45721 4.64750 4.60246 4.25116 3.25455 4.30647 4.82696 2.48010 4.01669 0.61229 3.21057 4.50182 4.70236 4.32237 4.14742 3.91942 3.74239 2.58290 5.21913 3.99936 33  L  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
34 2.95176 4.34736 4.48715 3.92862 3.38364 4.16964 4.55847 1.72559 3.81521 1.49111 3.24620 4.16648 4.49135 4.04367 4.01062 3.49157 2.53941 1.69254 5.16438 3.99613 34  l  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
35 3.64057 5.02416 4.22926 4.01361 2.35214 4.13664 3.17069 3.61657 3.86458 3.00194 4.26579 4.05560 4.60959 4.10694 3.99216 3.74852 3.93027 3.50018 4.03336 0.62549 35  Y  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
36 2.85310 4.25513 4.42500 3.85134 3.19549 4.03416 4.33525 1.86648 3.71894 1.70915 3.16920 4.06149 4.36046 3.91957 3.87490 3.34307 3.08676 1.61161 4.89213 2.77756 36  v  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
37 1.99784 4.25916 3.57759 3.18036 3.92653 3.20021 4.08126 3.19551 3.06262 2.98197 3.85664 3.35840 3.82894 3.36681 3.40395 1.90283 1.92400 2.30601 5.33319 4.10743 37  s  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89601 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
38 3.15153 4.46660 4.85274 4.29770 3.35450 4.47370 4.87082 1.79377 4.16379 1.51650 2.71916 4.51289 4.72220 4.35194 4.32950 3.81784 3.38762 1.26798 5.30397 4.17825 38  v  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
39 3.18886 4.48026 4.96702 4.48112 3.63969 4.57444 5.18429 1.04579 4.36944 2.11490 3.43296 4.68306 4.81406 4.62590 4.55366 3.97581 3.45605 1.31985 5.64605 4.44466 39  i  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.46576 0.95510
40 3.02825 5.54267 1.05958 2.18654 4.86135 3.27434 3.88156 4.44244 2.94341 3.95303 4.82466 2.16129 3.92430 3.66256 3.54993 2.81954 3.34148 4.00103 6.07039 4.55436 40  d  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89001 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
41 2.72219 5.08962 2.87023 2.00329 4.42134 3.43316 3.62441 3.86246 2.28615 3.37323 4.16185 2.92359 3.85061 2.40034 2.17180 2.20042 2.94972 3.47996 5.52183 4.17011 41  e  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
42 2.69432 5.08737 2.69520 2.38733 4.41722 3.43395 2.88633 3.86547 1.77130 3.36182 4.14537 2.91626 3.83476 2.71770 2.35648 2.47655 2.91881 3.47444 5.59311 4.15099 41  k  x  -  -
     2.68618 4.42225 2.77319 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89001 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 4)

```
43 2.60320 4.63632 2.86675 2.74901 4.52763 1.13376 4.12695 4.07134 3.07711 3.69178 4.54267 2.26698 3.81241 3.36129 3.46890 2.70318 3.04064 3.55403 5.79194 4.49135 42  q  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
44 2.73061 5.12029 2.77875 2.20600 4.43774 3.41276 3.20229 3.88712 1.96551 3.39955 4.18813 2.01669 3.84631 2.75910 2.76201 2.70990 2.73897 3.50146 5.55274 4.18499 43  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
45 3.19043 4.49229 4.93936 4.45846 3.61455 4.55063 5.16130 0.98282 4.33769 2.05523 3.41389 4.66252 4.85942 4.59779 4.52175 3.95511 3.46047 1.44029 5.62503 4.42322 44  i  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
46 2.79263 4.30644 4.02478 3.45244 3.35452 3.89219 4.18824 1.98561 2.69642 1.61823 3.26362 3.76546 4.24034 3.61257 3.55746 3.18069 3.02672 1.88499 4.98944 3.79590 45  l  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
47 2.64011 4.97439 2.63965 1.87516 4.24664 3.40151 3.61767 3.69146 2.12167 2.78652 4.03695 2.89818 3.81167 2.74637 2.85265 2.63425 2.87287 3.32790 5.44024 3.47670 46  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
48 2.93522 5.26295 2.61495 2.00832 4.56736 3.42344 3.15576 4.03499 2.44648 3.54451 4.40400 2.93073 3.94294 1.38696 2.82173 2.88934 3.18443 3.67462 5.68575 4.32791 47  q  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
49 2.60715 3.92764 2.56987 2.29666 3.32281 3.41426 3.61776 3.55836 1.97066 3.13935 3.93942 2.92870 3.80934 2.57349 2.86319 2.62261 2.83871 3.21585 5.35994 4.00976 49  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
50 2.52829 4.62326 2.57793 2.60802 4.32081 3.28626 3.91006 3.73627 2.80390 3.37452 4.19993 3.05274 3.81548 3.09771 3.24687 1.38031 2.36013 3.30794 5.60476 4.28276 50  s  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
51 3.19503 4.58110 4.54075 4.02336 2.38650 4.26616 4.12037 2.55260 3.87876 1.01067 3.15554 4.16008 4.54771 4.01569 4.01518 3.58910 3.41839 2.59627 4.42653 2.48020 51  l  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 5)

```
52 2.96076 4.95199 2.88656 2.80222 4.35872 3.37616 4.16275 4.13369 3.69325 3.75289 4.71266 0.77153 4.04408 3.44258 3.45107 3.02220 3.35515 3.72910 5.63560 4.29369 52  n  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
53 2.65510 4.50241 3.29118 2.16943 3.68500 3.56775 3.81156 2.25454 2.50296 2.68015 3.59636 3.21798 3.95811 3.05699 3.07833 2.81957 2.88886 2.17009 5.18455 3.84227 53  e  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2 77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
54 3.28820 4.58640 4.94239 4.45543 3.36777 4.61462 5.04051 1.06250 4.26796 1.51195 3.19220 4.66673 4.85680 4.46767 4.41956 4.00545 3.53563 1.86577 5.41235 4.23739 54  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
55 2.46012 4.96377 2.26099 2.35272 4.25740 3.05175 3.65071 3.69600 2.45881 3.26339 4.06068 2.43046 3.81642 2.61161 2.94404 2.64714 2.59434 2.60876 5.47144 4.10024 55  d  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
56 2.80085 5.13475 2.22651 2.38064 4.63071 1.66490 3.83772 4.12827 2.78599 3.67268 4.49339 1.76394 3.86936 3.00585 3.31502 2.77184 3.11961 3.69119 5.84239 4.41668 56  n  x  -  -
   2.69642 4.42249 2.77543 2.73077 3.46378 2.40513 3.72454 3.29359 2.67719 2.69379 4.24714 2.90332 2.73763 3.18131 2.89799 2.37911 2.77493 2.98542 4.58501 3.61391
   0.27438 1.46039 4.85247 1.62479 0.21934 0.48576 0.95510
57 2.63396 4.12803 4.00838 3.42971 2.84790 3.72694 4.01354 2.46248 3.32155 2.21833 2.90590 3.67180 4.09315 3.55546 3.53096 3.01172 2.35896 1.83669 4.71399 2.73430 65  v  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
58 2.91590 5.41705 1.32126 2.19789 4.76930 3.28431 3.80739 4.28912 2.78349 3.79476 4.62383 2.06895 3.21485 2.96680 3.35590 2.82972 3.20757 3.85401 5.95012 4.46832 66  d  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
59 3.64057 5.02418 4.22926 4.01381 2.35214 4.13864 3.77069 3.61657 3.86458 3.00194 4.26579 4.05560 4.60959 4.10694 3.99216 3.74852 3.93027 3.50018 4.60336 0.62549 67  y  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2 73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
60 2.99286 5.18478 3.36664 2.72396 4.55850 3.66289 2.17785 3.96102 1.71096 3.41861 4.26055 3.16468 4.02604 2.76990 1.67321 2.98219 3.16985 3.62743 5.47652 4.25160 68  r  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
61 2.34093 5.13345 2.52355 1.85416 4.44647 3.36589 3.62990 3.91497 2.09661 3.42606 4.19843 2.63524 3.81086 2.74990 2.90595 2.64818 2.72145 3.50931 5.58668 4.18220 69  e  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 6)

```
62 2.48544 4.57347 3.41224 2.88596 3.72927 3.60409 3.85159 2.95320 2.01994 1.69546 3.64259 3.30441 4.01782 3.69702 2.67595 2.91045 2.98780 2.75321 5.15357 3.91182 70  i  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
63 3.45721 4.84750 4.60246 4.25116 3.25455 4.30647 4.82696 2.48010 4.01669 0.61229 3.21657 4.50182 4.70238 4.32237 4.14742 3.91942 3.74239 2.58290 5.21913 3.99936 71  j  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
64 2.71333 5.17109 1.95200 2.13349 4.47775 3.35856 3.64618 3.94650 2.15524 3.45988 4.23714 2.41229 3.81965 2.76889 2.94778 2.66924 2.95579 3.04135 5.62159 4.20955 72  d  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
65 2.64887 5.01863 2.86833 2.60354 4.32062 3.40597 3.60693 3.76624 2.10660 3.29934 4.07969 2.71688 3.81225 2.39481 2.79121 2.63442 2.55274 3.04158 5.47369 4.10469 73  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
66 3.18538 5.27840 3.64933 2.92466 4.77435 3.77621 3.66931 4.10150 1.41176 3.51234 4.38981 3.30355 4.13091 2.80143 1.25731 3.17472 3.33463 3.78606 5.52412 4.38668 74  r  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
67 2.72457 4.98902 2.63570 1.40384 4.42297 3.32148 3.77028 3.83561 2.60513 3.43101 4.25741 2.91023 3.85895 2.92796 3.06780 2.45938 2.44349 3.45320 5.63973 4.26680 75  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3 46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
68 2.63595 4.75169 3.07014 2.51694 3.97258 2.91310 3.65791 2.83301 1.95436 2.99797 3.63179 3.01832 3.85632 2.46757 2.83150 2.69085 2.86405 3.07782 5.25192 3.39922 76  k  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
69 2.48916 4.94580 2.47701 2.03428 4.22313 3.39166 3.62517 2.99267 2.41566 3.22589 4.01824 2.72100 3.80727 2.75695 2.89717 2.46248 2.62169 3.29935 5.43287 4.06528 77  e  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
70 2.63491 5.01841 3.24883 2.62946 4.34972 3.57758 3.63816 3.73599 1.87901 3.26515 3.35887 2.75610 3.95072 2.77557 1.59593 2.88405 3.63599 3.41033 5.41177 4.15272 78  r  x  -  -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 7)

```
71 2.47195 4.92870 2.57272 2.13761 4.20070 3.40317 3.61011 3.63770 2.13703 2.94014 3.99137 2.90385 3.80292 2.73912 2.85690 2.61571 2.68380 3.27929 5.40614 3.20686 79  k  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
72 2.62936 5.02697 2.63477 2.01559 4.31666 3.38983 2.97695 3.77225 2.16230 3.30373 4.07753 2.87621 3.79731 2.71888 2.83941 2.61186 2.52390 3.38631 5.47885 3.44834 80  e  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
73 1.51018 5.05907 2.12508 2.13253 4.43561 3.31485 3.78376 3.84993 2.69247 3.45428 4.28827 2.87009 3.86615 2.94848 3.26011 2.75404 3.05542 3.47835 5.67789 4.28493 81  a  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
74 3.18998 5.25168 3.64930 2.94657 4.73459 3.77275 3.68658 4.07151 1.63813 3.49742 4.38409 3.31980 4.13835 2.82434 1.68956 3.10835 3.34551 3.76276 5.51611 4.37890 82  r  x  -  -
   2.68619 4.42226 2.77521 2.73125 3.46355 2.40514 3.72496 3.29355 2.67742 2.69356 4.24691 2.90348 2.73741 3.18148 2.89802 2.37888 2.77495 2.98520 4.58478 3.61504
   0.12378 2.21990 4.85247 0.35237 1.21409 0.48576 0.95510
75 2.96751 5.24359 3.27341 2.45035 4.67881 3.63078 3.62424 4.05228 1.54771 3.48207 4.30517 3.11444 3.99865 2.27677 1.72186 2.93866 3.14764 3.69231 5.53677 4.30540 83  k  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
76 2.65983 4.84262 2.85538 2.29186 4.12540 3.39615 3.69731 3.54490 2.50412 2.62980 3.99303 2.17704 3.84873 2.86059 2.94638 2.06383 2.91223 3.21540 5.39078 4.05187 85  s  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.71255 0.48576 0.95510
77 3.80005 5.07613 4.50824 4.28358 2.91921 4.03876 4.25547 3.84369 4.00523 3.19850 4.46404 4.38300 4.57939 4.37912 4.05544 4.00104 4.11526 3.74076 0.49457 2.96912 86  W  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
78 2.69945 5.05214 2.84111 2.38497 4.36603 2.82433 3.63485 3.82799 2.04158 3.35316 4.14297 2.65776 3.83997 2.02574 2.75617 2.68301 2.67024 3.44690 5.51667 4.15885 87  q  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
79 2.41084 4.46535 3.23829 2.68833 3.68617 3.47893 3.74851 3.05849 2.46511 2.74376 3.61128 3.14565 3.88394 2.98929 3.05144 2.37272 2.23158 2.61431 5.07052 3.04295 88  t  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0 02418 4.13012 4.85247 0.61958 0.77255 0.46576 0.95510
80 2.81494 4.41988 2.81254 3.24846 3.60613 3.83837 4.22216 1.38393 3.25342 2.41920 3.51273 3.64185 4.24105 3.55458 3.59447 3.15466 3.07197 1.91003 5.22905 3.97894 89  i  x  -  -
   2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
   0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 8)

81 2.67861 4.92245 2.78493 1.80954 4.21586 2.47933 3.66648 3.63203 2.49015 3.22590 4.04535 2.92589 3.84357 2.63495 2.94766 2.69044 2.92818 2.70406 5.45358 4.10160 90 e x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
82 2.61279 4.85681 2.85711 2.43384 4.28432 2.94002 3.70335 3.72181 2.30534 3.29276 4.09278 2.15805 3.81105 2.88740 2.94922 2.15535 2.45968 3.33678 5.49639 4.14643 91 s x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
83 2.72026 4.32170 3.97227 3.55290 3.56572 3.67678 4.34460 1.22176 3.43621 2.44405 3.54953 3.78378 4.20204 3.77012 3.67736 2.41508 3.04884 2.14900 5.19776 3.93997 92 i x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
84 3.15084 5.25853 3.55595 2.88414 4.73719 3.74369 3.67714 4.08104 1.04392 3.50469 4.30040 3.27775 4.11269 2.81108 1.80464 3.14137 3.31222 3.76128 5.52670 4.37212 93 k x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
85 2.84824 5.36455 2.18900 1.42495 4.67055 2.60445 3.72681 4.16205 2.35347 3.65850 4.45979 2.78682 3.86113 2.86645 3.14065 2.77080 3.10869 3.74218 5.81596 4.36455 94 e x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
86 3.42830 4.77740 4.87895 4.34347 3.10062 4.61572 4.95574 2.26299 4.12423 0.74697 2.51065 4.62139 4.80982 4.30182 4.25409 3.99323 3.65705 2.41757 5.21169 4.10939 95 l x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61956 0.77255 0.48576 0.95510
87 3.22788 5.10401 3.41982 3.00288 4.63017 3.68014 3.93039 4.07793 0.13236 3.59045 4.54550 3.41299 4.16843 3.11262 2.51722 3.25743 3.46557 3.77395 5.58409 4.43801 96 k x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
88 2.96513 5.26272 2.46301 0.98476 4.71386 2.73652 3.90943 4.20023 2.82657 3.75640 4.62464 2.92111 3.94900 3.09011 3.38861 2.92077 3.27384 3.79622 5.87054 4.49938 97 e x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
89 1.82065 4.27948 3.45173 3.28272 4.51394 1.04368 4.41426 3.89663 3.43922 3.63055 4.48197 3.42314 3.79936 3.70852 3.72642 2.52547 2.85655 3.32530 5.83785 4.65272 98 g x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61956 0.71255 0.48576 0.95510
90 3.64057 5.02418 4.22926 4.01381 2.35214 4.13864 3.77069 3.61657 3.86458 3.00194 4.26579 4.05560 4.60959 4.10694 3.99216 3.74852 3.93027 3.50018 4.00336 0.62549 99 Y x - -
2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

FIG. 12 (Cont. 9)

```
 91  3.26048 4.55476 4.98132 4.43696 3.32865 4.60730 5.01669 1.56552 4.31521 1.16477 3.11714 4.66091 4.82415 4.46353 4.44832 3.96942 3.49732 1.64634 5.37058 4.26383  100 l x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 92  2.70435 4.86029 2.68659 2.05417 4.41223 3.27788 3.87917 3.85569 2.73999 3.47039 4.32069 2.98663 3.87656 3.06509 3.17089 1.30017 3.04278 3.45555 5.66611 4.31377  101 s x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 93  2.70785 4.83318 3.04787 2.54263 4.08402 3.00628 3.67724 3.40426 2.20389 2.39101 3.92955 3.03698 3.89151 1.96089 2.73092 2.75186 2.93700 3.18032 5.32271 4.02271  102 q x
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 94  1.75280 4.28358 4.10723 3.70111 3.80526 3.54524 4.53206 2.30375 3.62033 2.59346 3.70250 3.83946 4.16126 3.91880 3.86422 2.96847 3.01604 1.24875 5.45691 4.24246  103 v x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 95  3.16191 4.46421 4.92041 4.43504 3.66860 4.53189 5.15158 1.45173 4.32478 2.16912 3.46861 4.63861 4.84790 4.59398 4.51903 3.93111 3.43223 0.93126 5.69948 4.43547  104 v x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 96  2.62518 4.66087 3.04673 2.66169 3.99635 3.35212 1.74262 3.52581 2.60670 3.15550 4.01299 3.10673 3.88031 3.02909 2.97644 2.43271 2.39493 3.18457 5.32601 3.98404  105 h x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 97  2.87140 5.25143 2.82355 1.91537 4.61776 3.47799 3.65568 4.04668 1.55608 3.51426 4.32260 2.95391 3.91873 2.33002 2.56682 2.82627 3.08932 3.66232 5.61826 4.29262  106 k x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 98  3.28331 4.56287 4.93375 4.42982 3.38012 4.60831 5.04065 1.03834 4.26030 1.55899 3.20806 4.66097 4.85601 4.48855 4.41522 4.00119 3.53242 1.85295 5.42051 4.23799  107 i x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

 99  1.74403 4.25406 3.57784 3.11466 4.02133 3.14966 4.11072 3.32477 3.08128 3.08354 3.94442 3.35273 3.80482 3.38504 3.42342 1.92938 1.89266 2.58698 5.41039 4.18571  108 a x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

100  2.63939 5.06118 2.38329 2.23591 4.37935 3.39921 3.59000 3.83794 1.92022 3.34777 3.45485 2.87946 3.80010 2.52915 2.60239 2.61490 2.86901 3.43814 5.50235 4.12093  109 k x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

101  3.40802 4.75206 4.89281 4.34298 3.09177 4.61102 4.89555 2.26811 4.14666 0.85318 2.05087 4.61699 4.79472 4.23569 4.26957 3.97519 3.63132 2.42939 5.19732 4.11938  110 l x - ,
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 10)

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102  1.89885 4.30479 4.08663 3.53760 3.36936 3.86455 4.26724 1.92989 3.43123 2.12834 2.25774 3.82080 4.25203 3.70155 3.67942 3.17603 3.04101 2.14595 5.03642 3.84892  111 a x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

103  2.72308 4.26125 3.94724 3.39522 3.40942 3.79713 4.15237 1.61713 3.28152 2.36371 3.36562 3.70089 4.18164 3.17071 3.54497 3.09505 2.48066 1.85998 4.98990 3.78386  112 i x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

104  2.76691 5.27524 2.38873 1.75996 4.60049 3.37038 3.66484 4.07620 1.78426 3.55875 4.34201 2.83444 3.84941 2.52700 2.89071 2.72687 3.02816 3.65948 5.69474 4.28420  113 e x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

105  2.95918 4.83582 3.25149 2.86851 2.80840 3.69476 3.10709 3.46453 2.83924 3.00471 3.57559 2.28372 4.11890 3.20111 3.19227 3.02273 3.19770 3.22028 4.39179 1.53747  114 y x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

106  2.89850 5.09000 2.64972 2.47954 4.06957 3.38789 3.14823 3.99114 2.65774 3.52679 4.41348 1.20563 3.95359 3.05189 3.04798 2.89122 3.19623 3.62289 5.41673 3.93716  115 n x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

107  0.69787 4.34149 3.74420 3.55689 4.32856 3.15741 4.54559 3.49415 3.58037 3.36310 4.37276 3.63494 3.92180 3.89892 3.80733 2.69277 2.98047 3.10285 5.72925 4.56130  116 n x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

108  3.18490 4.47659 4.98069 4.49245 3.66045 4.58535 5.19662 1.14616 4.38628 2.14260 3.45113 4.69243 4.88125 4.64269 4.57136 3.96503 3.45077 1.18358 5.66079 4.45711  117 i x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

109  2.66320 4.38734 4.74783 4.24023 3.66676 4.35336 4.94107 1.28306 4.14107 2.25966 3.48476 4.44336 4.70184 4.40469 4.34432 3.72618 3.31452 1.21271 5.53026 4.32527  118 v x - ,
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

110  2.36642 4.34190 4.28900 3.91171 3.80190 3.74575 4.12579 2.09714 3.81466 2.49179 3.67494 4.04205 4.33346 4.12843 4.03632 3.18450 3.14165 0.95300 5.55542 4.33051  119 v x - -
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

111  3.19377 4.56167 4.72807 4.16397 3.18453 4.41145 4.74544 2.17289 4.02120 1.34583 1.66281 4.41618 4.65581 4.18618 4.17256 3.74660 3.42456 1.87766 5.17277 4.07520  120 l x - -
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

112  3.20025 5.34641 2.62970 0.70730 4.72300 3.45944 4.07932 4.22998 2.99463 3.80888 4.76014 3.11272 4.07885 3.29501 3.42421 3.16112 3.50923 3.89026 5.84535 4.57831  121 e x - -
     2.66618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 11)

113	2.95109	5.47729	1.24509	1.97712	4.80239	3.28413	3.81475	4.31066	2.81125	3.81961	4.65771	2.76049	3.89359	2.97724	3.39568	2.27336	3.24104	3.88255	5.98717	4.49495	122 d x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
114	3.42237	4.76594	4.89724	4.35186	3.09404	4.62295	4.90828	2.26495	4.14836	0.78969	2.27903	4.62855	4.80643	4.30396	4.27280	3.99197	3.64696	2.42634	5.20628	4.12282	123 l x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
115	2.62507	4.73296	2.75085	2.59164	4.42466	3.19969	3.97307	3.98260	2.86630	3.59231	4.43823	1.27357	3.85833	3.18078	3.27917	2.13438	3.01946	3.51149	5.70985	4.33742	124 n x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
116	2.63559	4.18304	3.83756	3.26823	2.28410	3.68093	3.29664	2.46598	3.17841	2.36294	3.00248	3.54956	4.05145	3.42979	3.43029	2.51489	2.86710	2.44694	4.58430	2.60932	125 f x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
117	2.36691	4.30415	3.42101	3.22074	4.45463	1.08162	4.34678	3.82969	3.33014	3.55988	4.41773	3.39733	3.80825	3.63121	3.62665	2.54279	2.11455	3.29410	5.78088	4.58033	126 g x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
118	3.60575	4.93403	4.59390	4.36420	0.66856	4.22851	4.01913	3.10592	4.26681	2.41943	3.80192	4.31977	4.67844	4.36513	4.32239	3.85744	3.90962	3.11441	4.20462	2.54461	127 f x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
119	2.76234	4.69607	3.36056	2.77515	3.83488	3.61590	3.77959	2.75143	1.53039	2.71446	2.95838	3.22770	3.99598	2.97938	2.76731	2.87934	2.96605	2.84641	5.21710	3.95895	128 k x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
120	2.47505	5.01033	2.37736	2.43489	4.38305	3.40130	3.69360	3.80967	2.39462	3.36487	4.17992	2.95244	3.86840	2.83451	1.62966	2.72874	2.98429	3.43887	5.53950	4.19375	129 r x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
121	2.57179	4.67107	2.98359	2.61699	4.28722	1.71403	3.83950	3.70189	2.18638	3.31110	4.13570	3.66493	3.83701	3.01580	3.00968	2.42676	2.58366	3.30008	5.53052	4.22941	130 g x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
122	2.90470	4.14616	3.56679	3.00806	2.55917	3.68660	3.80912	3.24881	2.55050	2.77975	3.82193	3.39420	4.10214	3.14388	1.33732	3.03012	3.14594	3.05680	4.89357	3.44616	131 r x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														
123	2.63652	4.61260	3.13383	2.62105	2.51562	3.48370	3.71686	3.20259	2.38940	2.85538	3.72080	3.09751	3.89075	2.41079	2.95514	2.23592	2.87383	2.92247	5.11759	3.80589	132 s x - -
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503	
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510														

FIG. 12 (Cont. 12)

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```
135 2.33546 5.01980 2.81761 2.25278 4.35135 3.39631 3.64485 3.79210 1.82812 3.33139 4.12392 2.65377 3.83306 2.77430 2.81629 2.26930 2.92434 3.41481 5.50898 4.14732 144 k x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
136 2.78580 4.62550 3.48721 2.88519 3.79469 3.63272 3.77194 3.13539 2.28557 2.76012 1.83049 3.29121 4.01543 3.00339 2.25618 2.93084 3.00387 2.91350 5.13196 3.90653 145 m x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
137 3.45721 4.84750 4.60246 4.25116 3.25455 4.30647 4.82696 2.48010 4.01669 0.61229 3.21057 4.50182 4.70238 4.32237 4.14742 3.91942 3.74239 2.58290 5.21913 3.99938 146 L x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
138 2.93817 2.87069 4.68481 4.18313 3.53946 4.10846 4.75953 1.06937 4.02841 2.21923 3.43070 4.30780 4.53502 4.28368 4.18839 3.50339 3.22267 1.79605 5.33129 4.11734 147 i x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
139 2.29882 5.27119 1.52870 2.23654 4.66776 3.28592 3.79149 4.15252 2.73369 3.68680 4.50728 2.04448 3.86822 2.94733 3.28322 2.78198 3.13348 3.72717 5.85985 4.40757 148 d x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
140 3.15064 5.25820 3.55508 2.88396 4.73664 3.74338 3.67737 4.08071 1.04270 3.50477 4.30035 3.27766 4.11261 2.81137 1.80766 3.14123 3.31217 3.76096 5.52670 4.37198 149 k x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
141 3.40816 4.75217 4.89292 4.34311 3.09177 4.61117 4.89570 2.26608 4.14676 0.85259 2.05263 4.61715 4.79484 4.29520 4.26967 3.97537 3.63147 2.42939 5.19740 4.11945 150 l x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
142 2.55945 4.68963 2.78100 2.56828 4.48999 2.37674 3.94151 3.96782 2.83943 3.55732 4.37155 1.55241 3.81925 3.12580 3.28472 2.12940 2.94710 3.47910 5.73885 4.39661 151 n x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
143 2.99914 4.90791 2.30480 2.76983 3.00848 3.60972 3.75167 3.52536 3.01195 3.09501 4.09139 3.22909 4.10908 3.27543 3.41615 3.05013 3.26541 3.28148 4.58528 1.27355 152 y x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
144 3.29556 4.62685 4.90595 4.33096 2.59248 4.53341 4.79702 2.24923 4.20724 0.91780 2.52035 4.55917 4.72247 4.27733 4.30484 3.86848 3.51279 2.14765 5.09987 4.60236 153 1 x - -
    2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
    0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
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FIG. 12 (Cont. 14)

145	2.17096 4.21419 3.84787 3.43154 3.79650 3.33039 4.28864 2.68630 3.37015 2.76848 3.74222 3.59524 3.96586 3.66357 3.63549 2.20214 2.86866 1.31191 5.31213 4.10059 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	154	v x - -
146	2.61873 4.56711 2.49746 2.62150 2.54151 3.48105 3.72676 3.12886 2.64418 2.44266 3.67062 2.87605 3.88802 2.96699 3.06722 2.50384 2.85697 2.86649 5.06674 3.74446 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	155	l x - -
147	2.95423 5.17668 2.96388 2.59181 4.58584 3.52071 3.72228 4.02499 1.18162 3.51906 4.36953 2.41695 3.98977 2.86856 2.48507 2.93851 3.18628 3.66369 5.59027 4.31134 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	156	k x - -
148	2.73726 5.22229 1.97298 2.08533 4.53610 3.35118 3.65602 4.01528 2.05145 3.51394 4.29017 2.59530 3.82520 2.77878 2.96801 2.68413 2.50700 3.59923 5.66699 4.24509 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	157	d x - -
149	2.03309 4.62433 3.24659 2.71186 3.89367 3.50560 3.77919 2.58029 1.84218 2.87182 3.77503 3.16740 3.93039 2.98235 2.88438 2.78157 2.91657 2.84118 5.23942 3.96807 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	158	k x - -
150	2.65436 5.05521 2.60574 2.32501 4.37480 3.05953 3.62231 3.83346 2.03664 3.35850 4.13392 2.86843 2.77613 2.57821 2.86968 2.26831 2.89393 3.43870 5.52887 4.14122 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	159	k x - -
151	2.45516 4.65515 2.83251 2.27433 3.85749 3.45616 3.67372 2.74916 2.51886 2.89673 3.73760 3.02814 3.45744 2.69890 2.96217 2.67199 2.82671 2.56871 5.18488 3.44681 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	160	e x - -
152	2.54095 5.22751 2.05657 2.09956 4.53900 2.72452 3.23685 4.02352 2.47061 3.51907 4.29107 2.42978 3.81873 2.54406 2.98671 2.67398 2.97396 3.60276 5.67128 4.24276 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	161	d x - -
153	2.51942 5.11682 2.63318 1.62723 4.44834 3.36106 3.68488 3.90247 2.47948 3.43681 4.23572 2.86400 3.84374 2.35245 2.95156 2.31369 2.99051 3.51410 5.61415 4.22408 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	162	e x - -
154	2.16985 4.61145 3.09632 2.40836 3.88792 3.41610 3.72368 3.27105 2.57488 2.54815 3.77386 3.06033 2.71256 2.91875 3.00480 2.47280 2.65145 2.97467 5.22034 3.92506 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	163	a x - -
155	2.39475 2.97704 3.96472 3.69029 4.37241 0.84249 4.55555 3.75317 3.63702 3.52262 4.40740 3.66215 3.86165 3.92661 3.84678 2.60642 2.90713 3.24795 5.69952 4.57833 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510	164	g x - -

FIG. 12 (Cont. 15)

156	2.35097 4.30977 3.31116 3.15916 4.52972 1.07068 4.35135 4.02206 3.36211 3.69210 4.51868 3.35191 3.78837 3.63052 3.67333 1.93335 2.85784 3.40671 5.84392 4.61899 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	165 g x - -
157	2.65545 4.41259 4.63599 4.08371 3.36969 4.29418 4.70034 1.63146 3.96644 1.32243 3.20362 4.31394 4.59335 4.17608 4.14456 3.62911 3.28863 1.76260 5.23685 4.08810 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	166 l x - -
158	2.71956 4.33890 3.73638 3.22868 3.16705 3.68619 3.94680 2.70117 3.09712 1.42761 3.38439 3.54582 4.09791 3.42579 3.36745 2.47016 2.97375 2.53951 4.74178 3.01118 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	167 l x - -
159	2.76768 5.12938 2.96240 2.44606 4.47331 3.47820 2.87686 3.90813 1.93578 3.39755 4.18948 2.07407 3.87874 2.51671 2.40370 2.74215 2.98362 3.52743 5.51969 4.19068 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	168 k x - -
160	1.37585 4.29292 3.39773 3.07118 4.34644 1.98110 4.18978 3.75830 3.14512 3.43045 4.25147 3.30470 2.56866 3.43442 3.49819 2.48834 2.78835 3.23771 5.67187 4.44252 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	169 a x - -
161	3.00229 4.40597 4.40912 3.87549 2.39074 4.07601 3.96792 2.13483 3.74136 1.81418 3.27223 3.98839 4.39754 3.69088 3.88141 3.38420 3.22936 2.49214 4.32296 1.64239 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	170 y x - -
162	3.11472 5.09110 3.10202 2.86933 4.29287 3.55534 4.02691 3.97194 2.67386 3.44747 4.47621 3.32828 4.11766 0.83657 2.94936 3.15533 3.41531 3.68785 5.52206 4.23682 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	171 q x - -
163	3.25990 4.63815 4.76095 4.25894 3.23718 4.43615 4.86912 2.13515 4.07832 0.90310 3.07228 4.50043 4.72826 4.29385 4.23416 3.82510 3.51880 1.81519 5.27504 4.12362 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	172 l x - -
164	1.85833 4.22873 3.57849 3.21306 4.23610 3.05494 4.23617 3.57026 3.20999 3.31182 4.15620 3.38055 3.77537 3.51029 3.52734 1.99244 1.38254 3.10000 5.60168 4.39007 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	173 t x - -
165	2.46320 4.89465 2.81374 2.25659 4.17168 3.07741 3.67031 3.60327 2.48486 3.19186 4.00181 1.97943 3.83093 2.82114 2.95375 2.50772 2.89837 3.26106 5.41776 3.35455 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	174 n x - -
166	2.64394 4.99445 2.89192 2.26253 4.28999 3.41096 3.19632 3.73287 1.95430 3.27279 4.05561 2.53671 3.02376 2.72996 2.78768 2.63460 2.87405 2.98815 5.45289 4.08923 2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503 0.62418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95310	175 k x - -

FIG. 12 (Cont. 16)

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178  3.01803  4.75986  3.74392  3.67988  4.80222  0.42314  4.77677  4.47208  3.89576  4.69108  5.07443  3.90927  4.15863  4.20000  4.09622  3.19854  3.51923  3.95051  5.82154  4.91540  189 G x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

179  3.06155  4.39329  4.78365  4.23263  2.16021  4.33412  4.67246  1.62941  4.10928  1.90751  3.18890  4.39366  4.62034  4.26944  4.23350  3.67468  3.30113  1.43472  5.10032  3.86900  190 v x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

180  3.32382  4.61950  4.98332  4.45979  3.28753  4.64349  5.03155  1.26517  4.29749  1.21826  3.09768  4.69351  4.85713  4.47191  4.42929  4.02526  3.56291  1.96875  5.35776  4.22264  191 l x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

181  3.73549  4.95573  4.74114  4.44606  0.87363  4.48219  3.56098  3.36562  4.28496  2.67234  3.96179  4.17516  4.78968  4.23640  4.29912  3.86180  3.95914  3.31077  3.69386  1.60022  192 f x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

182  3.79040  5.00485  4.71402  4.42808  1.60446  4.49364  3.52625  3.47869  4.27039  2.78842  4.07242  4.15578  4.80367  4.22561  4.29484  3.87395  4.01326  3.40624  3.65813  0.83263  193 y x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

183  3.19145  4.47275  4.91126  4.44612  3.53672  4.59547  5.088621.46782  4.33971  1.73199  3.33123  4.65784  4.84848  4.55234  4.50677  3.96712  3.43973  1.13773  5.52283  4.35470  194 v x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

184  2.66032  4.70358  2.89201  2.71275  4.37649  3.24289  4.03083  3.91373  2.90724  3.53686  4.40607  2.30578  1.27476  3.25574  3.28575  2.74778  3.05511  3.47429  5.65434  4.33562  195 p x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

185  1.11177  4.43010  3.15158  2.91416  4.29503  3.13620  4.12215  3.66913  3.04358  3.39689  4.26035  2.38394  3.82043  3.36664  3.39932  2.59726  2.89066  3.22396  5.63577  4.35750  196 a x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

186  1.66707  4.29549  3.57254  3.09600  3.53286  2.93819  3.96373  3.02262  3.00832  2.73304  3.64079  3.39727  3.92560  3.34213  3.31374  2.74437  2.85584  2.75315  2.31534  3.70083  197 a x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

187  3.03636  4.67833  3.74688  3.27660  1.97138  3.88747  3.62560  3.22234  3.23320  2.77787  3.80630  2.01833  4.26594  3.48346  3.53813  3.18004  3.27114  3.02649  4.08829  1.71684  198 y x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.02418  4.13012  4.85247  0.61958  0.77255  0.48576  0.95510

188  2.57369  4.41659  3.68150  3.45563  4.22088  3.24488  4.45012  3.38072  3.40378  3.23478  4.27482  3.61620  3.97187  3.78716  3.64444  2.77866  0.79961  3.04671  5.63585  4.43998  199 t x - -
     2.68962  4.42820  2.76946  2.72989  3.44175  2.41163  3.73534  3.28233  2.67108  2.69357  4.24270  2.90231  2.74776  3.18548  2.89789  2.38231  2.77429  2.98869  4.58393  3.60445
     0.77820  0.62931  4.85247  4.93718  0.00720  0.48576  0.95510

189  2.34772  4.94441  2.66757  2.37110  4.27936  3.37475  3.63060  3.72421  2.00200  3.27535  4.05913  2.90201  3.04389  2.75731  2.87717  2.32649  2.66578  3.34373  5.46649  4.09730  388 k x - -
     2.68618  4.42225  2.77519  2.73123  3.46354  2.40513  3.72494  3.29354  2.67741  2.69355  4.24690  2.90347  2.73739  3.18146  2.89801  2.37887  2.77519  2.98518  4.58477  3.61503
     0.06622  4.13012  3.03667  0.61958  0.77255  0.48576  0.95510
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FIG. 12 (Cont. 18)

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202	2.62393	3.52496	3.07354	2.59904	3.87091	3.41411	2.46101	3.42614	2.54719	3.05316	3.90265	1.95736	3.87304	2.93930	2.94760	2.70504	2.89868	3.11113	5.21456	3.85323	403 n x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
203	3.21397	4.53943	4.82960	4.32329	2.42039	4.45252	4.69425	1.69496	4.19513	1.81950	3.17868	4.49220	4.72731	4.34887	4.32368	3.82245	3.45900	2.01124	5.03219	3.69984	404 i x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
204	0.94185	4.23506	3.56526	3.33878	4.42626	2.18549	4.40088	3.78137	3.42743	3.53049	4.38019	3.44407	3.78668	3.70336	3.70886	2.50027	2.81745	3.23903	5.77230	4.58914	405 a x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
205	2.85684	4.77935	3.46508	2.83873	3.97616	3.65384	3.72719	3.32276	1.84885	1.78242	3.84399	3.25604	4.02534	2.92215	2.17052	2.95660	3.06030	3.03576	5.22701	4.00132	406 l x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
206	3.14028	5.23801	3.50077	2.87515	4.70334	3.72380	3.69471	4.06176	0.98585	3.49959	4.38085	3.27386	4.10904	2.85323	1.96379	3.13550	3.31219	3.74318	5.52822	4.36511	407 k x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
207	2.67885	4.47227	3.58015	3.25655	2.39212	1.16523	4.12073	3.29709	3.29298	2.96697	3.96895	3.52945	4.02182	3.58712	3.59356	2.87133	3.06501	3.02217	5.00616	3.57046	408 g x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
208	2.64729	4.23826	3.74810	2.86016	3.31643	3.68552	3.96290	2.37556	3.08388	1.68329	2.72124	3.51936	4.05544	3.37606	3.36860	2.72203	2.88008	2.39000	4.84572	3.63819	409 i x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
209	2.88509	4.27476	4.44696	3.59929	2.19846	3.97358	3.92049	2.61294	3.74414	1.61599	2.87834	3.95197	4.30903	3.87098	3.83636	3.28211	3.11239	2.50277	2.65199	2.43994	410 i x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.12378	4.13012	2.29911	0.61958	0.77255	0.48576	0.95510																		
210	2.42592	3.64613	3.42248	2.60414	3.44651	3.53744	3.79551	2.59699	2.80627	2.37899	3.40537	3.26812	3.91994	2.82266	3.16409	2.78334	2.58673	2.25173	4.89111	3.65402	411 v x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
211	3.17764	4.50887	4.80002	4.23209	3.16618	4.45226	4.79088	1.78751	4.11385	1.20554	2.33995	4.47167	4.67000	4.24133	4.24664	3.78832	3.40376	1.66347	5.17380	4.69414	412 l x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.46513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
212	2.64523	5.02815	2.42652	2.69815	4.32939	3.37640	3.58960	3.77911	2.32216	2.92228	4.08849	2.86230	3.79336	2.54985	2.26075	2.62461	2.87684	3.39700	5.47593	4.10230	413 e x - -				
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		

FIG. 12 (Cont. 20)

```
213  2.30673 4.81816 3.04461 2.49501 4.08454 3.44943 3.62735 3.47609 1.93304 3.07005 3.32416 2.99355 3.84576 2.36092 2.68787 2.69182 2.88226 3.16310 5.30601 4.00145  414 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02668 4.03302 4.75536 0.61958 0.77255 0.58952 0.80876

214  3.19591 4.51569 4.81138 4.26499 3.27809 4.50469 4.88072 1.17895 4.11789 1.57119 2.71737 4.51549 4.73600 4.31505 4.27910 3.85900 3.43250 1.89082 5.29473 4.16284  415 i x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02668 4.03302 4.75536 0.61958 0.77255 0.42595 0.80876

215  2.47118 5.05350 2.64260 2.34706 4.37713 3.39666 3.60997 3.83157 1.82834 3.35018 4.12420 2.70189 3.81055 2.72699 2.59653 2.31677 2.88985 3.43768 5.51192 4.13451  416 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

216  2.71968 5.19949 2.48828 1.90371 4.52281 3.36913 3.63478 3.99630 2.01513 3.48905 4.25945 2.83710 3.82207 2.24433 2.87931 2.43146 2.95837 3.58113 5.63214 4.22307  417 e x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.12542 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

217  2.70563 5.08280 2.72526 1.81733 4.40005 3.37674 3.61504 3.84456 2.04084 3.36499 4.15850 2.86396 3.81892 2.50173 2.75650 2.49850 2.70999 3.46333 5.52640 4.15933  418 e x - -
     2.68621 4.42228 2.77523 2.73126 3.46357 2.40516 3.72498 3.29357 2.67744 2.69346 4.24693 2.90350 2.73743 3.18149 2.89804 2.37890 2.77523 2.98521 4.58480 3.61413
     0.48236 1.48149 1.86190 0.24325 1.53282 0.59106 0.80685

218  2.59353 4.79690 2.67462 2.39960 4.03523 3.38615 3.57486 3.46673 2.10043 3.05450 3.87241 2.71239 3.78392 2.72543 2.37286 2.61976 2.82132 3.14179 3.78491 3.93793  420 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.03139 3.87271 4.59565 0.61958 0.77255 0.62882 0.76190

219  2.52819 4.55478 3.02236 2.36450 3.27698 2.75979 3.67661 3.21874 2.54144 2.87223 3.71868 3.00594 3.25523 2.87609 2.97424 2.46189 2.52673 2.92315 5.16358 3.86760  421 e x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02940 3.93733 4.65968 0.61958 0.77255 0.38381 1.14338

220  2.81850 5.32045 1.81142 1.80479 4.62043 3.32155 3.71734 4.11544 2.60206 3.62089 4.41791 2.50029 3.85095 2.85519 3.14055 2.74846 2.31278 3.69823 5.78456 4.33646  422 e x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

221  2.48223 5.16696 2.08814 2.12032 4.49656 2.50746 3.66728 3.97234 2.49704 3.46585 4.26578 2.61630 3.23955 2.79405 3.01207 2.49575 2.96914 3.56038 5.65205 4.23362  423 d x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

222  2.73413 5.21474 1.79968 2.10692 4.52863 2.90567 3.65542 4.00740 2.28828 3.50728 4.28372 2.82078 3.82451 2.77834 2.71209 2.50434 2.97898 3.59247 5.66161 4.24109  424 d x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

223  2.53808 4.17721 3.75668 3.18631 2.49332 3.63941 3.92189 2.43396 3.10578 2.11880 3.28316 3.17384 4.01857 3.37495 3.38039 2.33561 2.83391 2.25094 4.76298 3.53175  425 l x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

224  2.47785 4.87401 2.55453 2.27144 4.13407 3.41474 3.61507 3.19068 2.10624 2.72206 3.93966 2.92825 3.80786 2.75106 2.70025 2.61975 2.65098 3.21651 5.36096 4.01982  426 k x x
     2.68618 4.42225 2.77519 2.73123 3.46354 2.40513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510
```

FIG. 12 (Cont. 21)

225	2.61126	4.94527	2.57241	2.37286	3.71167	3.40653	3.59976	3.66836	1.97769	3.22094	4.00500	2.90555	3.37250	2.72400	2.44551	2.60961	2.84179	3.30230	5.41233	3.55985	427	k	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.18397	2.69285	2.29911	0.43489	1.04225	0.48576	0.95510																		
226	2.18405	4.38164	3.37577	2.81062	3.53544	3.54756	3.79314	2.67818	2.45131	2.41847	3.11038	2.90063	3.92988	3.08382	3.13404	2.78686	2.82814	2.34777	4.96377	3.71691	428	a	x	-	-
	2.68606	4.42226	2.77520	2.73124	3.46355	2.40513	3.72495	3.29355	2.67741	2.69356	4.24690	2.90347	2.73740	3.18147	2.89801	2.37888	2.77520	2.98519	4.58478	3.61504					
	0.18397	2.69285	2.29911	0.43489	1.04225	0.48576	0.95510																		
227	2.57019	3.69781	2.80661	2.47625	3.49110	3.41201	3.64072	3.31706	2.01030	2.94744	3.77805	2.98079	3.33001	2.81253	2.90900	2.49197	2.80698	3.01391	5.21612	3.90115	430	k	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
228	2.81510	4.36656	4.00610	3.47175	3.23340	3.64296	4.19674	2.40289	3.29514	1.24901	2.79858	3.77015	4.22661	3.61756	3.53563	2.82539	3.07208	2.33413	4.95414	3.73407	431	i	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.08766	4.03302	2.71491	0.61958	0.77255	0.58952	0.80876																		
229	1.88128	4.80310	2.80372	2.22522	4.13838	3.34167	3.66684	3.53844	2.21992	3.14696	3.97800	2.92546	3.30399	2.83016	2.86040	2.66273	2.88978	3.20322	5.38230	4.05664	432	a	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02833	3.97368	4.69603	0.61958	0.77255	0.54804	0.86294																		
230	3.19616	4.50286	4.86442	4.32672	3.32366	4.53213	4.94945	1.34551	4.20041	1.37100	3.12115	4.56623	4.76654	4.38591	4.35908	3.89430	3.43719	1.69436	5.35009	4.21795	433	i	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
231	2.51435	4.60512	3.01948	2.57219	4.11628	3.27251	3.75749	3.52378	2.32853	3.14618	3.97173	3.02765	3.09031	2.93367	2.96803	1.83219	2.35460	3.15447	5.38847	4.08634	434	s	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
232	2.81965	5.02540	2.66137	2.42816	3.97065	3.36788	2.54257	3.85565	2.53077	3.39462	4.26560	1.57684	3.89853	2.94122	2.92218	2.81332	3.09474	3.50124	5.31188	3.83925	435	n	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
233	2.46168	4.99954	3.01886	2.47397	4.33955	3.46290	3.60057	3.75117	1.65945	3.27553	4.08408	2.97450	3.32043	2.50538	2.36729	2.72481	2.94535	3.39639	5.42850	4.12348	436	k	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.58952	0.80876																		
234	2.49023	5.28394	2.04462	1.56446	4.58573	3.29199	3.68128	4.06467	2.33890	3.57462	4.37638	2.75902	3.82022	2.82053	3.07841	2.72239	3.04985	3.65464	5.73993	4.29860	437	e	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02668	4.03302	4.75536	0.61958	0.77255	0.42595	1.05886																		
235	3.61543	4.88773	4.69185	4.34603	1.69853	4.38489	3.63739	3.27255	4.14768	2.58034	3.86001	4.16132	4.71632	4.18449	4.19510	3.79028	3.84818	3.22423	1.17687	2.08425	438	w	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		
236	3.44236	4.76578	4.90063	4.39881	2.22305	4.58884	4.61150	2.36138	4.25018	0.81644	2.97248	4.57671	4.79474	4.32575	4.34281	3.96640	3.66809	2.51693	4.83008	3.45127	439	l	x	-	-
	2.68618	4.42225	2.77519	2.73123	3.46354	2.40513	3.72494	3.29354	2.67741	2.69355	4.24690	2.90347	2.73739	3.18146	2.89801	2.37887	2.77519	2.98518	4.58477	3.61503					
	0.02418	4.13012	4.85247	0.61958	0.77255	0.48576	0.95510																		

FIG. 12 (Cont. 22)

```
237  2.63160 5.06455 2.57753 1.99902 4.37430 3.37882 3.59680 3.44694 2.25291 3.35417 4.11835 2.51129 3.79111 2.56360 2.84777 2.44737 2.65383 3.43672 5.51681 4.12214   440 e x - -
     2.68618 4.42225 2.77519 2.73123 3.46357 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

238  3.76851 4.97197 4.76815 4.48195 0.92854 4.58209 3.54206 3.41176 4.31842 2.71679 4.00275 4.18108 4.80514 4.25081 4.32077 3.88017 3.98943 3.35066 3.66965 1.45400   441 f x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

239  2.20908 4.38551 4.18048 3.62120 3.34372 3.92886 4.31537 2.03681 3.51539 2.97243 1.90374 3.89237 4.29696 3.76814 3.74852 3.23813 3.06757 1.98602 5.03631 3.85870   442 m x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

240  3.11472 5.09110 3.10202 2.86933 4.29287 3.55534 4.02691 3.97194 2.67386 3.44747 4.47621 3.32828 4.11766 0.83657 2.94936 3.15533 3.41531 3.68785 5.52206 4.23682   443 q x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

241  2.31978 4.92267 2.82856 2.13516 4.22950 3.38204 3.65440 3.66362 2.44724 3.23543 4.03605 2.63709 3.81932 2.61691 2.91664 2.45243 2.21674 3.30457 5.44694 4.08783   444 e x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

242  2.70209 4.96265 3.06674 2.49556 4.26277 3.08126 3.61768 3.67682 1.82627 2.94084 4.03017 2.99155 3.86524 2.39896 2.29187 2.70957 2.92053 3.04634 5.40245 4.08740   445 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

243  2.61458 5.00553 2.54503 2.34441 4.30786 3.07626 3.59030 3.76125 2.13203 3.29065 3.50711 2.88290 2.91215 2.56670 2.59515 2.59954 2.84571 3.37367 5.46373 4.08504   446 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.02418 4.13012 4.85247 0.61958 0.77255 0.48576 0.95510

244  2.59290 4.41084 2.99664 2.76200 2.91713 3.18688 3.76435 2.55316 2.40903 2.62745 3.50897 3.20445 3.91327 3.04861 3.11605 2.76595 2.82478 2.69289 4.95676 2.77598   447 k x - -
     2.68618 4.42225 2.77519 2.73123 3.46354 2.46513 3.72494 3.29354 2.67741 2.69355 4.24690 2.90347 2.73739 3.18146 2.89801 2.37887 2.77519 2.98518 4.58477 3.61503
     0.01634 4.12228       * 0.61958 0.77255 0.00000       *
```

FIG. 12 (Cont. 23)

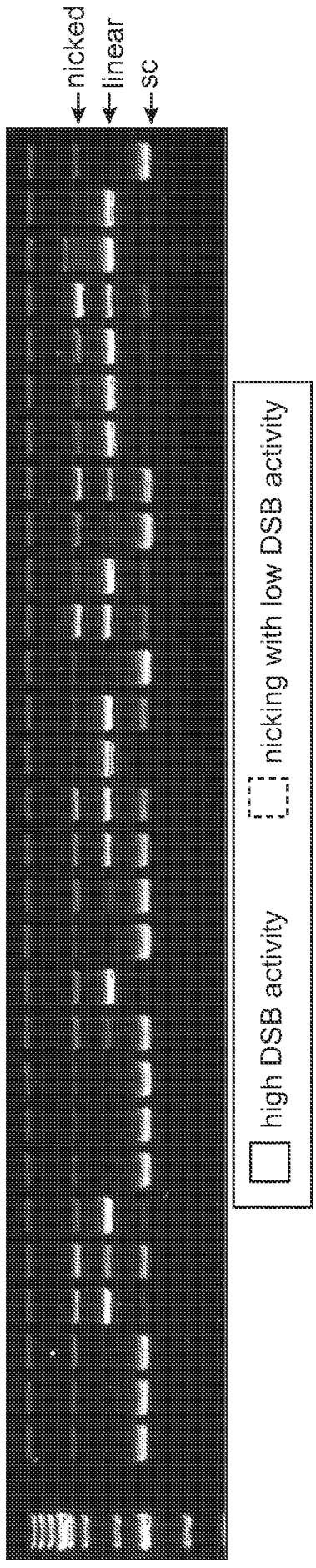

nicked
linear
sc
48 47 46 45 44 43 42 41 39 38 37 36 35 34 33 32 31 30 29 28 27 26 25 24 22 21 20 12 --
high DSB activity
nicking with low DSB activity

FIG. 13A

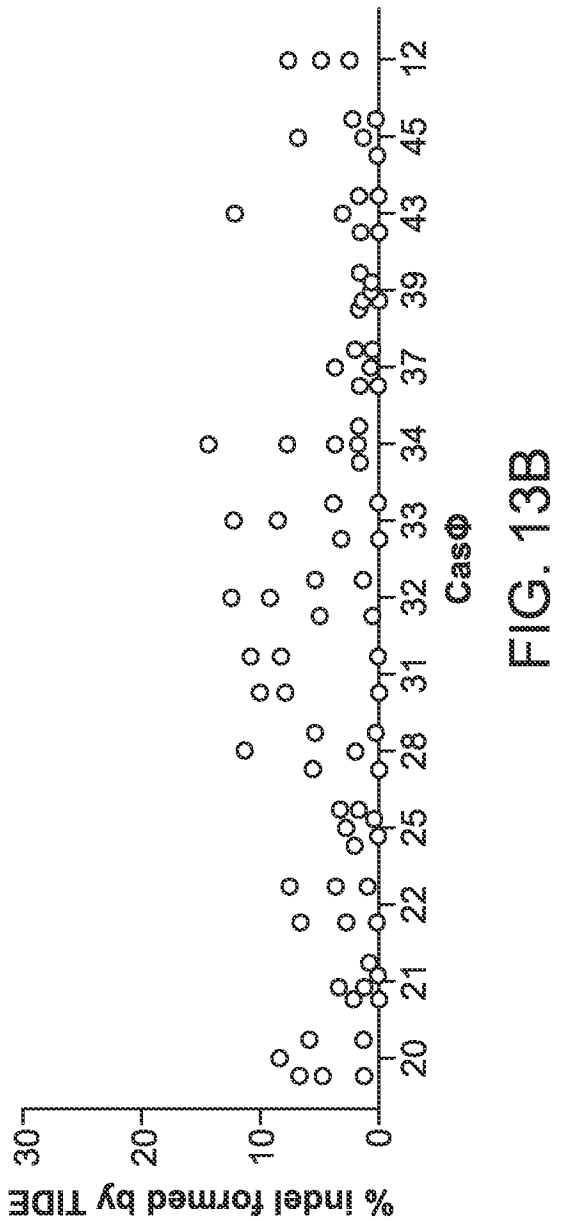


FIG. 13B

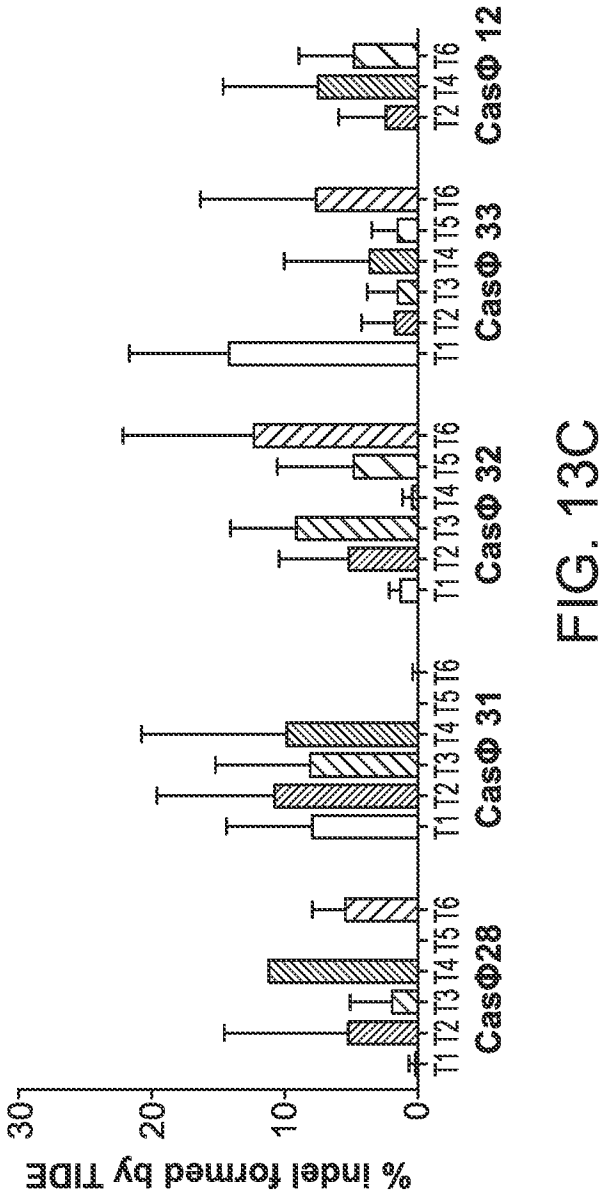


FIG. 13C

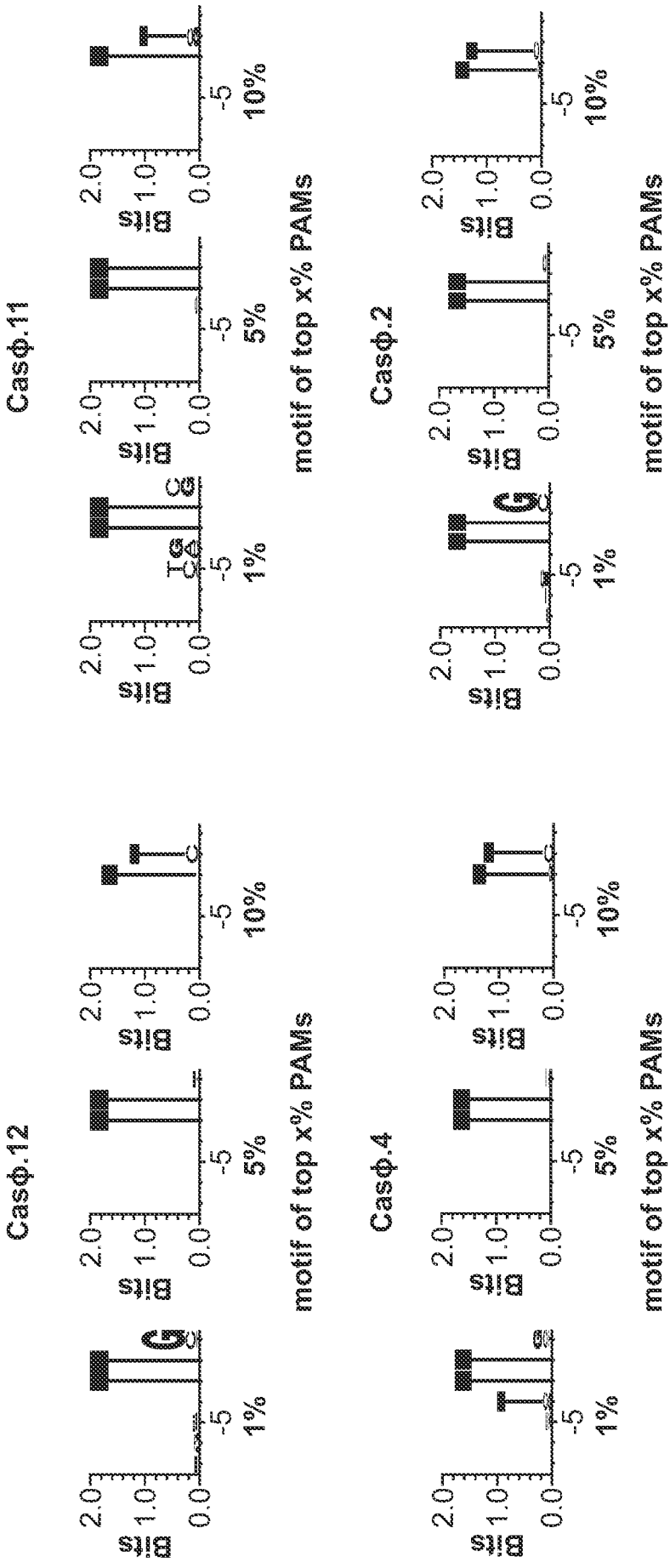

Casϕ.11
Casϕ.2
Casϕ.12
Casϕ.4
bits
2.0
1.0
0.0
-5
1%
5%
10%
motif of top x% PAMs

FIG. 14A

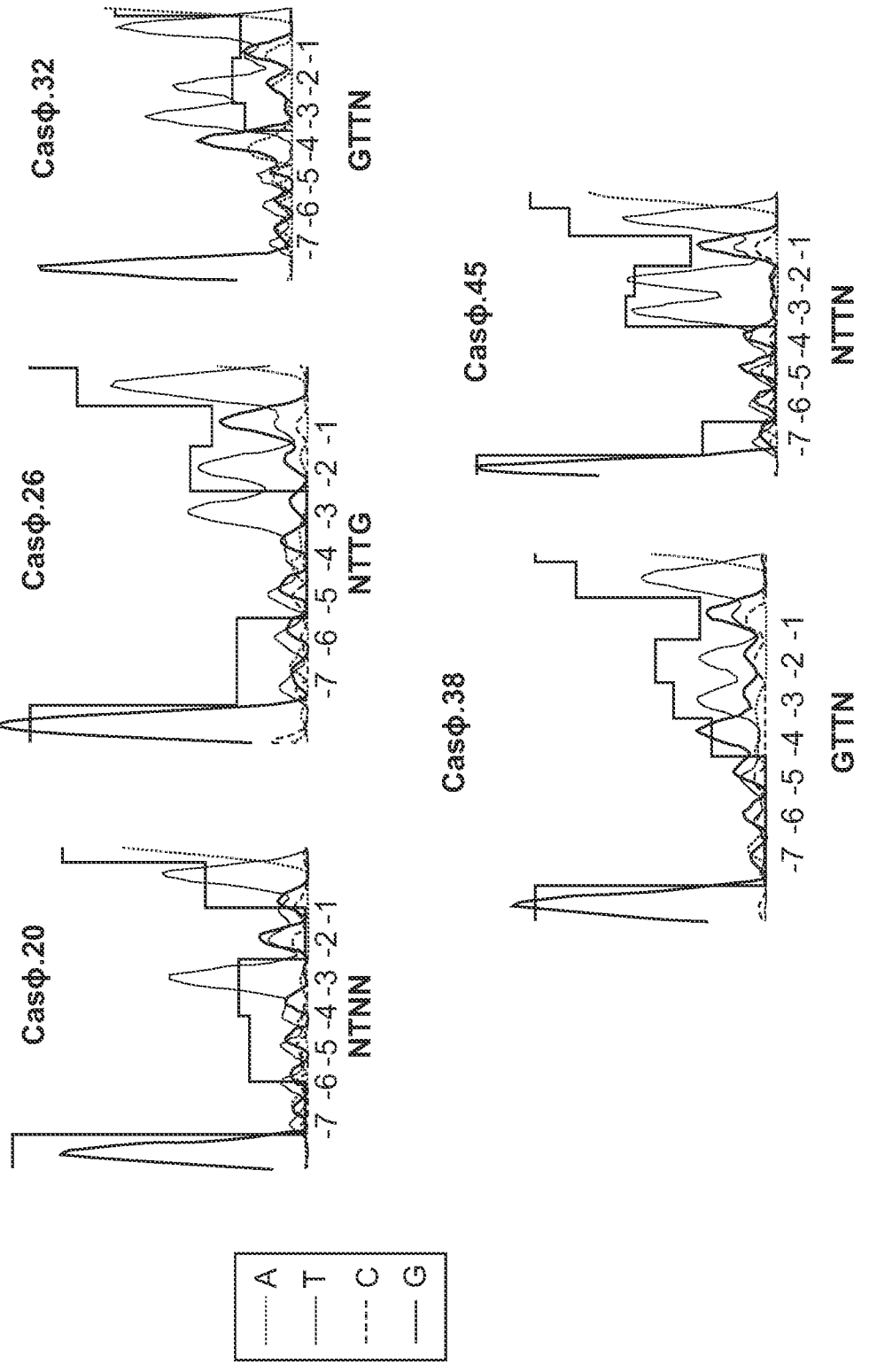

Casϕ.20
NTNN
Casϕ.26
NTTG
Casϕ.32
GTTN
Casϕ.38
GTTN
Casϕ.45
NTTN
-7 -6 -5 -4 -3 -2 -1
A
T
C
G

FIG. 14B

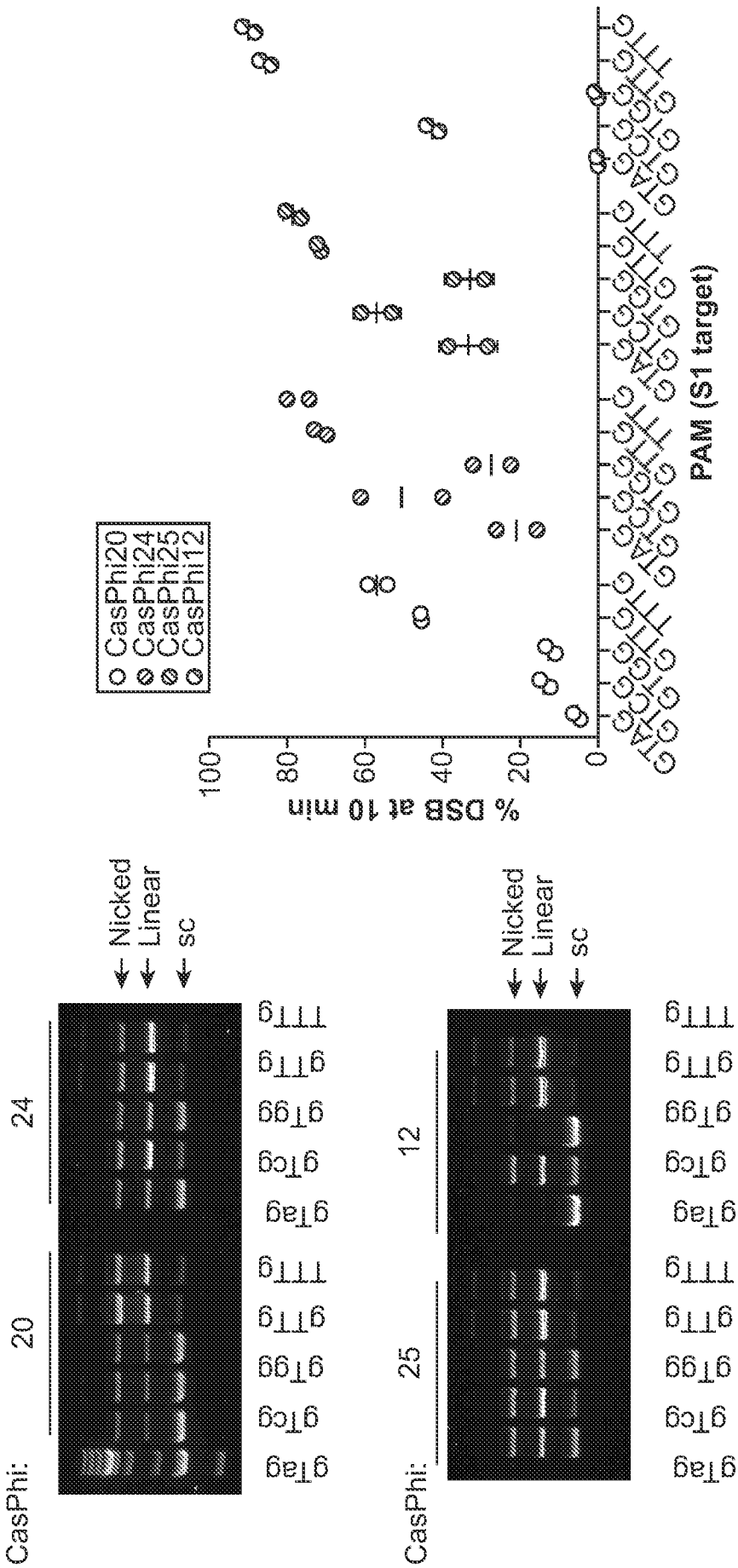


FIG. 14C

FIG. 14D

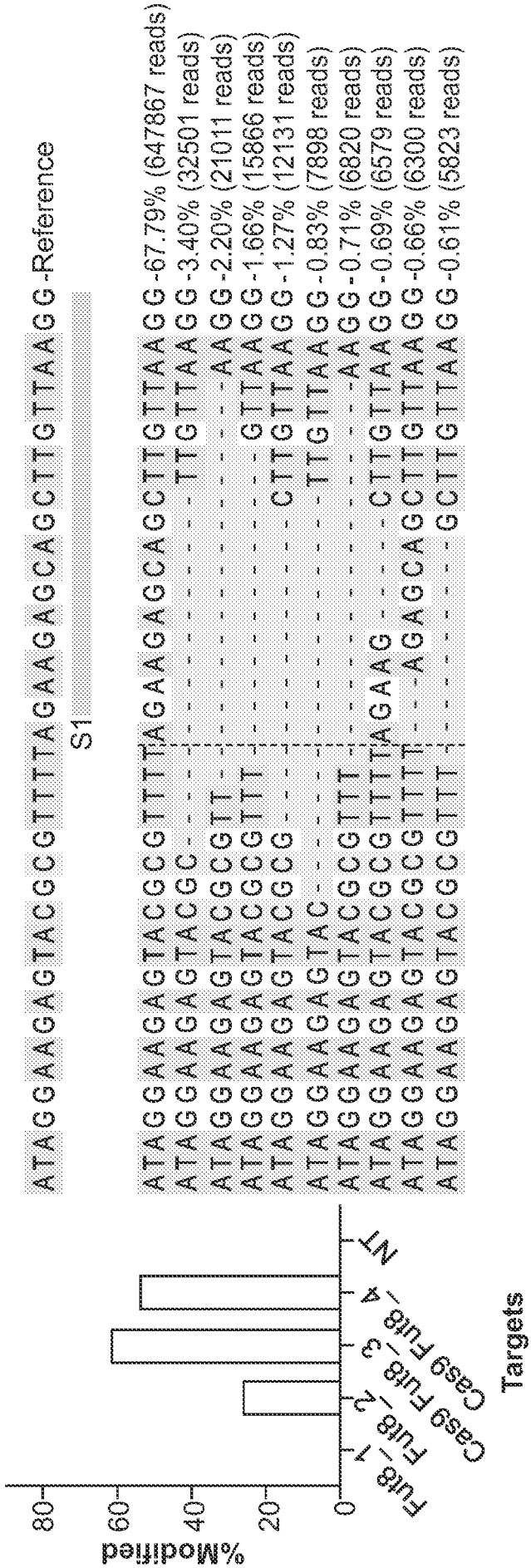

ATAGGAAGAGTACGCGTTTTAGAAGAGCAGCTTGTTAAGG-Reference
S1
ATAGGAAGAGTACGCGTTTTAGAAGAGCAGCTTGTTAAGG-67.79% (647867 reads)
ATAGGAAGAGTACGC----------TTGTTAAGG-3.40% (32501 reads)
ATAGGAAGAGTACGCGTT----------AAGG-2.20% (21011 reads)
ATAGGAAGAGTACGCGTTT----------GTTAAGG-1.66% (15866 reads)
ATAGGAAGAGTACGCG----------CTTGTTAAGG-1.27% (12131 reads)
ATAGGAAGAGTAC----------TTGTTAAGG-0.83% (7898 reads)
ATAGGAAGAGTACGCGTTT----------AAGG-0.71% (6820 reads)
ATAGGAAGAGTACGCGTTTTAGAAG----------CTTGTTAAGG-0.69% (6579 reads)
ATAGGAAGAGTACGCGTTTT----AGAGCAGCTTGTTAAGG-0.66% (6300 reads)
ATAGGAAGAGTACGCGTTT----------GCTTGTTAAGG-0.61% (5823 reads)
%Modified
0
20
40
60
80
Fulb_1
Fulb_2
Cas9 Fulb_3
Cas9 Fulb_4
NT
Targets

FIG. 15

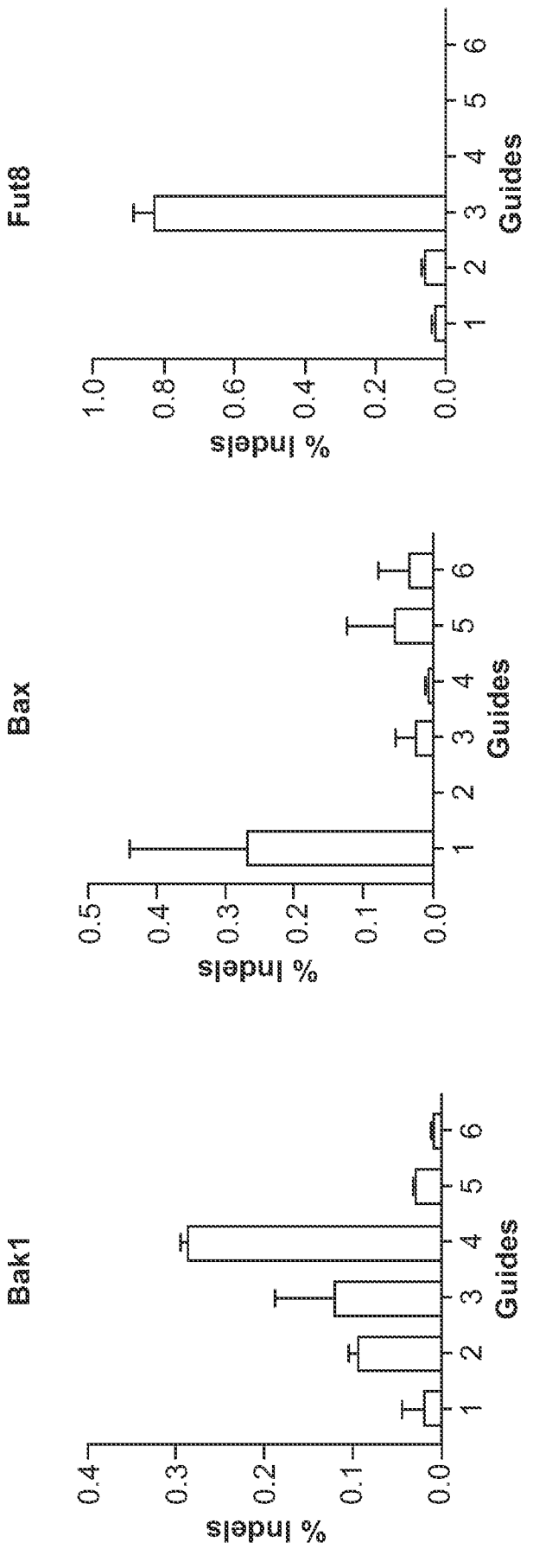

Fut8
% Indels
0.0
0.2
0.4
0.6
0.8
1.0
1 2 3 4 5 6
Guides
Bax
% Indels
0.0
0.1
0.2
0.3
0.4
0.5
1 2 3 4 5 6
Guides
Bak1
% Indels
0.0
0.1
0.2
0.3
0.4
1 2 3 4 5 6
Guides

FIG. 16A

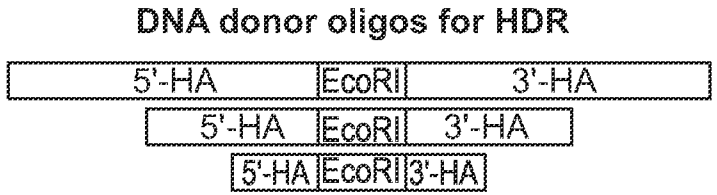


FIG. 16B

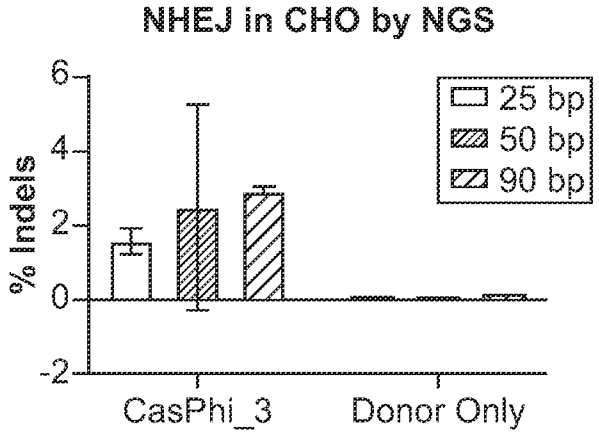


FIG. 16C

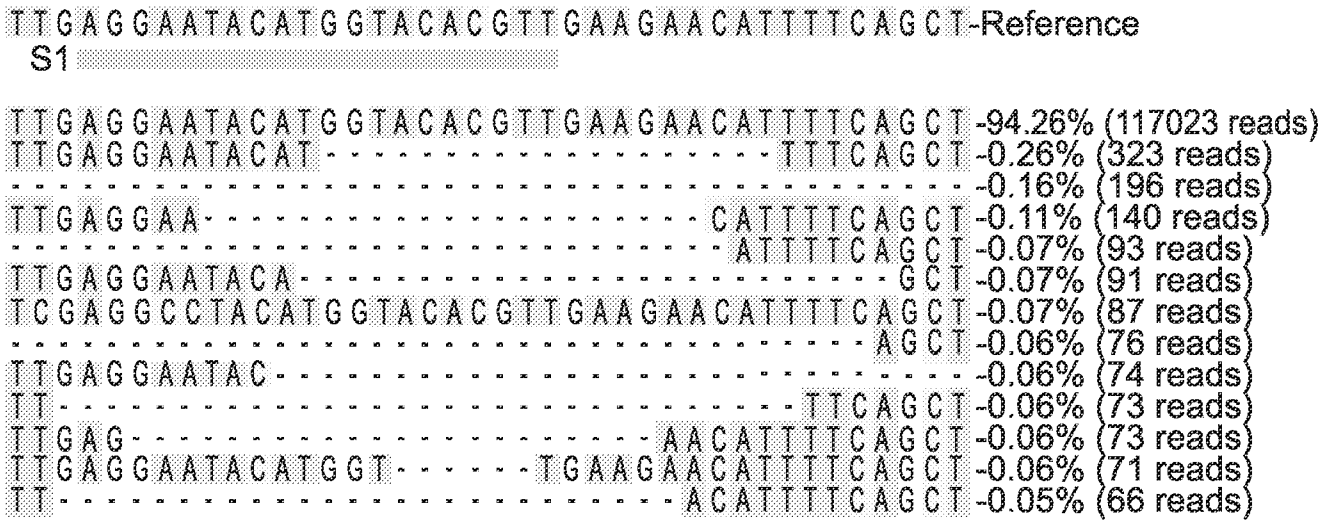


FIG. 16D

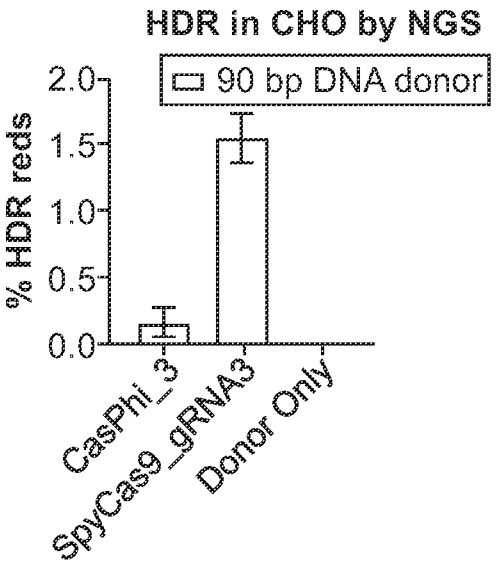


FIG. 16E

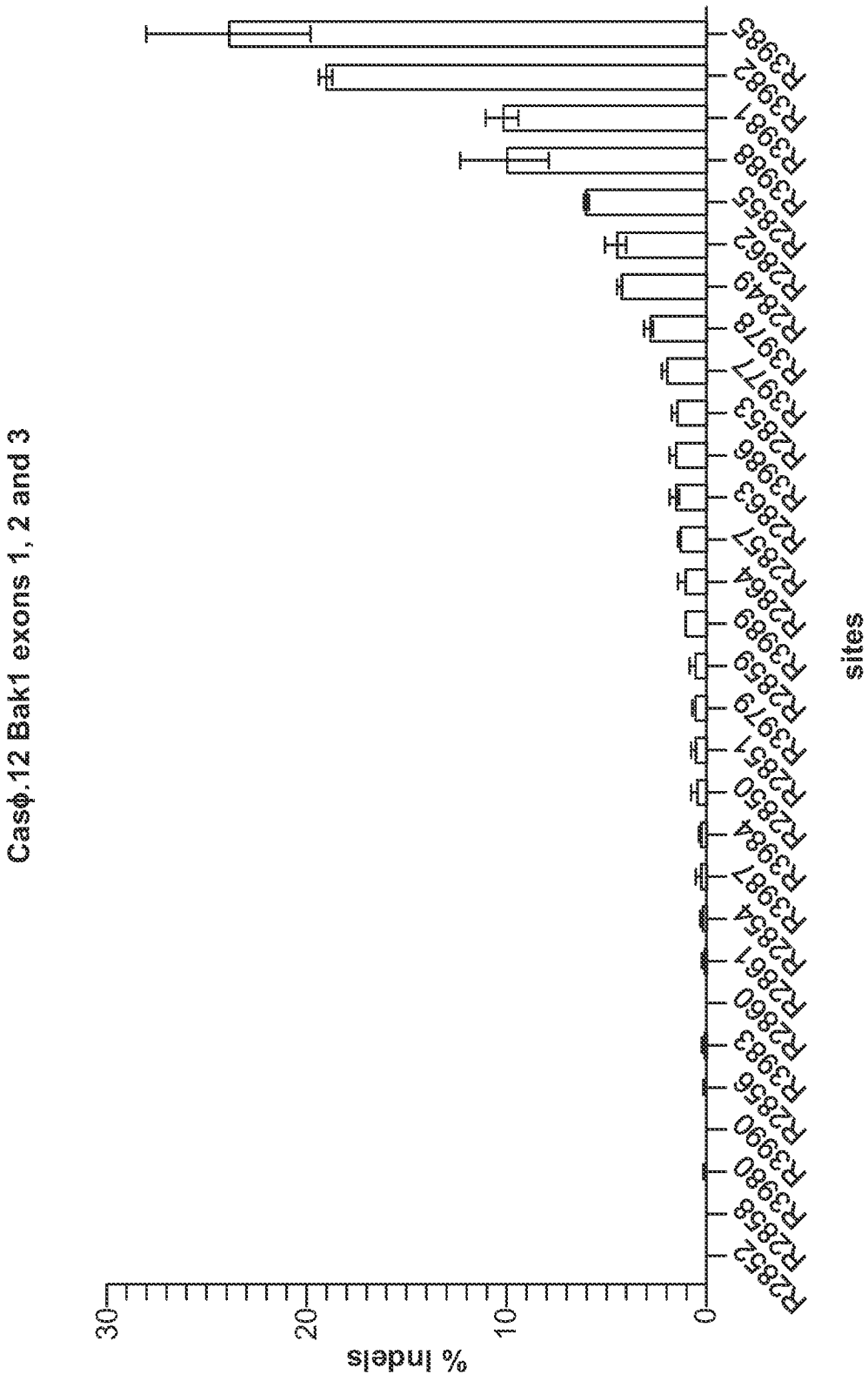

Casϕ.12 Bak1 exons 1, 2 and 3
% Indels
30
20
10
0
sites
R28652
R28658
R39980
R39990
R28656
R39983
R28660
R28661
R28654
R39987
R39984
R28650
R28651
R39979
R28659
R39989
R28664
R28657
R28663
R39986
R28653
R39977
R39978
R28649
R28662
R28655
R39988
R39981
R39982
R39985

FIG. 16F

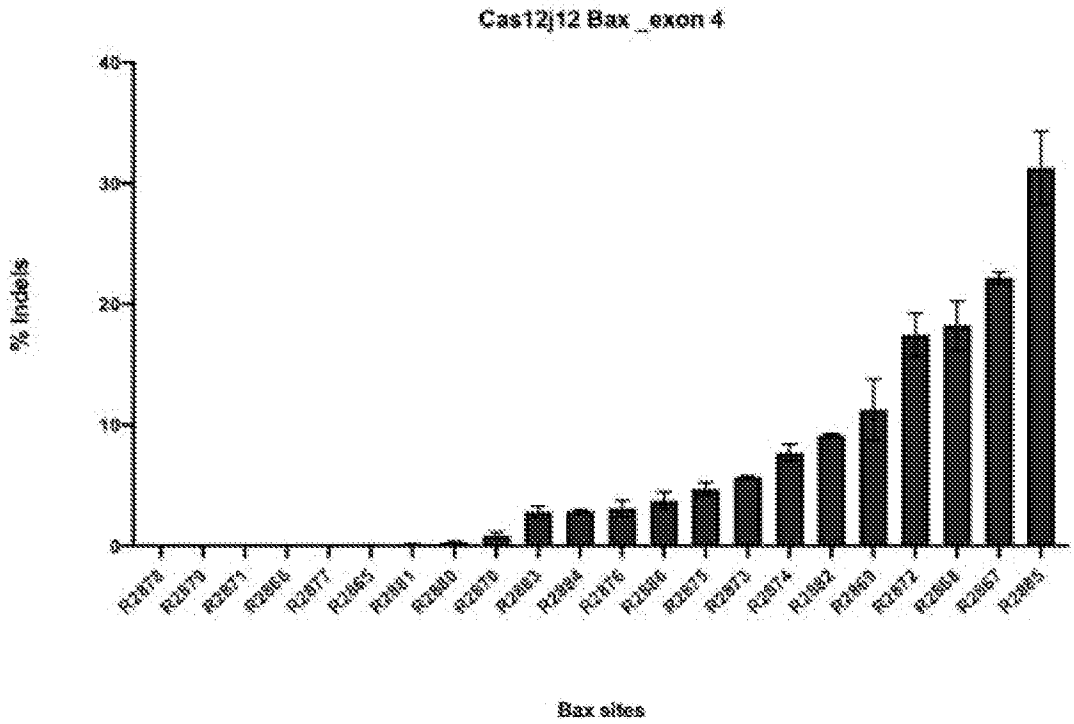


FIG. 16G

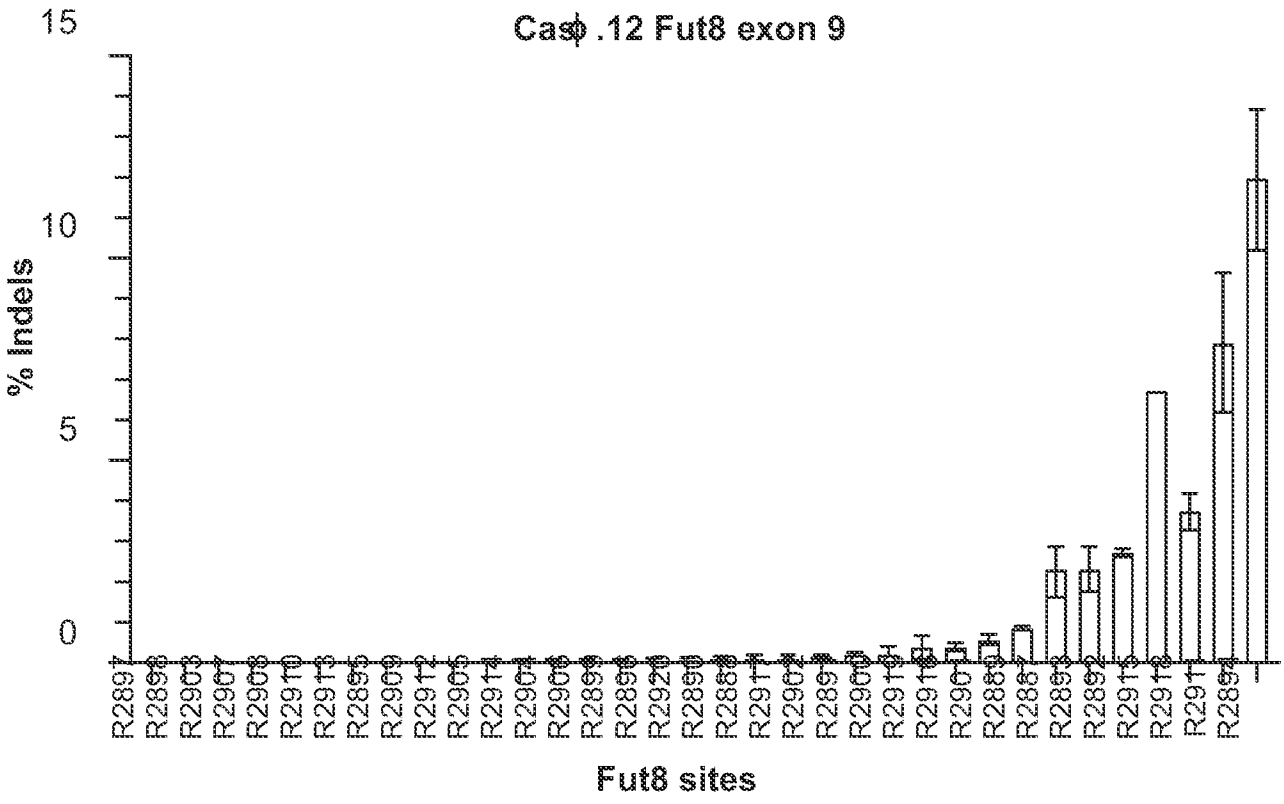


FIG. 16H

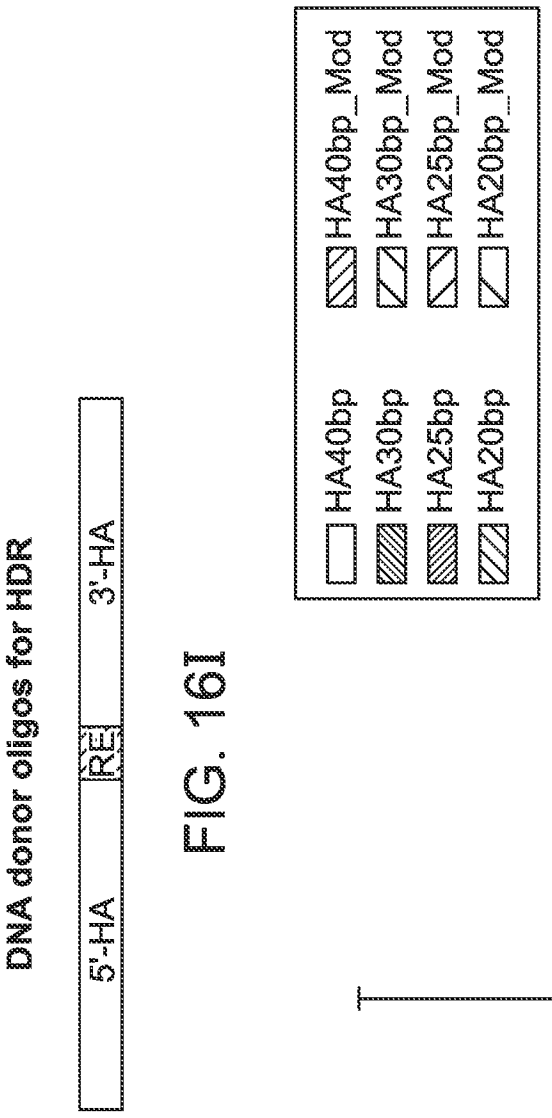


FIG. 16I

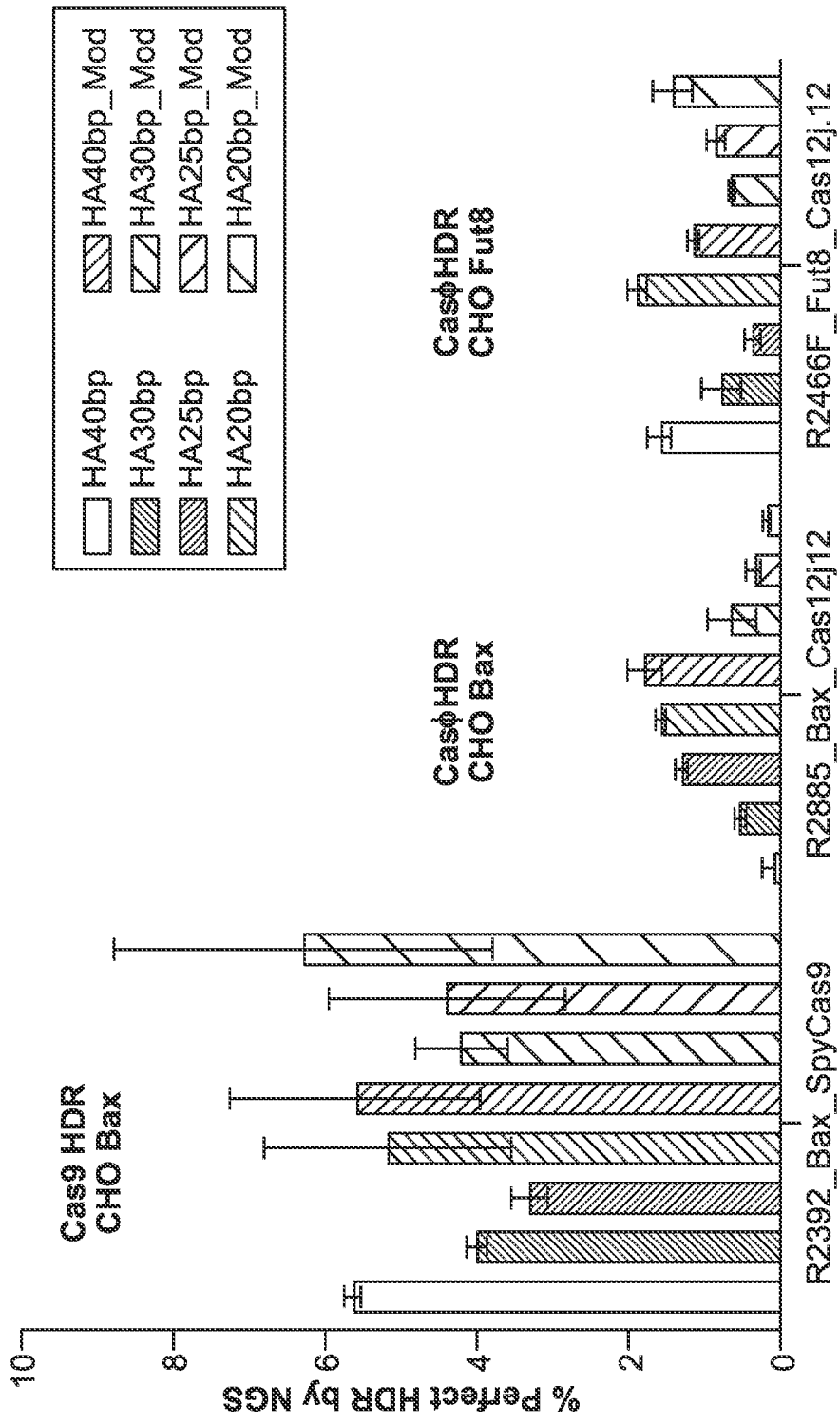


FIG. 16J

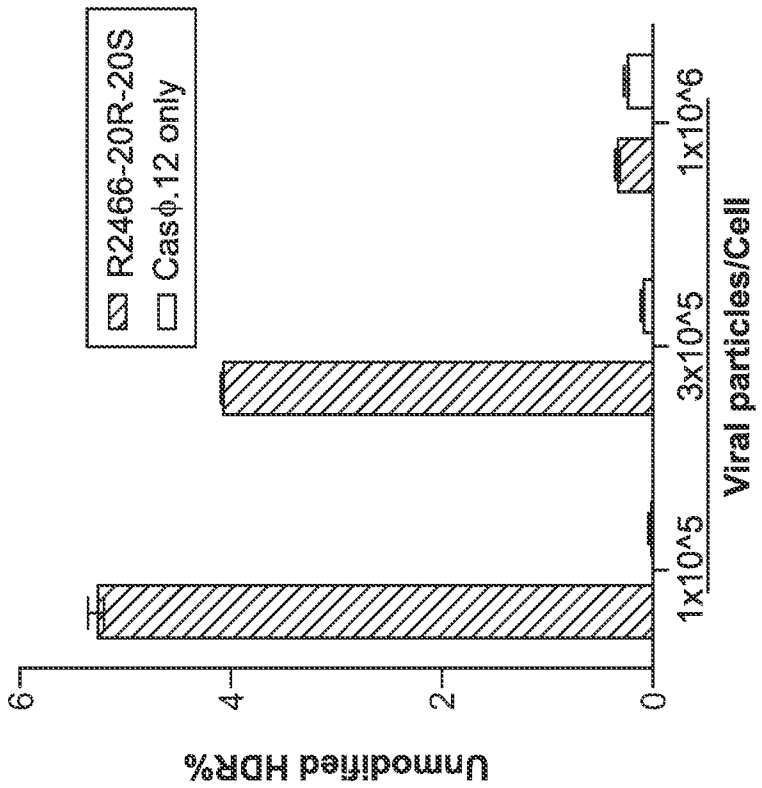


FIG. 16L

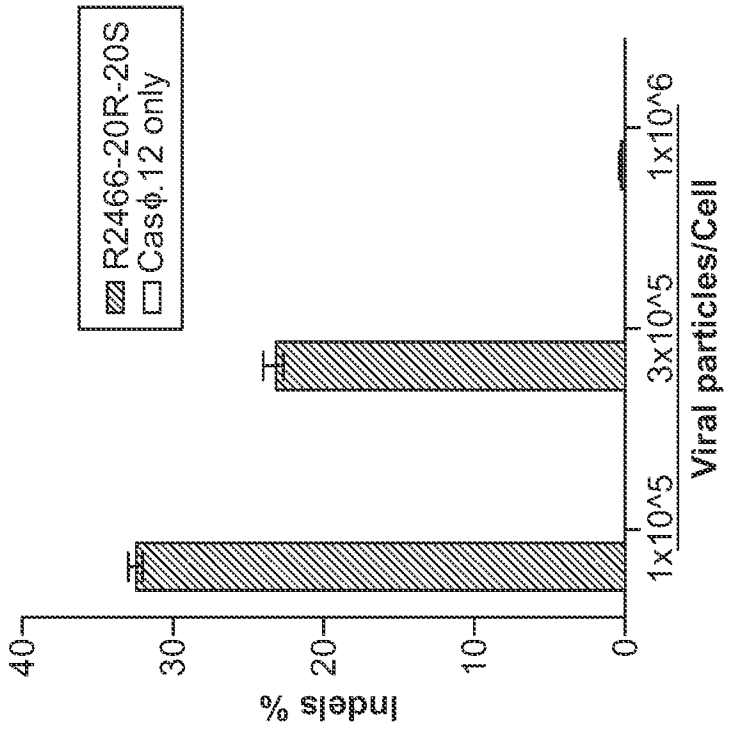


FIG. 16K

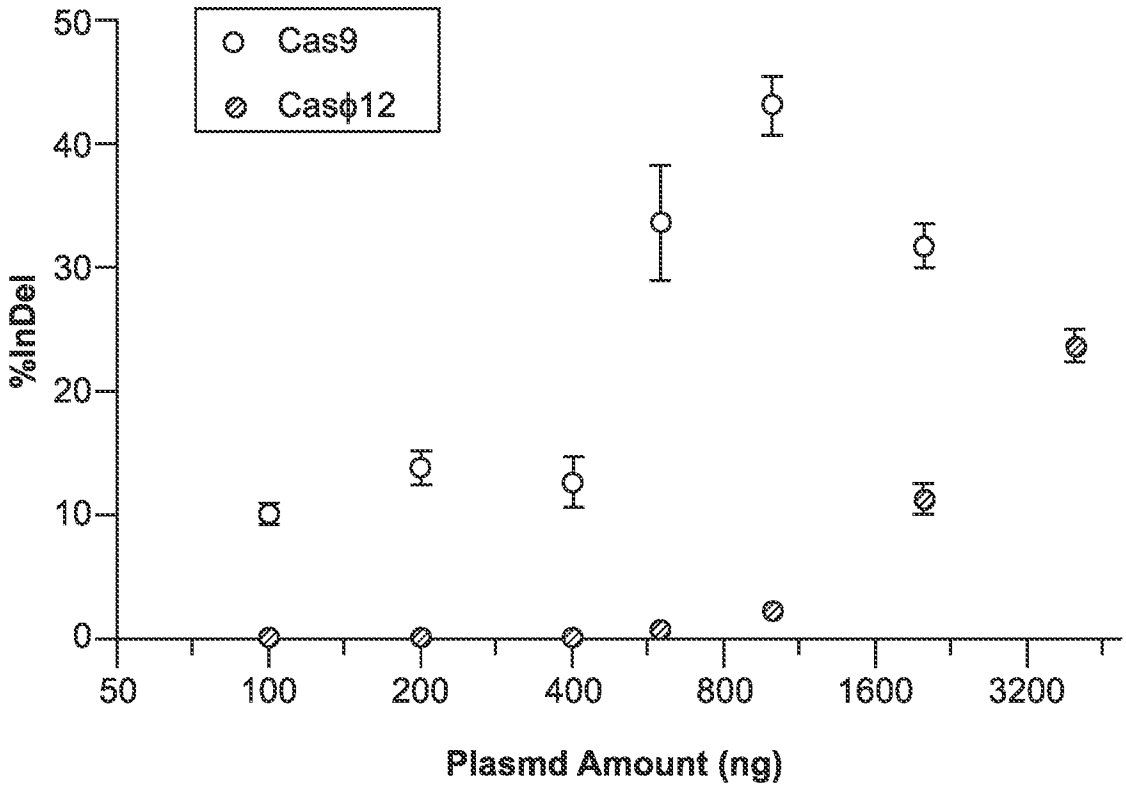

Cas9
Casϕ12
%InDel
0
10
20
30
40
50
50
100
200
400
800
1600
3200
Plasmd Amount (ng)

FIG. 17

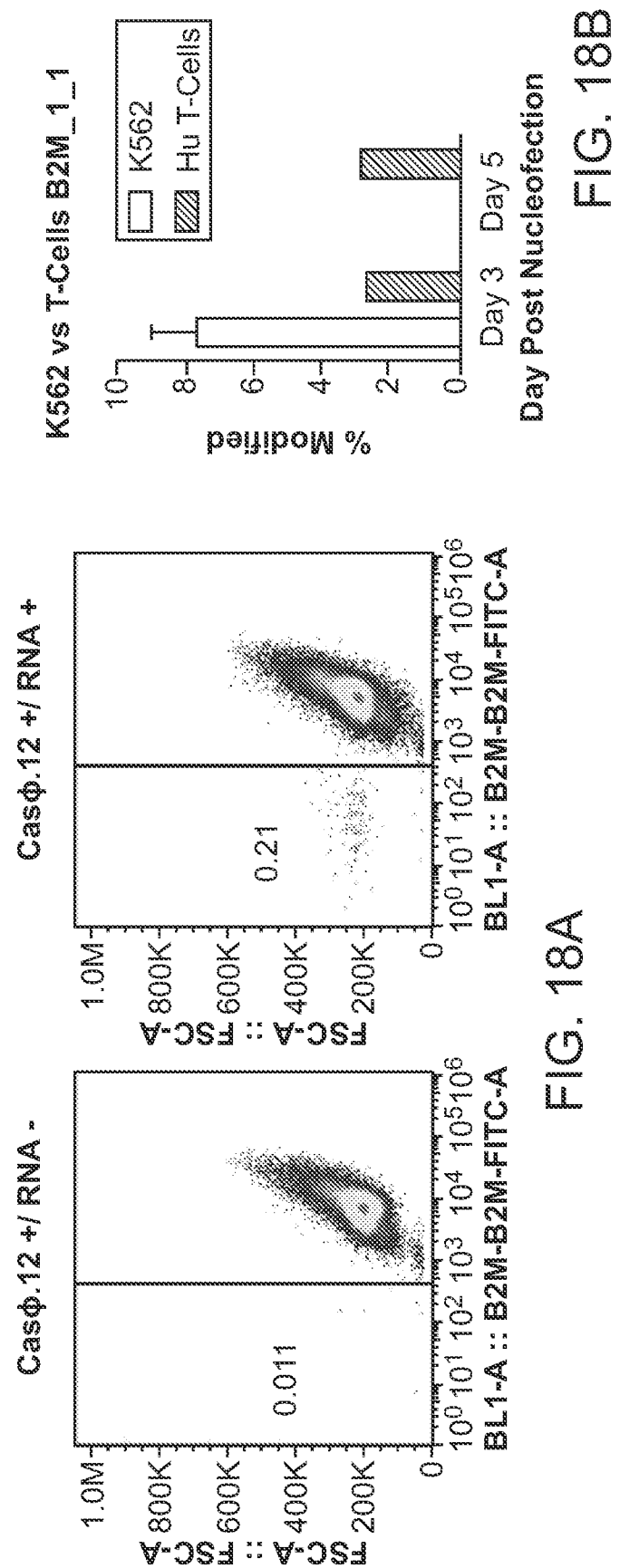


FIG. 18A

FIG. 18B

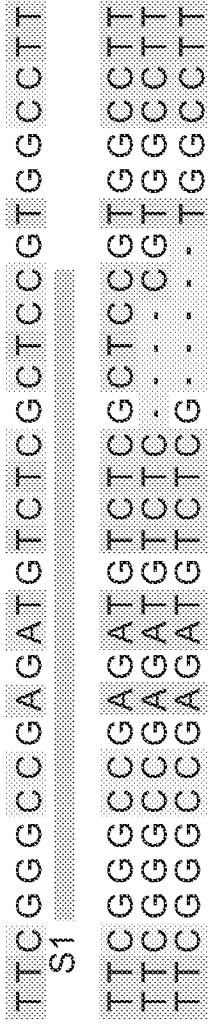


FIG. 18C

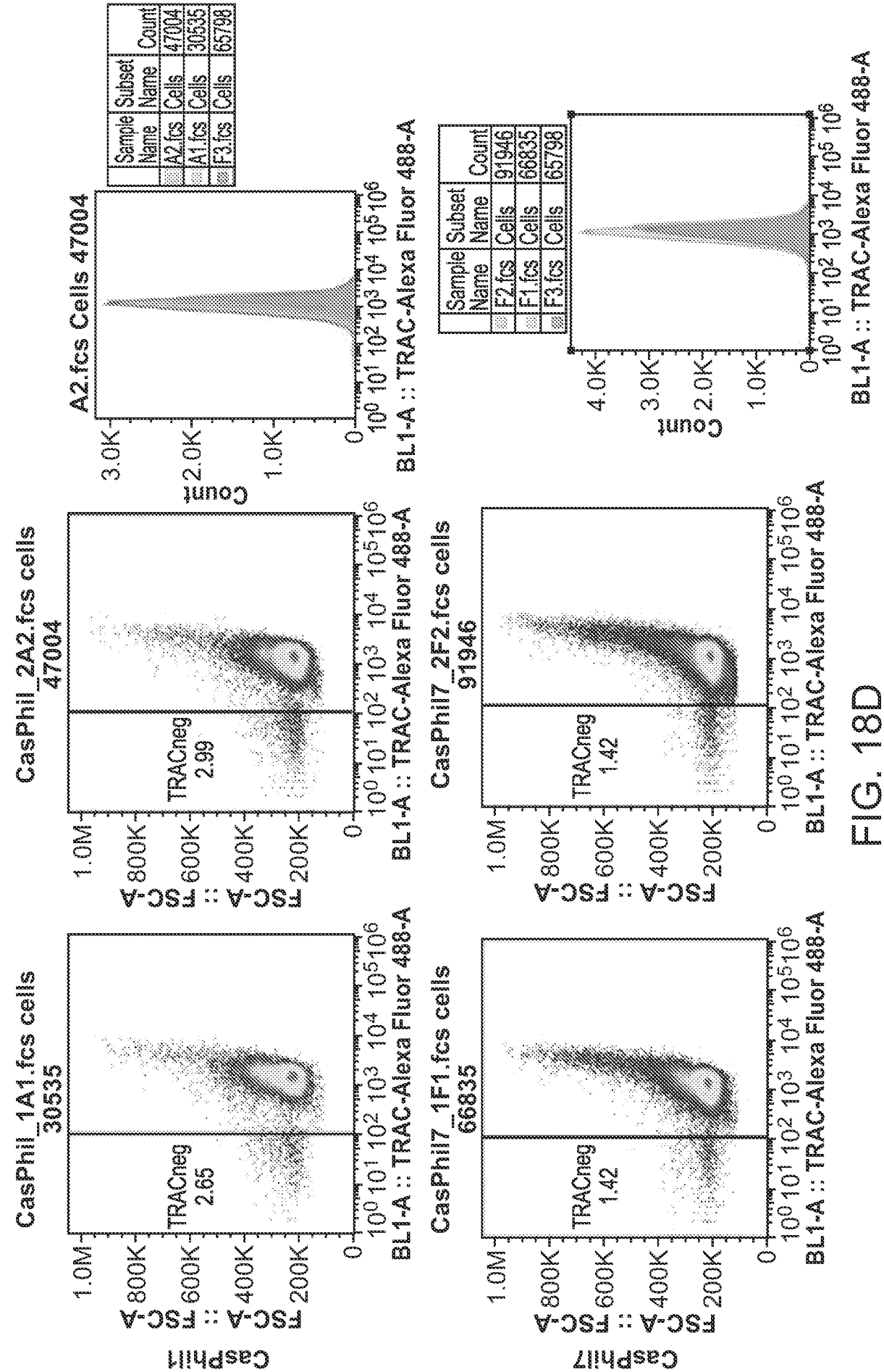

CasPhil_1A1.fcs cells
30535
TRACneg
2.65
CasPhil_2A2.fcs cells
47004
TRACneg
2.99
CasPhil7_1F1.fcs cells
66835
TRACneg
1.42
CasPhil7_2F2.fcs cells
91946
TRACneg
1.42
FSC-A :: TRAC-Alexa Fluor 488-A
BL1-A :: TRAC-Alexa Fluor 488-A
CasPhil1
CasPhil7
A2.fcs Cells 47004
Count
Sample Name | Subset Name | Count
A2.fcs | Cells | 47004
A1.fcs | Cells | 30535
F3.fcs | Cells | 65798
Sample Name | Subset Name | Count
F2.fcs | Cells | 91946
F1.fcs | Cells | 66835
F3.fcs | Cells | 65798

FIG. 18D

bold Substitutions
Insertions
- Deletions
----- Predicted cleavage position
CasPhi1
AGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAA-Reference
S1
AGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAA-95.16% (31757 reads)
AGAACCCTGACCCTGCCGT-A-ACCAGCTGAGAGACTCTAA-0.64% (212 reads)
AGAACCCTGACCCTGCCTTGTACCAGCTGAGAGACTCTAA-0.58% (193 reads)
AGAACCCTGACCCTGCCC---A-AGCTGAGAGACTCTAA-0.20% (68 reads)
AGAACCCTGACCCTG-----A-ACCAGCTGAGAGACTCTAA-0.10% (35 reads)
AGAACCCTGACCCT-----A-ACCAGCTGAGAGACTCTAA-0.08% (27 reads)
AGAACCCTGACCCTGCCGTGT CCAGCTGAGAGACTCTAA-0.08% (27 reads)
AGAACCCTGACCCTGCCGTGTCCGTGTACCAGCTGAGAGACTCTAA-0.08% (26 reads)
AGAACCCTGACCCTG TACCAGCTGAGAGACTCTAA-0.07% (23 reads)
AGAACCCTGACCCTGCCGTG-----A-0.07% (22 reads)
AG-----A-0.06% (21 reads)
AGCCTGACCCTGCCGCCGTGTACCAGCTGAGAGACTCTAA-0.06% (21 reads)
AGAACCCTGACCCTG---TGTACCAGCTGAGAGACTCTAA-0.06% (19 reads)
AGAACCCTGACCCTGCCGTGTAACAGCTGAGAGACTCTAA-0.06% (19 reads)
AGAACCCTGACCCTGCCGTG-----CTGAGAGACTCTAA-0.06% (19 reads)
AGAACCCTGACCCTGCCGTGTACCGTGTACCAGCTGAGAG-0.05% (18 reads)
AGAACCCTGACCCTGCCGTGT-----CTGAGAGACTCTAA-0.05% (17 reads)
AGA-----0.05% (17 reads)
CasPhi7
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAA-Reference
S1
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCAA-96.41% (49752 reads)
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACTT-AA-0.39% (201 reads)
CAGACAAAACTGTGCT---T-TGAGGTCTATGGACTTCAA-0.21% (110 reads)
CAGACAAAACTGTGCTAGA-T---GGTCTATGGACTTCAA-0.21% (108 reads)
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTCA--0.14% (71 reads)
CAGACAAAACTGTGCTA--CATGAGGTCTATGGACTTCAA-0.10% (53 reads)
CAGACAAAACTGTGCTAG-T---GTCTATGGACTTCAA-0.10% (53 reads)
CAGACAAAACTGTGCTAGACATGAGGTCTATGGTCTTCAA-0.09% (48 reads)
CAGACAAAACTGTGCTA--T-TGAGGTCTATGGACTTCAA-0.09% (45 reads)
CAGACAAAACTGTGCTAGACATG--TCTATGGACTTCAA-0.07% (36 reads)
CAGACAAAACTGTGCTAGAC---GTCTATGGACTTCAA-0.07% (36 reads)
CAGACAAAACTGTGCTAGACTAGACATGAGGTCTATGGAC-0.07% (35 reads)
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACT-CAA-0.07% (34 reads)
AGACAAAACTGTGCGTAGACATGAGGTCTATGGACTTCAA-0.06% (29 reads)
CAGACAAAACTGTGCTAGACATGAGGTCTATGGACTTTCA-0.05% (26 reads)

FIG. 18E

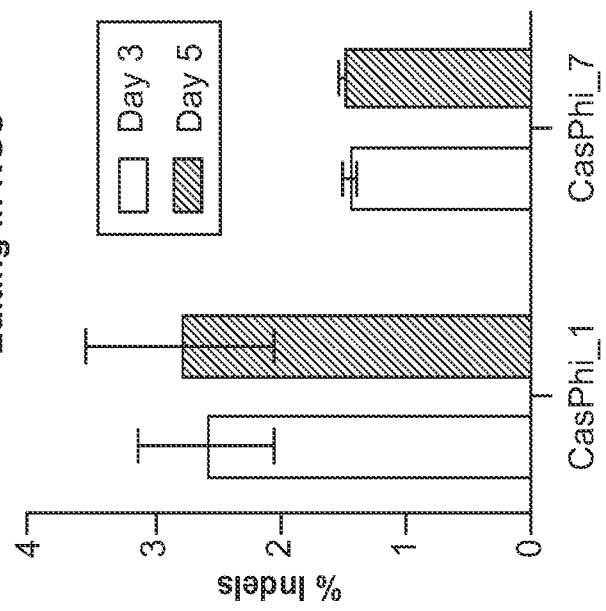


FIG. 18F

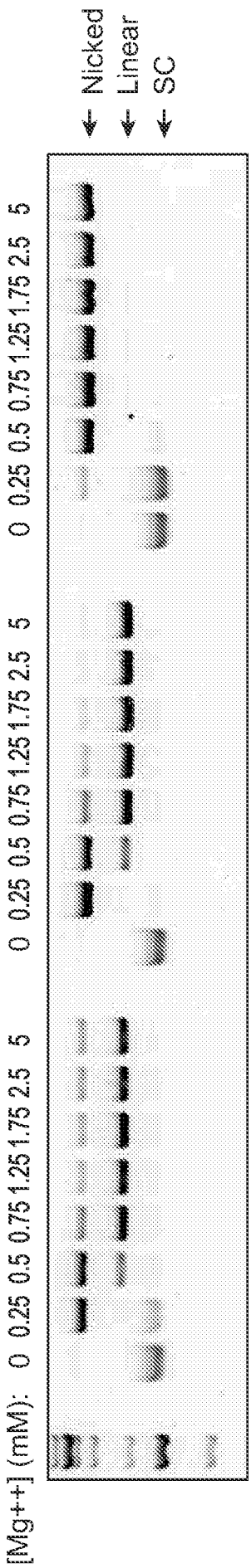


FIG. 19

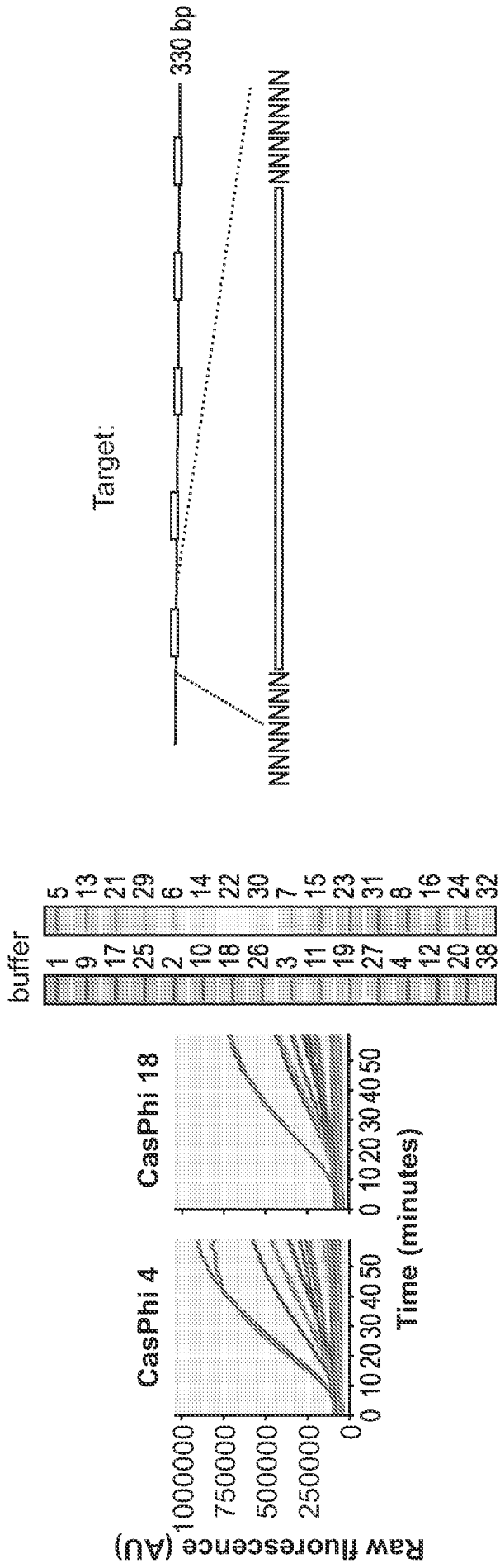

Raw fluorescence (AU)
1000000
750000
500000
250000
0
CasPhi 4
CasPhi 18
0 10 20 30 40 50
Time (minutes)
buffer
1 9 17 25 2 10 18 26 3 11 19 27 4 12 20 38
5 13 21 29 6 14 22 30 7 15 23 31 8 16 24 32
Target:
330 bp
NNNNNNN
NNNNNNN

FIG. 20

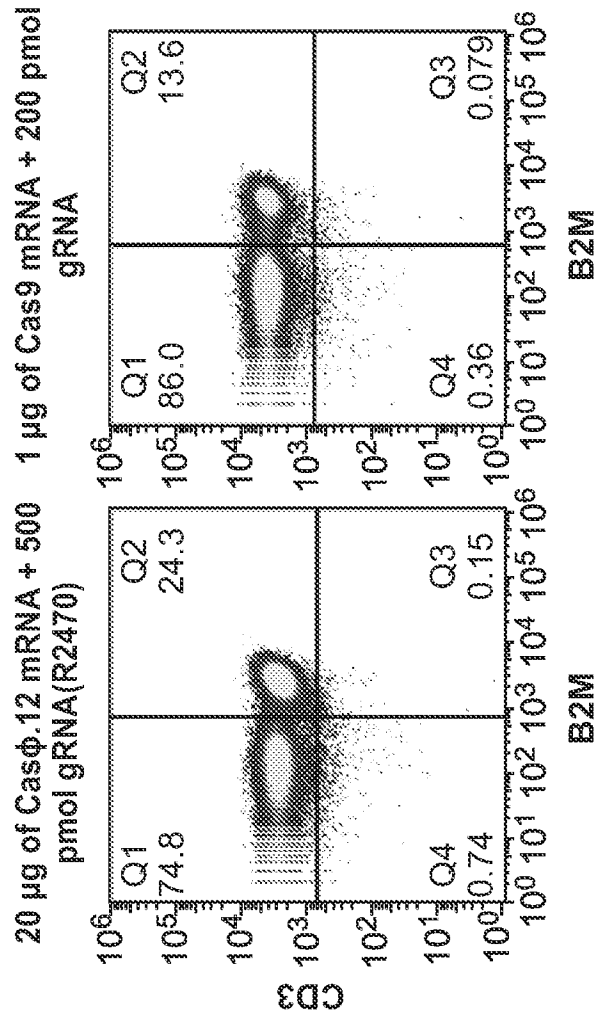


FIG. 21B

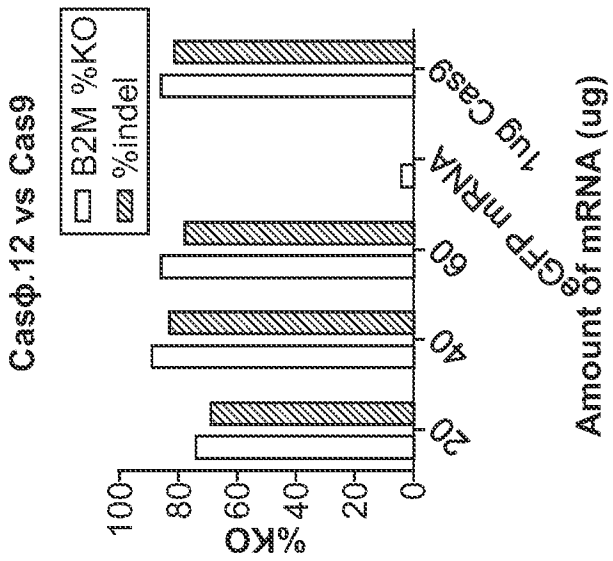


FIG. 21A

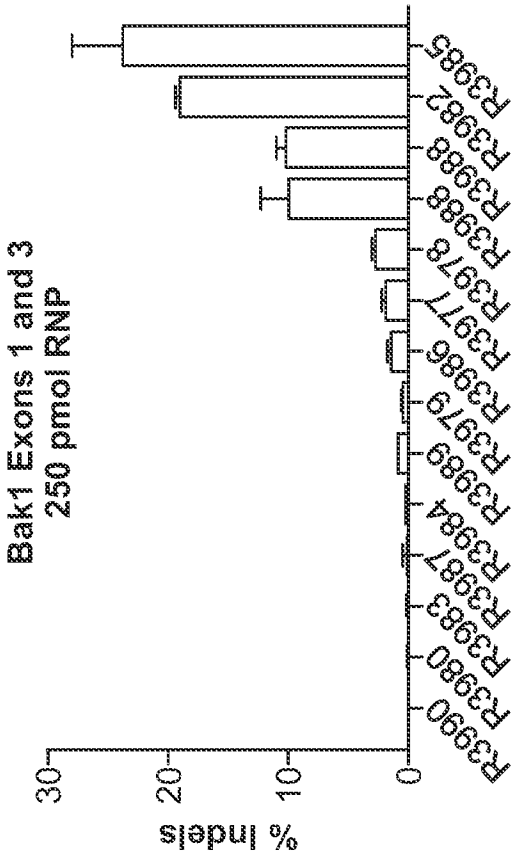


FIG. 22B

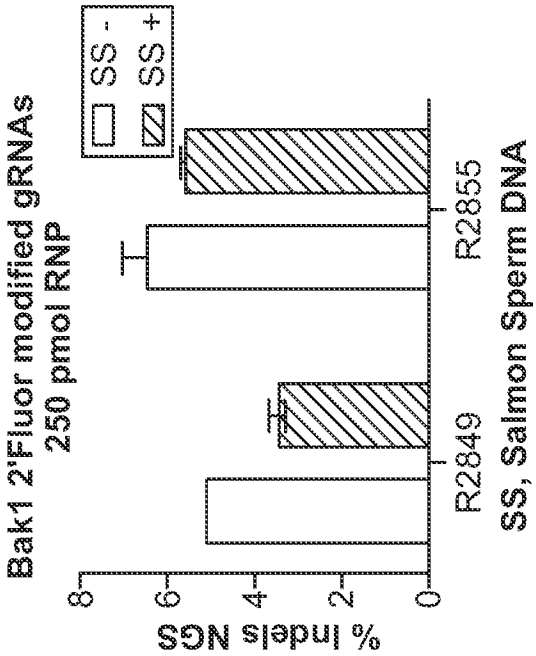


FIG. 22A

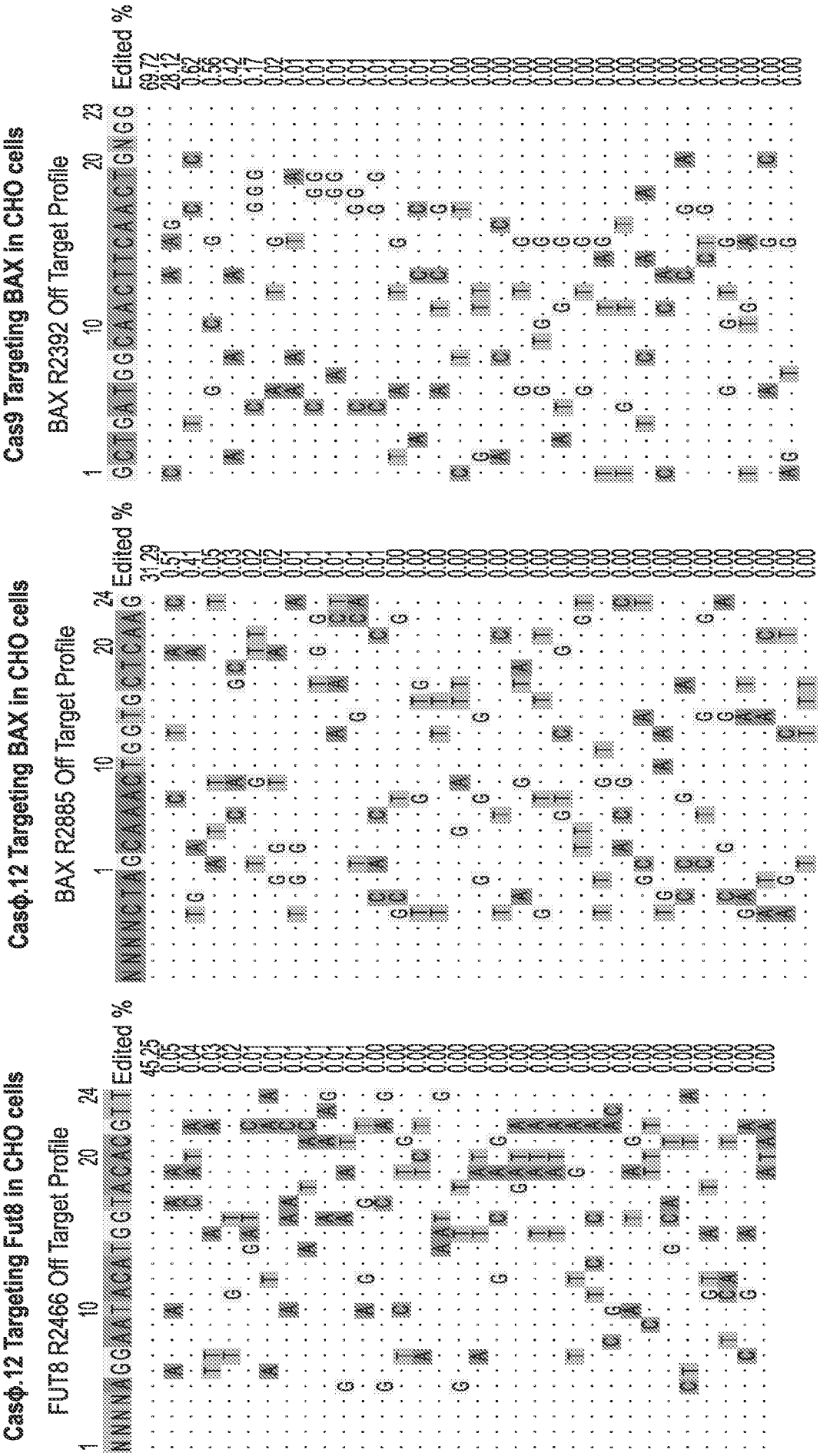
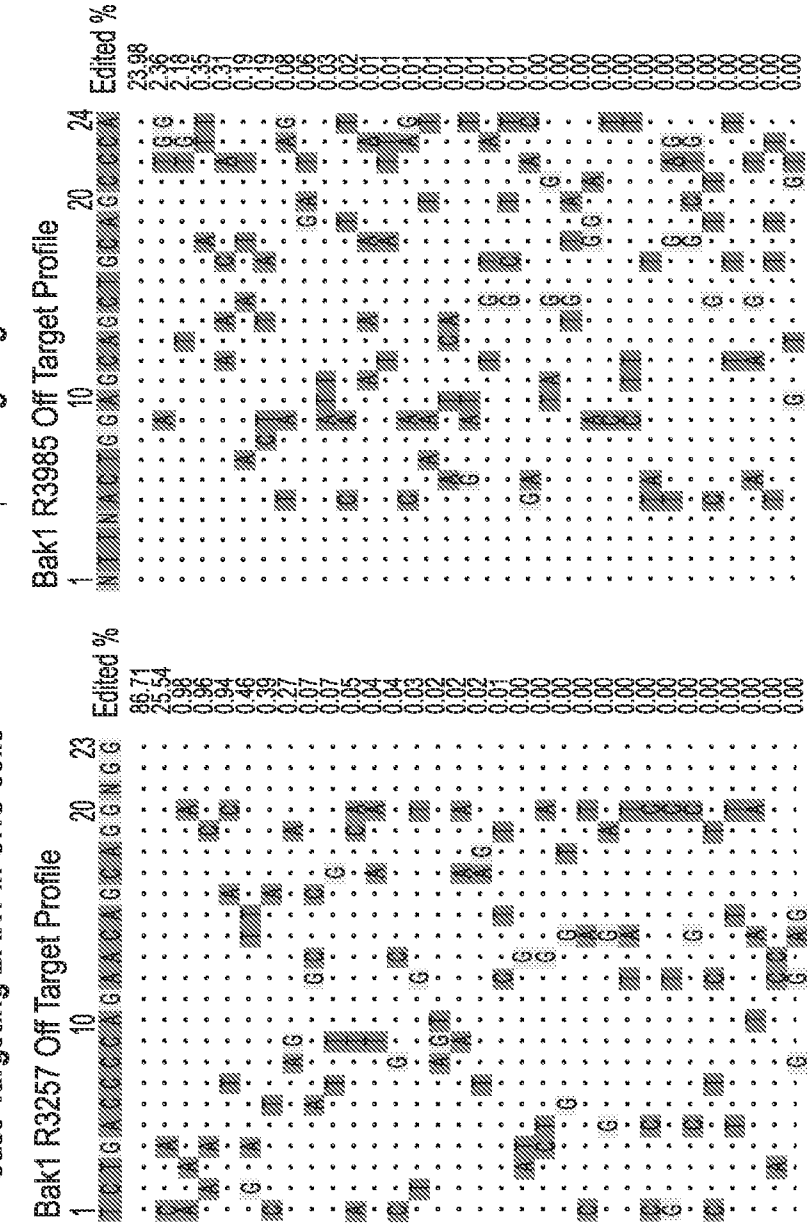


FIG. 23A

FIG. 23B

FIG. 23C


Cas9 Targeting Fut8 in CHO cells
FUT8 R2393 Off Target Profile
Edited %
76.88
0.30
0.23
0.04
0.03
0.02
0.02
0.01
0.01
0.00
0.00
0.00

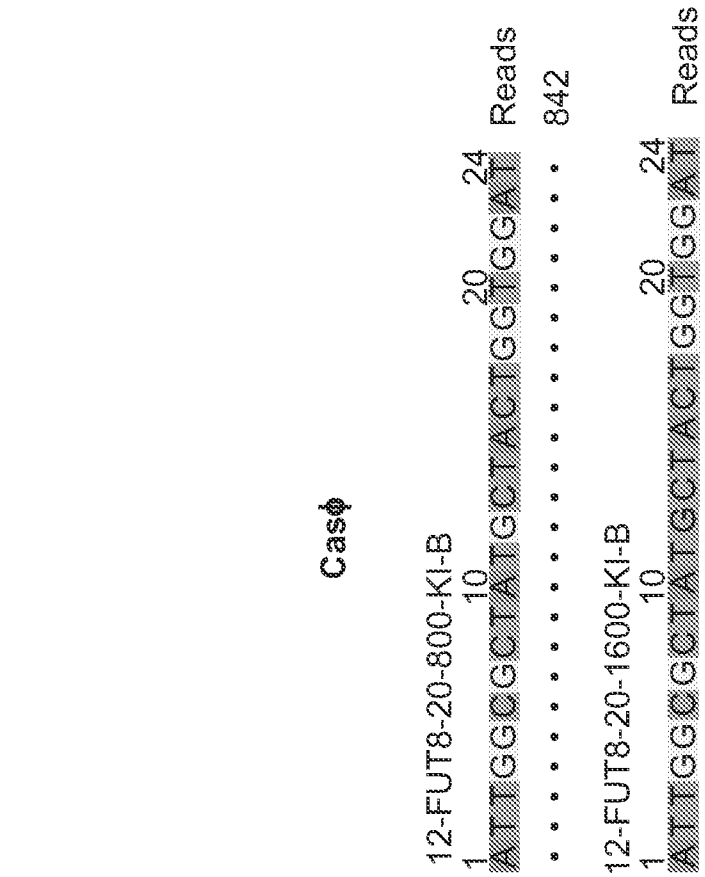


FIG. 23H

FIG. 23G

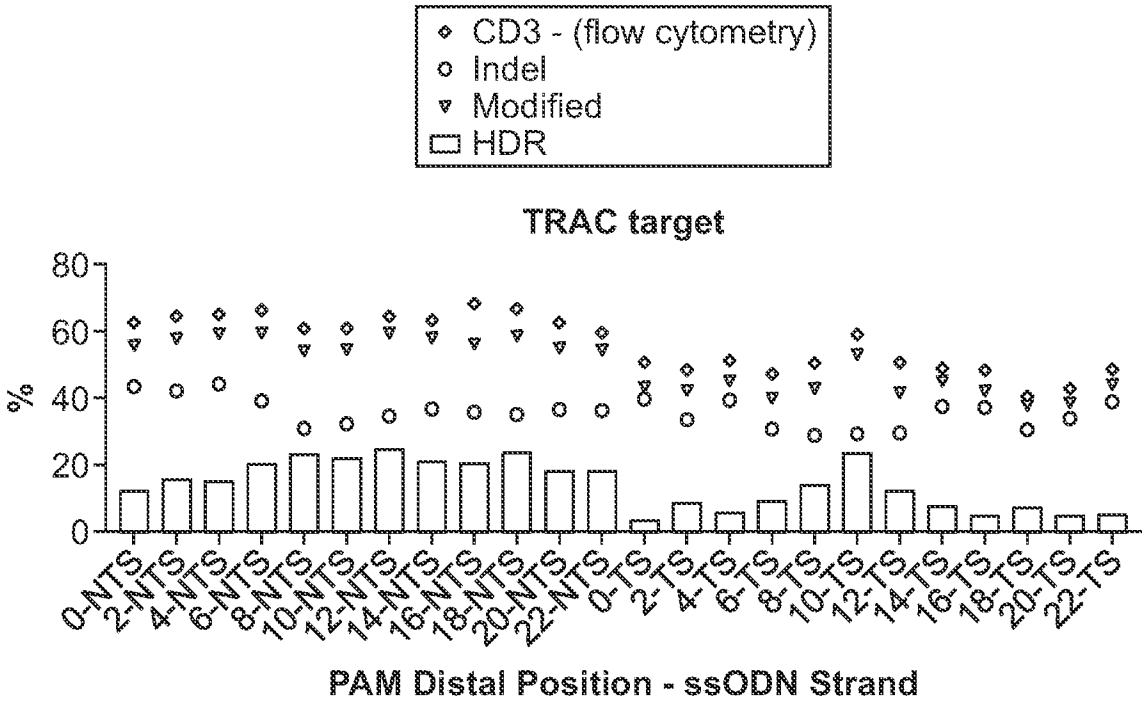


FIG. 24A

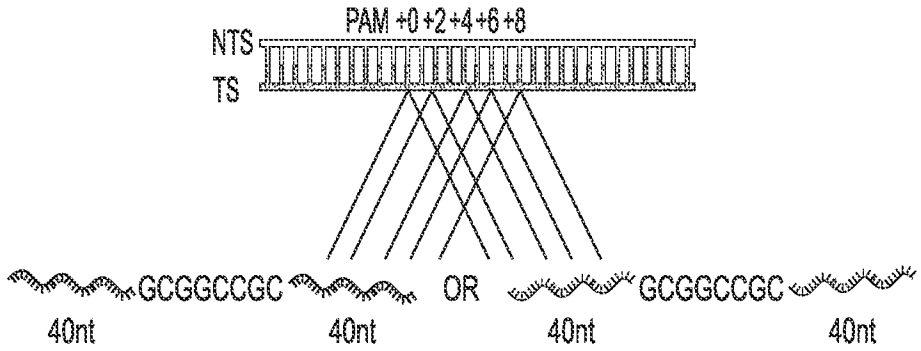


FIG. 24B

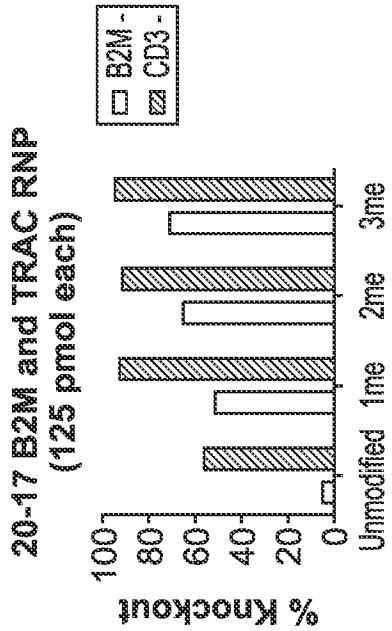


FIG. 25A

FIG. 25B

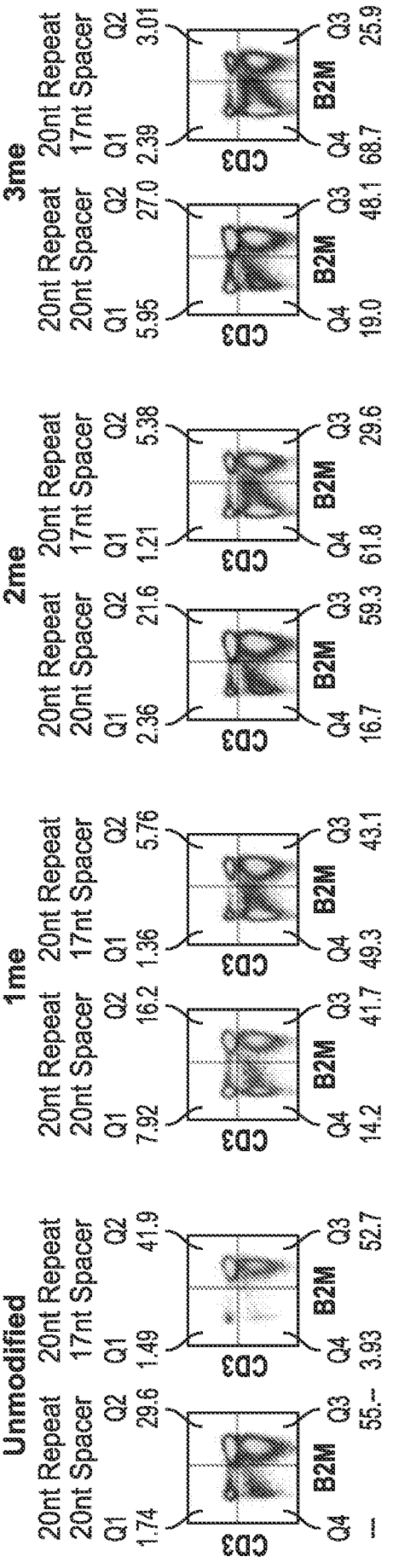


FIG. 25C

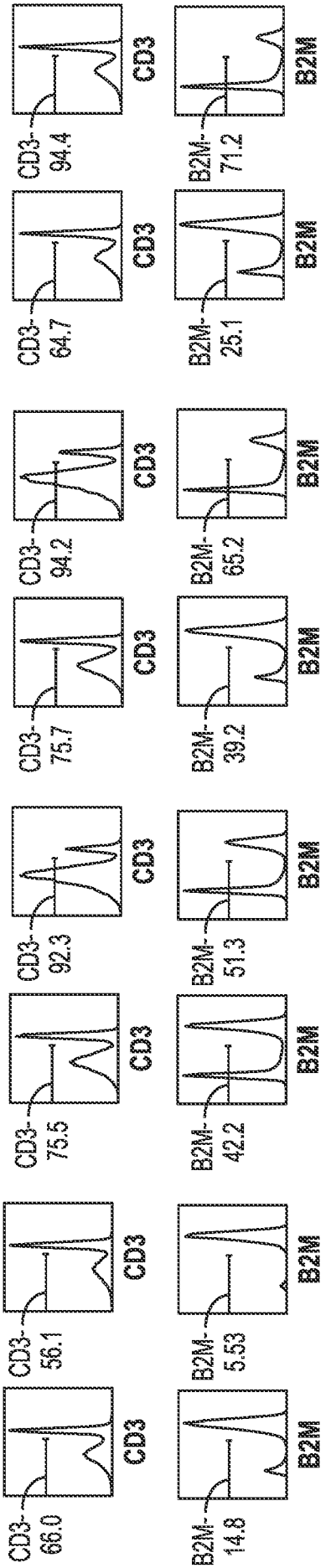

CD3-
66.0
CD3
B2M-
14.8
B2M
CD3-
56.1
CD3
B2M-
5.53
B2M
CD3-
75.5
CD3
B2M-
42.2
B2M
CD3-
92.3
CD3
B2M-
51.3
B2M
CD3-
75.7
CD3
B2M-
39.2
B2M
CD3-
94.2
CD3
B2M-
65.2
B2M
CD3-
64.7
CD3
B2M-
25.1
B2M
CD3-
94.4
CD3
B2M-
71.2
B2M

FIG. 25C (Cont.)

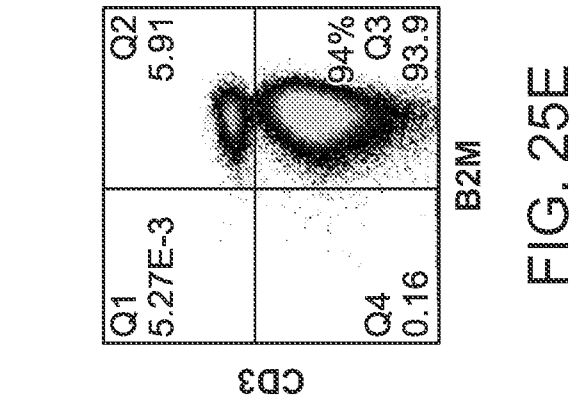


FIG. 25E

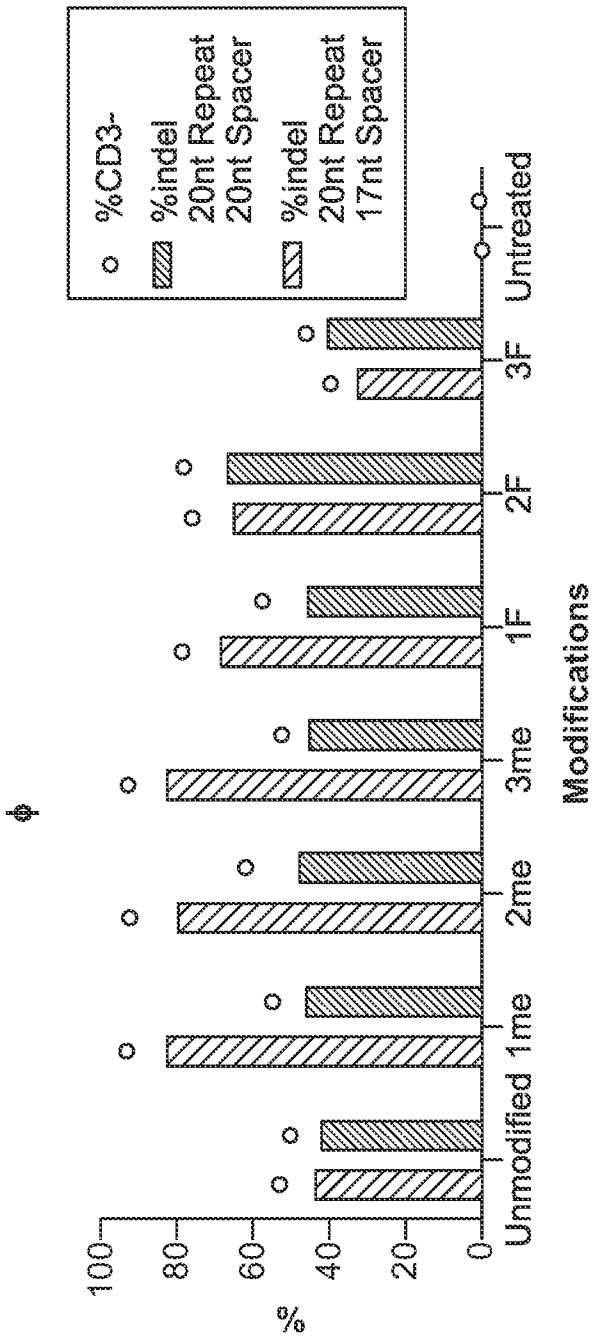


FIG. 25D

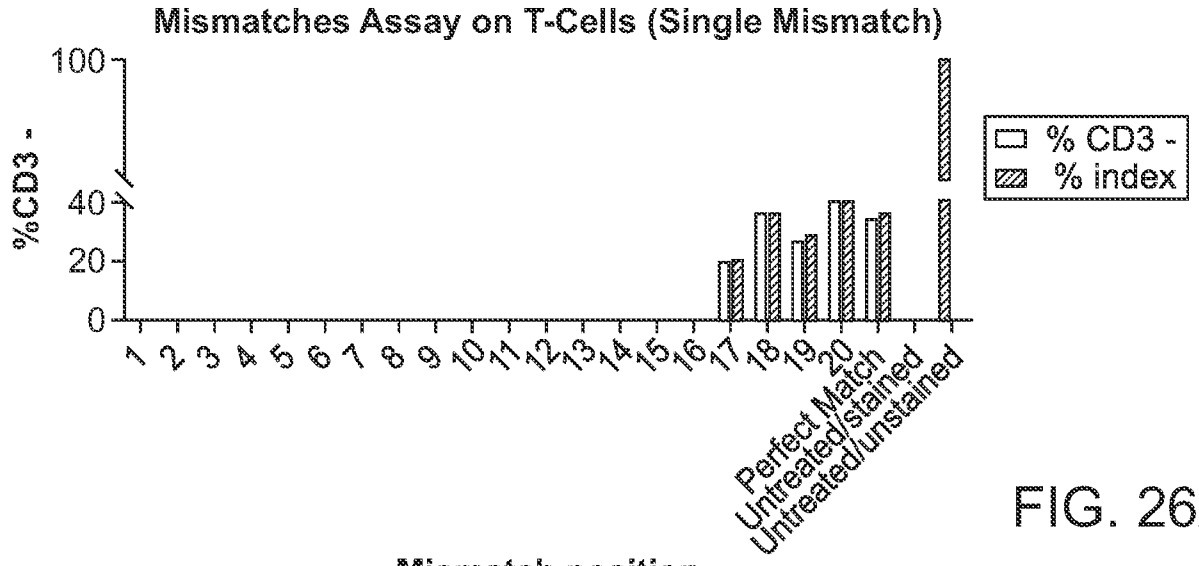


FIG. 26A

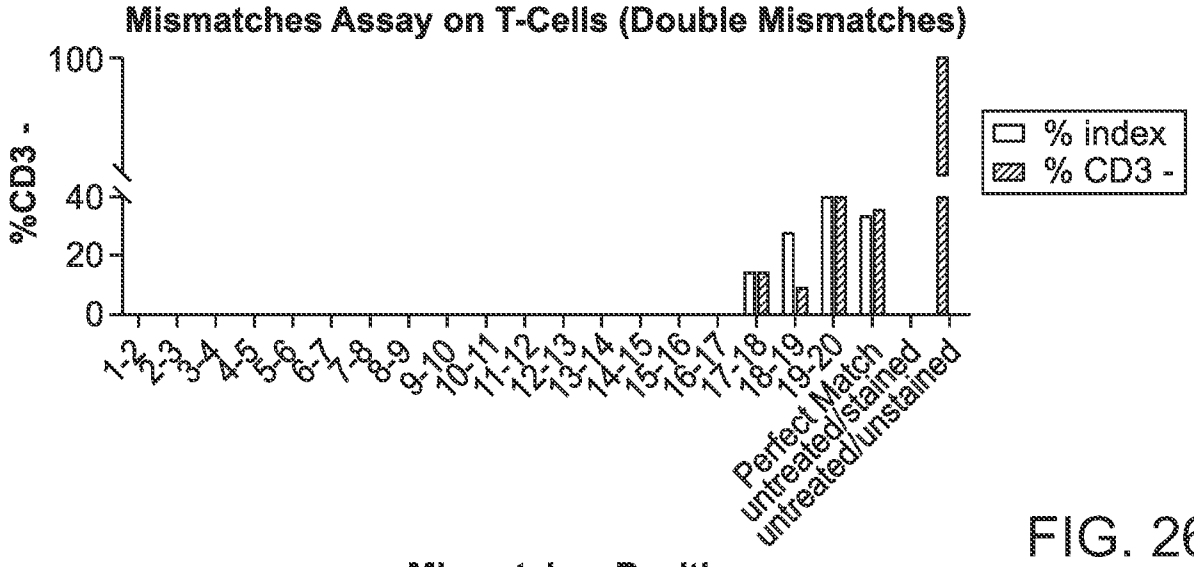


FIG. 26B

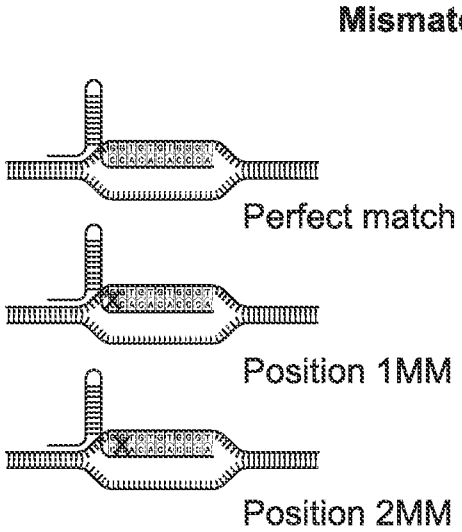


FIG. 26C

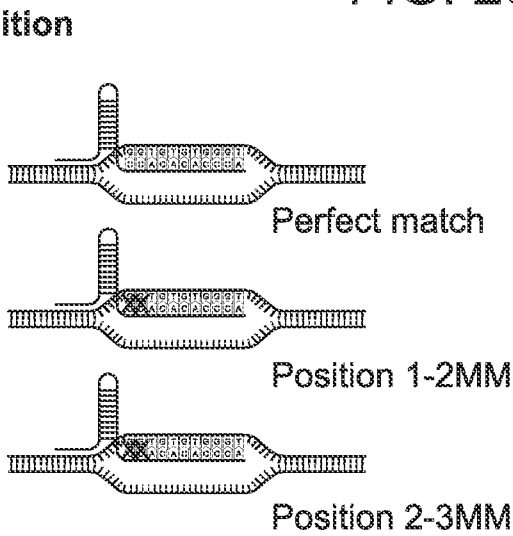


FIG. 26D

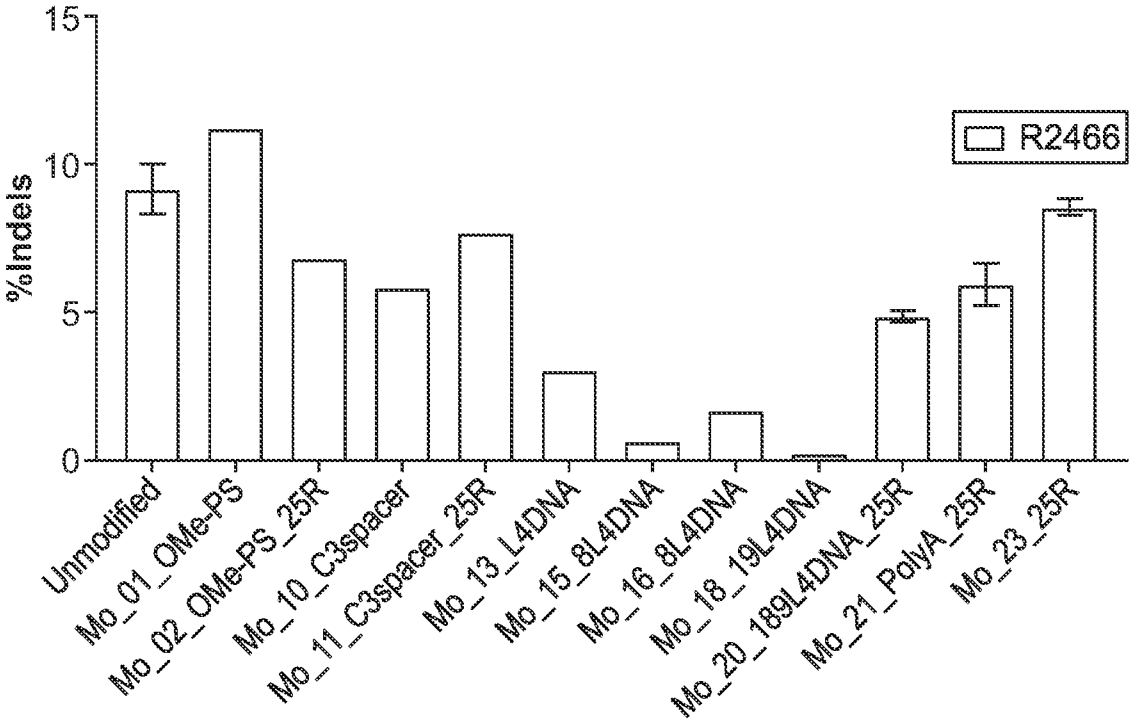


FIG. 27A

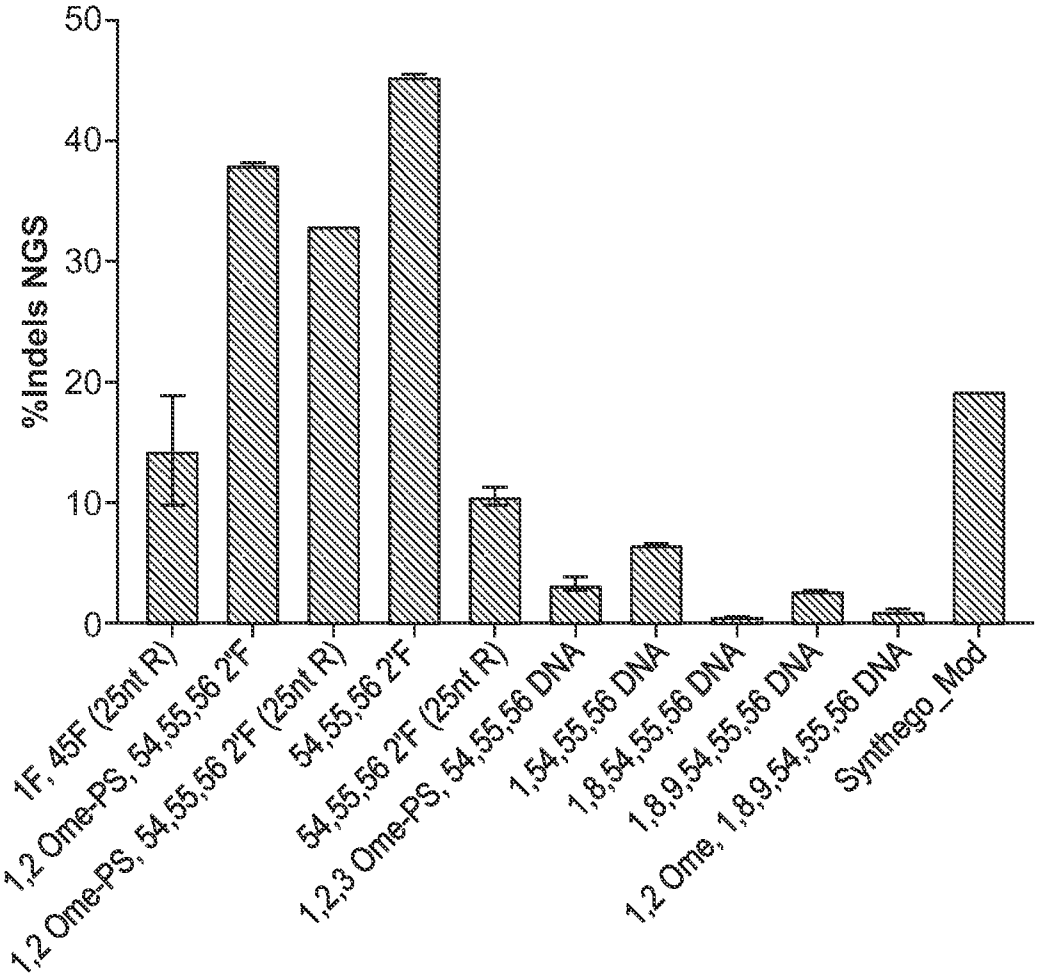


FIG. 27B

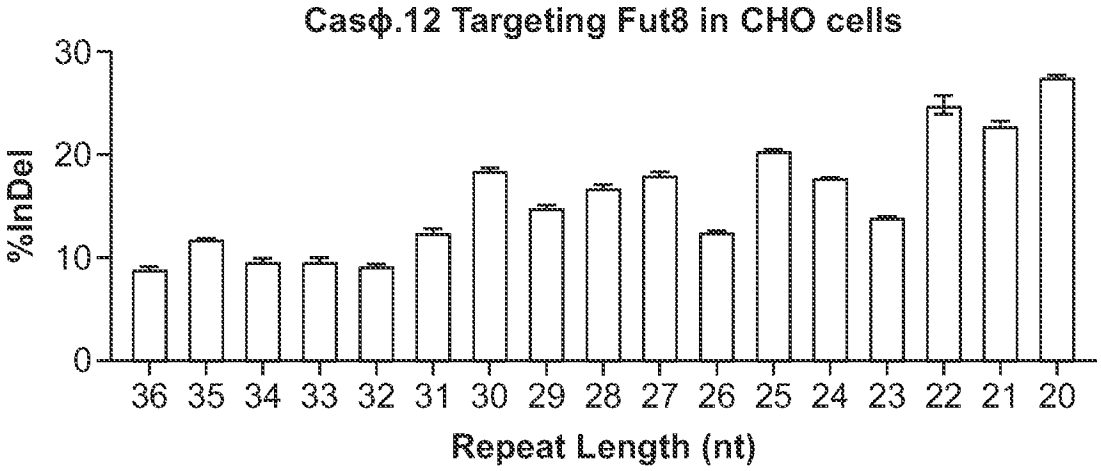


FIG. 28A

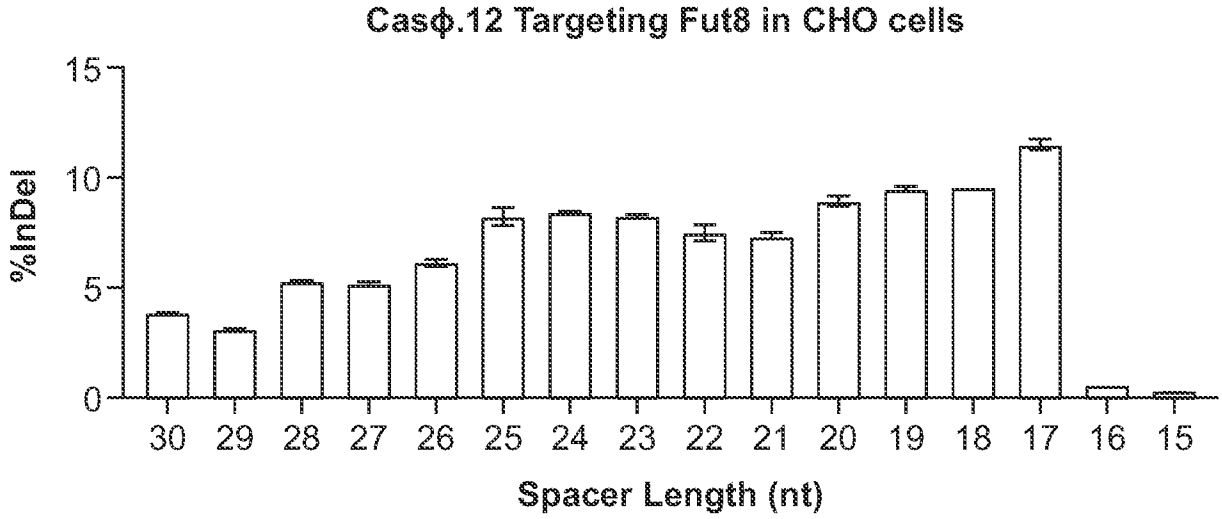


FIG. 28B

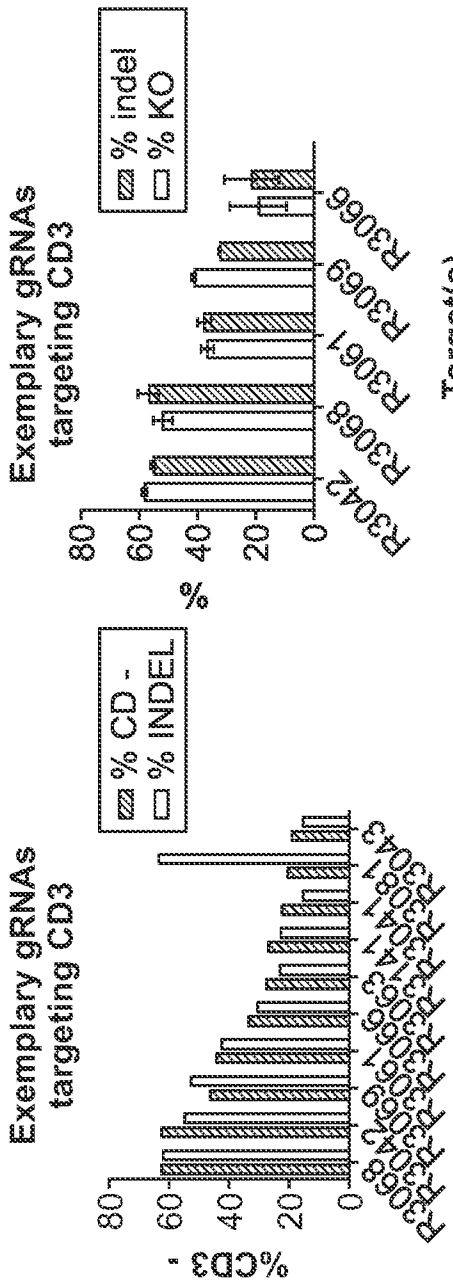


FIG. 29A

FIG. 29B

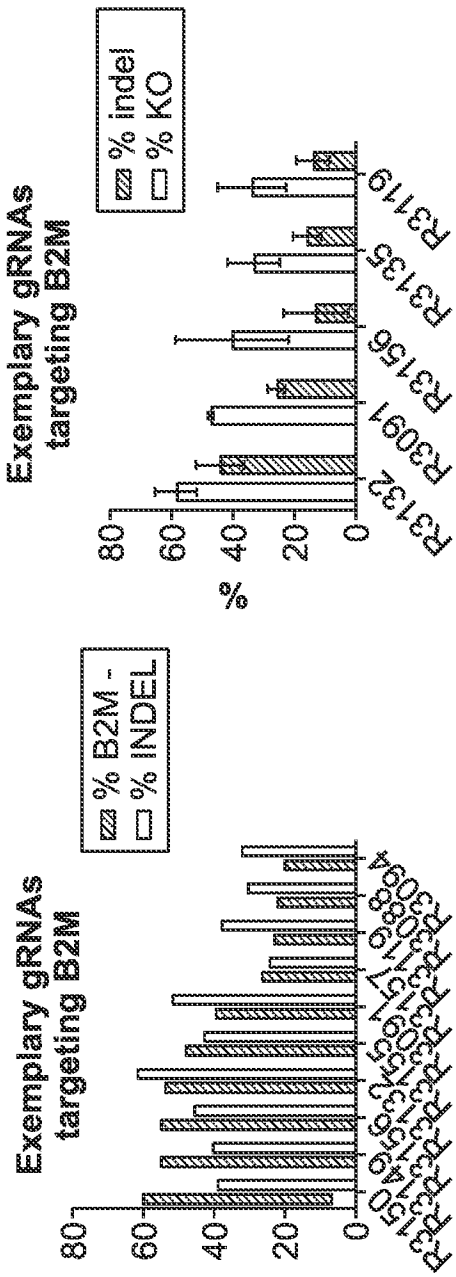


FIG. 29C

FIG. 29D

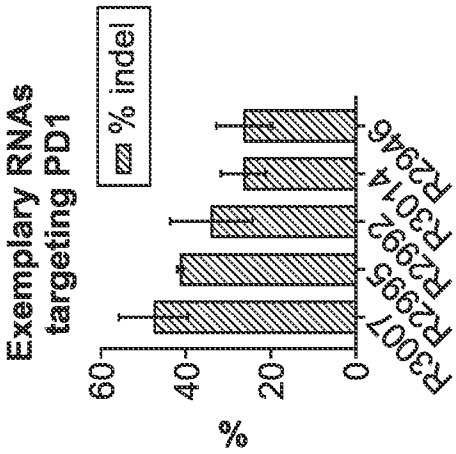


FIG. 29E

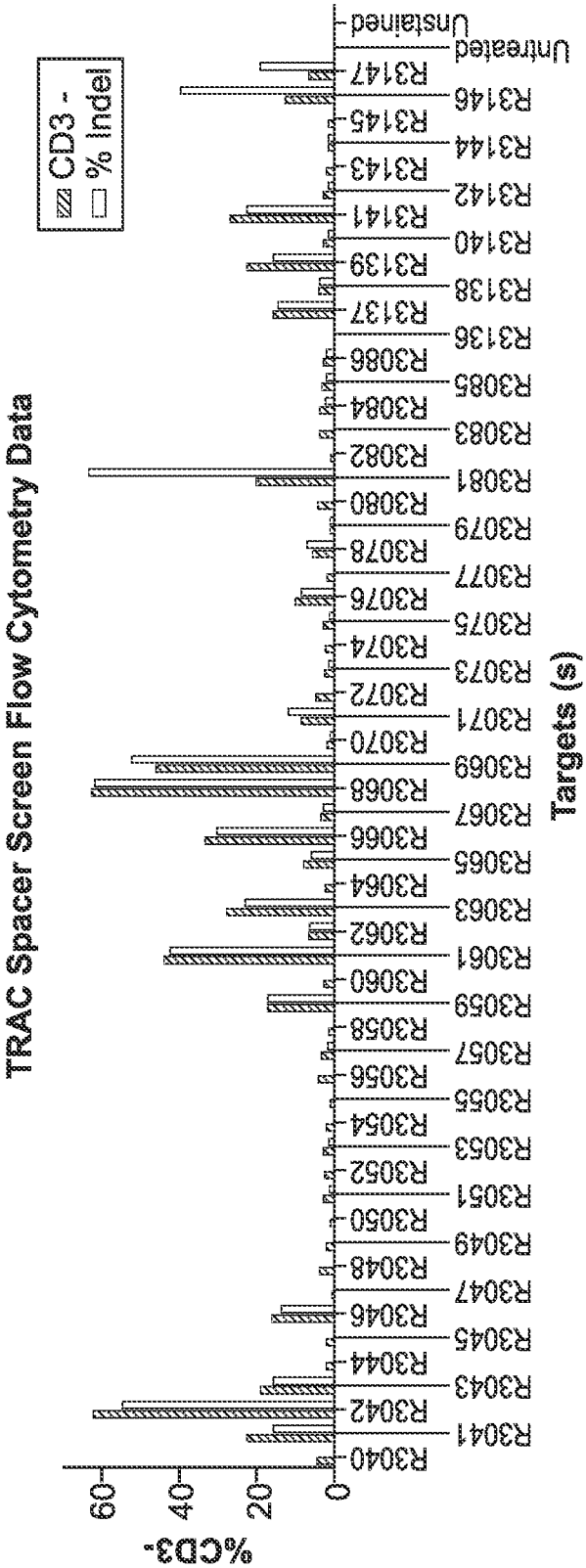


FIG. 29F

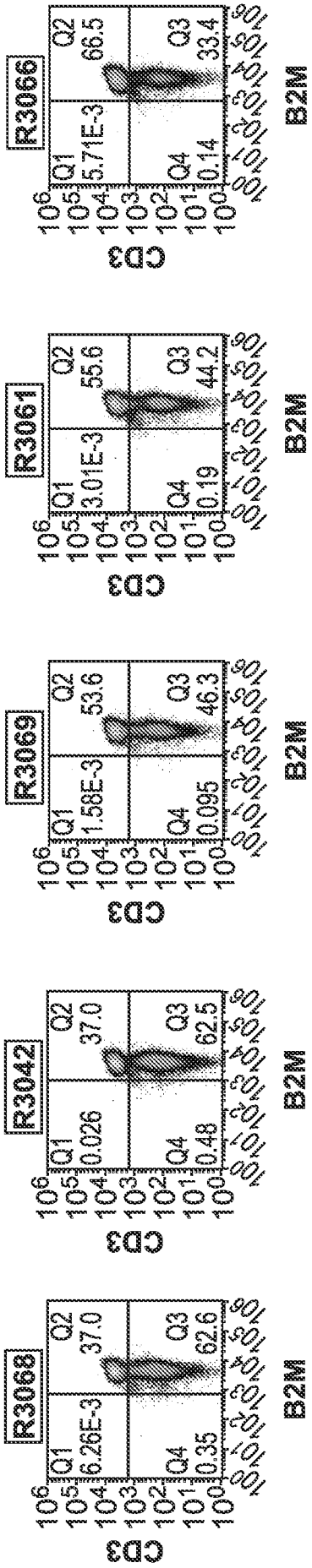


FIG. 29G

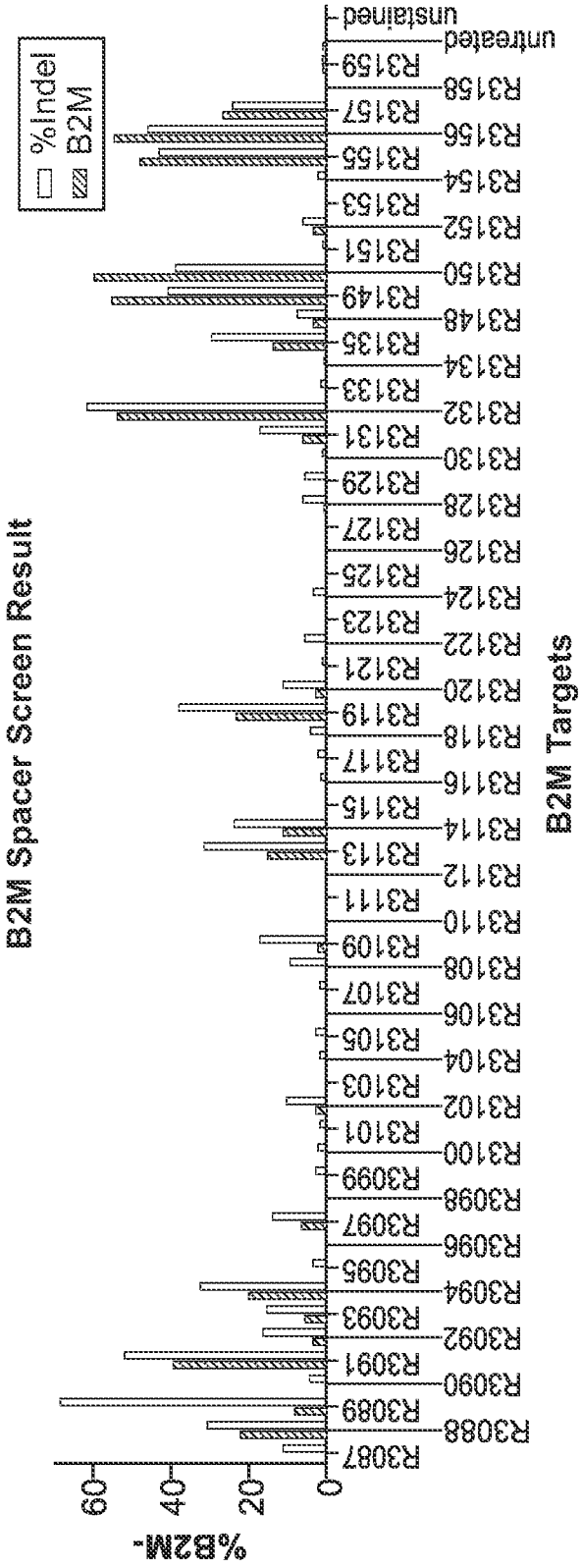


FIG. 29H

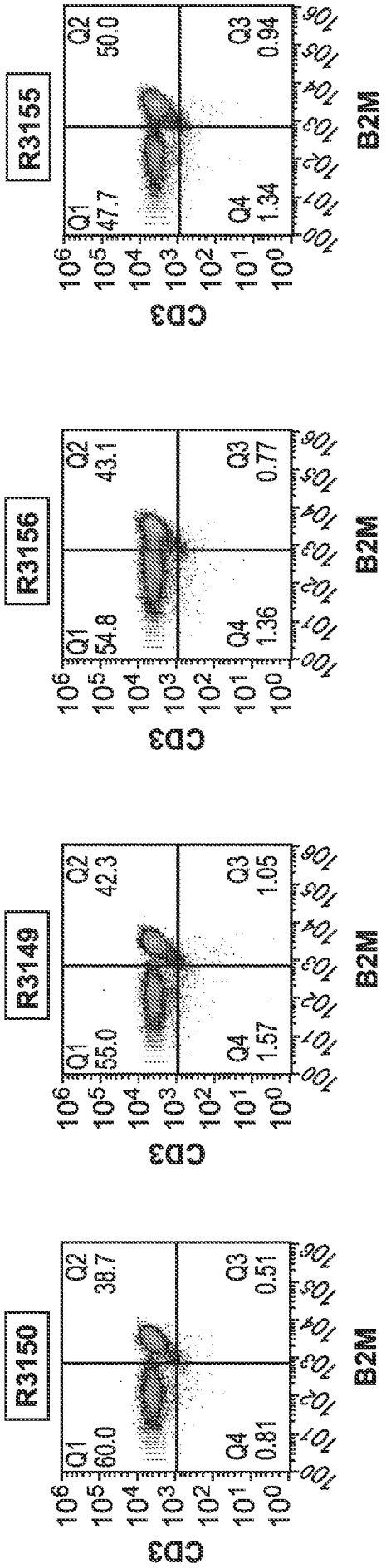


FIG. 29I

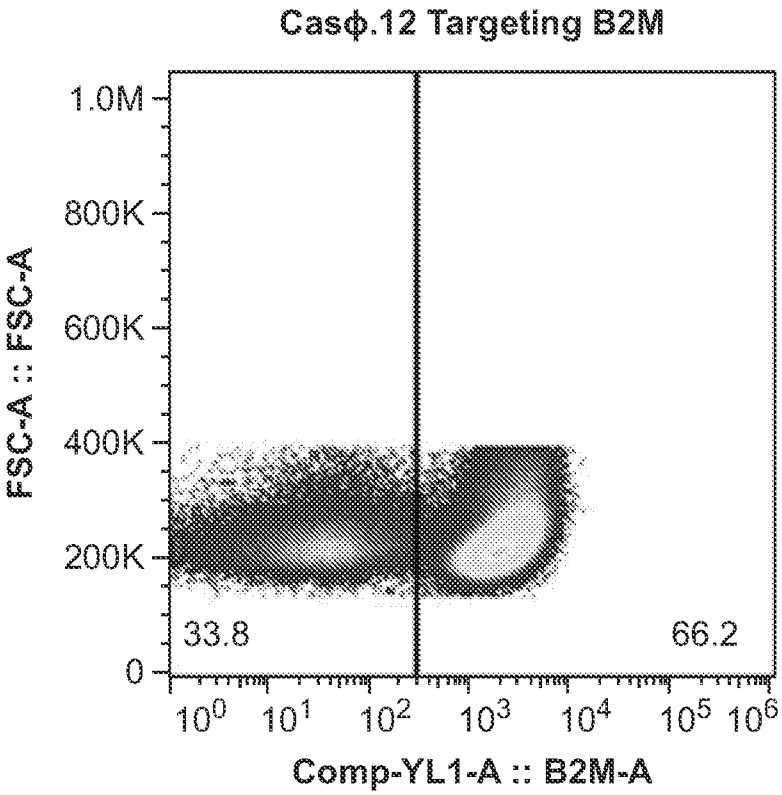


FIG. 30A

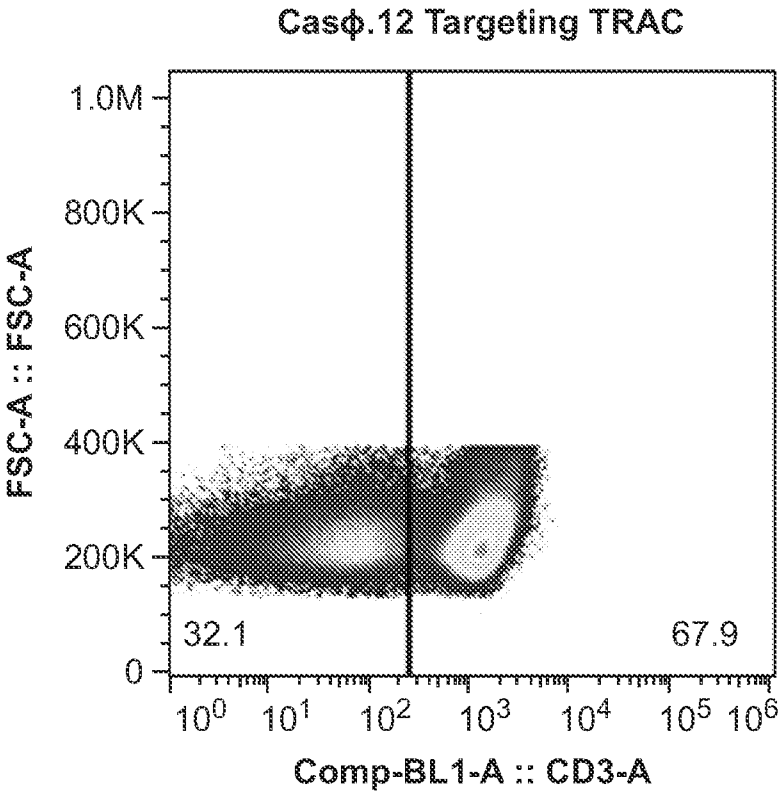


FIG. 30B

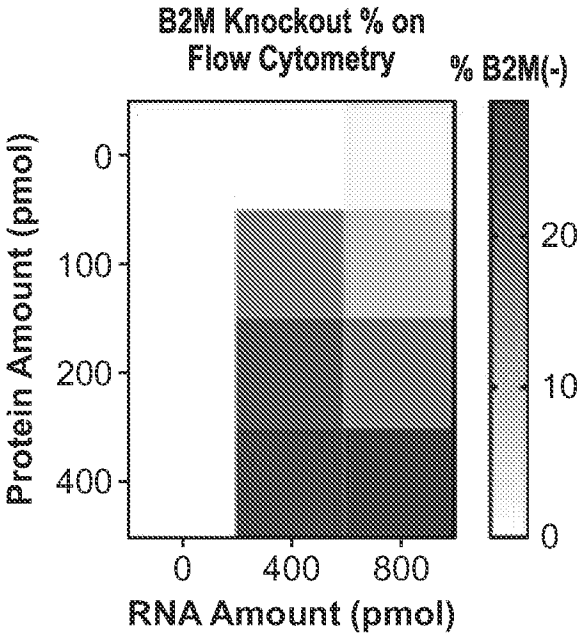


FIG. 30C

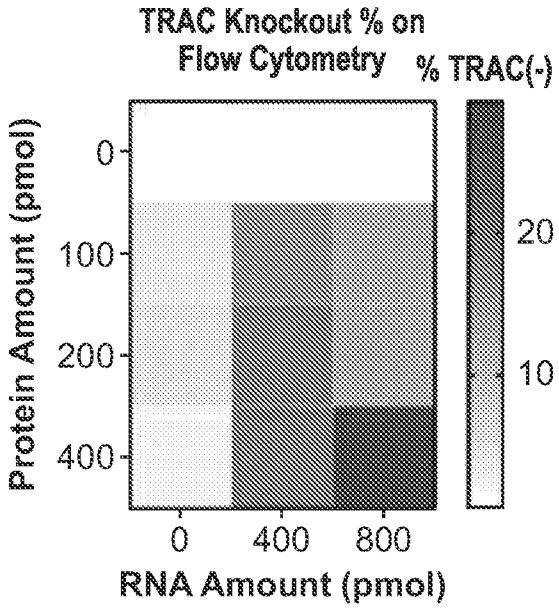


FIG. 30D

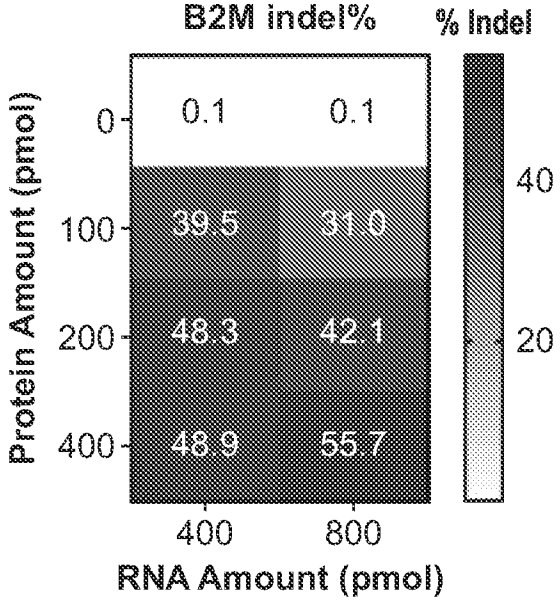


FIG. 30E

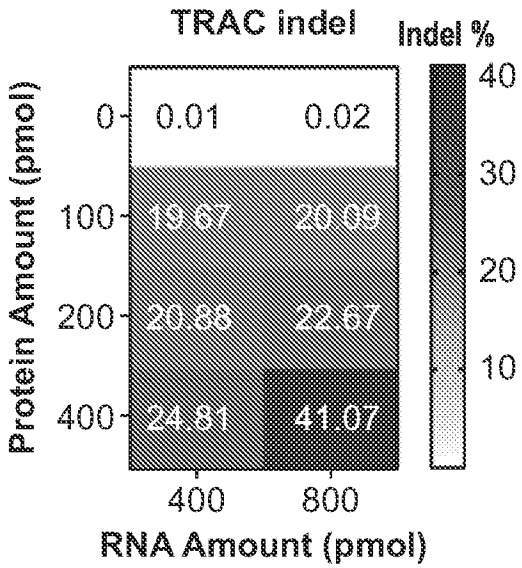


FIG. 30F

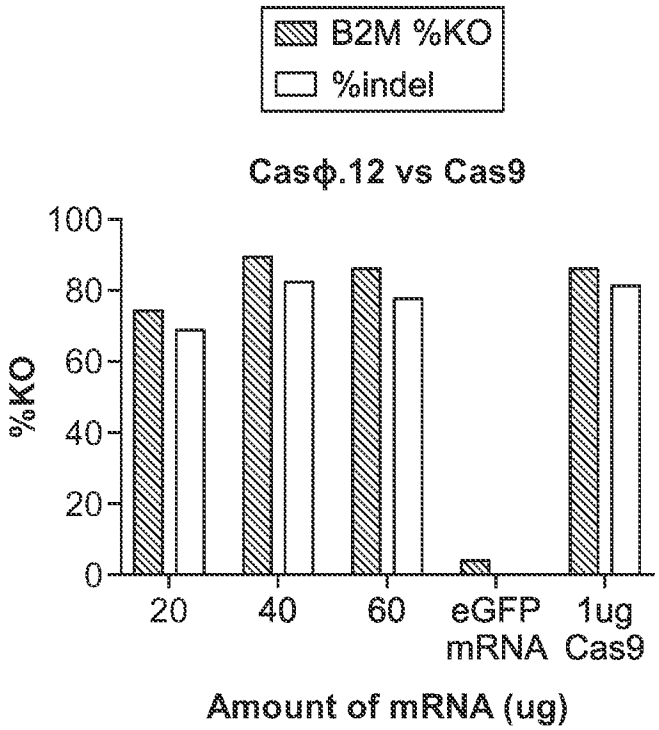


FIG. 30G

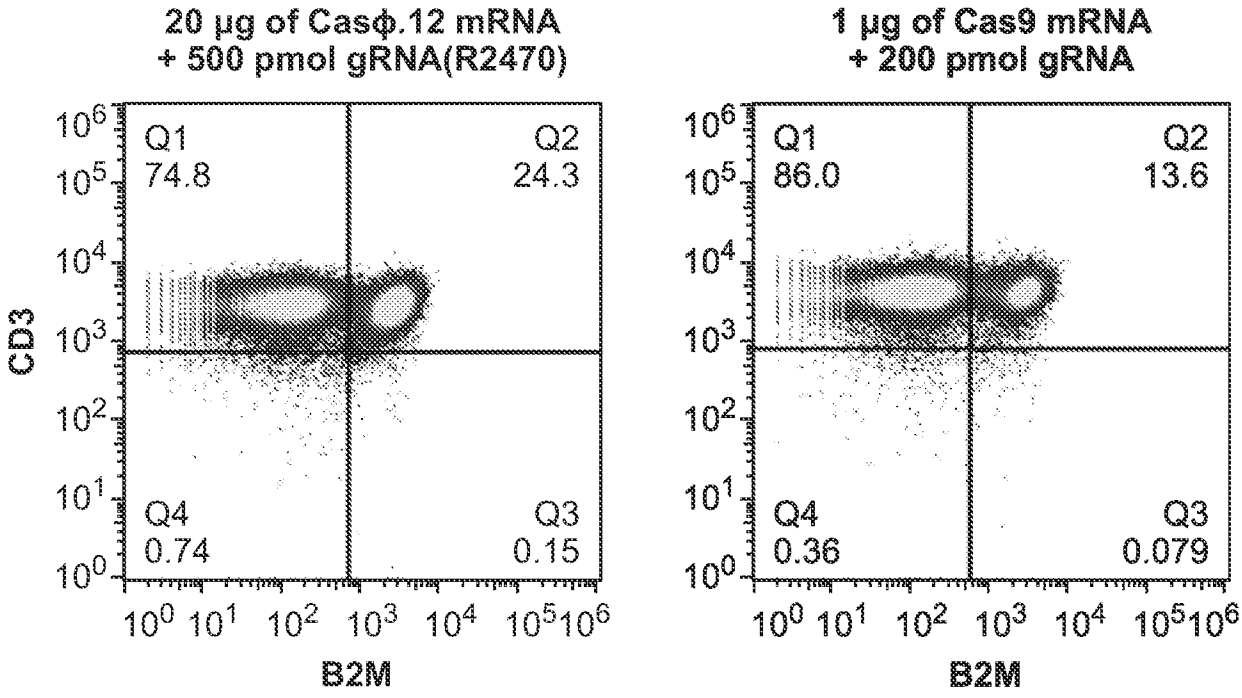


FIG. 30H

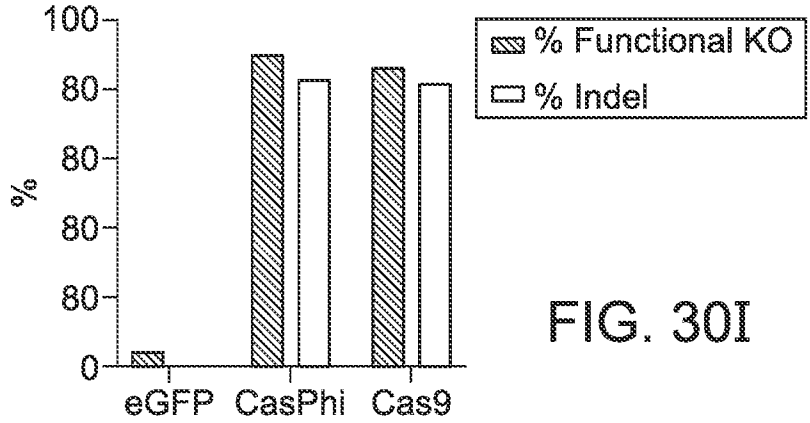
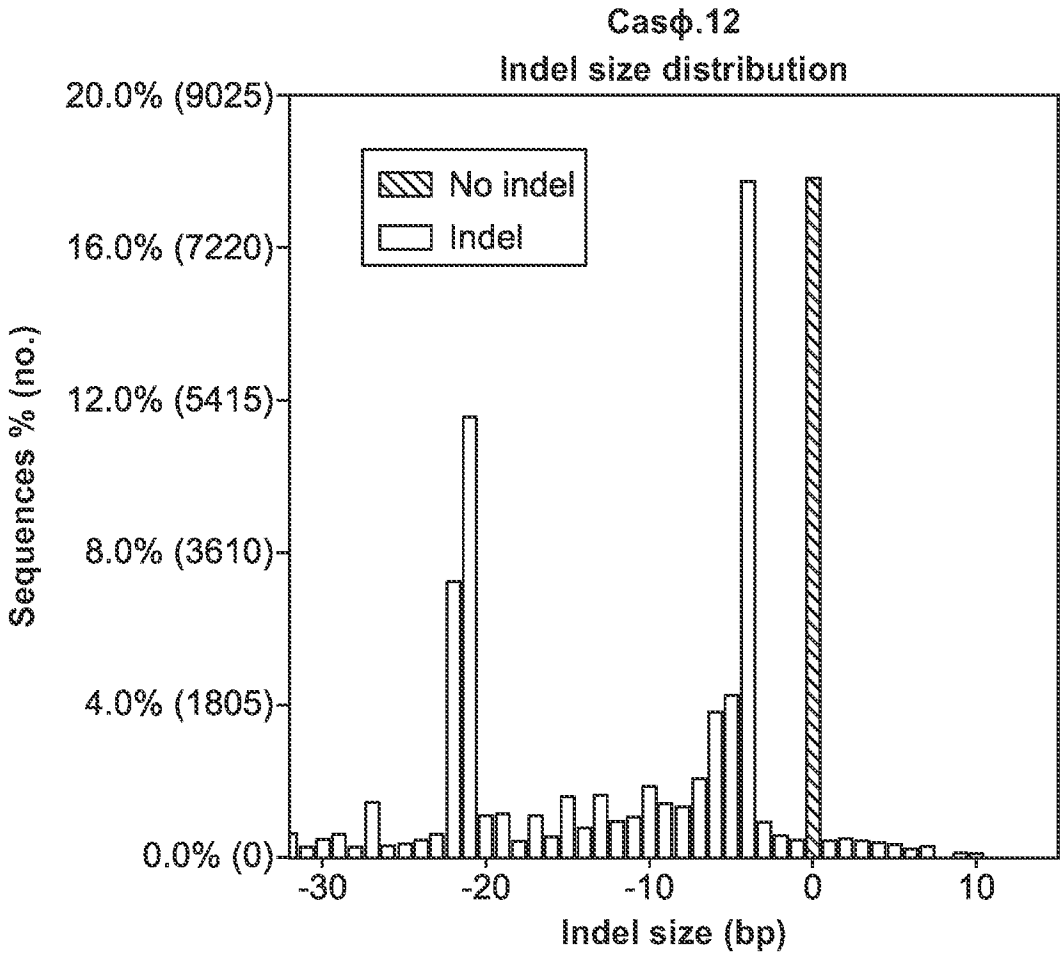


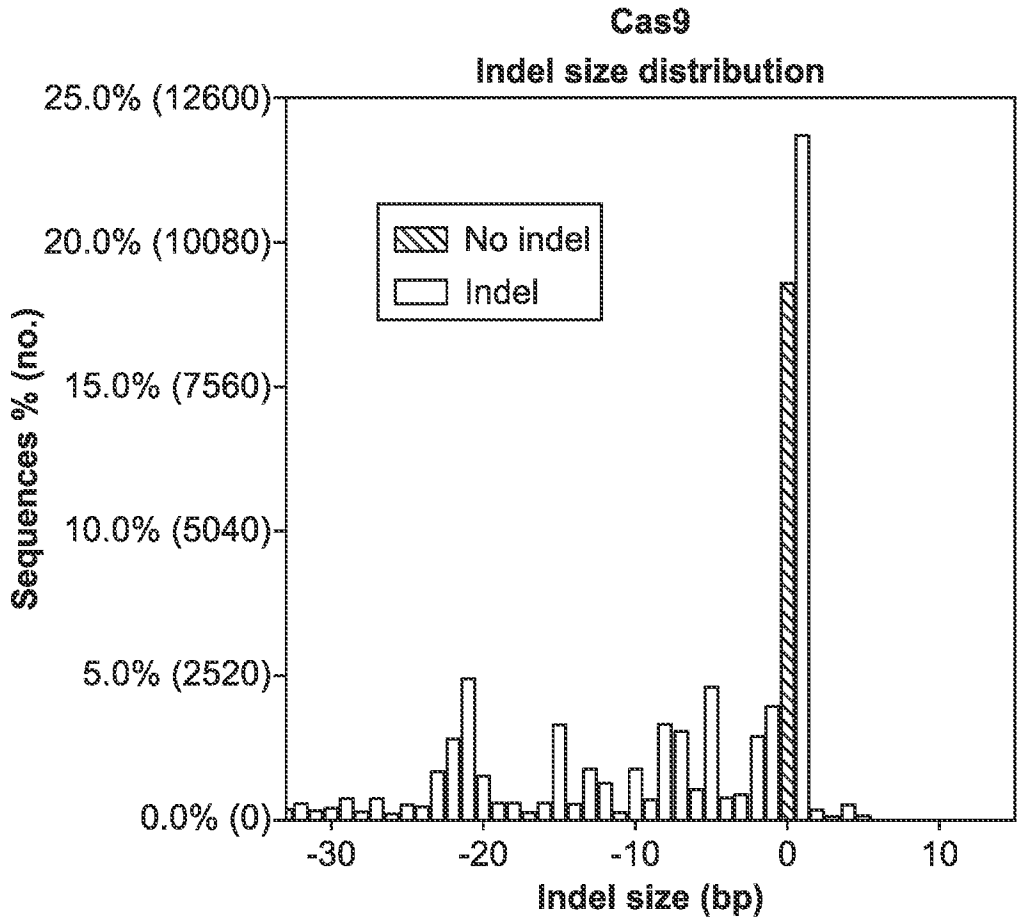
FIG. 30I



TTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCT -Reference
S1

TTCGGGCCGAGATGTCTC--|--CGTGGCCTTAGCTGTGCT -16.69% (7530 reads)
TTCGGGCC----------|---------TTAGCTGTGCT -10.90% (4918 reads)
TTCGGGCCGAGATGTCTCGC|TCCGTGGCCTTAGCTGTGCT -9.21% (4156 reads)
TTCGGGCCGAGATGT-----|----------GCT -4.77% (2154 reads)
TTCGGGCCGAGATGTC----|--CGTGGCCTTAGCTGTGCT -3.10% (1399 reads)

FIG. 30J


Cas9
Indel size distribution
25.0% (12600)
20.0% (10080)
15.0% (7560)
10.0% (5040)
5.0% (2520)
0.0% (0)
Sequences % (no.)
No indel
Indel
-30
-20
-10
0
10
Indel size (bp)


TCGGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTC-Reference
S1
TCGGGGCCGAGATGTCTCGCTTCCGTGGCCTTAGCTGTGCT-22.94% (11561 reads)
TCGGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTC-16.11% (8121 reads)
TCGGGGCC----------T TAGCTGTGCTC-4.58% (2309 reads)
TCGGGCCGAGATGTCTCG----TGGCCTTAGCTGTGCTC-3.86% (1943 reads)
TCGGGCCGAGATGTCTCGC-CCGTGGCCTTAGCTGTGCTC-2.70% (1363 reads)

FIG. 30J (Cont.)

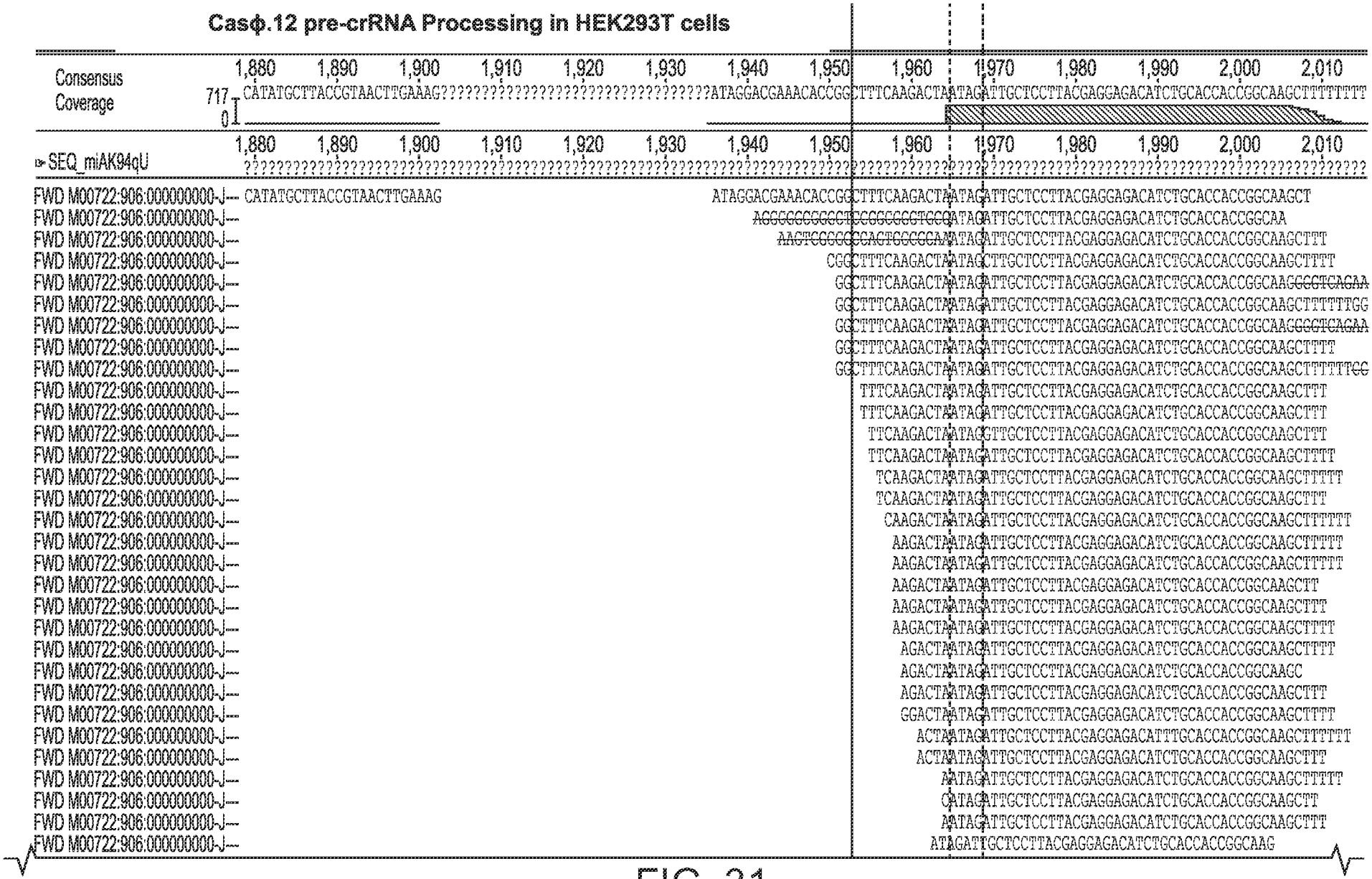

Casϕ.12 pre-crRNA Processing in HEK293T cells
Consensus
Coverage
717
0
1,880 1,890 1,900 1,910 1,920 1,930 1,940 1,950 1,960 1,970 1,980 1,990 2,000 2,010
CATATGCTTACCGTAACTTGAAAG??????????????????????????????????ATAGGACGAAACACCGGCTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACATCTGCACCACCGGCAAGCTTTTTTTT
SEQ_miAK94qU
FWD M00722:906:000000000-J---

FIG. 31

Full 36nt Repeat Length
Optimized Repeat Length
Mature 24nt Repeat Length

FIG. 31 (Cont.)

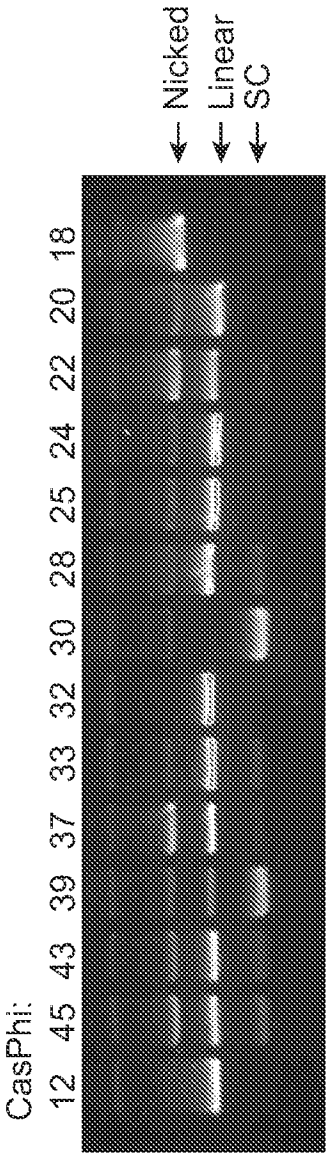


FIG. 32A

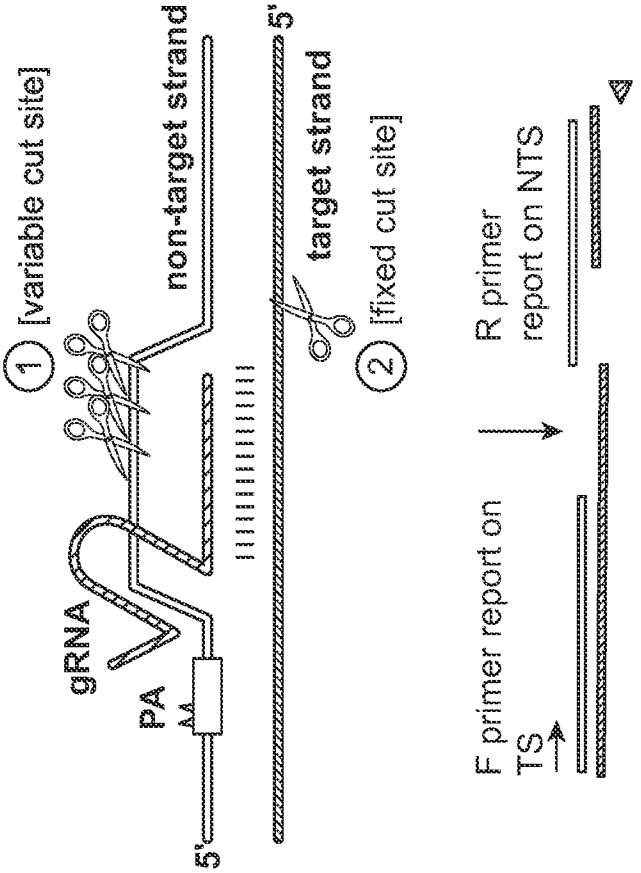


FIG. 32B

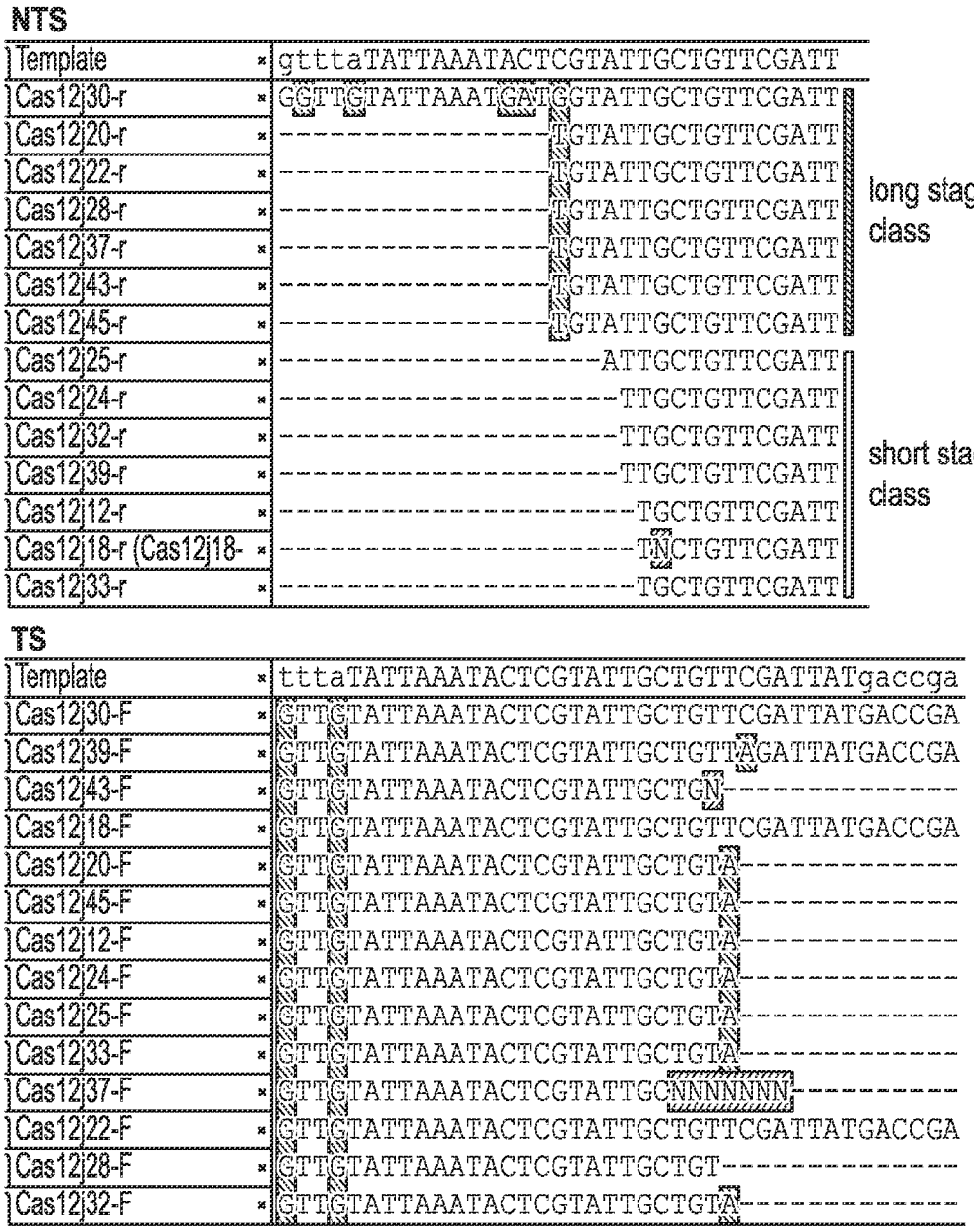


FIG. 32C

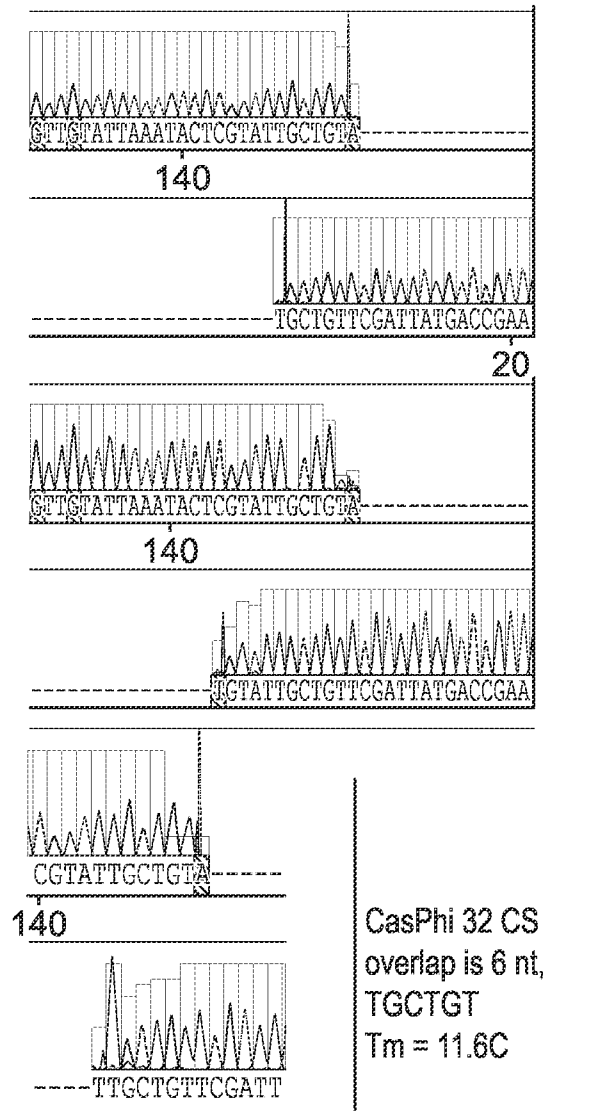


FIG. 32D

System	TS CS	NTS CS	# bp overlap
CasPhi20	21 or 22	12	9-10
CasPhi22	22	12	10
CasPhi28	21	12	9
CasPhi37	22	12	10
CasPhi43	21 or 22	12	9-10
CasPhi45	21 or 22	12	9-10
CasPhi12	22	17	5
CasPhi24	22	16	6
CasPhi25	22	16	6
CasPhi32	22	16	6
CasPhi33	22	17	5
CasPhi18	NA	18	NA

FIG. 32E

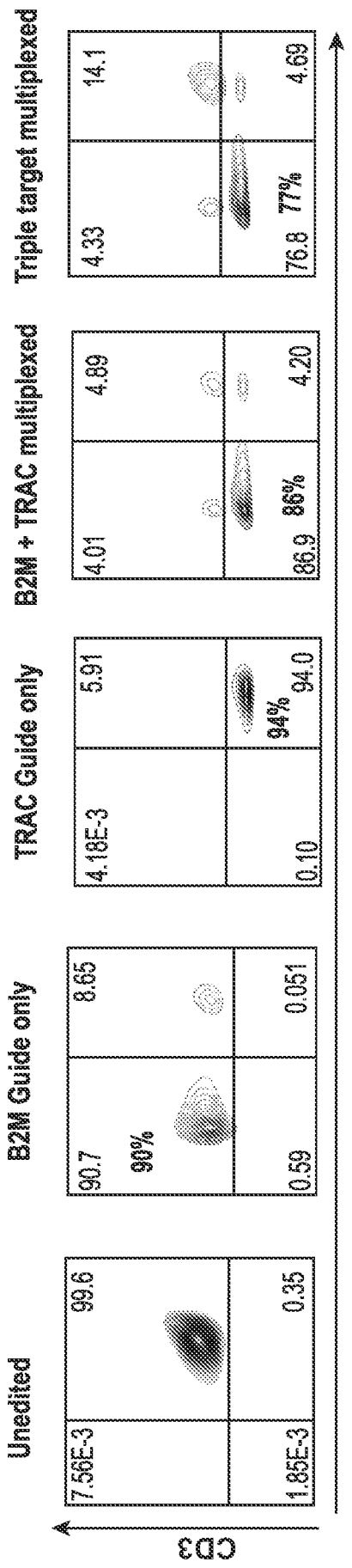

Unedited
B2M Guide only
TRAC Guide only
B2M + TRAC multiplexed
Triple target multiplexed
CD3
B2M
7.56E-3
99.6
1.85E-3
0.35
90.7
8.65
90%
0.59
0.051
4.18E-3
5.91
0.10
94%
94.0
4.01
4.89
86.9
86%
4.20
4.33
14.1
76.8
77%
4.69

FIG. 33

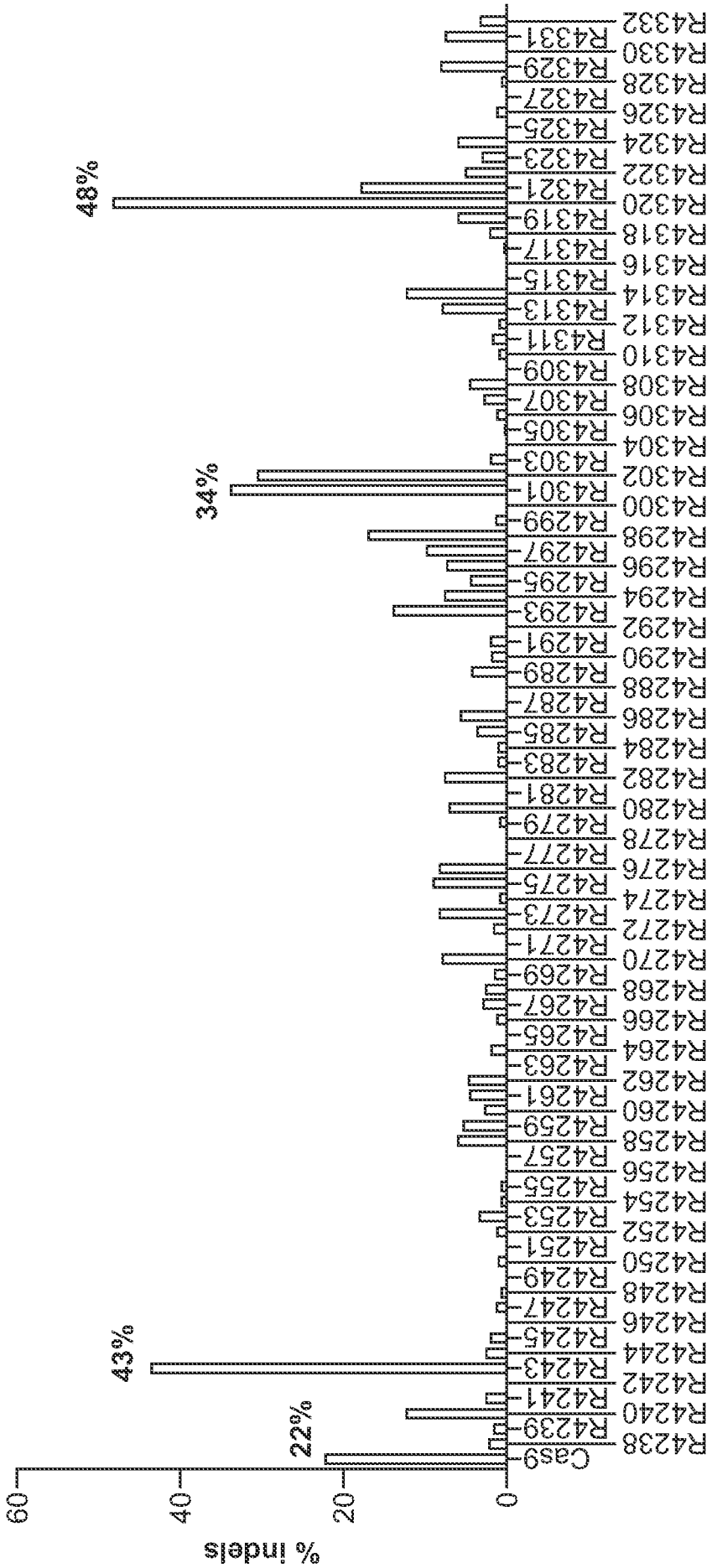

% indels
0
20
40
60
Cas9
22%
43%
34%
48%
R4238
R4239
R4240
R4241
R4242
R4243
R4244
R4245
R4246
R4247
R4248
R4249
R4250
R4251
R4252
R4253
R4254
R4255
R4256
R4257
R4258
R4259
R4260
R4261
R4262
R4263
R4264
R4265
R4266
R4267
R4268
R4269
R4270
R4271
R4272
R4273
R4274
R4275
R4276
R4277
R4278
R4279
R4280
R4281
R4282
R4283
R4284
R4285
R4286
R4287
R4288
R4289
R4290
R4291
R4292
R4293
R4294
R4295
R4296
R4297
R4298
R4299
R4300
R4301
R4302
R4303
R4304
R4305
R4306
R4307
R4308
R4309
R4310
R4311
R4312
R4313
R4314
R4315
R4316
R4317
R4318
R4319
R4320
R4321
R4322
R4323
R4324
R4325
R4326
R4327
R4328
R4329
R4330
R4331
R4332

FIG. 34

pAAV U6 crRNA EFS CasPhi (7395 bp)

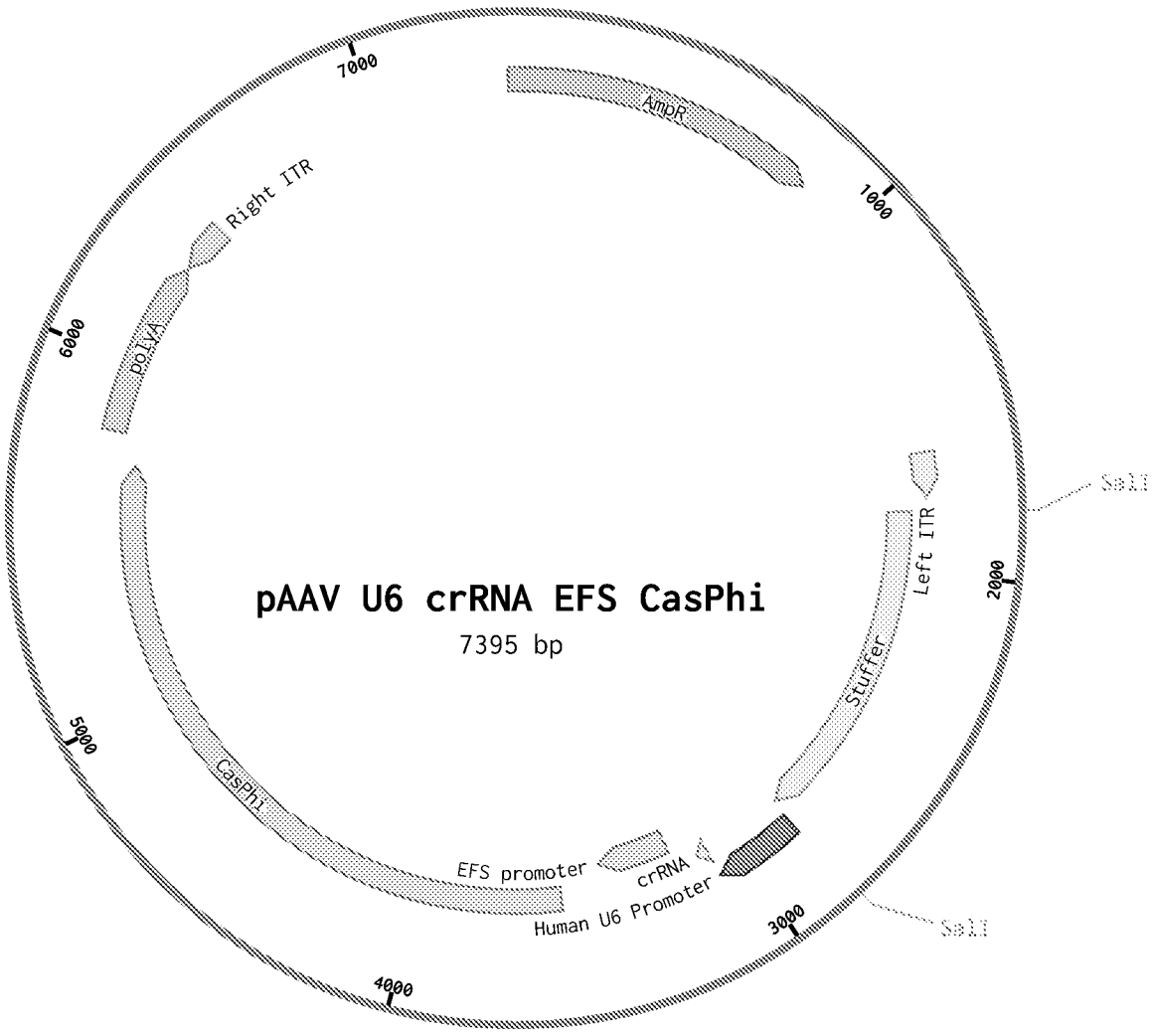


FIG. 35A

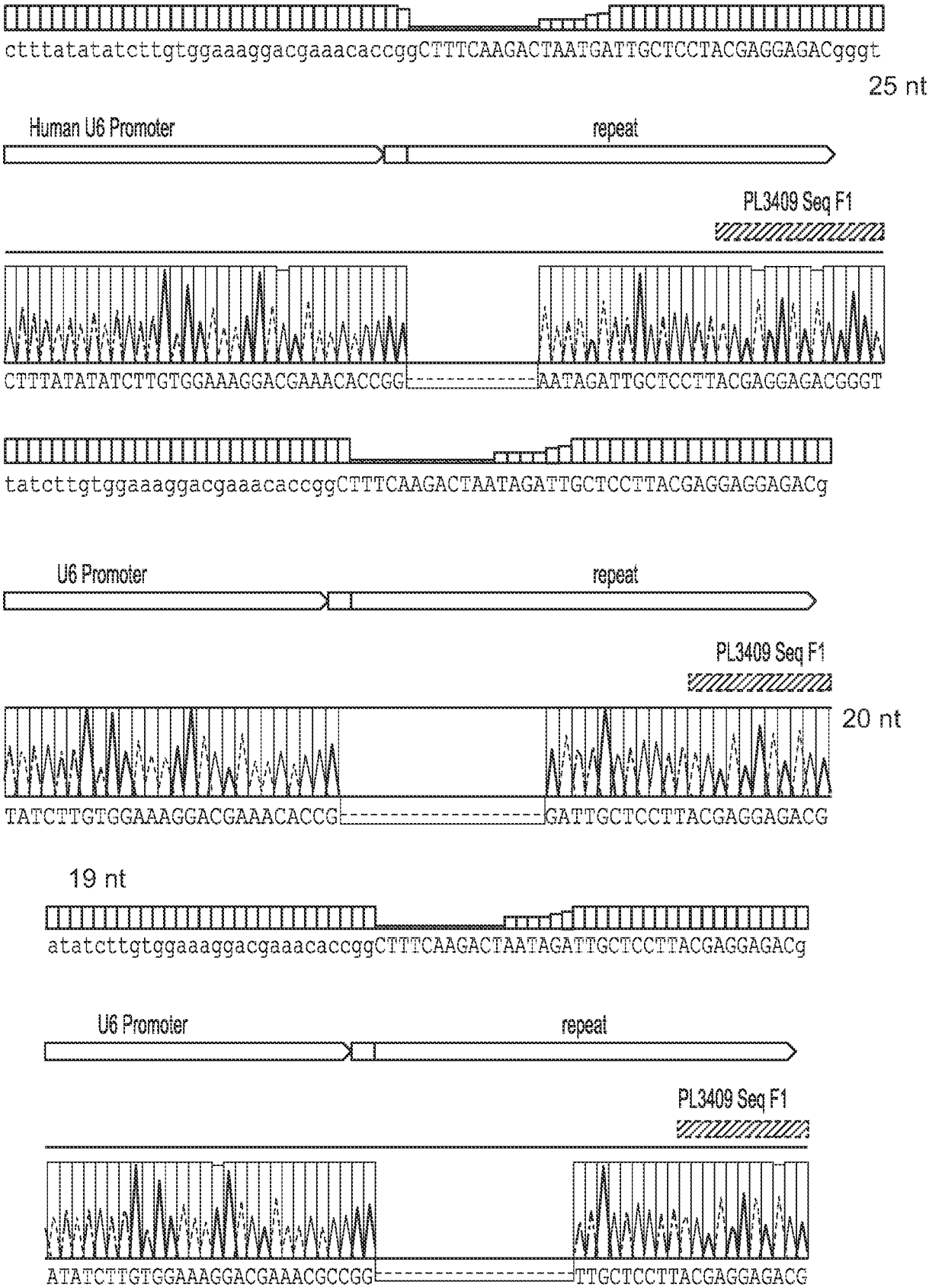

ctttatatatcttgtggaaaggacgaaacaccggCTTTCAAGACTAATGATTGCTCCTACGAGGAGACgggt
25 nt
Human U6 Promoter
repeat
PL3409 Seq F1
CTTTATATATCTTGTGGAAAGGACGAAACACCGG
AATAGATTGCTCCTTACGAGGAGACGGGT
tatcttgtggaaaggacgaaacaccggCTTTCAAGACTAATAGATTGCTCCTTACGAGGAGGAGACg
U6 Promoter
repeat
PL3409 Seq F1
20 nt
TATCTTGTGGAAAGGACGAAACACCG
GATTGCTCCTTACGAGGAGACG
19 nt
atatcttgtggaaaggacgaaacaccggCTTTCAAGACTAATAGATTGCTCCTTACGAGGAGACg
U6 Promoter
repeat
PL3409 Seq F1
ATATCTTGTGGAAAGGACGAAACGCCGG
TTGCTCCTTACGAGGAGACG

FIG. 35B

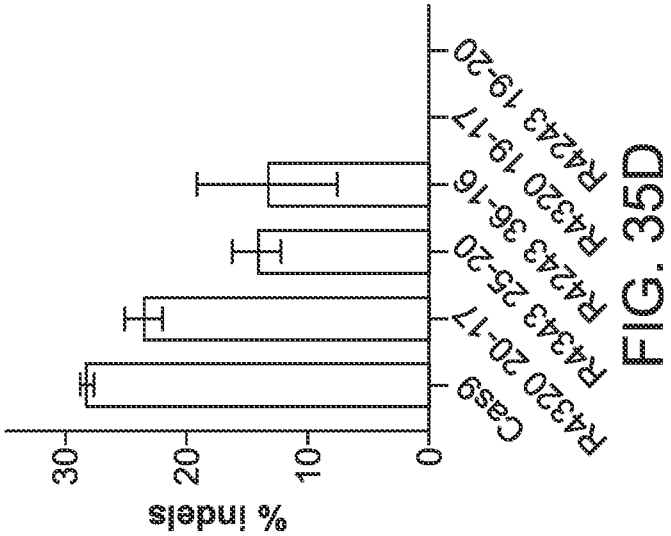


FIG. 35D

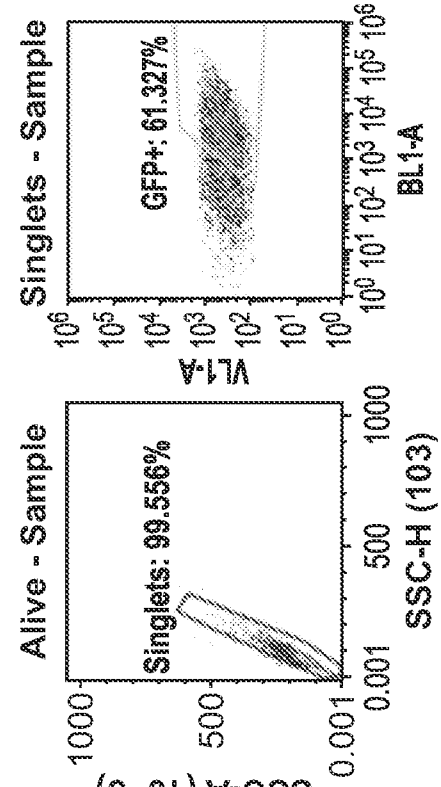


FIG. 35C

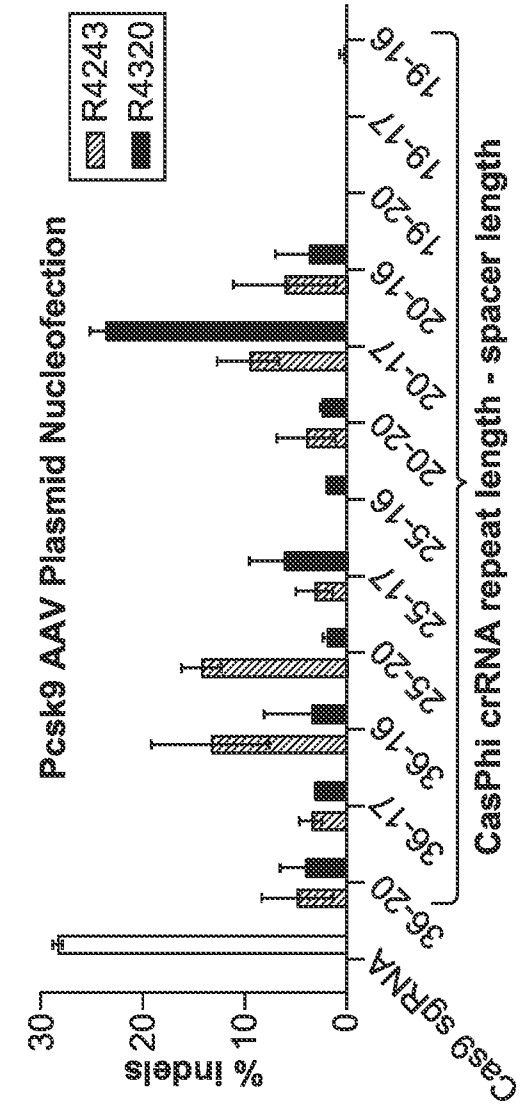


FIG. 35F

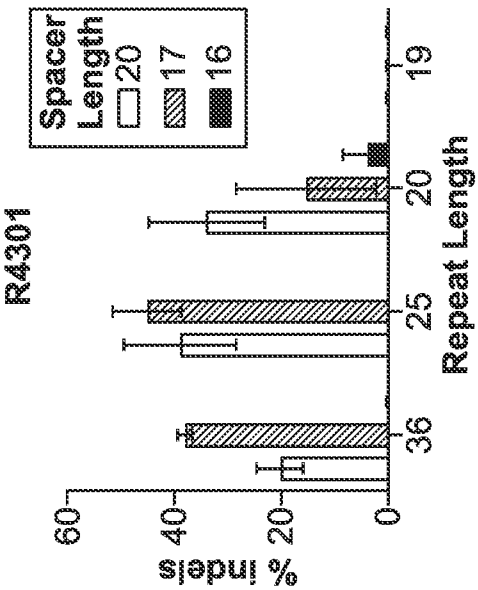


FIG. 35E

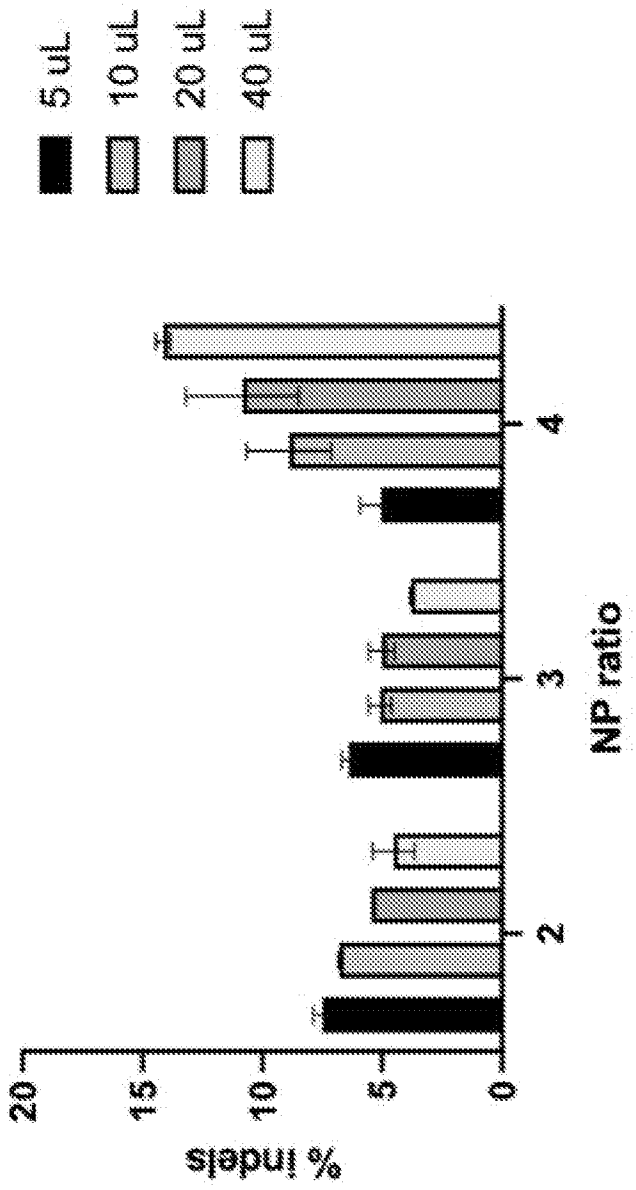


FIG. 36

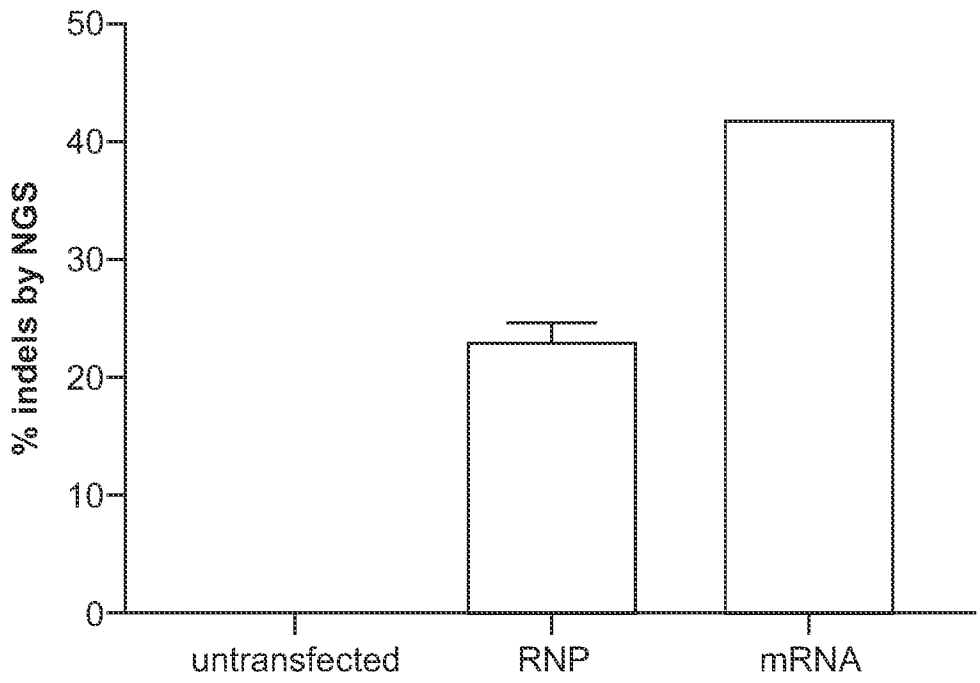


FIG. 37

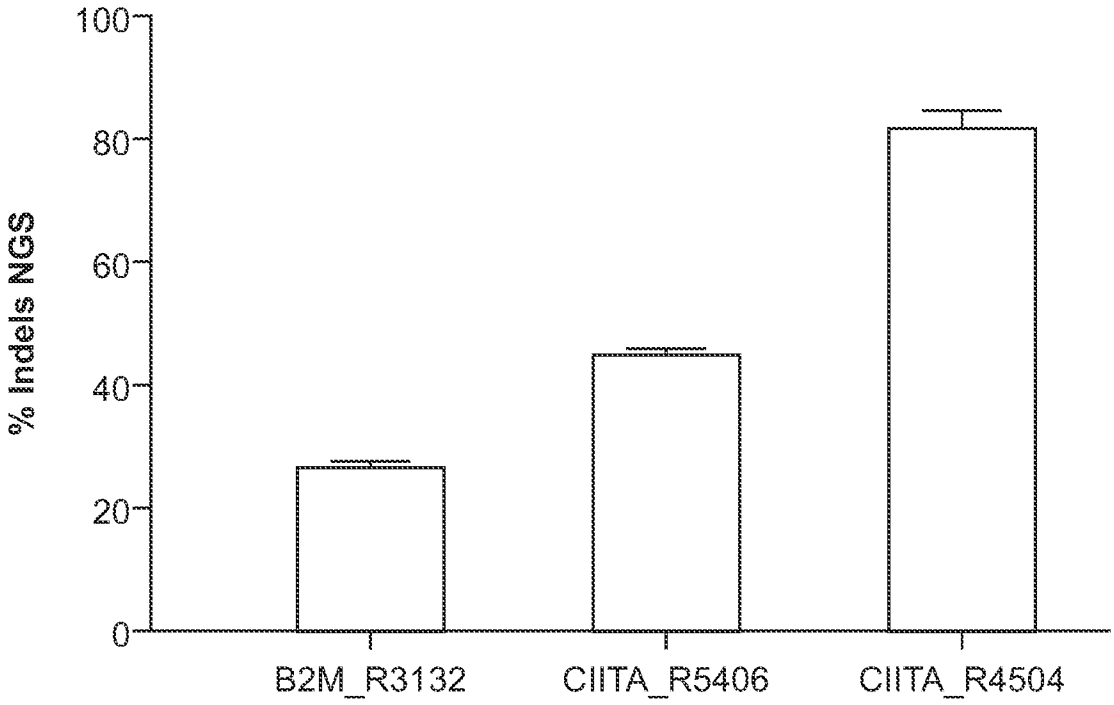


FIG. 38

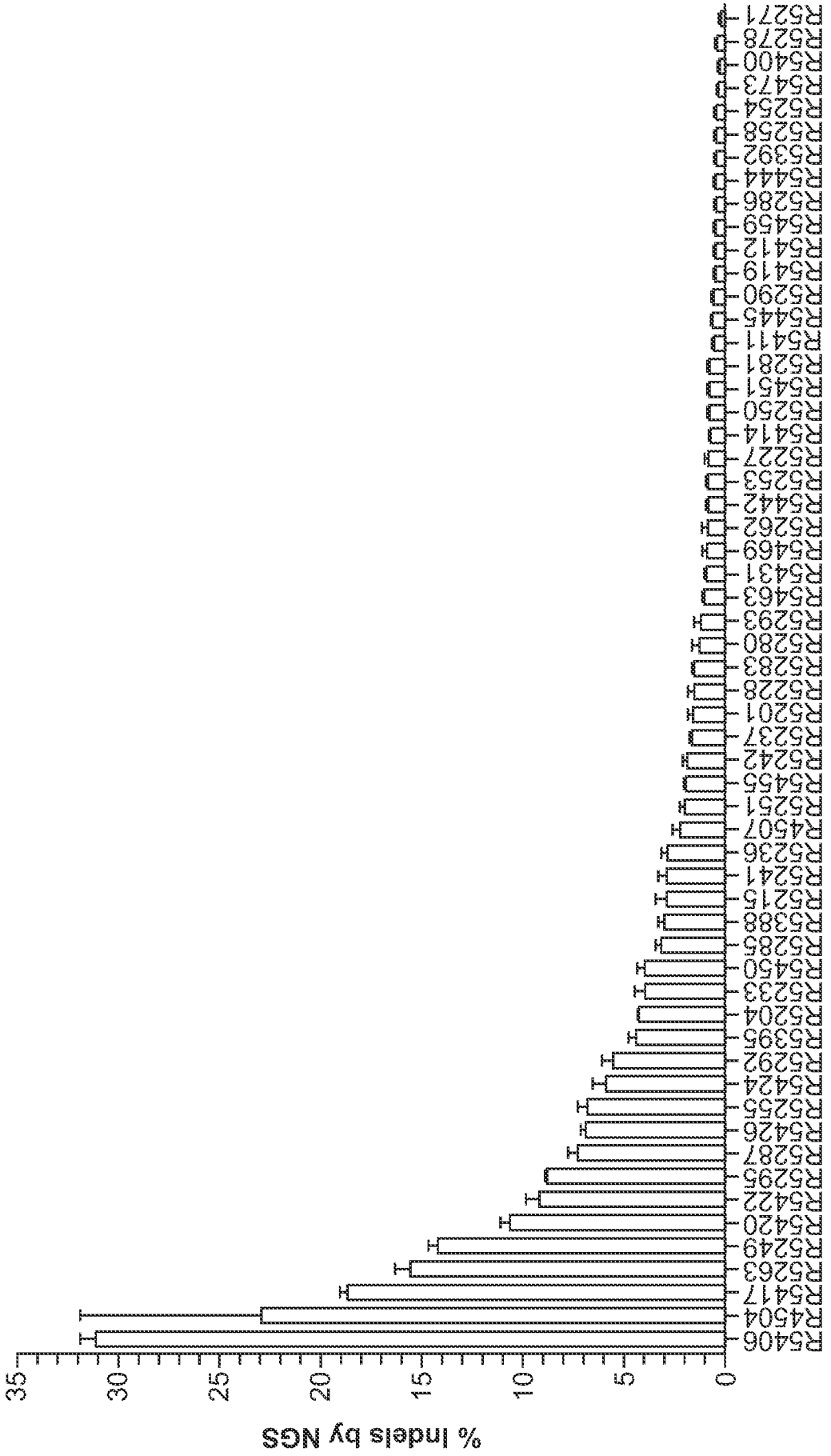

% Indels by NGS
0
5
10
15
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R5406
R4504
R5417
R5263
R5249
R5420
R5422
R5295
R5287
R5426
R5255
R5424
R5292
R5395
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R4506
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R5388
R5215
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R5290
R5419
R5412
R5459
R5286
R5444
R5392
R5258
R5254
R5473
R5400
R5278
R5271

FIG. 39

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/35781

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C12N 15/113; C12N 9/22; C12N 15/63 (2021.01)

CPC - C12N 15/102; C12N 15/113; C12N 2310/20; C12N 9/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0175128 A1 (EDITAS, INC.) 17 April 2015 (17.04.2015). Especially claim 1, SEQ ID NO: 49446	170-173
A	US 2014/0113376 A1 (SOREK et al.) 24 April 2014 (24.04.2014). Especially SEQ ID NO: 1120	1-17, 20-91, 115-117, 133, 136, 139, 142, 145, 148, 151, 154/(133,136,139,142,145,148,151) 179-183, 251/(1-17,90,91), 263-265
A	US 2011/0275135 A1 (LEE et al.) 10 November 2011 (10.11.2011). Especially SEQ ID NO: 2143	1-17, 20-91, 115-117, 133, 136, 139, 142, 145, 148, 151, 154/(133,136,139,142,145,148,151) 179-183, 251/(1-17,90,91), 263-265

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2021	OCT 25 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/35781

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/0108791 A1 (EDGERTON) 19 May 2005 (19.05.2005) Especially SEQ ID NO: 3340	1-17, 20-91, 115-117, 133, 136, 139, 142, 145, 148, 151, 154/(133,136,139,142,145,148,151) 179-183, 187--191, (203-203)/(187-191),251/(1-17,90,91), 263-265
A	US 2018/0155716 A1 (THE BROAD INSTITUTE, INC.) 7 June 2018 (07.06.2018) Especially SEQ ID NO: 1142	1-17, 20-91, 115-117, 133, 136, 139, 142, 145, 148, 151, 154/(133,136,139,142,145,148,151) 179-183, 192-196, (203-204)/(192-196),251/(1-17,90,91), 263-265
A	US 2015/0259693 A1 (BATTELLE ENERGY ALLIANCE, LLC) 17 September 2015 (17.09.2015). Especially SEQ ID NO: 2143	1-17, 20-91, 115-117, 133, 136, 139, 142, 145, 148, 151, 154/(133,136,139,142,145,148,151) 179-183, 197-202, (203-204)/(197-202),251/(1-17,90,91), 263-265
A	YAN et al. Functionally diverse type V CRISPR-Cas systems. Science. 4 January 2019 Vol 363 No 6422 Page 88-91. Especially abstract, PDF pg 1 col 1 para 2, PDF pg 2 Fig 1A, PDF pg 3 col 3 para 1.	90, 116, 134, 135, 137, 138, 140, 141, 143, 144, 146, 147, 149,150, 152, 153, 154/(134,135,137,138,140,141,143,144,146,147,149,150,152,153), 224-231
A	Pfam. Family: RuvC_1 (PF18516) [online] 20 December 2018 [retrieved 16 September 2021]. Available on the internet: < URL: https://www.google.com/search?q=pfam+pf18516&sxsrf=AOaemvlWR5ViLL2k312Vmyfp7ZFIJl2ehQ%3A1631827303641&source=lnt&tbs=cdr%3A1%2Ccd_min%3A%2Ccd_max%3A06%2F02%2F2020&tbm=> and <URL: https://pfam.xfam.org/family/RuvC_1>. Especially pg 1.	116, 134, 137, 140, 143, 146, 149, 152, 154/(134,137,140,143,146,149,152)
A	Pfam07282: OrfB_Zn_ribbon [online] 11 May 2020 [retrieved 16 September 2021]. Available on the internet: <URL: https://www.google.com/search?q=PFAM+%28PF07282%29+transposase+&sxsrf=AOaemvLwv4NzMmWkTNjlw6mVEM4hO03ZOA%3A1631896829262&source=hp&ei=_cREYe2bDcuT0PEPsbe-uAk&iflsig=ALswAMAAAAAYUTTDZHQljtRRjhbzEZaYIMFb6SYz92G&oq=PFAM+%28PF07282%29+transposase+&gs_lcp=Cgdnd3Mtd2l6EAMyBwgjEK4CECc6BwgjEOoCECdQtRNYtRNgpSRoAXAAeACAAVCIAVCSAQExmAEAoAECoAEBsAEK&sclient=gwswiz&ved=0ahUKEwitjsO2uYbzAhXLCTQIHbGbD5cQ4dUDCAk&uact=5> and <URL: https://www.ncbi.nlm.nih.gov/Structure/cdd/PF07282>. Especially pg 1.	116, 134, 137, 140, 143, 146, 149, 152 154/(134,137,140,143,146,149,152)
A	WO 2017/184786 A1 (THE BROAD INSTITUTE, INC.) 26 October 2017 (26.10.2017). Especially para [0056]	247-250
X,P	WO 2020/181101 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 September 2020 (10.09.2020). Especially entire document, claims 1-2, SEQ ID NOs: 109-111, 120.	1, 20, 45, 63, 82, 90, 115-117, 133, 135, 136, 138, 139, 141, 142, 144, 145, 148, 150, 151, 153, 179, 187, 192, 197, 224-230
X,P	PAUSCH et al. CRISPR-Cas-Phi from huge phages is a hypercompact genome editor. Science.17 July 2020 Vol 369, No 6501, Pages 333-337. Especially entire document.	90, 135, 138, 141, 144, 147, 150, 153, 224-230

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/35781

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/35781

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

232-246, 251/(18,19,92-95,157-159,207-209,234-236), 252-262, 266-276

3. ☒ Claims Nos.: 18, 19, 92-109, 111-114, 118-132, 155-169, 174-178, 184-186, 205-223, [cont. above]
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.