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(54) Title: PROGRAMMABLE NUCLEASE IMPROVEMENTS AND COMPOSITIONS AND METHODS FOR NUCLEIC ACID AMPLIFICATION AND DETECTION

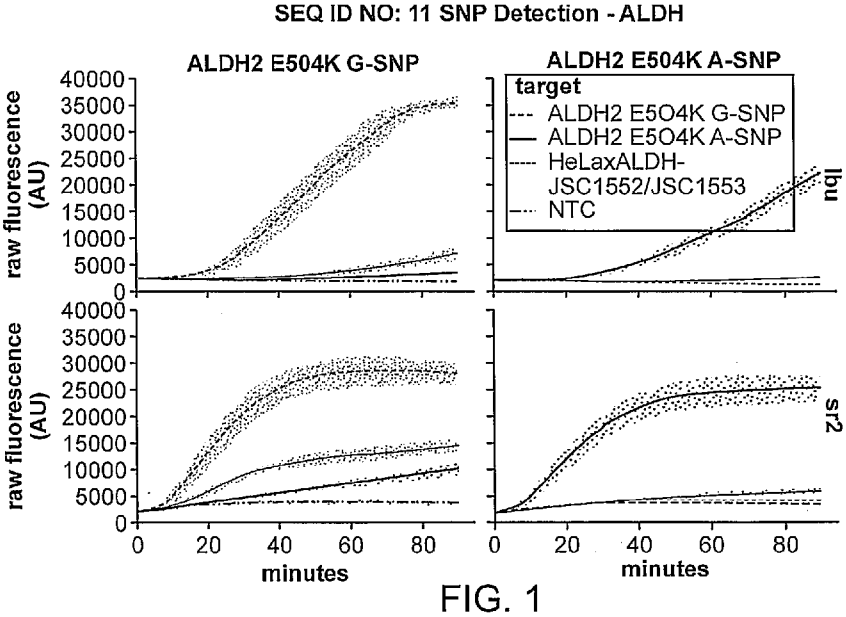

FIG. 1

(57) Abstract: Disclosed herein are compositions, kits, and methods related to improved Cas activity. Through compositions and kits disclosed herein and practice of methods disclosed herein, one attains improved Cas activity such as Cas12 activity relative to Cas proteins in the art such as LbCas12. Further described herein are methods to detect target nucleic acid using a programmable nuclease system. Often, the target nucleic acids are present in at low frequency in the sample. Provided herein are methods for enriching these target nucleic acids for detection. Also described herein are methods to insert a PAM sequence into a target sequence of interest for use in a detection comprising a programmable nuclease.

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PROGRAMMABLE NUCLEASE IMPROVEMENTS AND COMPOSITIONS AND METHODS FOR NUCLEIC ACID AMPLIFICATION AND DETECTION

CROSS-REFERENCE

[0001] The present application claims priority to and the benefit from U.S. Provisional Application Nos. 62/788,706 filed January 4, 2019, 62/894,515 filed August 30, 2019, 62/944,939 filed December 6, 2019, 62/788,704 filed January 4, 2019, 62/795,463 filed January 22, 2019, 62/863,166 filed June 18, 2019, 62/881,801 filed August 1, 2019, and 62/944,933 filed December 6, 2019, the entire contents of each of which are herein incorporated by reference.

BACKGROUND

[0002] CRISPR/Cas-based diagnostics can be very useful for early detection of nucleic acids associated with disease, however, there still exists a need for formulations of Cas proteins and reagents that exhibit optimal activity in diagnostic assays.

[0003] Assaying of a target nucleic acid comprising a mutation can be difficult, especially in the presence of a nucleic acid comprising a variant of the mutation because the mutation is the only difference between the sequences of these nucleic acids. This becomes more difficult when the mutation is a single nucleotide mutation. Additionally, it is often difficult to assay for the target nucleic acid comprising the mutation when the sample comprising the target nucleic acid also comprises more of the nucleic acid comprising the variant of the mutation than the target nucleic acid comprising the mutation. Therefore, there is a need for enhanced detection of a target nucleic acid with a mutation in a sample also comprising a nucleic acid comprising a variant of the mutation.

[0004] There are many target nucleic acids of interest that do not encode for the PAM sequence. However, a target nucleic acid is may need a PAM sequence for binding and trans cleavage activation of some programmable nucleases complexed with a guide nucleic acid. Therefore, there is a need for strategies to allow for binding and trans cleavage activation of the programmable nucleases complexed with a guide nucleic acid using any target nucleic sequence of interest.

SUMMARY

[0005] In various aspects, the present disclosure provides a composition comprising a programmable nuclease having at least 60% sequence identity to SEQ ID NO: 11 and a non-naturally occurring guide nucleic acid.

[0006] In some aspects, the programmable nuclease comprises a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute. In some aspects, the programmable nuclease recognizes a protospacer adjacent motif of YYN.

[0007] In various aspects, the present disclosure provides a composition comprising programmable nuclease having a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute and a non-naturally occurring guide nucleic acid.

[0008] In some aspects, the programmable nuclease recognizes a protospacer adjacent motif of YYN.

[0009] In various aspects, the present disclosure provides a composition comprising a non-naturally occurring guide nucleic acid and a programmable nuclease, wherein the programmable nuclease comprises a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute and recognizes a protospacer adjacent motif of YYN.

[0010] In some aspects, the programmable nuclease is a Type V programmable nuclease. In some aspects, the programmable nuclease is a Cas12 nuclease. In some aspects, the programmable nuclease comprises three partial RuvC domains. In some aspects, the programmable nuclease comprises a RuvC-I subdomain, a RuvC-II subdomain, and a RuvC-III subdomain.

[0011] In some aspects, the programmable nuclease has at least 60% sequence identity to SEQ ID NO: 11. In some aspects, the programmable nuclease has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% sequence identity to SEQ ID NO: 11. In some aspects, the programmable nuclease is SEQ ID NO: 11.

[0012] In some aspects, the Y is a C or T nucleotide. In some aspects, the N is any nucleotide. In some aspects, the composition further comprises a buffer. In some aspects, the buffer comprises a buffering agent, a salt, a crowding agent, a detergent, or any combination thereof.

[0013] In some aspects, the buffering agent is at a concentration of from 5 mM to 100 mM. In some aspects, the buffering agent is at a concentration of from 10 mM to 40 mM. In some aspects, the buffering agent is at a concentration of about 20 mM. In some aspects, the salt is from 5 mM to 100 mM. In some aspects, the the salt is from 5 mM to 10 mM. In some aspects, the crowding agent is from 0.5% (v/v) to 2% (v/v). In some aspects, the crowding agent is about 1% (v/v). In some aspects, the detergent is about 2% (v/v) or less In some aspects, the detergent is about 0.00016% (v/v). In some aspects, the buffering agent is HEPES. In some aspects, the salt is potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof.

[0014] In some aspects, the crowding agent is glycerol. In some aspects, the detergent is Tween, Triton-X, or any combination thereof. In some aspects, a pH of the composition is from 7 to 8. In some aspects, a pH of the composition is 7.5. In some aspects, the composition is at a temperature of from 25°C to 45°C. In some aspects, the programmable nuclease exhibits catalytic activity at a temperature of from 25°C to 45°C. In some aspects, the programmable nuclease exhibits catalytic activity after heating the composition to a temperature of greater than 45°C and restoring the temperature to from 25°C to 45°C.

[0015] In various aspects, the present disclosure provides a method of assaying for a segment of a target nucleic acid in a sample, the method comprising: contacting the sample to: a detector nucleic acid; and any of the above described compositions, wherein the guide nucleic acid hybridizes to a segment of the target nucleic acid; and assaying for a signal produced by cleavage of the detector nucleic acid.

[0016] In various aspects, the present disclosure provides a method of assaying for a segment of a target nucleic acid in a sample from a subject comprising: contacting the sample comprising a population of nucleic acids to: a guide nucleic acid that hybridizes to the segment of the target nucleic acid; a detector nucleic acid; and a Cas12 nuclease that cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid; and assaying for a signal produced by cleavage of the detector nucleic acid, wherein the signal is at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid and wherein the subject has a disease when the segment of the target nucleic acid is present.

[0017] In some aspects, the method further comprising administering a treatment for the disease.

[0018] In various aspects, the present disclosure provides a method of assaying for a segment of a target nucleic acid comprising: contacting a sample comprising a population of nucleic acids, wherein the population comprises at least one nucleic acid comprising a segment having less than 100% sequence identity to the segment of the target nucleic acid and having no less than 50% sequence identity to the segment of the target nucleic acid to: a guide nucleic acid that hybridizes to the segment of the target nucleic acid; a detector nucleic acid; and a Cas12 nuclease that cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid; and assaying for a signal produced by cleavage of the detector nucleic acid, wherein the signal is at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

[0019] In some aspects, the segment of the at least one nucleic acid comprises at least two base mutations compared to the segment of the target nucleic acid. In some aspects, the segment of the at least one nucleic acid comprises from one to ten base mutations compared to the segment of the target nucleic acid. In some aspects, the segment of the at least one nucleic acid comprises one base mutation compared to the segment of the target nucleic acid. In some aspects, the signal is from two-fold to 20-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some aspects, the signal is from two-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some aspects, the signal is from five-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

[0020] In some aspects, the guide nucleic acid is reverse complementary to the segment of the target nucleic acid In some aspects, the guide nucleic acid and the second guide nucleic acid lack synthetic mismatches. In some aspects, the guide nucleic acid is at least 10 bases. In some aspects, the guide nucleic acid is from 10 to 50 bases. In some aspects, the guide nucleic acid is at least 25 bases. In some aspects, the target nucleic acid is in the population of nucleic acids at a minor allele frequency of 10% or less. In some aspects, the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 10%. In some aspects, the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 5%. In some aspects, the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 1%.

[0021] In some aspects, the Cas12 nuclease is Cas12a, Cas12b, Cas12c, CasY, or Cas12e. In some aspects, the Cas12 nuclease is Cas12a. In some aspects, the Cas12 nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602. In some aspects, the Cas12 nuclease has at least 60% sequence identity to SEQ ID NO: 11.

[0022] In some aspects, the contacting is carried out in a buffer comprising a buffering agent, a salt, a crowding agent, a detergent, a reducing agent, a competitor, or any combination thereof. In some aspects, the buffering agent is at a concentration of from 5 mM to 100 mM. In some aspects, the buffering agent is at a concentration of from 10 mM to 30 mM. In some aspects, the salt is from 5 mM to 100 mM. In some aspects, the salt is from 5 mM to 10 mM. In some aspects, the crowding agent is from 0.5% (v/v) to 10% (v/v). In some aspects, the crowding agent

is from 1% (v/v) to 5% (v/v). In some aspects, the detergent is at 2% (v/v) or less In some aspects, the reducing agent is from 0.01 mM to 100 mM. In some aspects, the reducing agent is from 0.1 mM to 10 mM. In some aspects, the reducing agent is from 0.5 mM to 2 mM. In some aspects, the competitor is from 1 ug/ml to 100 ug/ml. In some aspects, the competitor is from 40 ug/ml to 60 ug/ml.

[0023] In some aspects, the buffering agent is HEPES, Tris, or any combination thereof. In some aspects, the salt is potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof. In some aspects, the crowding agent is glycerol. In some aspects, the detergent is Tween, Triton-X, or any combination thereof. In some aspects, the reducing agent is DTT. In some aspects, the competitor is heparin. In some aspects, a pH of the composition is from 7 to 8.

[0024] In some aspects, the method further comprises amplifying the target nucleic acid before the contacting. In some aspects, the amplifying the target nucleic acid before the contacting comprises using a blocking primer. In some aspects, the target nucleic acid segment comprises a single nucleotide mutation. In some aspects, the blocking primer binds to a nucleic acid comprising encoding the wild type sequence of the target nucleic acid segment. In some aspects, the amplifying comprises COLD-PCR. In some aspects, the COLD-PCR comprises full COLD-PCR. In some aspects, the COLD-PCR comprises fast COLD-PCR. In some aspects, the amplifying comprises fast COLD-PCR. In some aspects, the amplifying comprises allele-specific PCR. In some aspects, the amplifying further comprises COLD-PCR.

[0025] In various aspects, the present disclosure provides a composition comprising a programmable nuclease and a buffer, wherein the buffer comprises a salt at less than about 110 mM and wherein the buffer comprises a pH of from 7 to 8.

[0026] In some aspects, the salt is from 1 mM to 110 mM. In some aspects, the salt is from 1 mM to 60 mM. In some aspects, the salt is from 1 mM to 10 mM. In some aspects, the salt is at about 105 mM. In some aspects, the salt is at about 55 mM. In some aspects, the salt is at about 7 mM. In some aspects, the salt comprises potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, potassium chloride, or any combination thereof. In some aspects, the salt comprises potassium acetate and magnesium acetate. In some aspects, the salt comprises sodium chloride and magnesium chloride. In some aspects, the salt comprises potassium chloride and magnesium chloride.

[0027] In some aspects, the pH comprises about 7.5. In some aspects, the pH comprises about 8. In some aspects, the buffer comprises a crowding agent or a competitor. In some aspects, the crowding agent is present from 1% (v/v) to 10% (v/v). In some aspects, the crowding agent or

the competitor is present from 1% (v/v) to 5% (v/v). In some aspects, the crowding agent or the competitor is present at about 5% (v/v). In some aspects, the crowding agent or the competitor is present at about 1% (v/v). In some aspects, the crowding agent or the competitor is present from 1 ug/mL to 100 ug/ml. In some aspects, the crowding agent or the competitor is present from 30 ug/ml to 70 ug/ml. In some aspects, the crowding agent or the competitor is present at about 50 ug/ml. In some aspects, the crowding agent or the competitor is present from 1 mM to 50 mM. In some aspects, the crowding agent or the competitor is present from 10 mM to 30 mM. In some aspects, the crowding agent or the competitor is present at about 20 mM.

[0028] In some aspects, the crowding agent or the competitor is selected from the group consisting of: glycerol, heparin, bovine serum albumin, imidazole, and any combination thereof. In some aspects, the crowding agent or the competitor comprises glycerol. In some aspects, the crowding agent or the competitor comprises glycerol and heparin. In some aspects, the crowding agent or the competitor comprises glycerol, bovine serum albumin, and imidazole. In some aspects, the buffer comprises a buffering agent. In some aspects, the buffering agent is present from 1 mM to 50 mM. In some aspects, the buffering agent is present from 1 mM to 30 mM. In some aspects, the buffering agent is present at about 20 mM. In some aspects, the buffering agent is HEPES. In some aspects, the buffering agent is Tris.

[0029] In some aspects, the buffer comprises a detergent. In some aspects, the detergent is present from 0.00001% (v/v) to 0.1% (v/v). In some aspects, the detergent is present from 0.00001% (v/v) to 0.01% (v/v). In some aspects, the detergent is at about 0.00016% (v/v). In some aspects, the detergent is at about 0.01% (v/v). In some aspects, the detergent is Triton-X. In some aspects, the detergent is IGEPAL CA-630. In some aspects, the buffer comprises a reducing agent. In some aspects, the reducing agent is present from 0.01 mM to 100 mM. In some aspects, the reducing agent is present from 0.1 mM to 10 mM. In some aspects, the reducing agent is present at about 1 mM. In some aspects, the reducing agent is DTT.

[0030] In some aspects, the programmable nuclease comprises a RuvC domain. In some aspects, the programmable nuclease comprises a Type V Cas protein. In some aspects, the programmable nuclease is a Cas12 protein. In some aspects, the Cas12 protein is Cas12a, Cas12b, Cas12c, CasY, or Cas12e. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 1. In some aspects, the

programmable nuclease has at least 60%, at least 70%, 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. In some aspects, the programmable nuclease comprises at least two HEPN domains.

[0031] In some aspects, the programmable nuclease is a Type VI Cas protein. In some aspects, the programmable nuclease is a Cas13 protein. In some aspects, the Cas13 protein is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 103 – SEQ ID NO: 137. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 104.

[0032] In some aspects, the programmable nuclease has at least 60%, at least 70%, 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 and the buffer comprises about 20 mM HEPES, about 2 mM potassium acetate, about 5 mM magnesium acetate, about 1% glycerol, about 0.00016% Triton-X, and a pH of about 7.5. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 1 and the buffer comprises about 20 mM Tris, about 100 mM sodium chloride, about 5 mM magnesium chloride, about 5% glycerol, about 50 ug/mL heparin, about 1 mM DTT, and a pH of about 8. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 104 and the buffer comprises about 50 mM potassium chloride, about 5 mM magnesium chloride, about 10 ug/ml bovine serum albumin, about 5% (v/v) glycerol, about 20 mM imidazole, about 0.01% (v/v) IGEPAL CA-630, and a pH of about 7.5. In some aspects, the composition further comprises a guide nucleic acid. In some aspects, the composition further comprises a detector nucleic acid.

[0033] In various aspects, the present disclosure provides a composition comprising: a nucleic acid from a sample, wherein the sample comprises a PAM and a segment that hybridizes to a guide nucleic acid, wherein the PAM has a sequence of dUdUdUN; a guide nucleic acid that hybridizes to the segment of the nucleic acid; and a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid.

[0034] In some aspects, the composition further comprises a primer, wherein the primer comprises a first region that is reverse complementary to the PAM and a second region that is reverse complementary to a first segment of the nucleic acid.

[0035] In various aspects, the present disclosure provides a method of assaying for a target nucleic acid in a sample, wherein the target nucleic acid lacks a PAM, the method comprising: amplifying the target nucleic acid from a sample using a primer comprising a first region that is reverse complementary to a PAM and a second region that is reverse complementary to a first segment of the target nucleic acid, wherein the PAM is dUdUdUN, thereby producing a PAM target nucleic acid; contacting the PAM target nucleic acid to: a guide nucleic acid that hybridizes to a segment of the PAM target nucleic acid; a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to a segment of the PAM target nucleic acid; and a detector nucleic acid; and assaying for a signal produced by cleavage of the detector nucleic acid.

[0036] In some aspects, the second region comprises from 4 to 12 bases. In some aspects, the second region comprises from 4 to 10 bases. In some aspects, the second region comprises from 4 to 7 bases. In some aspects, the amplifying comprises thermal cycling amplification. In some aspects, the amplifying comprises isothermal amplification. In some aspects, the isothermal amplification comprises isothermal recombinase polymerase amplification (RPA), transcription mediated amplification (TMA), strand displacement amplification (SDA), helicase dependent amplification (HDA), loop mediated amplification (LAMP), rolling circle amplification (RCA), single primer isothermal amplification (SPIA), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), improved multiple displacement amplification (IMDA), or nucleic acid sequence-based amplification (NASBA). In some aspects, the isothermal amplification comprises loop mediated amplification (LAMP).

[0037] In some aspects, a sequence of the primer and a sequence of the guide nucleic acid overlap by 50% or less. In some aspects, a sequence of the primer and a sequence of the guide nucleic acid do not overlap. In some aspects, the primer is a forward primer, a reverse primer, a forward inner primer, or a reverse inner primer. In some aspects, the segment of the nucleic acid or the segment of the target nucleic acid comprises at least one base mutation compared to at least one other segment of a nucleic acid in the sample. In some aspects, the at least one base mutation is no more than 13 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid. In some aspects, the at least one base mutation is no more than 10 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid. In some aspects, the at least one base mutation is no more than 9 nucleotides 3' of the PAM in the nucleic acid or in the PAM

target nucleic acid. In some aspects, the at least one base mutation is no more than 8 nucleotides 3' of the PAM in the nucleic acid or in the PAM target nucleic acid. In some aspects, the at least one base mutation is a single nucleotide polymorphism.

[0038] In some aspects, the programmable nuclease comprises a RuvC domain. In some aspects, the programmable nuclease comprises three partial RuvC domains. In some aspects, wherein the programmable nuclease comprises a RuvC-I subdomain, a RuvC-II subdomain, and a RuvC-III subdomain. In some aspects, the programmable nuclease comprises a Type V Cas protein. In some aspects, the programmable nuclease is a Cas12 protein. In some aspects, the Cas12 protein is Cas12a, Cas12b, Cas12c, CasY, or Cas12e. In some aspects, the programmable nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, SEQ ID NO: 571 – SEQ ID NO: 602.

[0039] In various aspects, the present disclosure provides a Cas12 nuclease for use in diagnosis, wherein the Cas12 nuclease detects the segment of the target nucleic acid according to any of the above methods.

[0040] In some aspects, the present disclosure provides for the use of any of the above compositions in diagnosis.

[0041] In various aspects, the present disclosure provides for a programmable nuclease for use in diagnosis, wherein the programmable nuclease detects the target nucleic acid according to any of the above described methods.

[0042] Provided herein are embodiments related to improved Cas12, Cas13, and Cas14 proteins and related compositions and methods of use. Embodiments are summarized in part in the claims as listed herein.

[0043] In various aspects, the present disclosure provides a programmable nuclease that elicits maximal reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 100nM.

[0044] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, said protein elicits maximal reporter activity following contacting to a target template at least 50% faster than LbCas12a at a given target template concentration. In some aspects, said protein elicits maximal reporter activity following contacting to a target template at least 2x faster than LbCas12a at a given target template concentration. In some aspects, said protein elicits maximal reporter activity following contacting to a target template at least 4x faster than LbCas12a at a given target template concentration. In some aspects, said protein elicits no greater than 33% of maximal reporter

activity following contacting to a template differing from a target template by a single base at a template concentration of 100nM. In some aspects, the protein elicits maximal reporter activity in a composition comprising at least one component selected from the list consisting of acetate, heparin, dithiothreitol (DTT), triton-X, TCEP, BSA, NP-40, imidazole, MOPS, HEPES and DIPSO.

[0045] In some aspects, the template is unamplified. In some aspects, the template is amplified prior to contacting. In some aspects, the contacting is performed in an activity buffer (5X: 600mM NaCl, 25mM MgCl2, 100mM Tris pH 7.5, 5% (v/v) glycerol). In some aspects, the contacting is performed at about 25 °C. In some aspects, the contacting is performed at about 37 °C.

[0046] In various aspects, the present disclosure provides a programmable nuclease reaction buffer comprising at least one component selected from the list consisting of acetate, heparin, dithiothreitol (DTT), triton-X, TCEP, BSA, NP-40, imidazole, MOPS, HEPES and DIPSO.

[0047] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the programmable nuclease in said reaction buffer elicits no greater than 33% of maximal reporter activity following contacting to a template differing from a target template by a single base. In some aspects, the reaction buffer comprises no greater than 150 mM NaCl. In some aspects, the reaction buffer comprises no greater than 100 mM NaCl. In some aspects, the reaction buffer comprises no greater than 50 mM NaCl. In some aspects, the reaction buffer comprises no greater than 25 mM NaCl.

[0048] In various aspects, the present disclosure provides a programmable nuclease reaction buffer comprising at least one component selected from the list consisting of DMSO, polyvinyl alcohol, polyvinylpyrrolidone, and polypropylene glycol.

[0049] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the programmable nuclease in said reaction buffer elicits no greater than 33% of maximal reporter activity following contacting to a no-template control. In some aspects, the reaction buffer comprises no greater than 150mM NaCl. In some aspects, the reaction buffer comprises no greater than 100 mM NaCl. In some aspects, the reaction buffer comprises no greater than 50 mM NaCl. In some aspects, the reaction buffer comprises no greater than 25 mM NaCl.

[0050] In various aspects, the present disclosure provides a programmable nuclease that elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1nM in an activity buffer (5X :600mM NaCl, 25mM MgCl2, 100mM Tris pH 7.5, 5% (v/v) glycerol).

[0051] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the Cas12 protein elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1pM. In some aspects, the Cas12 protein elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1fM.

[0052] In various aspects, the present disclosure provides a programmable nuclease that exhibits at least 90% target cleavage in no more than 60 minutes.

[0053] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the Cas12 protein exhibits at least 90% target cleavage in no more than 15 minutes. In some aspects, an activity buffer (5X :600mM NaCl, 25mM MgCl2, 100mM Tris pH 7.5, 5% (v/v) glycerol) exhibits said target cleavage. In some aspects, said target cleavage is effected at a Cas12 concentration of 100nM. In some aspects, said target cleavage is effected at a target concentration of 15nM. In some aspects, said target cleavage is effected at a guide RNA concentration of 125nM. In some aspects, said target cleavage is effected at a temperature of about 25 °C. In some aspects, said target cleavage is effected at a temperature of about 37 °C.

[0054] In various aspects, the present disclosure provides a programmable nuclease that exhibits no more than 10% target cleavage in 60 minutes.

[0055] In some aspects, the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, an activity buffer (5X :600mM NaCl, 25mM MgCl2, 100mM Tris pH 7.5, 5% (v/v) glycerol) exhibits said target cleavage. In some aspects, said target cleavage is effected at a Cas12 concentration of 100nM. In some aspects, said target cleavage is effected at a target concentration of 15nM. In some aspects, said target cleavage is effected at a guide RNA concentration of 125nM. In some aspects, said target cleavage is effected at a temperature of about 25 °C. In some aspects, said target cleavage is effected at a temperature of about 37 °C.

[0056] In various aspects, the present disclosure provides a composition comprising a first programmable nuclease population and a second programmable nuclease population, wherein the first programmable nuclease population and the second programmable nuclease population do not share a common PAM sequence.

[0057] In some aspects, the composition comprises a third programmable nuclease population, wherein none of the first programmable nuclease population, the second programmable nuclease population, and the third programmable nuclease population share a common PAM sequence. In some aspects, the composition comprises a fourth programmable nuclease population, wherein

none of the first programmable nuclease population, the second programmable nuclease population, the third programmable nuclease population, and the programmable nuclease Cas12 population share a common PAM sequence. In some aspects, the first programmable nuclease, the second programmable nuclease, or a combination thereof comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the third programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. In some aspects, the fourth programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein.

[0058] In various aspects, the present disclosure provides a method for cleaving a unique site of a nucleic acid molecule, comprising designing a guide nucleic acid to cleave the unique site of the nucleic acid molecule and contacting the guide nucleic acid to a programmable nuclease and to the unique site of the nucleic acid molecule, thereby cleaving the unique site of the nucleic acid molecule.

[0059] In some aspects, a PAM sequence is not considered in the designing of the guide nucleic acid. In some aspects, the programmable nuclease comprises a Cas protein. In some aspects, the Cas protein is Cas14.

[0060] In various aspects, the present disclosure provides a method of sequence specific cleavage of a nucleic acid molecule in a sample comprising contacting to a first PAM independent nuclease to a flank on one side of a cleavage site the nucleic acid molecule and a second PAM independent nuclease to a flank on the other side of the cleavage site of the nucleic acid molecule.

[0061] In some aspects, the method further comprises contacting the sample to a DNA fragment for sequence specific break repair. In some aspects, the PAM independent nuclease is a Cas protein. In some aspects, the Cas protein is a nickase. In some aspects, the Cas protein is Cas14.

[0062] In various aspects, the present disclosure provides a method of detecting a presence or an absence of a target nucleic acid in a sample, the method comprising: contacting a first volume to a second volume, wherein the first volume comprises the sample and the second volume comprises: i) a guide nucleic acid having at least 10 nucleotides reverse complementary to a target nucleic acid in the sample; and ii) a programmable nuclease activated upon binding of the guide nucleic acid to the target nucleic acid; iii) a reporter comprising a nucleic acid and a detection moiety, wherein the second volume is at least 4-fold greater than the first volume; and detecting the presence or the absence of the target nucleic acid by measuring a signal produced by cleavage of the nucleic acid of the reporter, wherein cleavage occurs when the programmable nuclease is activated.

[0063] In some aspects, the first volume comprises from 1 μL to 10 μL. In some aspects, the first volume comprises from 1 μL to 5 μL. In some aspects, the first volume comprises about 2 μL. In some aspects, the first volume comprises about 4 μL. In some aspects, the second volume comprises from 5 μL to 40 μL. In some aspects, the second volume comprises from 10 μL to 30 μL. In some aspects, the second volume comprises about 20 μL. In some aspects, the second volume comprises about 30 μL.

[0064] In some aspects, the sample first volume comprises a buffer for cell lysis, a buffer for amplification, a primer, a polymerase, target nucleic acid, a non-target nucleic acid, a single-stranded DNA, a double-stranded DNA, a salt, a buffering agent, an NTP, a dNTP, or any combination thereof. In some aspects, the sample is a biological sample comprising 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.

[0065] In some aspects, the programmable nuclease is a programmable Type V CRISPR/Cas enzyme. In some aspects, the programmable Type V CRISPR/Cas enzyme is a programmable Cas12 nuclease. In some aspects, the programmable Cas12 nuclease is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. In some aspects, the programmable Type V CRISPR/Cas enzyme is a programmable Cas14 nuclease. In some aspects, the programmable Cas14 nuclease is Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. In some aspects, the programmable nuclease is a programmable Type VI CRISPR/Cas enzyme. In some aspects, the programmable Type VI CRISPR/Cas enzyme is a programmable Cas13 nuclease. In some aspects, the programmable Cas13 nuclease is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e.

[0066] In various aspects, the present disclosure provides a method of designing a plurality of primers for amplification of a target nucleic acid, the method comprising: providing a target nucleic acid, herein a guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between an F1c region and a B1 region or between an F1 and a B1c region; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[0067] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5'

of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between the F1c region and a B1 region or between an F1 region and the B1c region; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and

[0068] measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[0069] In some aspects, the sequence between the F1c region and the B1 region or the sequence between the B1c region and the F1 region is at least 50% reverse complementary to the guide nucleic acid sequence. In some aspects, the guide nucleic acid sequence is reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, or a combination thereof. In some aspects, the guide nucleic acid does not hybridize to the forward inner primer and the backward inner primer.

[0070] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1 region and 5' of the F1c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1 region and 5' of the B1c region. In some aspects, the 3' end of the target nucleic acid is 5' of the 5' end of the F3c region or the 3' end of the target nucleic acid is 5' of the 5' end of the B3c region. In some aspects, the 3' end of the target nucleic acid is 5' of the 5' end of the F2c region or 3' end of the target nucleic acid is 5' of the 5' end of the B2c region. In some aspects, the target nucleic acid is between the F1c region and the B1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the F2c region, or wherein the target nucleic acid is between the B1c region and the F1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the B2c region.

[0071] In some aspects, the guide nucleic acid has a sequence reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. In some aspects, the guide nucleic acid sequence does not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof.

[0072] In some aspects, the guide nucleic acid sequence has a sequence reverse complementary to no more than 50% of a sequence of an F3c region, an F2c region, the F1c region, the B1c

region, an B2c region, an B3c region, or any combination thereof. In some aspects, the guide nucleic acid sequence does not hybridize to a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof.

[0073] In various aspects, the present disclosure provides a method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a B2 region and a B1 region or between an F2 region and an F1 region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[0074] In various aspects, the present disclosure provides a method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a F1c region and an F2c region or between a B1c region and a B2c region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[0075] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between a B2 region and a B1 region or between the F2 region and an F1 region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[0076] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5'

of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between the F1c region and an F2c region or between the B1c region and a B2c region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[0077] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B2 region and 5' of the B1 region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F2 region and 5' of the F1 region. In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1c region and 5' of the B2c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1c region and 5' of the F2c region.

[0078] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some aspects, the PAM and the PFS are 5' of the 5' end of the F1c region, 5' of the 5' end of the B1c region, 3' of the 3' end of the F3 region, 3' of the 3' end of the B3 region, 3' of the 3' end of the F2 region, 3' of the 3' end of the B2 region, or any combination thereof.

[0079] In some aspects, the PAM and the PFS do not overlap the F2 region, the B3 region, the F1c region, the F2 region, the B1c region, the B2 region, or any combination thereof. In some aspects, the PAM and the PFS do not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof.

[0080] In some aspects, the plurality of primers further comprises a loop forward primer. In some aspects, the plurality of primers further comprises a loop backward primer. In some aspects, the loop forward primer is between an F1c region and an F2c region. In some aspects, the loop backward primer is between a B1c region and a B2c region.

[0081] In some aspects, the target nucleic acid comprises a single nucleotide polymorphism (SNP). In some aspects, the single nucleotide polymorphism (SNP) comprises a HERC2 SNP. In some aspects, the single nucleotide polymorphism (SNP) is associated with an increased risk or decreased risk of cancer. In some aspects, the target nucleic acid comprises a single nucleotide polymorphism (SNP), and wherein the detectable signal is higher in the presence of a guide nucleic acid that is 100% complementary to the target nucleic acid comprising the single

nucleotide polymorphism (SNP) than in the presence of a guide nucleic acid that is less than 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP).

[0082] In some aspects, the plurality of primers and the guide nucleic acid are present together in a sample comprising the target nucleic acid. In some aspects, the contacting the sample to the plurality of primers results in amplifying the target nucleic acid. In some aspects, the amplifying and the contacting the sample to the guide nucleic acid occurs at the same time. In other aspects, the amplifying and the contacting the sample to the guide nucleic acid occur at different times. In some aspects, the method further comprises providing a polymerase, a dATP, a dTTP, a dGTP, a dCTP, or any combination thereof.

[0083] The present disclosure provides methods of detecting a target nucleic acid using a programmable nuclease.

[0084] In some aspects, the present disclosure provides a method of assaying for a target nucleic acid in a sample, comprising: 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, 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 detector nucleic acids of a population of detector 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. Often, the target nucleic acid is from 0.05% to 20% of total nucleic acids in the sample. Various strategies, such as amplifying the target nucleic to insert a PAM sequence, COLD-PCR, allele-specific PCR, targeting the nucleic acid with a protein, or targeting other nucleic acids with protein can be used to enrich for the target nucleic acid in the sample. Additionally, a buffer comprising NaCl and heparin enhances the specificity of the programmable nuclease in the methods provided herein.

[0085] In various aspects, the present disclosure provides a method of assaying for a target nucleic acid in a sample, comprising: 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, 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 detector nucleic acids of a population of detector 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.

[0086] In some aspects, the target nucleic acid is from 0.05% to 20% of total nucleic acids in the sample. In further aspects, the target nucleic acid is from 0.1% to 10% of total nucleic acids in the sample. In still further aspects, the target nucleic acid is from 0.1% to 5% of total nucleic acids in the sample. In some aspects, the contacting is performed in a buffer comprising heparin and NaCl. In further aspects, the NaCl is 100 mM NaCl. In some aspects, the heparin is 50 ug/ml heparin.

[0087] In some aspects, the sample comprises at least one nucleic acid comprising at least 80% sequence identity to the segment of the target nucleic acid. In further aspects, the sample comprises at least one nucleic acid comprising at least 90% sequence identity to the segment of the target nucleic acid. In still further aspects, the sample comprises at least one nucleic acid comprising at least 99% sequence identity to the segment of the target nucleic acid.

[0088] In some aspects, the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 50% sequence identity to the segment of the target nucleic acid. In some aspects, the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 80% sequence identity to the segment of the target nucleic acid. In some aspects, the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 90% sequence identity to the segment of the target nucleic acid.

[0089] In some aspects, the target nucleic acid comprises a single nucleotide mutation. In further aspects, the segment of the target nucleic acid comprises a single nucleotide mutation. In some aspects, the single nucleotide mutation is a synonymous substitution or a nonsynonymous substitution. In some aspects, the nonsynonymous substitution is a missense substitution or a nonsense point mutation.

[0090] In other aspects, the target nucleic acid comprises a deletion. In further aspects, the segment of the target nucleic acid comprises a deletion. In some aspects, the deletion comprises a deletion of from 1 to 50 nucleotides. In some aspects, the deletion comprises a deletion of from 9 to 21 nucleotides.

[0091] In some aspects, the method further comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target

nucleic acid having a PAM sequence adjacent to target sequence of an amplification product before the contacting. In some aspects, the primer is a forward primer comprising the sequence encoding the PAM and has 1-8 nucleotides from the 3' end of the sequence encoding the PAM. In some aspects, the primer is a forward primer comprising the sequence encoding the PAM and has 4 nucleotides from the 3' end of the sequence encoding the PAM.

[0092] In other aspects, the primer is a forward primer comprising the sequence encoding the PAM and has 5 nucleotides from the 3' end of the sequence encoding the PAM. In still other aspects, the primer is a forward primer comprising the sequence encoding the PAM and has 6 nucleotides from the 3' end of the sequence encoding the PAM.

[0093] In some aspects, the segment of the target nucleic acid comprises the single nucleotide mutation at 5-9 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In some aspects, the segment of the target nucleic acid comprises the single nucleotide mutation at 6 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In other aspects, the segment of the target nucleic acid comprises the single nucleotide mutation at 7 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In still other aspects, the segment of the target nucleic acid comprises the single nucleotide mutation at 8 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM.

[0094] In some aspects, the segment of the target nucleic acid comprises the deletion at 5-9 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In some aspects, the segment of the target nucleic acid comprises the deletion at 6 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In other aspects, the segment of the target nucleic acid comprises the deletion at 7 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. In still other aspects, the segment of the target nucleic acid comprises the deletion at 8 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM.

[0095] In some aspects, the method further comprises amplifying the target nucleic acid before the contacting. In some aspects, the amplifying the target nucleic acid before the contacting comprises using a blocking primer. In some aspects, the blocking primer binds to a nucleic acid comprising encoding the wild type sequence of the target nucleic acid segment. In some aspects, the amplifying comprises COLD-PCR.

[0096] In further aspects, the COLD-PCR comprises full COLD-PCR. In some aspects, the COLD-PCR comprises fast COLD-PCR. In some aspects, the amplifying comprises fast COLD-PCR. In some aspects, the amplifying comprises allele-specific PCR. In some aspects, the amplifying further comprises COLD-PCR.

[0097] In some aspects, the method further comprises removing a nucleic acid comprising at least 50% sequence identity to the target nucleic acid by binding a protein to the nucleic acid before the contacting. In some aspects, the protein is an antibody. In some aspects, the protein is a programmable nuclease without endonuclease activity. In some aspects, the method further comprises binding a protein to the target nucleic acid to remove other nucleic acids of the sample. In some aspects, the other nucleic acids comprise a nucleic acid comprising at least 50% sequence identity to the target nucleic acid. In some aspects, the protein is attached to a surface. In some aspects, the removing of the other nucleic acids comprises washing away nucleic acids that are not bound to the protein. In some aspects, the protein is an antibody. In some aspects, the protein is a programmable nuclease without endonuclease activity.

[0098] In some aspects, the programmable nuclease is a target nucleic acid activated effector protein that exhibits sequence independent cleavage upon activation. In some aspects, the programmable nuclease is an RNA guided nuclease. In some aspects, the programmable nuclease comprises a Cas nuclease. In some aspects, the Cas nuclease is Cas13. In further aspects, the Cas13 is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. In other aspects, the Cas nuclease is Cas12. In further aspects, the Cas12 is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. In some aspects, the Cas nuclease is Cas14. In further aspects, the Cas14 is Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. In some aspects, the Cas nuclease is Csm1, Cas9, C2c4, C2c8, C2c5, C2c10, or C2c9.

[0099] In some aspects, the guide nucleic acid comprises a crRNA. In some aspects, the guide nucleic acid comprises a crRNA and a tracrRNA. In some aspects, cleavage of at least one detector nucleic acid yields a signal. In some aspects, cleavage of at least one detector nucleic acid activates a photoexcitable fluorophore. In some aspects, cleavage of at least one detector nucleic acid deactivates a photoexcitable fluorophore. In some aspects, the signal is present prior to detector nucleic acid cleavage. In some aspects, the signal is absent prior to detector nucleic acid cleavage.

[00100] In some aspects, the sample comprises 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. In some aspects, the single nucleotide mutation is a single nucleotide polymorphism.

[00101] In various aspects, the present disclosure provides a method, comprising: contacting a programmable nuclease comprising a polypeptide having endonuclease activity and a guide nucleic acid to a target nucleic acid in a buffer comprising heparin. In some aspects, the heparin is present at a concentration of from 1 to 100 ug/ml heparin. In further aspects, the heparin is present at a concentration of from 40 to 60 ug/ml heparin. In still further aspects, the heparin is present at a concentration 50 ug/ml heparin.

[00102] In some aspects, the buffer comprises NaCl. In further aspects, the NaCl is present at a concentration of from 1 to 200 mM NaCl. In still further aspects, the NaCl is present at a concentration of from 80 to 120 mM NaCl. In still further aspects, the NaCl is present at a concentration of 100 mM NaCl.

[00103] In some aspects, the target nucleic acid is a substrate target nucleic acid. In some aspects, the substrate nucleic acid comprises a cancer allele. In further aspects, the cancer allele is present at a low concentration relative to a wild type allele. In some aspects, the substrate target nucleic acid comprises a splice variant. In some aspects, the substrate target nucleic acid comprises an edited base. In some aspects, the substrate target nucleic acid comprises a bisulfite-treated base. In some aspects, the substrate target nucleic acid comprises a segment that is reverse complementary to a segment of the guide nucleic acid.

[00104] In various aspects, the present disclosure provides a method of designing a plurality of primers for amplification of a target nucleic acid, the method comprising: providing a target nucleic acid, herein a guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between an F1c region and a B1 region or between an F1 and a B1c region; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[00105] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the

target nucleic acid is between the F1c region and a B1 region or between an F1 region and the B1c region; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and

[00106] measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[00107] In some aspects, the sequence between the F1c region and the B1 region or the sequence between the B1c region and the F1 region is at least 50% reverse complementary to the guide nucleic acid sequence. In some aspects, the guide nucleic acid sequence is reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, or a combination thereof. In some aspects, the guide nucleic acid does not hybridize to the forward inner primer and the backward inner primer.

[00108] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1 region and 5' of the F1c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1 region and 5' of the B1c region. In some aspects, the 3' end of the target nucleic acid is 5' of the 5' end of the F3c region or the 3' end of the target nucleic acid is 5' of the 5' end of the B3c region. In some aspects, the 3' end of the target nucleic acid is 5' of the 5' end of the F2c region or 3' end of the target nucleic acid is 5' of the 5' end of the B2c region. In some aspects, the target nucleic acid is between the F1c region and the B1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the F2c region, or wherein the target nucleic acid is between the B1c region and the F1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the B2c region.

[00109] In some aspects, the guide nucleic acid has a sequence reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. In some aspects, the guide nucleic acid sequence does not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof.

[00110] In some aspects, the guide nucleic acid sequence has a sequence reverse complementary to no more than 50% of a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. In some aspects, the guide nucleic acid sequence does not hybridize to a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof.

[00111] In various aspects, the present disclosure provides a method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a B2 region and a B1 region or between an F2 region and an F1 region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[00112] In various aspects, the present disclosure provides a method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a F1c region and an F2c region or between a B1c region and a B2c region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region.

[00113] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between a B2 region and a B1 region or between the F2 region and an F1 region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[00114] In various aspects, the present disclosure provides a method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer

primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between the F1c region and an F2c region or between the B1c region and a B2c region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[00115] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B2 region and 5' of the B1 region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F2 region and 5' of the F1 region. In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1c region and 5' of the B2c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1c region and 5' of the F2c region.

[00116] In some aspects, a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some aspects, the PAM and the PFS are 5' of the 5' end of the F1c region, 5' of the 5' end of the B1c region, 3' of the 3' end of the F3 region, 3' of the 3' end of the B3 region, 3' of the 3' end of the F2 region, 3' of the 3' end of the B2 region, or any combination thereof.

[00117] In some aspects, the PAM and the PFS do not overlap the F2 region, the B3 region, the F1c region, the F2 region, the B1c region, the B2 region, or any combination thereof. In some aspects, the PAM and the PFS do not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof.

[00118] In some aspects, the plurality of primers further comprises a loop forward primer. In some aspects, the plurality of primers further comprises a loop backward primer. In some aspects, the loop forward primer is between an F1c region and an F2c region. In some aspects, the loop backward primer is between a B1c region and a B2c region.

[00119] In some aspects, the target nucleic acid comprises a single nucleotide polymorphism (SNP). In some aspects, the single nucleotide polymorphism (SNP) comprises a HERC2 SNP. In some aspects, the single nucleotide polymorphism (SNP) is associated with an increased risk or decreased risk of cancer. In some aspects, the target nucleic acid comprises a single nucleotide polymorphism (SNP), and wherein the detectable signal is higher in the presence of a guide nucleic acid that is 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP) than in the presence of a guide nucleic

acid that is less than 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP).

[00120] In some aspects, the plurality of primers and the guide nucleic acid are present together in a sample comprising the target nucleic acid. In some aspects, the amplifying and the contacting the sample to the guide nucleic acid occurs at the same time. In other aspects, the amplifying and the contacting the sample to the guide nucleic acid occur at different times. In some aspects, the method further comprises providing a polymerase, a dATP, a dTTP, a dGTP, a dCTP, or any combination thereof.

[00121] The present disclosure provides an amplification method for inserting a PAM sequence into a target nucleic acid.

[00122] In some aspects, the present disclosure provides a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprising amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. Often, the PAM comprises a sequence encoding dUdUdUN. Sometimes the PAM comprises a sequence encoding TTTN. The programmable nuclease is, for example, Cas12. The present disclosure further provides the number of nucleotides in a nucleotide extension of the forward primer used to produce the PAM target nucleic acid, as well as the location of the mutation or mismatch in the PAM target nucleic acid.

INCORPORATION BY REFERENCE

[00123] 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.

BRIEF DESCRIPTION OF THE DRAWINGS

[00124] The 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. The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[00125] **FIG. 1** shows an improved SNP detection enzyme and method. At left is shown ALDH2 E540K G-SNP, while at right one sees ALDH E540K A-SNP. The ALDH2 G-SNP was detected with a G-SNP gRNA (SEQ ID NO: 425, UAAUUUCUACUAAGUGUAGAUACUUCAGUGUAUGCCUGCAG), and the ALDH2 A-SNP was detected with an A-SNP gRNA (SEQ ID NO: 426, UAAUUUCUACUAAGUGUAGAUACUUUAGUGUAUGCCUGCAG). LbCas12a (SEQ ID NO: 1) is shown at top, while a representative improved enzyme, a Cas12 variant corresponding to (SEQ ID NO: 11), is shown at bottom.

[00126] **FIG. 2** shows the first of a series of experiments to assess buffer contents for detection using a Cas12 variant (SEQ ID NO: 11).

[00127] **FIG. 3** shows improvements conveyed by inclusion of acetate at concentrations of about 0, 10, 20, 37, 75, 150, 300 and 600 mM, from left to right on detection using a Cas12 variant (SEQ ID NO: 11).

[00128] **FIG. 4** shows an improvement in SNP specificity upon inclusion of heparin in a reaction buffer when detected with a Cas12 variant (SEQ ID NO: 11).

[00129] **FIG. 5** shows optimization for a number of buffer additives, such as heparin, DTT, NP-40, and BSA (from left to right) over a series of 8 iterative dilutions when detected with a Cas12 variant (SEQ ID NO: 11) and a gRNA of SEQ ID NO: 423.

[00130] **FIG. 6** shows base sensitivity for alleles having bases A, C, G, or T, at a SNP position, for LbCas12a (SEQ ID NO: 1), top, and a representative improved enzyme, a Cas12 variant corresponding to (SEQ ID NO: 11), below. Target sequences corresponding to SEQ ID NO: 431 – SEQ ID NO: 438 were detected. The A SNP allele was detected using a gRNA of SEQ ID NO: 427 (GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUGGCAGGCCAAACUGCUGGGU). The C SNP allele was detected using a gRNA of SEQ ID NO: 428 (GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUGGCCGGCCAAACUGCUGG

GU). The G SNP allele was detected using a gRNA of SEQ ID NO: 429 (GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUGGCGGGCCAAACUGCUGG GU). The T SNP allele was detected using a gRNA of SEQ ID NO: 430 (GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUGGCUGGCCAAACUGCUGG GU).

[00131] **FIG. 7** shows template optimization for an improved enzyme, a Cas12 variant corresponding to SEQ ID NO: 11, as disclosed herein. Templates comprising a C SNP allele (SEQ ID NO: 440, GGGCATGAGCTGCGTGATGA) or a T SNP allele (SEQ ID NO: 441, GGGCATGAGCTGCATGATGA) were detected using gRNAs directed to the C SNP (SEQ ID NO: 423) or the T SNP allele (SEQ ID NO: 439, UAAUUUCUACUAAGUGUAGAUUCAUCAUGCAGCUCAUGCCC). Primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397 were used to amplify the target sequence and insert a PAM sequence.

[00132] **FIG. 8** shows base sensitivity of an improved enzyme, a Cas12 variant corresponding to SEQ ID NO: 11, for each allele having bases A, C, G, or T, for an EGFR SNP as disclosed herein. EGFR target sequences corresponding to SEQ ID NO: 444 – SEQ ID NO: 447 were detected. Primers corresponding to SEQ ID NO: 442 (ACCACATGCAGGAAGGTCAG) and SEQ ID NO: 443 (AGAAGGACTCCATTGCTGC) were used to amplify the target sequences. The A SNP allele was detected using a gRNA of SEQ ID NO: 427. The C SNP allele was detected using a gRNA of SEQ ID NO: 428. The G SNP allele was detected using a gRNA of SEQ ID NO: 429. The T SNP allele was detected using a gRNA of SEQ ID NO: 430.

[00133] **FIG. 9** shows an assessment of buffer additives and their effect on detection using a Cas12 variant (SEQ ID NO: 11).

[00134] **FIG. 10** shows trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 586, SEQ ID NO: 581, SEQ ID NO: 576, SEQ ID NO: 587, SEQ ID NO: 578, SEQ ID NO: 572, SEQ ID NO: 575, SEQ ID NO: 11, SEQ ID NO: 573, SEQ ID NO: 589, and SEQ ID NO: 583, and of LbCas12a (SEQ ID NO: 1) on targets containing various PAMs, double and single mismatched substrates. Target dsDNA was obtained by annealing complementary ssDNA primers with 2:1 ratio of non-target strand to target strand in hybridization buffer (50 mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA) This ensures double-stranded DNA is being detected instead of single-stranded DNA. PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

[00135] **FIG. 11** shows trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, and SEQ ID NO: 599 – SEQ ID NO: 602 on targets containing various PAMs, double and single mismatched substrates. PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

[00136] **FIG. 12** shows trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, and SEQ ID NO: 3 on targets containing various PAMs, double and single mismatched substrates. PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

[00137] **FIG. 13A**, **FIG. 13B**, and **FIG. 13C** show trans cleavage activity of various Cas12 orthologs corresponding to SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, and SEQ ID NO: 3 on PCR targets containing a TTTA PAM using various guide RNA repeat sequences. Activity was detected in the presence of different Cas12 variants and different pre-crRNAs corresponding to different Cas12 variants. Sequences of the pre-crRNAs are provided in **TABLE 30**.

[00138] **FIG. 14** shows activity of various Cas12 orthologs and other improved enzymes corresponding to SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 on a target PCR product. The negative control ("(-) control") is PCR product with no Cas12 added. The positive control is cleavage with a BamHI restriction enzyme ("BamHI"). Numbers above each lane correspond to the time in minutes before the reaction was quenched with 10mM EDTA. Lanes marked with "-" under each Cas12 ortholog correspond to negative control conditions with protein but no crRNA.

[00139] **FIG. 15** shows limit of detection (LOD) assay results indicating trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 572, SEQ ID NO: 576, SEQ ID NO: 11, SEQ ID NO: 582, SEQ ID NO: 583, SEQ ID NO: 587, SEQ ID NO: 1, SEQ ID NO: 591, SEQ ID NO: 595, SEQ ID NO: 597, SEQ ID NO: 600, SEQ ID NO: 601, and SEQ ID NO: 2 in the presence of various activator concentrations (shown on the left).

[00140] **FIG. 16A** and **FIG. 16B** show trans cleavage activity of various Cas12 orthologs corresponding to SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence of various salt concentrations.

[00141] **FIG. 17A** and **FIG. 17B** show trans cleavage activity of various Cas12 orthologs corresponding to SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, and SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence of various salt concentrations.

[00142] **FIG. 18** shows activity of three programmable nucleases, a Cas12 variant (SEQ ID NO: 11), LbCas12a (SEQ ID NO: 1), and LbuCas13a (SEQ ID NO: 104, also referred to herein as Lbu C2C2). The results show that the functional range for the Cas12 variant (SEQ ID NO: 11) is between 25°C and 45°C, with maximal activity at 35°C.

[00143] **FIG. 19** shows the results of incubating two Cas12 proteins, SEQ ID NO: 1 and SEQ ID NO: 11, for 15 minutes at 45°C, 50°C, 55°C, 60°C, 65°C, or 70°C and then decreasing the reaction temperature to 37°C.

[00144] **FIG. 20** shows that the stability of a Cas12 variant (SEQ ID NO: 11) at elevated temperatures is dependent on the buffer composition.

[00145] **FIG. 21** shows graphs of activity of a Cas13 (SEQ ID NO: 104), as measured by fluorescence, with (left graph) and without (right graph) activator over time.

[00146] **FIG. 22** shows inhibition of Cas13a (SEQ ID NO: 104) activity by SDS and urea.

[00147] **FIG. 22A** shows the Cas13a (SEQ ID NO: 104) detection assay performed in the presence of 0-200 mM urea.

[00148] **FIG. 22B** shows complete inhibition of Cas13a (SEQ ID NO: 104) upon addition of 0.1% or greater amounts of SDS to the reaction (left graph shows with activator and right graph shows without activator).

[00149] **FIG. 23** shows the performance of Cas13a (SEQ ID NO: 104) in DETECTR reactions with varying concentrations of salt.

[00150] **FIG. 23A** shows the results of varying the concentration of NaCl in a Cas13a (SEQ ID NO: 104) DETECTR reaction.

[00151] **FIG. 23B** shows the results of varying the concentration of KCl in a Cas13a (SEQ ID NO: 104) DETECTR reaction.

[00152] **FIG. 24** shows optimization of DTT concentration in a Cas13a (SEQ ID NO: 104) DETECTR assay.

[00153] **FIG. 24A** shows activity of a Cas13a (SEQ ID NO: 104) at varying DTT concentration in NaCl.

[00154] **FIG. 24B** shows activity of a Cas13a (SEQ ID NO: 104) at varying DTT concentrations in KCl. The orange bar indicates buffer conditions with 50 mM KCl and no DTT. In addition to the indicated KCl and DTT concentration, each buffer condition also contained 20

mM HEPES pH 6.8, 5 mM $MgCl_2$, 10 μg/mL BSA, 100 μg/mL tRNA, 0.01% Igepal Ca-630 (NP-40), and 5% Glycerol).

[00155] **FIG. 25** shows the activity of Cas13a (SEQ ID NO: 104) in the DETECTR assay, as measured by fluorescence, for each of the tested reporters.

[00156] **FIG. 26** shows Cas13a (SEQ ID NO: 104) activity in the DETECTR assay, as measured by fluorescence, for each of the tested conditions.

[00157] **FIG. 27** shows Cas13a (SEQ ID NO: 104) performance in the DETECTR assay, as measured by fluorescence, for each of the five commercially available buffers and a HEPES pH 6.8 buffer ("Normal," 20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol).

[00158] **FIG. 28** shows a comparison of the a HEPES pH 6.8 buffer ("Original Buffer," 20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol) to an high performance buffer ("MBuffer1," 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 μg/μL BSA, 0.01% Igepal Ca-630, and 5% glycerol) for a Cas13a (SEQ ID NO: 104) DETECTR assay with serially diluted target RNAs and run at 37°C for 30 minutes.

[00159] **FIG. 29** shows that 5% glycerol in an high performance buffer ("MBuffer1," left graph, 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 μg/μL BSA, 0.01% Igepal Ca-630, and 5% glycerol) increases performance of a Cas13a (SEQ ID NO: 104) DETECTR assay in comparison to an identical buffer without glycerol (right graph).

[00160] **FIG. 30** shows a gradient chart of Cas13a (SEQ ID NO: 104) activity in the DETECTR assay, as measured by fluorescence, (darker squares indicate increased Cas13a activity) versus varying NP-40 concentration along the x-axis and varying BSA concentration along the y-axis. In addition to the indicated concentrations of NP-40 and BSA, each buffer contained 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, and 5% glycerol.

[00161] **FIG. 31** shows Cas13a (SEQ ID NO: 104) performance in DETECTR assays, as measured by fluorescence, versus the different additives tested.

[00162] **FIG. 32** shows the results of screening 84 different buffer and pH combinations to determine the optimal buffer for LbCas12a (SEQ ID NO: 1) activity in DETECTR assays, as measured by fluorescence.

[00163] **FIG. 33** shows LbCas12a (SEQ ID NO: 1) performance in DETECTR assays, as measured by fluorescence, in each of the tested conditions.

[00164] **FIG. 34** shows a Cas12 variant (SEQ ID NO: 11) performance in DETECTR assays, as measured by fluorescence, for each of the tested conditions (buffer type and pH).

[00165] **FIG. 35** shows a Cas12 variant (SEQ ID NO: 11) performance in DETECTR assays, as measured by fluorescence, for the various salt types and concentrations tested.

[00166] **FIG. 36** shows a Cas12 variant (SEQ ID NO: 11) performance in DETECTR assays, as measured by fluorescence (darker squares indicate greater fluorescence and more activity), versus heparin concentration on the x-axis and KOAc buffer concentration on the y-axis.

[00167] **FIG. 37** shows that specific compounds inhibited the performance of the Cas12 variant (SEQ ID NO: 11) DETECTR assay including: benzamidine hydrochloride, beryllium sulfate, manganese chloride, potassium bromide, sodium iodine, zinc chloride, di-ammonium hydrogen phosphate, tri-lithium citrate, tri-sodium citrate, cadmium chloride, copper chloride, yttrium chloride, 1-6 diaminohexane, 1-8-diaminooctane, ammonium fluoride, and ammonium sulfate.

[00168] **FIG. 38** shows the results of evaluating SNP sensitivity along target sequences for a Cas12 variant (SEQ ID NO: 11).

[00169] **FIG. 39** shows the results of evaluating SNP sensitivity along target sequences for a Cas12 variant (SEQ ID NO: 11) .

[00170] **FIG. 40** shows schemes for designing primers for loop mediated isothermal amplification (LAMP) of a target nucleic acid sequence. Regions denoted by "c" are reverse complementary to the corresponding region not denoted by "c" (e.g., region F3c is reverse complementary to region F3).

[00171] **FIG. 41A, FIG. 41B, FIG. 41C, and FIG. 41D** show schematics of exemplary configurations of various regions of a nucleic acid sequence that correspond to or anneal LAMP primers or guide RNA sequences, or that comprise protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for amplification and detection by LAMP and DETECTR.

[00172] **FIG. 41A** shows a schematic of an exemplary arrangement of the guide RNA (gRNA) with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA is reverse complementary to a sequence of the target nucleic acid, which is between an F1c region (a region reverse complementary to an F1 region) and a B1 region.

[00173] **FIG. 41B** shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA is partially reverse complementary to a sequence of the target nucleic acid, which is between an F1c region and a B1 region. For

example, the target nucleic acid comprises a sequence between an F1c region and a B1 region that is reverse complementary to at least 60% of a guide nucleic acid. In this arrangement, the guide RNA is not reverse complementary to the forward inner primer or the backward inner primer shown in **FIG. 40**.

[00174] **FIG. 41C** shows a schematic of an exemplary arrangement of the guide RNA with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA hybridizes to a sequence of the target nucleic acid, which is within the loop region between the B1 region and the B2 region. The primer sequences do not contain and are not reverse complementary to the PAM or PFS.

[00175] **FIG. 41D** shows a schematic of an exemplary arrangement of the guide RNA with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA hybridizes to a sequence of the target nucleic acid, which is within the loop region between the F2c region and F1c region. The forward inner primer, backward inner primer, forward outer primer, and backward outer primer sequences do not contain and are not reverse complementary to the PAM or PFS.

[00176] **FIG. 42A, FIG. 42B, and FIG. 42C** show schematics of exemplary configurations of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers or guide RNA sequences, or comprise protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for combined LAMP and DETECTR for amplification and detection, respectively. At the right, the schematics also show corresponding fluorescence data using the LAMP amplification and guide RNA sequences to detect the presence of a target nucleic acid sequence, where a fluorescence signal is the output of the DETECTR reaction and indicates presence of the target nucleic acid.

[00177] **FIG. 42A** shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 205, SEQ ID NO: 206, and SEQ ID NO: 249 – SEQ ID NO: 252) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 overlaps with the B2c region and is, thus, reverse complementary to the B2 region. gRNA2 overlaps with the B1 region and is, thus, reverse complementary to the B1c region. gRNA3 partially overlaps with the B3 region and partially overlaps with the B2 region and is, thus, partially reverse complementary to the B3c region and partially reverse complementary to the B2c region. The complementary regions (B1c, B2c, B3c, F1c, F2c, and F3c) are not depicted, but correspond to the regions shown in **FIG. 40**. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000

genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid.

[00178] **FIG. 42B** shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 202, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 253 – SEQ ID NO: 255) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 overlaps with the B1c region and is, thus, reverse complementary to the B1 region. gRNA2 overlaps with the LF region and is, thus, reverse complementary to the LFc region. gRNA 3 partially overlaps with the B2 region and partially overlaps with the LBc region and is, thus, partially reverse complementary to the B2c region and is partially reverse complementary to the LB region. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000 genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid.

[00179] **FIG. 42C** shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 255 – SEQ ID NO: 260) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 overlaps with the B1c region and is, thus, reverse complementary to the B1 region. gRNA2 partially overlaps with the LF region and partially overlaps with the F2c region and is, thus, partially reverse complementary to the LFc region and partially reverse complementary to the F2 region. gRNA3 overlaps with the B2 and is, thus, reverse complementary to the B2c region. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000 genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid.

[00180] **FIG. 43A** shows a detailed breakdown of the arrangement and sequences of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers or guide RNA sequences, or comprise protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for the LAMP and DETECTR assays shown in **FIG. 42A**.

[00181] **FIG. 43B** shows a detailed breakdown of the arrangement and sequences of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers or guide RNA sequences, or comprise protospacer-adjacent motif (PAM) or protospacer flanking site

(PFS), and target nucleic acid sequences for the LAMP and DETECTR assays shown in **FIG. 42B**.

[00182] **FIG. 43C** shows a detailed breakdown of the arrangement and sequences of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers or guide RNA sequences, or comprise protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for the LAMP and DETECTR assays shown in **FIG. 42C**.

[00183] **FIG. 44** shows the time to result of a reverse-transcription LAMP (RT-LAMP) reaction detected using a DNA binding dye. Amplification was performed using primer sets #1 - #10. Sequences of the primer sets are provided in **TABLE 10**.

[00184] **FIG. 45** shows fluorescence signal from a DETECTR reaction following a five-minute incubation with products from RT-LAMP reactions. Amplification was performed using primer sets #1 - #10. Sequences of the primer sets are provided in **TABLE 10**. LAMP primer sets #1-6 were designed for use with guide RNA #2 (SEQ ID NO: 240), and LAMP primer sets #7-10 were designed for use with guide RNA #1 (SEQ ID NO: 239).

[00185] **FIG. 46** shows detection of sequences from influenza A virus (IAV) using SYTO 9 (a DNA binding dye) following RT-LAMP amplification with LAMP primer sets 1, 2, 4, 5, 6, 7, 8, 9, 10, or a negative control. Sequences of the primer sets are provided in **TABLE 12**.

[00186] **FIG. 47** shows the time to amplification of an influenza B virus (IBV) target sequence following RT-LAMP amplification. Amplification was detected using SYTO 9 in the presence of increasing concentrations of target sequence (0, 100, 1000, 10,000, or 100,000 genome copies of the target sequence per reaction).

[00187] **FIG. 48** shows the time to amplification of an IAV target sequence following LAMP amplification with different primer sets.

[00188] **FIG. 49** shows detection of target nucleic acid sequences from influenza A virus (IAV) using DETECTR following RT-LAMP amplification with LAMP primer sets 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, or a negative control. Ten reactions were performed per primer set. DETECTR signal was measured as a function of an amount of target sequence present in the reaction. Sequences of the primer sets are provided in **TABLE 12**.

[00189] **FIG. 50** shows a scheme for designing primers for LAMP amplification of a target nucleic acid sequence and detection of a single nucleotide polymorphism (SNP) in the target nucleic acid sequence. In an exemplary arrangement, the SNP of the target nucleic acid is positioned between the F1c region and the B1 region.

[00190] **FIG. 51** shows schematics of exemplary arrangements of LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acids with a SNP for methods of LAMP amplification of a target nucleic acid and detection of the target nucleic acid using DETECTR.

[00191] **FIG. 51A** shows a schematic of an exemplary arrangement of the guide RNA with respect to various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between an F1c region and a B1 region. The entirety of the guide RNA sequence may be between the F1c region and the B1c region. The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00192] **FIG. 51B** shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between an F1c region and a B1 region and the target nucleic acid comprises a sequence between an F1c region and a B1 region that is reverse complementary to at least 60% of a guide nucleic acid. In this example, the guide RNA is not reverse complementary to the forward inner primer or the backward inner primer . The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00193] **FIG. 51C** shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between the F1c region and the B1 region and the entirety of the guide RNA sequence is between the F1c region and the B1 region. The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00194] **FIG. 52** shows an exemplary sequence of a nucleic acid comprising two PAM sites and a HERC2 SNP. The positions of two gRNAs targeting the HERC2 A SNP allele at either position 9 with respect to a first PAM site (SEQ ID NO: 245) or at position 14 with respect to a second PAM site (SEQ ID NO: 247) are shown. The position of a SNP is indicated with a triangle.

[00195] **FIG. 53** shows results from DETECTR reactions to detect a HERC2 SNP at position 9 with respect to a first PAM site or position 14 with respect to a second PAM site following LAMP amplification. Fluorescence signal, indicative of detection of the target sequence, was measured over time in the presence of a target sequence comprising either a G

allele or an A allele in HERC2. The target sequence was detected using a guide RNA (crRNA only) to detect either the A allele or the G allele (SEQ ID NO: 245 – SEQ ID NO: 248).

[00196] **FIG. 54** shows a heatmap of fluorescence from a DETECTR reaction following LAMP amplification of the target nucleic acid sequence. The DETECTR reaction differentiated between two HERC2 SNP alleles at position 9 with respect to the PAM, using guide RNAs (crRNA only) specific for the A allele (SEQ ID NO: 245, "R570 A SNP") or the G allele (SEQ ID NO: 246, "R571 G SNP"). Positive detection is indicated by a high fluorescence value in the DETECTR reaction.

[00197] **FIG. 55** shows combined LAMP amplification of a target nucleic acid by LAMP and detection of the target nucleic acid by DETECTR. Detection was carried out visually with DETECTR by illuminating the samples with a red LED. Each reaction contained a target nucleic acid sequence comprising a SNP allele for either a blue eye phenotype ("Blue Eye") or a brown eye phenotype ("Brown Eye"). Samples "Brown *" and "Blue *" were an A allele positive control and a G allele positive control, respectively. A position 9 guide RNA for either the brown eye phenotype (SEQ ID NO: 245, "Br") or the blue eye phenotype (SEQ ID NO: 246, "Bl") was used for each LAMP DETECTR reaction.

[00198] **FIG. 56A, FIG. 56B, FIG. 56C, FIG. 56D, FIG. 56E, FIG. 56F, FIG. 56G, and FIG. 56H** show high sensitivity and high specificity buffers for LbCas12a (SEQ ID NO: 1). In the presence of 50 μg/ml heparin and 100 mM salt, LbCas12a has improved targeting specificity and enhanced SNP discrimination capabilities. Target sequences were detected using a crRNA directed to the EGFR wild type sequence (SEQ ID NO: 448, UAAUUUCUACUAAGUGUAGAUGGCUGGCCAAACUGCUGGGU) or a crRNA directed to the EGFR mutant sequence (G SNP, SEQ ID NO: 449, UAAUUUCUACUAAGUGUAGAUGGCGGGCCAAACUGCUGGGU). In the absence of heparin and salt, Cas12a has improved sensitivity. For all SNP-related studies, high specificity buffer was used.

[00199] **FIG. 57** shows a schematic of PCR primers and guide RNA targeting sequence for EGFR T790M SNP. The forward primer represents a "PAMplification primer" (SEQ ID NO: 396), which embeds a PAM sequence ('TTTV') upstream of the targeting sequence and includes a 6nt 3' extension for priming. The PAM sequence is required for Cas12a-gRNA to recognize the matching DNA target. In this schematic, the guide RNA is designed to target the mismatch located 7 nucleotides (nt) downstream of the 5' end of the target sequence (SEQ ID NO: 400). This guide RNA/primer design is used for **FIG. 59 – FIG. 61**.

[00200] **FIG. 58A, FIG. 58B, and FIG. 58C** show the PAM forward (F) primer (also referred to as a PAMplification primer) used in amplification. PAM F primers with varying 3' extensions (4 nt, 5nt, 6 nt, SEQ ID NO: 394, SEQ ID NO: 395, and SEQ ID NO: 396, respectively) were tested with guide RNA targeting T790M with mismatch at the 7th position (SEQ ID NO: 400). The PAM F primer with 6nt extension (SEQ ID NO: 396) demonstrated optimal detection with the guide RNA. This PAM F primer was used for **FIG. 60 – FIG. 63**. The PAMplification primer produces dU-containing amplicons for detection of mutant sequences at low frequency. Cas12 guide RNAs were designed to target the T790M mutant allele (c.2369C>T, at guide mismatch position 7) in Horizon Discovery EGFR cfDNA standards at 0-5% minor allele frequencies (MAF) with 2ng input DNA. PAMplification primers include 4-6 nt extensions at the 3'end downstream of the embedded PAM. n = 3 technical replicates; bars represent mean ± SD.

[00201] **FIG. 59A – FIG. 59C** illustrate that Cas12 guide RNAs designed to target a wild type sequence ("WT" C SNP allele) and sequence comprising a T790M T SNP allele show specific Cas12-based detection in the presence of cognate single nucleotide polymorphism (SNP). Targets were detected with a crRNA directed to the wild type sequence (SEQ ID NO: 423) or a crRNA directed to the T SNP allele sequence (SEQ ID NO: 439). Time courses show activation of the WT or mutant crRNA only in the presence of the matching target (**FIG. 59A** and **FIG. 59B**). Heatmap represents time course data at t = 60 min (**FIG. 4C**) n = 3 technical replicates; synthetic oligo targets; bars represent mean ± SD.

[00202] **FIG. 60A – FIG. 60D** show Cas12a can detect down to 0.1-1% minor allele frequency (MAF) of EGFR T790M (T SNP allele) in mock cfDNA samples (Horizon Discovery), with 2 ng total DNA input and a PCR pre-amplification step. Targets were detected with a crRNA directed to the wild type sequence (SEQ ID NO: 423) or a crRNA directed to the T SNP allele sequence (SEQ ID NO: 439). Detection of WT (C SNP allele) and mutant allele at t = 90 min with low frequency EGFR standards (**FIG. 60A**). Bar graphs of mutant allele detection only (**FIG. 60B**). Heatmap representation of WT and mutant allele detection (**FIG. 60C**). The detection of low frequency SNPs using PAMplification with 6nt extension and dU-containing amplicons. Cas12a can detect down to 0.1-1% minor allele frequency (MAF) of EGFR T790M in mock cfDNA samples (Horizon Discovery), with 2 ng total DNA input. n = 3 replicates, two-tailed Student's t-test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; bars represent mean plus SD. **FIG. 60D** shows the different percentages of the WT and mutant allele in sample in a single test tube as pictorial representation of the percentage of MAF in the samples tested.

[00203] **FIG. 61** shows limit of detection studies illustrating that 2 ng total DNA is the minimum input allowed for detection of 0.1-1% minor allele frequency (MAF) of EGFR T790M (T SNP allele) in mock cfDNA samples (Horizon Discovery) with a PCR pre-amplification step. n = 3 replicates, two-tailed Student's t-test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; bars represent mean plus SD. Targets were detected using a crRNA directed to T SNP allele (SEQ ID NO: 403). Targets were amplified using primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397.

[00204] **FIG. 62** shows a table of **FIG. 61** assay parameters.

[00205] **FIG. 63A – FIG. 63D** show a blocking primer strategy.

[00206] **FIG. 63A** shows how the blocking primer blocks the forward primer from binding to the WT nucleic acid for amplification.

[00207] **FIG. 63B** shows how the mutation in SNP does not result in the binding of the blocking primer, and therefore allowing the forward primer to bind to the SNP nucleic acid for amplification.

[00208] **FIG. 63C and FIG. 63D** show the detection of the EGFR C SNP using an input of 6 ng and the detection of the EGFR T SNP using an input of 6 ng, respectively, after amplification using the blocking primer strategy of **FIG. 64A** and **FIG. 64B**. PAMplification and blocking primers are provided in **TABLE 16**.

[00209] **FIG. 64A – FIG. 64B** and **FIG. 65A – FIG. 65B** illustrate COLD-PCR strategies. **FIG. 64A** shows a full COLD-PCR strategy. **FIG. 64B** shows a fast COLD-PCR strategy.

[00210] **FIG. 65A** shows the detection of the EGFR C SNP using an input of 6 ng and a crRNA corresponding to SEQ ID NO: 423 and LbCas12a (SEQ ID NO: 1) after amplification using COLD-PCR. **FIG. 65B** shows the detection of the EGFR C SNP using an input of 6 ng the detection of the EGFR T SNP using an input of 6 ng and a crRNA corresponding to SEQ ID NO: 439 and LbCas12a (SEQ ID NO: 1) after amplification using COLD-PCR. COLD-PCR was performed using primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397.

[00211] **FIG. 66A – FIG. 66B** show experimental data illustrating that LbCas12a (SEQ ID NO: 1) can detect as low as 0.1% minor allele frequency (MAF) of EGFR L858R G SNP allele in synthetic DNA samples (Gblock), with a 1 nM total DNA input and a cold-PCR pre-amplification step. **FIG. 66A** shows detection of the mutant allele using a gRNA corresponding to SEQ ID NO: 429 and **FIG. 66B** shows detection of the WT allele using a gRNA corresponding to SEQ ID NO: 430 at t = 40 minutes. (n = 3 replicates, two-tailed Student's t-test; bars represent mean plus SD). Target sequences were amplified using primers corresponding to

SEQ ID NO: 450 (GGCAGCCAGGAACGTACTG) and SEQ ID NO: 451 (CCTTCTGCATGGTATTCTTTCTCTTCC).

[00212] **FIG. 67** shows the results of an EGFR exon 19 deletion Guide Screen using LbCas12a (SEQ ID NO: 1). Twenty-six guides corresponding to SEQ ID NO: 480 – SEQ ID NO: 506 were designed and screened on 1 nM synthetic DNA (Twist fragments). Two guides (SEQ ID NO: 493 and SEQ ID NO: 499) yielded DETECTR signals similar to wild-type. The remaining 24 guides showed activity greater than wild-type with three standing out with the highest DETECTR activity (SEQ ID NO: 485, SEQ ID NO: 488, and SEQ ID NO: 490). Targets corresponding to SEQ ID NO: 452 – SEQ ID NO: 477 and SEQ ID NO: 479 were detected. Target sequences are provided in **TABLE 21**.

[00213] **FIG. 68A – FIG. 68B** and **FIG. 69A – FIG. 69B** show the PAM forward primer (also referred to as a PAMplification primer). The single nucleotide mismatch was anchored at positions 3-8 or 5-8 nt downstream of the PAM. PAMplification primers with 2nt or 4nt extensions at the 3'end were tested for their ability to discriminate the non-cognate target containing a single nucleotide mismatch/polymorphisph (SNP). Here, a 4nt PAMplification 3'extension is better at SNP detection compared to the 2nt extension. The mismatch position is optimal around positions 6 ("6nt mm"), 7 ("7nt mm") or 8 ("8nt mm"). Primers used in this assay are provided in **TABLE 22**. Targets were detected using LbCas12a (SEQ ID NO: 1) and a gRNA corresponding to SEQ ID NO: 264 (UAAUUUCUACUAAGUGUAGAUAACUUGACAUUUAAUGCUCA).

[00214] **FIG. 70A – FIG. 70B** show that Cas12 recognizes dU-containing PAM and target sequences from 100 nM to 10 pM. **FIG. 70A**: WT SNP-targeting guide RNA; **FIG. 70B**: mutant SNP-targeting guide RNA. Left to right for both **FIG. 70A** and **FIG. 70B**: (top left) WT sequence with dT-containing target, (top middle) mutant sequence with dT-containing target, (top right) mutant sequence with dU-containing PAM and target, (bottom left) no target, (bottom right) mutant sequence with dT-containing PAM and dU-containing target. Cas12 (SEQ ID NO: 1) is capable of SNP detection with dU-containing sequences (both PAM and target) without compromising sensitivity. Primers used in this assay are provided in **TABLE 22**.

[00215] **FIG. 71A – FIG. 71B** show the detection of ALDH2 WT allele from human genomic DNA (SEQ ID NO: 417) with dU-containing amplicons with Cas12. The ALDH2 gene was amplified from human saliva containing the WT allele using Taq master mix containing dUTP in place of dTTP, such that all T nucleotides with the annotated ALDH2 target sequence shown in **FIG. 71A** have been replaced by U nucleotides. The amplicon was added directly to a Cas12 DETECTR assay. Cas12 guide RNAs targeting the ALDH2 WT allele detected only the

cognate WT sequence and not the mutant allele, demonstrating that Cas12 is capable of SNP detection with dU-containing targets. **FIG. 71B** shows a DETECTR reaction of an ALDH2 target nucleic acid sequence amplified with dUTPs using LbCas12a (SEQ ID NO: 1). Fluorescence was measured over time in the presences of the wild type nucleic acid sequence ("WT SNP"), a sequence with a point mutation ("Mutant SNP"), or a negative control without the target nucleic acid sequence.

[00216] **FIG. 72** shows detection of amplified HERC2 genomic DNA using a Cas12 variant (SEQ ID NO: 11) in the presence of increasing amounts of LAMP amplified DNA ("LAMP.Amplicon"). Each detection reaction was performed in the presence of 0 μL (negative control) of LAMP amplified DNA or from 1 μL to 14 μL LAMP amplified DNA per 20 μL reaction.

[00217] **FIG. 73** shows a schematic of addition of an artificial PAM to LAMP FIP or BIP primers. PAMs were introduced at different positions within the LAMP primer, and gRNAs were designed relative to each PAM for use in CRISPR-based detection assays of target nucleic acids.

[00218] **FIG. 74** shows LAMP amplification of a target human genomic DNA (HERC2, SEQ ID NO: 416) with an FIP primer having PAM sequences at varying positions to introduce an artificial PAM in the HERC2 target nucleic acid. Amplification was monitored using a SYTO9 DNA binding dye. The target was amplified using primers corresponding to SEQ ID NO: 233 – SEQ ID NO: 234 and SEQ ID NO: 236 – SEQ ID NO: 238 with a variable FIP depending on the position of the artificially introduced PAM. FIPs corresponding to SEQ ID NO: 265 – SEQ ID NO: 281 were used to insert artificial PAMs at position 1 – position 17, respectively. The FIP corresponding to SEQ ID NO: 235 was used to amplify the target without introducing a PAM.

[00219] **FIG. 75** shows detection of a target nucleic acid with an artificially introduced PAM using a Cas12 variant (SEQ ID NO: 11). gRNAs corresponding to SEQ ID NO: 283 – SEQ ID NO: 299 were used to detect target nucleic acids with artificially introduced PAMs at position 1 – position 17, respectively.

[00220] **FIG. 76** shows detection of single point mutations at different positions along a nucleic acid sequence using a SEQ ID NO: 11 programmable nuclease. Point mutations corresponding to all possible nucleic acids were inserted at different positions within either a HERC2 target sequence (top, wild type sequence corresponding to SEQ ID NO: 416) or an ALDH2 target sequence (bottom, wild type sequence corresponding to SEQ ID NO: 417). The HERC2 sequence was detected using a gRNA corresponding to SEQ ID NO: 246 (top plot) and

the ALDH sequence was detected using a gRNA corresponding to SEQ ID NO: 425 (bottom plot).

[00221] **FIG. 77** shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native PAM using a SEQ ID NO: 11 programmable nuclease. Guide RNAs corresponding to SEQ ID NO: 300 – SEQ ID NO: 319 were directed to the wild type (SEQ ID NO: 415, "WT") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 320 – SEQ ID NO: 339 were directed to the wild type ("WT") sequence on the reverse strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 340 – SEQ ID NO: 359 were directed to the mutant (SEQ ID NO: 414, "rs738409") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 360 – SEQ ID NO: 379 were directed to the mutant ("rs738409") sequence on the reverse strand at position 1 – position 20, respectively. Each gRNA was used to detect four different target sequences corresponding to the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a point mutation at a first site (SEQ ID NO: 413, "rs738408"), a sequence with a point mutation at a second site (SEQ ID NO: 414, "rs738409"), or a sequence with point mutations at both the first site and the second site (SEQ ID NO: 412, "rs738409+rs738408").

[00222] **FIG. 78** shows detection of single and double mutations in a target nucleic acid sequence using a SEQ ID NO: 11 programmable nuclease. Target sequences corresponding to SEQ ID NO: 412 – SEQ ID NO: 415 were detected.

[00223] **FIG. 79** shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native PAM using a SEQ ID NO: 11 programmable nuclease. Target sequences corresponding to SEQ ID NO: 412 – SEQ ID NO: 415 were detected. A sample without a target sequence (non-target control, "NTC") was used as a negative control. Sequences were detected using pooled gRNAs directed to either the wild type sequence (SEQ ID NO: 301 and SEQ ID NO: 421, "WT DETECTR") or the sequence containing a mutation at the second position (SEQ ID NO: 341 and SEQ ID NO: 422, "rs738409 DETECTR").

[00224] **FIG. 80** shows detection of single point mutations at different positions along a nucleic acid sequence using LbCas12a (SEQ ID NO: 1). Point mutations corresponding to all possible nucleic acids were inserted at different positions within either a HERC2 target sequence (top, wild type sequence corresponding to SEQ ID NO: 416) or an ALDH2 target sequence (bottom, wild type sequence corresponding to SEQ ID NO: 417). The HERC2 sequence was detected using a gRNA corresponding to SEQ ID NO: 246 (top plot) and the ALDH sequence was detected using a gRNA corresponding to SEQ ID NO: 425 (bottom plot).

[00225] **FIG. 81** shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native PAM using LbCas12a (SEQ ID NO: 1). Guide RNAs corresponding to SEQ ID NO: 300 – SEQ ID NO: 319 were directed to the wild type (SEQ ID NO: 415, "WT") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 320 – SEQ ID NO: 339 were directed to the wild type ("WT") sequence on the reverse strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 340 – SEQ ID NO: 359 were directed to the mutant (SEQ ID NO: 414, "rs738409") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 360 – SEQ ID NO: 379 were directed to the mutant ("rs738409") sequence on the reverse strand at position 1 – position 20, respectively. Each gRNA was used to detect four different target sequences corresponding to the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a point mutation at a first site (SEQ ID NO: 413, "rs738408"), a sequence with a point mutation at a second site (SEQ ID NO: 414, "rs738409"), or a sequence with point mutations at both the first site and the second site (SEQ ID NO: 412, "rs738409+rs738408").

[00226] **FIG. 82** shows detection of single point mutations at different positions along target RNA sequence (SEQ ID NO: 748, UGGACAAAGCGUCUACGCUGCAGUCCUCGCUCACUGGGCA) using LbuCas13a (SEQ ID NO: 104). Data is not shown for wild type positions (black circles labeled with "WT"). Detection of the wild type sequence is shown in the square marked "WT" at SNP position 1. Detection of a negative control (water) is shown in the sqare marked "None" at position "None." The targets were detected using a gRNA corresponding to SEQ ID NO: 507 (GGCCACCCCAAAAAUGAAGGGGACUAAAACAAGCGAGGACUGCAGCGUAGA).

[00227] **FIG. 83** shows detection of single point mutations at different positions along target ssDNA (SEQ ID NO: 749, TTTTGGACAAAGCGTCTACGCTGCAGTCCTCGCTCACTGGGCACGGTG) sequence using LbuCas13a (SEQ ID NO: 104). Data is not shown for wild type positions (black circles labeled with "WT"). Detection of the wild type sequence is shown in the square marked "WT" at SNP position 1. Detection of a negative control (water) is shown in the sqare marked "None" at position "None." The targets were detected using a gRNA corresponding to SEQ ID NO: 507.

[00228] **FIG. 84** shows detection of a *Chlamydia trachomatis* target nucleic acid sequence with LbuCas13a (SEQ ID NO: 104) following polymerase chain reaction (PCR) amplification and *in vitro* transcription (IVT) of samples that were either positive or negative for Chlamydia. Targets were detected with either a gRNA targeted to Chlamydia 5S rRNA (SEQ ID NO: 418), a

gRNA targeted to Chlamydia 16S rRNA (SEQ ID NO: 419), or an off-target gRNA (SEQ ID NO: 420).

[00229] **FIG. 85** shows heatmaps of the fluorescence detected in **FIG. 84** (right). Panels on the right indicate the maximum fluorescent rate detected with either a gRNA targeting a Chlamydia 16S RNA sequence (SEQ ID NO: 419, "16S gRNA"), a gRNA targeting a Chlamydia 5S RNA sequence (SEQ ID NO: 418, "5S gRNA"), or a gRNA not directed to a Chlamydia target sequence (SEQ ID NO: 420, "off-target gRNA"). Shaded boxes in the left column ("Ct") indicate that the sample was positive for Chlamydia.

[00230] **FIG. 86** shows trans cleavage rates of different Cas12 variants upon complex formation with a gRNA and a target sequence comprising different PAM sequences. Individual plots show trans cleavage rates for each Cas12 variant, and each plot illustrates the cleavage rate for target sequences comprising different PAM sequences. PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

[00231] **FIG. 87A** shows a schematic of a Cas protein, gRNA, and target sequence complex comprising either a single base pair mismatch (top) or a double base pair mismatch (bottom) between the gRNA and the target sequence.

[00232] **FIG. 87B** shows trans cleavage activity of different Cas12 programmable nuclease variants of SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, and SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 upon complex formation with a gRNA and a target sequence having either a single base pair mismatch (top) or a double base pair mismatch (bottom). Trans cleavage activity was tested for mismatches at different positions relative to the PAM sequence. Single or double mismatches were introduced in the first ("1MM"), fifth ("5MM"), tenth ("10MM"), fifteenth ("15MM"), and twentieth ("20MM") nucleotide position after the PAM (TTTA). PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

[00233] **FIG. 88** shows trans cleavage activity of different Cas12 variants of SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, and SEQ ID NO: 3 at different concentrations of NaCl.

[00234] **FIG. 89** shows urea PAGE gels of pre-crRNA processing activity of different Cas12 variants of SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence ("+") or absence ("-") of a Cas protein. Bands shown are RNA bands.

[00235] **FIG. 90** shows trans cleavage activity of different Cas12 variants of SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 649 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence of different crRNAs based on the native crRNAs found in the CRISPR locus for native Cas12 proteins. Pre-crRNA sequences are provided in **TABLE 30**. The target sequences is set forth in SEQ ID NO: 670.

[00236] **FIG. 91** shows cis cleavage activity of different Cas12 variants of SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 after incubation with a target nucleic acid sequence for 10 minutes. Cleavage with BamHI is shown as a cleavage positive control.

[00237] **FIG. 92** shows sequence alignments of the repeat region of different Cas12 variants aligned to the repeat sequence of LbCas12a (SEQ ID NO: 1). Repeat sequences of the Cas12 variants correspond to SEQ ID NO: 508 – SEQ ID NO: 520 and SEQ ID NO: 522 – SEQ ID NO: 536. The repeat sequence of LbCas12a corresponds to SEQ ID NO: 521. Repeat sequences are provided in **TABLE 30**.

[00238] **FIG. 93** shows the results of an assay comparing DETECTR assay efficiency for a Cas12 variant of SEQ ID NO: 11 with two different gRNAs. The gRNA contains either the LbCas12a repeat sequence ("gRNA #1," SEQ ID NO: 423, UAAUUUCUACUAAGUGUAGAUUCAUCACGCAGCUCAUGCCC) or the Cas12 variant repeat sequence ("gRNA #2," SEQ ID NO: 424, GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUUCAUCACGCAGCUCAUGCCC).

DETAILED DESCRIPTION

[00239] Disclosed herein are compositions, kits and methods related to improved Cas12 and other Cas protein activity. Through compositions and kits disclosed herein and practice of methods disclosed herein, one attains improved Cas activity such as Cas12 activity relative to Cas proteins in the art such as LbCas12a. Improved and in some cases high performance Cas12 proteins and conditions are disclosed herein.

[00240] The capability to quickly and accurately detect the presence of a target nucleic acid can provide valuable information associated with the presence of the target nucleic acid. For example, the capability to quickly and accurately detect the presence of an ailment provides valuable information and leads to actions to reduce the progression or transmission of the ailment. Detection of a target nucleic acid molecule encoding a specific sequence using a

programmable nuclease provides a method for efficiently and accurately detecting the presence of the nucleic acid molecule of interest. There exists a need for highly efficient, rapid, and accurate reactions for detecting whether a target nucleic acid is present in a sample. The present disclosure provides compositions and methods for detecting a target nucleic acid in a sample using a programmable nuclease in a reaction. The reaction is sometimes referred to as a DETECTR reaction. The present disclosure provides various methods, reagents, enzymes, and kits for rapid tests, which may quickly assess whether a target nucleic acid is present in a sample by using a programmable nuclease that can detect the presence of a nucleic acid of interest (e.g., a deoxyribonucleic acid or a deoxyribonucleic acid amplicon of the nucleic acid of interest, which can be the target deoxyribonucleic acid) and generating a detectable signal indicating the presence of said nucleic acid of interest. The methods or reagents may be used as a point of care diagnostic or as a lab test for detection of a target nucleic acid and, thereby, detection of a condition in a subject from which the sample was taken. The methods or reagents may be used in various sites or locations, such as in laboratories, in hospitals, in physician offices/laboratories (POLs), in clinics, at remotes sites, or at home. Sometimes, the present disclosure provides various devices, systems, fluidic devices, and kits for consumer genetic use or for over the counter use.

[00241] The methods of the present disclosure include providing a programmable nuclease and a guide nucleic acid, wherein the guide nucleic acid is reverse complementary to a target nucleic acid of interest. The target nucleic acid may be a segment of a nucleic acid sequence of interest. The target nucleic acid may be a gene or a segment of a gene. When the guide nucleic acid hybridizes to the target nucleic acid of interest, the programmable nuclease is activated and exhibits sequence-independent cleavage of a nucleic acid of a reporter. The reporter further comprises a detection moiety, which is released upon sequence-independent cleavage of the nucleic acid of the reporter, and produces a detectable signal. The detectable signal can be measured and quantified to determine the presence or absence of the target nucleic acid in the sample and further quantify the target nucleic acid when present.

[00242] Detecting target nucleic acids in a sample using these methods is highly unpredictable, as the reaction itself can comprise reagents that inhibit sequence-independent cleavage by an activated programmable nuclease. For example, a sample comprising the target nucleic acid may first need to be lysed. The sample can be further subject to various sample prep steps including filtration, amplification, reverse transcription, and in vitro transcription. Each of these steps can allow for reagents that may inhibit an activated programmable nuclease from sequence independent cleavage of the nucleic acid of a reporter, thereby dampening the

detectable signal. As one example, enzymes and/or salts in the buffers for lysing a sample may inhibit an activated programmable nuclease from sequence independent cleavage of the nucleic acid of a reporter. As another example, salts in the buffer for amplification, reverse transcription, and/or transcription of a target nucleic acid may inhibit an activated programmable nuclease from sequence independent cleavage of the nucleic acid of a reporter. As another example, the pH in the buffer of the unlysed sample, the lysis buffer, or the buffer for amplification, reverse transcription, and/or transcription of a target nucleic acid may inhibit an activated programmable nuclease from sequence independent cleavage of the nucleic acid of a reporter. In yet another example, amplification of a target nucleic acid comprises excess primer and may generate ssDNA that outcompete the nucleic acid of a reporter for cleavage by the activated programmable nuclease, thereby dampening the detectable signal. The compositions and methods disclosed herein identify volumes of the detection reaction to volumes of the sample, which provide for a strong detectable signal (in the presence of the target nucleic acid), thereby alleviating dampened detectable signals. The compositions and methods disclosed herein also identify ratios of the nucleic acid of the reporter to target and non-target nucleic acids, which provide for a strong detectable signal (in the presence of the target nucleic acid), thereby alleviating dampened detectable signals.

[00243] Also disclosed herein are methods of assaying for a target nucleic acid. The compositions, kits and methods related to improved Cas12 and other Cas protein activity 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 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 detector nucleic acids (also referred to herein as "nucleic acid of the reporter") of a population of detector 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. 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.

[00244] The segment of the target nucleic acid often comprises a single nucleotide mutation wherein the single nucleotide mutation comprises 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 segment of 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 single nucleotide mutation or SNP is associated with a disease such as cancer or a genetic disorder. The single nucleotide mutation or SNP 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. 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 (e.g., a substitution which does not change the amino acid sequence of an encoded protein). The segment of the target nucleic acid often comprises a deletion, for example a deletion of one or more base pairs from an exon sequence. The deletion can be associated with a phenotype of the sample or a phenotype of the organism from which the sample was taken. The deletion, in some cases, is associated with altered phenotype from wild type phenotype. Often, the deletion is associated with a disease such as cancer or a genetic disorder. The 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. The target nucleic acid can be DNA or RNA. Assaying of a target nucleic acid can be used to diagnose or identify diseases associated with target nucleic acid. The methods described herein use a programmable nuclease, such as the CRISPR/Cas system, to detect a target nucleic acid.

[00245] Often, a method disclosed herein comprises: contacting a programmable nuclease comprising a polypeptide having endonuclease activity and a guide nucleic acid to a target nucleic acid in a buffer comprising heparin. The heparin is present, for example, at a concentration of from 1 to 100 μg/ml heparin. Often, the heparin is present at a concentration of from 40 to 60 μg/ml heparin. Sometimes, the heparin is present at a concentration 50 μg/ml heparin. Often, the buffer comprises NaCl. The NaCl is present, for example, at a concentration of from 1 to 200 mM NaCl. Sometimes, the NaCl is present at a concentration of from 80 to 120 mM NaCl. Often, the NaCl is present at a concentration of 100 mM NaCl. The target nucleic acid can be a substrate target nucleic acid. Sometimes, the substrate nucleic acid comprises a cancer allele. Often, the cancer allele is present at a low concentration relative to a wild type allele. Sometimes, the substrate target nucleic acid comprises a splice variant. The substrate target nucleic acid often comprises an edited base. The substrate target nucleic acid sometimes comprises a bisulfite-treated base. Often, the substrate target nucleic acid comprises a segment that is reverse complementary to a segment of the guide nucleic acid.

[00246] Assaying of a target nucleic acid comprising a single nucleotide mutation can be difficult, especially in the presence of a nucleic acid comprising a variant of the single nucleotide mutation because there is only one nucleotide difference between the sequences of these nucleic acids. Additionally, it is often difficult to assay for the target nucleic acid comprising the single nucleotide mutation when the sample comprising the target nucleic acid also comprises more of the nucleic acid comprising the variant of the single nucleotide mutation than the target nucleic acid comprising the single nucleotide mutation. Often, the variant is the wild type variant of the single nucleotide mutation. Sometimes, the single nucleotide mutation is the wild variant of a SNP.

[00247] The methods described herein can enhance the assay detection a target nucleic acid. For example, a buffer comprising heparin and NaCl increases the discrimination of a programmable nuclease between the target nucleic acid comprising a single nucleotide mutation and other nucleic acids comprising a variant of the single nucleotide mutation.

[00248] Amplification methods can also enhance the assay detection of the target nucleic acid. For example, a PAM target nucleic acid comprising a sequence encoding a PAM sequence (e.g., TTTN or dUdUdUN) is produced by amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product. Often, the primer is the forward primer comprises the sequence encoding the PAM and has 1-8 nucleotides

from the 3'end of the sequence encoding the PAM. Often, the single nucleotide mutation in the target nucleic acid sequence is 5-9 nucleotides downstream of the 5' end of the target nucleic acid segment wherein the target nucleic acid segment is a segment that binds to a segment of the guide nucleic acid that is reverse complementary to it and comprises the sequence encoding the PAM. Additional amplification strategies for enhancing the assay detection of the target nucleic acid include, but are not limited to, amplification with a blocking primer, wherein the blocking primer binds to variant of the single nucleotide mutation of the target nucleic acid, co-amplification at lower denaturation temperature-PCR (COLD-PCR), such as full COLD-PCR and fast COLD-PCR, allele-specific PCR, targeting the nucleic acids comprising a variant of the single nucleotide mutation with a protein allowing for their removal, or targeting the target nucleic acids with a protein allowing for the removal of the other nucleic acids, or any combination thereof.

[00249] Further disclosed herein are methods of assaying for a target nucleic acid, wherein the target nucleic acid segment lacks a PAM sequence. For example, a method of assaying for a target nucleic acid in a sample comprising: producing a PAM target nucleic acid comprising a sequence encoding a PAM by amplifying the target nucleic acid of the sample using primers comprising the encoding the PAM; contacting the PAM target nucleic acid to a complex comprising a guide nucleic acid comprising a segment that is reverse complementary to a segment of the PAM 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 PAM target nucleic acid; and assaying for a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids, wherein the signal indicates a presence of the target nucleic acid in the sample and wherein the absence of the signal indicates an absence of the target nucleic acid in the sample. Sometimes, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence

of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. The sequence encoding the PAM can comprise TTTN. Sometimes, the sequence encoding the PAM comprises dUdUdUN. Often, a forward primer of the primers comprises the sequence encoding the PAM and has one to ten nucleotides from the 3' end of the sequence encoding the PAM. These nucleotides can be referred to as extension nucleotides. In some embodiments, extensions with 10 nucleotides or fewer may produce specific detection with the guide RNA. In some embodiments, extensions with greater than 12 nucleotides may self-activate, resulting in reduced detection specificity for the target sequence. Extensions between 5 nucleotides and 10 nucleotides may provide sufficient overlap with the target sequence to anneal to the target sequence with an annealing temperature amenable to detection. In some embodiments, an extension may comprise 4, 5, 6, 7, 8, 9, or 10 nucleotides from the 3' end of the sequence encoding the PAM. Sometimes, a mismatch for single nucleotide polymorphism (SNP) detection is 3-10 nucleotides downstream of the PAM in PAM target nucleic acid. This allows for detection of any target nucleic acid by a programmable nuclease. The methods described herein use a programmable nuclease, such as the CRISPR/Cas system, to detect a target nucleic acid. Often, the programmable nuclease is Cas12.

[00250] A target nucleic acid is required to have a PAM sequence for binding and trans cleavage activation of some programmable nucleases complexed with a guide nucleic acid. However, there are many target nucleic acids of interest that do not encode for the PAM sequence. Therefore, there is a need for strategies to allow for binding and trans cleavage activation of the programmable nucleases complexed with a guide nucleic acid using any target nucleic sequence of interest.

[00251] The methods describe herein use amplification techniques to insert a PAM sequence into the target nucleic acid for recognition by the programmable nuclease complexed with the guide nucleic acid.

Sample

[00252] A number of samples are consistent with the compositions and methods disclosed herein. The samples, as described herein, are compatible with the DETECTR assay methods disclosed herein. The samples, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) 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, which has been developed to improve the function of the programmable nuclease (e.g., a programmable nuclease and a buffer with low salt

(about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The samples, 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. Described herein are sample that contain deoxyribonucleic acid (DNA), ribonucleic acid (RNA), or both, which can be detected using a programmable nuclease, such as a Type V CRISPR/Cas enzyme (e.g., a Cas12 such as Cas12 is a Cas12a, Cas12b, Cas12c, Cas12d (also referred to as CasY), or Cas12e or a Cas14 such as Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h) or a Type VI CRISPR enzyme (e.g., a Cas13 such as Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e). 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).

[00253] 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.

[00254] 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.

[00255] 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.

[00256] 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.

[00257] 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.

[00258] 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.

[00259] 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 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.

[00260] 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.

[00261] 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.

[00262] 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.

[00263] 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.

[00264] 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.

[00265] 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.

[00266] 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.

[00267] 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.

[00268] 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 comprisis 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.

[00269] 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.

[00270] 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.

[00271] Additionally, target nucleic acid can be an amplified nucleic acid of interest, which can bind to the guide nucleic acid of a programmable nuclease, such as a DNA-activated programmable RNA nuclease. 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, a 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.

[00272] 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.

[00273] 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.

[00274] 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.

Reagents for Detection of Target Nucleic Acids

[00275] Disclosed herein are methods of assaying for a target nucleic acid as described herein. The reagents for detection of target nucleic acids, as described herein, are compatible with the DETECTR assay methods disclosed herein. The reagents for detection of target nucleic acids, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The reagents for detection of target nucleic acids, 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The reagents for detection of target nucleic acids, 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. 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, 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 detector nucleic acids of a population of detector 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.

[00276] The methods of assaying for a target nucleic acid described herein may further comprise introducing a PAM sequence into a target nucleic acid segment that lacks a PAM sequence. For example, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. A PAM-dependent sequence specific nuclease, often, is a programmable nuclease. Sometimes, a PAM-dependent sequence specific nuclease is a PAM-dependent sequence specific endonuclease.

[00277] A number of reagents are consistent with the compositions and methods disclosed herein. The reagents described herein for detecting a disease, cancer, or genetic disorder comprise a guide nucleic acid targeting the target nucleic acid segment indicative of a disease, cancer, or genetic disorder. 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 detected (e.g., the target DNA hybridizes to the guide nucleic) using a programmable nuclease 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 (e.g., the target DNA hybridizes to the guide nucleic) using a programmable nuclease and other reagents disclosed herein. Additionally, detection of multiple target nucleic acids is possible using two or more programmable nucleases 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).

Programmable Nucleases

[00278] The programmable nucleases disclosed herein (*e.g.*, a type V or VI CRISPR enzyme) enable the detection of target nucleic acids (*e.g.*, DNA or RNA). Additionally, detection by a first programmable nuclease, which can cleave RNA reporters, allows for multiplexing with programmable nucleases, which cleave DNA reporters.

[00279] The detection of the target nucleic acid is facilitated by a programmable nuclease. A programmable nuclease can comprise a programmable nuclease capable of being activated when complexed with a guide nucleic acid and target nucleic acid. The programmable nuclease can become activated after binding of a guide nucleic acid to a target nucleic, in which the activated programmable nuclease can cleave the target nucleic acid and exhibits sequence-independent cleavage activity. Sequence-independent cleavage activity, also referred to herein as "trans cleavage activity" or "collateral cleavage activity", can be non-specific cleavage of nearby single-stranded nucleic acids by the activated programmable nuclease, such as trans cleavage of nucleic acids in a reporter, where the reporter also comprises a detection moiety. The reporter may comprise a detector nucleic acid. Once the nucleic acid of the reporter is cleaved by the activated programmable nuclease, the detection moiety is released from the nucleic acid of the reporter, and generates a detectable signal. Often the detection moiety is at least one of a fluorophore, a dye, a polypeptide, or a nucleic acid. Sometimes the detection moiety binds to a capture molecule immobilized on a solid surface. The detectable signal can be visualized on the solid surface to assess the presence, the absence, or level of presence of the target nucleic acid. A detectable signal can be a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorometric, etc.), or piezo-electric signal. Often, the detectable signal is present prior to cleavage of the nucleic acid of the reporter and changes upon cleavage of the nucleic acid of the reporter. Sometimes, the signal is absent prior to cleavage of the nucleic acid of the reporter and is present upon cleavage of the nucleic acid of the reporter. The detectable signal can be immobilized on a solid surface for detection. The programmable nuclease can be a DNA-activated programmable RNA nuclease, a DNA-activated programmable DNA nuclease, or an RNA-activated programmable RNA nuclease. A DNA-activated programmable RNA nuclease is a programmable nuclease, which upon hybridization of its guide nucleic acid to a target DNA, exhibits sequence-independent cleavage of a reporter having a RNA (an RNA reporter). A DNA-activated programmable DNA nuclease is a programmable nuclease, which upon hybridization of its guide nucleic acid to a target DNA, exhibits sequence-independent cleavage of a reporter

having a DNA (a DNA reporter). A RNA-activated programmable RNA nuclease is a programmable nuclease, which upon hybridization of its guide nucleic acid to a target RNA, exhibits sequence-independent cleavage of a reporter having a RNA (a RNA reporter). The DNA-activated programmable DNA nuclease can be a Type V CRISPR/Cas enzyme (e.g., Cas12). The DNA-activated programmable RNA nuclease can be a Type VI CRISPR/Cas enzyme (e.g., Cas13). The RNA-activated programmable RNA nuclease can be a Type VI CRISPR/Cas enzyme (e.g., Cas13).

[00280] The programmable nucleases disclosed herein may elicit reporter activity upon cleavage of the nucleic acid of the reporter. Reporter activity refers to transcollatoral cleavage activity of a detector nucleic acid. A reporter activity may be a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorometric, etc.), or piezo-electric signal. For example, cleavage of the nucleic acid of the reporter by the programmable nuclease may elicity a fluorescent signal. A reporter activity may increase or decrease over time in response to a programmable nuclease trans cleavage activity. A reporter activity may accumulate over time in response to a programmable nuclease trans cleavage activity. A maximal reporter activity may occur when a reporter signal (e.g., a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorometric, etc.), or piezo-electric signal) is highest within a designated assay. In some embodiments, a maximal reporter signal may occur when a reporter signal reaches a maximum signal, after which the reporter signal decreases. In some embodiments, a maximal reporter signal may occur when a reporter signal increases to saturation after which the signal is no longer increasing.

[00281] 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.

[00282] In some embodiments, the Type V CRISPR/Cas enzyme is a programmable Cas12 nuclease. Type V CRISPR/Cas enzymes (*e.g.*, Cas12 or Cas14) lack an HNH domain. A Cas12 nuclease of the present disclosure cleaves a nucleic acid via a single catalytic RuvC domain. This single catalytic RuvC domain includes 3 partial RuvC domains (RuvC-I, RuvC-II, and RuvC-III, also referred to herein as subdomains) that are not contiguous with respect to the primary amino acid sequence of the Cas12 protein, but form an RuvC domain once the protein is produced and folds. In some embodiments, a programmable nuclease comprises three partial RuvC domains. In some embodiments, a programmable nuclease comprises an RuvC-I subdomain, an RuvC-II subdomain, and an RuvC-III subdomain. The RuvC domain is within a nuclease, or "NUC" lobe of the protein, and the Cas12 nucleases further comprise a recognition,

or "REC" lobe. The REC and NUC lobes are connected by a bridge helix and the Cas12 proteins additionally include two domains for PAM recognition termed the PAM interacting (PI) domain and the wedge (WED) domain. (Murugan et al., Mol Cell. 2017 Oct 5; 68(1): 15–25). In some embodiments, a Cas12 protein (e.g., a programmable nuclease having a sequence with at least 60% sequence identity to SEQ ID NO: 11) may recognize a PAM having a sequence of YYN, where N represents any nucleic acid and (e.g., A, T, C, G, or U) Y represents any pyrimidine (e.g., C or T). In some embodiments, a Cas12 protein may recongnize a PAM having a sequence of YR, where Y represents any pyrimidine (e.g., C or T) and R represents any purine (e.g., A or G). A programmable Cas12 nuclease can be a Cas12a (also referred to as Cpf1) protein, a Cas12b protein, Cas12c protein, Cas12d protein (also referred to as a CasY protein), or a Cas12e protein. For example, the programmable Cas12 nuclease may be a Cas12a. A programmable Cas12 nuclease can be a Cas12 variant. In some cases, a suitable Cas12 protein comprises an amino acid sequence having 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%, at least 92%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to any one of the Cas12 proteins or Cas12 variants provided in **TABLE 1** (e.g., any one of SEQ ID NO: 1 – SEQ ID NO: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602). For example, a suitable Cas12 protein comprises a sequence with at least 60% sequence identity to SEQ ID NO: 11. In some embodiments, a suitable Cas12 protein comprises a sequence with 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%, amino acid sequence identity to SEQ ID NO: 11. In some embodiments, a suitable Cas12 protein comprises a sequence with 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%, amino acid sequence identity to SEQ ID NO: 1. For example, a suitable Cas12 protein may comprise an amino acid sequence having 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%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%, amino acid sequence identity to SEQ ID NO: 11. A Cas12 protein can have a sequence as set forth in SEQ ID NO: 11. In some embodiments, a Cas12 nuclease may have at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602.

TABLE 1 – Cas12 Sequences

SEQ ID NO	Description	Sequence
SEQ ID NO: 1	Lachnospiraceae bacterium ND2006 (LbCas12a)	MSKLEKFTNCYSLSKTLRFKAIPVGKTQENIDNKRLLVEDEKRAEDYKGV KKLLDRYYLSFINDVLHSIKLKNLNNYISLFRKKTRTEKENKELENLEINLR KEIAKAFKGNEGYKSLFKKDIIETILPEFLDDKDEIALVNSFNGFTTAFTGF FDNRENMFSEEAKSTSIAFRCINENLTRYISNMDIFEKVDAIFDKHEVQEIK EKILNSDYDVEDFFEGEFFNFVLTQEGIDVYNAIIGGFVTESGEKIKGLNEY INLYNQKTKQKLPKFKPLYKQVLSDRESLSFYGEGYTSDEEVLEVFRNTL NKNSEIFSSIKKLEKLFKNFDEYSSAGIFVKNGPAISTISKDIFGEWNVIRDK WNAEYDDIHLKKKAVVTEKYEDDRRKSFKKIGSFSLEQLQEYADADLSV VEKLKEIIIQKVDEIYKVYGSSEKLFDADFVLEKSLKKNDAVVAIMKDLLD SVKSFENYIKAFFGEGKETNRDESFYGDFVLAYDILLKVDHIYDAIRNYVT QKPYSKDKFKLYFQNPQFMGGWDKDKETDYRATILRYGSKYYLAIMDK KYAKCLQKIDKDDVNGNYEKINYKLLPGPNKMLPKVFFSKKWMAYYNP SEDIQKIYKNGTFKKGDMFNLNDCHKLIDFFKDSISRYPKWSNAYDFNFSE TEKYKDIAGFYREVEEQGYKVSFESASKKEVDKLVEEGKLYMFQIYNKD FSDKSHGTPNLHTMYFKLLFDENNHGQIRLSGGAELFMRRASLKKEELVV HPANSPIANKNPDNPKKTTTLSYDVYKDKRFSEDQYELHIPIAINKCPKNIF KINTEVRVLLKHDDNPYVIGIDRGERNLLYIVVVDGKGNIVEQYSLNEIIN NFNGIRIKTDYHSLLDKKEKERFEARQNWTSIENIKELKAGYISQVVHKIC ELVEKYDAVIALEDLNSGFKNSRVKVEKQVYQKFEKMLIDKLNYMVDK KSNPCATGGALKGYQITNKFESFKSMSTQNGFIFYIPAWLTSKIDPSTGFV NLLKTKYTSIADSKKFISSFDRIMYVPEEDLFEFALDYKNFSRTDADYIKK WKLYSYGNRIRIFRNPKKNNVFDWEEVCLTSAYKELFNKYGINYQQGDIR ALLCEQSDKAFYSSFMALMSLMLQMRNSITGRTDVDFLISPVKNSDGIFY DSRNYEAQENAILPKNADANGAYNIARKVLWAIGQFKKAEDEKLDKVKI AISNKEWLEYAQTSVKH
SEQ ID NO: 2	Acidaminococcus sp. BV316 (AsCas12a)	MTQFEGFTNLYQVSKTLRFELIPQGKTLKHIQEQGFIEEDKARNDHYKELK PIIDRIYKTYADQCLQLVQLDWENLSAAIDSYRKEKTEETRNALIEEQATY RNAIHDYFIGRTDNLTDAINKRHAEIYKGLFKAELFNGKVLKQLGTVTTTE HENALLRSFDKFTTYFSGFYENRKNVFSAEDISTAIPHRIVQDNFPKFKENC HIFTRLITAVPSLREHFENVKKAIGIFVSTSIEEVFSFPFYNQLLTQTQIDLYN QLLGGISREAGTEKIKGLNEVLNLAIQKNDETAHIIASLPHRFIPLFKQILSD RNTLSFILEEFKSDEEVIQSFCKYKTLLRNENVLETAEALFNELNSIDLTHIF ISHKKLETISSALCDHWDTLRNALYERRISELTGKITKSAKEKVQRSLKHE DINLQEIISAAGKELSEAFKQKTSEILSHAHAALDQPLPTTLKKQEEKEILK SQLDSLLGLYHLLDWFAVDESNEVDPEFSARLTGIKLEMEPSLSFYNKAR NYATKKPYSVEKFKLNFQMPTLASGWDVNKEKNNGAILFVKNGLYYLGI MPKQKGRYKALSFEPTEKTSEGFDKMYYDYFPDAAKMIPKCSTQLKAVT AHFQTHTTPILLSNNFIEPLEITKEIYDLNNPEKEPKKFQTAYAKKTGDQKG YREALCKWIDFTRDFLSKYTKTTSIDLSSLRPSSQYKDLGEYYAELNPLLY HISFQRIAEKEIMDAVETGKLYLFQIYNKDFAKGHHGKPNLHTLYWTGLF SPENLAKTSIKLNGQAELFYRPKSRMKRMAHRLGEKMLNKKLKDQKTPI PDTLYQELYDYVNHRLSHDLSDEARALLPNVITKEVSHEIIKDRRFTSDKF FFHVPITLNYQAANSPSKFNQRVNAYLKEHPETPIIGIDRGERNLIYITVIDS TGKILEQRSLNTIQQFDYQKKLDNREKERVAARQAWSVVGTIKDLKQGY LSQVIHEIVDLMIHYQAVVVLENLNFGFKSKRTGIAEKAVYQQFEKMLID KLNCLVLKDYPAEKVGGVLNPYQLTDQFTSFAKMGTQSGFLFYVPAPYT SKIDPLTGFVDPFVWKTIKNHESRKHFLEGFDFLHYDVKTGDFILHFKMN RNLSFQRGLPGFMPAWDIVFEKNETQFDAKGTPFIAGKRIVPVIENHRFTG RYRDLYPANELIALLEEKGIVFRDGSNILPKLLENDDSHAIDTMVALIRSVL QMRNSNAATGEDYINSPVRDLNGVCFDSRFQNPEWPMDADANGAYHIA LKGQLLLNHLKESKDLKLQNGISNQDWLAYIQELRN
SEQ ID	Francisella novicida	MSIYQEFVNKYSLSKTLRFELIPQGKTLENIKARGLILDDEKRAKDYKKAK QIIDKYHQFFIEEILSSVCISEDLLQNYSDVYFKLKKSDDDNLQKDFKSAKD

SEQ ID NO	Description	Sequence
NO: 3	U112 (FnCas12a)	TIKKQISEYIKDSEKFKNLFNQNLIDAKKGQESDLILWLKQSKDNGIELFKA NSDITDIDEALEIIKSFKGWTTYFKGFHENRKNVYSSNDIPTSIIYRIVDDNL PKFLENKAKYESLKDKAPEAINYEQIKKDLAEELTFDIDYKTSEVNQRVFS LDEVFEIANFNNYLNQSGITKFNTIIGGKFVNGENTKRKGINEYINLYSQQI NDKTLKKYKMSVLFKQILSDTESKSFVIDKLEDDSDVVTTMQSFYEQIAA FKTVEEKSIKETLSLLFDDLKAQKLDLSKIYFKNDKSLTDLSQQVFDDYSV IGTAVLEYITQQIAPKNLDNPSKKEQELIAKKTEKAKYLSLETIKLALEEFN KHRDIDKQCRFEEILANFAAIPMIFDEIAQNKDNLAQISIKYQNQGKKDLL QASAEDDVKAIKDLLDQTNNLLHKLKIFHISQSEDKANILDKDEHFYLVFE ECYFELANIVPLYNKIRNYITQKPYSDEKFKLNFENSTLANGWDKNKEPD NTAILFIKDDKYYLGVMNKKNNKIFDDKAIKENKGEGYKKIVYKLLPGAN KMLPKVFFSAKSIKFYNPSEDILRIRNHSTHTKNGSPQKGYEKFEFNIEDCR KFIDFYKQSISKHPEWKDFGFRFSDTQRYNSIDEFYREVENQGYKLTFENIS ESYIDSVVNQGKLYLFQIYNKDFSAYSKGRPNLHTLYWKALFDERNLQD VVYKLNGEAELFYRKQSIPKKITHPAKEAIANKNKDNPKKESVFEYDLIK DKRFTEDKFFFHCPITINFKSSGANKFNDEINLLLKEKANDVHILSIDRGER HLAYYTLVDGKGNIIKQDTFNIIGNDRMKTNYHDKLAAIEKDRDSARKD WKKINNIKEMKEGYLSQVVHEIAKLVIEYNAIVVFEDLNFGFKRGRFKVE KQVYQKLEKMLIEKLNYLVFKDNEFDKTGGVLRAYQLTAPFETFKKMGK QTGIIYYVPAGFTSKICPVTGFVNQLYPKYESVSKSQEFFSKFDKICYNLDK GYFEFSFDYKNFGDKAAKGKWTIASFGSRLINFRNSDKNHNWDTREVYP TKELEKLLKDYSIEYGHGECIKAAICGESDKKFFAKLTSVLNTILQMRNSK TGTELDYLISPVADVNGNFFDSRQAPKNMPQDADANGAYHIGLKGLMLL GRIKNNQEGKKLNLVIKNEEYFEFVQNRNN
SEQ ID NO: 4	Porphyromonas macacae (PmCas12a)	MKTQHFFEDFTSLYSLSKTIRFELKPIGKTLENIKKNGLIRRDEQRLDDYEK LKKVIDEYHEDFIANILSSFSFSEEILQSYIQNLSESEARAKIEKTMRDTLAK AFSEDERYKSIFKKELVKKDIPVWCPAYKSLCKKFDNFTTSLVPFHENRK NLYTSNEITASIPYRIVHVNLPKFIQNIEALCELQKKMGADLYLEMMENLR NVWPSFVKTPDDLCNLKTYNHLMVQSSISEYNRFVGGYSTEDGTKHQGI NEWINIYRQRNKEMRLPGLVFLHKQILAKVDSSSFISDTLENDDQVFCVLR QFRKLFWNTVSSKEDDAASLKDLFCGLSGYDPEAIYVSDAHLATISKNIFD RWNYISDAIRRKTEVLMPRKKESVERYAEKISKQIKKRQSYSLAELDDLL AHYSEESLPAGFSLLSYFTSLGGQKYLVSDGEVILYEEGSNIWDEVLIAFR DLQVILDKDFTEKKLGKDEEAVSVIKKALDSALRLRKFFDLLSGTGAEIRR DSSFYALYTDRMDKLKGLLKMYDKVRNYLTKKPYSIEKFKLHFDNPSLL SGWDKNKELNNLSVIFRQNGYYYLGIMTPKGKNLFKTLPKLGAEEMFYE KMEYKQIAEPMLMLPKVFFPKKTKPAFAPDQSVVDIYNKKTFKTGQKGF NKKDLYRLIDFYKEALTVHEWKLFNFSFSPTEQYRNIGEFFDEVREQAYK VSMVNVPASYIDEAVENGKLYLFQIYNKDFSPYSKGIPNLHTLYWKALFS EQNQSRVYKLCGGGELFYRKASLHMQDTTVHPKGISIHKKNLNKKGETS LFNYDLVKDKRFTEDKFFFHVPISINYKNKKITNVNQMVRDYIAQNDDLQ IIGIDRGERNLLYISRIDTRGNLLEQFSLNVIESDKGDLRTDYQKILGDREQE RLRRRQEWKSIESIKDLKDGYMSQVVHKICNMVVEHKAIVVLENLNLSF MKGRKKVEKSVYEKFERMLVDKLNYLVVDKKNLSNEPGGLYAAYQLTN PLFSFEELHRYPQSGILFFVDPWNTSLTDPSTGFVNLLGRINYTNVGDARK FFDRFNAIRYDGKGNILFDLDLSRFDVRVETQRKLWTLTTFGSRIAKSKKS GKWMVERIENLSLCFLELFEQFNIGYRVEKDLKKAILSQDRKEFYVRLIYL FNLMMQIRNSDGEEDYILSPALNEKNLQFDSRLIEAKDLPVDADANGAYN VARKGLMVVQRIKRGDHESIHRIGRAQWLRYVQEGIVE
SEQ ID NO: 5	Moraxella bovoculi 237 (MbCas12a)	MLFQDFTHLYPLSKTVRFELKPIDRTLEHIHAKNFLSQDETMADMHQKVK VILDDYHRDFIADMMGEVKLTKLAEFYDVYLKFRKNPKDDELQKQLKDL QAVLRKEIVKPIGNGGKYKAGYDRLFGAKLFKDGKELGDLAKFVIAQEG ESSPKLAHLAHFEKFSTYFTGFHDNRKNMYSDEDKHTAIAYRLIHENLPRF

SEQ ID NO	Description	Sequence
		IDNLQILTTIKQKHSALYDQIINELTASGLDVSLASHLDGYHKLLTQEGITA YNTLLGGISGEAGSPKIQGINELINSHHNQHCHKSERIAKLRPLHKQILSDG MSVSFLPSKFADDSEMCQAVNEFYRHYADVFAKVQSLFDGFDDHQKDGI YVEHKNLNELSKQAFGDFALLGRVLDGYYVDVVNPEFNERFAKAKTDN AKAKLTKEKDKFIKGVHSLASLEQAIEHYTARHDDESVQAGKLGQYFKH GLAGVDNPIQKIHNNHSTIKGFLERERPAGERALPKIKSGKNPEMTQLRQL KELLDNALNVAHFAKLLTTKTTLDNQDGNFYGEFGVLYDELAKIPTLYN KVRDYLSQKPFSTEKYKLNFGNPTLLNGWDLNKEKDNFGVILQKDGCYY LALLDKAHKKVFDNAPNTGKSIYQKMIYKYLEVRKQFPKVFFSKEAIAIN YHPSKELVEIKDKGRQRSDDERLKLYRFILECLKIHPKYDKKFEGAIGDIQ LFKKDKKGREVPISEKDLFDKINGIFSSKPKLEMEDFFIGEFKRYNPSQDLV DQYNIYKKIDSNDNRKKENFYNNHPKFKKDLVRYYYESMCKHEEWEESF EFSKKLQDIGCYVDVNELFTEIETRRLNYKISFCNINADYIDELVEQGQLYL FQIYNKDFSPKAHGKPNLHTLYFKALFSEDNLADPIYKLNGEAQIFYRKAS LDMNETTIHRAGEVLENKNPDNPKKRQFVYDIIKDKRYTQDKFMLHVPIT MNFGVQGMTIKEFNKKVNQSIQQYDEVNVIGIDRGERHLLYLTVINSKGEI LEQCSLNDITTASANGTQMTTPYHKILDKREIERLNARVGWGEIETIKELK SGYLSHVVHQISQLMLKYNAIVVLEDLNFGFKRGRFKVEKQIYQNFENAL IKKLNHLVLKDKADDEIGSYKNALQLTNNFTDLKSIGKQTGFLFYVPAWN TSKIDPETGFVDLLKPRYENIAQSQAFFGKFDKICYNADKDYFEFHIDYAK FTDKAKNSRQIWTICSHGDKRYVYDKTANQNKGAAKGINVNDELKSLFA RHHINEKQPNLVMDICQNNDKEFHKSLMYLLKTLLALRYSNASSDEDFIL SPVANDEGVFFNSALADDTQPQNADANGAYHIALKGLWLLNELKNSDDL NKVKLAIDNQTWLNFAQNR
SEQ ID NO: 6	Moraxella bovoculi AAX08_00205 (Mb2Cas12a)	MGIHGVPAALFQDFTHLYPLSKTVRFELKPIGRTLEHIHAKNFLSQDETMA DMYQKVKVILDDYHRDFIADMMGEVKLTKLAEFYDVYLKFRKNPKDDG LQKQLKDLQAVLRKESVKPIGSGGKYKTGYDRLFGAKLFKDGKELGDLA KFVIAQEGESSPKLAHLAHFEKFSTYFTGFHDNRKNMYSDEDKHTAIAYR LIHENLPRFIDNLQILTTIKQKHSALYDQIINELTASGLDVSLASHLDGYHK LLTQEGITAYNRIIGEVNGYTNKHNQICHKSERIAKLRPLHKQILSDGMGV SFLPSKFADDSEMCQAVNEFYRHYTDVFAKVQSLFDGFDDHQKDGIYVE HKNLNELSKQAFGDFALLGRVLDGYYVDVVNPEFNERFAKAKTDNAKA KLTKEKDKFIKGVHSLASLEQAIEHHTARHDDESVQAGKLGQYFKHGLA GVDNPIQKIHNNHSTIKGFLERERPAGERALPKIKSGKNPEMTQLRQLKEL LDNALNVAHFAKLLTTKTTLDNQDGNFYGEFGVLYDELAKIPTLYNKVR DYLSQKPFSTEKYKLNFGNPTLLNGWDLNKEKDNFGVILQKDGCYYLAL LDKAHKKVFDNAPNTGKNVYQKMVYKLLPGPNKMLPKVFFAKSNLDYY NPSAELLDKYAKGTHKKGDNFNLKDCHALIDFFKAGINKHPEWQHFGFK FSPTSSYRDLSDFYREVEPQGYQVKFVDINADYIDELVEQGKLYLFQIYNK DFSPKAHGKPNLHTLYFKALFSEDNLADPIYKLNGEAQIFYRKASLDMNE TTIHRAGEVLENKNPDNPKKRQFVYDIIKDKRYTQDKFMLHVPITMNFGV QGMTIKEFNKKVNQSIQQYDEVNVIGIDRGERHLLYLTVINSKGEILEQRS LNDITTASANGTQVTTPYHKILDKREIERLNARVGWGEIETIKELKSGYLS HVVHQINQLMLKYNAIVVLEDLNFGFKRGRFKVEKQIYQNFENALIKKLN HLVLKDKADDEIGSYKNALQLTNNFTDLKSIGKQTGFLFYVPAWNTSKID PETGFVDLLKPRYENIAQSQAFFGKFDKICYNTDKGYFEFHIDYAKFTDKA KNSRQKWAICSHGDKRYVYDKTANQNKGAAKGINVNDELKSLFARYHI NDKQPNLVMDICQNNDKEFHKSLMCLLKTLLALRYSNASSDEDFILSPVA NDEGVFFNSALADDTQPQNADANGAYHIALKGLWLLNELKNSDDLNKV KLAIDNQTWLNFAQNR
SEQ ID	Moraxella bovoculi AAX11_00	MGIHGVPAALFQDFTHLYPLSKTVRFELKPIGKTLEHIHAKNFLNQDETM ADMYQKVKAILDDYHRDFIADMMGEVKLTKLAEFYDVYLKFRKNPKDD GLQKQLKDLQAVLRKEIVKPIGNGGKYKAGYDRLFGAKLFKDGKELGDL

SEQ ID NO	Description	Sequence
NO: 7	205 (Mb3Cas12a)	AKFVIAQEGESSPKLAHLAHFEKFSTYFTGFHDNRKNMYSDEDKHTAIAY RLIHENLPRFIDNLQILATIKQKHSALYDQIINELTASGLDVSLASHLDGYH KLLTQEGITAYNTLLGGISGEAGSRKIQGINELINSHHNQHCHKSERIAKLR PLHKQILSDGMGVSFLPSKFADDSEVCQAVNEFYRHYADVFAKVQSLFD GFDDYQKDGIYVEYKNLNELSKQAFGDFALLGRVLDGYYVDVVNPEFNE RFAKAKTDNAKAKLTKEKDKFIKGVHSLASLEQAIEHYTARHDDESVQA GKLGQYFKHGLAGVDNPIQKIHNNHSTIKGFLERERPAGERALPKIKSDKS PEIRQLKELLDNALNVAHFAKLLTTKTTLHNQDGNFYGEFGALYDELAKI ATLYNKVRDYLSQKPFSTEKYKLNFGNPTLLNGWDLNKEKDNFGVILQK DGCYYLALLDKAHKKVFDNAPNTGKSVYQKMIYKLLPGPNKMLPKVFF AKSNLDYYNPSAELLDKYAQGTHKKGDNFNLKDCHALIDFFKAGINKHP EWQHFGFKFSPTSSYQDLSDFYREVEPQGYQVKFVDINADYINELVEQGQ LYLFQIYNKDFSPKAHGKPNLHTLYFKALFSEDNLVNPIYKLNGEAEIFYR KASLDMNETTIHRAGEVLENKNPDNPKKRQFVYDIIKDKRYTQDKFMLH VPITMNFGVQGMTIKEFNKKVNQSIQQYDEVNVIGIDRGERHLLYLTVINS KGEILEQRSLNDITTASANGTQMTTPYHKILDKREIERLNARVGWGEIETI KELKSGYLSHVVHQISQLMLKYNAIVVLEDLNFGFKRGRFKVEKQIYQNF ENALIKKLNHLVLKDKADDEIGSYKNALQLTNNFTDLKSIGKQTGFLFYV PAWNTSKIDPETGFVDLLKPRYENIAQSQAFFGKFDKICYNADRGYFEFHI DYAKFNDKAKNSRQIWKICSHGDKRYVYDKTANQNKGATIGVNVNDEL KSLFTRYHINDKQPNLVMDICQNNDKEFHKSLMYLLKTLLALRYSNASSD EDFILSPVANDEGVFFNSALADDTQPQNADANGAYHIALKGLWLLNELK NSDDLNKVKLAIDNQTWLNFAQNR
SEQ ID NO: 8	Thiomicrospira sp. XS5 (TsCas12a)	MGIHGVPAATKTFDSEFFNLYSLQKTVRFELKPVGETASFVEDFKNEGLK RVVSEDERRAVDYQKVKEIIDDYHRDFIEESLNYFPEQVSKDALEQAFHL YQKLKAAKVEEREKALKEWEALQKKLREKVVKCFSDSNKARFSRIDKKE LIKEDLINWLVAQNREDDIPTVETFNNFTTYFTGFHENRKNIYSKDDHATA ISFRLIHENLPKFFDNVISFNKLKEGFPELKFDKVKEDLEVDYDLKHAFEIE YFVNFVTQAGIDQYNYLLGGKTLEDGTKKQGMNEQINLFKQQQTRDKAR QIPKLIPLFKQILSERTESQSFIPKQFESDQELFDSLQKLHNNCQDKFTVLQQ AILGLAEADLKKVFIKTSDLNALSNTIFGNYSVFSDALNLYKESLKTKKAQ EAFEKLPAHSIHDLIQYLEQFNSSLDAEKQQSTDTVLNYFIKTDELYSRFIK STSEAFTQVQPLFELEALSSKRRPPESEDEGAKGQEGFEQIKRIKAYLDTL MEAVHFAKPLYLVKGRKMIEGLDKDQSFYEAFEMAYQELESLIIPIYNKA RSYLSRKPFKADKFKINFDNNTLLSGWDANKETANASILFKKDGLYYLGI MPKGKTFLFDYFVSSEDSEKLKQRRQKTAEEALAQDGESYFEKIRYKLLP GASKMLPKVFFSNKNIGFYNPSDDILRIRNTASHTKNGTPQKGHSKVEFNL NDCHKMIDFFKSSIQKHPEWGSFGFTFSDTSDFEDMSAFYREVENQGYVIS FDKIKETYIQSQVEQGNLYLFQIYNKDFSPYSKGKPNLHTLYWKALFEEA NLNNVVAKLNGEAEIFFRRHSIKASDKVVHPANQAIDNKNPHTEKTQSTF EYDLVKDKRYTQDKFFFHVPISLNFKAQGVSKFNDKVNGFLKGNPDVNII GIDRGERHLLYFTVVNQKGEILVQESLNTLMSDKGHVNDYQQKLDKKEQ ERDAARKSWTTVENIKELKEGYLSHVVHKLAHLIIKYNAIVCLEDLNFGF KRGRFKVEKQVYQKFEKALIDKLNYLVFKEKELGEVGHYLTAYQLTAPF ESFKKLGKQSGILFYVPADYTSKIDPTTGFVNFLDLRYQSVEKAKQLLSDF NAIRFNSVQNYFEFEIDYKKLTPKRKVGTQSKWVICTYGDVRYQNRRNQ KGHWETEEVNVTEKLKALFASDSKTTTVIDYANDDNLIDVILEQDKASFF KELLWLLKLTMTLRHSKIKSEDDFILSPVKNEQGEFYDSRKAGEVWPKDA DANGAYHIALKGLWNLQQINQWEKGKTLNLAIKNQDWFSFIQEKPYQE
SEQ ID NO: 9	Butyrivibrio sp. NC3005 (BsCas12a)	MGIHGVPAAYYQNLTKKYPVSKTIRNELIPIGKTLENIRKNNILESDVKRK QDYEHVKGIMDEYHKQLINEALDNYMLPSLNQAAEIYLKKHVDVEDREE FKKTQDLLRREVTGRLKEHENYTKIGKKDILDLLEKLPSISEEDYNALESF RNFYTYFTSYNKVRENLYSDEEKSSTVAYRLINENLPKFLDNIKSYAFVKA

SEQ ID NO	Description	Sequence
		AGVLADCIEEEEQDALFMVETFNMTLTQEGIDMYNYQIGKVNSAINLYNQ KNHKVEEFKKIPKMKVLYKQILSDREEVFIGEFKDDETLLSSIGAYGNVL MTYLKSEKINIFFDALRESEGKNVYVKNDLSKTTMSNIVFGSWSAFDELL NQEYDLANENKKKDDKYFEKRQKELKKNKSYTLEQMSNLSKEDISPIEN YIERISEDIEKICIYNGEFEKIVVNEHDSSRKLSKNIKAVKVIKDYLDSIKEL EHDIKLINGSGQELEKNLVVYVGQEEALEQLRPVDSLYNLTRNYLTKKPF STEKVKLNFNKSTLLNGWDKNKETDNLGILFFKDGKYYLGIMNTTANKA FVNPPAAKTENVFKKVDYKLLPGSNKMLPKVFFAKSNIGYYNPSTELYSN YKKGTHKKGPSFSIDDCHNLIDFFKESIKKHEDWSKFGFEFSDTADYRDIS EFYREVEKQGYKLTFTDIDESYINDLIEKNELYLFQIYNKDFSEYSKGKLN LHTLYFMMLFDQRNLDNVVYKLNGEAEVFYRPASIAENELVIHKAGEGIK NKNPNRAKVKETSTFSYDIVKDKRYSKYKFTLHIPITMNFGVDEVRRFND VINNALRTDDNVNVIGIDRGERNLLYVVVINSEGKILEQISLNSIINKEYDIE TNYHALLDEREDDRNKARKDWNTIENIKELKTGYLSQVVNVVAKLVLKY NAIICLEDLNFGFKRGRQKVEKQVYQKFEKMLIEKLNYLVIDKSREQVSPE KMGGALNALQLTSKFKSFAELGKQSGIIYYVPAYLTSKIDPTTGFVNLFYI KYENIEKAKQFFDGFDFIRFNKKDDMFEFSFDYKSFTQKACGIRSKWIVYT NGERIIKYPNPEKNNLFDEKVINVTDEIKGLFKQYRIPYENGEDIKEIIISKA EADFYKRLFRLLHQTLQMRNSTSDGTRDYIISPVKNDRGEFFCSEFSEGTM PKDADANGAYNIARKGLWVLEQIRQKDEGEKVNLSMTNAEWLKYAQLH LL
SEQ ID NO: 10	AacCas12b	MAVKSIKVKLRLDDMPEIRAGLWKLHKEVNAGVRYYTEWLSLLRQENL YRRSPNGDGEQECDKTAEECKAELLERLRARQVENGHRGPAGSDDELLQ LARQLYELLVPQAIGAKGDAQQIARKFLSPLADKDAVGGLGIAKAGNKP RWVRMREAGEPGWEEEKEKAETRKSADRTADVLRALADFGLKPLMRVY TDSEMSSVEWKPLRKGQAVRTWDRDMFQQAIERMMSWESWNQRVGQE YAKLVEQKNRFEQKNFVGQEHLVHLVNQLQQDMKEASPGLESKEQTAH YVTGRALRGSDKVFEKWGKLAPDAPFDLYDAEIKNVQRRNTRRFGSHDL FAKLAEPEYQALWREDASFLTRYAVYNSILRKLNHAKMFATFTLPDATA HPIWTRFDKLGGNLHQYTFLFNEFGERRHAIRFHKLLKVENGVAREVDD VTVPISMSEQLDNLLPRDPNEPIALYFRDYGAEQHFTGEFGGAKIQCRRDQ LAHMHRRRGARDVYLNVSVRVQSQSEARGERRPPYAAVFRLVGDNHRA FVHFDKLSDYLAEHPDDGKLGSEGLLSGLRVMSVDLGLRTSASISVFRVA RKDELKPNSKGRVPFFFPIKGNDNLVAVHERSQLLKLPGETESKDLRAIRE ERQRTLRQLRTQLAYLRLLVRCGSEDVGRRERSWAKLIEQPVDAANHMT PDWREAFENELQKLKSLHGICSDKEWMDAVYESVRRVWRHMGKQVRD WRKDVRSGERPKIRGYAKDVVGGNSIEQIEYLERQYKFLKSWSFFGKVSG QVIRAEKGSRFAITLREHIDHAKEDRLKKLADRIIMEALGYVYALDERGK GKWVAKYPPCQLILLEELSEYQFNNDRPPSENNQLMQWSHRGVFQELIN QAQVHDLLVGTMYAAFSSRFDARTGAPGIRCRRVPARCTQEHNPEPFPW WLNKFVVEHTLDACPLRADDLIPTGEGEIFVSPFSAEEGDFHQIHADLNAA QNLQQRLWSDFDISQIRLRCDWGEVDGELVLIPRLTGKRTADSYSNKVFY TNTGVTYYERERGKKRRKVFAQEKLSEEEAELLVEADEAREKSVVLMRD PSGIINRGNWTRQKEFWSMVNQRIEGYLVKQIRSRVPLQDSACENTGDI
SEQ ID NO: 11	Cas12 Variant	MKKIDNFVGCYPVSKTLRFKAIPIGKTQENIEKKRLVEEDEVRAKDYKAV KKLIDRYHREFIEGVLDNVKLDGLEEYYMLFNKSDREESDNKKIEIMEERF RRVISKSFKNNEEYKKIFSKKIIEEILPNYIKDEEEKELVKGFKGFYTAFVG YAQNRENMYSDEKKSTAISYRIVNENMPRFITNIKVFEKAKSILDVDKINEI NEYILNNDYYVDDFFNIDFFNYVLNQKGIDIYNAIIGGIVTGDGRKIQGLNE CINLYNQENKKIRLPQFKPLYKQILSESESMSFYIDEIESDDMLIDMLKESL QIDSTINNAIDDLKVLFNNIFDYDLSGIFINNGLPITTISNDVYGQWSTISDG WNERYDVLSNAKDKESEKYFEKRRKEYKKVKSFSISDLQELGGKDLSICK KINEIISEMIDDYKSKIEEIQYLFDIKELEKPLVTDLNKIELIKNSLDGLKRIE

SEQ ID NO	Description	Sequence
		RYVIPFLGTGKEQNRDEVFYGYFIKCIDAIKEIDGVYNKTRNYLTKKPYSK DKFKLYFENPQLMGGWDRNKESDYRSTLLRKNGKYYVAIIDKSSSNCMM NIEEDENDNYEKINYKLLPGPNKMLPKVFFSKKNREYFAPSKEIERIYSTG TFKKDTNFVKKDCENLITFYKDSLDRHEDWSKSFDFSFKESSAYRDISEFY RDVEKQGYRVSFDLLSSNAVNTLVEEGKLYLFQLYNKDFSEKSHGIPNLH TMYFRSLFDDNNKGNIRLNGGAEMFMRRASLNKQDVTVHKANQPIKNK NLLNPKKTTTLPYDVYKDKRFTEDQYEVHIPITMNKVPNNPYKINHMVRE QLVKDDNPYVIGIDRGERNLIYVVVVDGQGHIVEQLSLNEIINENNGISIRT DYHTLLDAKERERDESRKQWKQIENIKELKEGYISQVVHKICELVEKYDA VIALEDLNSGFKNSRVKVEKQVYQKFEKMLITKLNYMVDKKKDYNKPG GVLNGYQLTTQFESFSKMGTQNGIMFYIPAWLTSKMDPTTGFVDLLKPK YKNKADAQKFFSQFDSIRYDNQEDAFVFKVNYTKFPRTDADYNKEWEIY TNGERIRVFRNPKKNNEYDYETVNVSERMKELFDSYDLLYDKGELKETIC EMEESKFFEELIKLFRLTLQMRNSISGRTDVDYLISPVKNSNGYFYNSNDY KKEGAKYPKDADANGAYNIARKVLWAIEQFKMADEDKLDKTKISIKNQE WLEYAQTHCE
SEQ ID NO: 282	CasY3	MKAKKSFYNQKRKFGKRGYRLHDERIAYSGGIGSMRSIKYELKDSYGIAG LRNRIADATISDNKWLYGNINLNDYLEWRSSKTDKQIEDGDRESSLLGFW LEALRLGFVFSKQSHAPNDFNETALQDLFETLDDDLKHVLDRKKWCDFIK IGTPKTNDQGRLKKQIKNLLKGNKREEIEKTLNESDDELKEKINRIADVFA KNKSDKYTIFKLDKPNTEKYPRINDVQVAFFCHPDFEEITERDRTKTLDLII NRFNKRYEITENKKDDKTSNRMALYSLNQGYIPRVLNDLFLFVKDNEDDF SQFLSDLENFFSFSNEQIKIIKERLKKLKKYAEPIPGKPQLADKWDDYASDF GGKLESWYSNRIEKLKKIPESVSDLRNNLEKIRNVLKKQNNASKILELSQK IIEYIRDYGVSFEKPEIIKFSWINKTKDGQKKVFYVAKMADREFIEKLDLW MADLRSQLNEYNQDNKVSFKKKGKKIEELGVLDFALNKAKKNKSTKNE NGWQQKLSESIQSAPLFFGEGNRVRNEEVYNLKDLLFSEIKNVENILMSSE AEDLKNIKIEYKEDGAKKGNYVLNVLARFYARFNEDGYGGWNKVKTVL ENIAREAGTDFSKYGNNNNRNAGRFYLNGRERQVFTLIKFEKSITVEKILE LVKLPSLLDEAYRDLVNENKNHKLRDVIQLSKTIMALVLSHSDKEKQIGG NYIHSKLSGYNALISKRDFISRYSVQTTNGTQCKLAIGKGKSKKGNEIDRY FYAFQFFKNDDSKINLKVIKNNSHKNIDFNDNENKINALQVYSSNYQIQFL DWFFEKHQGKKTSLEVGGSFTIAEKSLTIDWSGSNPRVGFKRSDTEEKRV FVSQPFTLIPDDEDKERRKERMIKTKNRFIGIDIGEYGLAWSLIEVDNGDK NNRGIRQLESGFITDNQQQVLKKNVKSWRQNQIRQTFTSPDTKIARLRESL IGSYKNQLESLMVAKKANLSFEYEVSGFEVGGKRVAKIYDSIKRGSVRKK DNNSQNDQSWGKKGINEWSFETTAAGTSQFCTHCKRWSSLAIVDIEEYEL KDYNDNLFKVKINDGEVRLLGKKGWRSGEKIKGKELFGPVKDAMRPNV DGLGMKIVKRKYLKLDLRDWVSRYGNMAIFICPYVDCHHISHADKQAAF NIAVRGYLKSVNPDRAIKHGDKGLSRDFLCQEEGKLNFEQIGLLI
SEQ ID NO: 571	Cas12 variant	MATLVSFTKQYQVQKTLRFELIPQGKTQANIDAKGFINDDLKRDENYMK VKGVIDELHKNFIEQTLVNVDYDWRSLATAIKNYRKDRSDTNKKNLEKT QEAARKEIIAWFEGKRGNSAFKNNQKSFYGKLFKKELFSEILRSDDLEYDE ETQDAIACFDKFTTYFVGFHENRKNMYSTEAKSTSVAYRVVNENFSKFLS NCEAFSVLEAVCPNVLVEAEQELHLHKAFSDLKLSDVFKVEAYNKYLSQ TGIDYYNQIIGGISSAEGVRKIRGVNEVVNNAIQQNDELKVALRNKQFTM VQLFKQILSDRSTLSFVSEQFTSDQEVITVVKQFNDDIVNNKVLAVVKTLF ENFNSYDLEKIYINSKELASVSNALLKDWSKIRNAVLENKIIELGANPPKT KISAVEKEVKNKDFSIAELASYNDKYLDKEGNDKEICSIANVVLEAVGAL EIMLAESLPADLKTLENKNKVKGILDAYENLLHLLNYFKVSAVNDVDLAF YGAFEKVYVDISGVMPLYNKVRNYATKKPYSVEKFKLNFAMPTLADGW DKNKERDNGSIILLKDGQYYLGVMNPQNKPVIDNAVCNDAKGYQKMVY KMFPEISKMVTKCSTQLNAVKAHFEDNTNDFVLDDTDKFISDLTITKEIYD

SEQ ID NO	Description	Sequence
		LNNVLYDGKKKFQIDYLRNTGDFAGYHKALETWIDFVKEFLSKYRSTAIY DLTTLLPTNYYEKLDVFYSDVNNLCYKIDYENISVEQVNEWVEEGNLYLF KIYNKDFATGSTGKPNLHTMYWNAVFAEENLHDVVVKLNGGAELFYRP KSNMPKVEHRVGEKLVNRKNVNGEPIADSVHKEIYAYANGKISKSELSEN AQEELPLAIIKDVKHNITKDKRYLSDKYFFHVPITLNYKANGNPSAFNTKV QAFLKNNPDVNIIGIDRGERNLLYVVVIDQQGNIIDKKQVSYNKVNGYDY YEKLNQREKERIEARQSWGAVGKIKELKEGYLSLVVREIADMMVKYNAI VVMENLNAGFKRVRGGIAEKAVYQKFEKMLIDKLNYLVFKDVEAKEAG GVLNAYQLTDKFDSFEKMGNQSGFLFYVPAAYTSKIDPVTGFANVFSTKH ITNTEAKKEFICSFNSLRYDEAKDKFVLECDLNKFKIVANSHIKNWKFIIGG KRIVYNSKNKTYMEKYPCEDLKATLNASGIDFSSSEIINLLKNVPANREYG KLFDETYWAIMNTLQMRNSNALTGEDYIISAVADDNEKVFDSRTCGAELP KDADANGAYHIALKGLYLLQRIDISEEGEKVDLSIKNEEWFKFVQQKEYA R
SEQ ID NO: 572	Cas12 variant	MKEQFINRYPLSKTLRFSLIPVGETENNFNKNLLLKKDKQRAENYEKVKC YIDRFHKEYIESVLSKARIEKVNEYANLYWKSNKDDSDIKAMESLENDMR KQISKQLTSTEIYKKRLFGKELICEDLPSFLTDKDERETVECFRSFTTYFKG FNTNRENMYSSDGKSTAIAYRCINDNLPRFLDNVKSFQKVFDNLSDETITK LNTDLYNIFGRNIEDIFSVDYFEFVLTQSGIEIYNSMIGGYTCSDKTKIQGL NECINLYNQQVAKNEKSKKLPLMKPLYKQILSEKDSVSFIPEKFNSDNEVL HAIDDYYTGHIGDFDLLTELLQSLNTYNANGIFVKSGVAITDISNGAFNSW NVLRSAWNEKYEALHPVTSKTKIDKYIEKQDKIYKAIKSFSLFELQSLGNE NGNEITDWYISSINESNSKIKEAYLQAQKLLNSDYEKSYNKRLYKNEKAT ELVKNLLDAIKEFQKLIKPLNGTGKEENKDELFYGKFTSYYDSIADIDRLY DKVRNYITQKPYSKDKIKLNFDNPQLLGGWDKNKESDYRTVLLHKDGLY YLAVMDKSHSKAFVDAPEITSDDKDYYEKMEYKLLPGPNKMLPKVFFAS KNIDTFQPSDRILDIRKRESFKKGATFNKAECHEFIDYFKDSIKKHDDWSQ FGFKFSPTESYNDISEFYREISDQGYSVRFNKISKNYIDGLVNNGYIYLFQIY NKDFSKYSKGTPNLHTLYFKMLFDERNLSNVVYKLNGEAEMFYREASIG DKEKITHYANQPIKNKNPDNEKKESVFEYDIVKDKRFTKRQFSLHLPITINF KAHGQEFLNYDVRKAVKYKDDNYVIGIDRGERNLIYISVINSNGEIVEQM SLNEIISDNGHKVDYQKLLDTKEKERDKARKNWTSVENIKELKEGYISQV VHKICELVIKYDAVIAMEDLNFGFKRGRFPVEKQVYQKFENMLISKLNLLI DKKAEPTEDGGLLRAYQLTNKFDGVNKAKQNGIIFYVPAWDTSKIDPAT GFVNLLKPKCNTSVPEAKKLFETIDDIKYNANTDMFEFYIDYSKFPRCNSD FKKSWTVCTNSSRILTFRNKEKNNKWDNKQIVLTDEFKSLFNEFGIDYKG NLKDSILSISNADFYRRLIKLLSLTLQMRNSITGSTLPEDDYLISPVANKSGE FYDSRNYKGTNAALPCDADANGAYNIARKALWAINVLKDTPDDMLNKA KLSITNAEWLEYTQK
SEQ ID NO: 573	Cas12 variant	MNNPRGAFGGFTNLYSLSKTLRFELKPYLEIPEGEKGKLFGDDKEYYKNC KTYTEYYLKKANKEYYDNEKVKNTDLQLVNFLHDERIEDAYQVLKPVFD TLHEEFITDSLESAEAKKIDFGNYYGLYEKQKSEQNKDEKKKIDKPLETER GKLRKAFTPIYEAEGKNLKNKAGKEKKDKDILKESGFKVLIEAGILKYIKN NIDEFADKKLKNNEGKEITKKDIETALGAENIEGIFDGFFTYFSGFNQNREN YYSTEEKATAVASRIVDENLSKFCDNILLYRKNENDYLKIFNFLKNKGKD LKLKNSKFGKENEPEFIPAYDMKNDEKSFSVADFVNCLSQGEIEKYNAKI ANANYLINLYNQNKDGNSSKLSMFKILYKQIGCGEKKDFIKTIKDNAELK QILEKACEAGKKYFIRGKSEDGGVSNIFDFTDYIQSHENYKGVYWSDKAI NTISGKYFANWDTLKNKLGDAKVFNKNTGEDKADVKYKVPQAVMLSEL FAVLDDNAGEDWREKGIFFKASLFEGDQNKSEIIKNANRPSQALLKMICD DMESLAKNFIDSGDKILKISDRDYQKDENKQKIKNWLDNALWINQILKYF KVKANKIKGDSIDARIDSGLDMLVFSSDNPAEDYDMIRNYLTQKPQDEIN KLKLNFENSSLAGGWDENKEKDNSCIILKDEQDKQYLAVMKYENTKVFE

SEQ ID NO	Description	Sequence
		QKNSQLYIADNAAWKKMIYKLVPGASKTLPKVFFSKKWTANRPTPSDIV EIYQKGSFKKENVDFNDKKEKDESRKEKNREKIIAELQKTCWMDIRYNID GKIESAKYVNKEKLAKLIDFYKENLKKYPSEEESWDRLFAFGFSDTKSYK SIDQFYIEVDKQGYKLEFVTINKARLDEYVRDGKIYLFEIRSRDNNLVNGE EKTSAKNLQTIYWNAAFGGDDNKPKLNGEAEIFYRPAIAENKLNKKKDK NGKEIIDGYRFSKEKFIFHCPITLNFCLKETKINDKLNAALAKPENGQGVYF LGIDRGEKHLAYYSLVNQKGEILEQGTLNLPFLDKNGKSRSIKVEKKSFEK DSNGGIIKDKDGNDKIKIEFVECWNYNDLLDARAGDRDYARKNWTTIGTI KELKDGYISQVVRKIVDLSIYKNTETKEFREMPAFIVLEDLNIGFKRGRQKI EKQVYQKLELALAKKLNFLVDKKADIGEIGSVTKAIQLTPPVNNFGDMEN RKQFGNMLYIRADYTSQTDPATGWRKSIYLKSGSESNVKEQIEKSFFDIRY ESGDYCFEYRDRHGKMWQLYSSKNGVSLDRFHGERNNSKNVWESEKQP LNEMLDILFDEKRFDKSKSLYEQMFKGGVALTRLPKEINKKDKPAWESLR FVIILIQQIRNTGKNGDDRNGDFIQSPVRDEKTGEHFDSRIYLDKEQKGEK ADLPTSGDANGAYNIARKGIVVAEHIKRGFDKLYISDEEWDTWLAGDEIW DKWLKENRESLTKTRK
SEQ ID NO: 574	Cas12 variant	MNGNRIIVYREFVGVTPVAKTLRNELRPIGHTQEHIIHNGLIQEDELRQEKS TELKNIMDDYYREYIDKSLSGVTDLDFTLLFELMNLVQSSPSKDNKKALE KEQSKMREQICTHMQSDSNYKNIFNAKFLKEILPDFIKNYNQYDAKDKAG KLETLALFNGFSTYFTDFFEKRKNVFTKEAVSTSIAYRIVHENSLTFLANM TSYKKISEKALDEIEVIEKNNQDKMGDWELNQIFNPDFYNMVLIQSGIDFY NEICGVVNAHMNLYCQQTKNNYNLFKMRKLHKQILAYTSTSFEVPKMFE DDMSVYNAVNAFIDETEKGNIIGKLKDIVNKYDELDEKRIYISKDFYETLS CFMSGNWNLITGCVENFYDENIHAKGKSKEEKVKKAVKEDKYKSINDVN DLVEKYIDEKERNEFKNSNAKQYIREISNIITDTETAHLEYDEHISLIESEEK ADEMKKRLDMYMNMYHWAKAFIVDEVLDRDEMFYSDIDDIYNILENIVP LYNRVRNYVTQKPYNSKKIKLNFQSPTLANGWSQSKEFDNNAIILIRDNK YYLAIFNAKNKPDKKIIQGNSDKKNDNDYKKMVYNLLPGANKMLPKVFL SKKGIETFKPSDYIISGYNAHKHIKTSENFDISFCRDLIDYFKNSIEKHAEWR KYEFKFSATDSYNDISEFYREVEMQGYRIDWTYISEADINKLDEEGKIYLF QIYNKDFAENSTGKENLHTMYFKNIFSEENLKDIIIKLNGQAELFYRRASV KNPVKHKKDSVLVNKTYKNQLDNGDVVRIPIPDDIYNEIYKMYNGYIKEN DLSEAAKEYLDKVEVRTAQKDIVKDYRYTVDKYFIHTPITINYKVTARNN VNDMAVKYIAQNDDIHVIGIDRGERNLIYISVIDSHGNIVKQKSYNILNNY DYKKKLVEKEKTREYARKNWKSIGNIKELKEGYISGVVHEIAMLMVEYN AIIAMEDLNYGFKRGRFKVERQVYQKFESMLINKLNYFASKGKSVDEPGG LLKGYQLTYVPDNIKNLGKQCGVIFYVPAAFTSKIDPSTGFISAFNFKSIST NASRKQFFMQFDEIRYCAEKDMFSFGFDYNNFDTYNITMSKTQWTVYTN GERLQSEFNNARRTGKTKSINLTETIKLLLEDNEINYADGHDVRIDMEKM DEDKNSEFFAQLLSLYKLTVQMRNSYTEAEEQEKGISYDKIISPVINDEGE FFDSDNYKESDDKECKMPKDADANGAYCIALKGLYEVLKIKSEWTEDGF DRNCLKLPHAEWLDFIQNKRYE
SEQ ID NO: 575	Cas12 variant	MKKIDSFVNYYPLSKTLRFSLIPVGKTEDNFNAKLLLEEDEKRAIEYEKVK RYIDRYHKHFIETVLANFHLDDVNEYAELYYKAGKDDKDLKYMEKLEG KMRKSISAAFTKDKKYKEIFGQEIIKNILPEFLENEDEKESVKMFQGFFTYF TGFNDNRKNMYTHEAQTTAISYRCINENLPKFLDNVQSFAKIKESISSDIM NKLDEVCMDLYGVYAQDMFCTDYFSFVLSQSGIDRYNNIIGGYVDDKGV KIQGINEYINLYNQQVDEKNKRLPLMKKLYKQILIEKESISFIPEKFESDNIV INAISDYYHNNVENLFDDFNKLFNEFSEYDDNGIFVTSGLAVTDISNAVFG SWNIISDSWNEEYKDSHPMKKTTNAEKYYEDMKKEYKKNLSFTIAELQR LGEAGCNDECKGDIKEYYKTTVAEKIENIKNAYEISKDLLASDYEKSNDK KLCKNDSAISLLKNLLDSIKDLEKTIKPLLGTGKEENKDDVFYGKFTNLYE MISEIDRLYDKVRNYVTQKPYSKDKIKLNFENPQHLGGWDKNKERDYRS

SEQ ID NO	Description	Sequence
		VLLKKEDKYYLAIMDKSNNKAFIDFPDDGECYEKIEYKLLPGPNKMLPKV FFASSNIEYFAPSKKILEIRSRESFKKGDMFNLKDCHEFIDFFKESIKKHED WSQFGFEFSPTEKYNDISEFYNEVKIQGYSLKYKNVSKKYIDELIECGQLY LFQIYNKDFSVYAKGNPNLHTMYFKMLFDERNLANVVYQLNGGAEMFY RKASIKDSEKIVHHANQPIKNKNADNVKKESVFEYDIIKDKRFTKRQFSIHI PITLNFKAKGQNFINNDVRMALKKADENYVIGIDRGERNLLYICVINSKGE IVEQKSLNEIIGDNGYRVDYHKLLDKKEAERDEARKSWGTIENIKELKEG YLSQIVHEISKLVIKYDAVIAIEDLNSGFKKGRFKVEKQVYQKFENMLCTK LNYLVDKNADANECGGLLKAYQLTNKEDGANRGRQNGIIFSVPAWLTSK IDPVTGFADLLRPKYKSVSESVEFISKIDNIRYNSKEDYFEFDIDYSKFPNST ASYKKKWTVCTYGERIINVRNKEKNNMWDNKTIVLTDEFKKLFADFGVD VSKNIKESVLAIDSKDFYYRFINLLANTLQLRNSEVGNVDVDYLISPVKGV DGSFYDSRLVKEKTLPENADANGAYNIARKALWAIDVLKQTKDEELKNA NLSIKNAEWLEYVQK
SEQ ID NO: 576	Cas12 variant	MRTMVTFEDFTKQYQVSKTLRFELIPQGKTLENMKRDGIISVDRQRNEDY QKAKGILDKLYKYILDFTMETVVIDWEALATATEEFRKSKDKKTYEKVQS KIRTALLEHVKKQKVGTEDLFKGMFSSKIITGEVLAAFPEIRLSDEENLILE KFKDFTTYFTGFFENRKNVFTDEALSTSFTYRLVNDNFIKFFDNCIVFKNV VNISPHMAKSLETCASDLGIFPGVSLEEVFSISFYNRLLTQTGIDQFNQLLG GISGKEGEHKQQGLNEIINLAMQQNLEVKEVLKNKAHRFTPLFKQILSDRS TMSFIPDAFADDDEVLSAVDAYRKYLSEKNIGDRAFQLISDMEAYSPELM RIGGKYVSVLSQLLFYSWSEIRDGVKAYKESLITGKKTKKELENIDKEIKY GVTLQEIKEALPKKDIYEEVKKYAMSVVKDYHAGLAEPLPEKIETDDERA SIKHIMDSMLGLYRFLEYFSHDSIEDTDPVFGECLDTILDDMNETVPLYNK VRNFSTRKVYSTEKFKLNFNNSSLANGWDKNKEQANGAILLRKEGEYFL GIFNSKNKPKLVSDGGAGIGYEKMIYKQFPDFKKMLPKCTISLKDTKAHF QKSDEDFTLQTDKFEKSIVITKQIYDLGTQTVNGKKKFQVDYPRLTGDME GYRAALKEWIDFGKEFIQAYTSTAIYDTSLFRDSSDYPDLPSFYKDVDNIC YKLTFEWIPDAVIDDCIDDGSLYLFKLHNKDFSSGSIGKPNLHTLYWKALF EEENLSDVVVKLNGQAELFYRPKSLTRPVVHEEGEVIINKTTSTGLPVPDD VYVELSKFVRNGKKGNLTDKAKNWLDKVTVRKMPHAITKDRRFTVDKF FFHVPITLNYKADSSPYRFNDFVRQYIKDCSDVKIIGIDRGERNLIYAVVID GKGNIIEQRSFNTVGTYNYQEKLEQKEKERQTARQDWATVTKIKDLKKG YLSAVVHELSKMIVKYKAIVALENLNVGFKRMRGGIAERSVYQQFEKALI DKLNYLVFKDEEQSGYGGVLNAYQLTDKFESFSKMGQQTGFLFYVPAAY TSKIDPLTGFINPFSWKHVKNREDRRNFLNLFSKLYYDVNTHDFVLAYHH SNKDSKYTIKGNWEIADWDILIQENKEVFGKTGTPYCVGKRIVYMDDSTT GHNRMCAYYPHTELKKLLSEYGIEYTSGQDLLKIIQEFDDDKLVKGLFYII KAALQMRNSNSETGEDYISSPIEGRPGICFDSRAEADTLPYDADANGAFHI AMKGLLLTERIRNDDKLAISNEEWLNYIQEMRG
SEQ ID NO: 577	Cas12 variant	MNKDIRKNFTDFVGISEIQKTLRFILIPIGKTAQNIDKYNMFEDDEIRHEYY PILKEACDDFYRNHIDQQFENLELDWSKLDEALASEDRDLINETRATYRQ VLFNRLKNSVDIKGDSKKNKTLSLESSDKNLGKKKTKNTFQYNFNDLFK AKLIKAILPLYIEYIYEGEKLENAKKALKMYNRFTSRLSNFWQARANIFTD DEISTGSPYRLVNDNFTIFRINNSIYTKNKPFIEEDILEFEKKLKSKKIIKDFE SVDDYFTVNAFNKLCTQNGIDKYNSILGGFTTKEREKVKGLNELFNLAQQ SINKGKKGEYRKNIRLGKLTKLKKQILAISDSTSFLIEQIEDDQDLYNKIKD FFELLLKEEIENENIFTQYANLQKLIEQADLSKIYINAKHLNKISHQVTGKW DSLNKGIALLLENININEESKEKSEVISNGQTKDISSEAYKRYLQIQSEEKDI ERLRTQIYFSLEDLEKALDLVLIDENMDRSDKSILSYVQSPDLNVNFERDL TDLYSRIMKLEENNEKLLANHSAIDLIKEFLDLIMLRYSRWQILFCDSNYE LDQTFYPIYDAVMEILSNIIRLYNLARNYLSRKPDRMKKKKINFNNPTLAD GWSESKIPDNSSMLFIKDGMYYLGIIKNRAAYSELLEAESLQSSEKKKSEN

SEQ ID NO	Description	Sequence
		SSYERMNYHFLPDAFRSIPKSSIAMKAVKEHFEINQKTADLLLDTDKFSKP LRITKEIFDMQYVDLHKNKKKYQVDYLRDTGDKKGYRKALNTWLNFCK DFISKYKGRNLFDYSKIKDADHYETVNEFYNDVDKYSYHIFFTSVAETTV EKFISEGKLYLFQLYNKDFSPHSTGKPNLHTIYWRALFSEENLTSKNIKLN GQAEIFFRPKQIETPFTHKKGSILVNRFDVNGNPIPINVYQEIKGFKNNVIK WDDLNKTTQEGLENDQYLYFESEFEIIKDRRYTEDQLFFHVPISFNWDIGS NPKINDLATQYIVNSNDIHIIGIDRGENHLIYYSVIDLQGAIVEQGSLNTITE YTENKFLNNKTNNLRKIPYKDILQQREDERADARIKWHAIDKIKDLKDGY LGQIVHFLAKLIIKYNAIVILEDLNYGFKRGRFKVERQVYQKFEMALMKK LNVLVFKDYDIDEIGGPLKPWQLTRPIDSYERMGRQNGILFYVPAAYTSA VDPVTGFANLFYLNNVKNSEKFHFFSKFESIKYHSDQDMFSFAFDYNNFG TTTRINDLSKSKWQVFTNHERSVWNNKEKNYVTQNLTDLIKKLLRTYNIE FKNNQNVLDSILKIENNTDKENFARELFRLFRLTIQLRNTTVNENNTEITEN ELDYIISPVKDKNGNFFDSRDELKNLPDNGDANGAYNIARKGLLYIEQLQE SIKTGKLPTLSISTLDWFNYIMK
SEQ ID NO: 578	Cas12 variant	MTPIFCNFVVYQIMLFNNNININVKTMNKKHLSDFTNLFPVSKTLRFRLEP QGKTMENIVKAQTIETDEERSHDYEKTKEYIDDYHRQFIDDTLDKFAFKV ESTGNNDSLQDYLDAYLSANDNRTKQTEEIQTNLRKAIVSAFKMQPQFNL LFKKEMVKHLLPQFVDTDDKKRIVAKFNDFTTYFTGFFTNRENMYSDEA KSTSIAYRIVNQNLIKFVENMLTFKSHILPILPQEQLATLYDDFKEYLNVAS IAEMFELDHFSIVLTQRQIEVYNSVIGGRKDENNKQIKPGLNQYINQHNQA VKDKSARLPLLKPLFNQILSEKAGVSFLPKQFKSASEVVKSLNEAYAELSP VLAAIQDVVTNITDYDCNGIFIKNDLGLTDIAQRFYGNYDAVKRGLRNQY ELETPMHNGQKAEKYEEQVAKHLKSIESVSLAQINQVVTDGGDICDYFKA FGATDDGDIQRENLLASINNAHTAISPVLNKENANDNELRKNTMLIKDLL DAIKRLQWFAKPLLGAGDETNKDQVFYGKFEPLYNQLDETISPLYDKVRS YLTKKPYSLDKFKINFEKSNLLGGWDPGADRKYQYNAVILRKDNDFYLGI MRDEATSKRKCIQVLDCNDEGLDENFEKVEYKQIKPSQNMPRCAFAKKE CEENADIMELKRKKNAKSYNTNKDDKNALIRHYQRYLDRTYPEFGFVYK DADEYDTVKAFTDSMDSQDYKLSFLQVSETGLNKLVDEGDLYLFKITNK DFSSYAKGRPNLHTIYWRMLFDPKNLANVVYKLEGKAEVFFRRKSLAST TTHKAKQAIKNKSRYNEAVKPQSTFDYDIIKDRRFTADKFEFHVPIKMNF KAAGWNSTRLTNEVREFIKSQGVRHIIGIDRGERHLLYLTMIDMDGNIVK QCSLNAPAQDNARASEVDYHQLLDSKEADRLAARRNWGTIENIKELKQG YLSQVVHLLATMMVDNDAILVLENLNAGFMRGRQKVEKSVYQKFEKML IDKLNYIVDKGQSPDKPTGALHAVQLTGLYSDFNKSNMKRANVRQCGFV FYIPAWNTSKIDPVTGFVNLFDTHLSSMGEIKAFFSKFDSIRYNQDKGWFE FKFDYSRFTTRAEGCRTQWTVCTYGERIWTHRSKNQNNQFVNDTVNVTQ QMLQLLQDCGIDPNGNLKEAIANIDSKKSLETLLHLFKLTVQMRNSVTGS EVDYMISPVADERGHFFDSRESDEHLPANADANGAFNIARKGLMVVRQI MATDDVSKIKFAVSNKDWLRFAQHIDD
SEQ ID NO: 579	Cas12 variant	MNKGGYVIMEKMTEKNRWENQFRITKTIKEEIIPTGYTKVNLQRVNMLK REMERNEDLKKMKEICDEYYRNMIDVSLRLEQVRTLGWESLIHKYRMLN KDEKEIKALEKEQEDLRKKISKGFGEKKAWTGEQFIKKILPQYLMDHYTG EELEEKLRIVKKFKGCTMFLSTFFKNRENIFTDKPIHTAVGHRITSENAMLF AANINTYEKMESNVTLEIERLQREFWRRGINISEIFTDAYYVNVLTQKQIE AYNKICGDINQHMNEYCQKQKLKFSEFRMRELKKQILAVVGEHFEIPEKI ESTKEVYRELNEYYESLKELHGQFEELKSVQLKYSQIYVQKKGYDRISRYI GGQWDLIQECMKKDCASGMKGTKKNHDAKIEEEVAKVKYQSIEHIQKLV CTYEEDRGHKVTDYVDEFIVSVCDLLGADHIITRDGERIELPLQYEPGTDL LKNDTINQRRLSDIKTILDWHMDMLEWLKTFLVNDLVIKDEEFYMAIEEL NERMQCVISVYNRIRNYVTQKGYEPEKIRICFDKGTILTGWTTGDNYQYS GFLLMRNDKYYLGIINTNEKSVRKILDGNEECKDENDYIRVGYHLINDAS

SEQ ID NO	Description	Sequence
		KQLPRIFVMPKAGKKSEILMKDEQCDYIWDGYCHNKHNESKEFMRELID YYKRSIMNYDKWEGYCFKFSSTESYDNMQDFYKEVREQSYNISFSYINEN VLEQLDKDGKIYLFQVYNKDFAAGSTGTPNLHTMYLQNLFSSQNLELKR LRLGGNAELFYRPGTEKDVTHRKGSILVDRTYVREEKDGIEVRDTVPEKE YLEIYRYLNGKQKGDLSESAKQWLDKVHYREAPCDIIKDKRYAQEKYFL HFSVEINPNAEGQTALNDNVRRWLSEEEDIHVIGIDRGERNLIYVSLMDGK GRIKDQKSYNIVNSGNKEPVDYLAKLKVREKERDEARRNWKAIGKIKDIK TGYLSYVVHEIVEMAVREKAIIVMEDLNYGFKRGRFKVERQVYQKFEEM LINKLNYVVDKQLSVDEPGGLLRGYQLAFIPKDKKSSMRQNGIVFYVPAG YTSKIDPTTGFVNIFKFPQFGKGDDDGNGKDYDKIRAFFGKFDEIRYECDE KVTADNTREVKERYRFDFDYSKFETHLVHMKKTKWTVYAEGERIKRKK VGNYWTSEVISDIALRMSNTLNIAGIEYKDGHNLVNEICALRGKQAGIILN ELLEIVRLTVQLRNSTTEGDVDERDEIISPVLNEKYGCFYHSTEYKQQNGD VLPKDADANGAYCIGLKGIYEIRQIKNKWKEDMTKGEGKALNEGMRISH DQWFEFIQNMNKGE
SEQ ID NO: 580	Cas12 variant	MNELVKNRCKQTKTICQKLIPIGKTRETIEKYNLMEIDRKIAANKELMNKL FSLIAGKHINDTLSKCTDLDFEPLLTSLSSLNNAKENDRDNLREYYDSVFE EKKTLAEEISSRLTAVKFAGKDFFTKNIPDFLETYEGDDKNEMSELVSLVI ENTVTAGYVKKLEKIDRSMEYRLVSGTVVKRVLTDNADIYEKNIEKAKD FDYGVLNIDEASQFTTLVAKDYANYLTADGIAIYNVGIGKINLALNEYCQ KNKEYSYNKLALLPLQKMLYGEKLSLFEKLEDFTSDEELINSYNKFAKTV NESGLAEIIKKAVPSYDEIVIKPNKISNYSNSITGHWSLVNRIMKDYLENNG IKNADKYMEKGLTLSEIGDALENKNIKHSDFISNLINDLGHTYTEIKENKES LKKDESVNALIIKKELDMLLSILQNLKVFDIDNEMFDTGFGIEVSKAIEILG YGVPLYNKIRNYITKKPDPKKKFMTKFGSATIGTGITTSVEGSKKATFLKD GDAVFLLLYNTAGCKANNVSVSNLADLINSSLEIENSGKCYQKMIYQTPG DIKKQIPRVFVYKSEDDDLIKDFKAGLHKTDLSFLNGRLIPYLKEAFATHE TYKNYTFSYRNSYESYDEFCEHMSEQAYILEWKWIDKKLIDDLVEDGSLL MFRVWNRFMKKKEGKISKHAKIVNELFSDENASNAAIKLLSVFDIFYRDK QIDNPIVHKAGTTLYNKRTKDGEVIVDYTTMVKNKEKRPNVYTTTKKYDI IKDRRYTEEQFEIHLHVNIGKEENKEKLETSKVINEKKNTLVVTRSNEHLL YVVIFDENDNILLKKSLNTVKGMNFKSKLEVVEIQKKENMQSWKTVGSN QALMEGYLSFAIKEIADLVKEYDAILVLEQNSVGKNILNERVYTRFKEMLI TNLSLDVDYENKDFYSYTELGGKVASWRDCVTNGICIQVPSAYKYKDPT TSFSTISMYAKTTAEKSKKLKQIKSFKYNRERGLFELVIAKGVGLENNIVC DSFGSRSIIENDISKEVSCTLKIEKYLIDAGIEYNDEKEVLKDLDTAAKTDA VHKAVTLLLKCFNESPDGRYYISPCGEHFTLCDAPEVLSAINYYIRSRYIRE QIVEGVKKMEYKKTILLAK
SEQ ID NO: 581	Cas12 variant	MNYKTGLEDFIGKESLSKTLRNALIPTESTKIHMEEMGVIRDDELRAEKQQ ELKEIMDDYYRTFIEEKLGQIQGIQWNSLFQKMEETMEDISVRKDLDKIQN EKRKEICCYFTSDKRFKDLFNAKLITDILPNFIKDNKEYTEEEKAEKEQTRV LFQRFATAFTNYFNQRRNNFSEDNISTAISFRIVNENSEIHLQNMRAFQRIE QQYPEEVCGMEEEYKDMLQEWQMKHIYSVDFYDRELTQPGIEYYNGICG KINEHMNQFCQKNRINKNDFRMKKLHKQILCKKSSYYEIPFRFESDQEVY DALNEFIKTMKKKEIIRRCVHLGQECDDYDLGKIYISSNKYEQISNALYGS WDTIRKCIKEEYMDALPGKGEKKEEKAEAAAKKEEYRSIADIDKIISLYGS EMDRTISAKKCITEICDMAGQISIDPLVCNSDIKLLQNKEKTTEIKTILDSFL HVYQWGQTFIVSDIIEKDSYFYSELEDVLEDFEGITTLYNHVRSYVTQKPY STVKFKLHFGSPTLANGWSQSKEYDNNAILLMRDQKFYLGIFNVRNKPD KQIIKGHEKEEKGDYKKMIYNLLPGPSKMLPKVFITSRSGQETYKPSKHIL DGYNEKRHIKSSPKFDLGYCWDLIDYYKECIHKHPDWKNYDFHFSDTKD YEDISGFYREVEMQGYQIKWTYISADEIQKLDEKGQIFLFQIYNKDFSVHS TGKDNLHTMYLKNLFSEENLKDIVLKLNGEAELFFRKASIKTPIVHKKGSV

SEQ ID NO	Description	Sequence
		LVNRSYTQTVGNKEIRVSIPEEYYTEIYNYLNHIGKGKLSSEAQRYLDEGK IKSFTATKDIVKNYRYCCDHYFLHLPITINFKAKSDVAVNERTLAYIAKKE DIHIIGIDRGERNLLYISVVDVHGNIREQRSFNIVNGYDYQQKLKDREKSR DAARKNWEEIEKIKELKEGYLSMVIHYIAQLVVKYNAVVAMEDLNYGFK TGRFKVERQVYQKFETMLIEKLHYLVFKDREVCEEGGVLRGYQLTYIPES LKKVGKQCGFIFYVPAGYTSKIDPTTGFVNLFSFKNLTNRESRQDFVGKFD EIRYDRDKKMFEFSFDYNNYIKKGTILASTKWKVYTNGTRLKKIVVNGKY TSQSMEVELTDAMEKMLQRAGIEYHDGKDLKGQIVEKGIEAEIIDIFRLTV QMRNSRSESEDREYDRLISPVLNDKGEFFDTATADKTLPQDADANGAYCI ALKGLYEVKQIKENWKENEQFPRNKLVQDNKTWFDFMQKKRYL
SEQ ID NO: 582	Cas12 variant	MEDKQFLERYKEFIGLNSLSKTLRNSLIPVGSTLKHIQEYGILEEDSLRAQK REELKGIMDDYYRNYIEMHLRDVHDIDWNELFEALTEVKKNQTDDAKKR LEKIQEKKRKEIYQYLSDDAVFSEMFKEKMISGILPDFIRCNEGYSEEEKEE KLKTVALFHRFTSSFNDFFLNRKNVFTKEAIVTAIGYRVVHENAEIFLENM VAFQNIQKSAESQISIIERKNEHYFMEWKLSHIFTADYYMMLMTQKAIEH YNEMCGVVNQQMREYCQKEKKNWNLYRMKRLHKQILSNASTSFKIPEK YENDAEVYESVNSFLQNVMEKTVMERIAVLKNSTDNFDLSKIYITAPYYE KISNYLCGSWNTITDCLTHYYEQQIAGKGARKDQKVKAAVKADKWKSLS EIEQLLKEYARAEEVKRKPEEYIAEIENIVSLKEAHLLEYHPEVNLIENEKY ATEIKDVLDNYMELFHWMKWFYIEEAVEKEVNFYGELDDLYEEIKDIVPL YNKVRNYVTQKPYSDTKIKLNFGTPTLANGWSKSKEYDYNAILLQKDGK YYMGIFNPIQKPEKEIIEGHSQPLEGNEYKKMVYYYLPSANKMLPKVLLS KKGMEIYQPSEYIINGYKERRHIKSEEKFDLQFCHDLIDYFKSGIERNSDW KVFGFDFSDTDTYQDISGFYREVEDQGYKIDWTYIKEADIDRLNEEGKLY LFQIYNKDFSEKSTGRENLHTMYLKNLFSEENVREQVLKLNGEAEIFFRKS SVKKPIIHKKGTMLVNRTYMEEVNGNSVRRNIPEKEYQEIYNYKNHRLKG ELSTEAKKYLEKAVCHETKKDIVKDYRYSVDKFFIHLPITINYRASGKETL NSVAQRYIAHQNDMHVIGIDRGERNLIYVSVINMQGEIKEQKSFNIINEFN YKEKLKEREQSRGAARRNWKEIGQIKDLKEGYLSGVIHEIAKMMIKYHAI IAMEDLNYGFKRGRFKVERQVYQKFENMLIQKLNYLVFKDRPADEDGGV LRGYQLAYIPDSVKKMGRQCGMIFYVPAAFTSKIDPTTGFVDIFKHKVYT TEQAKREFILSFDEICYDVERQLFRFTFDYANFVTQNVTLARNNWTIYTNG TRAQKEFGNGRMRDKEDYNPKDKMVELLESEGIEFKSGKNLLPALKKVS NAKVFEELQKIVRFTVQLRNSKSEENDVDYDHVISPVLNEEGNFFDSSKY KNKEEKKESLLPVDADANGAYCIALKGLYIMQAIQKNWSEEKALSPDVL RLNNNDWFDYIQNKRYR
SEQ ID NO: 583	Cas12 variant	MEKSLNDFIGLYSVSKTLRFELKPVSETLENIKKFHFLEEDKKKANDYKD VKKIIDNYHKYFIDDVLKNASFNWKKLEEAIREYNKNKSDDSALVAEQK KLGDAILKLFTSDKRYKALTAATPKELFESILPDWFGEQCNQDLNKAALK TFQKFTSYFTGFQENRKNVYSAEAIPTAVPYRIVNDNFPKFLQNVLIFKTIQ EKCPQIIDEVEKELSSYLGKEKLAGIFTLESFNKYLGQGGKENQRGIDFYN QIIGGVVEKEGGINLRGVNQFLNLYWQQHPDFTKEDRRIKMVPLYKQILS DRSSLSFKIESIENDEELKNALLECADKLELKNDEKKSIFEEVCDLFSSVKN LDLSGIYINRKDINSVSRILTGDWSWLQSRMNVYAEEKFTTKAEKARWQK SLDDEGENKSKGFYSLTDLNEVLEYSSENVAETDIRITDYFEHRCRYYVD KETEMFVQGSELVALSLQEMCDDILKKRKAMNTVLENLSSENKLREKTD DVAVIKEYLDAVQELLHRIKPLKVNGVGDSTFYSVYDSIYSALSEVISVYN KTRNYITKKAASPEKYKLNFDNPTLADGWDLNKEQANTSVILRKDGMFY LGIMNPKNKPKFAEKYDCGNESCYEKMIYKQFDATKQIPKCSTQKKEVQ KYFLSGATEPYILNDKKSFKSELIITKDIWFMNNHVWDGEKFVPKRDNET RPKKFQIGYFKQTGDFDGYKNALSNWISFCKNFLQSYLSATVYDYNFKNS EEYEGLDEFYNYLNATCYKLNFINIPETEINKMVSEGKLYLFQIYNKDFAS GSTGMPNMHTLYWKNLFSDENLKNVCLKLNGEAELFYRPAGIKEPVIHK

SEQ ID NO	Description	Sequence
		EGSYLVNRTTEDGESIPEKIYFEIYKNANGKLEKLSDEAQNYISNHEVVIK KAGHEIIKDRHYTEPKFLFHVPLTINFKASGNSYSINENVRKFLKNNPDVNI IGLDRGERHLIYLSLINQKGEIIKQFTFNEVERNKNGRTIKVNYHEKLDQRE KERDAARKSWQAIGKIAELKEGYLSAVIHQLTKLMVEYNAVVVMEDLNF GFKRGRFHVEKQVYQKFEHILIDKSNYLVFKDRGLNEPGGVLNGYQIAGQ FESFQKLGKQSGMLFYVPAGYTSKIDPKTGFVSMMNFKDLTNVHKKRDF FSKFDNIHYDEANGSFVFTFDYKKFDGKAKEEMKLTKWSVYSRDKRIVY FAKTKSYEDVLPTEKLQKIFESNGIDYKSGNNIQDSVMAIGADLKEGAKPS KEISDFWDGLLSNFKLILQMRNSNARTGEDYIISPVMADDGTFFDSREEFK KGEDAKLPLDADANGAYHIALKGLSLINKINLSKDEELKKFDMKISNADW FKFAQEKNYAK
SEQ ID NO: 584	Cas12 variant	MEEKKMSKIEKFIGKYKISKTLRFRAVPVGKTQDNIEKKGILEKDKKRSED YEKVKAYLDSLHRDFIENTLKKVKLNELNEYACLFFSGTKDDGDKKKME KLEEKMRKTISNEFCNDEMYKKIFSEKILSENNEEDVSDIVSSYKGFFTSLN GYVNNRKNLYVSDAKPTSIAYRCINENLPKFLRNVECYKKVVQVIPKEQI EYMSNNLNLSPYRIEDCFNIDFFEFCLSQGGIDLYNTFIGGYSKKDGTKVQ GINEIVNLYNQKNKKDKEKYKLPQFTPLFKQILSDRDTKSFSIEKLENIYEV VELVKKSYSDEMFDDIETVFSNLNYYDASGIYVKNGPAITHISMNLTKDW ATIRNNWNYEYDEKHSTKKNKNIEKYEDTRNTMYKKIDSFTLEYISRLVG KDIDELVKYFENEVANFVMDIKKTYSKLTPLFDRCQKENFDISEDEVNDIK GYLDNVKLLESFMKSFTINGKENNIDYVFYGKFTDDYDKLHEFDHIYNKV RNYITTSRKPYKLDKYKLYFDNPQLLGGWDINKEKDYRTVMLTKDGKYY FAIIDKGEHPFDNIPKDYFDNNGYYKKIIYRQIPNAAKYLSSKQIVPQNPPE EVKRILDKKKADSKSLTEEEKNIFIDYIKSDFLKNYKLLFDKNNNPYFNFA FRESSTYESLNEFFEDVERQAYSVRYENLPADYIDNLVNEGKIYLFEIYSK DFSEYSKGTNNLHTMYFKALFDNDNLKNTVFKLSGNAELFIRPASIKKDE LVIHPKNQLLQNKNPLNPKKQSIFDYDLVKDKRFFENQYMLHISIEINKNE RDAKKIKNINEMVRKELKDSDDNYIIGIDRGERNLLYVCVINSAGKIVEQM SLNEIINEYNGIKHTVDYQGLLDKCEKERNAQRQSWKSIENIKELKDGYIS QVVHKLCQLVEKYDAIIAMENLNGGFKRGRTKFEKQVYQKFENKLINKM EYMADKKRKTTENGGILRGYQLTNGCINNSYQNGFIFYVPAWLTSKIDPT TGFVDLLKPKYTNVEEAHLWINKFNSITYDKKLDMFAFNINYSQFPRADI DYRKIWTFYTNGYRIETFRNSEKNNEFDWKEVHLTSVIKKLLEEYQINYIS GKNIIDDLIQIKDKPFWNSFIKYIRLTLQMRNSITGRTDVDYIISPVINNEGT FYDSRKDLDEITLPQDADANGAYNIARKALWIIEKLKESPDEELNKVKLAI TQREWLEYAQINI
SEQ ID NO: 585	Cas12 variant	MIIHNCYIGGSFMKKIDSFTNCYSLSKTLRFKLIPIGATQSNFDLNKMLDED KKRAENYSKAKSIIDKYHRFFIDKVLSSVTENKAFDSFLEDVRAYAELYY RSNKDDSDKASMKTLESKMRKFIALALQSDEGFKDLFGQNLIKKTLPEFL ESDTDKEIIAEFDGFSTYFTGFFNNRKNMYSADDQPTAISYRCINDNLPKFL DNVRTFKNSDVASILNDNLKILNEDFDGIYGTSAEDVFNVDYFPFVLSQK GIEAYNSILGGYTNSDGSKIKGLNEYINLYNQKNENIHRIPKMKQLFKQILS ERESVSFIPEKFDSDDDVLSSINDYYLERDGGKVLSIEKTVEKIEKLFSAVT DYSTDGIFVKNAAELTAVCSGAFGYWGTVQNAWNNEYDALNGYKETEK YIDKRKKAYKSIESFSLADIQKYADVSESSETNAEVTEWLRNEIKEKCNLA VQGYESSKDLISKPYTESKKLFNNDNAVELIKNALDSVKELENVLRLLLGT GKEESKDENFYGEFLPCYERICEVDSLYDKVRNYMTQKLYKTDKIKLNFQ NPQFLGGWDRNKEADYSAVLLRRNSLYYIAIMPSGYKRVFEKIPAPKADE TVYEKVIYKLLPGPNKMLPKVFFSKKGIETFNPPKEILEKYELGTHKTGDG FNLDDCHALIDYFKSALDVHSDWSNFGFRFSDTSTYKNIADFYNEVKNQG YKITFCDVPQSYINELVDEGKLYLFQLYNKDFSEHSKGTPNLHTLYFKML FDERNLENVVFKLNGEAEMFYREASISKDDMIVHPKNQPIKNKNEQNSRK QSTFEYDIVKDRRYTVDQFMLHIPITLNFTANGGTNINNEVRKALKDCDK

SEQ ID NO	Description	Sequence
		NYVIGIDRGERNLLYICVVDSEGRIIEQYSLNEIINEYNGNTYSTDYHALLD KKEKERLESRKAWKTVENIKELKEGYISQVVHKICELVEKYDAVIVMEDL NLGFKQGRSGKFEKSVYQKFEKMLIDKLNYFADKKKSPEEIGSVLNAYQL TNAFESFEKMGKQNGFIFYVPAYLTSKIDPTTGFADLLHPSSKQSKESMRD FVGRFDSITFNKTENYFEFELDYNKFPRCNTDYRKKWTVCTYGSRIKTFR NPEKNSEWDNKTVELTPAFMALFEKYSIDVNGDIKAQIMSVDKKDFFVEL IGLLRLTLQMRNSETGKVDRDYLISPVKNSEGVFYNSDDYKGIENASLPK DADANGAYNIARKGLWIIEQIKACENDAELNKIRLAISNAEWLEYAQKK
SEQ ID NO: 586	Cas12 variant	MKEQFVNQYPISKTLRFSLIPIGKTEENFNKNLLLKEDEKKAEEYQKVKGY IDRYHKFFIETALCNINFEGFEEYSLLYYKCSKDDNDLKTMEDIEIKLRKQI SKTMTSHKLYKDLFGENMIKTILPNFLDSDEEKNSLEMFRGFYTYFSGFNT NRKNMYTEEAKSTSIAYRCINDNLPKFLDNSKSFEKIKCALNKEELKAKN EEFYEIFQIYATDIFNIDFFNFVLTQPGIDKYNGIIGGYTCSDGTKVQGLNEII NLYNQQIAKDDKSKRLPLLKMLYKQILSDRETVSFIPEKFSSDNEVLESIN NYFSKNVSNAIKSLKELFQGFEAYNMNGIFISSGVAITDLSNAVFGDWNAI STAWEKAYFETNPPKKNKSQEKYEEELKANYKKIKSFSLDEIQRLGSIAKS PDSIGSVAEYYKITVTEKIDNITELYDGSKELLNCNYSESYDKKLIKNDTVI EKVKTLLDAVKSLEKLIKPLVGTGKEDKDELFYGTFLPLYTSLSAVDRLY DKVRNYATQKPYSKDKIKLNFNCSSFLSGWATDYSSNGGLIFEKDGLYYL GIVNKKFTTEEIDYLQQNADENPAQRIVYDFQKPDNKNTPRLFIRSKGTNY SPSVKEYNLPVEEIVELYDKRYFTTEYRNKNPELYKASLVKLIDYFKLGFT RHESYRHYDFKWKKSEEYNDISEFYKDVEISCYSLKQEKINYNTLLNFVA ENRIYLFQIYNKDFSKYSKGTPNLHTRYFKALFDENNLSDVVFKLNGGSE MFFRKASIKDNEKVVHPANQPIDNKNPDNSKKQSTFDYELIKDKRFTKHQ FSIHIPITMNFKARGRDFINNDIRKAIKSEYKPYVIGIDRGERNLIYISVINNN GEIVEQMSLNDIISDNGYKVDYQRLLDRKEKERDNARKSWGTIENIKELK EGYISQVIHKICELVIKYDAVIAMEDLNFGFKRGRFNVEKQVYQKFENMLI SKLNYLCDKKSEANSEGGLLKAYQLTNKFDGVNKGKQNGIIFYVPAWLT SKIDPVTGFVDLLHPKYISVEETHSLFEKLDDIRYNFEKDMFEFDIDYSKLP KCNADFKQKWTVCTNADRIMTFRNSEKNNEWDNKRILLSDEFKRLFEEF GIDYCHNLKNKILSISNKDFCYRFIKLFALTMQMRNSITGSTNPEDDYLISP VRDENGVFYDSRNFIGSKAGLPIDADANGAYNIARKGLWAINAIKSTADD MLDKVDLSISNAKWLEYVQK
SEQ ID NO: 587	Cas12 variant	MADLSQFTHKYQVPKTLRFELIPQGKTLENLSAYGMVADDKQRSENYKK LKPVIDRIYKYFIEESLKNTNLDWNPLYEAIREYRKEKTTATITNLKEQQDI CRRAIASRFEGKVPDKGDKSVKDFNKKQSKLFKELFGKELFTDSVLEQLP GVSLSDEDKALLKSFDKFTTYFVGFYDNRKNVFSSDDISTGIPHRLVQENF PKFIDNCDDYKRLVLVAPELKEKLEKAAEATKIFEDVSLDEIFSIKFYNRLL QQNQIDQFNQLLGGIAGAPGTPKIQGLNETLNLSMQQDKTLEQKLKSVPH RFSPLYKQILSDRSSLSFIPESFSCDAEVLLAVQEYLDNLKTEHVIEDLKEV FNRLTTLDLKHIYVNSTKVTAFSQALFGDWNLCREQLRVYKMSNGNEKI TKKALGELESWLKNSDIAFTELQEALADEALPAKVNLKVQEAISGLNEQM AKSLPKELKIPEEKEELKALLDAIQEVYHTLEWFIVSDDVETDTDFYVPLK ETLQIIQPIIPLYNKVRNFATQKPYSVEKFKLNFANPTLADGWDENKEQQN CAVLFQKGNNYYLGILNPKNKPDFDNVDTEKQGNCYQKMVYKQFPDFS KMMPKCTTQLKEVKQHFEGKDSDYILNNKNFIKPLTITREVYDLNNVLYD GKKKFQIDYLRKTKDEDGYYTALHTWIDFAKKFVASYKSTSIYDTSTILPP EKYEKLNEFYGALDNLFYQIKFENIPEEIIDTYVEDGKLFLFQIYNKDFAAG ATGAPNLHTIYWKAVFDPENVKDVVVKLNGQAELFYRPKSNMDVIRHK VGEKLVNRTLKDGSILTDELHKELYLYANGSLKKGLSEDAKIILDKNLAVI YDVHHEIVKDRRFTTDKFFFHVPLTLNYKCDKNPVKFNAEVQEYLKENP DTYVIGIDRGERNLIYAVVIDPKGRIVEQKSFNVINGFDYHGKLDQREKER VKARQAWTAVGKIKELKQGYLSLVVHEISKMMVRYQAVVVLENLNVGF

SEQ ID NO	Description	Sequence
		KRVRSGIAEKAVYQQFEKMLINKLNYLMFKDAGGTEPGSVLNAYQLTDR FESFAKMGLQTGFLFYIPAAFTSKIDPATGFVDPFRWGAIKTLADKREFLS GFESLKFDSTTGNFILHFDVSKNKNFQKKLEGFVPDWDIIIEANKMKTGKG ATYIAGKRIEFVRDNNSQGHYEDYLPCNALAETLRQCDIPYEEGKDILPLIL EKNDSKLLHSVFKVVRLTLQMRNSNAETGEDYISSPVEDVSGSCFDSRME NEKLPKDADANGAYHIALKGMLALERLRKDEKMAISNNDWLNYIQEKR A
SEQ ID NO: 588	Cas12 variant	MTNFDNFTKKYVNSKTIRLEAIPVGKTLKNIEKMGFIAADRQRDEDYQKA KSVIDHIYKAFMDDCLKDLFLDWDPLYEAVVACWRERSPEGRQALQIMQ ADYRKKIADRFRNHELYGSLFTKKIFDGSVAQRLPDLEQSAEEKSLLSNFN KFTSYFRDFFDKRKRLFSDDEKHSAIAYRLINENFLKFVANCEAFRRMTER VPELREKLQNTGSLQVYNGLALDEVFSADFYNQLIVQKQIDLYNQLIGGIA GEPGTPNIQGLNATINLALQGDSSLHEKLAGIPHRFNPLYKQILSDVSTLSF VPSAFQSDGEMLAAVRGFKVQLESGRVLQNVRRLFNGLETEADLSRVYV NNSKLAAFSSMFFGRWNLCSDALFAWKKGKQKKITNKKLTEIKKWLKNS DIAIAEIQEAFGEDFPRGKINEKIQAQADALHSQLALPIPENLKALCAKDGL KSMLDTVLGLYRMLQWFIVGDDNEKDSDFYFGLGKILGSLDPVLVLYNR VRNYITKKPYSLTKFRLNFDNSQLLNGWDENNLDTNCASIFIKDGKYYLG ISNKNNRPQFDTVATSGKSGYQRMVYKQFANWGRDLPHSTTQMKKVKK HFSASDADYVLDGDKFIRPLIITKEIFDLNNVKFNGKKKLQVDYLRNTGD REGYTHALHTWINFAKDFCACYKSTSIYDISCLRPTDQYDNLMDFYADLG NLSHRIVWQTIPEEAIDNYVEQGQLFLFQLYNKDFAPGADGKPNLHTLYW KAVFNPENLEDVVVKLNGKAELFYRPRSNMDVVRHKVGEKLVNRKLKN GLTLPSRLHEEIYRYVNGTLNKDLSADARSVLPLAVVRDVQHEIIKDRRFT ADKFFFHASLTFNFKSSDKPVGFNEDVREYLREHPDTYVVGVDRGERNLI YIVVIDPQGNIVEQRSFNMINGIDYWSLLDQKEKERVEAKQAWETVGKIK DLKCGYLSFLIHEITKIIIKYHAVVILENLSLGFKRVRTGIAEKAVYQQFER MLVTKLGYVVFKDRAGKAPGGVLNAYQLTDNTRTAENTGIQNGFLFYVP AAFTSRVDPATGFFDFYDWGKIKTATDKKNFIAGFNSVRYERSTGDFIVH VGAKNLAVRRVAEDVRTEWDIVIEANVRKMGIDGNSYISGKRIRYRSGEQ GHGQYENHLPCQELIRALQQYGIQYETGKDILPAILQQDDAKLTDTVFDV FRLALQMRNTSAETGEDYFNSVVRDRSGRCFDTRRAEAAMPKEADAND AYHIALKGLFVLEKLRKGESIGIKNTEWLRYVQQRHS
SEQ ID NO: 589	Cas12 variant	MENYGGFTGLYPLQKTLKFELRPQGRTMEHLVSSNFFEEDRDRAEKYKIV KKVIDNYHKDFINECLSKRSFDWTPLMKTSEKYYASKEKNGKKKQDLDQ KIIPTIENLSEKDRKELELEQKRMRKEIVSVFKEDKRFKYLFSEKLFSILLKD EDYSKEKLTEKEILALKSFNKFSGYFIGLHKNRANFYSEGDESTAIAYRIV NENFPKFLSNLKKYREVCEKYPEIIQDAEQSLAGLNIKMDDIFPMENFNKV MTQDGIDLYNLAIGGKAQALGEKQKGLNEFLNEVNQSYKKGNDRIRMTP LFKQILSERTSYSYILDAFDDNSQLITSINGFFTEVEKDKEGNTFDRAVGLI ASYMKYDLSRVYIRKADLNKVSMEIFGSWERLGGLLRIFKSELYGDVNAE KTSKKVDKWLNSGEFSLSDVINAIAGSKSAETFDEYILKMRVARGEIDNA LEKIKCINGNFSEDENSKMIIKAILDSVQRLFHLFSSFQVRADFSQDGDFYA EYNEIYEKLFAIVPLYNRVRNYLTKNNLSMKKIKLNFKNPALANGWDLN KEYDNTAVIFLREGKYYLGIMNPSKKKNIKFEEGSGTGPFYKKMAYKLLP DPNKMLPKVFFAKKNINYYNPSDEIVKGYKAGKYKKGENFDIDFCHKLID FFKESIQKNEDWRAFNYLFSATESYKDISDFYSEVEDQGYRMYFLNVPVA NIDEYVEKGDLFLFQIYNKDFASGAKGNKDMHTIYWNAAFSDENLRNVV VKLNGEAELFYRDKSIIEPICHKKGEMLVNRTCFDKTPVPDKIHKELFDYH NGRAKTLSIEAKGYLDRVGVFQASYEIIKDRRYSENKMYFHVPLKLNFKA DGKKNLNKMVIEKFLSDKDVHIIGIDRGERNLLYYSVIDRRGNIIDQDSLNI IDGFDYQKKLGQREIERREARQSWNSIGKIKDLKEGYLSKAVHKVSKMVL EYNAIVVLEDLNFGFKRGRFKVEKQVYQKFEKMLIDKLNYLVFKEVLDS

SEQ ID NO	Description	Sequence
		RDAGGVLNAYQLTTQLESFNKLGKQSGILFYVPAAYTSKIDPTTGFVSLFN TSRIESDSEKKDFLSGFDSIVYSAKDGGIFAFKFDYRNRNFQREKTDHKNI WTVYTNGDRIKYKGRMKGYEITSPTKRIKDVLSSSGIRYDDGQELRDSIIQ SGNKVLINEVYNSFIDTLQMRNSDGEQDYIISPVKNRNGEFFRTDPDRREL PVDADANGAYHIALRGELLMQKIAEDFDPKSDKFTMPKMEHKDWFEFM QTRGD
SEQ ID NO: 590	Cas12 variant	MLHAFTNQYQLSKTLRFGATLKEDEKKCKSHEELKGFVDISYENMKSSA TIAESLNENELVKKCERCYSEIVKFHNAWEKIYYRTDQIAVYKDFYRQLS RKARFDAGKQNSQLITLASLCGMYQGAKLSRYITNYWKDNITRQKSFLK DFSQQLHQYTRALEKSDKAHTKPNLINFNKTFMVLANLVNEIVIPLSNGAI SFPNISKLEDGEESHLIEFALNDYSQLSELIGELKDAIATNGGYTPFAKVTL NHYTAEQKPHVFKNDIDAKIRELKLIGLVETLKGKSSEQIEEYFSNLDKFST YNDRNQSVIVRTQCFKYKPIPFLVKHQLAKYISEPNGWDEDAVAKVLDA VGAIRSPAHDYANNQEGFDLNHYPIKVAFDYAWEQLANSLYTTVTFPQE MCEKYLNSIYGCEVSKEPVFKFYADLLYIRKNLAVLEHKNNLPSNQEEFIC KINNTFENIVLPYKISQFETYKKDILAWINDGHDHKKYTDAKQQLGFIRGG LKGRIKAEEVSQKDKYGKIKSYYENPYTKLTNEFKQISSTYGKTFAELRD KFKEKNEITKITHFGIIIEDKNRDRYLLASELKHEQINHVSTILNKLDKSSEFI TYQVKSLTSKTLIKLIKNHTTKKGAISPYADFHTSKTGFNKNEIEKNWDNY KREQVLVEYVKDCLTDSTMAKNQNWAEFGWNFEKCNSYEDIEHEIDQKS YLLQSDTISKQSIASLVEGGCLLLPIINQDITSKERKDKNQFSKDWNHIFEG SKEFRLHPEFAVSYRTPIEGYPVQKRYGRLQFVCAFNAHIVPQNGEFINLK KQIENFNDEDVQKRNVTEFNKKVNHALSDKEYVVIGIDRGLKQLATLCVL DKRGKILGDFEIYKKEFVRAEKRSESHWEHTQAETRHILDLSNLRVETTIE GKKVLVDQSLTLVKKNRDTPDEEATEENKQKIKLKQLSYIRKLQHKMQT NEQDVLDLINNEPSDEEFKKRIEGLISSFGEGQKYADLPINTMREMISDLQ GVIARGNNQTEKNKIIELDAADNLKQGIVANMIGIVNYIFAKYSYKAYISL EDLSRAYGGAKSGYDGRYLPSTSQDEDVDFKEQQNQMLAGLGTYQFFE MQLLKKLQKIQSDNTVLRFVPAFRSADNYRNILRLEETKYKSKPFGVVHFI DPKFTSKKCPVCSKTNVYRDKDDILVCKECGFRSDSQLKERENNIHYIHN GDDNGAYHIALKSVENLIQMK
SEQ ID NO: 591	Cas12 variant	MKNGINLFKTKTTKTKGVDMEKYQITKTIRFKLLPDNAHEIVEKVKSLKT SNVDELMDEVKNVHLKGLELLFALKKYFYFDGNQCKSFKSTLEIKARWL RLYTPDQYYLKKSSKNSYQLKSLSYFKDVFNDWLFNWEESVSELAIIYEK YKICQHQRDSRADIALLIKKLSMKEYFPFISDLIDCVNDKNSNKTFLMKLS EELSVLLEKCNSRALPYQSNGIVVGKASLNYYTVSKSEKMLQNEYEDVC QSLDKNYDITEMKVILYKEKLDNLNFKDVTIANAYNLLKENKALQKRLFS EYVSQGKVLSLIKTELPLFSNINDNDFEKYKEWSNEIKKLADKKNTFCKKT QQDKIKDIQNKISELKKKRGALFQYKFTSFQKHCDNYKKVAVQYGKLKA RKKAIEKDEIEANLLRYWSVILEQEDKHSLVLIPKNNAKDAKQYIETINTK GGKYIIHHLDSLTLRALNKLCFNAVDIEKGQMVRENTFYQGIKEEFERNKI NCDNQGVLKIQGLYSFKTEGGQINEKEAVEFFKEVLKSNYAREVLNLPYD LESNIFQKEYTNLDQFRQDLEKCCYALHSKIGKDDLDEFTRRFEAQVFDIT SIDLKSKKEKTKTTGEMKKHTQLWLEFWKGAIEQNFATRVNPELSIFWRA PKSSREKKYGKGSDLYDPNKNNRYLYEQYTLALTITENAGSHFKDIAFKD TSKIKEAIKEFNMSLSQSKYCFGIDRGNAELVSLCLIKNEKDFPFEKFPVYR LRDLTYQGDFKDKHDQMRYGVAIKNISYFIDQEDLFEKNNLSAIDMTTAK LIKNKIVLNGDVLTYLKLKEETAKHKLTQFFQGSSINKNSRVYFDEDENVF KITTNRNHNPEEIIYFYRGEYGAIKNKNDLEDILNEYLCKMETGESEIVLLN RVNHLRDAISANIVGILSYLIDLFPETIVALENLAKGTIDRHVSQSYENITRR FEWALYRKLLNKQLAPPELKENILLREGDDKIDQFGIIHFVEEKNTSKDCP NCRKTTQQTNDNKFKEKKFVCKSCGFDTSKDRKGMDSLNSPDTVAAYN VARKKFES

SEQ ID NO	Description	Sequence
SEQ ID NO: 592	Cas12 variant	MAKETKEFKTFDDFTNLYEVQKTLRFELEAVPETEIVLENRGIWYKRDKK RADEKPIVKFYMDILHREFTDEALEKIKESGVLNLSGYFKLFEELRRLQNH GANTKEEKKLKLEEIRAKKREISNELSQIRRVFSVRGFDVVDSDWKKKYTI EGKKIKNDKSKTYLILSENILNFLENRFTSKEVERLRSIDKKHVEDYGNVV NSGGENIFATFKGFFGYFDSLIKNRENFYETDGKAGRVATRSVDENLNFF AENLHIFSTDLPKALKDDLSDTQKAIFERSYYKNCLLQKDIKSYNLIIGDIN KEINKHRQQRDTKIKFLNTLFKQILSIEEKEQYKHIEINNDEDLIRAIRDFISL NESKISEGTKIFNQFIQRCLQKEDLGQIYLPKDSVNTIAHRIFKPWDEIMAL FDRKYFVSLEEIKDLTESSVWKERVLEESKTKSLIFKDTHIHTIISGQEIFSN FILILEKEYKNQFSGFISETRRGKAAFVGYDESLKNLRATIKWFEGKNLKL SETEKVEWIKAIKDYADAALRIFQMTKYLWLPVVGDEEDKDYLRIKAEID QLTKDNDFYNKINAFIDGYKPEPFIYRSSFQEYLTRRPFSTDKFKINFENSR LLDGWDKDMIDDRMGILLQRDGDYFLGILNKEDRHCLDNLVDVKSEDK NSYALMQFKQLTGLYRQLPRMAFPKKKQPVLEANAEIKKIKEDFDFLQK QKKEREVNVNVVFDNKKLNLLINHYAEFLKENYKDEKCYDFSLLNKEKV YESLSDFYADVDKITYSLSFIQVSIDQLIKTGKILLFRLKNKDLLKGSLGQN KNLHTYYFHALFERENLSQGRIRLGAQAEIFFRPASIEKEKDKNRSNALKK SPKTRYVKEILKNKRYSEDKVFLHLPIQLNADAYDLPSINQNVFEFIKNRQ EKVKIIGIDRGEKNLAYYSVISQNSNGKIKIEEPPRDLNLGYLEPLDELENK RQDERKAWQSISEIKSKRDGYISYAVSKIVELMLKYQAIIVLEDLSGKFKR SRMKFEKAPYQQLELALIKKLNYLVKKNSKSGKPGHYLSAYQLTEPVGS YKEMGKQTGIIFYTQAGYTSRTCPTCGWRKRVQGLYYKDRTSAQRRFDP KTGVKIFYDSVNDRFVFQYHPVYEQKELKEWDKEIYSDVTRIRWNNEEK KNNEYRKGDITLKIKRLFRDRGIDLSRNINEQLVNVGDASFWEELINLLRLI TEIRNIDNENNRDFIECPHCHFQSENGFHGVAWNGDANGAYNIARKGLLI TKAVCDPEKNVGDITWSDLKVDMKDWDAATDEWAKKNPEK
SEQ ID NO: 593	Cas12 variant	MENEKIFSDLTNRYQVVKTLPFELKPVPRTRVLLGLDNPNKGEIFSKDRER AENFTIIKKYIDRLHSLFINESLKKADIDFSNFYKQYGKNINTKNNKNIDDD NDINDDEKEDSENDNLKKYRQEIANLFNKSKYKSWVNVGKDGDKISGML FEKGLIDLLRTHFSDNLNEDIEIPELFSNKKIKDTRKLKEIINSFGKDGKDGQ NFTTYFSVSFHNNRKNYYKSDGKMGRVSTRIVDENLERFCKNIYLYKEIIG KNEIKEIFSGNWDIYLQKKPNFSNDKTYKKLDEFKNDKYDWEMIFRDVNS YNKYFLQSDIEFYNYIRGKLNQDINEYNGKKRDSKEKINSQFENLRNQVH GEKKNYDDDFEIDEDNIIQFINEIFVRHNQNKMRFSEKLFSDFIDLLMVDN GDKLDKVYFSQKAVENAIARYYFVEETTNEGREPLLISLLLQNAGKDRKK LSNKPIKLGDIKFVLDQANNKPAEDIFKNRYVLSESNNDGIINANDKNHW ANLLRLIKKDFYFHKDNLIKSQDKLALETKYNKGSDEGERQIETIKNFAES AKAILRMTKYFDLRKNGVIQNVIGGKDPIHEEVDKYFDGDVLSGEESCRIS KYYDALRNFITKKAWSADKIILNFDCSEFLGGWDRSQEQKKRGIILRHRD GDEERYYLAVLGKNGKQYFENRTLFKGCESSDWQKIEYNVIQKPHMSLP KNLITPFFKKDKITNERFIDRSKKGAKALIEIDINPSDEFLNNYNLGKHTKE NLDKSFLCDYFKYLMDAIAKYYKGEFNFNFPDVSNFDNTQPFYSFIEKNA YSIKYFGISSKEIEKLIADCYYKEDVYLFQIYCKDFEIDPKIGKAKYGNEFR TKAEIRKSKGEEAGNENLNTKYFKLLFDEKNLKNQNGIVYKLNGGAKMF YRPSSIKKDEKIDGKWRYKEDKYSLNITITCNFSSKKDDLSIDKDINKKIAE VNANSDFRIISIDRGEKNLAYCCVMDENANILDIKSLNRITRYDKNGKAIK EKNMFHEVKDGKLCYGEPVYDFYKDYQNLLDEREIKRLVNRRSWNVIED IKNLKKGYVALLINYICKAVVIAINEGKYPIIVLESLDKGMLHNRVKIEKQI YRGVEEGLVRKLNYFVDKKTDNVLNAWQLLAKFETVGSSLDRKKQLGII FYVDPGYTSITCPCCGFRQRKYIKAERAEENFKEIKIKFDGKRYSFAYDYR CIDDNGKEKSKEDIIYSNVKRLLRSGRNGRAVQIEDVTDELTNLFKKHNIN IEQDINEQLAGKDNKFWKQLLWWFNAIEQIRNTQSLRRKFNTEENKLEILE NNDCDFILCPHCYFDSNKDKFQNKIWNGDANGAFNIGRKGIIDIFEIKKHQ

SEQ ID NO	Description	Sequence
		RMLSDFMEQWGIDKLPKANGGNQAVIEIVKNDKKYNLCILNNKKIPYYC LRIGKEKIDSIADDRKCNQLPDLMVNWKKWDMWLDKWGK
SEQ ID NO: 594	Cas12 variant	MPEVKNVFQDFTNLYELSKTLRFELKPVPETEKILELNAAKTKKFPKDLY RAENFEIIKKYTDELHRTYIRETLNNVNIDYLKFLEIFRINGKKKNEMTDEN EESDENNEKDDIQKIKKELRSKIGNLFNKWNNDKDNKFKDWVKIDVGKK EKEVSGDLFGKELITILKNYFKNKLDSKVNVPMLFFNEQEIKNGEAKKQR KLEAVFENFDKFTTYFTDSFYNNRKNYYKTEGRVGQVATRIIDENLPRFC SNLIAFNEVVSLYSTLLNNFDLGWKEYLNEKKINQTWVEKFELSNYDWK ALFNDVNYYNQCLLQEGIDKYNYIIKKLNKDINEYTQNKYKSVEKGNNN NPDINFFQKLHKQIHGERDFKLIEIDIDENNIFTKILPEFILHSDMKLMTKID EEVGVEEIVGAERIIKIFIKQELKDLEKIYLSRRAIETISAKWFHSWETLKDL ILGYLNKDLLESKKRKKVPDFVDFNIIKIVLENNKDDYKDLFKRKYFEAD KNEFVDWIDSSGGTKKLEFGGENWINFLNVFEYEFGTLLTEYKKNKNALL YLIDKKIDYDKNNEVGQTAAIKNFADSALGIFRMVSYFALRKKGVMVEP KNGKDEIFYAFVDRYLDGDDNDREEQNKIVQYYNTLRNFVTQKAWSIDK VRLCFDCGEFLKGWDKDKIHERLGIILRNNNKFYLGILNKNHKQIFIKIKSH DNNNFYYVIYDYKQLNNVYRQIPRLAFPSRSVKKGDAYMLRAIQERKKK FFLEDEEFIELQEIKNEYDKIGNDLSKEKLTKLIEYYKKVVISNYSSLYNVS NLNNKKFNSINEFNQYVENLMYSLIPTRISPDFIKEKISKGELYLFQIYNKD FELDESIGKEKFGEDFAPVIMDGKNNLHTEYFKLLFNDSNLKNPNGVVFK LSGGAKMFYRPATENLPIKKDRDGNIIKNKKGENVIVGQRYKEDKYFLHL PIILNFVNKGKNYSINDMVNKAITNASDDQDKFRIIGLDRGEKHLVYYSVI NERQEIIEIGSLNNISRKDNKGEIIEEKNWYHDKFGNIEKEPTKEYHKDYHN LLDQREIERLKSRQSWEKIENIKELKEGYISAVINKICNLVIKAIKENKIPIV ALENLNSGMKRGRIKIDKQIYQKLELKLAKKLNFLVDKKEKNYLSAWQF TPKIETFSGDIEKKNQVGIIFYVDPAFTSATCPNCGFRKRIKMDPQNAKKKI KDMEITYENGIYKFDYPIENGENDVVYSDVERLKWDNEKKKVIKTKNVS DDFGKLFEDIKDKNNLKKELLSIGEENKEFWKEFSRCFNLLLRIRNSKLIK RKLNDDTGKVEIIADDDLADRDRDFIYCPQCHFHSEGGDVFGEFVKKKYL GKDNFEFNGDANGAYNIARKTIIAVNKIKDYQLGLNHFIEKYRISELPNNG KDKKNIFYNNNSYILSFFEVQDEKFRKVKVYGLKKDGDRQIIQKKEMWY RRYPDIFVNNKEWDKFVQNKS
SEQ ID NO: 595	Cas12 variant	MLFFMSTDITNKPREKGVFDNFTNLYEFSKTLTFGLIPLKWDDNKKMIVE DEDFSVLRKYGVIEEDKRIAESIKIAKFYLNILHRELIGKVLGSLKFEKKNL ENYDRLLGEIEKNNKNENISEDKKKEIRKNFKKELSIAQDILLKKVGEVFE SNGSGILSSKNCLDELTKRFTRQEVDKLRRENKDIGVEYPDVAYREKDGK EETKSFFAMDVGYLDDFHKNRKQLYSVKGKKNSLGRRILDNFEIFCKNK KLYEKYKNLDIDFSEIERNFNLTLEKVFDFDNYNERLTQEGLDEYAKILGG ESNKQERTANIHGLNQIINLYIQKKQSEQKAEQKETGKKKIKFNKKDYPTF TCLQKQILSQVFRKEIIIESDRDLIRELKFFVEESKEKVDKARGIIEFLLNHEE NDIDLAMVYLPKSKINSFVYKVFKEPQDFLSVFQDGASNLDFVSFDKIKTH LENNKLTYKIFFKTLIKENHDFESFLILLQQEIDLLIDGGETVTLGGKKESIT SLDEKKNRLKEKLGWFEGKVRENEKMKDEEEGEFCSTVLAYSQAVLNIT KRAEIFWLNEKQDAKVGEDNKDMIFYKKFDEFADDGFAPFFYFDKFGNY LKRRSRNTTKEIKLHFGNDDLLEGWDMNKEPEYWSFILRDRNQYYLGIG KKDGEIFHKKLGNSVEAVKEAYELENEADFYEKIDYKQLNIDRFEGIAFPK KTKTEEAFRQVCKKRADEFLGGDTYEFKILLAIKKEYDDFKARRQKEKD WDSKFSKEKMSKLIEYYITCLGKRDDWKRFNLNFRQPKEYEDRSDFVRHI QRQAYWIDPRKVSKDYVDKKVAEGEMFLFKVHNKDFYDFERKSEDKKN HTANLFTQYLLELFSCENIKNIKSKDLIESIFELDGKAEIRFRPKTDDVKLKI YQKKGKDVTYADKRDGNKEKEVIQHRRFAKDALTLHLKIRLNFGKHVNL FDFNKLVNTELFAKVPVKILGMDRGENNLIYYCFLDEHGEIENGKCGSLN RVGEQIITLEDDKKVKEPVDYFQLLVDREGQRDWEQKNWQKMTRIKDLK

SEQ ID NO	Description	Sequence
		KAYLGNVVSWISKEMLSGIKEGVVTIGVLEDLNSNFKRTRFFRERQVYQG FEKALVNKLGYLVDKKYDNYRNVYQFAPIVDSVEEMEKNKQIGTLVYVP ASYTSKICPHPKCGWRERLYMKNSASKEKIVGLLKSDGIKISYDQKNDRF YFEYQWEQEHKSDGKKKKYSGVDKVFSNVSRMRWDVEQKKSIDFVDGT DGSITNKLKSLLKGKGIELDNINQQIVNQQKELGVEFFQSIIFYFNLIMQIRN YDKEKSGSEADYIQCPSCLFDSRKPEMNGKLSAITNGDANGAYNIARKGF MQLCRIRENPQEPMKLITNREWDEAVREWDIYSAAQKIPVLSEEN
SEQ ID NO: 596	Cas12 variant	MTIKKHKPFTNFECLTPVQKTLRFRLIPVGRTTEFVKCRNIIEADRKRSEM YPLLKELADRFYREFMTDQLSNLLFDWSPLVEALLLARNNTDPRENQRIA SLVRDEQKKYRTLLLKRLSGQVDRNGTPLPKNTASVNKKYYDDLFKARF VTETLPAYLEHLKNKPDGRISDELFDAYKDALDSYQKFTSRLTNFWQARK NIFTDEDIATGFAYRIVHEIVPDYLFNRRVYEQHKLDFPEPLDLLETELKKK NLIANDESLDALFTIPAINRLLTQKGVDLHNAVIGGFFTDDHTKVQGFNEL ANLKNQTLKNVSDNSEIKPVGKMTRLKKHILSISESTSFLFEQIESDDDLLA RIIEFNNTLSEPDIDGLSIADINDQLYNIMTGVDPSTILVHARNLNKLSHEAS LSWNRLRDGLYQMATESPYREDERFKRYIDASEEERDLSKLKNDIYFSLQ ELQFALDQSIDLEEEATPTEDIFLPFEFPGMDLKSELTVLFRSIEQLISSETKL IGNPDAIATIKKYLDAIMARYSIWNLLSCEAVELQDDLFYPEYDRVMGSLS NIILLYNLARNYLSRKPSSKEKFRLNFDKPTLADGWSESKVPDNFSVLLRK DDLFYLGILKDRKAYRVLSYENCDETAKNIKGYYERMIYHFSPDAYRMIP KCSTARKDVKKHFGEQGETTGYTLYPGASNFVKPFTIPYEIYRLQTELVN DKKRYQADYLKQTEDEEGYRQAVTAWIDFCKSYLESYEGTSTFDYSHLL KSEDYEDVNQFYADVDRASYSIYFEKVSVDLIHTMVDRGDLYLFQLYNK DFSPHSTGKPNLHTMYWRALFSNDNLQNNTIKLNGQAELFYRPKQVEQP TVHLQGSYLLNRFDKHGDVIPAGLYCEIYNHINERHPEGYTLSEEATQGLL DGRFVYREAPFELVKDKRYTEDQLFLHVPLEFNWTASANVPFENLANEYI KKDSDLHIIGIDRGERNLLYYSVINLQGDIVKQGSLNTLIQQTTLKGETVER QIPYQSMLKQREDERAEARQNWQSIDRIKDLKEGYLSHVIYKLSRLIIKYH AIVVMENLNVGFKRGRFKVERQVYQKFEVALINKLNALSFKEYEPNELG GVMRPWQLARRVVSPEDTRSQNGIVFYVPASYTSIVDPVTGFANLFYLNR IRNKDLNSFYGHFQEIRYDHEFDRFIFRFNYADFGVFCRIKNVPSRTWNLV SGERKAFNPKRRMIEKRDTTDEIKKALEAHGIAYQNEQNLLPLLLENENLL ARIHRSFRLVLQLRNSDSDRDDIVSPALDKENNTFDSGQQPYESSLPINAD ANGAYNIARKGLLLVDKVKNDKRAVLSNREWFEYLMAEE
SEQ ID NO: 597	Cas12 variant	MENKDYSLSRFTKQYQNSKTVRFALTPIGRTEEYIIQNQYIEAARRKNQA YKIVKPIIDEKFRSMIDDVLTHCEKQDWVTLDKLILQYQNNKCRENMDAL AEQQEEIRKNISEEFTKSDEYKNFFGKEDSKKLFKIFLPEYLNQINASESDK EAVNEFQKFKTYFSNFLIVRADIFKADNKHNTIPYRIVNENFMIFAGNKRT FSNIIRLIPNALEEIAKDGMKKEEWSFYNIQNVDSWFEPDSFQMCMSQKGI QKYNFIIGLVNSYINLYTQQNPQATEVKRSRLKLRMLHKQILSDRVNPSW LPEQFKEGEEGEKQIYEAILALENDLIKNCFDKKYDLWIQSIDIQNPRIYIA ASEMARVSSALHMGWNGLNDVRKTILLKSDKKQAKVEKILKQDVSLKD LSDTLNRYADIYKEEQIPSLYQYIEYGSELLQDCAITRKEYHDLLNGNSNT LSLNQNEKLIEGLKAYLDSYQAIVHFLNVFIVGDELDKDTDFYAELDGLV ESLSEIVPLYNKVRNYITRKVYSLDKMRIMFERSDFLGGWGQSFDTKEAL LFQKDNLYYIGIIEKKYTNMDVEYLHEGIKEGNRAIRFIYNFQKADNKNIP RTFIRSKGTNYAPAVRKYNLPIESIIDIYDVGKFKTNYKKINEKEYYESLEK LIDYFKDGILKNENYKKFHFNWKPSNEYENINEFYNDTNNACFLLEKEEIN YDHLKEQANQGKIYLFQISSKDFNEGSKGTPNLQTMYWRELFSNQNCKD GVIKLCGGASIYMRDASIKQPVVHRKNAWLINKWYKVNGQNVVIPDNTY VKFTKIAQERMNEDELTPQERQLWNSGLIQKKKATHDIMKDRRFTKKQY MLHAPLTINYKQQDSPRYFNEKVRSFLKDNPDINIIGIDRGEKNLIYITIIDQ KGNILKGMQKSFNQIEEKGKEGRTIDYYSKLESVEARHDAARKNWKQIG

SEQ ID NO	Description	Sequence
		TIRELKEGYLSQVVHEITQLMIQYNAVIVMENLNMGFKKGRMKVEKSVY QKFEKMLIDKMNYLAFKRDMQGNAIDPYEVGGVMNGYQLTDRFTSFAD MGSQNGFIFYVPAAYTSVIDPVTGFVNVFQKTEFKTNDFLHRFDSISWND KEQSFVFTFDYQNFKCNGTCYQNKWSLYADVDRIETIIKNNQVDRIEPCN PNQKLIDFFDKKGIIYRDGHNIVDDLEKYDSKTISEIIHNFKLILQLRNSMR NPDTGEIIDYIASPVMHNEERFDSRKRNPELPQDADANGAYHIALKGLMF LQKINEYADSDGNMDNRKLKITNEEWFKYMQTRKEHTYF
SEQ ID NO: 598	Cas12 variant	MSNKTSSITTTNKLSYTGFHNNGKQSKTLMFELKPIGRTTEHLDRKGYLA DDIDRAESYKTFKEIADNFHKNLIEESLATFTFSDTLKDYFDLWLSPVRTN EDTPKLRKMEAKLRKELSSALKQHPSFAATSSGKRLIDEALYPNASDKER QCLDRFKGRSSYLDSYTEVRSFIYTDLCKHNTIAYRVVNENLKIYLENILA YEKLMQTAVNGKLETVKEMFHDLYPTFSMDISIFFTSYGFDYCLSQNAIT RYNILLGGWSDDNGIHHKGLNNYINEYNQTVPRNKRLPKLNKLQKMILSE ENSMSFIIDKFENDVDLANAIRYWLKNCQFDALNLLIWTLDVHYNLDEIH FKNDNQGKNISDLSQALFKNHHVIRDAWDYDYDIVNAKAKSRQKPERYA EKRDKAFKKINSFSLSYLANILSQYDNQYANFVAQFKTRISVHIQNVQQMI ADKTLDMRLDPLMLLKSISSDTKLVEDIKRVLDSLKDMQRMLTPLLGEGT EPNRDAMFYSDFEPLMNYVDTLTPLYNKVRNYITKKPYSTKKTSLYFGAS NFGSGFDVTKLPVSHTIIMRDKGCYYLAVIDNNKLIDKLYDHNDNDGYEY MVYKQIPSPIKYFSLKNILPQDPPDDIRQLLEDRKNGAKWSHDDETRFIDYI VNEFLPTYPPIHDKNGNPYFSWKFKNPDEYESLNEFFDDVSKQAYQTSFR FVSRDFVDDAVENGDIFFFQIYNQDFSPASHGKPSPHTLWFRALFSDVNLE TKDIRLKGNATAYFRPASIFYTDEKWRKGHHYEQLKNKFKFPIIKDKRYA LDKFFFHITLEINCNATVEKYFNNRVNEEIRKADRYNILAINRGERNLLYA VVMDQDGTILEQKSFNIIKSELPNKTVKETDYWKKLHAREKERDTARKS WKSIECIKDLKKGYLSYVVKTITDMMFEYNAVLVMENLDIEMKRSRQKIE KNVYAQFQNAIIQKLSMYVNKDIDLHIARTAPGGTLNPYQLTYIPASRTKT PKQNGFVFFLNPWNITEIDPTTGFVDLFQTCFRTKNEYKDFFAKFKDIRYN EAQGWFEFDTDYTYFRDKEKAGKRTRWNICSYGTRLRRFRNPDKNYAED AMTVYPTQMLKDLFDEYNIPYAPASAKSTSISIKDDIIQIDKLDFYKKLLYI LKLIVQLRNTSPSSTEQEDDYIISPVINEDTNWFYDSRDYNEESLLPCNTDA NGAYSLALKCNMVIDRIKNTIPGEPVDMYISNADWLDARQ
SEQ ID NO: 599	Cas12 variant	MNSKTSIFDFSNIFGRDITLRFKLTPVTINSKGEVKDANGADPYRPYLSADE ELQEQYELLKTAIDAYHQMYIDKKLKHILCLPLTEKGKDGVEHDTAKSKF VKSCLAYIKDYGEKDKKRQTADLRTFISRVFADDNISSLPPYKVKSDFITK TLRQWLEQPDTKVEKKEAILDLIEKNGSKLYANCQGLLEARQRLYEKDG KSTSVPYRCIDRNLPRFSKDYHLFEKILGDCSDVFDFEQLDKDFSEELKGIA RLSGIRVESVREVFQPLLYLAYLNQEGIQYLNTIIGTKKEKGTSALGLNEYI NQYNQKQGIKKKKDGIPMLNKLNNQILFGDEVFIETLAEHKEAIPVIKKVV SSLGKLGAFDGECHENKLYQFLLSLSSYAGNIYVNTKVVAQISSSLWGDY SILYDAVKHDKNGRLIQKSVTLGELNEKIERLKLEDNRDAFEYFRRSQVK DVVHGSSNVGVFEQLKNCYNDFVEKKILKCSFFSEDQVLVIQRLFDSILSL QRIFKVFCPSLYEVDSDGLFVAKFSDYWNVLRGFDKDYDLLRNLFKRKP YSTDKIRVHFGLSNLMDGFVDSWTDKKDKGTQYNGYILRQAHSFVDENT SKELQEFQRYNYYLVISGNVRLFREKGNALVCEKKKEKLVASDEFSGFER FDYYQSSINNFNREFKRLTGRDRKSFTDEILQNEGKKELKSTYIENLIKVA KSMKRLTALQNLVSDEKVRKYSENLDYETLSAEIGQILATGRERKYVPVS TNEMKNLLKSSKNNKGEEVRTFMFRISNKDLSYAETMQKGERKSHGAEN MHTMYFRALLDTLQNTFDIGTGTVYFRKASDKRKMKYDEKNPTHRKGD ELAFKNPYNKGKKKSVFGYDLIKDRRYTKDSYLFHLSITQNYQKKGNAE DLNAMVRDYIRTQEDLRVIGIDRGERNLLYATMIDGEGHILAQKSFNVIG YQGTTASGESFQVETDYHQLLNEKAEKMRSLQREWKEMDKIQDMKDGY LSVVVHELAKMVVENNAIIVMEDLNMGFMESRQSQLANVYQKFEEKLR

SEQ ID NO	Description	Sequence
		NKLQFYVDKRKRNDEPSGLYHALQLAGTETKDNQNGFIFYIPAWNTSKID SVTGFVNLFNLKYTNIKDAKAFFSTFEKIEKNVETGHYDFTFSYSSMARK KMAKRMDGTRDSWTISTHGSRIVREQKGNYWEYREIESLTSEFDALFEKY SIDTRCRLKEAIDKCGEAEFFKELIRLMKWTLQLRNYDDRGNDYIVSPVC YRGNEYYCSLDYDNEEGMCISKIPCQMPKDADANGAFNIARKGLMLCER LKKGEKIGVIKGTEWLQYVQNMSERYVGMV
SEQ ID NO: 600	Cas12 variant	MINTMEQPKKSIWDEFTNLYSLQKTLRFELKPQGKTKELVRTLFINPEEHH HKLISDDLELSKNYKKVKKLIDCMHRNIINNVLSKHQFTGEELKKLDKNS NAEDNDTETDNADKKDPFAKIRERLTKALNEESKIMFDNKLLNPKKGKN KGECELKKWMDKAEDKYFELGNNEKIDKEAVKADMERLEGFFTYFGGF NKNRENVYSSKKIATAIPFRIIHDNFPIFKKNIENYKKITEKHPELAKLLNEK GANEIFQLEHFNKCLTQDGIDVYNNEKLGIIAKEQGKEQDKGINQLINEYA QKKNKEIKENAKGGEKPKKIKIAVFDKLKKQILSISKTKSFQFEVFEDTSDI INGINKRYTFLTEAKEGMSIVDEIKKIIGSVGDEKYSLDEIYLKEKFISTLSK KLFNYSRYIEVALEKWYDDRYDDKINKSGTDKRKFISAKQFSITSIQDAIN YYLEKYEKDEELSKKYTGKNIIVDYFKNPTITIEHKQKEEVISEEKDLFKEL EVRRNVIQHILNGDYKKDLKEEKQQDGDSEKVKAFLDALLEFNYILNPFII KDKNLRKEQEKDEEFYNEIKKLQESIFEAEILDLYNQTRNYITKKPYKLDK FKLTFGSGYFLSGWSNDMEEREGSILIKYNEDRSKNYYLIIMAKPLTDDDK KQLFSDNGTHSKICIYEFQKMDMKNFPRMFINSKGSNPAPAIEKYNLPIKTI WADYQKYKNLNQKGKDKFLEENPDFRHNLIGYFKICAEKHESLAPFKHQ FSSIWKPTKEYENLAQFYKDTLEACYNLKFENVNFDNISQLVSSGKLHLF KIHNKDFNPGSTGKKNLHTLYWEMLFDEKNLQDVIFKLSGGAELFYREAS ILKNKIIHKIGEKVLKKFFKLPDGKLEPVPAESIKNLSAYFRKELPEHELTEI DRKYIDNYSIIGKKDDKLGIMKDERFTVDKIQFHCPITINFKSKNKNFINDD VLEYLHKRDDVHIIGLDRGERHLIYLTMINKDGKIVDNMQFSLNELQRRY KINGNEEIQKINYQKLLDTREVSRTEARRNWQTIENIKNLKEGYLSLIVHQ LAKLMIEKNAIVVMENLNYGFKDSRARVEKQIYQKFESILIKKLQYLVMD KNNLYDSGGVLSAYQLTNQEVPAYKYISKQNGFLFYVPPDYTSKIDPETG FINLLDTRYYSRKNAVALLNKFDKIYYDRDNKYFRFDFDYNSTDSNGNK NFDKLRVDISELTRTKWSVCSHPAKRSITVQINNKWVRQPINDVTDKLIKL FEDKQIGYESGKCLKDEILKVEDAKFFEDLLRYLSVLLALRHTYTENGVE YDLIISSVEKAPGSNEFFVSGKDNNLPANADANGAYNIARKGLWLLRKLD EIDNQELAIKKFNELKHAKEIKKNGEESKEDKGDRKRKKKWVSQWCPNK EWLAFAQSMQDVSEK
SEQ ID NO: 601	Cas12 variant	MNNGTNNFQNFIGISSLQKTLRNALIPTETTQQFIVKNGIIKEDELRGENRQ ILKDIMDDYYRGFISETLSSIDDIDWTSLFEKMEIQLKNGDNKDTLIKEQTE YRKAIHKKFANDDRFKNMFSAKLISDILPEFVIHNNNYSASEKEEKTQVIK LFSRFATSFKDYFKNRANCFSADDISSSSCHRIVNDNAEIFFSNALVYRRIV KSLSNDDINKISGDMKDSLKEMSLEEIYSYEKYGEFITQEGISFYNDICGKV NSFMNLYCQKNKENKNLYKLQKLHKQILCIADTSYEVPYKFESDEEVYQS VNGFLDNISSKHIVERLRKIGDNYNGYNLDKIYIVSKFYESVSQKTYRDWE TINTALEIHYNNILPGNGKSKADKVKKAVKNDLQKSITEINELVSNYKLCS DDNIKAETYIHEISHILNNFEAQELKYNPEIHLVESELKASELKNVLDVIMN AFHWCSVFMTEELVDKDNNFYAELEEIYDEIYPVISLYNLVRNYVTQKPY STKKIKLNFGIPTLADGWSKSKEYSNNAIILMRDNLYYLGIFNAKNKPDKK IIEGNTSENKGDYKKMIYNLLPGPNKMIPKVFLSSKTGVETYKPSAYILEG YKQNKHIKSSKDFDITFCHDLIDYFKNCIAIHPEWKNFGFDFSDTSTYEDIS GFYREVELQGYKIDWTYISEKDIDLLQEKGQLYLFQIYNKDFSKKSTGND NLHTMYLKNLFSEENLKDIVLKLNGEAEIFFRKSSIKNPIIHKKGSILVNRT YEAEEKDQFGNIQIVRKNIPENIYQELYKYFNDKSDKELSDEAAKLKNVV GHHEAATNIVKDYRYTYDKYFLHMPITINFKANKTGFINDRILQYIAKEKD LHVIGIDRGERNLIYVSVIDTCGNIVEQKSFNIVNGYDYQIKLKQQEGARQI

SEQ ID NO	Description	Sequence
		ARKEWKEIGKIKEIKEGYLSLVIHEISKMVIKYNAIIAMEDLSYGFKKGRFK VERQVYQKFETMLINKLNYLVFKDISITENGGLLKGYQLTYIPDKLKNVG HQCGCIFYVPAAYTSKIDPTTGFVNIFKFKDLTVDAKREFIKKFDSIRYDSE KNLFCFTFDYNNFITQNTVMSKSSWSVYTYGVRIKRRFVNGRFSNESDTID ITKDMEKTLEMTDINWRDGHDLRQDIIDYEIVQHIFEIFRLTVQMRNSLSE LEDRDYDRLISPVLNENNIFYDSAKAGDALPKDADANGAYCIALKGLYEI KQITENWKEDGKFSRDKLKISNKDWFDFIQNKRYL
SEQ ID NO: 602	Cas12 variant	MSNLNTFISPEFTGKIKMTKSLKVSMIPIGETEHWIAKHKVFEKDRELFDK NLKARPILDEFIKYTVSRALPNLLFDFEAYYLVKKDRTKARAFEKELAKT VTDLILKEMDELKSASLIDSADFVKTTLKKFAGTHDIPGLSRIEAIESLEAA SKLTALNGKFNTSRIAIINTLIPKRIIENFDIYLSNMEKIRNVYESGEFGFLFE RYPDTLLFMEPANYRTVCSPEAIEDYNRFISGYGDSTESWIKGFNQELSEA SNSSKSSNGGVRRYSLIKPLHKQHLFETKKFFTFASISSDDDVRELINSVKG STEDACLNALAFFSSSDPKTLFVKGSYLHTLSAFLYGSANSYILPERIKEGE KARLTAEYDSVAKKTKAVTTRYNVAMNNISKKINEKIFSLADIDAYCCDI SKRRSVREILLGIMQEMYAAVYGENGKWSNIEAEAVLDSKTKIWKAKNG AVAKAVNDYLTAILEIRKFIRPFALRMEELEELGLDTSSALDAGEITNTLFE AVRAQKLVHAYLTRNDADIALSTQVYFGGTQKAAASWWNYETGDIQNR QIALAKKDGMYYFIGTFDERGSYSIEPASPGEDYYEMLDVKKGQDANKQI KKVLFSNKAIREHFADSSNDYVITTKVNSPITVRREIFDKYQAGEFKLTSQ KIRKGDLVGEKEMTYYREYMDLLFQMAKGYTEYSRFNMDTLLPIEEYDT ENDLLDDVNTNTIDYRWVRISAACIDDGVRNGDIFVFRAQTSSMYGKREN KKGYTGLFLELVSDENLLVTRGMSLNSAMSIYYRAKVHDAITVHKKGDV LVNKFTNARERIPENSYKAICAFYNSGKSIEELTIEDRDWLAKATTRICSGE IIKDRRYTKNQYSISISYNINRSVNNRKRVDLATIVDDTASAGRIISVTRGT KDLVYYTVIDDGGSVIEARSLNVINGINYAKMLAQISEERHDSNANFDIPK RVETIKEAYCAFAVHEIISAALKHNALIVVELISDAIKDKYSLLDNQVFLKF ENVLKNCLMSVKVKGARGMEPGSISNPLQLCNADDKSFRNGILYQIPSSYI NICPVTGYADIIDYYNIVSAGDIRNFFVRFENIVYNKEKARFEFSFDLKNIPI KLEKCPDRTKWTVLGRGEITTYDPLTKSNHYVFDAAQMLAETVSKEGLD PCANIVEHIDELSAATLKKMFNTFRNIAKGIVSECDEVPVSYYKSPVIDEA DIKNKSLDNKSISEIKCYNLDEKARYYLALAKSSSDGENKNRYVSSTAIEW LNYIQEKRTHE

[00283] Alternatively, the Type V CRISPR/Cas enzyme is a programmable Cas14 nuclease. A Cas14 protein of the present disclosure includes 3 partial RuvC domains (RuvC-I, RuvC-II, and RuvC-III, also referred to herein as subdomains) that are not contiguous with respect to the primary amino acid sequence of the Cas14 protein, but form a RuvC domain once the protein is produced and folds. A naturally occurring Cas14 protein functions as an endonuclease that catalyzes cleavage at a specific sequence in a target nucleic acid. A programmable Cas14 nuclease can be a Cas14a protein, a Cas14b protein, a Cas14c protein, a Cas14d protein, a Cas14e protein, a Cas14f protein, a Cas14g protein, a Cas14h protein, or a Cas14u protein. In some cases, a suitable Cas14 protein comprises an amino acid sequence having 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%, at least 92%, at least 95%, at least 97%, at least 98%, at least 99%, or 100%, amino acid sequence identity to any one of SEQ ID NO: 12 – SEQ ID NO: 102.

TABLE 2 – Cas14 Sequences

SEQ ID NO	Sequence
SEQ ID NO: 12	MEVQKTVMKTLSLRILRPLYSQEIEKEIKEEKERRKQAGGTGELDGGFYKKLEKKHSEM FSFDRLNLLLNQLQREIAKVYNHAISELYIATIAQGNKSNKHYISSIVYNRAYGYFYNAY IALGICSKVEANFRSNELLTQQSALPTAKSDNFPIVLHKQKGAEGEDGGFRISTEGSDLIF EIPIPFYEYNGENRKEPYKWVKKGGQKPVLKLILSTFRRQRNKGWAKDEGTDAEIRKVT EGKYQVSQIEINRGKKLGEHQKWFANFSIEQPIYERKPNRSIVGGLDVGIRSPLVCAINNS FSRYSVDSNDVFKFSKQVFAFRRRLLSKNSLKRKGHGAAHKLEPITEMTEKNDKFRKKI IERWAKEVTNFFVKNQVGIVQIEDLSTMKDREDHFFNQYLRGFWPYYQMQTLIENKLK EYGIEVKRVQAKYTSQLCSNPNCRYWNNYFNFEYRKVNKFPKFKCEKCNLEISADYNA ARNLSTPDIEKFVAKATKGINLPEK
SEQ ID NO: 13	MEEAKTVSKTLSLRILRPLYSAEIEKEIKEEKERRKQGGKSGELDSGFYKKLEKKHTQM FGWDKLNLMLSQLQRQIARVFNQSISELYIETVIQGKKSNKHYTSKIVYNRAYSVFYNA YLALGITSKVEANFRSTELLMQKSSLPTAKSDNFPILLHKQKGVEGEEGGFKISADGNDL IFEIPIPFYEYDSANKKEPFKWIKKGGQKPTIKLILSTFRRQRNKGWAKDEGTDAEIRKVI EGKYQVSHIEINRGKKLGDHQKWFVNFTIEQPIYERKLDKNIIGGIDVGIKSPLVCAVNN SFARYSVDSNDVLKFSKQAFAFRRRLLSKNSLKRSGHGSKNKLDPITRMTEKNDRFRKK IIERWAKEVTNFFIKNQVGTVQIEDLSTMKDRQDNFFNQYLRGFWPYYQMQNLIENKL KEYGIETKRIKARYTSQLCSNPSCRHWNSYFSFDHRKTNNFPKFKCEKCALEISADYNA ARNISTPDIEKFVAKATKGINLPDKNENVILE
SEQ ID NO: 14	MAKNTITKTLKLRIVRPYNSAEVEKIVADEKNNREKIALEKNKDKVKEACSKHLKVAA YCTTQVERNACLFCKARKLDDKFYQKLRGQFPDAVFWQEISEIFRQLQKQAAEIYNQSL IELYYEIFIKGKGIANASSVEHYLSDVCYTRAAELFKNAAIASGLRSKIKSNFRLKELKN MKSGLPTTKSDNFPIPLVKQKGGQYTGFEISNHNSDFIIKIPFGRWQVKKEIDKYRPWEK FDFEQVQKSPKPISLLLSTQRRKRNKGWSKDEGTEAEIKKVMNGDYQTSYIEVKRGSKI GEKSAWMLNLSIDVPKIDKGVDPSIIGGIDVGVKSPLVCAINNAFSRYSISDNDLFHFNK KMFARRRILLKKNRHKRAGHGAKNKLKPITILTEKSERFRKKLIERWACEIADFFIKNKV GTVQMENLESMKRKEDSYFNIRLRGFWPYAEMQNKIEFKLKQYGIEIRKVAPNNTSKT CSKCGHLNNYFNFEYRKKNKFPHFKCEKCNFKENADYNAALNISNPKLKSTKEEP
SEQ ID NO: 15	MERQKVPQIRKIVRVVPLRILRPKYSDVIENALKKFKEKGDDTNTNDFWRAIRDRDTEF FRKELNFSEDEINQLERDTLFRVGLDNRVLFSYFDFLQEKLMKDYNKIISKLFINRQSKSS FENDLTDEEVEELIEKDVTPFYGAYIGKGIKSVIKSNLGGKFIKSVKIDRETKKVTKLTAI NIGLMGLPVAKSDTFPIKIIKTNPDYITFQKSTKENLQKIEDYETGIEYGDLLVQITIPWFK NENKDFSLIKTKEAIEYYKLNGVGKKDLLNINLVLTTYHIRKKKSWQIDGSSQSLVREM ANGELEEKWKSFFDTFIKKYGDEGKSALVKRRVNKKSRAKGEKGRELNLDERIKRLYD SIKAKSFPSEINLIPENYKWKLHFSIEIPPMVNDIDSNLYGGIDFGEQNIATLCVKNIEKDD YDFLTIYGNDLLKHAQASYARRRIMRVQDEYKARGHGKSRKTKAQEDYSERMQKLRQ KITERLVKQISDFFLWRNKFHMAVCSLRYEDLNTLYKGESVKAKRMRQFINKQQLFNGI ERKLKDYNSEIYVNSRYPHYTSRLCSKCGKLNLYFDFLKFRTKNIIIRKNPDGSEIKYMPF FICEFCGWKQAGDKNASANIADKDYQDKLNKEKEFCNIRKPKSKKEDIGEENEEERDYS RRFNRNSFIYNSLKKDNKLNQEKLFDEWKNQLKRKIDGRNKFEPKEYKDRFSYLFAYY QEIIKNESES
SEQ ID NO: 16	MVPTELITKTLQLRVIRPLYFEEIEKELAELKEQKEKEFEETNSLLLESKKIDAKSLKKLK RKARSSAAVEFWKIAKEKYPDILTKPEMEFIFSEMQKMMARFYNKSMTNIFIEMNNDEK VNPLSLISKASTEANQVIKCSSISSGLNRKIAGSINKTKFKQVRDGLISLPTARTETFPISFY KSTANKDEIPISKINLPSEEEADLTITLPFPFFEIKKEKKGQKAYSYFNIIEKSGRSNNKIDL LLSTHRRQRRKGWKEEGGTSAEIRRLMEGEFDKEWEIYLGEAEKSEKAKNDLIKNMTR GKLSKDIKEQLEDIQVKYFSDNNVESWNDLSKEQKQELSKLRKKKVEELKDWKHVKEI LKTRAKIGWVELKRGKRQRDRNKWFVNITITRPPFINKELDDTKFGGIDLGVKVPFVCA

SEQ ID NO	Sequence
	VHGSPARLIIKENEILQFNKMVSARNRQITKDSEQRKGRGKKNKFIKKEIFNERNELFRK KIIERWANQIVKFFEDQKCATVQIENLESFDRTSYK
SEQ ID NO: 17	MKSDTKDKKIIIHQTKTLSLRIVKPQSIPMEEFTDLVRYHQMIIFPVYNNGAIDLYKKLFK AKIQKGNEARAIKYFMNKIVYAPIANTVKNSYIALGYSTKMQSSFSGKRLWDLRFGEAT PPTIKADFPLPFYNQSGFKVSSENGEFIIGIPFGQYTKKTVSDIEKKTSFAWDKFTLEDTTK KTLIELLLSTKTRKMNEGWKNNEGTEAEIKRVMDGTYQVTSLEILQRDDSWFVNFNIA YDSLKKQPDRDKIAGIHMGITRPLTAVIYNNKYRALSIYPNTVMHLTQKQLARIKEQRT NSKYATGGHGRNAKVTGTDTLSEAYRQRRKKIIEDWIASIVKFAINNEIGTIYLEDISNTN SFFAAREQKLIYLEDISNTNSFLSTYKYPISAISDTLQHKLEEKAIQVIRKKAYYVNQICSL CGHYNKGFTYQFRRKNKFPKMKCQGCLEATSTEFNAAANVANPDYEKLLIKHGLLQL KK
SEQ ID NO: 18	MSTITRQVRLSPTPEQSRLLMAHCQQYISTVNVLVAAFDSEVLTGKVSTKDFRAALPSA VKNQALRDAQSVFKRSVELGCLPVLKKPHCQWNNQNWRVEGDQLILPICKDGKTQQE RFRCAAVALEGKAGILRIKKKRGKWIADLTVTQEDAPESSGSAIMGVDLGIKVPAVAHI GGKGTRFFGNGRSQRSMRRRFYARRKTLQKAKKLRAVRKSKGKEARWMKTINHQLSR QIVNHAHALGVGTIKIEALQGIRKGTTRKSRGAAARKNNRMTNTWSFSQLTLFITYKAQ RQGITVEQVDPAYTSQDCPACRARNGAQDRTYVCSECGWRGHRDTVGAINISRRAGLS GHRRGATGA
SEQ ID NO: 19	MIAQKTIKIKLNPTKEQIIKLNSIIEEYIKVSNFTAKKIAEIQESFTDSGLTQGTCSECGKEK TYRKYHLLKKDNKLFCITCYKRKYSQFTLQKVEFQNKTGLRNVAKLPKTYYTNAIRFA SDTFSGFDEIIKKKQNRLNSIQNRLNFWKELLYNPSNRNEIKIKVVKYAPKTDTREHPHY YSEAEIKGRIKRLEKQLKKFKMPKYPEFTSETISLQRELYSWKNPDELKISSITDKNESMN YYGKEYLKRYIDLINSQTPQILLEKENNSFYLCFPITKNIEMPKIDDTFEPVGIDWGITRNI AVVSILDSKTKKPKFVKFYSAGYILGKRKHYKSLRKHFGQKKRQDKINKLGTKEDRFID SNIHKLAFLIVKEIRNHSNKPIILMENITDNREEAEKSMRQNILLHSVKSRLQNYIAYKAL WNNIPTNLVKPEHTSQICNRCGHQDRENRPKGSKLFKCVKCNYMSNADFNASINIARKF YIGEYEPFYKDNEKMKSGVNSISM
SEQ ID NO: 20	LKLSEQENITTGVKFKLKLDKETSEGLNDYFDEYGKAINFAIKVIQKELAEDRFAGKVRL DENKKPLLNEDGKKIWDFPNEFCSCGKQVNRYVNGKSLCQECYKNKFTEYGIRKRMYS AKGRKAEQDINIKNSTNKISKTHFNYAIREAFILDKSIKKQRKERFRRLREMKKKLQEFIE IRDGNKILCPKIEKQRVERYIHPSWINKEKKLEDFRGYSMSNVLGKIKILDRNIKREEKSL KEKGQINFKARRLMLDKSVKFLNDNKISFTISKNLPKEYELDLPEKEKRLNWLKEKIKII KNQKPKYAYLLRKDDNFYLQYTLETEFNLKEDYSGIVGIDRGVSHIAVYTFVHNNGKN ERPLFLNSSEILRLKNLQKERDRFLRRKHNKKRKKSNMRNIEKKIQLILHNYSKQIVDFA KNKNAFIVFEKLEKPKKNRSKMSKKSQYKLSQFTFKKLSDLVDYKAKREGIKVLYISPE YTSKECSHCGEKVNTQRPFNGNSSLFKCNKCGVELNADYNASINIAKKGLNILNSTN
SEQ ID NO: 21	MEESIITGVKFKLRIDKETTKKLNEYFDEYGKAINFAVKIIQKELADDRFAGKAKLDQNK NPILDENGKKIYEFPDEFCSCGKQVNKYVNNKPFCQECYKIRFTENGIRKRMYSAKGRK AEHKINILNSTNKISKTHFNYAIREAFILDKSIKKQRKKRNERLRESKKRLQQFIDMRDG KREICPTIKGQKVDRFIHPSWITKDKKLEDFRGYTLSIINSKIKILDRNIKREEKSLKEKGQ IIFKAKRLMLDKSIRFVGDRKVLFTISKTLPKEYELDLPSKEKRLNWLKEKIEIIKNQKPK YAYLLRKNIESEKKPNYEYYLQYTLEIKPELKDFYDGAIGIDRGINHIAVCTFISNDGKVT PPKFFSSGEILRLKNLQKERDRFLLRKHNKNRKKGNMRVIENKINLILHRYSKQIVDMA KKLNASIVFEELGRIGKSRTKMKKSQRYKLSLFIFKKLSDLVDYKSRREGIRVTYVPPEY TSKECSHCGEKVNTQRPFNGNYSLFKCNKCGIQLNSDYNASINIAKKGLKIPNST
SEQ ID NO: 22	LWTIVIGDFIEMPKQDLVTTGIKFKLDVDKETRKKLDDYFDEYGKAINFAVKIIQKNLKE DRFAGKIALGEDKKPLLDKDGKKIYNYPNESCSCGNQVRRYVNAKPFCVDCYKLKFTE NGIRKRMYSARGRKADSDINIKNSTNKISKTHFNYAIREGFILDKSLKKQRSKRIKKLLEL KRKLQEFIDIRQGQMVLCPKIKNQRVDKFIHPSWLKRDKKLEEFRGYSLSVVEGKIKIFN RNILREEDSLRQRGHVNFKANRIMLDKSVRFLDGGKVNFNLNKGLPKEYLLDLPKKEN KLSWLNEKISLIKLQKPKYAYLLRREGSFFIQYTIENVPKTFSDYLGAIGIDRGISHIAVCT FVSKNGVNKAPVFFSSGEILKLKSLQKQRDLFLRGKHNKIRKKSNMRNIDNKINLILHKY SRNIVNLAKSEKAFIVFEKLEKIKKSRFKMSKSLQYKLSQFTFKKLSDLVEYKAKIEGIK

SEQ ID NO	Sequence
	VDYVPPEYTSKECSHCGEKVDTQRPFNGNSSLFKCNKCRVQLNADYNASINIAKKSLNI SN
SEQ ID NO: 23	MSKTTISVKLKIIDLSSEKKEFLDNYFNEYAKATTFCQLRIRRLLRNTHWLGKKEKSSKK WIFESGICDLCGENKELVNEDRNSGEPAKICKRCYNGRYGNQMIRKLFVSTKKREVQEN MDIRRVAKLNNTHYHRIPEEAFDMIKAADTAEKRRKKNVEYDKKRQMEFIEMFNDEK KRAARPKKPNERETRYVHISKLESPSKGYTLNGIKRKIDGMGKKIERAEKGLSRKKIFGY QGNRIKLDSNWVRFDLAESEITIPSLFKEMKLRITGPTNVHSKSGQIYFAEWFERINKQPN NYCYLIRKTSSNGKYEYYLQYTYEAEVEANKEYAGCLGVDIGCSKLAAAVYYDSKNK KAQKPIEIFTNPIKKIKMRREKLIKLLSRVKVRHRRRKLMQLSKTEPIIDYTCHKTARKIV EMANTAKAFISMENLETGIKQKQQARETKKQKFYRNMFLFRKLSKLIEYKALLKGIKIV YVKPDYTSQTCSSCGADKEKTERPSQAIFRCLNPTCRYYQRDINADFNAAVNIAKKALN NTEVVTTLL
SEQ ID NO: 24	MARAKNQPYQKLTTTTGIKFKLDLSEEEGKRFDEYFSEYAKAVNFCAKVIYQLRKNLK FAGKKELAAKEWKFEISNCDFCNKQKEIYYKNIANGQKVCKGCHRTNFSDNAIRKKMI PVKGRKVESKFNIHNTTKKISGTHRHWAFEDAADIIESMDKQRKEKQKRLRREKRKLSY FFELFGDPAKRYELPKVGKQRVPRYLHKIIDKDSLTKKRGYSLSYIKNKIKISERNIERDE KSLRKASPIAFGARKIKMSKLDPKRAFDLENNVFKIPGKVIKGQYKFFGTNVANEHGKK FYKDRISKILAGKPKYFYLLRKKVAESDGNPIFEYYVQWSIDTETPAITSYDNILGIDAGI TNLATTVLIPKNLSAEHCSHCGNNHVKPIFTKFFSGKELKAIKIKSRKQKYFLRGKHNKL VKIKRIRPIEQKVDGYCHVVSKQIVEMAKERNSCIALEKLEKPKKSKFRQRRREKYAVS MFVFKKLATFIKYKAAREGIEIIPVEPEGTSYTCSHCKNAQNNQRPYFKPNSKKSWTSM FKCGKCGIELNSDYNAAFNIAQKALNMTSA
SEQ ID NO: 25	MDEKHFFCSYCNKELKISKNLINKISKGSIREDEAVSKAISIHNKKEHSLILGIKFKLFIEN KLDKKKLNEYFDNYSKAVTFAARIFDKIRSPYKFIGLKDKNTKKWTFPKAKCVFCLEEK EVAYANEKDNSKICTECYLKEFGENGIRKKIYSTRGRKVEPKYNIFNSTKELSSTHYNYA IRDAFQLLDALKKQRQKKLKSIFNQKLRLKEFEDIFSDPQKRIELSLKPHQREKRYIHLSK SGQESINRGYTLRFVRGKIKSLTRNIEREEKSLRKKTPIHFKGNRLMIFPAGIKFDFASNK VKISISKNLPNEFNFSGTNVKNEHGKSFFKSRIELIKTQKPKYAYVLRKIKREYSKLRNYE IEKIRLENPNADLCDFYLQYTIETESRNNEEINGIIGIDRGITNLACLVLLKKGDKKPSGVK FYKGNKILGMKIAYRKHLYLLKGKRNKLRKQRQIRAIEPKINLILHQISKDIVKIAKEKNF AIALEQLEKPKKARFAQRKKEKYKLALFTFKNLSTLIEYKSKREGIPVIYVPPEKTSQMC SHCAINGDEHVDTQRPYKKPNAQKPSYSLFKCNKCGIELNADYNAAFNIAQKGLKTLM LNHSH
SEQ ID NO: 26	MLQTLLVKLDPSKEQYKMLYETMERFNEACNQIAETVFAIHSANKIEVQKTVYYPIREK FGLSAQLTILAIRKVCEAYKRDKSIKPEFRLDGALVYDQRVLSWKGLDKVSLVTLQGRQ IIPIKFGDYQKARMDRIRGQADLILVKGVFYLCVVVEVSEESPYDPKGVLGVDLGIKNLA VDSDGEVHSGEQTTNTRERLDSLKARLQSKGTKSAKRHLKKLSGRMAKFSKDVNHCIS KKLVAKAKGTLMSIALEDLQGIRDRVTVRKAQRRNLHTWNFGLLRMFVDYKAKIAGV PLVFVDPRNTSRTCPSCGHVAKANRPTRDEFRCVSCGFAGAADHIAAMNIAFRAEVSQP IVTRFFVQSQAPSFRVG
SEQ ID NO: 27	MDEEPDSAEPNLAPISVKLKLVKLDGEKLAALNDYFNEYAKAVNFCELKMQKIRKNLV NIRGTYLKEKKAWINQTGECCICKKIDELRCEDKNPDINGKICKKCYNGRYGNQMIRKL FVSTNKRAVPKSLDIRKVARLHNTHYHRIPPEAADIIKAIETAERKRRNRILFDERRYNEL KDALENEEKRVARPKKPKEREVRYVPISKKDTPSKGYTMNALVRKVSGMAKKIERAKR NLNKRKKIEYLGRRILLDKNWVRFDFDKSEISIPTMKEFFGEMRFEITGPSNVMSPNGRE YFTKWFDRIKAQPDNYCYLLRKESEDETDFYLQYTWRPDAHPKKDYTGCLGIDIGGSK LASAVYFDADKNRAKQPIQIFSNPIGKWKTKRQKVIKVLSKAAVRHKTKKLESLRNIEP RIDVHCHRIARKIVGMALAANAFISMENLEGGIREKQKAKETKKQKFSRNMFVFRKLSK LIEYKALMEGVKVVYIVPDYTSQLCSSCGTNNTKRPKQAIFMCQNTECRYFGKNINADF NAAINIAKKALNRKDIVRELS
SEQ ID	MEKNNSEQTSITTGIKFKLKLDKETKEKLNNYFDEYGKAINFAVRIIQMQLNDDRLAGK YKRDEKGKPILGEDGKKILEIPNDFCSCGNQVNHYVNGVSFCQECYKKRFSENGIRKRM YSAKGRKAEQDINIKNSTNKISKTHFNYAIREAFNLDKSIKKQREKRFKKLKDMKRKLQ

SEQ ID NO	Sequence
NO: 28	EFLEIRDGKRVICPKIEKQKVERYIHPSWINKEKKLEEFRGYSLSIVNSKIKSFDRNIQREE KSLKEKGQINFKAQRLMLDKSVKFLKDNKVSFTISKELPKTFELDLPKKEKKLNWLNEK LEIIKNQKPKYAYLLRKENNIFLQYTLDSIPEIHSEYSGAVGIDRGVSHIAVYTFLDKDGK NERPFFLSSSGILRLKNLQKERDKFLRKKHNKIRKKGNMRNIEQKINLILHEYSKQIVNF AKDKNAFIVFELLEKPKKSRERMSKKIQYKLSQFTFKKLSDLVDYKAKREGIKVIYVEP AYTSKDCSHCGERVNTQRPFNGNFSLFKCNKCGIVLNSDYNASLNIARKGLNISAN
SEQ ID NO: 29	MAEEKFFFCEKCNKDIKIPKNYINKQGAEEKARAKHEHRVHALILGIKFKIYPKKEDISK LNDYFDEYAKAVTFTAKIVDKLKAPFLFAGKRDKDTSKKKWVFPVDKCSFCKEKTEIN YRTKQGKNICNSCYLTEFGEQGLLEKIYATKGRKVSSSFNLFNSTKKLTGTHNNYVVKE SLQLLDALKKQRSKRLKKLSNTRRKLKQFEEMFEKEDKRFQLPLKEKQRELRFIHVSQK DRATEFKGYTMNKIKSKIKVLRRNIEREQRSLNRKSPVFFRGTRIRLSPSVQFDDKDNKI KLTLSKELPKEYSFSGLNVANEHGRKFFAEKLKLIKENKSKYAYLLRRQVNKNNKKPIY DYYLQYTVEFLPNIITNYNGILGIDRGINTLACIVLLENKKEKPSFVKFFSGKGILNLKNK RRKQLYFLKGVHNKYRKQQKIRPIEPRIDQILHDISKQIIDLAKEKRVAISLEQLEKPQKP KFRQSRKAKYKLSQFNFKTLSNYIDYKAKKEGIRVIYIAPEMTSQNCSRCAMKNDLHVN TQRPYKNTSSLFKCNKCGVELNADYNAAFNIAQKGLKILNS
SEQ ID NO: 30	MISLKLKLLPDEEQKKLLDEMFWKWASICTRVGFGRADKEDLKPPKDAEGVWFSLTQL NQANTDINDLREAMKHQKHRLEYEKNRLEAQRDDTQDALKNPDRREISTKRKDLFRPK ASVEKGFLKLKYHQERYWVRRLKEINKLIERKTKTLIKIEKGRIKFKATRITLHQGSFKIR FGDKPAFLIKALSGKNQIDAPFVVVPEQPICGSVVNSKKYLDEITTNFLAYSVNAMLFGL SRSEEMLLKAKRPEKIKKKEEKLAKKQSAFENKKKELQKLLGRELTQQEEAIIEETRNQF FQDFEVKITKQYSELLSKIANELKQKNDFLKVNKYPILLRKPLKKAKSKKINNLSPSEWK YYLQFGVKPLLKQKSRRKSRNVLGIDRGLKHLLAVTVLEPDKKTFVWNKLYPNPITGW KWRRRKLLRSLKRLKRRIKSQKHETIHENQTRKKLKSLQGRIDDLLHNISRKIVETAKEY DAVIVVEDLQSMRQHGRSKGNRLKTLNYALSLFDYANVMQLIKYKAGIEGIQIYDVKP AGTSQNCAYCLLAQRDSHEYKRSQENSKIGVCLNPNCQNHKKQIDADLNAARVIASCY ALKINDSQPFGTRKRFKKRTTN
SEQ ID NO: 31	METLSLKLKLNPSKEQLLVLDKMFWKWASICTRLGLKKAEMSDLEPPKDAEGVWFSK TQLNQANTDVNDLRKAMQHQGKRIEYELDKVENRRNEIQEMLEKPDRRDISPNRKDLF RPKAAVEKGYLKLKYHKLGYWSKELKTANKLIERKRKTLAKIDAGKMKFKPTRISLHT NSFRIKFGEEPKIALSTTSKHEKIELPLITSLQRPLKTSCAKKSKTYLDAAILNFLAYSTNA ALFGLSRSEEMLLKAKKPEKIEKRDRKLATKRESFDKKLKTLEKLLERKLSEKEKSVFK RKQTEFFDKFCITLDETYVEALHRIAEELVSKNKYLEIKKYPVLLRKPESRLRSKKLKNL KPEDWTYYIQFGFQPLLDTPKPIKTKTVLGIDRGVRHLLAVSIFDPRTKTFTFNRLYSNPI VDWKWRRRKLLRSIKRLKRRLKSEKHVHLHENQFKAKLRSLEGRIEDHFHNLSKEIVD LAKENNSVIVVENLGGMRQHGRGRGKWLKALNYALSHFDYAKVMQLIKYKAELAGV FVYDVAPAGTSINCAYCLLNDKDASNYTRGKVINGKKNTKIGECKTCKKEFDADLNAA RVIALCYEKRLNDPQPFGTRKQFKPKKP
SEQ ID NO: 32	MKALKLQLIPTRKQYKILDEMFWKWASLANRVSQKGESKETLAPKKDIQKIQFNATQL NQIEKDIKDLRGAMKEQQKQKERLLLQIQERRSTISEMLNDDNNKERDPHRPLNFRPKG WRKFHTSKHWVGELSKILRQEDRVKKTIERIVAGKISFKPKRIGIWSSNYKINFFKRKISI NPLNSKGFELTLMTEPTQDLIGKNGGKSVLNNKRYLDDSIKSLLMFALHSRFFGLNNTD TYLLGGKINPSLVKYYKKNQDMGEFGREIVEKFERKLKQEINEQQKKIIMSQIKEQYSN RDSAFNKDYLGLINEFSEVFNQRKSERAEYLLDSFEDKIKQIKQEIGESLNISDWDFLIDE AKKAYGYEEGFTEYVYSKRYLEILNKIVKAVLITDIYFDLRKYPILLRKPLDKIKKISNLK PDEWSYYIQFGYDSINPVQLMSTDKFLGIDRGLTHLLAYSVFDKEKKEFIINQLEPNPIM GWKWKLRKVKRSLQHLERRIRAQKMVKLPENQMKKKLKSIEPKIEVHYHNISRKIVNL AKDYNASIVVESLEGGGLKQHGRKKNARNRSLNYALSLFDYGKIASLIKYKADLEGVP MYEVLPAYTSQQCAKCVLEKGSFVDPEIIGYVEDIGIKGSLLDSLFEGTELSSIQVLKKIK NKIELSARDNHNKEINLILKYNFKGLVIVRGQDKEEIAEHPIKEINGKFAILDFVYKRGKE KVGKKGNQKVRYTGNKKVGYCSKHGQVDADLNASRVIALCKYLDINDPILFGEQRKSF K

SEQ ID NO	Sequence
SEQ ID NO: 33	MVTRAIKLKLDPTKNQYKLLNEMFWKWASLANRFSQKGASKETLAPKDGTQKIQFNA TQLNQIKKDVDDLRGAMEKQGKQKERLLIQIQERLLTISEILRDDSKKEKDPHRPQNFRP FGWRRFHTSAYWSSEASKLTRQVDRVRRTIERIKAGKINFKPKRIGLWSSTYKINFLKKK INISPLKSKSFELDLITEPQQKIIGKEGGKSVANSKKYLDDSIKSLLIFAIKSRLFGLNNKD KPLFENIITPNLVRYHKKGQEQENFKKEVIKKFENKLKKEISQKQKEIIFSQIERQYENRD ATFSEDYLRAISEFSEIFNQRKKERAKELLNSFNEKIRQLKKEVNGNISEEDLKILEVEAE KAYNYENGFIEWEYSEQFLGVLEKIARAVLISDNYFDLKKYPILIRKPTNKSKKITNLKPE EWDYYIQFGYGLINSPMKIETKNFMGIDRGLTHLLAYSIFDRDSEKFTINQLELNPIKGW KWKLRKVKRSLQHLERRMRAQKGVKLPENQMKKRLKSIEPKIESYYHNLSRKIVNLAK ANNASIVVESLEGGGLKQHGRKKNSRHRALNYALSLFDYGKIASLIKYKSDLEGVPMY EVLPAYTSQQCAKCVLKKGSFVEPEIIGYIEEIGFKENLLTLLFEDTGLSSVQVLKKSKNK MTLSARDKEGKMVDLVLKYNFKGLVISQEKKKEEIVEFPIKEIDGKFAVLDSAYKRGKE RISKKGNQKLVYTGNKKVGYCSVHGQVDADLNASRVIALCKYLGINEPIVFGEQRKSF K
SEQ ID NO: 34	LDLITEPIQPHKSSSLRSKEFLEYQISDFLNFSLHSLFFGLASNEGPLVDFKIYDKIVIPKPE ERFPKKESEEGKKLDSFDKRVEEYYSDKLEKKIERKLNTEEKNVIDREKTRIWGEVNKL EEIRSIIDEINEIKKQKHISEKSKLLGEKWKKVNNIQETLLSQEYVSLISNLSDELTNKKKE LLAKKYSKFDDKIKKIKEDYGLEFDENTIKKEGEKAFLNPDKFSKYQFSSSYLKLIGEIAR SLITYKGFLDLNKYPIIFRKPINKVKKIHNLEPDEWKYYIQFGYEQINNPKLETENILGIDR GLTHILAYSVFEPRSSKFILNKLEPNPIEGWKWKLRKLRRSIQNLERRWRAQDNVKLPE NQMKKNLRSIEDKVENLYHNLSRKIVDLAKEKNACIVFEKLEGQGMKQHGRKKSDRL RGLNYKLSLFDYGKIAKLIKYKAEIEGIPIYRIDSAYTSQNCAKCVLESRRFAQPEEISCL DDFKEGDNLDKRILEGTGLVEAKIYKKLLKEKKEDFEIEEDIAMFDTKKVIKENKEKTVI LDYVYTRRKEIIGTNHKKNIKGIAKYTGNTKIGYCMKHGQVDADLNASRTIALCKNFDI NNPEIWK
SEQ ID NO: 35	MSDESLVSSEDKLAIKIKIVPNAEQAKMLDEMFKKWSSICNRISRGKEDIETLRPDEGKE LQFNSTQLNSATMDVSDLKKAMARQGERLEAEVSKLRGRYETIDASLRDPSRRHTNPQ KPSSFYPSDWDISGRLTPRFHTARHYSTELRKLKAKEDKMLKTINKIKNGKIVFKPKRIT LWPSSVNMAFKGSRLLLKPFANGFEMELPIVISPQKTADGKSQKASAEYMRNALLGLA GYSINQLLFGMNRSQKMLANAKKPEKVEKFLEQMKNKDANFDKKIKALEGKWLLDRK LKESEKSSIAVVRTKFFKSGKVELNEDYLKLLKHMANEILERDGFVNLNKYPILSRKPM KRYKQKNIDNLKPNMWKYYIQFGYEPIFERKASGKPKNIMGIDRGLTHLLAVAVFSPDQ QKFLFNHLESNPIMHWKWKLRKIRRSIQHMERRIRAEKNKHIHEAQLKKRLGSIEEKTE QHYHIVSSKIINWAIEYEAAIVLESLSHMKQRGGKKSVRTRALNYALSLFDYEKVARLIT YKARIRGIPVYDVLPGMTSKTCATCLLNGSQGAYVRGLETTKAAGKATKRKNMKIGKC MVCNSSENSMIDADLNAARVIAICKYKNLNDPQPAGSRKVFKRF
SEQ ID NO: 36	MLALKLKIMPTEKQAEILDAMFWKWASICSRIAKMKKKVSVKENKKELSKKIPSNSDI WFSKTQLCQAEVDVGDHKKALKNFEKRQESLLDELKYKVKAINEVINDESKREIDPNN PSKFRIKDSTKKGNLNSPKFFTLKKWQKILQENEKRIKKKESTIEKLKRGNIFFNPTKISL HEEEYSINFGSSKLLLNCFYKYNKKSGINSDQLENKFNEFQNGLNIICSPLQPIRGSSKRSF EFIRNSIINFLMYSLYAKLFGIPRSVKALMKSNKDENKLKLEEKLKKKKSSFNKTVKEFE KMIGRKLSDNESKILNDESKKFFEIIKSNNKYIPSEEYLKLLKDISEEIYNSNIDFKPYKYSI LIRKPLSKFKSKKLYNLKPTDYKYYLQLSYEPFSKQLIATKTILGIDRGLKHLLAVSVFDP SQNKFVYNKLIKNPVFKWKKRYHDLKRSIRNRERRIRALTGVHIHENQLIKKLKSMKNK INVLYHNVSKNIVDLAKKYESTIVLERLENLKQHGRSKGKRYKKLNYVLSNFDYKKIES LISYKAKKEGVPVSNINPKYTSKTCAKCLLEVNQLSELKNEYNRDSKNSKIGICNIHGQI DADLNAARVIALCYSKNLNEPHFK
SEQ ID NO: 37	VINLFGYKFALYPNKTQEELLNKHLGECGWLYNKAIEQNEYYKADSNIEEAQKKFELLP DKNSDEAKVLRGNISKDNYVYRTLVKKKKSEINVQIRKAVVLRPAETIRNLAKVKKKG LSVGRLKFIPIREWDVLPFKQSDQIRLEENYLILEPYGRLKFKMHRPLLGKPKTFCIKRTA TDRWTISFSTEYDDSNMRKNDGGQVGIDVGLKTHLRLSNENPDEDPRYPNPKIWKRYD RRLTILQRRISKSKKLGKNRTRLRLRLSRLWEKIRNSRADLIQNETYEILSENKLIAIEDLN VKGMQEKKDKKGRKGRTRAQEKGLHRSISDAAFSEFRRVLEYKAKRFGSEVKPVSAID

SEQ ID NO	Sequence
	SSKECHNCGNKKGMPLESRIYECPKCGLKIDRDLNSAKVILARATGVRPGSNARADTKI SATAGASVQTEGTVSEDFRQQMETSDQKPMQGEGSKEPPMNPEHKSSGRGSKHVNIGC KNKVGLYNEDENSRSTEKQIMDENRSTTEDMVEIGALHSPVLTT
SEQ ID NO: 38	MIASIDYEAVSQALIVFEFKAKGKDSQYQAIDEAIRSYRFIRNSCLRYWMDNKKVGKYD LNKYCKVLAKQYPFANKLNSQARQSAAECSWSAISRFYDNCKRKVSGKKGFPKFKKH ARSVEYKTSGWKLSENRKAITFTDKNGIGKLKLKGTYDLHFSQLEDMKRVRLVRRADG YYVQFCISVDVKVETEPTGKAIGLDVGIKYFLADSSGNTIENPQFYRKAEKKLNRANRR KSKKYIRGVKPQSKNYHKARCRYARKHLRVSRQRKEYCKRVAYCVIHSNDVVAYEDL NVKGMVKNRHLAKSISDVAWSTFRHWLEYFAIKYGKLTIPVAPHNTSQNCSNCDKKVP KSLSTRTHICHHCGYSEDRDVNAAKNILKKALSTVGQTGSLKLGEIEPLLVLEQSCTRKF DL
SEQ ID NO: 39	LAEENTLHLTLAMSLPLNDLPENRTRSELWRRQWLPQKKLSLLLGVNQSVRKAAADCL RWFEPYQELLWWEPTDPDGKKLLDKEGRPIKRTAGHMRVLRKLEEIAPFRGYQLGSAV KNGLRHKVADLLLSYAKRKLDPQFTDKTSYPSIGDQFPIVWTGAFVCYEQSITGQLYLY LPLFPRGSHQEDITNNYDPDRGPALQVFGEKEIARLSRSTSGLLLPLQFDKWGEATFIRG ENNPPTWKATHRRSDKKWLSEVLLREKDFQPKRVELLVRNGRIFVNVACEIPTKPLLEV ENFMGVSFGLEHLVTVVVINRDGNVVHQRQEPARRYEKTYFARLERLRRRGGPFSQEL ETFHYRQVAQIVEEALRFKSVPAVEQVGNIPKGRYNPRLNLRLSYWPFGKLADLTSYKA VKEGLPKPYSVYSATAKMLCSTCGAANKEGDQPISLKGPTVYCGNCGTRHNTGFNTAL NLARRAQELFVKGVVAR
SEQ ID NO: 40	MSQSLLKWHDMAGRDKDASRSLQKSAVEGVLLHLTASHRVALEMLEKSVSQTVAVT MEAAQQRLVIVLEDDPTKATSRKRVISADLQFTREEFGSLPNWAQKLASTCPEIATKYA DKHINSIRIAWGVAKESTNGDAVEQKLQWQIRLLDVTMFLQQLVLQLADKALLEQIPSS IRGGIGQEVAQQVTSHIQLLDSGTVLKAELPTISDRNSELARKQWEDAIQTVCTYALPFS RERARILDPGKYAAEDPRGDRLINIDPMWARVLKGPTVKSLPLLFVSGSSIRIVKLTLPR KHAAGHKHTFTATYLVLPVSREWINSLPGTVQEKVQWWKKPDVLATQELLVGKGALK KSANTLVIPISAGKKRFFNHILPALQRGFPLQWQRIVGRSYRRPATHRKWFAQLTIGYTN PSSLPEMALGIHFGMKDILWWALADKQGNILKDGSIPGNSILDFSLQEKGKIERQQKAG KNVAGKKYGKSLLNATYRVVNGVLEFSKGISAEHASQPIGLGLETIRFVDKASGSSPVN ARHSNWNYGQLSGIFANKAGPAGFSVTEITLKKAQRDLSDAEQARVLAIEATKRFASRI KRLATKRKDDTLFV
SEQ ID NO: 41	VEPVEKERFYYRTYTFRLDGQPRTQNLTTQSGWGLLTKAVLDNTKHYWEIVHHARIAN QPIVFENPVIDEQGNPKLNKLGQPRFWKRPISDIVNQLRALFENQNPYQLGSSLIQGTYW DVAENLASWYALNKEYLAGTATWGEPSFPEPHPLTEINQWMPLTFSSGKVVRLLKNAS GRYFIGLPILGENNPCYRMRTIEKLIPCDGKGRVTSGSLILFPLVGIYAQQHRRMTDICESI RTEKGKLAWAQVSIDYVREVDKRRRMRRTRKSQGWIQGPWQEVFILRLVLAHKAPKL YKPRCFAGISLGPKTLASCVILDQDERVVEKQQWSGSELLSLIHQGEERLRSLREQSKPT WNAAYRKQLKSLINTQVFTIVTFLRERGAAVRLESIARVRKSTPAPPVNFLLSHWAYRQ ITERLKDLAIRNGMPLTHSNGSYGVRFTCSQCGATNQGIKDPTKYKVDIESETFLCSICSH REIAAVNTATNLAKQLLDE
SEQ ID NO: 42	MNDTETSETLTSHRTVCAHLHVVGETGSLPRLVEAALAELITLNGRATQALLSLAKNGL VLRRDKEENLIAAELTLPCRKNKYADVAAKAGEPILATRINNKGKLVTKKWYGEGNSY HIVRFTPETGMFTVRVFDRYAFDEELLHLHSEVVFGSDLPKGIKAKTDSLPANFLQAVFT SFLELPFQGFPDIVVKPAMKQAAEQLLSYVQLEAGENQQAEYPDTNERDPELRLVEWQ KSLHELSVRTEPFEFVRARDIDYYAETDRRGNRFVNITPEWTKFAESPFARRLPLKIPPEF CILLRRKTEGHAKIPNRIYLGLQIFDGVTPDSTLGVLATAEDGKLFWWHDHLDEFSNLE GKPEPKLKNKPQLLMVSLEYDREQRFEESVGGDRKICLVTLKETRNFRRGWNGRILGIH FQHNPVITWALMDHDAEVLEKGFIEGNAFLGKALDKQALNEYLQKGGKWVGDRSFGN KLKGITHTLASLIVRLAREKDAWIALEEISWVQKQSADSVANHEIVEQPHHSLTR
SEQ ID NO: 43	MNDTETSETLTSHRTVCAHLHVVGETGSLPRLVEAALAELITLNGRATQALLSLAKNGL VLRRDKEENLIAAELTLPCRKNKYADVAAKAGEPILATRINNKGKLVTKKWYGEGNSY HIVRFTPETGMFTVRVFDRYAFDEELLHLHSEVVFGSDLPKGIKAKTDSLPANFLQAVFT SFLELPFQGFPDIVVKPAMKQAAEQLLSYVQLEAGENQQAEYPDTNERDPELRLVEWQ

SEQ ID NO	Sequence
	KSLHELSVRTEPFEFVRARDIDYYAETDRRGNRFVNITPEWTKFAESPFARRLPLKIPPEF CILLRRKTEGHAKIPNRIYLGLQIFDGVTPDSTLGVLATAEDGKLFWWHDHLDEFSNLE GKPEPKLKNKPQLLMVSLEYDREQRFEESVGGDRKICLVTLKETRNFRRGRHGHTRTD RLPAGNTLWRADFATSAEVAAPKWNGRILGIHFQHNPVITWALMDHDAEVLEKGFIEG NAFLGKALDKQALNEYLQKGGKWVGDRSFGNKLKGITHTLASLIVRLAREKDAWIALE EISWVQKQSADSVANRRFSMWNYSRLATLIEWLGTDIATRDCGTAAPLAHKVSDYLTH FTCPECGACRKAGQKKEIADTVRAGDILTCRKCGFSGPIPDNFIAEFVAKKALERMLKK KPV
SEQ ID NO: 44	MAKRNFGEKSEALYRAVRFEVRPSKEELSILLAVSEVLRMLFNSALAERQQVFTEFIASL YAELKSASVPEEISEIRKKLREAYKEHSISLFDQINALTARRVEDEAFASVTRNWQEETL DALDGAYKSFLSLRRKGDYDAHSPRSRDSGFFQKIPGRSGFKIGEGRIALSCGAGRKLSF PIPDYQQGRLAETTKLKKFELYRDQPNLAKSGRFWISVVYELPKPEATTCQSEQVAFVA LGASSIGVVSQRGEEVIALWRSDKHWVPKIEAVEERMKRRVKGSRGWLRLLNSGKRR MHMISSRQHVQDEREIVDYLVRNHGSHFVVTELVVRSKEGKLADSSKPERGGSLGLNW AAQNTGSLSRLVRQLEEKVKEHGGSVRKHKLTLTEAPPARGAENKLWMARKLRESFL KEV
SEQ ID NO: 45	LAKNDEKELLYQSVKFEIYPDESKIRVLTRVSNILVLVWNSALGERRARFELYIAPLYEE LKKFPRKSAESNALRQKIREGYKEHIPTFFDQLKKLLTPMRKEDPALLGSVPRAYQEETL NTLNGSFVSFMTLRRNNDMDAKPPKGRAEDRFHEISGRSGFKIDGSEFVLSTKEQKLRF PIPNYQLEKLKEAKQIKKFTLYQSRDRRFWISIAYEIELPDQRPFNPEEVIYIAFGASSIGVI SPEGEKVIDFWRPDKHWKPKIKEVENRMRSCKKGSRAWKKRAAARRKMYAMTQRQQ KLNHREIVASLLRLGFHFVVTEYTVRSKPGKLADGSNPKRGGAPQGFNWSAQNTGSFG EFILWLKQKVKEQGGTVQTFRLVLGQSERPEKRGRDNKIEMVRLLREKYLESQTIVV
SEQ ID NO: 46	MAKGKKKEGKPLYRAVRFEIFPTSDQITLFLRVSKNLQQVWNEAWQERQSCYEQFFGSI YERIGQAKKRAQEAGFSEVWENEAKKGLNKKLRQQEISMQLVSEKESLLQELSIAFQEH GVTLYDQINGLTARRIIGEFALIPRNWQEETLDSLDGSFKSFLALRKNGDPDAKPPRQRV SENSFYKIPGRSGFKVSNGQIYLSFGKIGQTLTSVIPEFQLKRLETAIKLKKFELCRDERD MAKPGRFWISVAYEIPKPEKVPVVSKQITYLAIGASRLGVVSPKGEFCLNLPRSDYHWK PQINALQERLEGVVKGSRKWKKRMAACTRMFAKLGHQQKQHGQYEVVKKLLRHGVH FVVTELKVRSKPGALADASKSDRKGSPTGPNWSAQNTGNIARLIQKLTDKASEHGGTVI KRNPPLLSLEERQLPDAQRKIFIAKKLREEFLADQK
SEQ ID NO: 47	MAKREKKDDVVLRGTKMRIYPTDRQVTLMDMWRRRCISLWNLLLNLETAAYGAKNT RSKLGWRSIWARVVEENHAKALIVYQHGKCKKDGSFVLKRDGTVKHPPRERFPGDRKI LLGLFDALRHTLDKGAKCKCNVNQPYALTRAWLDETGHGARTADIIAWLKDFKGECD CTAISTAAKYCPAPPTAELLTKIKRAAPADDLPVDQAILLDLFGALRGGLKQKECDHTH ARTVAYFEKHELAGRAEDILAWLIAHGGTCDCKIVEEAANHCPGPRLFIWEHELAMIM ARLKAEPRTEWIGDLPSHAAQTVVKDLVKALQTMLKERAKAAAGDESARKTGFPKFK KQAYAAGSVYFPNTTMFFDVAAGRVQLPNGCGSMRCEIPRQLVAELLERNLKPGLVIG AQLGLLGGRIWRQGDRWYLSCQWERPQPTLLPKTGRTAGVKIAASIVFTTYDNRGQTK EYPMPPADKKLTAVHLVAGKQNSRALEAQKEKEKKLKARKERLRLGKLEKGHDPNAL KPLKRPRVRRSKLFYKSAARLAACEAIERDRRDGFLHRVTNEIVHKFDAVSVQKMSVA PMMRRQKQKEKQIESKKNEAKKEDNGAAKKPRNLKPVRKLLRHVAMARGRQFLEYK YNDLRGPGSVLIADRLEPEVQECSRCGTKNPQMKDGRRLLRCIGVLPDGTDCDAVLPR NRNAARNAEKRLRKHREAHNA
SEQ ID NO: 48	MNEVLPIPAVGEDAADTIMRGSKMRIYPSVRQAATMDLWRRRCIQLWNLLLELEQAAY SGENRRTQIGWRSIWATVVEDSHAEAVRVAREGKKRKDGTFRKAPSGKEIPPLDPAML AKIQRQMNGAVDVDPKTGEVTPAQPRLFMWEHELQKIMARLKQAPRTHWIDDLPSHA AQSVVKDLIKALQAMLRERKKRASGIGGRDTGFPKFKKNRYAAGSVYFANTQLRFEAK RGKAGDPDAVRGEFARVKLPNGVGWMECRMPRHINAAHAYAQATLMGGRIWRQGE NWYLSCQWKMPKPAPLPRAGRTAAIKIAAAIPITTVDNRGQTREYAMPPIDRERIAAHA AAGRAQSRALEARKRRAKKREAYAKKRHAKKLERGIAAKPPGRARIKLSPGFYAAAA KLAKLEAEDANAREAWLHEITTQIVRNFDVIAVPRMEVAKLMKKPEPPEEKEEQVKAP

SEQ ID NO	Sequence
	WQGKRRSLKAARVMMRRTAMALIQTTLKYKAVDLRGPQAYEEIAPLDVTAAACSGCG VLKPEWKMARAKGREIMRCQEPLPGGKTCNTVLTYTRNSARVIGRELAVRLAERQKA
SEQ ID NO: 49	MTTQKTYNFCFYDQRFFELSKEAGEVYSRSLEEFWKIYDETGVWLSKFDLQKHMRNKL ERKLLHSDSFLGAMQQVHANLASWKQAKKVVPDACPPRKPKFLQAILFKKSQIKYKNG FLRLTLGTEKEFLYLKWDINIPLPIYGSVTYSKTRGWKINLCLETEVEQKNLSENKYLSID LGVKRVATIFDGENTITLSGKKFMGLMHYRNKLNGKTQSRLSHKKKGSNNYKKIQRAK RKTTDRLLNIQKEMLHKYSSFIVNYAIRNDIGNIIIGDNSSTHDSPNMRGKTNQKISQNPE QKLKNYIKYKFESISGRVDIVPEPYTSRKCPHCKNIKKSSPKGRTYKCKKCGFIFDRDGV GAINIYNENVSFGQIISPGRIRSLTEPIGMKFHNEIYFKSYVAA
SEQ ID NO: 50	MSVRSFQARVECDKQTMEHLWRTHKVFNERLPEIIKILFKMKRGECGQNDKQKSLYKS ISQSILEANAQNADYLLNSVSIKGWKPGTAKKYRNASFTWADDAAKLSSQGIHVYDKK QVLGDLPGMMSQMVCRQSVEAISGHIELTKKWEKEHNEWLKEKEKWESEDEHKKYL DLREKFEQFEQSIGGKITKRRGRWHLYLKWLSDNPDFAAWRGNKAVINPLSEKAQIRIN KAKPNKKNSVERDEFFKANPEMKALDNLHGYYERNFVRRRKTKKNPDGFDHKPTFTL PHPTIHPRWFVFNKPKTNPEGYRKLILPKKAGDLGSLEMRLLTGEKNKGNYPDDWISVK FKADPRLSLIRPVKGRRVVRKGKEQGQTKETDSYEFFDKHLKKWRPAKLSGVKLIFPDK TPKAAYLYFTCDIPDEPLTETAKKIQWLETGDVTKKGKKRKKKVLPHGLVSCAVDLSM RRGTTGFATLCRYENGKIHILRSRNLWVGYKEGKGCHPYRWTEGPDLGHIAKHKREIRI LRSKRGKPVKGEESHIDLQKHIDYMGEDRFKKAARTIVNFALNTENAASKNGFYPRAD VLLLENLEGLIPDAEKERGINRALAGWNRRHLVERVIEMAKDAGFKRRVFEIPPYGTSQ VCSKCGALGRRYSIIRENNRREIRFGYVEKLFACPNCGYCANADHNASVNLNRRFLIED SFKSYYDWKRLSEKKQKEEIETIESKLMDKLCAMHKISRGSISK
SEQ ID NO: 51	MHLWRTHCVFNQRLPALLKRLFAMRRGEVGGNEAQRQVYQRVAQFVLARDAKDSVD LLNAVSLRKRSANSAFKKKATISCNGQAREVTGEEVFAEAVALASKGVFAYDKDDMR AGLPDSLFQPLTRDAVACMRSHEELVATWKKEYREWRDRKSEWEAEPEHALYLNLRP KFEEGEAARGGRFRKRAERDHAYLDWLEANPQLAAWRRKAPPAVVPIDEAGKRRIAR AKAWKQASVRAEEFWKRNPELHALHKIHVQYLREFVRPRRTRRNKRREGFKQRPTFT MPDPVRHPRWCLFNAPQTSPQGYRLLRLPQSRRTVGSVELRLLTGPSDGAGFPDAWVN VRFKADPRLAQLRPVKVPRTVTRGKNKGAKVEADGFRYYDDQLLIERDAQVSGVKLL FRDIRMAPFADKPIEDRLLSATPYLVFAVEIKDEARTERAKAIRFDETSELTKSGKKRKT LPAGLVSVAVDLDTRGVGFLTRAVIGVPEIQQTHHGVRLLQSRYVAVGQVEARASGEA EWSPGPDLAHIARHKREIRRLRQLRGKPVKGERSHVRLQAHIDRMGEDRFKKAARKIV NEALRGSNPAAGDPYTRADVLLYESLETLLPDAERERGINRALLRWNRAKLIEHLKRM CDDAGIRHFPVSPFGTSQVCSKCGALGRRYSLARENGRAVIRFGWVERLFACPNPECPG RRPDRPDRPFTCNSDHNASVNLHRVFALGDQAVAAFRALAPRDSPARTLAVKRVEDTL RPQLMRVHKLADAGVDSPF
SEQ ID NO: 52	MATLVYRYGVRAHGSARQQDAVVSDPAMLEQLRLGHELRNALVGVQHRYEDGKRAV WSGFASVAAADHRVTTGETAVAELEKQARAEHSADRTAATRQGTAESLKAARAAVK QARADRKAAMAAVAEQAKPKIQALGDDRDAEIKDLYRRFCQDGVLLPRCGRCAGDLR SDGDCTDCGAAHEPRKLYWATYNAIREDHQTAVKLVEAKRKAGQPARLRFRRWTGD GTLTVQLQRMHGPACRCVTCAEKLTRRARKTDPQAPAVAADPAYPPTDPPRDPALLAS GQGKWRNVLQLGTWIPPGEWSAMSRAERRRVGRSHIGWQLGGGRQLTLPVQLHRQM PADADVAMAQLTRVRVGGRHRMSVALTAKLPDPPQVQGLPPVALHLGWRQRPDGSL RVATWACPQPLDLPPAVADVVVSHGGRWGEVIMPARWLADAEVPPRLLGRRDKAME PVLEALADWLEAHTEACTARMTPALVRRWRSQGRLAGLTNRWRGQPPTGSAEILTYLE AWRIQDKLLWERESHLRRRLAARRDDAWRRVASWLARHAGVLVVDDADIAELRRRD DPADTDPTMPASAAQAARARAALAAPGRLRHLATITATRDGLGVHTVASAGLTRLHR KCGHQAQPDPRYAASAVVTCPGCGNGYDQDYNAAMLMLDRQQQP
SEQ ID NO: 53	MSRVELHRAYKFRLYPTPAQVAELAEWERQLRRLYNLAHSQRLAAMQRHVRPKSPGV LKSECLSCGAVAVAEIGTDGKAKKTVKHAVGCSVLECRSCGGSPDAEGRTAHTAACSF VDYYRQGREMTQLLEEDDQLARVVCSARQETLRDLEKAWQRWHKMPGFGKPHFKKR IDSCRIYFSTPKSWAVDLGYLSFTGVASSVGRIKIRQDRVWPGDAKFSSCHVVRDVDEW YAVFPLTFTKEIEKPKGGAVGINRGAVHAIADSTGRVVDSPKFYARSLGVIRHRARLLD

SEQ ID NO	Sequence
	RKVPFGRAVKPSPTKYHGLPKADIDAAAARVNASPGRLVYEARARGSIAAAEAHLAAL VLPAPRQTSQLPSEGRNRERARRFLALAHQRVRRQREWFLHNESAHYAQSYTKIAIED WSTKEMTSSEPRDAEEMKRVTRARNRSILDVGWYELGRQIAYKSEATGAEFAKVDPGL RETETHVPEAIVRERDVDVSGMLRGEAGISGTCSRCGGLLRASASGHADAECEVCLHVE VGDVNAAVNVLKRAMFPGAAPPSKEKAKVTIGIKGRKKKRAA
SEQ ID NO: 54	MSRVELHRAYKFRLYPTPVQVAELSEWERQLRRLYNLGHEQRLLTLTRHLRPKSPGVL KGECLSCDSTQVQEVGADGRPKTTVRHAEQCPTLACRSCGALRDAEGRTAHTVACAF VDYYRQGREMTELLAADDQLARVVCSARQEVLRDLDKAWQRWRKMPGFGKPRFKRR TDSCRIYFSTPKAWKLEGGHLSFTGAATTVGAIKMRQDRNWPASVQFSSCHVVRDVDE WYAVFPLTFVAEVARPKGGAVGINRGAVHAIADSTGRVVDSPRYYARALGVIRHRARL FDRKVPSGHAVKPSPTKYRGLSAIEVDRVARATGFTPGRVVTEALNRGGVAYAECALA AIAVLGHGPERPLTSDGRNREKARKFLALAHQRVRRQREWFLHNESAHYARTYSKIAIE DWSTKEMTASEPQGEETRRVTRSRNRSILDVGWYELGRQLAYKTEATGAEFAQVDPGL KETETNVPKAIADARDVDVSGMLRGEAGISGTCSKCGGLLRAPASGHADAECEICLNVE VGDVNAAVNVLKRAMFPGDAPPASGEKPKVSIGIKGRQKKKKAA
SEQ ID NO: 55	MEAIATGMSPERRVELGILPGSVELKRAYKFRLYPMKVQQAELSEWERQLRRLYNLAH EQRLAALLRYRDWDFQKGACPSCRVAVPGVHTAACDHVDYFRQAREMTQLLEVDAQ LSRVICCARQEVLRDLDKAWQRWRKKLGGRPRFKRRTDSCRIYLSTPKHWEIAGRYLR LSGLASSVGEIRIEQDRAFPEGALLSSCSIVRDVDEWYACLPLTFTQPIERAPHRSVGLNR GVVHALADSDGRVVDSPKFFERALATVQKRSRDLARKVSGSRNAHKARIKLAKAHQR VRRQRAAFLHQESAYYSKGFDLVALEDMSVRKMTATAGEAPEMGRGAQRDLNRGILD VGWYELARQIDYKRLAHGGELLRVDPGQTTPLACVTEEQPARGISSACAVCGIPLARPA SGNARMRCTACGSSQVGDVNAAENVLTRALSSAPSGPKSPKASIKIKGRQKRLGTPAN RAGEASGGDPPVRGPVEGGTLAYVVEPVSESQSDT
SEQ ID NO: 56	MTVRTYKYRAYPTPEQAEALTSWLRFASQLYNAALEHRKNAWGRHDAHGRGFRFWD GDAAPRKKSDPPGRWVYRGGGGAHISKNDQGKLLTEFRREHAELLPPGMPALVQHEV LARLERSMAAFFQRATKGQKAGYPRWRSEHRYDSLTFGLTSPSKERFDPETGESLGRG KTVGAGTYHNGDLRLTGLGELRILEHRRIPMGAIPKSVIVRRSGKRWFVSIAMEMPSVE PAASGRPAVGLDMGVVTWGTAFTADTSAAAALVADLRRMATDPSDCRRLEELEREAA QLSEVLAHCRARGLDPARPRRCPKELTKLYRRSLHRLGELDRACARIRRRLQAAHDIAE PVPDEAGSAVLIEGSNAGMRHARRVARTQRRVARRTRAGHAHSNRRKKAVQAYARA KERERSARGDHRHKVSRALVRQFEEISVEALDIKQLTVAPEHNPDPQPDLPAHVQRRRN RGELDAAWGAFFAALDYKAADAGGRVARKPAPHTTQECARCGTLVPKPISLRVHRCP ACGYTAPRTVNSARNVLQRPLEEPGRAGPSGANGRGVPHAVA
SEQ ID NO: 57	MNCRYRYRIYPTPGQRQSLARLFGCVRVVWNDALFLCRQSEKLPKNSELQKLCITQAK KTEARGWLGQVSAIPLQQSVADLGVAFKNFFQSRSGKRKGKKVNPPRVKRRNNRQGA RFTRGGFKVKTSKVYLARIGDIKIKWSRPLPSEPSSVTVIKDCAGQYFLSFVVEVKPEIKP PKNPSIGIDLGLKTFASCSNGEKIDSPDYSRLYRKLKRCQRRLAKRQRGSKRRERMRVK VAKLNAQIRDKRKDFLHKLSTKVVNENQVIALEDLNVGGMLKNRKLSRAISQAGWYE FRSLCEGKAEKHNRDFRVISRWEPTSQVCSECGYRWGKIDLSVRSIVCINCGVEHDRDD NASVNIEQAGLKVGVGHTHDSKRTGSACKTSNGAVCVEPSTHREYVQLTLFDW
SEQ ID NO: 58	MKSRWTFRCYPTPEQEQHLARTFGCVRFVWNWALRARTDAFRAGERIGYPATDKALT LLKQQPETVWLNEVSSVCLQQALRDLQVAFSNFFDKRAAHPSFKRKEARQSANYTERG FSFDHERRILKLAKIGAIKVKWSRKAIPHPSSIRLIRTASGKYFVSLVVETQPAPMPETGE SVGVDFGVARLATLSNGERISNPKHGAKWQRRLAFYQKRLARATKGSKRRMRIKRHV ARIHEKIGNSRSDTLHKLSTDLVTRFDLICVEDLNLRGMVKNHSLARSLHDASIGSAIRM IEEKAERYGKNVVKIDRWFPSSKTCSDCGHIVEQLPLNVREWTCPECGTTHDRDANAA ANILAVGQTVSAHGGTVRRSRAKASERKSQRSANRQGVNRA
SEQ ID NO: 59	KEPLNIGKTAKAVFKEIDPTSLNRAANYDASIELNCKECKFKPFKNVKRYEFNFYNNWY RCNPNSCLQSTYKAQVRKVEIGYEKLKNEILTQMQYYPWFGRLYQNFFHDERDKMTSL DEIQVIGVQNKVFFNTVEKAWREIIKKRFKDNKETMETIPELKHAAGHGKRKLSNKSLL RRRFAFVQKSFKFVDNSDVSYRSFSNNIACVLPSRIGVDLGGVISRNPKREYIPQEISFNA FWKQHEGLKKGRNIEIQSVQYKGETVKRIEADTGEDKAWGKNRQRRFTSLILKLVPKQ

SEQ ID NO	Sequence
	GGKKVWKYPEKRNEGNYEYFPIPIEFILDSGETSIRFGGDEGEAGKQKHLVIPFNDSKAT PLASQQTLLENSRFNAEVKSCIGLAIYANYFYGYARNYVISSIYHKNSKNGQAITAIYLES IAHNYVKAIERQLQNLLLNLRDFSFMESHKKELKKYFGGDLEGTGGAQKRREKEEKIEK EIEQSYLPRLIRLSLTKMVTKQVEM
SEQ ID NO: 60	ELIVNENKDPLNIGKTAKAVFKEIDPTSINRAANYDASIELACKECKFKPFNNTKRHDFS FYSNWHRCSPNSCLQSTYRAKIRKTEIGYEKLKNEILNQMQYYPWFGRLYQNFFNDQR DKMTSLDEIQVTGVQNKIFFNTVEKAWREIIKKRFRDNKETMRTIPDLKNKSGHGSRKL SNKSLLRRRFAFAQKSFKLVDNSDVSYRAFSNNVACVLPSKIGVDIGGIINKDLKREYIP QEITFNVFWKQHDGLKKGRNIEIHSVQYKGEIVKRIEADTGEDKAWGKNRQRRFTSLIL KITPKQGGKKIWKFPEKKNASDYEYFPIPIEFILDNGDASIKFGGEEGEVGKQKHLLIPFN DSKATPLSSKQMLLETSRFNAEVKSTIGLALYANYFVSYARNYVIKSTYHKNSKKGQIV TEIYLESISQNFVRAIQRQLQSLMLNLKDWGFMQTHKKELKKYFGSDLEGSKGGQKRR EKEEKIEKEIEASYLPRLIRLSLTKSVTKAEEM
SEQ ID NO: 61	PEEKTSKLKPNSINLAANYDANEKFNCKECKFHPFKNKKRYEFNFYNNLHGCKSCTKST NNPAVKRIEIGYQKLKFEIKNQMEAYPWFGRLRINFYSDEKRKMSELNEMQVTGVKNK IFFDAIECAWREILKKRFRESKETLITIPKLKNKAGHGARKHRNKKLLIRRRAFMKKNFH FLDNDSISYRSFANNIACVLPSKVGVDIGGIISPDVGKDIKPVDISLNLMWASKEGIKSGR KVEIYSTQYDGNMVKKIEAETGEDKSWGKNRKRRQTSLLLSIPKPSKQVQEFDFKEWP RYKDIEKKVQWRGFPIKIIFDSNHNSIEFGTYQGGKQKVLPIPFNDSKTTPLGSKMNKLE KLRFNSKIKSRLGSAIAANKFLEAARTYCVDSLYHEVSSANAIGKGKIFIEYYLEILSQNY IEAAQKQLQRFIESIEQWFVADPFQGRLKQYFKDDLKRAKCFLCANREVQTTCYAAVK LHKSCAEKVKDKNKELAIKERNNKEDAVIKEVEASNYPRVIRLKLTKTITNKAM
SEQ ID NO: 62	SESENKIIEQYYAFLYSFRDKYEKPEFKNRGDIKRKLQNKWEDFLKEQNLKNDKKLSNY IFSNRNFRRSYDREEENEEGIDEKKSKPKRINCFEKEKNLKDQYDKDAINASANKDGAQ KWGCFECIFFPMYKIESGDPNKRIIINKTRFKLFDFYLNLKGCKSCLRSTYHPYRSNVYIE SNYDKLKREIGNFLQQKNIFQRMRKAKVSEGKYLTNLDEYRLSCVAMHFKNRWLFFDS IQKVLRETIKQRLKQMRESYDEQAKTKRSKGHGRAKYEDQVRMIRRRAYSAQAHKLL DNGYITLFDYDDKEINKVCLTAINQEGFDIGGYLNSDIDNVMPPIEISFHLKWKYNEPILN IESPFSKAKISDYLRKIREDLNLERGKEGKARSKKNVRRKVLASKGEDGYKKIFTDFFSK WKEELEGNAMERVLSQSSGDIQWSKKKRIHYTTLVLNINLLDKKGVGNLKYYEIAEKT KILSFDKNENKFWPITIQVLLDGYEIGTEYDEIKQLNEKTSKQFTIYDPNTKIIKIPFTDSK AVPLGMLGINIATLKTVKKTERDIKVSKIFKGGLNSKIVSKIGKGIYAGYFPTVDKEILEE VEEDTLDNEFSSKSQRNIFLKSIIKNYDKMLKEQLFDFYSFLVRNDLGVRFLTDRELQNI EDESFNLEKRFFETDRDRIARWFDNTNTDDGKEKFKKLANEIVDSYKPRLIRLPVVRVIK RIQPVKQREM
SEQ ID NO: 63	KYSTRDFSELNEIQVTACKQDEFFKVIQNAWREIIKKRFLENRENFIEKKIFKNKKGRGK RQESDKTIQRNRASVMKNFQLIENEKIILRAPSGHVACVFPVKVGLDIGGFKTDDLEKNI FPPRTITINVFWKNRDRQRKGRKLEVWGIKARTKLIEKVHKWDKLEEVKKKRLKSLEQ KQEKSLDNWSEVNNDSFYKVQIDELQEKIDKSLKGRTMNKILDNKAKESKEAEGLYIE WEKDFEGEMLRRIEASTGGEEKWGKRRQRRHTSLLLDIKNNSRGSKEIINFYSYAKQGK KEKKIEFFPFPLTITLDAEEESPLNIKSIPIEDKNATSKYFSIPFTETRATPLSILGDRVQKFK TKNISGAIKRNLGSSISSCKIVQNAETSAKSILSLPNVKEDNNMEIFINTMSKNYFRAMM KQMESFIFEMEPKTLIDPYKEKAIKWFEVAASSRAKRKLKKLSKADIKKSELLLSNTEEF EKEKQEKLEALEKEIEEFYLPRIVRLQLTKTILETPVM
SEQ ID NO: 64	KKLQLLGHKILLKEYDPNAVNAAANFETSTAELCGQCKMKPFKNKRRFQYTFGKNYH GCLSCIQNVYYAKKRIVQIAKEELKHQLTDSIASIPYKYTSLFSNTNSIDELYILKQERAA FFSNTNSIDELYITGIENNIAFKVISAIWDEIIKKRRQRYAESLTDTGTVKANRGHGGTAY KSNTRQEKIRALQKQTLHMVTNPYISLARYKNNYIVATLPRTIGMHIGAIKDRDPQKKL SDYAINFNVFWSDDRQLIELSTVQYTGDMVRKIEAETGENNKWGENMKRTKTSLLLEIL TKKTTDELTFKDWAFSTKKEIDSVTKKTYQGFPIGIIFEGNESSVKFGSQNYFPLPFDAKI TPPTAEGFRLDWLRKGSFSSQMKTSYGLAIYSNKVTNAIPAYVIKNMFYKIARAENGKQ IKAKFLKKYLDIAGNNYVPFIIMQHYRVLDTFEEMPISQPKVIRLSLTKTQHIIIKKDKTDS KM

SEQ ID NO	Sequence
SEQ ID NO: 65	NTSNLINLGKKAINISANYDANLEVGCKNCKFLSSNGNFPRQTNVKEGCHSCEKSTYEP SIYLVKIGERKAKYDVLDSLKKFTFQSLKYQSKKSMKSRNKKPKELKEFVIFANKNKAF DVIQKSYNHLILQIKKEINRMNSKKRKKNHKRRLFRDREKQLNKLRLIESSNLFLPRENK GNNHVFTYVAIHSVGRDIGVIGSYDEKLNFETELTYQLYFNDDKRLLYAYKPKQNKIIKI KEKLWNLRKEKEPLDLEYEKPLNKSITFSIKNDNLFKVSKDLMLRRAKFNIQGKEKLSK EERKINRDLIKIKGLVNSMSYGRFDELKKEKNIWSPHIYREVRQKEIKPCLIKNGDRIEIFE QLKKKMERLRRFREKRQKKISKDLIFAERIAYNFHTKSIKNTSNKINIDQEAKRGKASYM RKRIGYETFKNKYCEQCLSKGNVYRNVQKGCSCFENPFDWIKKGDENLLPKKNEDLRV KGAFRDEALEKQIVKIAFNIAKGYEDFYDNLGESTEKDLKLKFKVGTTINEQESLKL
SEQ ID NO: 66	TSNPIKLGKKAINISANYDSNLQIGCKNCKFLSYNGNFPRQTNVKEGCHSCEKSTYEPPV YTVRIGERRSKYDVLDSLKKFIFLSLKYRQSKKMKTRSKGIRGLEEFVISANLKKAMDVI QKSYRHLILNIKNEIVRMNGKKRNKNHKRLLFRDREKQLNKLRLIEGSSFFKPPTVKGD NSIFTCVAIHNIGRDIGIAGDYFDKLEPKIELTYQLYYEYNPKKESEINKRLLYAYKPKQN KIIEIKEKLWNLRKEKSPLDLEYEKPLTKSITFLVKRDGVFRISKDLMLRKAKFIIQGKEK LSKEERKINRDLIKIKSNIISLTYGRFDELKKDKTIWSPHIFRDVKQGKITPCIERKGDRMD IFQQLRKKSERLRENRKKRQKKISKDLIFAERIAYNFHTKSIKNTSNLINIKHEAKRGKAS YMRKRIGNETFRIKYCEQCFPKNNVYKNVQKGCSCFEDPFEYIKKGNEDLIPNKNQDLK AKGAFRDDALEKQIIKVAFNIAKGYEDFYENLKKTTEKDIRLKFKVGTIISEEM
SEQ ID NO: 67	NNSINLSKKAINISANYDANLQVRCKNCKFLSSNGNFPRQTDVKEGCHSCEKSTYEPPV YDVKIGEIKAKYEVLDSLKKFTFQSLKYQLSKSMKFRSKKIKELKEFVIFAKESKALNVI NRSYKHLILNIKNDINRMNSKKRIKNHKGRLFLDRQKQLSKLKLIEGSSFFVPAKNVGN KSVFTCVAIHSIGRDIGIAGLYDSFTKPVNEITYQIFFSGERRLLYAYKPKQLKILSIKENL WSLKNEKKPLDLLYEKPLGKNLNFNVKGGDLFRVSKDLMIRNAKFNVHGRQRLSDEE RLINRNFIKIKGEVVSLSYGRFEELKKDRKLWSPHIFKDVRQNKIKPCLVMQGQRIDIFE QLKRKLELLKKIRKSRQKKLSKDLIFGERIAYNFHTKSIKNTSNKINIDSDAKRGRASYM RKRIGNETFKLKYCDVCFPKANVYRRVQNGCSCSENPYNYIKKGDKDLLPKKDEGLAI KGAFRDEKLNKQIIKVAFNIAKGYEDFYDDLKKRTEKDVDLKFKIGTTVLDQKPMEIFD GIVITWL
SEQ ID NO: 68	LLTTVVETNNLAKKAINVAANFDANIDRQYYRCTPNLCRFIAQSPRETKEKDAGCSSCT QSTYDPKVYVIKIGKLLAKYEILKSLKRFLFMNRYFKQKKTERAQQKQKIGTELNEMSIF AKATNAMEVIKRATKHCTYDIIPETKSLQMLKRRRHRVKVRSLLKILKERRMKIKKIPN TFIEIPKQAKKNKSDYYVAAALKSCGIDVGLCGAYEKNAEVEAEYTYQLYYEYKGNSS TKRILYCYNNPQKNIREFWEAFYIQGSKSHVNTPGTIRLKMEKFLSPITIESEALDFRVWN SDLKIRNGQYGFIKKRSLGKEAREIKKGMGDIKRKIGNLTYGKSPSELKSIHVYRTEREN PKKPRAARKKEDNFMEIFEMQRKKDYEVNKKRRKEATDAAKIMDFAEEPIRHYHTNNL KAVRRIDMNEQVERKKTSVFLKRIMQNGYRGNYCRKCIKAPEGSNRDENVLEKNEGCL DCIGSEFIWKKSSKEKKGLWHTNRLLRRIRLQCFTTAKAYENFYNDLFEKKESSLDIIKL KVSITTKSM
SEQ ID NO: 69	ASTMNLAKQAINFAANYDSNLEIGCKGCKFMSTWSKKSNPKFYPRQNNQANKCHSCT YSTGEPEVPIIEIGERAAKYKIFTALKKFVFMSVAYKERRRQRFKSKKPKELKELAICSNR EKAMEVIQKSVVHCYGDVKQEIPRIRKIKVLKNHKGRLFYKQKRSKIKIAKLEKGSFFK TFIPKVHNNGCHSCHEASLNKPILVTTALNTIGADIGLINDYSTIAPTETDISWQVYYEFIP NGDSEAVKKRLLYFYKPKGALIKSIRDKYFKKGHENAVNTGFFKYQGKIVKGPIKFVNN ELDFARKPDLKSMKIKRAGFAIPSAKRLSKEDREINRESIKIKNKIYSLSYGRKKTLSDKD IIKHLYRPVRQKGVKPLEYRKAPDGFLEFFYSLKRKERRLRKQKEKRQKDMSEIIDAAD EFAWHRHTGSIKKTTNHINFKSEVKRGKVPIMKKRIANDSFNTRHCGKCVKQGNAINK YYIEKQKNCFDCNSIEFKWEKAALEKKGAFKLNKRLQYIVKACFNVAKAYESFYEDFR KGEEESLDLKFKIGTTTTLKQYPQNKARAM
SEQ ID NO: 70	HSHNLMLTKLGKQAINFAANYDANLEIGCKNCKFLSYSPKQANPKKYPRQTDVHEDGN IACHSCMQSTKEPPVYIVPIGERKSKYEILTSLNKFTFLALKYKEKKRQAFRAKKPKELQ ELAIAFNKEKAIKVIDKSIQHLILNIKPEIARIQRQKRLKNRKGKLLYLHKRYAIKMGLIK NGKYFKVGSPKKDGKKLLVLCALNTIGRDIGIIGNIEENNRSETEITYQLYFDCLDANPN ELRIKEIEYNRLKSYERKIKRLVYAYKPKQTKILEIRSKFFSKGHENKVNTGSFNFENPLN

SEQ ID NO	Sequence
	KSISIKVKNSAFDFKIGAPFIMLRNGKFHIPTKKRLSKEEREINRTLSKIKGRVFRLTYGRN ISEQGSKSLHIYRKERQHPKLSLEIRKQPDSFIDEFEKLRLKQNFISKLKKQRQKKLADLL QFADRIAYNYHTSSLEKTSNFINYKPEVKRGRTSYIKKRIGNEGFEKLYCETCIKSNDKE NAYAVEKEELCFVCKAKPFTWKKTNKDKLGIFKYPSRIKDFIRAAFTVAKSYNDFYENL KKKDLKNEIFLKFKIGLILSHEKKNHISIAKSVAEDERISGKSIKNILNKSIKLEKNCYSCFF HKEDM
SEQ ID NO: 71	SLERVIDKRNLAKKAINIAANFDANINKGFYRCETNQCMFIAQKPRKTNNTGCSSCLQST YDPVIYVVKVGEMLAKYEILKSLKRFVFMNRSFKQKKTEKAKQKERIGGELNEMSIFA NAALAMGVIKRAIRHCHVDIRPEINRLSELKKTKHRVAAKSLVKIVKQRKTKWKGIPNS FIQIPQKARNKDADFYVASALKSGGIDIGLCGTYDKKPHADPRWTYQLYFDTEDESEKR LLYCYNDPQAKIRDFWKTFYERGNPSMVNSPGTIEFRMEGFFEKMTPISIESKDFDFRV WNKDLLIRRGLYEIKKRKNLNRKAREIKKAMGSVKRVLANMTYGKSPTDKKSIPVYRV EREKPKKPRAVRKEENELADKLENYRREDFLIRNRRKREATEIAKIIDAAEPPIRHYHTN HLRAVKRIDLSKPVARKNTSVFLKRIMQNGYRGNYCKKCIKGNIDPNKDECRLEDIKKC ICCEGTQNIWAKKEKLYTGRINVLNKRIKQMKLECFNVAKAYENFYDNLAALKEGDLK VLKLKVSIPALNPEASDPEEDM
SEQ ID NO: 405	NASINLGKRAINLSANYDSNLVIGCKNCKFLSFNGNFPRQTNVREGCHSCDKSTYAPEV YIVKIGERKAKYDVLDSLKKFTFQSLKYQIKKSMRERSKKPKELLEFVIFANKDKAFNVI QKSYEHLILNIKQEINRMNGKKRIKNHKKRLFKDREKQLNKLRLIGSSSLFFPRENKGDK DLFTYVAIHSVGRDIGVAGSYESHIEPISDLTYQLFINNEKRLLYAYKPKQNKIIELKENL WNLKKEKKPLDLEFTKPLEKSITFSVKNDKLFKVSKDLMLRQAKFNIQGKEKLSKEERQ INRDFSKIKSNVISLSYGRFEELKKEKNIWSPHIYREVKQKEIKPCIVRKGDRIELFEQLKR KMDKLKKFRKERQKKISKDLNFAERIAYNFHTKSIKNTSNKINIDQEAKRGKASYMRKR IGNESFRKKYCEQCFSVGNVYHNVQNGCSCFDNPIELIKKGDEGLIPKGKEDRKYKGAL RDDNLQMQIIRVAFNIAKGYEDFYNNLKEKTEKDLKLKFKIGTTISTQESNNKEM
SEQ ID NO: 72	SNLIKLGKQAINFAANYDANLEVGCKNCKFLSSTNKYPRQTNVHLDNKMACRSCNQST MEPAIYIVRIGEKKAKYDIYNSLTKFNFQSLKYKAKRSQRFKPKQPKELQELSIAVRKEK ALDIIQKSIDHLIQDIRPEIPRIKQQKRYKNHVGKLFYLQKRRKNKLNLIGKGSFFKVFSP KEKKNELLVICALTNIGRDIGLIGNYNTIINPLFEVTYQLYYDYIPKKNNKNVQRRLLYA YKSKNEKILKLKEAFFKRGHENAVNLGSFSYEKPLEKSLTLKIKNDKDDFQVSPSLRIRT GRFFVPSKRNLSRQEREINRRLVKIKSKIKNMTYGKFETARDKQSVHIFRLERQKEKLPL QFRKDEKEFMEEFQKLKRRTNSLKKLRKSRQKKLADLLQLSEKVVYNNHTGTLKKTSN FLNFSSSVKRGKTAYIKELLGQEGFETLYCSNCINKGQKTRYNIETKEKCFSCKDVPFVW KKKSTDKDRKGAFLFPAKLKDVIKATFTVAKAYEDFYDNLKSIDEKKPYIKFKIGLILAH VRHEHKARAKEEAGQKNIYNKPIKIDKNCKECFFFKEEAM
SEQ ID NO: 73	NTTRKKFRKRTGFPQSDNIKLAYCSAIVRAANLDADIQKKHNQCNPNLCVGIKSNEQSR KYEHSDRQALLCYACNQSTGAPKVDYIQIGEIGAKYKILQMVNAYDFLSLAYNLTKLR NGKSRGHQRMSQLDEVVIVADYEKATEVIKRSINHLLDDIRGQLSKLKKRTQNEHITEH KQSKIRRKLRKLSRLLKRRRWKWGTIPNPYLKNWVFTKKDPELVTVALLHKLGRDIGL VNRSKRRSKQKLLPKVGFQLYYKWESPSLNNIKKSKAKKLPKRLLIPYKNVKLFDNKQ KLENAIKSLLESYQKTIKVEFDQFFQNRTEEIIAEEQQTLERGLLKQLEKKKNEFASQKK ALKEEKKKIKEPRKAKLLMEESRSLGFLMANVSYALFNTTIEDLYKKSNVVSGCIPQEP VVVFPADIQNKGSLAKILFAPKDGFRIKFSGQHLTIRTAKFKIRGKEIKILTKTKREILKNI EKLRRVWYREQHYKLKLFGKEVSAKPRFLDKRKTSIERRDPNKLADQTDDRQAELRNK EYELRHKQHKMAERLDNIDTNAQNLQTLSFWVGEADKPPKLDEKDARGFGVRTCISA WKWFMEDLLKKQEEDPLLKLKLSIM
SEQ ID NO: 74	PKKPKFQKRTGFPQPDNLRKEYCLAIVRAANLDADFEKKCTKCEGIKTNKKGNIVKGRT YNSADKDNLLCYACNISTGAPAVDYVFVGALEAKYKILQMVKAYDFHSLAYNLAKLW KGRGRGHQRMGGLNEVVIVSNNEKALDVIEKSLNHFHDEIRGELSRLKAKFQNEHLHV HKESKLRRKLRKISRLLKRRRWKWDVIPNSYLRNFTFTKTRPDFISVALLHRVGRDIGLV TKTKIPKPTDLLPQFGFQIYYTWDEPKLNKLKKSRLRSEPKRLLVPYKKIELYKNKSVLE EAIRHLAEVYTEDLTICFKDFFETQKRKFVSKEKESLKRELLKELTKLKKDFSERKTALK RDRKEIKEPKKAKLLMEESRSLGFLAANTSYALFNLIAADLYTKSKKACSTKLPRQLSTI

SEQ ID NO	Sequence
	LPLEIKEHKSTTSLAIKPEEGFKIRFSNTHLSIRTPKFKMKGADIKALTKRKREILKNATKL EKSWYGLKHYKLKLYGKEVAAKPRFLDKRNPSIDRRDPKELMEQIENRRNEVKDLEYE IRKGQHQMAKRLDNVDTNAQNLQTKSFWVGEADKPPELDSMEAKKLGLRTCISAWK WFMKDLVLLQEKSPNLKLKLSLTEM
SEQ ID NO: 75	KFSKRQEGFLIPDNIDLYKCLAIVRSANLDADVQGHKSCYGVKKNGTYRVKQNGKKGV KEKGRKYVFDLIAFKGNIEKIPHEAIEEKDQGRVIVLGKFNYKLILNIEKNHNDRASLEIK NKIKKLVQISSLETGEFLSDLLSGKIGIDEVYGIIEPDVFSGKELVCKACQQSTYAPLVEY MPVGELDAKYKILSAIKGYDFLSLAYNLSRNRANKKRGHQKLGGGELSEVVISANYDK ALNVIKRSINHYHVEIKPEISKLKKKMQNEPLKVMKQARIRRELHQLSRKVKRLKWKW GMIPNPELQNIIFEKKEKDFVSYALLHTLGRDIGLFKDTSMLQVPNISDYGFQIYYSWED PKLNSIKKIKDLPKRLLIPYKRLDFYIDTILVAKVIKNLIELYRKSYVVYETFGEEYGYAKK AEDILFDWDSINLSEGIEQKIQKIKDEFSDLLYEARESKRQNFVESFENILGLYDKNFASD RNSYQEKIQSMIIKKQQENIEQKLKREFKEVIERGFEGMDQNKKYYKVLSPNIKGGLLY TDTNNLGFFRSHLAFMLLSKISDDLYRKNNLVSKGGNKGILDQTPETMLTLEFGKSNLP NISIKRKFFNIKYNSSWIGIRKPKFSIKGAVIREITKKVRDEQRLIKSLEGVWHKSTHFKR WGKPRFNLPRHPDREKNNDDNLMESITSRREQIQLLLREKQKQQEKMAGRLDKIDKEIQ NLQTANFQIKQIDKKPALTEKSEGKQSVRNALSAWKWFMEDLIKYQKRTPILQLKLAK M
SEQ ID NO: 76	KFSKRQEGFVIPENIGLYKCLAIVRSANLDADVQGHVSCYGVKKNGTYVLKQNGKKSI REKGRKYASDLVAFKGDIEKIPFEVIEEKKKEQSIVLGKFNYKLVLDVMKGEKDRASLT MKNKSKKLVQVSSLGTDEFLLTLLNEKFGIEEIYGIIEPEVFSGKKLVCKACQQSTYAPL VEYMPVGELDSKYKILSAIKGYDFLSLAYNLARHRSNKKRGHQKLGGGELSEVVISAN NAKALNVIKRSLNHYYSEIKPEISKLRKKMQNEPLKVGKQARMRRELHQLSRKVKRLK WKWGKIPNLELQNITFKESDRDFISYALLHTLGRDIGMFNKTEIKMPSNILGYGFQIYYD WEEPKLNTIKKSKNTPKRILIPYKKLDFYNDSILVARAIKELVGLFQESYEWEIFGNEYN YAKEAEVELIKLDEESINGNVEKKLQRIKENFSNLLEKAREKKRQNFIESFESIARLYDES FTADRNEYQREIQSFIIEKQKQSIEKKLKNEFKKIVEKKFNEQEQGKKHYRVLNPTIINEF LPKDKNNLGFLRSKIAFILLSKISDDLYKKSNAVSKGGEKGIIKQQPETILDLEFSKSKLPS INIKKKLFNIKYTSSWLGIRKPKFNIKGAKIREITRRVRDVQRTLKSAESSWYASTHFRR WGFPRFNQPRHPDKEKKSDDRLIESITLLREQIQILLREKQKGQKEMAGRLDDVDKKIQ NLQTANFQIKQTGDKPALTEKSAGKQSFRNALSAWKWFMENLLKYQNKTPDLKLKIA RTVM
SEQ ID NO: 77	KWIEPNNIDFNKCLAITRSANLDADVQGHKMCYGIKTNGTYKAIGKINKKHNTGIIEKR RTYVYDLIVTKEKNEKIVKKTDFMAIDEEIEFDEKKEKLLKKYIKAEVLGTGELIRKDLN DGEKFDDLCSIEEPQAFRRSELVCKACNQSTYASDIRYIPIGEIEAKYKILKAIKGYDFLSL KYNLGRLRDSKKRGHQKMGQGELKEFVICANKEKALDVIKRSLNHYLNEVKDEISRLN KKMQNEPLKVNDQARWRRELNQISRRLKRLKWKWGEIPNPELKNLIFKSSRPEFVSYA LIHTLGRDIGLINETELKPNNIQEYGFQIYYKWEDPELNHIKKVKNIPKRFIIPYKNLDLFG KYTILSRAIEGILKLYSSSFQYKSFKDPNLFAKEGEKKITNEDFELGYDEKIKKIKDDFKS YKKALLEKKKNTLEDSLNSILSVYEQSLLTEQINNVKKWKEGLLKSKESIHKQKKIENIE DIISRIEELKNVEGWIRTKERDIVNKEETNLKREIKKELKDSYYEEVRKDFSDLKKGEESE KKPFREEPKPIVIKDYIKFDVLPGENSALGFFLSHLSFNLFDSIQYELFEKSRLSSSKHPQIP ETILDL
SEQ ID NO: 78	FRKFVKRSGAPQPDNLNKYKCIAIVRAANLDADIMSNESSNCVMCKGIKMNKRKTAKG AAKTTELGRVYAGQSGNLLCTACTKSTMGPLVDYVPIGRIRAKYTILRAVKEYDFLSLA YNLARTRVSKKGGRQKMHSLSELVIAAEYEIAWNIIKSSVIHYHQETKEEISGLRKKLQA EHIHKNKEARIRREMHQISRRIKRLKWKWHMIPNSELHNFLFKQQDPSFVAVALLHTLG RDIGMINKPKGSAKREFIPEYGFQIYYKWMNPKLNDINKQKYRKMPKRSLIPYKNLNVF GDRELIENAMHKLLKLYDENLEVKGSKFFKTRVVAISSKESEKLKRDLLWKGELAKIKK DFNADKNKMQELFKEVKEPKKANALMKQSRNMGFLLQNISYGALGLLANRMYEASA KQSKGDATKQPSIVIPLEMEFGNAFPKLLLRSGKFAMNVSSPWLTIRKPKFVIKGNKIKN ITKLMKDEKAKLKRLETSYHRATHFRPTLRGSIDWDSPYFSSPKQPNTHRRSPDRLSADI

SEQ ID NO	Sequence
	TEYRGRLKSVEAELREGQRAMAKKLDSVDMTASNLQTSNFQLEKGEDPRLTEIDEKGR SIRNCISSWKKFMEDLMKAQEANPVIKIKIALKDESSVLSEDSM
SEQ ID NO: 79	KFHPENLNKSYCLAIVRAANLDADIQGHINCIGIKSNKSDRNYENKLESLQNVELLCKA CTKSTYKPNINSVPVGEKKAKYSILSEIKKYDFNSLVYNLKKYRKGKSRGHQKLNELRE LVITSEYKKALDVINKSVNHYLVNIKNKMSKLKKILQNEHIHVGTLARIRRERNRISRKL DHYRKKWKFVPNKILKNYVFKNQSPDFVSVALLHKLGRDIGLITKTAILQKSFPEYSLQ LYYKYDTPKLNYLKKSKFKSLPKRILISYKYPKFDINSNYIEESIDKLLKLYEESPIYKNNS KIIEFFKKSEDNLIKSENDSLKRGIMKEFEKVTKNFSSKKKKLKEELKLKNEDKNSKMLA KVSRPIGFLKAYLSYMLFNIISNRIFEFSRKSSGRIPQLPSCIINLGNQFENFKNELQDSNIG SKKNYKYFCNLLLKSSGFNISYEEEHLSIKTPNFFINGRKLKEITSEKKKIRKENEQLIKQ WKKLTFFKPSNLNGKKTSDKIRFKSPNNPDIERKSEDNIVENIAKVKYKLEDLLSEQRKE FNKLAKKHDGVDVEAQCLQTKSFWIDSNSPIKKSLEKKNEKVSVKKKMKAIRSCISAW KWFMADLIEAQKETPMIKLKLALM
SEQ ID NO: 80	TTLVPSHLAGIEVMDETTSRNEDMIQKETSRSNEDENYLGVKNKCGINVHKSGRGSSKH EPNMPPEKSGEGQMPKQDSTEMQQRFDESVTGETQVSAGATASIKTDARANSGPRVGT ARALIVKASNLDRDIKLGCKPCEYIRSELPMGKKNGCNHCEKSSDIASVPKVESGFRKA KYELVRRFESFAADSISRHLGKEQARTRGKRGKKDKKEQMGKVNLDEIAILKNESLIEY TENQILDARSNRIKEWLRSLRLRLRTRNKGLKKSKSIRRQLITLRRDYRKWIKPNPYRPD EDPNENSLRLHTKLGVDIGVQGGDNKRMNSDDYETSFSITWRDTATRKICFTKPKGLLP RHMKFKLRGYPELILYNEELRIQDSQKFPLVDWERIPIFKLRGVSLGKKKVKALNRITEA PRLVVAKRIQVNIESKKKKVLTRYVYNDKSINGRLVKAEDSNKDPLLEFKKQAEEINSD AKYYENQEIAKNYLWGCEGLHKNLLEEQTKNPYLAFKYGFLNIV
SEQ ID NO: 81	LDFKRTCSQELVLLPEIEGLKLSGTQGVTSLAKKLINKAANVDRDESYGCHHCIHTRTSL SKPVKKDCNSCNQSTNHPAVPITLKGYKIAFYELWHRFTSWAVDSISKALHRNKVMGK VNLDEYAVVDNSHIVCYAVRKCYEKRQRSVRLHKRAYRCRAKHYNKSQPKVGRIYKK SKRRNARNLKKEAKRYFQPNEITNGSSDALFYKIGVDLGIAKGTPETEVKVDVSICFQV YYGDARRVLRVRKMDELQSFHLDYTGKLKLKGIGNKDTFTIAKRNESLKWGSTKYEVS RAHKKFKPFGKKGSVKRKCNDYFRSIASWSCEAASQRAQSNLKNAFPYQKALVKCYK NLDYKGVKKNDMWYRLCSNRIFRYSRIAEDIAQYQSDKGKAKFEFVILAQSVAEYDISA IM
SEQ ID NO: 82	VFLTDDKRKTALRKIRSAFRKTAEIALVRAQEADSLDRQAKKLTIETVSFGAPGAKNAFI GSLQGYNWNSHRANVPSSGSAKDVFRITELGLGIPQSAHEASIGKSFELVGNVVRYTAN LLSKGYKKGAVNKGAKQQREIKGKEQLSFDLISNGPISGDKLINGQKDALAWWLIDKM GFHIGLAMEPLSSPNTYGITLQAFWKRHTAPRRYSRGVIRQWQLPFGRQLAPLIHNFFRK KGASIPIVLTNASKKLAGKGVLLEQTALVDPKKWWQVKEQVTGPLSNIWERSVPLVLY TATFTHKHGAAHKRPLTLKVIRISSGSVFLLPLSKVTPGKLVRAWMPDINILRDGRPDEA AYKGPDLIRARERSFPLAYTCVTQIADEWQKRALESNRDSITPLEAKLVTGSDLLQIHST VQQAVEQGIGGRISSPIQELLAKDALQLVLQQLFMTVDLLRIQWQLKQEVADGNTSEK AVGWAIRISNIHKDAYKTAIEPCTSALKQAWNPLSGFEERTFQLDASIVRKRSTAKTPDD ELVIVLRQQAAEMTVAVTQSVSKELMELAVRHSATLHLLVGEVASKQLSRSADKDRG AMDHWKLLSQSM
SEQ ID NO: 83	EDLLQKALNTATNVAAIERHSCISCLFTESEIDVKYKTPDKIGQNTAGCQSCTFRVGYSG NSHTLPMGNRIALDKLRETIQRYAWHSLLFNVPPAPTSKRVRAISELRVAAGRERLFTVI TFVQTNILSKLQKRYAANWTPKSQERLSRLREEGQHILSLLESGSWQQKEVVREDQDLI VCSALTKPGLSIGAFCRPKYLKPAKHALVLRLIFVEQWPGQIWGQSKRTRRMRRRKDV ERVYDISVQAWALKGKETRISECIDTMRRHQQAYIGVLPFLILSGSTVRGKGDCPILKEIT RMRYCPNNEGLIPLGIFYRGSANKLLRVVKGSSFTLPMWQNIETLPHPEPFSPEGWTATG ALYEKNLAYWSALNEAVDWYTGQILSSGLQYPNQNEFLARLQNVIDSIPRKWFRPQGL KNLKPNGQEDIVPNEFVIPQNAIRAHHVIEWYHKTNDLVAKTLLGWGSQTTLNQTRPQ GDLRFTYTRYYFREKEVPEV
SEQ ID	VPKKKLMRELAKKAVFEAIFNDPIPGSFGCKRCTLIDGARVTDAIEKKQGAKRCAGCEP CTFHTLYDSVKHALPAATGCDRTAIDTGLWEILTALRSYNWMSFRRNAVSDASQKQV WSIEELAIWADKERALRVILSALTHTIGKLKNGFSRDGVWKGGKQLYENLAQKDLAKG

SEQ ID NO	Sequence
NO: 84	LFANGEIFGKELVEADHDMLAWTIVPNHQFHIGLIRGNWKPAAVEASTAFDARWLTNG APLRDTRTHGHRGRRFNRTEKLTVLCIKRDGGVSEEFRQERDYELSVMLLQPKNKLKPE PKGELNSFEDLHDHWWFLKGDEATALVGLTSDPTVGDFIQLGLYIRNPIKAHGETKRRL LICFEPPIKLPLRRAFPSEAFKTWEPTINVFRNGRRDTEAYYDIDRARVFEFPETRVSLEH LSKQWEVLRLEPDRENTDPYEAQQNEGAELQVYSLLQEAAQKMAPKVVIDPFGQFPLE LFSTFVAQLFNAPLSDTKAKIGKPLDSGFVVESHLHLLEEDFAYRDFVRVTFMGTEPTFR VIHYSNGEGYWKKTVLKGKNNIRTALIPEGAKAAVDAYKNKRCPLTLEAAILNEEKDR RLVLGNKALSLLAQTARGNLTILEALAAEVLRPLSGTEGVVHLHACVTRHSTLTESTET DNM
SEQ ID NO: 85	VEKLFSERLKRAMWLKNEAGRAPPAETLTLKHKRVSGGHEKVKEELQRVLRSLSGTNQ AAWNLGLSGGREPKSSDALKGEKSRVVLETVVFHSGHNRVLYDVIEREDQVHQRSSIM HMRRKGSNLLRLWGRSGKVRRKMREEVAEIKPVWHKDSRWLAIVEEGRQSVVGISSA GLAVFAVQESQCTTAEPKPLEYVVSIWFRGSKALNPQDRYLEFKKLKTTEALRGQQYD PIPFSLKRGAGCSLAIRGEGIKFGSRGPIKQFFGSDRSRPSHADYDGKRRLSLFSKYAGDL ADLTEEQWNRTVSAFAEDEVRRATLANIQDFLSISHEKYAERLKKRIESIEEPVSASKLE AYLSAIFETFVQQREALASNFLMRLVESVALLISLEEKSPRVEFRVARYLAESKEGFNRK AM
SEQ ID NO: 86	VVITQSELYKERLLRVMEIKNDRGRKEPRESQGLVLRFTQVTGGQEKVKQKLWLIFEGF SGTNQASWNFGQPAGGRKPNSGDALKGPKSRVTYETVVFHFGLRLLSAVIERHNLKQQ RQTMAYMKRRAAARKKWARSGKKCSRMRNEVEKIKPKWHKDPRWFDIVKEGEPSIV GISSAGFAIYIVEEPNFPRQDPLEIEYAISIWFRRDRSQYLTFKKIQKAEKLKELQYNPIPFR LKQEKTSLVFESGDIKFGSRGSIEHFRDEARGKPPKADMDNNRRLTMFSVFSGNLTNLT EEQYARPVSGLLAPDEKRMPTLLKKLQDFFTPIHEKYGERIKQRLANSEASKRPFKKLEE YLPAIYLEFRARREGLASNWVLVLINSVRTLVRIKSEDPYIEFKVSQYLLEKEDNKAL
SEQ ID NO: 87	KQDALFEERLKKAIFIKRQADPLQREELSLLPPNRKIVTGGHESAKDTLKQILRAINGTN QASWNPGTPSGKRDSKSADALAGPKSRVKLETVVFHVGHRLLKKVVEYQGHQKQQH GLKAFMRTCAAMRKKWKRSGKVVGELREQLANIQPKWHYDSRPLNLCFEGKPSVVGL RSAGIALYTIQKSVVPVKEPKPIEYAVSIWFRGPKAMDREDRCLEFKKLKIATELRKLQF EPIVSTLTQGIKGFSLYIQGNSVKFGSRGPIKYFSNESVRQRPPKADPDGNKRLALFSKFS GDLSDLTEEQWNRPILAFEGIIRRATLGNIQDYLTVGHEQFAISLEQLLSEKESVLQMSIE QQRLKKNLGKKAENEWVESFGAEQARKKAQGIREYISGFFQEYCSQREQWAENWVQQ LNKSVRLFLTIQDSTPFIEFRVARYLPKGEKKKGKAM
SEQ ID NO: 88	ANHAERHKRLRKEANRAANRNRPLVADCDTGDPLVGICRLLRRGDKMQPNKTGCRSC EQVEPELRDAILVSGPGRLDNYKYELFQRGRAMAVHRLLKRVPKLNRPKKAAGNDEK KAENKKSEIQKEKQKQRRMMPAVSMKQVSVADFKHVIENTVRHLFGDRRDREIAECA ALRAASKYFLKSRRVRPRKLPKLANPDHGKELKGLRLREKRAKLKKEKEKQAELARSN QKGAVLHVATLKKDAPPMPYEKTQGRNDYTTFVISAAIKVGATRGTKPLLTPQPREWQ CSLYWRDGQRWIRGGLLGLQAGIVLGPKLNRELLEAVLQRPIECRMSGCGNPLQVRGA AVDFFMTTNPFYVSGAAYAQKKFKPFGTKRASEDGAAAKAREKLMTQLAKVLDKVVT QAAHSPLDGIWETRPEAKLRAMIMALEHEWIFLRPGPCHNAAEEVIKCDCTGGHAILW ALIDEARGALEHKEFYAVTRAHTHDCEKQKLGGRLAGFLDLLIAQDVPLDDAPAARKI KTLLEATPPAPCYKAATSIATCDCEGKFDKLWAIIDATRAGHGTEDLWARTLAYPQNV NCKCKAGKDLTHRLADFLGLLIKRDGPFRERPPHKVTGDRKLVFSGDKKCKGHQYVIL AKAHNEEVVRAWISRWGLKSRTNKAGYAATELNLLLNWLSICRRRWMDMLTVQRDT PYIRMKTGRLVVDDKKERKAM
SEQ ID NO: 89	AKQREALRVALERGIVRASNRTYTLVTNCTKGGPLPEQCRMIERGKARAMKWEPKLV GCGSCAAATVDLPAIEEYAQPGRLDVAKYKLTTQILAMATRRMMVRAAKLSRRKGQW PAKVQEEKEEPPEPKKMLKAVEMRPVAIVDFNRVIQTTIEHLWAERANADEAELKALK AAAAYFGPSLKIRARGPPKAAIGRELKKAHRKKAYAERKKARRKRAELARSQARGAA AHAAIRERDIPPMAYERTQGRNDVTTIPIAAAIKIAATRGARPLPAPKPMKWQCSLYWN EGQRWIRGGMLTAQAYAHAANIHRPMRCEMWGVGNPLKVRAFEGRVADPDGAKGR KAEFRLQTNAFYVSGAAYRNKKFKPFGTDRGGIGSARKKRERLMAQLAKILDKVVSQA AHSPLDDIWHTRPAQKLRAMIKQLEHEWMFLRPQAPTVEGTKPDVDVAGNMQRQIKA

SEQ ID NO	Sequence
	LMAPDLPPIEKGSPAKRFTGDKRKKGERAVRVAEAHSDEVVTAWISRWGIQTRRNEGS YAAQELELLLNWLQICRRRWLDMTAAQRVSPYIRMKSGRMITDAADEGVAPIPLVENM
SEQ ID NO: 90	KSISGRSIKHMACLKDMLKSEITEIEEKQKKESLRKWDYYSKFSDEILFRRNLNVSANHD ANACYGCNPCAFLKEVYGFRIERRNNERIISYRRGLAGCKSCVQSTGYPPIEFVRRKFGA DKAMEIVREVLHRRNWGALARNIGREKEADPILGELNELLLVDARPYFGNKSAANETN LAFNVITRAAKKFRDEGMYDIHKQLDIHSEEGKVPKGRKSRLIRIERKHKAIHGLDPGET WRYPHCGKGEKYGVWLNRSRLIHIKGNEYRCLTAFGTTGRRMSLDVACSVLGHPLVK KKRKKGKKTVDGTELWQIKKATETLPEDPIDCTFYLYAAKPTKDPFILKVGSLKAPRW KKLHKDFFEYSDTEKTQGQEKGKRVVRRGKVPRILSLRPDAKFKVSIWDDPYNGKNKE GTLLRMELSGLDGAKKPLILKRYGEPNTKPKNFVFWRPHITPHPLTFTPKHDFGDPNKK TKRRRVFNREYYGHLNDLAKMEPNAKFFEDREVSNKKNPKAKNIRIQAKESLPNIVAK NGRWAAFDPNDSLWKLYLHWRGRRKTIKGGISQEFQEFKERLDLYKKHEDESEWKEK EKLWENHEKEWKKTLEIHGSIAEVSQRCVMQSMMGPLDGLVQKKDYVHIGQSSLKAA DDAWTFSANRYKKATGPKWGKISVSNLLYDANQANAELISQSISKYLSKQKDNQGCEG RKMKFLIKIIEPLRENFVKHTRWLHEMTQKDCEVRAQFSRVSM
SEQ ID NO: 91	FPSDVGADALKHVRMLQPRLTDEVRKVALTRAPSDRPALARFAAVAQDGLAFVRHLN VSANHDSNCTFPRDPRDPRRGPCEPNPCAFLREVWGFRIVARGNERALSYRRGLAGCKS CVQSTGFPSVPFHRIGADDCMRKLHEILKARNWRLLARNIGREREADPLLTELSEYLLV DARTYPDGAAPNSGRLAENVIKRAAKKFRDEGMRDIHAQLRVHSREGKVPKGRLQRL RRIERKHRAIHALDPGPSWEAEGSARAEVQGVAVYRSQLLRVGHHTQQIEPVGIVARTL FGVGRTDLDVAVSVLGAPLTKRKKGSKTLESTEDFRIAKARETRAEDKIEVAFVLYPTA SLLRDEIPKDAFPAMRIDRFLLKVGSVQADREILLQDDYYRFGDAEVKAGKNKGRTVT RPVKVPRLQALRPDAKFRVNVWADPFGAGDSPGTLLRLEVSGVTRRSQPLRLLRYGQP STQPANFLCWRPHRVPDPMTFTPRQKFGERRKNRRTRRPRVFERLYQVHIKHLAHLEPN RKWFEEARVSAQKWAKARAIRRKGAEDIPVVAPPAKRRWAALQPNAELWDLYAHDR EARKRFRGGRAAEGEEFKPRLNLYLAHEPEAEWESKRDRWERYEKKWTAVLEEHSRM CAVADRTLPQFLSDPLGARMDDKDYAFVGKSALAVAEAFVEEGTVERAQGNCSITAK KKFASNASRKRLSVANLLDVSDKADRALVFQAVRQYVQRQAENGGVEGRRMAFLRK LLAPLRQNFVCHTRWLHM
SEQ ID NO: 92	AARKKKRGKIGITVKAKEKSPPAAGPFMARKLVNVAANVDGVEVHLCVECEADAHGS ASARLLGGCRSCTGSIGAEGRLMGSVDVDRERVIAEPVHTETERLGPDVKAFEAGTAES KYAIQRGLEYWGVDLISRNRARTVRKMEEADRPESSTMEKTSWDEIAIKTYSQAYHAS ENHLFWERQRRVRQHALALFRRARERNRGESPLQSTQRPAPLVLAALHAEAAAISGRA RAEYVLRGPSANVRAAAADIDAKPLGHYKTPSPKVARGFPVKRDLLRARHRIVGLSRA YFKPSDVVRGTSDAIAHVAGRNIGVAGGKPKEIEKTFTLPFVAYWEDVDRVVHCSSFK ADGPWVRDQRIKIRGVSSAVGTFSLYGLDVAWSKPTSFYIRCSDIRKKFHPKGFGPMKH WRQWAKELDRLTEQRASCVVRALQDDEELLQTMERGQRYYDVFSCAATHATRGEAD PSGGCSRCELVSCGVAHKVTKKAKGDTGIEAVAVAGCSLCESKLVGPSKPRVHRQMA ALRQSHALNYLRRLQREWEALEAVQAPTPYLRFKYARHLEVRSM
SEQ ID NO: 93	AAKKKKQRGKIGISVKPKEGSAPPADGPFMARKLVNVAANVDGVEVNLCIECEADAH GSAPARLLGGCKSCTGSIGAEGRLMGSVDVDRADAIAKPVNTETEKLGPDVQAFEAGT AETKYALQRGLEYWGVDLISRNRSRTVRRTEEGQPESATMEKTSWDEIAIKSYTRAYH ASENHLFWERQRRVRQHALALFKRAKERNRGDSTLPREPGHGLVAIAALACEAYAVG GRNLAETVVRGPTFGTARAVRDVEIASLGRYKTPSPKVAHGSPVKRDFLRARHRIVGLA RAYYRPSDVVRGTSDAIAHVAGRNIGVAGGKPRAVEAVFTLPFVAYWEDVDRVVHCS SFQVSAPWNRDQRMKIAGVTTAAGTFSLHGGELKWAKPTSFYIRCSDTRRKFRPKGFG PMKRWRQWAKDLDRLVEQRASCVVRALQDDAALLETMERGQRYYDVFACAVTHAT RGEADRLAGCSRCALTPCQEAHRVTTKPRGDAGVEQVQTSDCSLCEGKLVGPSKPRLH RTLTLLRQEHGLNYLRRLQREWESLEAVQVPTPYLRFKYARHLEVRSM
SEQ ID NO: 94	TDSQSESVPEVVYALTGGEVPGRVPPDGGSAEGARNAPTGLRKQRGKIKISAKPSKPGS PASSLARTLVNEAANVDGVQSSGCATCRMRANGSAPRALPIGCVACASSIGRAPQEETV CALPTTQGPDVRLLEGGHALRKYDIQRALEYWGVDLIGRNLDRQAGRGMEPAEGATA TMKRVSMDELAVLDFGKSYYASEQHLFAARQRRVRQHAKALKIRAKHANRSGSVKRA

SEQ ID NO	Sequence
	LDRSRKQVTALAREFFKPSDVVRGDSDALAHVVGRNLGVSRHPAREIPQTFTLPLCAY WEDVDRVISCSSLLAGEPFARDQEIRIEGVSSALGSLRLYRGAIEWHKPTSLYIRCSDTRR KFRPRGGLKKRWRQWAKDLDRLVEQRACCIVRSLQADVELLQTMERAQRFYDVHDC AATHVGPVAVRCSPCAGKQFDWDRYRLLAALRQEHALNYLRRLQREWESLEAQQVK MPYLRFKYARKLEVSGPLIGLEVRREPSMGTAIAEM
SEQ ID NO: 95	AGTAGRRHGSLGARRSINIAGVTDRHGRWGCESCVYTRDQAGNRARCAPCDQSTYAP DVQEVTIGQRQAKYTIFLTLQSFSWTNTMRNNKRAAAGRSKRTTGKRIGQLAEIKITGV GLAHAHNVIQRSLQHNITKMWRAEKGKSKRVARLKKAKQLTKRRAYFRRRMSRQSRG NGFFRTGKGGIHAVAPVKIGLDVGMIASGSSEPADEQTVTLDAIWKGRKKKIRLIGAKG ELAVAACRFREQQTKGDKCIPLILQDGEVRWNQNNWQCHPKKLVPLCGLEVSRKFVSQ ADRLAQNKVASPLAARFDKTSVKGTLVESDFAAVLVNVTSIYQQCHAMLLRSQEPTPS LRVQRTITSM
SEQ ID NO: 96	GVRFSPAQSQVFFRTVIPQSVEARFAINMAAIHDAAGAFGCSVCRFEDRTPRNAKAVHG CSPCTRSTNRPDVFVLPVGAIKAKYDVFMRLLGFNWTHLNRRQAKRVTVRDRIGQLDE LAISMLTGKAKAVLKKSICHNVDKSFKAMRGSLKKLHRKASKTGKSQLRAKLSDLRER TNTTQEGSHVEGDSDVALNKIGLDVGLVGKPDYPSEESVEVVVCLYFVGKVLILDAQG RIRDMRAKQYDGFKIPIIQRGQLTVLSVKDLGKWSLVRQDYVLAGDLRFEPKISKDRKY AECVKRIALITLQASLGFKERIPYYVTKQVEIKNASHIAFVTEAIQNCAENFREMTEYLM KYQEKSPDLKVLLTQLM
SEQ ID NO: 97	RAVVGKVFLEQARRALNLATNFGTNHRTGCNGCYVTPGKLSIPQDGEKNAAGCTSCL MKATASYVSYPKPLGEKVAKYSTLDALKGFPWYSLRLNLRPNYRGKPINGVQEVAPVS KFRLAEEVIQAVQRYHFTELEQSFPGGRRRLRELRAFYTKEYRRAPEQRQHVVNGDRNI VVVTVLHELGFSVGMFNEVELLPKTPIECAVNVFIRGNRVLLEVRKPQFDKERLLVESL WKKDSRRHTAKWTPPNNEGRIFTAEGWKDFQLPLLLGSTSRSLRAIEKEGFVQLAPGRD PDYNNTIDEQHSGRPFLPLYLYLQGTISQEYCVFAGTWVIPFQDGISPYSTKDTFQPDLK RKAYSLLLDAVKHRLGNKVASGLQYGRFPAIEELKRLVRMHGATRKIPRGEKDLLKKG DPDTPEWWLLEQYPEFWRLCDAAAKRVSQNVGLLLSLKKQPLWQRRWLESRTRNEPL DNLPLSMALTLHLTNEEAL
SEQ ID NO: 98	AAVYSKFYIENHFKMGIPETLSRIRGPSIIQGFSVNENYINIAGVGDRDFIFGCKKCKYTR GKPSSKKINKCHPCKRSTYPEPVIDVRGSISEFKYKIYNKLKQEPNQSIKQNTKGRMNPS DHTSSNDGIIINGIDNRIAYNVIFSSYKHLMEKQINLLRDTTKRKARQIKKYNNSGKKKH SLRSQTKGNLKNRYHMLGMFKKGSLTITNEGDFITAVRKVGLDISLYKNESLNKQEVET ELCLNIKWGRTKSYTVSGYIPLPINIDWKLYLFEKETGLTLRLFGNKYKIQSKKFLIAQLF KPKRPPCADPVVKKAQKWSALNAHVQQMAGLFSDSHLLKRELKNRMHKQLDFKSLW VGTEDYIKWFEELSRSYVEGAEKSLEFFRQDYFCFNYTKQTTM
SEQ ID NO: 99	PQQQRDLMLMAANYDQDYGNGCGPCTVVASAAYRPDPQAQHGCKRHLRTLGASAVT HVGLGDRTATITALHRLRGPAALAARARAAQAASAPMTPDTDAPDDRRRLEAIDADDV VLVGAHRALWSAVRRWADDRRAALRRRLHSEREWLLKDQIRWAELYTLIEASGTPPQ GRWRNTLGALRGQSRWRRVLAPTMRATCAETHAELWDALAELVPEMAKDRRGLLRP PVEADALWRAPMIVEGWRGGHSVVVDAVAPPLDLPQPCAWTAVRLSGDPRQRWGLH LAVPPLGQVQPPDPLKATLAVSMRHRGGVRVRTLQAMAVDADAPMQRHLQVPLTLQR GGGLQWGIHSRGVRRREARSMASWEGPPIWTGLQLVNRWKGQGSALLAPDRPPDTPP YAPDAAVAPAQPDTKRARRTLKEACTVCRCAPGHMRQLQVTLTGDGTWRRFRLRAPQ GAKRKAEVLKVATQHDERIANYTAWYLKRPEHAAGCDTCDGDSRLDGACRGCRPLLV GDQCFRRYLDKIEADRDDGLAQIKPKAQEAVAAMAAKRDARAQKVAARAAKLSEAT GQRTAATRDASHEARAQKELEAVATEGTTVRHDAAAVSAFGSWVARKGDEYRHQVG VLANRLEHGLRLQELMAPDSVVADQQRASGHARVGYRYVLTAM
SEQ ID NO: 100	AVAHPVGRGNAGSPGARGPEELPRQLVNRASNVTRPATYGCAPCRHVRLSIPKPVLTG CRACEQTTHPAPKRAVRGGADAAKYDLAAFFAGWAADLEGRNRRRQVHAPLDPQPDP NHEPAVTLQKIDLAEVSIEEFQRVLARSVKHRHDGRASREREKARAYAQVAKKRRNSH AHGARTRRAVRRQTRAVRRAHRMGANSGEILVASGAEDPVPEAIDHAAQLRRRIRACA RDLEGLRHLSRRYLKTLEKPCRRPRAPDLGRARCHALVESLQAAERELEELRRCDSPDT AMRRLDAVLAAAAASTDATFATGWTVVGMDLGVAPRGSAAPEVSPMEMAISVFWRKG

SEQ ID NO	Sequence
	SRRVIVSKPIAGMPIRRHELIRLEGLGTLRLDGNHYTGAGVTKGRGLSEGTEPDFREKSP STLGFTLSDYRHESRWRPYGAKQGKTARQFFAAMSRELRALVEHQVLAPMGPPLLEAH ERRFETLLKGQDNKSIHAGGGGRYVWRGPPDSKKRPAADGDWFRFGRGHADHRGWA NKRHELAANYLQSAFRLWSTLAEAQEPTPYARYKYTRVTM
SEQ ID NO: 101	WDFLTLQVYERHTSPEVCVAGNSTKCASGTRKSDHTHGVGVKLGAQEINVSANDDRD HEVGCNICVISRVSLDIKGWRYGCESCVQSTPEWRSIVRFDRNHKEAKGECLSRFEYWG AQSIARSLKRNKLMGGVNLDELAIVQNENVVKTSLKHLFDKRKDRIQANLKAVKVRM RERRKSGRQRKALRRQCRKLKRYLRSYDPSDIKEGNSCSAFTKLGLDIGISPNKPPKIEP KVEVVFSLFYQGACDKIVTVSSPESPLPRSWKIKIDGIRALYVKSTKVKFGGRTFRAGQR NNRRKVRPPNVKKGKRKGSRSQFFNKFAVGLDAVSQQLPIASVQGLWGRAETKKAQTI CLKQLESNKPLKESQRCLFLADNWVVRVCGFLRALSQRQGPTPYIRYRYRCNM
SEQ ID NO: 102	ARNVGQRNASRQSKRESAKARSRRVTGGHASVTQGVALINAAANADRDHTTGCEPCT WERVNLPLQEVIHGCDSCTKSSPFWRDIKVVNKGYREAKEEIMRIASGISADHLSRALS HNKVMGRLNLDEVCILDFRTVLDTSLKHLTDSRSNGIKEHIRAVHRKIRMRRKSGKTAR ALRKQYFALRRQWKAGHKPNSIREGNSLTALRAVGFDVGVSEGTEPMPAPQTEVVLSV FYKGSATRILRISSPHPIAKRSWKVKIAGIKALKLIRREHDFSFGRETYNASQRAEKRKFS PHAARKDFFNSFAVQLDRLAQQLCVSSVENLWVTEPQQKLLTLAKDTAPYGIREGARF ADTRARLAWNWVFRVCGFTRALHQEQEPTPYCRFTWRSKM

[00284] In some embodiments, the Type VI CRISPR/Cas enzyme is a programmable Cas13 nuclease. The general architecture of a Cas13 protein includes an N-terminal domain and two HEPN (higher eukaryotes and prokaryotes nucleotide-binding) domains separated by two helical domains (Liu et al., Cell 2017 Jan 12;168(1-2):121-134.el2). The HEPN domains each comprise aR-X_4-H motif. Shared features across Cas13 proteins include that upon binding of the crRNA of the guide nucleic acid to a target nucleic acid, the protein undergoes a conformational change to bring together the HEPN domains and form a catalytically active RNase. (Tambe et al., Cell Rep. 2018 Jul 24; 24(4): 1025–1036.). Thus, two activatable HEPN domains are characteristic of a programmable Cas13 nuclease of the present disclosure. In some embodiments, a programmable nuclease (e.g., a Cas13 programmable nuclease) comprises at least two HEPN domains. However, programmable Cas13 nucleases also consistent with the present disclosure include Cas13 nucleases comprising mutations in the HEPN domain that enhance the Cas13 proteins cleavage efficiency or mutations that catalytically inactivate the HEPN domains. Programmable Cas13 nucleases consistent with the present disclosure also Cas13 nuclease comprising catalytic

[00285] A programmable Cas13 nuclease can be a Cas13a protein (also referred to as "c2c2"), a Cas13b protein, a Cas13c protein, a Cas13d protein, or a Cas13e protein. Example C2c2 proteins are set forth as SEQ ID NO: 103 - SEQ ID NO: 110. Example Cas13b proteins are set forth in SEQ ID NO: 128 – SEQ ID NO: 132. Example Cas13c proteins are set forth in SEQ ID NO: 133 – SEQ ID NO: 137. In some cases, a subject C2c2 protein includes an amino acid sequence having 80% or more (e.g., 85% or more, 90% or more, 95% or more, 98% or more, 99% or

more, 99.5% or more, or 100%) amino acid sequence identity with the amino acid sequence set forth in any one of SEQ ID NO: 103 - SEQ ID NO: 110. In some embodiments, a programmable nuclease has at least 60%, at least 70%, 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: 103 – SEQ ID NO: 137. In some embodiments, the programmable nuclease has at least 60%, at least 70%, 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: 104. In some cases, a suitable C2c2 polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Listeria seeligeri C2c2 amino acid sequence set forth in SEQ ID NO: 103. In some cases, a suitable C2c2 polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Leptotrichia buccalis C2c2 amino acid sequence set forth in SEQ ID NO: 104. In some cases, a suitable C2c2 polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Rhodobacter capsulatus C2c2 amino acid sequence set forth in SEQ ID NO: 106. In some cases, a suitable C2c2 polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Carnobacterium gallinarum C2c2 amino acid sequence set forth in SEQ ID NO: 107. In some cases, a suitable C2c2 polypeptide comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Herbinix hemicellulosilytica C2c2 amino acid sequence set forth in SEQ ID NO: 108. In some cases, the C2c2 protein includes an amino acid sequence having 80% or more amino acid sequence identity with the Leptotrichia buccalis (Lbu) C2c2 amino acid sequence set forth in SEQ ID NO: 104. In some cases, the C2c2 protein is a Leptotrichia buccalis (Lbu) C2c2 protein (e.g., see SEQ ID NO: 104). In some cases, the C2c2 protein includes the amino acid sequence set forth in any one of SEQ ID NOs: 103-104 and SEQ ID NOs: 106-110. In some cases, a C2c2 protein used in a method of the present disclosure is not a Leptotrichia shahii (Lsh) C2c2 protein. In some cases, a C2c2 protein used in a method of the present disclosure is not a C2c2 polypeptide having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the Lsh C2c2 polypeptide set forth in SEQ ID NO: 105.

TABLE 3 – Cas13 Sequences

SEQ ID NO	Description	Sequence
SEQ ID NO: 103	Listeria seeligeri C2c2 amino acid sequence	MWISIKTLIHHLGVLFFCDYMYNRREKKIIEVKTMRITKVEVDRKKV LISRDKNGGKLVYENEMQDNTEQIMHHKKSSFYKSVVNKTICRPEQ KQMKKLVHGLLQENSQEKIKVSDVTKLNISNFLNHRFKKSLYYFPE NSPDKSEEYRIEINLSQLLEDSLKKQQGTFICWESFSKDMELYINWA ENYISSKTKLIKKSIRNNRIQSTESRSGQLMDRYMKDILNKNKPFDIQ SVSEKYQLEKLTSALKATFKEAKKNDKEINYKLKSTLQNHERQIIEE LKENSELNQFNIEIRKHLETYFPIKKTNRKVGDIRNLEIGEIQKIVNHR LKNKIVQRILQEGKLASYEIESTVNSNSLQKIKIEEAFALKFINACLFA SNNLRNMVYPVCKKDILMIGEFKNSFKEIKHKKFIRQWSQFFSQEIT VDDIELASWGLRGAIAPIRNEIIHLKKHSWKKFFNNPTFKVKKSKIIN GKTKDVTSEFLYKETLFKDYFYSELDSVPELIINKMESSKILDYYSSD QLNQVFTIPNFELSLLTSAVPFAPSFKRVYLKGFDYQNQDEAQPDYN LKLNIYNEKAFNSEAFQAQYSLFKMVYYQVFLPQFTTNNDLFKSSV DFILTLNKERKGYAKAFQDIRKMNKDEKPSEYMSYIQSQLMLYQKK QEEKEKINHFEKFINQVFIKGFNSFIEKNRLTYICHPTKNTVPENDNIE IPFHTDMDDSNIAFWLMCKLLDAKQLSELRNEMIKFSCSLQSTEEIST FTKAREVIGLALLNGEKGCNDWKELFDDKEAWKKNMSLYVSEELL QSLPYTQEDGQTPVINRSIDLVKKYGTETILEKLFSSSDDYKVSAKDI AKLHEYDVTEKIAQQESLHKQWIEKPGLARDSAWTKKYQNVINDIS NYQWAKTKVELTQVRHLHQLTIDLLSRLAGYMSIADRDFQFSSNYI LERENSEYRVTSWILLSENKNKNKYNDYELYNLKNASIKVSSKNDP QLKVDLKQLRLTLEYLELFDNRLKEKRNNISHFNYLNGQLGNSILEL FDDARDVLSYDRKLKNAVSKSLKEILSSHGMEVTFKPLYQTNHHLK IDKLQPKKIHHLGEKSTVSSNQVSNEYCQLVRTLLTMK
SEQ ID NO: 104	Leptotrichia buccalis (Lbu) C2c2 amino acid sequence	MKVTKVGGISHKKYTSEGRLVKSESEENRTDERLSALLNMRLDMYI KNPSSTETKENQKRIGKLKKFFSNKMVYLKDNTLSLKNGKKENIDR EYSETDILESDVRDKKNFAVLKKIYLNENVNSEELEVFRNDIKKKLN KINSLKYSFEKNKANYQKINENNIEKVEGKSKRNIIYDYYRESAKRD AYVSNVKEAFDKLYKEEDIAKLVLEIENLTKLEKYKIREFYHEIIGRK NDKENFAKIIYEEIQNVNNMKELIEKVPDMSELKKSQVFYKYYLDK EELNDKNIKYAFCHFVEIEMSQLLKNYVYKRLSNISNDKIKRIFEYQ NLKKLIENKLLNKLDTYVRNCGKYNYYLQDGEIATSDFIARNRQNE AFLRNIIGVSSVAYFSLRNILETENENDITGRMRGKTVKNNKGEEKY VSGEVDKIYNENKKNEVKENLKMFYSYDFNMDNKNEIEDFFANIDE AISSIRHGIVHFNLELEGKDIFAFKNIAPSEISKKMFQNEINEKKLKLK IFRQLNSANVFRYLEKYKILNYLKRTRFEFVNKNIPFVPSFTKLYSRI DDLKNSLGIYWKTPKTNDDNKTKEIIDAQIYLLKNIYYGEFLNYFMS NNGNFFEISKEIIELNKNDKRNLKTGFYKLQKFEDIQEKIPKEYLANI QSLYMINAGNQDEEEKDTYIDFIQKIFLKGFMTYLANNGRLSLIYIGS DEETNTSLAEKKQEFDKFLKKYEQNNNIKIPYEINEFLREIKLGNILK YTERLNMFYLILKLLNHKELTNLKGSLEKYQSANKEEAFSDQLELIN LLNLDNNRVTEDFELEADEIGKFLDFNGNKVKDNKELKKFDTNKIY FDGENIIKHRAFYNIKKYGMLNLLEKIADKAGYKISIEELKKYSNKK NEIEKNHKMQENLHRKYARPRKDEKFTDEDYESYKQAIENIEEYTH LKNKVEFNELNLLQGLLLRILHRLVGYTSIWERDLRFRLKGEFPENQ YIEEIFNFENKKNVKYKGGQIVEKYIKFYKELHQNDEVKINKYSSAN IKVLKQEKKDLYIRNYIAHFNYIPHAEISLLEVLENLRKLLSYDRKLK NAVMKSVVDILKEYGFVATFKIGADKKIGIQTLESEKIVHLKNLKKK KLMTDRNSEELCKLVKIMFEYKMEEKKSEN
SEQ ID NO: 105	Leptotrichia shahii (Lsh) C2c2 protein	MGNLFGHKRWYEVRDKKDFKIKRKVKVKRNYDGNKYILNINENN NKEKIDNNKFIRKYINYKKNDNILKEFTRKFHAGNILFKLKGKEGIIR IENNDDFLETEEVVLYIEAYGKSEKLKALGITKKKIIDEAIRQGITKD DKKIEIKRQENEEEIEIDIRDEYTNKTLNDCSIILRIIENDELETKKSIYE

SEQ ID NO	Description	Sequence
		IFKNINMSLYKIIEKIIENETEKVFENRYYEEHLREKLLKDDKIDVILT NFMEIREKIKSNLEILGFVKFYLNVGGDKKKSKNKKMLVEKILNINV DLTVEDIADFVIKELEFWNITKRIEKVKKVNNEFLEKRRNRTYIKSY VLLDKHEKFKIERENKKDKIVKFFVENIKNNSIKEKIEKILAEFKIDEL IKKLEKELKKGNCDTEIFGIFKKHYKVNFDSKKFSKKSDEEKELYKII YRYLKGRIEKILVNEQKVRLKKMEKIEIEKILNESILSEKILKRVKQY TLEHIMYLGKLRHNDIDMTTVNTDDFSRLHAKEELDLELITFFASTN MELNKIFSRENINNDENIDFFGGDREKNYVLDKKILNSKIKIIRDLDFI DNKNNITNNFIRKFTKIGTNERNRILHAISKERDLQGTQDDYNKVINI IQNLKISDEEVSKALNLDVVFKDKKNIITKINDIKISEENNNDIKYLPS FSKVLPEILNLYRNNPKNEPFDTIETEKIVLNALIYVNKELYKKLILE DDLEENESKNIFLQELKKTLGNIDEIDENIIENYYKNAQISASKGNNK AIKKYQKKVIECYIGYLRKNYEELFDFSDFKMNIQEIKKQIKDINDN KTYERITVKTSDKTIVINDDFEYIISIFALLNSNAVINKIRNRFFATSV WLNTSEYQNIIDILDEIMQLNTLRNECITENWNLNLEEFIQKMKEIEK DFDDFKIQTKKEIFNNYYEDIKNNILTEFKDDINGCDVLEKKLEKIVI FDDETKFEIDKKSNILQDEQRKLSNINKKDLKKKVDQYIKDKDQEIK SKILCRIIFNSDFLKKYKKEIDNLIEDMESENENKFQEIYYPKERKNEL YIYKKNLFLNIGNPNFDKIYGLISNDIKMADAKFLFNIDGKNIRKNKI SEIDAILKNLNDKLNGYSKEYKEKYIKKLKENDDFFAKNIQNKNYK SFEKDYNRVSEYKKIRDLVEFNYLNKIESYLIDINWKLAIQMARFER DMHYIVNGLRELGIIKLSGYNTGISRAYPKRNGSDGFYTTTAYYKFF DEESYKKFEKICYGFGIDLSENSEINKPENESIRNYISHFYIVRNPFAD YSIAEQIDRVSNLLSYSTRYNNSTYASVFEVFKKDVNLDYDELKKKF KLIGNNDILERLMKPKKVSVLELESYNSDYIKNLIIELLTKIENTNDT L
SEQ ID NO: 106	Rhodobacter capsulatus C2c2 amino acid sequence	MQIGKVQGRTISEFGDPAGGLKRKISTDGKNRKELPAHLSSDPKALI GQWISGIDKIYRKPDSRKSDGKAIHSPTPSKMQFDARDDLGEAFWK LVSEAGLAQDSDYDQFKRRLHPYGDKFQPADSGAKLKFEADPPEPQ AFHGRWYGAMSKRGNDAKELAAALYEHLHVDEKRIDGQPKRNPK TDKFAPGLVVARALGIESSVLPRGMARLARNWGEEEIQTYFVVDVA ASVKEVAKAAVSAAQAFDPPRQVSGRSLSPKVGFALAEHLERVTGS KRCSFDPAAGPSVLALHDEVKKTYKRLCARGKNAARAFPADKTEL LALMRHTHENRVRNQMVRMGRVSEYRGQQAGDLAQSHYWTSAG QTEIKESEIFVRLWVGAFALAGRSMKAWIDPMGKIVNTEKNDRDLT AAVNIRQVISNKEMVAEAMARRGIYFGETPELDRLGAEGNEGFVFA LLRYLRGCRNQTFHLGARAGFLKEIRKELEKTRWGKAKEAEHVVL TDKTVAAIRAIIDNDAKALGARLLADLSGAFVAHYASKEHFSTLYSE IVKAVKDAPEVSSGLPRLKLLLKRADGVRGYVHGLRDTRKHAFAT KLPPPPAPRELDDPATKARYIALLRLYDGPFRAYASGITGTALAGPA ARAKEAATALAQSVNVTKAYSDVMEGRSSRLRPPNDGETLREYLS ALTGETATEFRVQIGYESDSENARKQAEFIENYRRDMLAFMFEDYIR AKGFDWILKIEPGATAMTRAPVLPEPIDTRGQYEHWQAALYLVMH FVPASDVSNLLHQLRKWEALQGKYELVQDGDATDQADARREALD LVKRFRDVLVLFLKTGEARFEGRAAPFDLKPFRALFANPATFDRLF MATPTTARPAEDDPEGDGASEPELRVARTLRGLRQIARYNHMAVLS DLFAKHKVRDEEVARLAEIEDETQEKSQIVAAQELRTDLHDKVMK CHPKTISPEERQSYAAAIKTIEEHRFLVGRVYLGDHLRLHRLMMDVI GRLIDYAGAYERDTGTFLINASKQLGAGADWAVTIAGAANTDART QTRKDLAHFNVLDRADGTPDLTALVNRAREMMAYDRKRKNAVPR SILDMLARLGLTLKWQMKDHLLQDATITQAAIKHLDKVRLTVGGP AAVTEARFSQDYLQMVAAVFNGSVQNPKPRRRDDGDAWHKPPKP ATAQSQPDQKPPNKAPSAGSRLPPPQVGEVYEGVVVKVIDTGSLGF

SEQ ID NO	Description	Sequence
		LAVEGVAGNIGLHISRLRRIREDAIIVGRRYRFRVEIYVPPKSNTSKL NAADLVRID
SEQ ID NO: 107	Carnobacterium gallinarum C2c2 amino acid sequence	MRITKVKIKLDNKLYQVTMQKEEKYGTLKLNEESRKSTAEILRLKK ASFNKSFHSKTINSQKENKNATIKKNGDYISQIFEKLVGVDTNKNIR KPKMSLTDLKDLPKKDLALFIKRKFKNDDIVEIKNLDLISLFYNALQ KVPGEHFTDESWADFCQEMMPYREYKNKFIERKIILLANSIEQNKGF SINPETFSKRKRVLHQWAIEVQERGDFSILDEKLSKLAEIYNFKKMC KRVQDELNDLEKSMKKGKNPEKEKEAYKKQKNFKIKTIWKDYPYK THIGLIEKIKENEELNQFNIEIGKYFEHYFPIKKERCTEDEPYYLNSETI ATTVNYQLKNALISYLMQIGKYKQFGLENQVLDSKKLQEIGIYEGF QTKFMDACVFATSSLKNIIEPMRSGDILGKREFKEAIATSSFVNYHHF FPYFPFELKGMKDRESELIPFGEQTEAKQMQNIWALRGSVQQIRNEI FHSFDKNQKFNLPQLDKSNFEFDASENSTGKSQSYIETDYKFLFEAE KNQLEQFFIERIKSSGALEYYPLKSLEKLFAKKEMKFSLGSQVVAFA PSYKKLVKKGHSYQTATEGTANYLGLSYYNRYELKEESFQAQYYL LKLIYQYVFLPNFSQGNSPAFRETVKAILRINKDEARKKMKKNKKFL RKYAFEQVREMEFKETPDQYMSYLQSEMREEKVRKAEKNDKGFEK NITMNFEKLLMQIFVKGFDVFLTTFAGKELLLSSEEKVIKETEISLSK KINEREKTLKASIQVEHQLVATNSAISYWLFCKLLDSRHLNELRNEM IKFKQSRIKFNHTQHAELIQNLLPIVELTILSNDYDEKNDSQNVDVSA YFEDKSLYETAPYVQTDDRTRVSFRPILKLEKYHTKSLIEALLKDNP QFRVAATDIQEWMHKREEIGELVEKRKNLHTEWAEGQQTLGAEKR EEYRDYCKKIDRFNWKANKVTLTYLSQLHYLITDLLGRMVGFSALF ERDLVYFSRSFSELGGETYHISDYKNLSGVLRLNAEVKPIKIKNIKVI DNEENPYKGNEPEVKPFLDRLHAYLENVIGIKAVHGKIRNQTAHLS VLQLELSMIESMNNLRDLMAYDRKLKNAVTKSMIKILDKHGMILKL KIDENHKNFEIESLIPKEIIHLKDKAIKTNQVSEEYCQLVLALLTTNPG NQLN
SEQ ID NO: 108	Herbinix hemicellulosilytica C2c2 amino acid sequence	MKLTRRRISGNSVDQKITAAFYRDMSQGLLYYDSEDNDCTDKVIES MDFERSWRGRILKNGEDDKNPFYMFVKGLVGSNDKIVCEPIDVDSD PDNLDILINKNLTGFGRNLKAPDSNDTLENLIRKIQAGIPEEEVLPEL KKIKEMIQKDIVNRKEQLLKSIKNNRIPFSLEGSKLVPSTKKMKWLF KLIDVPNKTFNEKMLEKYWEIYDYDKLKANITNRLDKTDKKARSIS RAVSEELREYHKNLRTNYNRFVSGDRPAAGLDNGGSAKYNPDKEE FLLFLKEVEQYFKKYFPVKSKHSNKSKDKSLVDKYKNYCSYKVVK KEVNRSIINQLVAGLIQQGKLLYYFYYNDTWQEDFLNSYGLSYIQV EEAFKKSVMTSLSWGINRLTSFFIDDSNTVKFDDITTKKAKEAIESNY FNKLRTCSRMQDHFKEKLAFFYPVYVKDKKDRPDDDIENLIVLVKN AIESVSYLRNRTFHFKESSLLELLKELDDKNSGQNKIDYSVAAEFIKR DIENLYDVFREQIRSLGIAEYYKADMISDCFKTCGLEFALYSPKNSL MPAFKNVYKRGANLNKAYIRDKGPKETGDQGQNSYKALEEYRELT WYIEVKNNDQSYNAYKNLLQLIYYHAFLPEVRENEALITDFINRTKE WNRKETEERLNTKNNKKHKNFDENDDITVNTYRYESIPDYQGESLD DYLKVLQRKQMARAKEVNEKEEGNNNYIQFIRDVVVWAFGAYLE NKLKNYKNELQPPLSKENIGLNDTLKELFPEEKVKSPFNIKCRFSIST FIDNKGKSTDNTSAEAVKTDGKEDEKDKKNIKRKDLLCFYLFLRLL DENEICKLQHQFIKYRCSLKERRFPGNRTKLEKETELLAELEELMEL VRFTMPSIPEISAKAESGYDTMIKKYFKDFIEKKVFKNPKTSNLYYH SDSKTPVTRKYMALLMRSAPLHLYKDIFKGYYLITKKECLEYIKLSN IIKDYQNSLNELHEQLERIKLKSEKQNGKDSLYLDKKDFYKVKEYV ENLEQVARYKHLQHKINFESLYRIFRIHVDIAARMVGYTQDWERDM HFLFKALVYNGVLEERRFEAIFNNNDDNNDGRIVKKIQNNLNNKNR ELVSMLCWNKKLNKNEFGAIIWKRNPIAHLNHFTQTEQNSKSSLES

SEQ ID NO	Description	Sequence
		LINSLRILLAYDRKRQNAVTKTINDLLLNDYHIRIKWEGRVDEGQIY FNIKEKEDIENEPIIHLKHLHKKDCYIYKNSYMFDKQKEWICNGIKEE VYDKSILKCIGNLFKFDYEDKNKSSANPKHT
SEQ ID NO: 109	Paludibacter propionicigenes C2c2 amino acid sequence	MRVSKVKVKDGGKDKMVLVHRKTTGAQLVYSGQPVSNETSNILPE KKRQSFDLSTLNKTIIKFDTAKKQKLNVDQYKIVEKIFKYPKQELPK QIKAEEILPFLNHKFQEPVKYWKNGKEESFNLTLLIVEAVQAQDKR KLQPYYDWKTWYIQTKSDLLKKSIENNRIDLTENLSKRKKALLAWE TEFTASGSIDLTHYHKVYMTDVLCKMLQDVKPLTDDKGKINTNAY HRGLKKALQNHQPAIFGTREVPNEANRADNQLSIYHLEVVKYLEHY FPIKTSKRRNTADDIAHYLKAQTLKTTIEKQLVNAIRANIIQQGKTNH HELKADTTSNDLIRIKTNEAFVLNLTGTCAFAANNIRNMVDNEQTN DILGKGDFIKSLLKDNTNSQLYSFFFGEGLSTNKAEKETQLWGIRGA VQQIRNNVNHYKKDALKTVFNISNFENPTITDPKQQTNYADTIYKA RFINELEKIPEAFAQQLKTGGAVSYYTIENLKSLLTTFQFSLCRSTIPF APGFKKVFNGGINYQNAKQDESFYELMLEQYLRKENFAEESYNAR YFMLKLIYNNLFLPGFTTDRKAFADSVGFVQMQNKKQAEKVNPRK KEAYAFEAVRPMTAADSIADYMAYVQSELMQEQNKKEEKVAEET RINFEKFVLQVFIKGFDSFLRAKEFDFVQMPQPQLTATASNQQKAD KLNQLEASITADCKLTPQYAKADDATHIAFYVFCKLLDAAHLSNLR NELIKFRESVNEFKFHHLLEIIEICLLSADVVPTDYRDLYSSEADCLA RLRPFIEQGADITNWSDLFVQSDKHSPVIHANIELSVKYGTTKLLEQI INKDTQFKTTEANFTAWNTAQKSIEQLIKQREDHHEQWVKAKNAD DKEKQERKREKSNFAQKFIEKHGDDYLDICDYINTYNWLDNKMHF VHLNRLHGLTIELLGRMAGFVALFDRDFQFFDEQQIADEFKLHGFV NLHSIDKKLNEVPTKKIKEIYDIRNKIIQINGNKINESVRANLIQFISSK RNYYNNAFLHVSNDEIKEKQMYDIRNHIAHFNYLTKDAADFSLIDLI NELRELLHYDRKLKNAVSKAFIDLFDKHGMILKLKLNADHKLKVES LEPKKIYHLGSSAKDKPEYQYCTNQVMMAYCNMCRSLLEMKK
SEQ ID NO: 110	Leptotrichia wadei (Lwa) C2c2 amino acid sequence	MYMKITKIDGVSHYKKQDKGILKKKWKDLDERKQREKIEARYNKQ IESKIYKEFFRLKNKKRIEKEEDQNIKSLYFFIKELYLNEKNEEWELK NINLEILDDKERVIKGYKFKEDVYFFKEGYKEYYLRILFNNLIEKVQ NENREKVRKNKEFLDLKEIFKKYKNRKIDLLLKSINNNKINLEYKKE NVNEEIYGINPTNDREMTFYELLKEIIEKKDEQKSILEEKLDNFDITNF LENIEKIFNEETEINIIKGKVLNELREYIKEKEENNSDNKLKQIYNLEL KKYIENNFSYKKQKSKSKNGKNDYLYLNFLKKIMFIEEVDEKKEIN KEKFKNKINSNFKNLFVQHILDYGKLLYYKENDEYIKNTGQLETKD LEYIKTKETLIRKMAVLVSFAANSYYNLFGRVSGDILGTEVVKSSKT NVIKVGSHIFKEKMLNYFFDFEIFDANKIVEILESISYSIYNVRNGVG HFNKLILGKYKKKDINTNKRIEEDLNNNEEIKGYFIKKRGEIERKVK EKFLSNNLQYYYSKEKIENYFEVYEFEILKRKIPFAPNFKR11KKGED LFNNKNNKKYEYFKNFDKNSAEEKKEFLKTRNFLLKELYYNNFYK EFLSKKEEFEKIVLEVKEEKKSRGNINNKKSGVSFQSIDDYDTKINIS DYIASIHKKEMERVEKYNEEKQKDTAKYIRDFVEEIFLTGFINYLEK DKRLHFLKEEFSILCNNNNNVVDFNININEEKIKEFLKENDSKTLNLY LFFNMIDSKRISEFRNELVKYKQFTKKRLDEEKEFLGIKIELYETLIEF VILTREKLDTKKSEEIDAWLVDKLYVKDSNEYKEYEEILKLFVDEKI LSSKEAPYYATDNKTPILLSNFEKTRKYGTQSFLSEIQSNYKYSKVE KENIEDYNKKEEIEQKKKSNIEKLQDLKVELHKKWEQNKITEKEIEK YNNTTRKINEYNYLKNKEELQNVYLLHEMLSDLLARNVAFFNKWE RDFKFIVIAIKQFLRENDKEKVNEFLNPPDNSKGKKVYFSVSKYKNT VENIDGIHKNFMNLIFLNNKFMNRKIDKMNCAIWVYFRNYIAHFLH LHTKNEKISLISQMNLLIKLFSYDKKVQNHILKSTKTLLEKYNIQINF

SEQ ID NO	Description	Sequence
		EISNDKNEVFKYKIKNRLYSKKGKMLGKNNKFEILENEFLENVKAM LEYSE
SEQ ID NO: 128	Bergeyella zoohelcum Cas13b	MENKTSLGNNIYYNPFKPQDKSYFAGYFNAAMENTDSVFRELGKR LKGKEYTSENFFDAIFKENISLVEYERYVKLLSDYFPMARLLDKKEV PIKERKENFKKNFKGIIKAVRDLRNFYTHKEHGEVEITDEIFGVLDE MLKSTVLTVKKKKVKTDKTKEILKKSIEKQLDILCQKKLEYLRDTA RKIEEKRRNQRERGEKELVAPFKYSDKRDDLIAAIYNDAFDVYIDK KKDSLKESSKAKYNTKSDPQQEEGDLKIPISKNGVVFLLSLFLTKQEI HAFKSKIAGFKATVIDEATVSEATVSHGKNSICFMATHEIFSHLAYK KLKRKVRTAEINYGEAENAEQLSVYAKETLMMQMLDELSKVPDVV YQNLSEDVQKTFIEDWNEYLKENNGDVGTMEEEQVIHPVIRKRYED KFNYFAIRFLDEFAQFPTLRFQVHLGNYLHDSRPKENLISDRRIKEKI TVFGRLSELEHKKALFIKNTETNEDREHYWEIFPNPNYDFPKENISV NDKDFPIAGSILDREKQPVAGKIGIKVKLLNQQYVSEVDKAVKAHQ LKQRKASKPSIQNIIEEIVPINESNPKEAIVFGGQPTAYLSMNDIHSILY EFFDKWEKKKEKLEKKGEKELRKEIGKELEKKIVGKIQAQIQQIIDK DTNAKILKPYQDGNSTAIDKEKLIKDLKQEQNILQKLKDEQTVREKE YNDFIAYQDKNREINKVRDRNHKQYLKDNLKRKYPEAPARKEVLY YREKGKVAVWLANDIKRFMPTDFKNEWKGEQHSLLQKSLAYYEQ CKEELKNLLPEKVFQHLPFKLGGYFQQKYLYQFYTCYLDKRLEYIS GLVQQAENFKSENKVFKKVENECFKFLKKQNYTHKELDARVQSIL GYPIFLERGFMDEKPTIIKGKTFKGNEALFADWFRYYKEYQNFQTFY DTENYPLVELEKKQADRKRKTKIYQQKKNDVFTLLMAKHIFKSVFK QDSIDQFSLEDLYQSREERLGNQERARQTGERNTNYIWNKTVDLKL CDGKITVENVKLKNVGDFIKYEYDQRVQAFLKYEENIEWQAFLIKE SKEEENYPYVVEREIEQYEKVRREELLKEVHLIEEYILEKVKDKEILK KGDNQNFKYYILNGLLKQLKNEDVESYKVFNLNTEPEDVNINQLKQ EATDLEQKAFVLTYIRNKFAHNQLPKKEFWDYCQEKYGKIEKEKTY AEYFAEVFKKEKEALIK
SEQ ID NO: 129	Prevotella intermedia Cas13b	MEDDKKTTDSIRYELKDKHFWAAFLNLARHNVYITVNHINKILEEG EINRDGYETTLKNTWNEIKDINKKDRLSKLIIKHFPFLEAATYRLNPT DTTKQKEEKQAEAQSLESLRKSFFVFIYKLRDLRNHYSHYKHSKSLE RPKFEEGLLEKMYNIFNASIRLVKEDYQYNKDINPDEDFKHLDRTEE EFNYYFTKDNEGNITESGLLFFVSLFLEKKDAIWMQQKLRGFKDNR ENKKKMTNEVFCRSRMLLPKLRLQSTQTQDWILLDMLNELIRCPKS LYERLREEDREKFRVPIEIADEDYDAEQEPFKNTLVRHQDRFPYFAL RYFDYNEIFTNLRFQIDLGTYHFSIYKKQIGDYKESHHLTHKLYGFE RIQEFTKQNRPDEWRKFVKTFNSFETSKEPYIPETTPHYHLENQKIGI RFRNDNDKIWPSLKTNSEKNEKSKYKLDKSFQAEAFLSVHELLPMM FYYLLLKTENTDNDNEIETKKKENKNDKQEKHKIEEIIENKITEIYAL YDTFANGEIKSIDELEEYCKGKDIEIGHLPKQMIAILKDEHKVMATE AERKQEEMLVDVQKSLESLDNQINEEIENVERKNSSLKSGKIASWL VNDMMRFQPVQKDNEGKPLNNSKANSTEYQLLQRTLAFFGSEHER LAPYFKQTKLIESSNPHPFLKDTEWEKCNNILSFYRSYLEAKKNFLES LKPEDWEKNQYFLKLKEPKTKPKTLVQGWKNGFNLPRGIFTEPIRK WFMKHRENITVAELKRVGLVAKVIPLFFSEEYKDSVQPFYNYHFNV GNINKPDEKNFLNCEERRELLRKKKDEFKKMTDKEKEENPSYLEFK SWNKFERELRLVRNQDIVTWLLCMELFNKKKIKELNVEKIYLKNIN TNTTKKEKNTEEKNGEEKNIKEKNNILNRIMPMRLPIKVYGRENFSK NKKKKIRRNTFFTVYIEEKGTKLLKQGNFKALERDRRLGGLFSFVKT PSKAESKSNTISKLRVEYELGEYQKARIEIIKDMLALEKTLIDKYNSL DTDNFNKMLTDWLELKGEPDKASFQNDVDLLIAVRNAFSHNQYPM

SEQ ID NO	Description	Sequence
		RNRIAFANINPFSLSSANTSEEKGLGIANQLKDKTHKTIEKIIEIEKPIE TKE
SEQ ID NO: 130	Prevotella buccae Cas13b	MQKQDKLFVDRKKNAIFAFPKYITIMENKEKPEPIYYELTDKHFWA AFLNLARHNVYTTINHINRRLEIAELKDDGYMMGIKGSWNEQAKK LDKKVRLRDLIMKHFPFLEAAAYEMTNSKSPNNKEQREKEQSEALS LNNLKNVLFIFLEKLQVLRNYYSHYKYSEESPKPIFETSLLKNMYKV FDANVRLVKRDYMHHENIDMQRDFTHLNRKKQVGRTKNIIDSPNF HYHFADKEGNMTIAGLLFFVSLFLDKKDAIWMQKKLKGFKDGRNL REQMTNEVFCRSRISLPKLKLENVQTKDWMQLDMLNELVRCPKSL YERLREKDRESFKVPFDIFSDDYNAEEEPFKNTLVRHQDRFPYFVLR YFDLNEIFEQLRFQIDLGTYHFSIYNKRIGDEDEVRHLTHHLYGFARI QDFAPQNQPEEWRKLVKDLDHFETSQEPYISKTAPHYHLENEKIGIK FCSAHNNLFPSLQTDKTCNGRSKFNLGTQFTAEAFLSVHELLPMMF YYLLLTKDYSRKESADKVEGIIRKEISNIYAIYDAFANNEINSIADLTR RLQNTNILQGHLPKQMISILKGRQKDMGKEAERKIGEMIDDTQRRL DLLCKQTNQKIRIGKRNAGLLKSGKIADWLVNDMMRFQPVQKDQN NIPINNSKANSTEYRMLQRALALFGSENFRLKAYFNQMNLVGNDNP HPFLAETQWEHQTNILSFYRNYLEARKKYLKGLKPQNWKQYQHFLI LKVQKTNRNTLVTGWKNSFNLPRGIFTQPIREWFEKHNNSKRIYDQI LSFDRVGFVAKAIPLYFAEEYKDNVQPFYDYPFNIGNRLKPKKRQFL DKKERVELWQKNKELFKNYPSEKKKTDLAYLDFLSWKKFERELRLI KNQDIVTWLMFKELFNMATVEGLKIGEIHLRDIDTNTANEESNNILN RIMPMKLPVKTYETDNKGNILKERPLATFYIEETETKVLKQGNFKAL VKDRRLNGLFSFAETTDLNLEEHPISKLSVDLELIKYQTTRISIFEMTL GLEKKLIDKYSTLPTDSFRNMLERWLQCKANRPELKNYVNSLIAVR NAFSHNQYPMYDATLFAEVKKFTLFPSVDTKKIELNIAPQLLEIVGK AIKEIEKSENKN
SEQ ID NO: 131	Porphyromonas gingivalis Cas13b	MNTVPASENKGQSRTVEDDPQYFGLYLNLARENLIEVESHVRIKFG KKKLNEESLKQSLLCDHLLSVDRWTKVYGHSRRYLPFLHYFDPDSQ IEKDHDSKTGVDPDSAQRLIRELYSLLDFLRNDFSHNRLDGTTFEHL EVSPDISSFITGTYSLACGRAQSRFAVFFKPDDFVLAKNRKEQLISVA DGKECLTVSGFAFFICLFLDREQASGMLSRIRGFKRTDENWARAVH ETFCDLCIRHPHDRLESSNTKEALLLDMLNELNRCPRILYDMLPEEE RAQFLPALDENSMNNLSENSLDEESRLLWDGSSDWAEALTKRIRHQ DRFPYLMLRFIEEMDLLKGIRFRVDLGEIELDSYSKKVGRNGEYDRT ITDHALAFGKLSDFQNEEEVSRMISGEASYPVRFSLFAPRYAIYDNKI GYCHTSDPVYPKSKTGEKRALSNPQSMGFISVHDLRKLLLMELLCE GSFSRMQSDFLRKANRILDETAEGKLQFSALFPEMRHRFIPPQNPKS KDRREKAETTLEKYKQEIKGRKDKLNSQLLSAFDMDQRQLPSRLLD EWMNIRPASHSVKLRTYVKQLNEDCRLRLRKFRKDGDGKARAIPL VGEMATFLSQDIVRMIISEETKKLITSAYYNEMQRSLAQYAGEENRR QFRAIVAELRLLDPSSGHPFLSATMETAHRYTEGFYKCYLEKKREW LAKIFYRPEQDENTKRRISVFFVPDGEARKLLPLLIRRRMKEQNDLQ DWIRNKQAHPIDLPSHLFDSKVMELLKVKDGKKKWNEAFKDWWS TKYPDGMQPFYGLRRELNIHGKSVSYIPSDGKKFADCYTHLMEKTV RDKKRELRTAGKPVPPDLAADIKRSFHRAVNEREFMLRLVQEDDRL MLMAINKMMTDREEDILPGLKNIDSILDEENQFSLAVHAKVLEKEG EGGDNSLSLVPATIEIKSKRKDWSKYIRYRYDRRVPGLMSHFPEHK ATLDEVKTLLGEYDRCRIKIFDWAFALEGAIMSDRDLKPYLHESSSR EGKSGEHSTLVKMLVEKKGCLTPDESQYLILIRNKAAHNQFPCAAE MPLIYRDVSAKVGSIEGSSAKDLPEGSSLVDSLWKKYEMIIRKILPIL DPENRFFGKLLNNMSQPINDL

SEQ ID NO	Description	Sequence
SEQ ID NO: 132	Bacteroides pyogenes Cas13b	MESIKNSQKSTGKTLQKDPPYFGLYLNMALLNVRKVENHIRKWLG DVALLPEKSGFHSLLTTDNLSSAKWTRFYYKSRKFLPFLEMFDSDK KSYENRRETAECLDTIDRQKISSLLKEVYGKLQDIRNAFSHYHIDDQ SVKHTALIISSEMHRFIENAYSFALQKTRARFTGVFVETDFLQAEEK GDNKKFFAIGGNEGIKLKDNALIFLICLFLDREEAFKFLSRATGFKST KEKGFLAVRETFCALCCRQPHERLLSVNPREALLMDMLNELNRCPD ILFEMLDEKDQKSFLPLLGEEEQAHILENSLNDELCEAIDDPFEMIAS LSKRVRYKNRFPYLMLRYIEEKNLLPFIRFRIDLGCLELASYPKKMG EENNYERSVTDHAMAFGRLTDFHNEDAVLQQITKGITDEVRFSLYA PRYAIYNNKIGFVRTSGSDKISFPTLKKKGGEGHCVAYTLQNTKSFG FISIYDLRKILLLSFLDKDKAKNIVSGLLEQCEKHWKDLSENLFDAIR TELQKEFPVPLIRYTLPRSKGGKLVSSKLADKQEKYESEFERRKEKL TEILSEKDFDLSQIPRRMIDEWLNVLPTSREKKLKGYVETLKLDCRE RLRVFEKREKGEHPLPPRIGEMATDLAKDIIRMVIDQGVKQRITSAY YSEIQRCLAQYAGDDNRRHLDSIIRELRLKDTKNGHPFLGKVLRPGL GHTEKLYQRYFEEKKEWLEATFYPAASPKRVPRFVNPPTGKQKELP LIIRNLMKERPEWRDWKQRKNSHPIDLPSQLFENEICRLLKDKIGKE PSGKLKWNEMFKLYWDKEFPNGMQRFYRCKRRVEVFDKVVEYEY SEEGGNYKKYYEALIDEVVRQKISSSKEKSKLQVEDLTLSVRRVFKR AINEKEYQLRLLCEDDRLLFMAVRDLYDWKEAQLDLDKIDNMLGE PVSVSQVIQLEGGQPDAVIKAECKLKDVSKLMRYCYDGRVKGLMP YFANHEATQEQVEMELRHYEDHRRRVFNWVFALEKSVLKNEKLRR FYEESQGGCEHRRCIDALRKASLVSEEEYEFLVHIRNKSAHNQFPDL EIGKLPPNVTSGFCECIWSKYKAIICRIIPFIDPERRFFGKLLEQK
SEQ ID NO: 133	Cas13c	MTEKKSIIFKNKSSVEIVKKDIFSQTPDNMIRNYKITLKISEKNPRVVE AEIEDLMNSTILKDGRRSARREKSMTERKLIEEKVAENYSLLANCPM EEVDSIKIYKIKRFLTYRSNMLLYFASINSFLCEGIKGKDNETEEIWH LKDNDVRKEKVKENFKNKLIQSTENYNSSLKNQIEEKEKLLRKESK KGAFYRTIIKKLQQERIKELSEKSLTEDCEKIIKLYSELRHPLMHYDY QYFENLFENKENSELTKNLNLDIFKSLPLVRKMKLNNKVNYLEDND TLFVLQKTKKAKTLYQIYDALCEQKNGFNKFINDFFVSDGEENTVF KQIINEKFQSEMEFLEKRISESEKKNEKLKKKFDSMKAHFHNINSED TKEAYFWDIHSSSNYKTKYNERKNLVNEYTELLGSSKEKKLLREEIT QINRKLLKLKQEMEEITKKNSLFRLEYKMKIAFGFLFCEFDGNISKF KDEFDASNQEKIIQYHKNGEKYLTYFLKEEEKEKFNLEKMQKIIQKT EEEDWLLPETKNNLFKFYLLTYLLLPYELKGDFLGFVKKHYYDIKN VDFMDENQNNIQVSQTVEKQEDYFYHKIRLFEKNTKKYEIVKYSIV PNEKLKQYFEDLGIDIKYLTGSVESGEKWLGENLGIDIKYLTVEQKS EVSEEKIKKFL
SEQ ID NO: 134	Cas13c	MEKDKKGEKIDISQEMIEEDLRKILILFSRLRHSMVHYDYEFYQALY SGKDFVISDKNNLENRMISQLLDLNIFKELSKVKLIKDKAISNYLDK NTTIHVLGQDIKAIRLLDIYRDICGSKNGFNKFINTMITISGEEDREYK EKVIEHFNKKMENLSTYLEKLEKQDNAKRNNKRVYNLLKQKLIEQ QKLKEWFGGPYVYDIHSSKRYKELYIERKKLVDRHSKLFEEGLDEK NKKELTKINDELSKLNSEMKEMTKLNSKYRLQYKLQLAFGFILEEF DLNIDTFINNFDKDKDLIISNFMKKRDIYLNRVLDRGDNRLKNIIKEY KFRDTEDIFCNDRDNNLVKLYILMYILLPVEIRGDFLGFVKKNYYD MKHVDFIDKKDKEDKDTFFHDLRLFEKNIRKLEITDYSLSSGFLSKE HKVDIEKKINDFINRNGAMKLPEDITIEEFNKSLILPIMKNYQINFKLL NDIEISALFKIAKDRSITFKQAIDEIKNEDIKKNSKKNDKNNHKDKNI NFTQLMKRALHEKIPYKAGMYQIRNNISHIDMEQLYIDPLNSYMNS NKNNITISEQIEKIIDVCVTGGVTGKELNNNIINDYYMKKEKLVFNL KLRKQNDIVSIESQEKNKREEFVFKKYGLDYKDGEINIIEVIQKVNSL

SEQ ID NO	Description	Sequence
		QEELRNIKETSKEKLKNKETLFRDISLINGTIRKNINFKIKEMVLDIVR MDEIRHINIHIYYKGENYTRSNIIKFKYAIDGENKKYYLKQHEINDIN LELKDKFVTLICNMDKHPNKNKQTINLESNYIQNVKFIIP
SEQ ID NO: 135	Cas13c	MENKGNNKKIDFDENYNILVAQIKEYFTKEIENYNNRIDNIIDKKEL LKYSEKKEESEKNKKLEELNKLKSQKLKILTDEEIKADVIKIIKIFSDL RHSLMHYEYKYFENLFENKKNEELAELLNLNLFKNLTLLRQMKIEN KTNYLEGREEFNIIGKNIKAKEVLGHYNLLAEQKNGFNNFINSFFVQ DGTENLEFKKLIDEHFVNAKKRLERNIKKSKKLEKELEKMEQHYQR LNCAYVWDIHTSTTYKKLYNKRKSLIEEYNKQINEIKDKEVITAINV ELLRIKKEMEEITKSNSLFRLKYKMQIAYAFLEIEFGGNIAKFKDEFD CSKMEEVQKYLKKGVKYLKYYKDKEAQKNYEFPFEEIFENKDTHN EEWLENTSENNLFKFYILTYLLLPMEFKGDFLGVVKKHYYDIKNVD FTDESEKELSQVQLDKMIGDSFFHKIRLFEKNTKRYEIIKYSILTSDEI KRYFRLLELDVPYFEYEKGTDEIGIFNKNIILTIFKYYQIIFRLYNDLEI HGLFNISSDLDKILRDLKSYGNKNINFREFLYVIKQNNNSSTEEEYRK IWENLEAKYLRLHLLTPEKEEIKTKTKEELEKLNEISNLRNGICHLNY KEIIEEILKTEISEKNKEATLNEKIRKVINFIKENELDKVELGFNFINDF FMKKEQFMFGQIKQVKEGNSDSITTERERKEKNNKKLKETYELNCD NLSEFYETSNNLRERANSSSLLEDSAFLKKIGLYKVKNNKVNSKVK DEEKRIENIKRKLLKDSSDIMGMYKAEVVKKLKEKLILIFKHDEEKR IYVTVYDTSKAVPENISKEILVKRNNSKEEYFFEDNNKKYVTEYYTL EITETNELKVIPAKKLEGKEFKTEKNKENKLMLNNHYCFNVKIIY
SEQ ID NO: 136	Cas13c	MEEIKHKKNKSSIIRVIVSNYDMTGIKEIKVLYQKQGGVDTFNLKTII NLESGNLEIISCKPKEREKYRYEFNCKTEINTISITKKDKVLKKEIRKY SLELYFKNEKKDTVVAKVTDLLKAPDKIEGERNHLRKLSSSTERKL LSKTLCKNYSEISKTPIEEIDSIKIYKIKRFLNYRSNFLIYFALINDFLC AGVKEDDINEVWLIQDKEHTAFLENRIEKITDYIFDKLSKDIENKKN QFEKRIKKYKTSLEELKTETLEKNKTFYIDSIKTKITNLENKITELSLY NSKESLKEDLIKIISIFTNLRHSLMHYDYKSFENLFENIENEELKNLLD LNLFKSIRMSDEFKTKNRTNYLDGTESFTIVKKHQNLKKLYTYYNN LCDKKNGFNTFINSFFVTDGIENTDFKNLIILHFEKEMEEYKKSIEYY KIKISNEKNKSKKEKLKEKIDLLQSELINMREHKNLLKQIYFFDIHNSI KYKELYSERKNLIEQYNLQINGVKDVTAINHINTKLLSLKNKMDKIT KQNSLYRLKYKLKIAYSFLMIEFDGDVSKFKNNFDPTNLEKRVEYL DKKEEYLNYTAPKNKFNFAKLEEELQKIQSTSEMGADYLNVSPENN LFKFYILTYIMLPVEFKGDFLGFVKNHYYNIKNVDFMDESLLDENEV DSNKLNEKIENLKDSSFFNKIRLFEKNIKKYEIVKYSVSTQENMKEY FKQLNLDIPYLDYKSTDEIGIFNKNMILPIFKYYQNVFKLCNDIEIHA LLALANKKQQNLEYAIYCCSKKNSLNYNELLKTFNRKTYQNLSFIR NKIAHLNYKELFSDLFNNELDLNTKVRCLIEFSQNNKFDQIDLGMNF INDYYMKKTRFIFNQRRLRDLNVPSKEKIIDGKRKQQNDSNNELLK KYGLSRTNIKDIFNKAWY
SEQ ID NO: 137	Cas13c	MKVRYRKQAQLDTFIIKTEIVNNDIFIKSIIEKAREKYRYSFLFDGEE KYHFKNKSSVEIVKNDIFSQTPDNMIRNYKITLKISEKNPRVVEAEIE DLMNSTILKDGRRSARREKSMTERKLIEEKVAENYSLLANCPIEEVD SIKIYKIKRFLTYRSNMLLYFASINSFLCEGIKGKDNETEEIWHLKDN DVRKEKVKENFKNKLIQSTENYNSSLKNQIEEKEKLSSKEFKKGAFY RTIIKKLQQERIKELSEKSLTEDCEKIIKLYSELRHPLMHYDYQYFEN LFENKENSELTKNLNLDIFKSLPLVRKMKLNNKVNYLEDNDTLFVL QKTKKAKTLYQIYDALCEQKNGFNKFINDFFVSDGEENTVFKQIINE KFQSEMEFLEKRISESEKKNEKLKKKLDSMKAHFRNINSEDTKEAYF WDIHSSRNYKTKYNERKNLVNEYTKLLGSSKEKKLLREEITKINRQL LKLKQEMEEITKKNSLFRLEYKMKIAFGFLFCEFDGNISKFKDEFDA

SEQ ID NO	Description	Sequence
		SNQEKIIQYHKNGEKYLTSFLKEEEKEKFNLEKMQKIIQKTEEEDWL LPETKNNLFKFYLLTYLLLPYELKGDFLGFVKKHYYDIKNVDFMDE NQNNIQVSQTVEKQEDYFYHKIRLFEKNTKKYEIVKYSIVPNEKLKQ YFEDLGIDIKYLTGSVESGEKWLGENLGIDIKYLTVEQKSEVSEEKN KKVSLKNNGMFNKTILLFVFKYYQIAFKLFNDIELYSLFFLREKSEKP FEVFLEELKDKMIGKQLNFGQLLYVVYEVLVKNKDLDKILSKKIDY RKDKSFSPEIAYLRNFLSHLNYSKFLDNFMKINTNKSDENKEVLIPSI KIQKMIQFIEKCNLQNQIDFDFNFVNDFYMRKEKMFFIQLKQIFPDIN STEKQKKSEKEEILRKRYHLINKKNEQIKDEHEAQSQLYEKILSLQKI FSCDKNNFYRRLKEEKLLFLEKQGKKKISMKEIKDKIASDISDLLGIL KKEITRDIKDKLTEKFRYCEEKLLNISFYNHQDKKKEEGIRVFLIRDK NSDNFKFESILDDGSNKIFISKNGKEITIQCCDKVLETLMIEKNTLKIS SNGKIISLIPHYSYSIDVKY

[00286] The programmable nuclease can be Cas13. Sometimes the Cas13 can be Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. In some cases, the programmable nuclease can be Mad7 or Mad2. In some cases, the programmable nuclease can be Cas12. Sometimes the Cas12 can be Cas12a, Cas12b, Cas12c, Cas12d (also referred to as CasY), or Cas12e. In some cases, the Cas12 can be a Cas12 variant (e.g., SEQ ID NO: 11), which is a specific protein variant within the Cas12 protein family/classification. In some cases, the programmable nuclease can be Csm1, Cas9, C2c4, C2c8, C2c5, C2c10, C2c9, or CasZ. Sometimes, the Csm1 can also be also called smCms1, miCms1, obCms1, or suCms1. Sometimes Cas13a can also be also called C2c2. Sometimes CasZ can also be called Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. The programmable nuclease can be a CRISPR-Cas (clustered regularly interspaced short palindromic repeats - CRISPR associated) nucleoprotein complex with trans cleavage activity, which can be activated by binding of a guide nucleic acid with a target nucleic acid. The CRISPR-Cas nucleoprotein complex can comprise a Cas protein (also referred to as a Cas nuclease) complexed with a guide nucleic acid, which can also be referred to as CRISPR enzyme. A guide nucleic acid can be a CRISPR RNA (crRNA). Sometimes, a guide nucleic acid comprises a crRNA and a trans-activating crRNA (tracrRNA). The CRISPR/Cas system used to detect a modified target nucleic acids can comprise CRISPR RNAs (crRNAs), trans-activating crRNAs (tracrRNAs), Cas proteins, and nucleic acids of a reporter.

[00287] The programmable nucleases described herein are capable of being activated when complexed with the guide nucleic acid and the target nucleic acid (*e.g.*, DNA). A programmable nuclease can be capable of being activated when complexed with a guide nucleic acid and the target deoxyribonucleotide. The programmable nuclease can be activated upon binding of the guide nucleic acid to its target nucleic acid and degrades non-specifically nucleic acid in its

environment. The programmable nuclease may have trans cleavage activity once activated. In some embodiments, an activated DNA-activated programmable RNA nuclease non-specifically degrades RNA in its environment (e.g., exhibits sequence-independent cleavage of RNA, such as RNA reporters). A DNA-activated programmable RNA nuclease can be a Cas protein (also referred to, interchangeably, as a Cas nuclease). A crRNA and Cas protein can form a CRISPR/Cas enzyme. In some embodiments, the DNA-activated programmable RNA nuclease is a Type VI CRISPR enzyme. Sometimes, the programmable nuclease is a type V CRISPR-Cas system. The programmable nuclease can be a type VI CRISPR-Cas system. Sometimes the programmable nuclease is a type III CRISPR-Cas system. In some embodiments, the DNA-activated programmable RNA nuclease is Cas13. Sometimes the Cas13 is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. In some cases, the DNA-activated programmable RNA nuclease is from at least one of *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).* Sometimes the DNA-activated programmable RNA nuclease is at least one of LbuCas13a, LwaCas13a, LbaCas13a, HheCas13a, PprCas13a, EreCas13a, CamCas13a, or LshCas13a.

[00288] In some embodiments, a programmable nuclease is capable of being activated by a target RNA to initiate trans cleavage of an RNA reporter and is capable of being activated by a target DNA to initiate trans cleavage of an RNA reporter, such as a Type VI CRISPR protein (e.g., Cas13). For example, Cas13a of the present disclosure can be activated by a target RNA to initiate trans cleavage activity of the Cas13a for the cleavage of an RNA reporter and can be activated by a target DNA to initiate trans cleavage activity of the Cas13a for trans cleavage of an RNA reporter.

[00289] The trans cleavage activity of the DNA-activated programmable RNA nuclease can be activated when the crRNA is complexed with the target deoxyribonucleic acid. The trans cleavage activity of the DNA-activated programmable RNA nuclease can be activated when the guide nucleic acid comprising a tracrRNA and crRNA are complexed with the target

deoxyribonucleic acid. The target deoxyribonucleic acid can be a DNA or reverse transcribed RNA, or an amplicon thereof. Preferably, the target deoxyribonucleic acid is single-stranded DNA. Thus, a Cas13a nuclease of the present disclosure can be activated by a target DNA to initiate trans cleavage activity of the Cas13a nuclease that cleaves an RNA reporter. For example, Cas13a nucleases disclosed herein are activated by the binding of the guide nucleic acid to a target DNA that was reverse transcribed from an RNA to cleave nucleic acids of a reporter in a sequence-independent manner. For example, Cas13a nucleases disclosed herein are activated by the binding of the guide nucleic acid to a target DNA that was amplified from a DNA to trans-collaterally cleave reporter molecules. The reporters can be RNA reporters. In some embodiments, the Cas13a recognizes and detects ssDNA and, further, trans cleaves RNA reporters. Multiple Cas13a isolates can recognize, be activated by, and detect target DNA as described herein, including ssDNA. For example, trans-collateral cleavage of RNA reporters can be activated in LbuCas13a or LwaCas13a by target DNA. Therefore, a DNA-activated programmable RNA nuclease can be used to detect target DNA by assaying for cleaved RNA reporters.

[00290] In some embodiments, the programmable nuclease may be 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 programmable nuclease may be 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 programmable nuclease may be 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.

[00291] A DNA-activated programmable RNA nuclease can be used to detect DNA at multiple pH values. A DNA-activated programmable RNA nuclease can be used to detect DNA at multiple pH values compared to an RNA-activated programmable RNA nuclease, such as a Cas13a complexed with a guide RNA that detects a target ribonucleic acid. For example, a

Cas13 protein that detects a target RNA may exhibit high cleavage activity at pH values from 7.9 to 8.2. A Cas13 protein that detects a target DNA can exhibit consistent cleavage across a wide range of pH conditions, such as from a pH of 6.8 to a pH of 8.2. In some embodiments, Cas13 ssDNA detection may exhibit high cleavage activity at pH values from 6 to 6.5, from 6.1 to 6.6, from 6.2 to 6.7, from 6.3 to 6.8, from 6.4 to 6.9, from 6.5 to 7, from 6.6 to 7.1, from 6.7 to 7.2, from 6.8 to 7.3, from 6.9 to 7.4, from 7 to 7.5, from 7.1 to 7.6, from 7.2 to 7.7, from 7.3 to 7.8, from 7.4 to 7.9, from 7.5 to 8, from 7.6 to 8.1, from 7.7 to 8.2, from 7.8 to 8.3, from 7.9 to 8.4, from 8 to 8.5, from 8.1 to 8.6, from 8.2 to 8.7, from 8.3 to 8.8, from 8.4 to 8.9, from 8.5 to 9, from 6 to 8, from 6.5 to 8, or from 7 to 8. Preferrably, Cas13 ssDNA detection may exhibit high cleavage activity at pH values from 7.0 to 8.0. More preferably, Cas13 ssDNA detection may exhibit high cleavage activity at pH 7.5.

[00292] In some embodiments, a programmable nuclease is capable of being activated by a target RNA to initiate trans cleavage of an RNA reporter and is capable of being activated by a target DNA to initiate trans cleavage of an RNA reporter, such as a Type VI CRISPR protein (e.g., Cas13). For example, Cas13a of the present disclosure can be activated by a target RNA to initiate trans cleavage activity of the Cas13a for the cleavage of an RNA reporter and can be activated by a target DNA to initiate trans cleavage activity of the Cas13a for trans cleavage of an RNA reporter. In some embodiments, target DNA binding preferences of a DNA-activated programmable RNA nuclease can be distinct from target RNA binding preferences of a RNA-activated programmable RNA nuclease. In some embodiments, target DNA binding preferences of a guide nucleic acid complexed with a DNA-activated programmable RNA nuclease can be distinct from target RNA binding preferences of a guide nucleic acid complexed with a RNA-activated programmable RNA nuclease. For example, guide RNA (gRNA) binding to a target DNA, and preferably a target ssDNA, may not necessarily correlate with the binding of the same gRNAs binding to a target RNA. For example, gRNAs can perform at a high level regardless of target nucleotide identity at a 3' position in a sequence of a target RNA. In some embodiments, gRNAs can perform at a high level in the absence of a G at a 3' position in a sequence of a target DNA. Furthermore, target DNA detected by a DNA-activated programmable RNA nuclease complexed with a guide nucleic acid as disclosed herein can be directly from organisms, or can be indirectly generated by nucleic acid amplification methods, such as PCR and LAMP of DNA or reverse transcription of RNA. Key steps for the sensitive detection of direct DNA by a DNA-activated programmable RNA nuclease, such as a Cas13a, can include: (1) production or isolation of DNA to concentrations above about 0.1 nM per reaction for in vitro diagnostics, (2) selection of a target DNA with the appropriate sequence features to enable DNA detection as

these some of these features are distinct from those required for target RNA detection, and (3) buffer composition that enhances DNA detection. The detection of DNA by a DNA-activated programmable RNA nuclease can be connected to a variety of readouts including fluorescence, lateral flow, electrochemistry, or any other readouts described herein. Multiplexing of a DNA-activated programmable RNA nuclease with a DNA-activated programmable DNA nuclease with RNA and DNA FQ-reporter molecules (each with a different color fluorophore), respectively, can enable detection of ssDNA or a combination of ssDNA and dsDNA, respectively. Multiplexing of different DNA-activated programmable RNA nuclease that have distinct RNA reporter cleavage preferences can enable additional multiplexing, such a first DNA-activated programmable RNA nuclease that preferentially cleaves uracil in an RNA reporter and a second DNA-activated programmable RNA nuclease that preferentially cleaves adenines in an RNA reporter. Methods for the generation of ssDNA for a DNA-activated programmable RNA nuclease-based detection or diagnostics can include (1) asymmetric PCR, (2) asymmetric isothermal amplification, such as RPA, LAMP, SDA, etc. (3) NEAR for the production of short ssDNA molecules, and (4) conversion of RNA targets into ssDNA by a reverse transcriptase followed by RNase H digestion. Thus, a DNA-activated programmable RNA nuclease detection of target DNA is compatible with the various systems, kits, compositions, reagents, and methods disclosed herein. Cas13a DNA detection can be employed in a DETECTR assay disclosed herein to provide CRISPR diagnostics leveraging Type VI systems (*e.g.*, Cas13) for the detection of a target DNA.

[00293] Some programmable nucleases can exhibit a high turnover rate. Turnover rate quantifies how many molecules of a detector nucleic acid each programmable nuclease is cleaving per minute. Programmable nucleases with a higher turnover rate are more efficient and transcollateral cleavage in the DETECTR assay methods disclosed herein.

[00294] Turnover rate is quantified as the max transcleaving velocity (max slope in a plot of signal versus time in a DETECTR assay) divided by the amount of programmable nuclease complexed with the guide nucleic acid present in the DETECTR assay, wherein the programmable nuclease is at saturation with respect to its active site for transcollateral cleavage of detector nucleic acids.

[00295] Turnover rate can be quantified with the following equation:

$$Turnover\ rate = \frac{maximum\ transcleaving\ velocity\ (\frac{AU}{min})/signal\ normalization\ factor\ (\frac{AU}{nM})}{concentration\ of\ programmanble\ nuclease\ complexed\ with\ guide\ nucleic\ acid\ (nM)}$$

[00296] Signal normalization factor is based on a standard curve and is the amount of signal produced from a known quantity of detector nucleic acid (substrate of transcollateral cleavage). The turnover rate is, thus, expressed as cleaved detector nucleic acid molecules per minute divided by the concentration of the programmable nuclease complexed with guide nucleic acid (can also be referred to as "nucleoprotein" or "ribonucleoprotein"). Therefore, a programmable nuclease with a high turnover rate exhibits superior and highly efficienct transcollateral cleavage of detector nucleic acids in the DETECTR assay methods disclosed herein. For example, a programmable nuclease having at least 60% sequence identity to SEQ ID NO: 11 can exhibit high a turnover rate of at least about 0.1 cleaved detector molecules per minute. A programmable nuclease having a sequence of SEQ ID NO: 11 exhibits a turnover rate of at least about 0.1 cleaved detector molecules per minute. For example, a programmable nuclease (e.g., SEQ ID NO: 11) that recognizes a PAM of YYN complexed with a guide nucleic acid comprises a turnover rate of at least about 0.1 cleaved detector molecules per minute. The programmable nuclease may be a Type V programmable nuclease. The programmable nuclease may be a Cas12 programmable nuclease. A programmble nuclease having a sequence of SEQ ID NO: 11 exhibits a turnover rate that is higher than the turnover rate of SEQ ID NO: 1. For example, a programmble nuclease having a sequence of SEQ ID NO: 11 can exhibit a turnover rate that is at least about 2-fold higher than the turnover rate of SEQ ID NO: 1. In some embodiments, a programmble nuclease having a sequence of SEQ ID NO: 11 can exhibit a turnover rate that is at about 2-fold higher than the turnover rate of SEQ ID NO: 1. In some embodiments, a programmable nuclease having a sequence of SEQ ID NO: 11 complexed with a guide nucleic acid can exhibit a turnover rate that is at least about 2-fold higher than the turnover rate of SEQ ID NO: 1 complexed with a guide nucleic acid. Thus, a programmable nuclease of SEQ ID NO: 11 is superior and more efficient at transcollateral cleavage in the DETECTR assay methods disclosed herein than a programmable nuclease of SEQ ID NO: 1.

[00297] In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 0.1 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 0.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 1 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 2 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 3 cleaved detector

molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 4 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 10 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 15 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 20 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 25 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 30 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 35 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 40 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 45 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 50 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 60 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 70 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 80 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 90 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 100 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 0.1 to 0.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 0.5 to 1 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 1 to 1.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 1.5 to 2 cleaved detector molecules per minute. In some embodiments, programmable

nucleases with a high turnover rate have a turnover rate of at least about 2 to 2.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 2.5 to 3 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 3 to 3.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 3.5 to 4 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 4 to 4.5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 4.5 to 5 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 5 to 10 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 10 to 15 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 15 to 20 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 20 to 25 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 25 to 30 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 30 to 35 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 35 to 40 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 40 to 45 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 45 to 50 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 50 to 60 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 60 to 70 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 70 to 80 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 80 to 90 cleaved detector molecules per minute. In some embodiments, programmable nucleases with a high turnover rate have a turnover rate of at least about 90 to 100 cleaved detector molecules per minute.

Guide Nucleic Acids

[00298] The reagents of this disclosure may comprise a guide nucleic acid. The guide nucleic acid can bind to a single stranded target nucleic acid or portion thereof as described herein. 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 and further comprising a mutation, such as a single nucleotide polymorphism (SNP), which can confer resistance to a treatment, such as antibiotic treatment. 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 synthezised.

[00299] A guide nucleic acid (gRNA) sequence (e.g., a non-naturally occurring gRNA) may hybridize to a target sequence of a target nucleic acid. The term "gRNA" may be used interchangeably with the term "crRNA." A gRNA comprises a repeat region corresponding to a specific programmable nuclease (e.g., a Cas protein), for example the repeat sequences provided in **TABLE 30**. In some embodiments, the repeat region may comprise mutations or truncations with respect to the repeat sequences in pre-crRNA. The repeat sequence interacts with the programmable nuclease (e.g., a Cas protein), allowing for the gRNA and the programmable nuclease to form a complex. This complex may be referred to as a nucleoprotein. A spacer sequence may be positioned 3' of the repeat region. 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. In some embodiments, a programmable nuclease (e.g., a Cas protein) may cleave a precursor RNA

("pre-crRNA") to produce a gRNA, also referred to as a "mature guide RNA." A programmable nuclease (e.g., a Cas protein) that cleaves pre-crRNA to produce a mature guide RNA is said to have pre-crRNA processing activity.

[00300] A guide nucleic acid can comprise a sequence that is, at least in part, reverse complementary to the sequence of a target nucleic acid. 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 synthezised.A guide nucleic acid can be a crRNA. Sometimes, a guide nucleic acid comprises a crRNA and tracrRNA. The guide nucleic acid can bind specifically to the target nucleic acid. 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.

[00301] 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 strain of HPV 16 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, 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.

Reporters

[00302] Described herein are reagents comprising a reporter. The reporter can comprise a single stranded nucleic acid and a detection moiety, wherein the nucleic acid is capable of being cleaved by the activated programmable nuclease, releasing the detection moiety, and, generating a detectable signal. As used herein, "reporter" is used interchangeably with "detector 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 "detector nucleic acid," the "reporter molecule," or the "nucleic acid of the reporter."

[00303] A major advantage of the compositions and methods disclosed herein is 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 may be inhibited in its ability to bind and cleave the reporter sequences. This is because the activated programmable nucleases 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.

[00304] A second significant advantage of the compositions and methods disclosed herein is the design of an excess volume comprising the guide nucleic acid, the programmable nuclease, 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.

[00305] As described herein, nucleic acid sequences comprising DNA may be detected using a DNA-activated programmable RNA nuclease, a DNA-activated programmable DNA nuclease, an RNA-activated programmable RNA nuclease, or any combination thereof, and other reagents disclosed herein. The DNA-activated programmable RNA nuclease may be activated and cleave an RNA reporter upon binding of a guide nucleic acid to a target DNA. In some cases, the reporter comprises a nucleic acid, which is a single-stranded nucleic acid sequence comprising ribonucleotides. Additionally, detection by a DNA-activated programmable RNA nuclease, which can cleave RNA reporters, allows for multiplexing with a DNA-activated programmable DNA nuclease that can cleave DNA reporters (e.g., Type V CRISPR enzyme). In some cases, the nucleic acid of a reporter is a single-stranded nucleic acid sequence comprising deoxyribonucleotides.

[00306] 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 ribonucleotide. 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 ribonucleotide. 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 ribonucleotide. 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 nucleic acid of a reporter is at least 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. In some cases, the nucleic acid of a reporter 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. For cleavage by a programmable nuclease comprising Cas13, a nucleic acid of a reporter can be 5, 8, or 10 nucleotides in length. For cleavage by a programmable nuclease comprising Cas12, a nucleic acid of a reporter can be 10 nucleotides in length.

[00307] The single stranded nucleic acid of a reporter comprises a detection moiety capable of generating a first detectable signal. Sometimes the detector 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 4 – Exemplary Single Stranded Nucleic Acids in a Reporter

5' Detection Moiety*	Sequence (SEQ ID NO)	3' Quencher*
/56-FAM/	rUrUrUrUrU (SEQ ID NO: 111)	/3IABkFQ/
/5IRD700/	rUrUrUrUrU (SEQ ID NO: 111)	/3IRQC1N/
/5TYE665/	rUrUrUrUrU (SEQ ID NO: 111)	/3IAbRQSp/
/5Alex594N/	rUrUrUrUrU (SEQ ID NO: 111)	/3IAbRQSp/
/5ATTO633N/	rUrUrUrUrU (SEQ ID NO: 111)	/3IAbRQSp/
/56-FAM/	rUrUrUrUrUrUrUrU(SEQ ID NO: 112)	/3IABkFQ/
/5IRD700/	rUrUrUrUrUrUrUrU(SEQ ID NO: 112)	/3IRQC1N/
/5TYE665/	rUrUrUrUrUrUrUrU(SEQ ID NO: 112)	/3IAbRQSp/
/5Alex594N/	rUrUrUrUrUrUrUrU(SEQ ID NO: 112)	/3IAbRQSp/
/5ATTO633N/	rUrUrUrUrUrUrUrU(SEQ ID NO: 112)	/3IAbRQSp/
/56-FAM/	rUrUrUrUrUrUrUrUrU(SEQ ID NO: 113)	/3IABkFQ/
/5IRD700/	rUrUrUrUrUrUrUrUrU(SEQ ID NO: 113)	/3IRQC1N/
/5TYE665/	rUrUrUrUrUrUrUrUrU(SEQ ID NO: 113)	/3IAbRQSp/
/5Alex594N/	rUrUrUrUrUrUrUrUrU(SEQ ID NO: 113)	/3IAbRQSp/
/5ATTO633N/	rUrUrUrUrUrUrUrUrU(SEQ ID NO: 113)	/3IAbRQSp/
/56-FAM/	TTTTrUrUTTTT(SEQ ID NO: 114)	/3IABkFQ/
/5IRD700/	TTTTrUrUTTTT(SEQ ID NO: 114)	/3IRQC1N/
/5TYE665/	TTTTrUrUTTTT(SEQ ID NO: 114)	/3IAbRQSp/
/5Alex594N/	TTTTrUrUTTTT(SEQ ID NO: 114)	/3IAbRQSp/
/5ATTO633N/	TTTTrUrUTTTT(SEQ ID NO: 114)	/3IAbRQSp/

5' Detection Moiety*	Sequence (SEQ ID NO)	3' Quencher*
/56-FAM/	TTrUrUTT(SEQ ID NO: 115)	/3IABkFQ/
/5IRD700/	TTrUrUTT(SEQ ID NO: 115)	/3IRQC1N/
/5TYE665/	TTrUrUTT(SEQ ID NO: 115)	/3IAbRQSp/
/5Alex594N/	TTrUrUTT(SEQ ID NO: 115)	/3IAbRQSp/
/5ATTO633N/	TTrUrUTT(SEQ ID NO: 115)	/3IAbRQSp/
/56-FAM/	TArArUGC(SEQ ID NO: 116)	/3IABkFQ/
/5IRD700/	TArArUGC(SEQ ID NO: 116)	/3IRQC1N/
/5TYE665/	TArArUGC(SEQ ID NO: 116)	/3IAbRQSp/
/5Alex594N/	TArArUGC(SEQ ID NO: 116)	/3IAbRQSp/
/5ATTO633N/	TArArUGC(SEQ ID NO: 116)	/3IAbRQSp/
/56-FAM/	TArUrGGC(SEQ ID NO: 117)	/3IABkFQ/
/5IRD700/	TArUrGGC(SEQ ID NO: 117)	/3IRQC1N/
/5TYE665/	TArUrGGC(SEQ ID NO: 117)	/3IAbRQSp/
/5Alex594N/	TArUrGGC(SEQ ID NO: 117)	/3IAbRQSp/
/5ATTO633N/	TArUrGGC(SEQ ID NO: 117)	/3IAbRQSp/
/56-FAM/	rUrUrUrUrU(SEQ ID NO: 118)	/3IABkFQ/
/5IRD700/	rUrUrUrUrU(SEQ ID NO: 118)	/3IRQC1N/
/5TYE665/	rUrUrUrUrU(SEQ ID NO: 118)	/3IAbRQSp/
/5Alex594N/	rUrUrUrUrU(SEQ ID NO: 118)	/3IAbRQSp/
/5ATTO633N/	rUrUrUrUrU(SEQ ID NO: 118)	/3IAbRQSp/
/56-FAM/	TTATTATT (SEQ ID NO: 119)	/3IABkFQ/
/56-FAM/	TTATTATT (SEQ ID NO: 119)	/3IABkFQ/
/5IRD700/	TTATTATT (SEQ ID NO: 119)	/3IRQC1N/
/5TYE665/	TTATTATT (SEQ ID NO: 119)	/3IAbRQSp/
/5Alex594N/	TTATTATT (SEQ ID NO: 119)	/3IAbRQSp/
/5ATTO633N/	TTATTATT (SEQ ID NO: 119)	/3IAbRQSp/
/56-FAM/	TTTTTT (SEQ ID NO: 120)	/3IABkFQ/
/56-FAM/	TTTTTTTT (SEQ ID NO: 121)	/3IABkFQ/
/56-FAM/	TTTTTTTTTT (SEQ ID NO: 122)	/3IABkFQ/
/56-FAM/	TTTTTTTTTTTT (SEQ ID NO: 123)	/3IABkFQ/
/56-FAM/	TTTTTTTTTTTTTT (SEQ ID NO: 124)	/3IABkFQ/
/56-FAM/	AAAAAA (SEQ ID NO: 125)	/3IABkFQ/
/56-FAM/	CCCCCC (SEQ ID NO: 126)	/3IABkFQ/
/56-FAM/	GGGGGG (SEQ ID NO: 127)	/3IABkFQ/
/56-FAM/	TTATTATT (SEQ ID NO: 119)	/3IABkFQ/

/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)
rU: uracil ribonucleotide
rG: guanine ribonucleotide
*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.

[00308] 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.

[00309] 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: 111 with a fluorophore that emits a fluorescence around 520 nm is used for testing in non-urine samples, and SEQ ID NO: 118 with a fluorophore that emits a fluorescence around 700 nm is used for testing in urine samples.

[00310] 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.

[00311] The generation of the detectable signal from the release of the detection moiety indicates that cleavage by the programmable nuclease 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.

[00312] 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.

[00313] 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.

[00314] 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.

[00315] 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.

[00316] 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, fom 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.

[00317] 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.

[00318] 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.

[00319] 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 synthezised. 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.

[00320] 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. In a preferred embodiment, a Cas13a programmable nuclease us used to detect the presence of a single-stranded DNA target nucleic acid. For example, a programmable nuclease is LbuCas13a that detects a target nucleic acid and a single stranded nucleic acid of a reporter comprises two adjacent uracil nucleotides with a green detectable moiety that is detected upon cleavage. As another example, a programmable nuclease is LbaCas13a that detects a target nucleic acid and a single-stranded nucleic acid of a reporter comprises two adjacent adenine nucleotides with a red detectable moiety that is detected upon cleavage.

Buffers

[00321] The reagents described herein can also include buffers, which are compatible with the methods, compositions, reagents, enzymes, and kits disclosed herein. Buffers can be referred to herein as a "high performance buffer" or an "activity buffer" and are compatible with different programmable nucleases described herein. Compositions including the high performance buffers and programmable nucleases described herein exhibit superior and efficienct transcollateral cleavage activity in the various methods described herein (e.g., DETECTR assay methods for assaying for a target nucleic acid). Any of the methods, compositions, reagents, enzymes, or kits disclosed herein may comprise a buffer (e.g., a high performanc buffer or an activity buffer). These buffers are 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 assay detection a target nucleic acid, such as enhancing 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, 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 detector nucleic acids of a population of detector 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. The buffer can increase the discrimination of the programmable nuclease of the segment of the target nucleic acid and the at least one nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid. For example, the buffer increases the discrimination between the segment of the target nucleic acid comprising a single nucleotide mutation and the at least one nucleic acid comprising a variant of the single nucleotide mutation of the segment of the target nucleic acid. Sometimes, the buffer increases the discrimination between the segment of the target nucleic acid comprising deletion and the at least one nucleic acid comprising a variant of the segment of the target nucleic acid. The methods as described herein can be performed in the buffer.

[00322] 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 agent include HEPES, Tris, and imidazole. A buffer may comprise HEPES, Tris, or any combination thereof. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) may comprise HEPES. A buffer may comprise HEPES, Tris, or any combination thereof. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) may comprise Tris. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a buffering agent at a concentration of from 10 mM to 40 mM. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a buffering agent at a concentration of about 20 mM. A buffer compatible with a programmable nuclease may comprise a buffering agent at a concentration of from 5 mM to 100 mM. A buffer compatible with a programmable nuclease may comprise a buffering agent at a concentration of from 10 mM to 30 mM. A composition (e.g., a composition comprising a programmable nuclease) may have a pH of from 7 to 8. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1 or SEQ ID NO: 11) may comprise a buffering agent at a concentration of from 1 mM to 50 mM. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1 or SEQ ID NO: 11) may comprise a buffering agent at a concentration of from 1 mM to 30 mM. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1 or SEQ ID NO: 11) may comprise a buffering agent at a concentration of about 20 mM.

[00323] Exemplary salts include NaCl, KCl, magenesium acetate, potassium acetate, and $MgCl_2$. A buffer may comprise potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt at a concentration

of from 5 mM to 100 mM. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt at a concentration of from 5 mM to 10 mM. 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 (e.g., SEQ ID NO: 11 or SEQ ID NO: 104) comprises a salt from 1 mM to 60 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt from 1 mM to 10 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a salt at about 105 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises a salt at about 55 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt at about 7 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt, wherein the salt comprises potassium acetate and magnesium acetate. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a salt, wherein the salt comprises sodium chloride and magnesium chloride. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises a salt, wherein the salt comprises potassium chloride and magnesium chloride.

[00324] 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. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a crowding agent at a concentration of from 0.5% (v/v) to 2% (v/v). In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a crowding agent at a concentration of about 1% (v/v). A buffer compatible with a programmable nuclease may comprise a crowding agent at a concentration of from 1% (v/v) to 5% (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).

[00325] 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 (e.g., SEQ ID NO: 11) may comprise Triton-X. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) may comprise IGEPAL CA-630. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a detergent at a concentration of 2% (v/v) or less. In some embodiments, a buffer

compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a detergent at a concentration of about 0.00016% (v/v). A buffer compatible with a programmable nuclease may comprise a deterget at a concentration of 2% (v/v) or less. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11 or SEQ ID NO: 104) may comprise a deterget at a concentration of from 0.00001% (v/v) to 0.01% (v/v). A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) may comprise a deterget at a concentration of about 0.01% (v/v).

[00326] Exemplary reducing agents comprise dithiothreitol (DTT), ß-mercaptoethanol (BME), or tris(2-carboxyethyl)phosphine (TCEP). A buffer may comprise DTT. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) 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 (e.g., SEQ ID NO: 1) may comprise a reducing agent at a concentration of from 0.01 mM to 100 mM. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) may comprise a reducing agent at a concentration of from 0.1 mM to 10 mM. A buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) may comprise a reducing agent at a concentration of about 1 mM.

[00327] A buffer compatible with a programmable nuclease may comprise a competitor. Exemplary competitors compete with the target nucleic acid or the detector nucleic acid for cleavage by the programmable nuclease. Exemplary competitors include heparin, and imidazole, and salmon sperm DNA. A buffer may comprise heparin. 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.

[00328] In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1, SEQ ID NO: 11, or SEQ ID NO: 104) comprises a crowding agent or a competitor. For example, the crowding agent is present from 1% (v/v) to 10% (v/v). In some embodiments, the crowding agent or competitor is present from 1% (v/v) to 5% (v/v). In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1, or SEQ ID NO: 104) comprises a crowding agent or competitor present at about to 5% (v/v). In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises a crowding agent or competitor present at about 1% (v/v). In some embodiments, a

buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a crowding agent or competitor present from 1 μg/mL to 100 μg/mL. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a crowding agent or competitor present from 30 μg/mL to 70 μg/mL. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a crowding agent or competitor present at about 50 μg/mL. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises a crowding agent or competitor present from 1 mM to 30 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises a crowding agent or competitor present from 1 mM to 50 mM. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises a crowding agent or competitor present at about 20 mM. The crowding agent or competitor may be selected from the group consisting of: glycerol, heparin, bovine serum albumin, imidazole, and any combination thereof. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11) comprises glycerol. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises glycerol and heparin. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 104) comprises glycerol, BSA, and imidazole.

[00329] Sometimes, a method used herein comprises: contacting a programmable nuclease comprising a polypeptide having endonuclease activity and a guide nucleic acid to a target nucleic acid in a buffer comprising heparin. The heparin is present, for example, at a concentration of from 1 to 100 μg/ml heparin. Often, the heparin is present at a concentration of from 40 to 60 μg/ml heparin. Sometimes, the heparin is present at a concentration 50 μg/ml heparin. Often, the buffer comprises NaCl. The NaCl is present, for example, at a concentration of from 1 to 200 mM NaCl. Sometimes, the NaCl is present at a concentration of from 80 to 120 mM NaCl. Often, the NaCl is present at a concentration of 100 mM NaCl.

[00330] In some embodiments, the buffer comprises heparin. The buffer can comprise 50 μg/ml heparin. Sometimes, the buffer comprises 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml, 25 μg/ml, 30 μg/ml, 35 μg/ml, 40 μg/ml, 45 μg/ml, 50 μg/ml, 55 μg/ml, 60 μg/ml, 65 μg/ml, 70 μg/ml, 75 μg/ml, 80 μg/ml, 85 μg/ml, 90 μg/ml, 95 μg/ml, or 100 μg/ml of heparin, or any value within these values. Often, the buffer comprises from 40 μg/ml to 60 μg/ml heparin. Often, a buffer may comprise from 40 μg/ml to 60 μg/ml heparin. Preferably, a specificity buffer may comprise 50 μg/ml heparin. Preferrably, a high sensitivity buffer may not contain heparin.

[00331] In some embodiments, the buffer comprises NaCl. The buffer can comprise 100 mM NaCl. Sometimes, the buffer comprises 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM,

80 mM, 85 mM, 90 mM, 95 mM, 100 mM, 105 mM, 110 mM, 115 mM, 120 mM, 125 mM, 130 mM, 140 mM, 150 mM, or 200 mM of NaCl, or any value within these values. Often, the buffer comprises from 75 mM to 125 mM NaCl. Preferrably, a high specificity buffer may comprise 100 mM NaCl. Preferrably, a high sensitivity buffer may not contain NaCl.

[00332] In some embodiments, the buffer comprises heparin and NaCl. The buffer can comprise 50 μg/ml heparin and 100 mM NaCl. Sometimes, the buffer comprises 5 μg/ml, 10 μg/ml, 15 μg/ml, 20 μg/ml, 25 μg/ml, 30 μg/ml, 35 μg/ml, 40 μg/ml, 45 μg/ml, 50 μg/ml, 55 μg/ml, 60 μg/ml, 65 μg/ml, 70 μg/ml, 75 μg/ml, 80 μg/ml, 85 μg/ml, 90 μg/ml, 95 μg/ml, or 100 μg/ml of heparin, or any value within these values, and 50 mM, 55 mM, 60 mM, 65 mM, 70 mM, 75 mM, 80 mM, 85 mM, 90 mM, 95 mM, 100 mM, 105 mM, 110 mM, 115 mM, 120 mM, 125 mM, 130 mM, 140 mM, 150 mM, or 200 mM of NaCl, or any value within these values. Preferrably, a high specificity buffer may comprise 100 mM NaCl and 50 μg/ml heparin. Preferrably, a high sensitivity buffer may not contain NaCl and may not contain heparin.

[00333] A high specificity buffer can comprise 20 mM Tris pH 8.0, 100 mM NaCl, 5 mM $MgCl_2$, 1 mm DTT, 5% (v/v) glycerol, and 50 μg/ml heparin.

[00334] In contrast a high sensitivity buffer comprises the high specificity buffer as described above, but without the heparin and NaCl (e.g., 20 mM Tris pH 8.0, 5 mM $MgCl_2$, 1 mm DTT, 5% (v/v) glycerol).

[00335] Sometimes, a method used herein comprises: contacting a programmable nuclease comprising a polypeptide having endonuclease activity and a guide nucleic acid to a target nucleic acid in a buffer comprising heparin. The heparin is present, for example, at a concentration of from 1 to 100 μg/ml heparin. Often, the heparin is present at a concentration of from 40 to 60 μg/ml heparin. Sometimes, the heparin is present at a concentration 50 μg/ml heparin. Often, the buffer comprises NaCl. The NaCl is present, for example, at a concentration of from 1 to 200 mM NaCl. Sometimes, the NaCl is present at a concentration of from 80 to 120 mM NaCl. Often, the NaCl is present at a concentration of 100 mM NaCl.

[00336] As described herein, nucleic acid sequences comprising DNA may be detected using a DNA-activated programmable RNA nuclease and other reagents disclosed herein. Additionally, detection by a DNA-activated programmable RNA nuclease, which can cleave RNA reporters, allows for multiplexing with other programmable nucleases, such as a DNA-activated programmable DNA nuclease that can cleave DNA reporters (e.g., Type V CRISPR enzyme). The methods described herein can also include the use of buffers, which are compatible with the methods disclosed herein. For example, a buffer comprises 20 mM HEPES pH 6.8, 50 mM KCl, 5 mM $MgCl_2$, and 5% (v/v) glycerol. In some instances the buffer comprises from 0 to 100, 0 to

75, 0 to 50, 0 to 25, 0 to 20, 0 to 10.0 to 5, 5 to 10.5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30, 15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM HEPES pH 6.8. The buffer can comprise to 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM KCl. Preferrably, a buffer may comprise 25 to 75 mM KCl. More preferably, a buffer may comprise 50 mM KCl. In other instances the buffer comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30, 15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM $MgCl_2$. Preferrably, a buffer may comprise 1 to 10 mM $MgCl_2$. More preferably, a buffer may comprise 5 mM $MgCl_2$. The buffer can comprise 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, 5 to 30% (v/v) glycerol. The buffer can comprise 0 to 30, 2 to 25, or 10 to 20% (v/v) glycerol. Preferrably, the buffer may comprise 0% (v/v) to 10% (v/v) glycerol. More preferably, a buffer may comprise 5% (v/v) glycerol. In an preferred example, a buffer may comprise 50 mM KCl, 5 mM $MgCl_2$, and 5% (v/v) glycerol.

[00337] In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a HEPES buffering agent. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a salt, wherein the salt comprises potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, potassium chloride, or any combination thereof. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a glycerol crowding agent. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a detergent, wherein the detergent is Tween, Triton-X, or any combination thereof. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a pH of from 7 to 8. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 11 or SEQ ID NO: 104) comprises a pH of about 7.5. In some embodiments, a buffer compatible with a programmable nuclease (e.g., SEQ ID NO: 1) comprises a pH of about 8.

[00338] In some embodiments, a buffer compatible with a programmable nuclease may comprise a salt at less than about 110 mM and wherein the buffer comprises a pH of from 7 to 8. In some embodiments, the salt is from 1 mM to 110 mM. In some embodiments, a buffer compatible with a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) comprises a pH of about 7.5. In some

embodiments, a buffer (e.g., a buffer comprising about 20 mM HEPES, about 2 mM potassium acetate, about 5 mM magnesium acetate, about 1% (v/v) glycerol, about 0.00016% (v/v) Triton-X, and a pH of about 7.5) is compatible with a programmable nuclease comprising at least 60%, at least 70%, 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. In some embodiments, a buffer (e.g., a buffer comprising about 20 mM Tris, about 100 mM sodium chloride, about 5 mM magnesium chloride, about 5% (v/v) glycerol, about 50 ug/mL heparin, about 1 mM DTT, and a pH of about 8) is compatible with a programmable nuclease comprising at least 60%, at least 70%, 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: 1. In some embodiments, a buffer (e.g., a buffer comprising about 50 mM potassium chloride, about 5 mM magnesium chloride, about 10 ug/ml bovine serum albumin, about 5% (v/v) glycerol, about 20 mM imidazole, about 0.01% (v/v) IGEPAL CA-630, and a pH of about 7.5) is compatible with a programmable nuclease comprising at least 60%, at least 70%, 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: 104. Any of the buffers or compositions described herein may comprise a guide nucleic acid (e.g., a non-naturally occurring guide nucleic acid). Any of the buffers or compositions described herein may comprise a detector nucleic acid.

[00339] As another example, a buffer comprises 100 mM Imidazole pH 7.5; 250 mM KCl, 25 mM $MgCl_2$, 50 µg/mL BSA, 0.05% (v/v) Igepal Ca-630, and 25% (v/v) Glycerol. In some instances the buffer comprises 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM Imidazole pH 7.5. In some instances, the buffer comprises 100 to 250, 100 to 200, or 150 to 200 mM Imidazole pH 7.5. Preferrably, the buffer may comprise 20 mM Imidazole pH 7.5. The buffer can comprise 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM KCl. Preferrably, a buffer may comprise 25 to 75 mM KCl. More preferably, a buffer may comprise 50 mM KCl. In other instances the buffer comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30,

15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM $MgCl_2$. Preferrably, a buffer may comprise 1 to 10 mM $MgCl_2$. More preferably, a buffer may comprise 5 mM $MgCl_2$. The buffer, in some instances, comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 50, 10 to 75, 10 to 100, 25 to 50, 25 to 75 25 to 100, 50 to 75, or 50 to 100 μg/mL BSA. In some instances, the buffer comprises 0 to 1, 0 to 0.5, 0 to 0.25, 0 to 0.01, 0 to 0.05, 0 to 0.025, 0 to 0.01, 0.01 to 0.025, 0.01 to 0.05, 0.01 to 0.1, 0.01 to 0.25, 0.01, to 0.5, 0.01 to 1, 0.025 to 0.05, 0.025 to 0.1, 0.025, to 0.5, 0.025 to 1, 0.05 to 0.1, 0.05 to 0.25, 0.05 to 0.5, 0.05 to 0.75, 0.05 to 1, 0.1 to 0.25, 0.1 to 0.5, or 0.1 to 1 % (v/v) Igepal Ca-630. The buffer can comprise 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, 5 to 30% (v/v) glycerol. The buffer can comprise 0 to 30, 2 to 25, or 10 to 20% (v/v) glycerol. Preferrably, the buffer may comprise 0% (v/v) to 10% (v/v) glycerol. More preferably, a buffer may comprise 5% (v/v) glycerol. While reagent (e.g., crowding agents or detergents) concentrations may be described in terms of percent volume per volume (v/v), a percent may also indicate percent weight per volume (w/v).

Stability

[00340] Present in this disclosure are stable compositions of the reagents and the programmable nuclease system for use in the methods as discussed herein. The reagents and programmable nuclease system described herein may be stable in various storage conditions including refrigerated, ambient, and accelerated conditions. Disclosed herein are stable reagents. The stability may be measured for the reagents and programmable nuclease system themselves or the reagents and programmable nuclease system present on the support medium.

[00341] In some instances, stable as used herein refers to a reagents having about 5% w/w or less total impurities at the end of a given storage period. Stability may be assessed by HPLC or any other known testing method. The stable reagents may have about 10% w/w, about 5% w/w, about 4% w/w, about 3% w/w, about 2% w/w, about 1% w/w, or about 0.5% w/w total impurities at the end of a given storage period. The stable reagents may have from 0.5% w/w to 10% w/w, from 1% w/w to 8% w/w, from 2% w/w to 7% w/w, or from 3% w/w to 5% w/w total impurities at the end of a given storage period.

[00342] In some embodiments, stable as used herein refers to a reagents and programmable nuclease system having about 10% or less loss of detection activity at the end of a given storage period and at a given storage condition. Detection activity can be assessed by known positive sample using a known method. Alternatively or combination, detection activity can be assessed by the sensitivity, accuracy, or specificity. In some embodiments, the stable reagents has about 10%, about 9%, about 8%, about 7%, about 6%, about 5%, about 4%, about 3%, about 2%, about

1%, or about 0.5% loss of detection activity at the end of a given storage period. In some embodiments, the stable reagents has from 0.5% to 10%, from 1% to 8%, from 2% to 7%, or from 3% to 5% loss of detection activity at the end of a given storage period.

[00343] In some embodiments, the stable composition has zero loss of detection activity at the end of a given storage period and at a given storage condition. The given storage condition may comprise humidity of equal to or less than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% relative humidity. The controlled storage environment may comprise humidity from 0% to 50% relative humidity, from 0% to 40% relative humidity, from 0% to 30% relative humidity, from 0% to 20% relative humidity, or from 0% to 10% relative humidity. The controlled storage environment may comprise humidity from 10% to 80%, from 10% to 70%, from 10% to 60%, from 20% to 50%, from 20% to 40%, or from 20% to 30% relative humidity. The controlled storage environment may comprise temperatures of about –100°C, about –80°C, about –20°C, about 4°C, about 25°C (room temperature), or about 40°C. The controlled storage environment may comprise temperatures from –80°C to 25°C, or from –100°C to 40°C. The controlled storage environment may comprise temperatures from –20°C to 40°C, from –20°C to 4°C, or from 4°C to 40°C. The controlled storage environment may protect the system or kit from light or from mechanical damage. The controlled storage environment may be sterile or aseptic or maintain the sterility of the light conduit. The controlled storage environment may be aseptic or sterile.

[00344] A kit of this disclosure can be packaged to be stored for extended periods of time prior to use. The kit or system may be packaged to avoid degradation of the kit or system. The packaging may include desiccants or other agents to control the humidity within the packaging. The packaging may protect the kit or system from mechanical damage or thermal damage. The packaging may protect the kit or system from contamination of the reagents and programmable nuclease system. The kit or system may be transported under conditions similar to the storage conditions that result in high stability of the reagent or little loss of reagent activity. The packaging may be configured to provide and maintain sterility of the kit. The kit can be compatible with standard manufacturing and shipping operations.

Multiplexing

[00345] The compositions and methods disclosed herein can be carried out for multiplexed detection. The compositions and methods for multiplexed detection are compatible with the DETECTR assay methods disclosed herein. The compositions and methods for multiplexed detection described here are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The

compositions and methods for multiplexed detection described here 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The compositions and methods for multiplexed detection described here 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. These methods of multiplexing are, for example, consistent with fluidic devices for detection of a target nucleic acid sequence within the sample, wherein the fluidic device may comprise multiple pumps, valves, reservoirs, and chambers for sample preparation, amplification of a target nucleic acid sequence within the sample, mixing with a programmable nuclease, and detection of a detectable signal arising from cleavage of the nucleic acids of a reporter by the programmable nuclease within the fluidic system itself.

[00346] The methods described herein can be multiplexed in a number of ways. These methods of multiplexing are, for example, consistent with the assay methods disclosed herein for detection of a target nucleic acid within the sample when the target nucleic acid, such as multiplexing 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, 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 detector nucleic acids of a population of detector 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. 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.

[00347] In some embodiments, the target nucleic acid for multiplexed detection lacks a PAM. A method of multiplexed assaying for a target nucleic acid segment in a sample, wherein the target

nucleic acid segment lacks a PAM sequence, may comprise amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample.

[00348] Methods consistent with the present disclosure include a multiplexing method of assaying for a target nucleic acid in a sample. A multiplexing method 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 (e.g., DNA) and a programmable nuclease (e.g., a DNA-activated programmable RNA nuclease, such as Cas13) that exhibits sequence-independent cleavage upon forming a complex comprising the segment of the guide nucleic acid binding to 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. As another example, multiplexing method of assaying for a target nucleic acid in a sample, for example, comprises: a) 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; b) contacting the complex to a substrate; c) contacting the substrate to a reagent that differentially reacts with a cleaved substrate; and d) assaying for a signal indicating cleavage of the substrate, 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. Often, the substrate is an enzyme-nucleic acid. Sometimes, the substrate is an enzyme substrate-nucleic acid.

[00349] Multiplexing can be either spatial multiplexing wherein multiple different target nucleic acids at the same time, but the reactions are spatially separated. Often, the multiple target nucleic acids are detected using the same programmable nuclease, but different guide nucleic acids. The

multiple target nucleic acids sometimes are detected using the different programmable nucleases. For example, a DNA-activated programmable RNA nuclease and a DNA-activated programmable DNA nuclease can both be used in a single assay to directly detect DNA targets encoding different sequences. The activated DNA-activated programmable RNA nuclease cleaves an RNA reporter, generating a first detectable signal and the activated DNA-activated programmable DNA nuclease cleaves a DNA reporter, generating a second detectable signal. In some embodiments, the first and second detectable signals are different, and those allow simultaneous detection of more than one target DNA sequences using two programmable nucleases. In some embodiments, the DNA-activated programmable DNA nuclease and the DNA-activated programmable RNA nuclease are complexed to a guide nucleic acid that hybridizes to the same target DNA. The activated DNA-activated programmable RNA nuclease cleaves an RNA reporter, generating a first detectable signal and the DNA-activated programmable DNA nuclease cleaves a DNA reporter, generating a second detectable signal. The first detectable signal and the second detectable signal can be the same, thus, allowing for signal amplification by cleavage of reporters by two different programmable nucleases that are activated by the same target DNA.

[00350] Sometimes, multiplexing can be single reaction multiplexing wherein multiple different target nucleic acids are detected in a single reaction volume. Often, at least two different programmable nucleases are used in single reaction multiplexing. For example, multiplexing can be enabled by immobilization of multiple categories of nucleic acids of a reporter within a fluidic system, to enable detection of multiple target nucleic acids within a single fluidic system. Multiplexing allows for detection of multiple target nucleic acids in one kit or system. In some cases, the multiple target nucleic acids comprise different target nucleic acids to a virus, a bacterium, or a pathogen responsible for one disease. In some cases, the multiple target nucleic acids comprise different target nucleic acids associated with a cancer or genetic disorder. Multiplexing for one disease, cancer, or genetic disorder increases at least one of sensitivity, specificity, or accuracy of the assay to detect the presence of the disease in the sample. In some cases, the multiple target nucleic acids comprise target nucleic acids directed to different viruses, bacteria, or pathogens responsible for more than one disease. In some cases, multiplexing allows for discrimination between multiple target nucleic acids, such as target nucleic acids that comprise different genotypes of the same bacteria or pathogen responsible for a disease, for example, for a wild-type genotype of a bacteria or pathogen and for genotype of a bacteria or pathogen comprising a mutation, such as a single nucleotide polymorphism (SNP) or deletion that can confer resistance to a treatment, such as antibiotic treatment. For example, multiplexing

comprises method of assaying comprising a single assay for a microorganism species using a first programmable nuclease and an antibiotic resistance pattern in a microorganism using a second programmable nuclease. Sometimes, multiplexing allows for discrimination between multiple target nucleic acids of different HPV strains, for example, HPV16 and HPV18. In some cases, the multiple target nucleic acids comprise target nucleic acids directed to different cancers or genetic disorders. Often, multiplexing allows for discrimination between multiple target nucleic acids, such as target nucleic acids that comprise different genotypes, for example, for a wild-type genotype and for SNP genotype. Multiplexing for multiple diseases, cancers, or genetic disorders provides the capability to test a panel of diseases from a single sample. For example, multiplexing for multiple diseases can be valuable in a broad panel testing of a new patient or in epidemiological surveys. Often multiplexing is used for identifying bacterial pathogens in sepsis or other diseases associated with multiple pathogens.

[00351] Furthermore, signals from multiplexing can be quantified. For example, a method of quantification for a disease panel comprises assaying for a plurality of unique target nucleic acids in a plurality of aliquots from a sample, assaying for a control nucleic acid control in a second aliquot of the sample, and quantifying a plurality of signals of the plurality of unique target nucleic acids by measuring signals produced by cleavage of nucleic acids of a reporter compared to the signal produced in the second aliquot. Often the plurality of unique target nucleic acids are from a plurality of bacterial pathogens in the sample. Sometimes the quantification of a signal of the plurality correlates with a concentration of a unique target nucleic acid of the plurality for the unique target nucleic acid of the plurality that produced the signal of the plurality. The disease panel can be for any communicable disease, such as sepsis.

[00352] The methods described herein can be multiplexed by various configurations of the reagents and the support medium. In some cases, the kit or system is designed to have multiple support mediums encased in a single housing. Sometimes, the multiple support mediums housed in a single housing share a single sample pad. The single sample pad may be connected to the support mediums in various designs such as a branching or a radial formation. Alternatively, each of the multiple support mediums has its own sample pad. In some cases, the kit or system is designed to have a single support medium encased in a housing, where the support medium comprises multiple detection spots for detecting multiple target nucleic acids. Sometimes, the reagents for multiplexed assays comprise multiple guide nucleic acids, multiple programmable nucleases, and multiple single stranded detector nucleic acids, where a combination of one of the guide nucleic acids, one of the programmable nucleases, and one of the single stranded detector nucleic acids detects one target nucleic acid and can provide a detection spot on the detection

region. In some cases, the combination of a guide nucleic acid, a programmable nuclease, and a single stranded detector nucleic acid configured to detect one target nucleic acid is mixed with at least one other combination in a single reagent chamber. In some cases, the combination of a guide nucleic acid, a programmable nuclease, and a single stranded detector nucleic acid configured to detect one target nucleic acid is mixed with at least one other combination on a single support medium. When these combinations of reagents are contacted with the sample, the reaction for the multiple target nucleic acids occurs simultaneously in the same medium or reagent chamber. Sometimes, this reacted sample is applied to the multiplexed support medium described herein.

[00353] In some cases, the combination of a guide nucleic acid, a programmable nuclease, and a single stranded detector nucleic acid configured to detect one target nucleic acid is provided in its own reagent chamber or its own support medium. In this case, multiple reagent chambers or support mediums are provided in the device, kit, or system, where one reagent chamber is designed to detect one target nucleic acid. In this case, multiple support mediums are used to detect the panel of diseases, cancers, or genetic disorders of interest.

[00354] In some instances, multiplexed detection detects at least 2 different target nucleic acids in a single reaction. In some instances, multiplexed detection detects at least 3 different target nucleic acids in a single reaction. In some instances, multiplexed detection detects at least 4 different target nucleic acids in a single reaction. In some instances, multiplexed detection detects at least 5 different target nucleic acids in a single reaction. In some cases, multiplexed detection detects at least 6, 7, 8, 9, or 10 different target nucleic acids in a single reaction. In some instances, the multiplexed kits detect at least 2 different target nucleic acids in a single kit. In some instances, the multiplexed kits detect at least 3 different target nucleic acids in a single kit. In some instances, the multiplexed kits detect at least 4 different target nucleic acids in a single kit. In some instances, the multiplexed kits detect at least 5 different target nucleic acids in a single kit. In some instances, the multiplexed kits detect at least 6, 7, 8, 9, or 10 different target nucleic acids in a single kit. In some instances, the multiplexed kits detect from 2 to 10, from 3 to 9, from 4 to 8, or from 5 to 7 different target nucleic acids in a single kit.

Detection Methods

[00355] Disclosed herein are methods of assaying for a target nucleic acid as described herein wherein a signal is detected. The methods of assaying for a target nucleic acid wherein a signal is detected are compatible with the DETECTR assay methods disclosed herein. The methods of assaying for a target nucleic acid wherein a signal is detected, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable

nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The methods of assaying for a target nucleic acid wherein a signal is detected, 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The methods of assaying for a target nucleic acid wherein a signal is detected, 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. A method of assaying for a segment of a target nucleic acid in a sample may comprise contacting the sample to a detector nucleic acid any of the compositions described herein (e.g., a composition comprising a programmable nuclease of SEQ ID NO: 11), wherein the guide nucleic acid hybridizes to a segment of the target nucleic acid , and assaying for a signal produced by cleavage of the detector nucleic acid. In some embodiments, the programmable nuclease (e.g., a Cas12 programmable nuclease) cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid. In some embodiments, the signal produced by cleavage of the detector nucleic acid may be at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid and wherein the subject has a disease when the segment of the target nucleic acid is present.

[00356] In some embodiments, the methods disclosed herein are methods of assaying for a target deoxyribonucleic acid as described herein using a DNA-activated programmable RNA nuclease wherein a signal is detected. For example, 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. In some embodiments, the sample comprises at least one nucleic acid

comprising at least 50% sequence identity to a segment of the target nucleic acid. As another example, a method of assaying for a target nucleic acid in a sample, for example, comprises: a) 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 DNA-activated programmable RNA 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; b) contacting the complex to a substrate; c) contacting the substrate to a reagent that differentially reacts with a cleaved substrate; and d) assaying for a signal indicating cleavage of the substrate, 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. Often, the substrate is an enzyme-nucleic acid. Sometimes, the substrate is an enzyme substrate-nucleic acid. As described herein, nucleic acid sequences comprising DNA may be detected using a DNA-activated programmable RNA nuclease and other reagents disclosed herein.

[00357] In some embodiments, a method of assaying for a target nucleic acid in a sample comprises a sample, wherein the target nucleic acid segment lacks a PAM. For example, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. A PAM-dependent sequence specific nuclease, often, is a programmable nuclease. Sometimes, a PAM-dependent sequence specific nuclease is a PAM-dependent sequence specific endonuclease.

[00358] Present in this disclosure are methods of assaying for a target nucleic acid as described herein. In some embodiments, the method is a method of assaying for a target deoxyribonucleic acid using a DNA-activated programmable RNA nuclease, wherein assaying comprises detecting cleavage of an RNA reporter. For example, 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 DNA-activated programmable RNA 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 (e.g. target deoxyribonucleic 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. As another example, a method of assaying for a target nucleic acid in a sample, for example, comprises: a) 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; b) contacting the complex to a substrate; c) contacting the substrate to a reagent that differentially reacts with a cleaved substrate; and d) assaying for a signal indicating cleavage of the substrate, 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. Often, the substrate is an enzyme-nucleic acid. Sometimes, the substrate is an enzyme substrate-nucleic acid.

[00359] A programmable nuclease can comprise a programmable nuclease capable of being activated when complexed with a guide nucleic acid and target nucleic acid. The programmable nuclease can become activated after binding of a guide nucleic acid with a target nucleic acid, in which the activated programmable nuclease can cleave the target nucleic acid and can have trans cleavage activity. Trans cleavage activity can be non-specific cleavage of nearby nucleic acids by the activated programmable nuclease, such as trans cleavage of detector nucleic acids with a detection moiety. Once the detector nucleic acid is cleaved by the activated programmable nuclease, the detection moiety can be released from the detector nucleic acid and can generate a signal. The signal can be immobilized on a support medium for detection. The signal can be visualized to assess whether a target nucleic acid comprises a modification.

[00360] 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. In some cases, the signal is generated by binding of the detection moiety to the capture molecule in the detection region, where the 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

detector nucleic acid. In some cases, the signal is generated directly by the cleavage event. Alternatively, or in combination, the signal is generated indirectly by the signal event. Sometimes the signal is not a fluorescent signal. In some instances, the 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.

[00361] In some cases, the threshold of detection, for a method of assaying of a target nucleic acid described herein 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, fom 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 minimum concentration at which a 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 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 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 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 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 target nucleic acid can be detected in a sample is in a range of from 1 pM to 10 pM. In some cases, methods described herein detect a target nucleic acid in a sample comprising a plurality of nucleic acids such as a plurality of non-target nucleic acids, where the 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.

[00362] In some cases, the methods described herein detect a target 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 methods described herein detect a target 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.

[00363] Some methods as described herein can be 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, 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 detector nucleic acids of a population of detector 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. Some methods as described herein can be a method of assaying for a target nucleic acid in a sample comprising: producing a PAM target nucleic acid comprising a sequence encoding a PAM by amplifying the target nucleic acid of the sample using primers comprising the encoding the PAM; contacting the PAM target nucleic acid to a complex comprising a guide nucleic acid comprising a segment that is reverse complementary to a segment of the PAM 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 PAM target nucleic acid; and assaying for a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids, wherein the signal indicates a presence of the target nucleic acid in the sample and wherein the absence of the signal indicates an absence of the target nucleic acid in the sample. The cleaving of the detector nucleic acid 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. In some cases, the cleavage efficiency is at least 40%, 50%, 60%, 70%, 80%, 90%, or 95% 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. The change in color may be a detectable colorimetric signal or a signal visible by eye. The signal can be detectable within 5 minutes of contacting the sample comprising the target nucleic acid to a guide nucleic acid complexed with programmable nuclease and a detector nucleic acid comprising a detection moiety, wherein the nucleic acid of the detector nucleic is cleaved by the activated nuclease. The 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.

[00364] The methods described herein can also include the use of buffers, which are compatible with the methods disclosed herein. For example, a buffer comprises 20 mM HEPES pH 6.8, 50 mM KCl, 5 mM $MgCl_2$, and 5% glycerol. In some instances the buffer comprises from 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10,5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30, 15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM HEPES pH 6.8. The buffer can comprise to 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to

50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM KCl. In other instances the buffer comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30, 15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM $MgCl_2$. The buffer can comprise 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, 5 to 30% glycerol.

[00365] As another example, a buffer comprises 100 mM Imidazole pH 7.5; 250 mM KCl, 25 mM $MgCl_2$, 50 μg/mL BSA, 0.05% Igepal Ca-630, and 25% Glycerol. In some instances the buffer comprises 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM Imidazole pH 7.5. The buffer can comprise to 0 to 500, 0 to 400, 0 to 300, 0 to 250, 0 to 200, 0 to 150, 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 5 to 150, 5 to 200, 5 to 250, 5 to 300, 5 to 400, 5 to 500, 25 to 50, 25 to 75, 25 to 100, 50 to 100, 50 150, 50 to 200, 50 to 250, 50 to 300, 100 to 200, 100 to 250, 100 to 300, or 150 to 250 mM KCl. In other instances the buffer comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, to 30, 5 to 40, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 30, 10 to 40, 10 to 50, 15 to 20, 15 to 25, 15 to 30, 15 to 4, 15 to 50, 20 to 25, 20 to 30, 20 to 40, or 20 to 50 mM $MgCl_2$. The buffer, in some instances, comprises 0 to 100, 0 to 75, 0 to 50, 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 50, 5 to 75, 5 to 100, 10 to 20, 10 to 50, 10 to 75, 10 to 100, 25 to 50, 25 to 75 25 to 100, 50 to 75, or 50 to 100 μg/mL BSA. In some instances, the buffer comprises 0 to 1, 0 to 0.5, 0 to 0.25, 0 to 0.01, 0 to 0.05, 0 to 0.025, 0 to 0.01, 0.01 to 0.025, 0.01 to 0.05, 0.01 to 0.1, 0.01 to 0.25, 0.01, to 0.5, 0.01 to 1, 0.025 to 0.05, 0.025 to 0.1, 0.025, to 0.5, 0.025 to 1, 0.05 to 0.1, 0.05 to 0.25, 0.05 to 0.5, 0.05 to 0.75, 0.05 to 1, 0.1 to 0.25, 0.1 to 0.5, or 0.1 to 1 % Igepal Ca-630. The buffer can comprise 0 to 25, 0 to 20, 0 to 10, 0 to 5, 5 to 10, 5 to 15, 5 to 20, 5 to 25, 5 to 30% glycerol.

[00366] The methods for detection of a target nucleic acid described herein further can comprises reagents protease treatment of the sample. The sample can be treated with protease, such as Protease K, before amplification or before assaying for a detectable signal. Often, a protease treatment is for no more than 15 minutes. Sometimes, the protease treatment is for no more than 1, 5, 10, 15, 20, 25, 30, or more minutes, or any value from 1 to 30 minutes.

Sometimes, the protease treatment is from 1 to 30, from 5 to 25, from 10 to 20, or from 10 to 15 minutes. Sometimes, the total time for the performing the method described herein is no greater than 3 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or any value from 3 hours to 20 minutes. Often, a method of nucleic acid detection from a raw sample comprises protease treating the sample for no more than 15 minutes, amplifying (can also be referred to as pre-amplifying) the sample for no more than 15 minutes, subjecting the sample to a programmable nuclease-mediated detection, and assaying nuclease mediated detection. The total time for performing this method, sometimes, is no greater than 3 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or any value from 3 hours to 20 minutes. Often, the protease treatment is Protease K. Often the amplifying is thermal cycling amplification. Sometimes the amplifying is isothermal amplification.

Enrichment of the Target Nucleic Acid using a Targeting Protein

[00367] Enriching for the target nucleic acid in methods described herein can also enhance the assay detection of the target nucleic acid, such as for 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, 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 detector nucleic acids of a population of detector 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. Often, the segment of the target nucleic acid of the methods described herein comprise a mutation and the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid comprise a variant of the mutation. In some embodiments, a target nucleic acid is enriched in a sample prior to or concurrent with detection of the target nucleic acid using any of the methods disclosed herein.

[00368] The compositions for enrichment of target nucleic acids and methods of use thereof, as described herein, are compatible with the DETECTR assay methods disclosed herein. The methods of assaying for a target nucleic acid wherein a signal is detected, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The compositions for

enrichment 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The compositions for enrichment 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.

[00369] A segment of the target nucleic acid may be enriched, for example, by depleting other nucleic acid species that do not correspond to the target nucleic acid from the sample. A segment of the target nucleic acid may be enriched, for example, by increasing the concentration of the target nucleic acid in the sample. In some cases, a nucleic acid species that does not correspond to the target nucleic acid may be a nucleic acid comprising a mutation relative to the target nucleic acid. In some cases, a nucleic acid species that does not correspond to the target nucleic acid may be a nucleic acid comprising a variation relative to the target nucleic acid. In some cases, the nucleic acid species that does not correspond to the target nucleic acid may be a region of a genome that does not comprise the target nucleic acid. In some cases, a nucleic acid species that does not correspond to the target nucleic acid may be a nucleic acid contaminant. The segment of the target nucleic acid in the sample may be enriched by targeting the nucleic acid species that does not correspond to the target nucleic acid with a protein that does not bind the segment of the target nucleic acid. For example, the protein may bind the nucleic acid comprising a mutation relative to the target nucleic acid but not to the segment of the target nucleic acid. Targeting the nucleic acid species that does not correspond to the target nucleic acid with the protein that does not bind the segment of the target nucleic acid may allow for the removal of the targeted nucleic acid. The segment of the target nucleic acid in the sample may be enriched by targeting the target nucleic acid with a protein that specifically binds the segment of the target nucleic acid. For example, the protein may bind the segment of target nucleic acid but not to a nucleic acid comprising a mutation relative to the target nucleic acid. Targeting the segment of the target nucleic acid with the protein that specifically binds the segment of the target nucleic acid may allow for the removal of the nucleic acids that are not targeted by the protein or isolation of the nucleic acids targeted by the protein. A protein may be targeted to the

segment of the target nucleic acid, or the protein may be targeted to a nucleic acid that does not correspond to the target nucleic acid, or any combination thereof, before the contacting of the methods described herein.

[00370] For enrichment of the segment of the target nucleic acid by targeting the nucleic acids comprising a variant or a mutation relative to the target nucleic acid with a protein, the protein can be an antibody that binds to the variant or the mutation of the nucleic acid. Often, the protein is a programmable nuclease without endonuclease activity. Sometimes, the protein is attached to a surface and the sample is passed through the protein attached to surface. The nucleic acids comprising the variant mutation are therefore removed from the flow through, leaving a sample with enriched target nucleic acid.

[00371] Alternatively, for enrichment of the segment of the target nucleic acid by targeting the segment of the target nucleic acid with a protein, the protein can be an antibody that binds to the target nucleic acid. Often, the protein is a programmable nuclease without endonuclease activity. Sometimes, the protein is attached to a surface and the sample is passed through the protein attached to surface. The target nucleic acids therefore bound to the protein and other nucleic acids are separated from the target nucleic acids in the flow through. The bound target nucleic acids can then be released from the protein, leaving a sample with the enriched segments of the target nucleic acids.

Detection of a Mutation in a Target Nucleic Acid

[00372] Disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for detection of a single nucleotide mutation (single nucleotide polymorphism, SNP) in a target nucleic acid. The compositions for detection of a mutation in a target nucleic acid and methods of use thereof, as described herein, are compatible with the DETECTR assay methods disclosed herein. The compositions for detection of a mutation in a target nucleic acid and methods of use thereof, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The compositions for detection of a mutation in a target nucleic acid 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The compositions for detection of a mutation in a target nucleic acid 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. 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. Sometimes, the methods can be used for detection of a deletion in a target nucleic acid. For example, 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, 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 detector nucleic acids of a population of detector 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. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

[00373] A target nucleic acid may be present in a heterogenous sample, for example a sample comprising the target nucleic acid and a nucleic acid with less than 100% sequence identity to the target nucleic acid (e.g., a target nucleic acid comprising a mutation and a nucleic acid that does not comprise the mutation). The target nucleic acid may be present in the heterogenous sample at a minor allele frequency of 10% or less. For example, the target nucleic acid may comprise less than 10% of the nucleic acid population comprising the target nucleic acid comprising a mutation and a nucleic acid that does not comprise the mutation. In some embodiments, the target nucleic acid may be present in the sample at a minor allele frequency of from 0.1% to 10%. In some embodiments, the target nucleic acid may be present in the sample at a minor allele frequency of from 0.1% to 5%. In some embodiments, the target nucleic acid may be present in the sample at a minor allele frequency of from 0.1% to 1%. In some embodiments, the segment of the nucleic acid or the segment of the target nucleic acid comprises at least one base mutation compared to at least one other segment of a nucleic acid in the sample. In some embodiments, the at least one base mutation is no more than 13 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid. In some embodiments, the at least one base mutation

is no more than 10 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid. In some embodiments, the at least one base mutation is no more than 9 nucleotides 3' of the PAM in the nucleic acid or in the PAM target nucleic acid. In some embodiments, the at least one base mutation is no more than 8 nucleotides 3' of the PAM in the nucleic acid or in the PAM target nucleic acid. In some embodiments, the at least one base mutation is a single nucleotide polymorphism.

[00374] Also disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for detection of a single nucleotide mutation in a target nucleic acid. For example, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation.

[00375] Methods described herein can be used to identify a mutation in a target nucleic acid. The methods can be used to identify a single nucleotide mutation of a target nucleic acid that affects the expression of a gene. A mutation that affects the expression of gene can be a single nucleotide mutation of a target nucleic acid within the gene, a single nucleotide mutation of a target nucleic acid comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a single nucleotide mutation of a nucleic acid associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. A mutation that affects the expression of a gene can be a deletion of one or more nucleic acids within the gene, a deletion of one or more target nucleic acids comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a deletion of one or more nucleic acids associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. Often, a status of a mutation is used to diagnose or identify diseases associated with the mutation of target nucleic acid. Detection of target nucleic acids

having a mutation are applicable to a number of fields, such as clinically, as a diagnostic, in laboratories as a research tool, and in agricultural applications. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

Disease Detection

[00376] Disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for disease detection. For example, 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, 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 detector nucleic acids of a population of detector 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. The detection of the signal can indicate the presence of the target nucleic acid. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion. In some embodiments, a method may further comprise administering a treatment for the disease being detected. Any of the methods described herein may be used in diagnosis, wherein a Cas12 nuclease detects a segment of a target nucleic acid. Any of the compositions described herein may be used in diagnosis. Any of the programmable nucleases described herein may be used in diagnosis, wherein the programmable nuclease detects the target nucleic acid.

[00377] Also disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for disease detection. For example, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in

the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation.

[00378] The methods as described herein can be used to identify or diagnose a cancer or genetic disorder associated with a mutation in a target nucleic acid. The methods can be used to identify a mutation of a target nucleic acid that affects the expression of a cancer gene. A cancer gene can be any gene whose aberrant expression is associated with cancer, such as overexpression of an oncogene, suppression of tumor suppressor gene, or dysregulation of a checkpoint inhibitor gene or gene associated with cellular growth, cellular metabolism, or the cell cycle. A mutation that affects the expression of cancer gene can be a mutation of a target nucleic acid within the cancer gene, a mutation of a target nucleic acid comprising RNA associated with the expression of a cancer gene, or a target nucleic acid comprising a mutation of a nucleic acid associated with regulation of expression of a cancer gene, such as an RNA or a promoter, enhancer, or repressor of the cancer gene. For example, a target nucleic acid comprising a mutation that affects a cancer gene can contribute to or lead to colon cancer, bladder cancer, stomach cancer, breast cancer, non-small-cell lung cancer, pancreatic cancer, esophageal cancer, cervical cancer, ovarian cancer, hepatocellular cancer, and acute myeloid leukemia. The target nucleic acid comprise a mutation of a cancer gene or RNA expressed from a cancer gene. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

[00379] The methods can be used to identify a mutation that affects the expression of a gene associated with a genetic disorder. A gene associated with a genetic disorder can be 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. A mutation that affects the expression of a gene associated with a genetic disorder can be mutation within the gene associated with a genetic disorder, a mutation of RNA associated with a gene of the genetic disorder, or a mutation of a nucleic acid associated with regulation of expression of a gene associated with a genetic disorder, such as an RNA or a promoter, enhancer, or repressor of the gene associated with the genetic disorder. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

[00380] Methods described herein can be used to identify a mutation in a target nucleic acid from a bacteria, virus, or microbe. The methods can be used to identify a mutation of a target nucleic acid that affects the expression of a gene. A mutation that affects the expression of

gene can be a mutation of a target nucleic acid within the gene, a mutation of a target nucleic acid comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a mutation of a nucleic acid associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. Sometimes, a status of a target nucleic acid mutation is used to determine a pathogenicity of a bacteria, virus, or microbe or treatment resistance, such as resistance to antibiotic treatment. Often, a status of a mutation is used to diagnose or identify diseases associated with the mutation of target nucleic acid sequences in the bacteria, virus, or microbe. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

Detection as a Research Tool

[00381] Disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used as a research tool. For example, 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, 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 detector nucleic acids of a population of detector 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. The detection of the signal can indicate the presence of the target nucleic acid. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

[00382] Also disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used as a research tool. For example, a method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population

of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation.

[00383] The methods as described herein can be used to identify a single nucleotide mutation in a target nucleic acid. The methods described herein can be used to identify a deletion in a target nucleic acid. The methods can be used to identify mutation of a target nucleic acid that affects the expression of a gene. A mutation that affects the expression of gene can be a single nucleotide mutation of a target nucleic acid within the gene, a mutation of a target nucleic acid comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a mutation of a nucleic acid associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. A mutation that affects the expression of gene can be a deletion of one or more nucleic acids within the gene, a deletion of one or more target nucleic acids comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a deletion of one or more nucleic acids associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

Detection for Agricultural Applications

[00384] Disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for agricultural applications. For example, 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, 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 detector nucleic acids of a population of detector 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. The detection of the signal can indicate the presence of the target nucleic acid. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

[00385] Also disclosed herein are methods of assaying for a target nucleic acid as described herein that can be used for agricultural applications. For example, a method of

assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. The detection of the signal can indicate the presence of the target nucleic acid. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation.

[00386] The methods as described herein can be used to identify a mutation in a target nucleic acid of a plant or of a bacteria, virus, or microbe associated with a plant or soil. The methods can be used to identify a mutation of a target nucleic acid that affects the expression of a gene. A mutation that affects the expression of gene can be a mutation of a target nucleic acid within the gene, a mutation of a target nucleic acid comprising RNA associated with the expression of a gene, or a target nucleic acid comprising a mutation of a nucleic acid associated with regulation of expression of a gene, such as an RNA or a promoter, enhancer, or repressor of the gene. Often, the mutation is a single nucleotide mutation. Alternatively, the mutation is a deletion.

Amplification of Target Nucleic Acids

[00387] 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 (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease 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 compositions comprising a programmable nuclease and a buffer, which has been developed to improve the function of the programmable nuclease (e.g., a programmable nuclease and a

buffer with low salt (about 110 mM or less) and a pH of 7 to 8) 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.

[00388] 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.

[00389] 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.

Amplification for insertion of a PAM sequence

[00390] Amplification methods can also enhance the assay detection of the target nucleic acid, such as enhancing 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, 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 detector nucleic acids of a population of detector 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. For example, amplification of a target nucleic acid with primers encoding a PAM sequence to insert the PAM sequence into the sequence of the target nucleic acid before the contacting. More specifically, a PAM target nucleic acid comprising a sequence

encoding a PAM sequence (e.g., TTTN or dUdUdUN) is produced by amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product. Often, a sequence encoding a PAM sequence is TTTN. Sometimes, a sequence encoding a PAM is dUdUdUN. This allows for any target nucleic acid to be used with a programmable nuclease (e.g., Cas12) that requires the target nucleic acid to comprise a sequence encoding a PAM for activation of the programmable nuclease complexed with the guide nucleic acid. This allows for any target nucleic acid to be used with a programmable nuclease (e.g., Cas12) that requires the target nucleic acid to comprise a sequence encoding a PAM for binding to the guide nucleic acid. One or more steps of the method as disclosed herein may be performed in a common reaction volume (e.g., a single reaction mixture). Often, the method as disclosed herein is performed in a common reaction volume.

[00391] Often, the primer is a forward primer. For example, the forward primer comprises the sequence encoding the PAM. Sometimes, the forward primer comprises from 1 to 20 nucleotides from the 3' end of the sequence encoding the PAM. Often, the forward primer comprises from 1 to 8 nucleotides from the 3' end of the sequence encoding the PAM. The forward primer can comprise 6 nucleotides from the 3' end of the sequence encoding the PAM. The forward primer can comprise 7 nucleotides from the 3' end of the sequence encoding the PAM. The forward primer can comprise 8 nucleotides from the 3' end of the sequence encoding the PAM. Sometimes, these nucleotides from the 3' end of the sequence encoding the PAM is referred are referred to extension nucleotides (e.g., 6 nucleotide extension).

[00392] Often, a mutation in the target nucleic acid amplified using the primer is located a certain number of nucleotides downstream of the 5' end of the target nucleic acid segment wherein the target nucleic acid segment is a segment that binds to a segment of the guide nucleic acid that is reverse complementary to it and comprises the sequence encoding the PAM. Sometimes, the mutation is a single nucleotide mutation or a SNP (e.g., a synonymous mutation or a non-synonymous mutation such as a missense substitution or a nonsense point mutation). Sometimes, the mutation is a deletion. Often, the mutation is from 3 to 20 nucleotides downstream of the target nucleic acid segment. Sometimes, the mutation is from 5 to 9 nucleotides downstream of the target nucleic acid segment. The mutation can be 6 nucleotides downstream of the target nucleic acid segment. The mutation can be 7 nucleotides downstream of the target nucleic acid segment. The mutation can be 8 nucleotides downstream of the target nucleic acid segment.

[00393] A method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprises amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. Often, a sequence encoding a PAM sequence is TTTN. Sometimes, a sequence encoding a PAM is dUdUdUN. This allows for any target nucleic acid to be used with a programmable nuclease (e.g., Cas12) that requires the target nucleic acid to comprise a sequence encoding a PAM for activation of the programmable nuclease complexed with the guide nucleic acid. One or more steps of the method as disclosed herein may be performed in a common reaction volume (e.g., a single reaction mixture). Often, the method as disclosed herein is performed in a common reaction volume.

[00394] Often, the forward primer comprises the sequence encoding the PAM. Sometimes, the PAM forward primer comprises from 1 to 20 nucleotides from the 3' end of the sequence encoding the PAM. Often, the PAM forward primer comprises from 1 to 8 nucleotides from the 3' end of the sequence encoding the PAM. Sometimes, the PAM forward primer comprises from 1 to 2 or 4 to 8 nucleotides from the 3' end of the sequence encoding the PAM. Often a PAM forward primer comprising from 1 to 2 or 4 to 8 nucleotides from the 3' end of the sequence encoding the PAM is a PAM sequence comprising dUdUdUN. The PAM forward primer can comprise 1 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 2 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 3 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 4 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 5 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 6 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 7 nucleotides from the 3' end of the sequence encoding the PAM. The PAM forward primer can comprise 8 nucleotides from the 3' end of the sequence encoding the PAM. Sometimes, these nucleotides

from the 3' end of the sequence encoding the PAM is referred are referred to extension nucleotides (e.g., 6 nucleotide extension).

[00395] Often, a mutation in the target nucleic acid (also referred to as the mismatch) amplified using PAM primers is located a certain number of nucleotides downstream of the 5' end of the PAM in PAM target nucleic acid. Sometimes, the mutation or mismatch is a single nucleotide mutation or a SNP. Often, the mismatch is from 3 to 20 nucleotides downstream of the PAM in PAM target nucleic acid. The mismatch can be from 3 to 10 nucleotides downstream of the PAM in PAM target nucleic acid. Sometimes, the mismatch is from 5 to 9 nucleotides downstream of the PAM in PAM target nucleic acid. The mutation can be 6 nucleotides downstream of the PAM in PAM target nucleic acid. The mutation can be 7 nucleotides downstream of the PAM in PAM target nucleic acid. The mutation can be 8 nucleotides downstream of the PAM in PAM target nucleic acid.

[00396] The amplification that produces the PAM target nucleic acid 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 amplification reaction is performed at a temperature of around 20-45°C. The 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 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. Sometimes, the amplification is performed with dTTP, dATP, dCTP, and dGTP. Often the amplification is performed with dUTP, dATP, dCTP, and dGTP. In some embodiments, an amplified target nucleic acid comprises dU nucleic acids.

[00397] The amplification that produces the PAM target nucleic acid can be thermal cycling amplification or isothermal amplification. The reagents for the amplification can comprise a recombinase, a oligonucleotide primer, a single-stranded DNA binding (SSB) protein, and a polymerase. The isothermal amplification can be transcription mediated amplification (TMA). Isothermal amplification can be helicase dependent amplification (HDA) or circular helicase dependent amplification (cHDA). In additional cases, isothermal amplification is strand displacement amplification (SDA). The isothermal amplification can be recombinase polymerase amplification (RPA). The isothermal amplification can be at least one of loop mediated amplification (LAMP) or the exponential amplification reaction (EXPAR). Isothermal 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). In a preferred embodiment, the isothermal amplification is LAMP.

[00398] Various compositions are compatible with the amplification methods described herein. In some embodiments, a composition may comprise a nucleic acid from a sample, wherein the nucleic acid comprises a PAM and a segment that hybridizes to a guide nucleic acid, wherein the PAM has a sequence of dUdUdUN, a guide nucleic acid that hybridizes to the segment of the nucleic acid, and a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid. A composition may further comprise a primer, wherein the primer comprises a first region that is reverse complementary to the PAM and a second region that is reverse complementary to a first segment of the nucleic acid.

[00399] Various methods of assaying are compatible with the amplification methods described herein. In some embodiments, a method of assaying for a target nucleic acid in a sample, wherein the target nucleic acid lacks a PAM may comprise amplifying the target nucleic acid from a sample using a primer comprising a first region that is reverse complementary to a PAM and a second region that is reverse complementary to a first segment of the target nucleic acid, wherein the PAM is dUdUdUN, thereby producing a PAM target nucleic acid, contacting the PAM target nucleic acid to a guide nucleic acid that hybridizes to a segment of the PAM target nucleic acid, a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to a segment of the PAM target nucleic acid, and a detector nucleic acid, and assaying for a signal produced by cleavage of the detector nucleic acid. In some embodiments, the second region comprises from 4 to 12 bases. In some embodiments, the second region comprises from 4 to 10 bases. In some embodiments, the second region comprises from 4 to 7 bases.

Amplification using Blocking Primer

[00400] Amplification methods can also enhance the assay detection of the target nucleic acid, such as enhancing 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, 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 detector nucleic acids of a population of detector 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.

[00401] The methods described herein may comprise amplifying the target prior to detection. In some embodiments, amplifying may comprise using a blocking primer. In some cases, amplification may be performed in the presence of a blocking primer to block amplification of a nucleic acid sequence comprising a mutation or a variation relative to the target nucleic acid. The mutation can be a single nucleotide mutation, a SNP, or a deletion. The variant can be the wild type variant of the mutation (e.g., the wild type variant of the single nucleotide mutation or the wild type variant of the SNP). For example, the blocking primer may bind to a nucleic acid region comprising the mutation relative to the target nucleic acid but may not bind to the target nucleic acid that does not comprise the mutation. In some embodiments, the blocking primer binds to a nucleic acid comprising encoding the wild type sequence of the target nucleic acid segment. Binding of the blocking primer to the nucleic acid region comprising the mutation may prevent amplification of the nucleic acid sequence comprising the mutation. Often, the blocking primer comprises a 3' phosphate. The blocking primer may be a primer incapable of initiating nucleic acid extension. The blocking primer may prevent binding of a primer that is capable of initiating nucleic acid extension. In some cases, the blocking primer can bind perfectly to the nucleic acid comprising the variant mutation. Amplification in the presence of the blocking primer may be performed before the contacting of the methods described herein.

[00402] The use of a blocking primer results in selective amplification of the target nucleic acid. This occurs using standard PCR conditions when a blocking primer is added with a forward primer and a reverse primer. The blocking primer and either the forward or the reverse primer encode at least part of a sequence that overlaps with the sequence of the blocking primer. In this PCR reaction, the blocking primer binds to a variant of the mutation of the target nucleic acid and blocks either the forward primer or the reverse primer (depending on which primer comprises the overlapping sequence with the blocking primer) from priming the extension of the nucleic acid comprising variant of the mutation of the target nucleic acid, and thus the nucleic acid comprising the variant of the mutation of the target nucleic acid is not amplified. In contrast, the blocking primer does not bind the mutation of the target nucleic acid and does not block either the forward primer or the reverse primer (depending on which primer comprises the overlapping sequence with the blocking primer) from priming the extension of the nucleic acid comprising variant of the mutation of the target nucleic acid, and thus the target nucleic acid is selectively amplified. This results in target nucleic acid enrichment in the before the contacting step of the methods described herein.

COLD-PCR Amplification

[00403] Amplification methods can also enhance the assay detection of the target nucleic acid, such as enhancing 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, 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 detector nucleic acids of a population of detector 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. For example, amplification is performed using co-amplification at lower denaturation temperature PCR (COLD-PCR), such as full COLD-PCR and fast COLD-PCR, before the contacting of the methods described herein. In some embodiments, amplifying comprises fast COLD-PCR. In some embodiments, amplifying comprises allele-specific COLD-PCR. In some embodiments, amplifying comprises COLD-PCR. Often, the target nucleic acid is from 0.05% to 20% of total nucleic acids in the sample in these methods.

[00404] The mismatches from mutations in the segment of the target nucleic acid, such as a single nucleotide mutation or a deletion, compared to a nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid, result in altering the melting temperature (Tm) of a double stranded DNA comprising the segment of the target nucleic acid. For example, a target nucleic acid comprising the segment of the target nucleic acid has a Tm that is from 0.1 to 5 C lower than the nucleic acid comprising at least 50% sequence identity to segment of the target nucleic acid. Both full COLD-PCR and fast COLD-PCR are based on this principle and can be used to selectively amplify the target nucleic acid comprising the mutation.

[00405] For performing amplification using full COLD-PCR, the sample comprising the segement of the target nucleic acid and nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid can undergo a denaturation step, such as denaturation at high temperature (e.g., about 94 °C or higher). Next, the temperature is changed to an intermediate annealing temperature that allows hybridization of the segment of the target nucleic acid and the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid to one another. After hybridization, the heteroduplexes of the segment of the target nucleic acid and the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid melt at lower temperatures for denaturation (at a Tc temperature which is a

critical temperature of the double stranded DNA that is lower than its Tm) while the homoduplexes of the segment of the target nucleic acid or the homoduplexes of the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid remain double stranded. Mismatched sequences (e.g., heteroduplexes of the segment of the target nucleic acid and the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid) may be selectively denatured at a critical temperature ("Tc," e.g., about 86.5 °C). Matched sequences (e.g., homoduplexes of the segment of the target nucleic acid or the homoduplexes of the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid) may remain double stranded during selective denaturation of the mismatched sequences. Primers can then anneal to the denatured strands and a DNA polymerase can extend these strands. Since only heteroduplexes of the segment of the target nucleic acid and the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid are available for amplification, a larger portion of the target nucleic acid is amplified and also becomes available for amplification is subsequent rounds. **FIG. 64A** illustrates an exemplary protocol of full COLD-PCR.

[00406] For performing amplification using fast COLD-PCR, the Tm of the segment of the target nucleic acid is lower than the Tm of the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid. Thus, fast COLD-PCR can enrich for segment of the target nucleic acids comprising a mutation that results in a lower Tm than the Tm of the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid. For fast COLD-PCR, the sample comprising the segment of the target nucleic acid and nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid can undergo a denaturation step, such as denaturation at high temperature (e.g., 94 °C). Next, the temperature is reduced so that primers can then anneal to the denatured strands of the segment of the target nucleic acid and a DNA polymerase can extend these strands. Since the segment of the target nucleic acid can be denatured at a lower temperature than the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid, the segment of the target nucleic acid is amplified while the nucleic acid comprising at least 50% sequence identity to segment of the target nucleic acid remains double stranded. Mutant sequences (e.g., the segment of the target nucleic acid comprising a mutation) may be selectively denatured at a critical temperature ("Tc," e.g., about 86.5 °C). Wild type sequences (e.g., the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid) may remain double stranded during selective denaturation of the mutant sequences. **FIG. 64B** illustrates an exemplary protocol of fast COLD-PCR.

[00407] In some embodiments, a composition comprising a Cas12 programmable nuclease (e.g., SEQ ID NO: 11) is at a temperature of from 25° C to 45° C. The Cas12 programmable nuclease (e.g., SEQ ID NO: 11) may exhibit catalytic activity at a temperature of from 25° C to 45° C. The Cas12 programmable nuclease (e.g., SEQ ID NO: 11) may exhibit catalytic activity after heating the composition to a temperature of greater than 45°C and restoring the temperature to a temperature of from 25° C to 45° C.

Allele specific PCR Amplification

[00408] Amplification methods can also enhance the assay detection of the target nucleic acid, such as enhancing 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, 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 detector nucleic acids of a population of detector 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. For example, amplification is performed using allele-specific PCR. Allele-specific PCR comprises using a common reverse primer and two forward allele-specific primers with different 3' ends to amplify the two allele-specific PCR products of different lengths. Often the forward primer for the segment of the target nucleic acid comprises the mutation at the 3' end of the primer and the forward primer for the nucleic acid comprising at least 50% sequence identity segment of the to the target nucleic acid comprises a variant of the mutation at the 3' end of the primer. The 3' end can cause a mismatch that will result in the primer not functioning as a primer under appropriate conditions. This allows for the choosing of conditions that allow for the amplification of the segment of the target nucleic acid but not the nucleic acid comprising at least 50% sequence identity to the segment of the target nucleic acid. Often, the products from the two different forward primers are also different lengths, so these two products can be separated based on their differing lengths using techniques, such as agarose gel electrophoresis. Therefore, the segment of the target nucleic can be enriched before the contacting in the method described herein.

[00409] Often, allele-specific PCR is combined with COLD-PCR. Sometimes, allele-specific PCR is combined with full COLD-PCR as described above. Sometimes, allele-specific PCR is combined with fast COLD-PCR as described above.

Primer and Guide Nucleic Acid Design for Amplification and Detection

[00410] A number of target amplification and detection methods are consistent with the methods, compositions, reagents, enzymes, and kits disclosed herein. The target amplification and detection methods, as described herein, are compatible with the DETECTR assay methods disclosed herein. The target amplification and detection methods, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The target amplification and detection methods, 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The target amplification and detection methods, 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. As described herein, a target nucleic acid may be detected using a DNA-activated programmable RNA nuclease (e.g., a Cas13), a DNA-activated programmable DNA nuclease (e.g., a Cas12), or an RNA-activated programmable RNA nuclease (e.g., a Cas13) and other reagents disclosed herein (e.g., RNA components). The target nucleic acid may be detected using DETECTR, as described herein. The target nucleic acid may be an RNA, reverse transcribed RNA, DNA, DNA amplicon, amplified DNA, synthetic nucleic acids, or nucleic acids found in biological or environmental samples. Amplification methods can also enhance the assay detection of the target nucleic acid sequence, such as enhancing a method of assaying for a target nucleic acid in a sample. In some cases, the target nucleic acid is amplified prior to or concurrent with detection. In some cases, the target nucleic acid is reverse transcribed prior to amplification. The target nucleic acid may be amplified via loop mediated isothermal amplification (LAMP) of a target nucleic acid sequence. In some cases, the nucleic acid is amplified using LAMP coupled with reverse transcription (RT-LAMP). The LAMP amplification may be performed independently, or the LAMP amplification may be coupled to DETECTR for detection of the target nucleic acid. The RT-LAMP amplification may be performed independently, or the RT-LAMP amplification may be coupled to DETECTR for detection of the target nucleic acid. The DETECTR reaction may be performed using any

method consistent with the methods disclosed herein.

Amplification and Detection Reaction Mixtures

[00411] In some embodiments, a LAMP amplification reaction comprises a plurality of primers, dNTPs, and a DNA polymerase. LAMP may be used to amplify DNA with high specificity under isothermal conditions. The DNA may be single stranded DNA or double stranded DNA. In some cases, a target nucleic acid comprising RNA may be reverse transcribed into DNA using a reverse transcriptase prior to LAMP amplification. A reverse transcription reaction may comprise primers, dNTPs, and a reverse transcriptase. In some cases, the reverse transcription reaction and the LAMP amplification reaction may be performed in the same reaction. A combined RT-LAMP reaction may comprise LAMP primers, reverse transcription primers, dNTPs, a reverse transcriptase, and a DNA polymerase. In some case, the LAMP primers may comprise the reverse transcription primers. In some embodiments, the dNTPs may comprise dTTP, dATP, dGTP, and dCTP. In some embodiments, the dNTPs may comprise dUTP, dATP, dGTP, and dCTP.

[00412] A target nucleic acid may be reverse transcribed prior to or concurrent with amplification. For example, an RNA target nucleic acid may be reverse transcribed into DNA. A reverse transcription reaction may comprise an RNA target nucleic acid, dNTPs, and a reverse transcriptase. In some embodiments, the dNTPs may comprise dTTP, dATP, dGTP, and dCTP. In some embodiments, the dNTPs may comprise dUTP, dATP, dGTP, and dCTP. Reverse transcription may be performed in the same reaction as LAMP amplification as a reverse transcription LAMP (RT-LAMP reaction). An amplified target nucleic acid may be transcribed using *in vitro* transcription (IVT) concurrent with or subsequent to amplification. The amplification may be LAMP, or the amplification may be RT-LAMP. An IVT reaction may comprise an amplified target nucleic acid, NTPs, and an RNA polymerase. In some embodiments, the amplified target nucleic acid comprises dU nucleic acids.

[00413] In some embodiments, an amplification reaction comprises an uracil-DNA glycosylase (UDG) enzyme. The UDG enzyme may be heat-activated (e.g., at about 50° C) to degrade any nucleic acid containing dU in the sample. For example, the heat-activated UDG enzyme may degrade contaminating DNA containing dU. The UDG enzyme may be heat-inactivated (e.g., at 95° C) after degradation of the nucleic acid containing dU and prior to amplification of the target nucleic acid. For example, the heat-inactivated UDG enzyme may be inactivated prior to amplifying a target nucleic acid sequence using dNTPs comprising dUTP. An active UDG enzyme may be added to an amplification reaction prior to amplification to degrade contaminating nucleic acids containing dU. In some embodiments, the UDG enzyme is removed

prior to amplification of the target nucleic acid. The UDG enzyme may also be present in an inactive state during amplification of the target nucleic acid using dUTPs. In some embodiments, active UDG enzyme is present in an amplification reaction using dNTPs that do not comprise dUTP.

[00414] A DETECTR reaction to detect the target nucleic acid sequence may comprise a guide nucleic acid comprising a segment that is reverse complementary to a segment of the target nucleic acid and a programmable nuclease. The programmable nuclease when activated, as described elsewhere herein, exhibits sequence-independent cleavage of a reporter (e.g., a nucleic acid comprising a moiety that becomes detectable upon cleavage of the nucleic acid by the programmable nuclease). The programmable nuclease is activated upon the guide nucleic acid hybridizing to the target nucleic acid. In some embodiments, the target nucleic acid comprises dU nucleic acids. A combined LAMP DETECTR reaction may comprise a plurality of primers, dNTPs, a DNA polymerase, a guide nucleic acid, a programmable nuclease, and a substrate nucleic acid. A combined RT-LAMP DETECTR reaction may comprise LAMP primers, reverse transcription primers, dNTPs, a reverse transcriptase, a DNA polymerase, a guide nucleic acid, a programmable nuclease, and a substrate nucleic acid. In some case, the LAMP primers may comprise the reverse transcription primers. LAMP and DETECTR can be carried out in the same sample volume. LAMP and DETECTR can be carried out concurrently in separate sample volumes or in the same sample volume. RT-LAMP and DETECTR can be carried out in the same sample volume. RT-LAMP and DETECTR can be carried out concurrently in separate sample volumes or in the same sample volume.

Primer Design for LAMP Amplification

[00415] A LAMP reaction may comprise a plurality of primers. A plurality of primers are designed to amplify a target nucleic acid sequence, which is shown in **FIG. 40** relative to various regions of a double stranded nucleic acid. The primers can anneal to or have sequences corresponding to these various regions. As shown in **FIG. 40**, the F1c region is 5' of the F2c region, and the F2c region is 5' of the F3c region. Additionally, the B1 region is 3' of the B2 region, and the B2 region is 3' of the B3 region. The F3c, F2c, F1c, B1, B2, and B3 regions are shown on the lower strand in **FIG. 40**. An F3 region is a sequence reverse complementary to the F3c region. An F2 region is a sequence reverse complementary to the F2c region. An F1 region is a sequence reverse complementary to the F1c region. The B1c region is a sequence reverse complementary to a B1 region. The B2c region is a sequence reverse complementary to a B2 region. The B3c region is a sequence reverse complementary to a B3 region. The target nucleic acid may be 5' of the F1c region and 3' of the B1 region, as shown in the top configuration of

FIG. 40. The target nucleic acid may be 5' of the B1c region and 3' of the F1 region, as shown in the bottom configuration of **FIG. 40**. In some embodiments, the target nucleic acid may be 5' of the F2c region and 3' of the F1c region. In some embodiments, the target nucleic acid may be 5' of the B2c region and 3' of the B1c region. In some embodiments, the target nucleic acid sequence may be 5' of the B1 region and 3' of the B2 region. In some embodiments, the target nucleic acid sequence may be 5' of the F1 region and 3' of the F2 region.

[00416] FIG. 40 also shows the structure and directionality of the various primers. The forward outer primer has a sequence of the F3 region. Thus, the forward outer primer anneals to the F3c region. The backward outer primer has a sequence of the B3 region. Thus, the backward outer primer anneals to the B3c region. The forward inner primer has a sequence of the F1c region 5' of a sequence of the F2 region. Thus, the F2 region of the forward inner primer anneals to the F2c region and the amplified sequence forms a loop held together via hybridization of the sequence of the F1c region in the forward inner primer and the F1. The backward inner primer has a sequence of a B1c region 5' of a sequence of the B2 region. Thus, the B2 region of the backward inner primer anneals to the B2c region and the amplified sequence forms a loop held together via hybridization of the sequence of the B1c region of the backward inner primer and the B1 region.

[00417] Further, as shown in **FIG. 40**, the plurality of primers may additionally include a loop forward primer (LF) and/or a loop backward primer (LB). LF is positioned 3' of the F1c region and 5' of the F2c region. LB is positioned 5' of the B2c region and 3' of the B1c region. The F1, F1c, F2, F2c, F3, F3c, B1, B1c, B2, B2c, B3, and/or B3c regions are illustrated in various arrangements relative to the target nucleic acid, the PAM, and the guide RNA (gRNA), as shown in any one of **FIG. 40 – FIG. 42 or FIG. 50 – FIG. 51**. The target nucleic acid may be within the nucleic acid strand comprising the B1, B2, B3, LF, F1c, F2c, F3c, and LBc regions. The target nucleic acid may be within the nucleic acid strand comprising the F1, F2, F3, LB, B1c, B2c, B3c, and LFc regions.

[00418] A set of LAMP primers may be designed for use in combination with a DETECTR reaction. The nucleic acid may comprise a region (e.g., a target nucleic acid), to which a guide RNA hybridizes. All or part of the guide RNA sequence may be reverse complementary to all or part of the target sequence. The target nucleic acid sequence may be adjacent to a protospacer adjacent motif (PAM) 3' of the target nucleic acid sequence. The PAM may promote interaction the programmable nuclease with the target nucleic acid. A PAM may adjacent to a DNA target nucleic acid sequence. The target nucleic acid sequence may be adjacent to a protospacer flanking site (PFS) 3' of the target nucleic acid sequence. The PFS may promote interaction the

programmable nuclease with the target nucleic acid. A PFS may be adjacent to an RNA target nucleic acid sequence. One or more of the guide RNA, the PAM or PFS, or the target nucleic acid sequence may be specifically positioned with respect to one or more of the F1, F1c, F2, F2c, F3, F3c, LF, LFc, LB, LBc, B1, B1c, B2, B2c, B3, and/or B3c regions.

[00419] In some cases, the guide RNA is reverse complementary to a sequence of the target nucleic acid, which is between an F1c region and a B1 region, as in **FIG. 41A**. In some cases, the guide RNA is reverse complementary to a sequence of the target nucleic acid, which is between a B1c region and an F1 region.

[00420] In some cases, the guide RNA is partially reverse complementary to a sequence of the target nucleic acid, which is between an F1c region and a B1 region, as in **FIG. 41B**. In some cases, the guide RNA is partially reverse complementary to a sequence of the target nucleic acid, which is between a B1c region and an F1 region. For example, the target nucleic acid comprises a sequence between an F1c region and a B1 region or a B1c region and an F1 region that is reverse complementary to at least 60% of a guide nucleic acid. In another example, the target nucleic acid comprises a sequence between an F1c region and a B1 region that is reverse complementary to 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%, at least 100%, from 5% to 100%, 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%, or from 95% to 100% of a guide nucleic acid. In this arrangement, the guide RNA is not reverse complementary to the forward inner primer or the backward inner primer shown in **FIG. 40**.

[00421] In some cases, the guide RNA is reverse complementary to no more than 50%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% of the forward inner primer, the backward inner primer, or a combination thereof. the sequence between the F1c region and the B1 region or the sequence between the B1c region and the F1 region is at least 50%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 99%, or 100% reverse complementary to the guide nucleic acid sequence. In some cases, the guide nucleic acid has a sequence reverse complementary to no more than 50%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% of the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. In some cases, the guide nucleic acid sequence has a sequence reverse complementary to no more than 50%, no more than 40%, no more than 35%, no more than 30%, no more than 25%, no more than 20%, no more than 15%, no more than 10%, or no more than 5% of a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. In some embodiments, a sequence of the primer and a sequence of the guide nucleic acid overlap by 50% or less. In some embodiments, a sequence of the primer and a sequence of the guide nucleic acid do not overlap. In some embodiments, the primer is a forward primer, a reverse primer, a forward inner primer, or a reverse inner primer.

[00422] In some cases, the region corresponding to the guide RNA sequence does not overlap or hybridize to any of the primers and may further not overlap with or hybridize to any of the regions shown in **FIG. 40 - FIG. 42 and FIG. 50 - FIG. 51.**

[00423] In some cases, all or a portion of the guide nucleic acid is reverse complementary to a sequence of the target nucleic acid in a loop region. For example, all or a portion of the sequence of the target nucleic acid that hybridizes to the gRNA may be located between the B1 and B2 regions, as shown in **FIG. 41C**. In another example, all or a portion of the sequence of the target nucleic acid that hybridizes to the gRNA may be located between the F2c and F1c regions, as shown in **FIG. 41D**. In some cases, all or a portion of the sequence of the target nucleic acid that hybridizes to the gRNA may be located between the F1 and F2 regions. In some cases, all or a portion of the sequence of the target nucleic acid that hybridizes to the gRNA may be located between the B2c and B1c regions.

[00424] In some cases, a LAMP primer set may be designed using a commercially available primer design software. A LAMP primer set may be designed for use in combination with a DETECR reaction, a reverse transcription reaction, or both. In some cases, a LAMP primer set

may be designed using distributed ledger technology (DLT), artificial intelligence (AI), extended reality (XR) and quantum computing, commonly called "DARQ." In some cases, a LAMP primer set may be designed using quenching of unincorporated amplification signal reporters (QUASR) (Ball et al., Anal Chem. 2016 Apr 5;88(7):3562-8. doi: 10.1021/acs.analchem.5b04054. Epub 2016 Mar 24.). These methods of designing a set of LAMP primers are provided by way of example only; other methods of designing a set of LAMP primers may be readily apparent to one skilled in the art and may be employed in any of the compositions, kits and methods described herein. Exemplary sets of LAMP primers for use in a combined RT-LAMP DETECTR reaction or LAMP-DETECTR to detect the presence of a nucleic acid sequence corresponding to a respiratory syncytial virus (RSV), an influenza A virus (IAV), an influenza B virus (IAV), or a HERC2 SNP are provided in **TABLE 5**.

TABLE 5 – Exemplary LAMP Primers

SEQ ID NO:	**Primer Name**	**Primer Set**	**Sequence**
SEQ ID NO: 138	F3 RSV-A-set13	#1	TGGAACAAGTTGTGGAGG
SEQ ID NO: 139	B3 RSV-A-set13	#1	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 140	FIP RSV-A-set13	#1	TAGTGATGCTTTTGGGTTGTTCAA TTGTATGAGTATGCTCAAAAATTG G
SEQ ID NO: 141	BIP RSV-A-set13	#1	GTGTAGTATTGGGCAATGCTGCTC CTTGGTGTACCTCTGT
SEQ ID NO: 142	LF RSV-A-set13	#1	TATGGTAGAATCCTGCTTCTCC
SEQ ID NO: 143	LB RSV-A-set13	#1	TGGCCTAGGCATAATGGGAGA
SEQ ID NO: 144	F3 RSV-A-set14	#2	AACAAGTTGTGGAGGTGTA
SEQ ID NO: 145	B3 RSV-A-set14	#2	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 146	FIP RSV-A-set14	#2	TAGTGATGCTTTTGGGTTGTTCAA GAGTATGCTCAAAAATTGGGTG

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 147	BIP RSV-A-set14	#2	GTATTGGGCAATGCTGCTGGCAT ATAGATCTTGATTCCTTGGTG
SEQ ID NO: 148	LF RSV-A-set14	#2	ATATGGTAGAATCCTGCTTCTC
SEQ ID NO: 149	LB RSV-A-set14	#2	CCTAGGCATAATGGGAGAATAC
SEQ ID NO: 144	F3 RSV-A-set15	#3	AACAAGTTGTGGAGGTGTA
SEQ ID NO: 145	B3 RSV-A-set15	#3	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 150	FIP RSV-A-set15	#3	ATAGTGATGCTTTTGGGTTGTTCA AGTATGCTCAAAAATTGGGTG
SEQ ID NO: 151	BIP RSV-A-set15	#3	GCTGCTGGCCTAGGCATAATGCA TCATATAGATCTTGATTCCTT
SEQ ID NO: 406	LF RSV-A-set15	#3	TATATGGTAGAATCCTGCTTCTC
SEQ ID NO: 152	LB RSV-A-set15	#3	GGGAGAATACAGAGGTACAC
SEQ ID NO: 153	F3 RSV-A-set16	#4	GGGTCTTAGCAAAATCAGTT
SEQ ID NO: 139	B3 RSV-A-set16	#4	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 154	FIP RSV-A-set16	#4	GAATCCTGCTTCTCCACCCAATTG ACACGCTAGTGTACAAGC
SEQ ID NO: 141	BIP RSV-A-set16	#4	GTGTAGTATTGGGCAATGCTGCTC CTTGGTGTACCTCTGT
SEQ ID NO: 155	LF RSV-A-set16	#4	CCTCCACAACTTGTTCCATTTCT
SEQ ID NO: 156	LB RSV-A-set16	#4	TGGCCTAGGCATAATGGGAG
SEQ ID NO: 157	F3 RSV-A-set17	#5	AAGCAGAAATGGAACAAGTT

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 145	B3 RSV-A-set17	#5	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 158	FIP RSV-A-set17	#5	TAGTGATGCTTTTGGGTTGTTCAG TGGAGGTGTATGAGTATGC
SEQ ID NO: 159	BIP RSV-A-set17	#5	GTAGTATTGGGCAATGCTGCTGAT ATAGATCTTGATTCCTTGGTG
SEQ ID NO: 160	LF RSV-A-set17	#5	TGCTTCTCCACCCAATTTTTGA
SEQ ID NO: 161	LB RSV-A-set17	#5	GCCTAGGCATAATGGGAGAATAC
SEQ ID NO: 153	F3 RSV-A-set18	#6	GGGTCTTAGCAAAATCAGTT
SEQ ID NO: 139	B3 RSV-A-set18	#6	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 162	FIP RSV-A-set18	#6	GAATCCTGCTTCTCCACCCAGACA CGCTAGTGTACAAGC
SEQ ID NO: 141	BIP RSV-A-set18	#6	GTGTAGTATTGGGCAATGCTGCTC CTTGGTGTACCTCTGT
SEQ ID NO: 155	LF RSV-A-set18	#6	CCTCCACAACTTGTTCCATTTCT
SEQ ID NO: 156	LB RSV-A-set18	#6	TGGCCTAGGCATAATGGGAG
SEQ ID NO: 163	F3 RSV-A-set19	#7	TACACAGCTGCTGTTCAA
SEQ ID NO: 164	B3 RSV-A-set19	#7	GGTAAATTTGCTGGGCATT
SEQ ID NO: 165	FIP RSV-A-set19	#7	TTGGAACATGGGCACCCATAAAT GTCCTAGAAAAAGACGATG
SEQ ID NO: 166	BIP RSV-A-set19	#7	CTAGTGAAACAAATATCCACACC CAGCACTGCACTTCTTGAGTT
SEQ ID NO: 167	LF RSV-A-set19	#7	TTGTAAGTGATGCAGGAT

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 168	LB RSV-A-set19	#7	AGGGACCCTCATTAAGAGTCATG
SEQ ID NO: 169	F3 RSV-A-set20	#8	ATACACAGCTGCTGTTCA
SEQ ID NO: 164	B3 RSV-A-set20	#8	GGTAAATTTGCTGGGCATT
SEQ ID NO: 170	FIP RSV-A-set20	#8	TCTGCTGGCATGGATGATTGAATG TCCTAGAAAAAGACGATG
SEQ ID NO: 166	BIP RSV-A-set20	#8	CTAGTGAAACAAATATCCACACC CAGCACTGCACTTCTTGAGTT
SEQ ID NO: 171	LF RSV-A-set20	#8	CCCATATTGTAAGTGATGCAGGA T
SEQ ID NO: 172	LB RSV-A-set20	#8	AGGGACCCTCATTAAGAGTCAT
SEQ ID NO: 169	F3 RSV-A-set21	#9	ATACACAGCTGCTGTTCA
SEQ ID NO: 173	B3 RSV-A-set21	#9	TGGTAAATTTGCTGGGCAT
SEQ ID NO: 170	FIP RSV-A-set21	#9	TCTGCTGGCATGGATGATTGAATG TCCTAGAAAAAGACGATG
SEQ ID NO: 174	BIP RSV-A-set21	#9	TGAAACAAATATCCACACCCAAG GGCACTGCACTTCTTGAGTT
SEQ ID NO: 175	LF RSV-A-set21	#9	CCATATTGTAAGTGATGCAGGAT
SEQ ID NO: 176	LB RSV-A-set21	#9	GACCCTCATTAAGAGTCATGAT
SEQ ID NO: 177	F3 RSV-A-set22	#10	AACATACGTGAACAAACTTCA
SEQ ID NO: 178	B3 RSV-A-set22	#10	GCACATATGGTAAATTTGCTGG
SEQ ID NO: 179	FIP RSV-A-set22	#10	ACCCATATTGTAAGTGATGCAGG ATAGGGCTCCACATACACAG

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 180	BIP RSV-A-set22	#10	CTAGTGAAACAAATATCCACACC CAAGCACTGCACTTCTTGAG
SEQ ID NO: 181	LF RSV-A-set22	#10	TTTCTAGGACATTGTATTGAACAG C
SEQ ID NO: 182	LB RSV-A-set22	#10	GGGACCCTCATTAAGAGTCATG
SEQ ID NO: 183	IAV-MP-F3	#1	GACTTGAAGATGTCTTTGC
SEQ ID NO: 184	IAV-MP B3	#1	TGTTGTTTGGGTCCCCATT
SEQ ID NO: 185	IAV-MP-FIP	#1	TTAGTCAGAGGTGACAGGATTGC AGATCTTGAGGCTCTC
SEQ ID NO: 186	IAV-MP-BIP	#1	TTGTGTTCACGCTCACCGTGTTTG GACAAAGCGTCTACG
SEQ ID NO: 187	IAV-MP FL	#1	GTCTTGTCTTTAGCCA
SEQ ID NO: 188	IAV-MP BL	#1	CAGTGAGCGAGGACTG
SEQ ID NO: 189	IAV F3 v2	#2	ACCGAGGTCGAAACGT
SEQ ID NO: 190	IAV B3 v2	#2	GGTCCCCATTCCCATTG
SEQ ID NO: 191	IAV FIP v2	#2	CAAAGACATCTTCAAGTCTCTGCG TTTTTTCTCTCTATCGTCCCGTCA
SEQ ID NO: 192	IAV BIP v2	#2	AATGGCTAAAGACAAGACCAATC CTTTTTTGTCTACGCTGCAGTCC
SEQ ID NO: 193	IAV LF v2	#2	CGATCTCGGCTTTGAGGG
SEQ ID NO: 194	IAV LB v2	#2	TCACCGTGCCCAGTGAG
SEQ ID NO: 195	IAV F3 v3	#3	CGAAAGCAGGTAGATATTGAAAG
SEQ ID NO: 196	IAV B3 v3	#3	TCTACGCTGCAGTCCTC
SEQ ID NO: 197	IAV FIP v3	#3	TCAAGTCTCTGCGCGATCTCTTTT TTGAGTCTTCTAACCGAGGT
SEQ ID NO: 198	IAV BIP v3	#3	AGATGTCTTTGCAGGGAAAAACA CTTTTTTCACAAATCCTAAAATCC CCTTAG

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 199	IAV LF v3	#3	GACGATAGAGAGAACGTACGTTTC
SEQ ID NO: 200	IAV LB v3	#3	AAGACCAATCCTGTCACCTCT
SEQ ID NO: 201	IAV-set4-F3	#4	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set4-B3	#4	CATTCCCATTGAGGGCATT
SEQ ID NO: 203	IAV-set4-FIP	#4	CTTCAAGTCTCTGCGCGATCTATGAGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set4-BIP	#4	TTGAGGCTCTCATGGAATGGCAGCGTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set4-LF	#4	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set4-LB	#4	ACAAGACCAATCCTGTCACC
SEQ ID NO: 201	IAV-set5-F3	#5	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set5-B3	#5	CATTCCCATTGAGGGCATT
SEQ ID NO: 207	IAV-set5-FIP	#5	TTCAAGTCTCTGCGCGATCTCATGAGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set5-BIP	#5	TTGAGGCTCTCATGGAATGGCAGCGTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set5-LF	#5	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set5-LB	#5	ACAAGACCAATCCTGTCACC
SEQ ID NO: 201	IAV-set6-F3	#6	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 208	IAV-set6-B3	#6	TTGGACAAAGCGTCTACG
SEQ ID NO: 203	IAV-set6-FIP	#6	CTTCAAGTCTCTGCGCGATCTATGAGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set6-BIP	#6	TTGAGGCTCTCATGGAATGGCAGCGTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set6-LF	#6	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set6-LB	#6	ACAAGACCAATCCTGTCACC

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 201	IAV-set7-F3	#7	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set7-B3	#7	CATTCCCATTGAGGGCATT
SEQ ID NO: 209	IAV-set7-FIP	#7	AAGTCTCTGCGCGATCTCGATGA GTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set7-BIP	#7	TTGAGGCTCTCATGGAATGGCAG CGTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set7-LF	#7	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set7-LB	#7	ACAAGACCAATCCTGTCACC
SEQ ID NO: 210	IAV-set8-F3	#8	TCTTCTAACCGAGGTCGAA
SEQ ID NO: 211	IAV-set8-B3	#8	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 212	IAV-set8-FIP	#8	TCAGAGGTGACAGGATTGGTCTG AAGATGTCTTTGCAGGGAA
SEQ ID NO: 213	IAV-set8-BIP	#8	TTGTGTTCACGCTCACCGTCATTC CCATTGAGGGCATT
SEQ ID NO: 214	IAV-set8-LF	#8	ATTCCATGAGAGCCTCAAGATC
SEQ ID NO: 215	IAV-set8-LB	#8	GAGGACTGCAGCGTAGAC
SEQ ID NO: 216	IAV-set9-F3	#9	TTCTCTCTATCGTCCCGTC
SEQ ID NO: 211	IAV-set9-B3	#9	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 217	IAV-set9-FIP	#9	CCCTTAGTCAGAGGTGACAGGAA CACAGATCTTGAGGCTCT
SEQ ID NO: 213	IAV-set9-BIP	#9	TTGTGTTCACGCTCACCGTCATTC CCATTGAGGGCATT
SEQ ID NO: 218	IAV-set9-LF	#9	GGTCTTGTCTTTAGCCATTCCA
SEQ ID NO: 215	IAV-set9-LB	#9	GAGGACTGCAGCGTAGAC
SEQ ID NO: 219	IAV-set10-F3	#10	GTCTTCTAACCGAGGTCGA
SEQ ID NO: 211	IAV-set10-B3	#10	CTGCTCTGTCCATGTTGTT

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 220	IAV-set10-FIP	#10	GAGGTGACAGGATTGGTCTTGTT GAAGATGTCTTTGCAGGG
SEQ ID NO: 213	IAV-set10-BIP	#10	TTGTGTTCACGCTCACCGTCATTC CCATTGAGGGCATT
SEQ ID NO: 214	IAV-set10-LF	#10	ATTCCATGAGAGCCTCAAGATC
SEQ ID NO: 215	IAV-set10-LB	#10	GAGGACTGCAGCGTAGAC
SEQ ID NO: 221	IAV-set11-F3	#11	AAGAAGACAAGAGATATGGC
SEQ ID NO: 222	IAV-set11-B3	#11	CAATTCGACACTAATTGATGGC
SEQ ID NO: 223	IAV-set11-FIP	#11	GTCTCCTTGCCCAATTAGCAAGCA TCAATGAACTGAGCA
SEQ ID NO: 224	IAV-set11-BIP	#11	GTGGTGTTGGTAATGAAACGAAG CTGTCTGGCTGTCAGTA
SEQ ID NO: 225	IAV-set11-LF	#11	ACATTAGCCTTCTCTCCTTT
SEQ ID NO: 226	IAV-set11-LB	#11	AACGGGACTCTAGCATACT
SEQ ID NO: 227	M605 F3 IBV LAMP	IBV	AGGGACATGAACAACAAAGA
SEQ ID NO: 228	M606 B3 IBV LAMP	IBV	CAAGTTTAGCAACAAGCCT
SEQ ID NO: 229	M607 FIP IBV LAMP	IBV	TCAGGGACAATACATTACGCATA TCGATAAAGGAGGAAGTAAACAC TCA
SEQ ID NO: 230	M608 BIP IBV LAMP	IBV	TAAACGGAACATTCCTCAAACAC CACTCTGGTCATATGCATTC
SEQ ID NO: 231	M609 LF IBV LAMP	IBV	TCAAACGGAACTTCCCTTCTTTC
SEQ ID NO: 232	M610 LB IBV LAMP	IBV	GGATACAAGTCCTTATCAACTCTG C
SEQ ID NO: 233	M948 F3 HERC2 set3	HERC2	CTTGTAATCAACATCAGGGTAA

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 234	M949 B3 HERC2 set3	HERC2	AGAAACGACAAGTAGACCATT
SEQ ID NO: 235	M950 FIP HERC2 set3	HERC2	CGCCTCTTGGATCAGACACATGTG TTAATACAAAGGTACAGGA
SEQ ID NO: 236	M951 BIP HERC2 set3	HERC2	CACGCTATCATCATCAGGGGCTG CTTCAAGTGTATATAAACTCAC
SEQ ID NO: 237	M952 LF HERC2 set3	HERC2	GAGAGCCATGAAGAACAAATTCT
SEQ ID NO: 238	M953 LB HERC2 set3	HERC2	CGAGGCTTCTCTTTGTTTTTAAT

[00425] A set of LAMP primers may be designed to introduce a PAM sequence into a target nucleic acid sequence that lacks a PAM sequence. The FIP primer may contain a PAM sequence that is not present in the target nucleic acid. The BIP primer may contain a PAM sequence that is not present in the target nucleic acid. The FIP primer may contain a sequence that is reverse complementary to a PAM sequence that is not present in the target nucleic acid. The BIP primer may contain a sequence that is reverse complementary to a PAM sequence that is not present in the target nucleic acid. The PAM sequence or the sequence complementary to the PAM sequence may be located within the FIP primer or the BIP primer at a distance in bases from the 5' end of the primer. For example, the PAM sequence may be located 0, 1, 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, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 bases from 5' end of the primer. In some embodiments, the PAM sequence may be located from 0 to 10, from 5 to 15, from 10 to 20, from 15 to 25, from 20 to 30, from 25 to 35, from 30 to 40 bases from 5' end of the primer.

[00426] A set of LAMP primers may be designed for use in combination with a DETECTR reaction to detect a single nucleotide polymorphism (SNP) in a target nucleic acid. In some embodiments, a sequence of the target nucleic acid comprising the SNP may be reverse complementary to all or a portion of the guide nucleic acid. For example, the SNP may be positioned within a sequence of the target nucleic acid that is reverse complementary to the guide RNA sequence, as illustrated in **FIG. 51C**. In some cases, the sequence of the target nucleic acid sequence comprising the SNP does not overlap with or is not reverse complementary to the primers or one or more of the F1, F1c, F2, F2c, F3, F3c, B1, B1c, B2, B2c, B3, B3c, LB, LBc, LF, or LFc regions shown in **FIG. 51**. The guide nucleic acid may be reverse complementary to a sequence of the target nucleic acid between the F1c and B1 regions, as illustrated in **FIG. 51A**.

The guide nucleic acid may be reverse complementary to a sequence of the target nucleic acid between the B1c and F1 regions. A guide nucleic acid may be partially reverse complementary to a sequence of the target nucleic acid between the F1c region and the B1 region, for example as illustrated in **FIG. 51B**. A guide nucleic acid may be partially reverse complementary to a sequence of the target nucleic acid between the B1c region and the F1 region. For example, the sequence of the target nucleic acid sequence having the SNP may be reverse complementary to 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%, at least 100%, from 5% to 100%, 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%, or from 95% to 100% of the guide nucleic acid. In some cases, the guide nucleic acid does not overlap with and/or is not reverse complementary to any of the plurality of primers or the F1, F1c, F2, F2c, F3, F3c, B1, B1c, B2, B2c, B3, B3c, LB, LBc, LF, or LFc regions. Exemplary sets of DETECTR gRNAs for use in a combined RT-LAMP DETECTR or LAMP-DETECTR reaction to detect the presence of a nucleic acid sequence corresponding to a respiratory syncytial virus (RSV), an influenza A virus (IAV), an influenza B virus (IAV), or a HERC2 SNP are provided in **TABLE 6**.

TABLE 6 – Exemplary DETECTR Guide RNAs

SEQ ID NO:	gRNA Name	Sequence
SEQ ID NO: 239	gRNA #1 (R1118)	UAAUUUCUACUAAGUGUAGAUCUUAUAAAA GAACUAGCCAA

SEQ ID NO:	gRNA Name	Sequence
SEQ ID NO: 240	gRNA #2 (R288)	UAAUUUCUACUAAGUGUAGAUACUCAAUUUCCUCACUUCUC
SEQ ID NO: 241	R283	UAAUUUCUACUAAGUGUAGAUUGUUCACGCUCACCGUGCCC
SEQ ID NO: 242	R781	UAAUUUCUACUAAGUGUAGAUGCCAUUCCAUGAGAGCCUCA
SEQ ID NO: 243	R782	UAAUUUCUACUAAGUGUAGAUGACAAAGCGUCUACGCUGCA
SEQ ID NO: 244	IBV (R778)	UAAUUUCUACUAAGUGUAGAUCUAACACUCUCAGGGACAAU
SEQ ID NO: 245	A SNP Position 9 (R570)	UAAUUUCUACUAAGUGUAGAUAGCAUUAAAUGUCAAGUUCU
SEQ ID NO: 246	G SNP Position 9 (R571)	UAAUUUCUACUAAGUGUAGAUAGCAUUAAGUGUCAAGUUCU
SEQ ID NO: 247	A SNP Position 14 (R1138)	UAAUUUCUACUAAGUGUAGAUAUUUGAGCAUUAAAUGUCAA
SEQ ID NO: 248	G SNP Position 14 (R1139)	UAAUUUCUACUAAGUGUAGAUAUUUGAGCAUUAAGUGUCAA

Amplification and Detection of a Single Nucleotide Polymorphism Allele

[00427] A DETECTR reaction may be used to detect the presence of a specific single nucleotide polymorphism (SNP) allele in a sample. The DETECTR reaction may produce a detectable signal, as described elsewhere herein, in the presence of a target nucleic acid comprising a specific SNP allele. The DETECTR reaction may not produce a signal in the absence of the target nucleic acid or in the presence of a nucleic acid sequence that does not comprise the specific SNP allele or comprises a different SNP allele. In some cases, a DETECTR reaction may comprise a guide RNA reverse complementary to a portion of a target nucleic acid sequence comprising a specific SNP allele. The guide RNA and the target nucleic acid comprising the specific SNP allele may bind to and activate a programmable nuclease, thereby producing a detectable signal as described elsewhere herein. The guide RNA and a nucleic acid sequence that does not comprise the specific SNP allele may not bind to or activate the programmable nuclease and may not produce a detectable signal. In some cases, a target nucleic acid sequence that may or may not comprise a specific SNP allele may be amplified using, for example, a LAMP amplification reaction. In some cases, the LAMP amplification reaction may be combined with a reverse transcription reaction, a DETECTR reaction, or both. For example, the LAMP reaction may be an RT-LAMP reaction, a LAMP DETECTR reaction, or an RT-LAMP DETECTR

reactions.

[00428] A method of assaying for a segment of a target nucleic acid may comprise contacting a sample comprising a population of nucleic acids, wherein the population comprises at least one nucleic acid comprising a segment having less than 100% sequence identity to the segment of the target nucleic acid and having no less than 50% sequence identity to the segment of the target nucleic acid to a guide nucleic acid that hybridizes to the segment of the target nucleic acid, a detector nucleic acid, and a Cas12 nuclease (e.g., SEQ ID NO: 1 or SEQ ID NO: 11) that cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid; and assaying for a signal produced by cleavage of the detector nucleic acid, wherein the signal is at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the segment of the at least one nucleic acid comprises at least two base mutations compared to the segment of the target nucleic acid. In some embodiments, the segment of the at least one nucleic acid comprises from one to ten base mutations compared to the segment of the target nucleic acid. In some embodiments, the segment of the at least one nucleic acid comprises one base mutation compared to the segment of the target nucleic acid. In some embodiments, the signal produced is from two-fold to 20-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from two-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from five-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 2 fold to 100 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 2 fold to 5 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 5 fold to 10 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 10 fold to 15 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 15 fold to 20 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks

the segment of the target nucleic acid. In some embodiments, the signal produced is from 20 fold to 25 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 25 fold to 30 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 30 fold to 35 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 35 fold to 40 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 40 fold to 45 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 45 fold to 50 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 50 fold to 60 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 60 fold to 70 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 70 fold to 80 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 80 fold to 90 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 90 fold to 100 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 100 fold to 200 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 2 fold to 10 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 20 fold to 40 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 2 fold to 50 fold greater when the

segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. In some embodiments, the signal produced is from 1.5 fold to 100 fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid. The guide may be reverse complementary to the segment of the target nucleic acid. In some embodiments, the guide nucleic acid and the second guide nucleic acid lack synthetic mismatches. A synthetic mismatch may be an additional mismatch between a target nucleic acid and a guide nucleic acid introduced into the guide nucleic acid to improve the single-base distinction capabilities of a programmable nuclease.

[00429] In some embodiments, the DETECTR reaction may be used to detect the presence of a specific SNP allele in a sample, wherein the SNP is located in a target nucleic acid sequence that lacks a PAM sequence. For example, the DETECTR reaction, wherein the target nucleic acid segment lacks a PAM sequence, comprises LAMP amplifying the target nucleic acid segment using a forward inner primer (FIP) or a backward inner primer (BIP) having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product; contacting the PAM target nucleic acid to PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one nucleic acid of the reporter of a population of nucleic acids of the reporters, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. The detection of the signal can indicate the presence of the target nucleic acid. Sometimes, the target nucleic acid comprises a mutation. Often, the mutation is a single nucleotide mutation.

[00430] The SNP may be positioned at a distance from a PAM sequence. The PAM sequence may be a native PAM sequence, or the PAM sequence may be a generated PAM sequence. The PAM sequence may be generated by amplification. In some embodiments, the SNP may be positioned 1, 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, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 bases from the PAM sequence. In some embodiments, the SNP may be positioned from 1 to 10, from 5 to 15, from 10 to 20, from 15 to 25, from 20 to 30, from 25 to 35, from 30 to 40 bases from the PAM sequence. The SNP may be positioned on the forward strand. The SNP may be positioned on the reverse strand.

[00431] A guide nucleic acid may be specific for an SNP allele. For example, a guide nucleic acid may increase the trans cleavage activity of a programmable nuclease more when contacted to a target nucleic acid comprising a specific SNP allele than when contacted to a target nucleic acid comprising a different SNP allele. In some embodiments, the guide nucleic acid may increase the trans cleavage activity of a programmable nuclease more when contacted to a target nucleic acid comprising an A nucleic acid at a SNP than when contacted to a target nucleic acid comprising a T, a C, or a G nucleic acid at the SNP. In some embodiments, the guide nucleic acid may increase the trans cleavage activity of a programmable nuclease more when contacted to a target nucleic acid comprising a T nucleic acid at a SNP than when contacted to a target nucleic acid comprising an A, a C, or a G nucleic acid at the SNP. In some embodiments, the guide nucleic acid may increase the trans cleavage activity of a programmable nuclease more when contacted to a target nucleic acid comprising a C nucleic acid at a SNP than when contacted to a target nucleic acid comprising an A, a T, or a G nucleic acid at the SNP. In some embodiments, the guide nucleic acid may increase the trans cleavage activity of a programmable nuclease more when contacted to a target nucleic acid comprising a G nucleic acid at a SNP than when contacted to a target nucleic acid comprising an A, a C, or a T nucleic acid at the SNP. In some embodiments, the guide nucleic acid may be specific for a first SNP allele at a first SNP and a second SNP allele at a second SNP site. The programmable nuclease may be a Cas12, a Cas13, or a Cas14.

[00432] A DETECTR reaction, as described elsewhere herein, may produce a detectable signal specifically in the presence of a target nucleic acid sequence comprising a specific SNP allele. For example, the DETECTR reaction may produce a detectable signal in the presence of a target nucleic acid comprising a G nucleic acid at a location of a SNP but not in the presence of a nucleic acid comprising a C, a T, or an A nucleic acid at the location of the SNP. The DETECTR reaction may produce a detectable signal in the presence of a target nucleic acid comprising a T nucleic acid at a location of a SNP but not in the presence of a nucleic acid comprising a G, a C, or an A nucleic acid at the location of the SNP. The DETECTR reaction may produce a detectable signal in the presence of a target nucleic acid comprising a C nucleic acid at a location of a SNP but not in the presence of a nucleic acid comprising a G, a T, or an A nucleic acid at the location of the SNP. The DETECTR reaction may produce a detectable signal in the presence of a target nucleic acid comprising an A nucleic acid at a location of a SNP but not in the presence of a nucleic acid comprising a G, a T, or a C nucleic acid at the location of the SNP. In addition to the DETECTR reaction, the target nucleic acid having the SNP may be concurrently, sequentially, concurrently together in a sample, or sequentially together in a sample be carried

out alongside LAMP or RT-LAMP. For example, the reactions can comprise LAMP and DETECTR reactions, or RT-LAMP and DETECTR reactions. Performing a DETECTR reaction in combination with a LAMP reaction may result in an increased detectable signal as compared to the DETECTR reaction in the absence of the LAMP reaction.

[00433] In some cases, the detectable signal produced in the DETECTR reaction may be higher in the presence of a target nucleic acid comprising a specific SNP allele than in the presence of a nucleic acid that does not comprise the specific SNP allele. In some cases, the DETECTR reaction may produce a detectable signal that is at least 1-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 100-fold, at least 200-fold, at least 300-fold, at last 400-fold, at least 500-fold, at least 1000-fold, at least 2000-fold, at least 3000-fold, at least 4000-fold, at least 5000-fold, at least 6000-fold, at least 7000-fold, at least 8000-fold, at least 9000-fold, at least 10000-fold, at least 50000-fold, at least 100000-fold, at least 500000-fold, or at least 1000000-fold greater in the presence of a target nucleic acid comprising a specific SNP allele than in the presence of a nucleic acid that does not comprise the specific SNP allele. In some cases, the DETECTR reaction may produce a detectable signal that is from 1-fold to 2-fold, from 2-fold to 3-fold, from 3-fold to 4-fold, from 4-fold to 5-fold, from 5-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 100-fold, from 100-fold to 500-fold, from 500-fold to 1000-fold, from 1000-fold to 10,000-fold, from 10,000-fold to 100,000-fold, or from 100,000-fold to 1,000,000-fold greater in the presence of a target nucleic acid comprising a specific SNP allele than in the presence of a nucleic acid that does not comprise the specific SNP allele.

[00434] A DETECTR reaction may be used to detect the presence of a SNP allele associated with a disease or a condition in a nucleic acid sample. The DETECTR reaction may be used to detect the presence of a SNP allele associated with an increased likelihood of developing a disease or a condition in a nucleic acid sample. The DETECTR reaction may be used to detect the presence of a SNP allele associated with a phenotype in a nucleic acid sample. For example, a DETECTR reaction may be used to detect a SNP allele associated with a disease such as phenylketonuria (PKU), cystic fibrosis, sickle-cell anemia, albinism, Huntington's disease, myotonic dystrophy type 1, hypercholesterolemia, neurofibromatosis, polycystic kidney disease, hemophilia, muscular dystrophy, hypophosphatemic rickets, Rett's syndrome, or spermatogenic failure. A SNP allele associated with a disease may be in a gene such as phenylalanine hydroxylase (PAH) gene, cystic fibrosis transmembrane conductance regulator (CFTR) gene, a ß-globin gene, a Huntingtin gene, a dystrophin (DMD) gene, an apolipoprotein B (APOB) gene,

a low-density lipoprotein receptor (LDLR) gene, a low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) gene, a proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, a neurofibromin (NF1) gene, a PKD1 gene, an PKD2 gene, a coagulation factor VIII (F8) gene, a coagulation factor IX (F9) gene, a myotonic dystrophy protein kinase (DMPK) gene, a phosphate regulating endopeptidase homolog X-linked (PHEX) gene, or a methyl CpG binding protein 2 (MECP) gene. A DETECTR reaction may be used to detect a SNP allele associated with an increased risk of cancer, for example bladder cancer, brain cancer, breast cancer, cervical cancer, colon cancer, colorectal cancer, gallbladder cancer, stomach cancer, leukemia, liver cancer, lung cancer, oral cancer, esophageal cancer, ovarian cancer, pancreatic cancer, prostate cancer, skin cancer, testicular cancer, thyroid cancer, neuroblastoma, or lymphoma. A DETECTR reaction may be used to detect a SNP allele associated with an increased risk of a disease, for example Alzheimer's disease, Parkinson's disease, amyloidosis, heterochromatosis, celiac disease, macular degeneration, or hypercholesterolemia. A DETECTR reaction may be used to detect a SNP allele associated with a phenotype, for example, eye color, hair color, height, skin color, race, alcohol flush reaction, caffeine consumption, deep sleep, genetic weight, lactose intolerance, muscle composition, saturated fat and weight, or sleep movement.

[00435] A target nucleic acid may be amplified prior to detection (e.g., detection using a DETECTR reaction). The target nucleic acid may be amplified using any of the amplification methods or reagents described herein. The DETECTR reaction may comprise detecting the presence of a target nucleic acid comprising a specific SNP allele at a SNP of interest. In some cases, the target nucleic acid comprises a sequence variation that is not of interest near the SNP of interest. The sequence variation may comprise a second SNP, a heterogenous sequence, or a region of low sequence conservation. The sequence variation may be near the SNP of interest. For example, the sequence variation may overlap with an annealing region for a gRNA directed to detect a specific allele of the SNP of interest. In some embodiments, the target nucleic acid may be amplified prior to or concurrent with detection to reduce or remove the sequence variation that is not of interest while preserving the SNP of interest. For example, amplification to remove the sequence variation may be performed using a primer that overlaps with or anneals to a region of the nucleic acid comprising the sequence variation that is not of interest. The primer may not overlap or anneal to the region comprising the SNP of interest. In some cases, the primer overlaps with a region that corresponds to or anneals to the gRNA. Amplification using the primer that overlaps the sequence variation that is not of interest may increase the homogeneity of the nucleic acid sequence at the site of the variation that is not of interest while maintaining the heterogeneity of the nucleic acid at the SNP of interest. For example,

amplification may be used to overwrite the sequence variation that is not of interest. In some cases, amplification to increase the homogeneity of the nucleic acid sequence may be used improve species-level detection of a target nucleic acid wherein the gRNA is target to a region of low or imperfect sequence conservation.

Detection/Visualization Devices

[00436] A number of detection or visualization devices and methods are consistent with the methods, compositions, reagents, enzymes, and kits disclosed herein for assaying for a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids. The methods disclosed herein are, for example, consistent with fluidic devices for detection of a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids, wherein the fluidic device may comprise multiple pumps, valves, reservoirs, and chambers for sample preparation, amplification of a target nucleic acid within the sample, mixing with a programmable nuclease, and detection of a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids by the programmable nuclease within the fluidic system itself. For example, the fluidic device may comprise an incubation and detection chamber or a stand-alone detection chamber, in which a colorimetric, fluorescence, electrochemical, or electrochemiluminesence signal is generated for detection. The detection can be analyzed using various methods.

[00437] As described herein, a target nucleic acid comprising DNA may be detected using a DNA-activated programmable RNA nuclease and other reagents disclosed herein. A DNA-activated programmable RNA nuclease may also be multiplexed as described herein. Sometimes, the signal generated for detection is a calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric signal. 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 a 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. Sometimes, the nucleic acid of a reporter is a protein-nucleic acid. Often, the protein-nucleic acid is an enzyme-

nucleic acid. The detection/visualization can be analyzed using various methods, as further described below.

[00438] The results from the detection region from a completed assay can be detected or visualized and analyzed in various ways. In some cases, the positive control spot and the detection spot in the detection region is visible by eye, and the results can be read by the user. In some cases, the positive control spot and the detection spot in the detection region is visualized by an imaging device. Often, the imaging device is a digital camera, such a digital camera on a mobile device. The mobile device may have a software program or a mobile application that can capture an image of the support medium, identify the assay being performed, detect the detection region and the detection spot, provide image properties of the detection spot, analyze the image properties of the detection spot, and provide a result. Alternatively, or in combination, the imaging device can capture fluorescence, ultraviolet (UV), infrared (IR), or visible wavelength signals. The imaging device may have an excitation source to provide the excitation energy and captures the emitted signals. In some cases, the excitation source can be a camera flash and optionally a filter. In some cases, the imaging device is used together with an imaging box that is placed over the support medium to create a dark room to improve imaging. The imaging box can be a cardboard box that the imaging device can fit into before imaging. In some instances, the imaging box has optical lenses, mirrors, filters, or other optical elements to aid in generating a more focused excitation signal or to capture a more focused emission signal. Often, the imaging box and the imaging device are small, handheld, and portable to facilitate the transport and use of the assay in remote or low resource settings.

[00439] The assay described herein can be visualized and analyzed by a mobile application (app) or a software program. Using the graphic user interface (GUI) of the app or program, an individual can take an image of the support medium, including the detection region, barcode, reference color scale, and fiduciary markers on the housing, using a camera on a mobile device. The program or app reads the barcode or identifiable label for the test type, locate the fiduciary marker to orient the sample, and read the detectable signals, compare against the reference color grid, and determine the presence or absence of the target nucleic acid, which indicates the presence of the gene, virus, or the agent responsible for the disease, cancer, or genetic disorder. The mobile application can present the results of the test to the individual. The mobile application can store the test results in the mobile application. The mobile application can communicate with a remote device and transfer the data of the test results. The test results can be viewable remotely from the remote device by another individual, including a healthcare

professional. A remote user can access the results and use the information to recommend action for treatment, intervention, clean up of an environment.

[00440] The methods for detection of a target nucleic acid described herein further can comprises reagents protease treatment of the sample. The sample can be treated with protease, such as Protease K, before amplification or before assaying for a detectable signal. Often, a protease treatment is for no more than 15 minutes. Sometimes, the protease treatment is for no more than 1, 5, 10, 15, 20, 25, 30, or more minutes, or any value from 1 to 30 minutes. Sometimes, the protease treatment is from 1 to 30, from 5 to 25, from 10 to 20, or from 10 to 15 minutes. Sometimes, the total time for the performing the method described herein is no greater than 3 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or any value from 3 hours to 20 minutes. Often, a method of nucleic acid detection from a raw sample comprises protease treating the sample for no more than 15 minutes, amplifying (can also be referred to as pre-amplifying) the sample for no more than 15 minutes, subjecting the sample to a programmable nuclease-mediated detection, and assaying nuclease mediated detection. The total time for performing this method, sometimes, is no greater than 3 hours, 2 hours, 1 hour, 50 minutes, 40 minutes, 30 minutes, 20 minutes, or any value from 3 hours to 20 minutes. Often, the protease treatment is Protease K. Often the amplifying is thermal cycling amplification. Sometimes the amplifying is isothermal amplification.

Fluidic Devices

[00441] Disclosed herein are various fluidic devices for assaying for a signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids. The fluidic devices described herein can be used to monitor the signal indicating cleavage of at least some detector nucleic acids of a population of detector nucleic acids that occurs when a target nucleic acid in samples binding to a programmable nuclease complexed with a guide nucleic acid, thereby allowing initiating cleavage of detector nucleic acids that produce a signal upon cleavage. All samples and reagents disclosed herein are compatible for use with a fluidic device disclosed below. Any programmable nuclease, such as any Cas nuclease described herein, are compatible for use with a fluidic device disclosed below. Support mediums and housing disclosed herein are also compatible for use in conjunction with the fluidic devices disclosed below. Multiplexing detection, as described throughout the present disclosure, can be carried out within the fluidic devices disclosed herein. Compositions and methods for detection and visualization disclosed herein are also compatible for use within the below described fluidic systems.

[00442] A workflow of a method for assaying a target nucleic acid in a sample within a fluidic device can include sample preparation, nucleic acid amplification, incubation with a programmable nuclease, and/or detection (readout). For example, a step 1 is sample preparation, a step 2 is nucleic acid amplification, a step 3 is programmable nuclease incubation, and a step 4 is detection (readout). In some embodiments, amplification comprises producing a PAM target nucleic acid. Sometimes, steps 1 and 2 are optional. Steps 3 and 4 can occur concurrently, if incubation and detection of programmable nuclease activity are within the same chamber. Sample preparation and amplification can be carried out within a fluidic device described herein or, alternatively, can be carried out prior to introduction into the fluidic device. As mentioned above, sample preparation of any nucleic acid amplification are optional, and can be excluded. In further cases, programmable nuclease reaction incubation and detection (readout) can be performed sequentially (one after another) or concurrently (at the same time). In some embodiments, sample preparation and/or amplification can be performed within a first fluidic device and then the sample can be transferred to a second fluidic device to carry out Steps 3 and 4 and, optionally, Step 2.

[00443] A fluidic device for sample preparation can be referred to as a filtration device. In some embodiments, the filtration device for sample preparation resembles a syringe or, comprises, similar functional elements to a syringe. For example, a functional element of the filtration device for sample preparation includes a narrow tip for collection of liquid samples. Liquid samples can include blood, saliva, urine, or any other biological fluid. Liquid samples can also include liquid tissue homogenates. The tip, for collection of liquid samples, can be manufactured from glass, metal, plastic, or other biocompatible materials. The tip may be replaced with a glass capillary that may serve as a metering apparatus for the amount of biological sample added downstream to the fluidic device. For some samples, *e.g.*, blood, the capillary may be the only fluidic device required for sample preparation. Another functional element of the filtration device for sample preparation may include a channel that can carry volumes from nL to mL, containing lysis buffers compatible with the programmable nuclease reaction downstream of this process. The channel may be manufactured from metal, plastic, or other biocompatible materials. The channel may be large enough to hold an entire fecal, buccal, or other biological sample collection swab. The filtration device may further contain a solution of reagents that will lyse the cells in each type of samples and release the nucleic acids so that they are accessible to the programmable nuclease. Active ingredients of the solution may be chaotropic agents, detergents, salts, and can be of high osmolality, ionic strength and pH. Chaotropic agents or chaotropes are substances that disrupt the three-dimensional structure in

macromolecules such as proteins, DNA, or RNA. One example protocol comprises a 4 M guanidinium isothiocyanate, 25 mM sodium citrate.$2H_20$, 0.5% (w/v) sodium lauryl sarcosinate, and 0.1 M β-mercaptoethanol), but numerous commercial buffers for different cellular targets may also be used. Alkaline buffers may also be used for cells with hard shells, particularly for environmental samples. Detergents such as sodium dodecyl sulphate (SDS) and cetyl trimethylammonium bromide (CTAB) may also be implemented to chemical lysis buffers. Cell lysis may also be performed by physical, mechanical, thermal or enzymatic means, in addition to chemically-induced cell lysis mentioned previously. The device may include more complex architecture depending on the type of sample, such as nanoscale barbs, nanowires, sonication capability in a separate chamber of the device, integrated laser, integrated heater, for example, a Peltier-type heater, or a thin-film planar heater, and/or microcapillary probes for electrical lysis. Any samples described herein can be used in this workflow. For example samples may include liquid samples collected from a subject being tested for a condition of interest. The sample preparation fluidic device can process different types of biological sample: finger-prick blood, urine or swabs with fecal, cheek or other collection.

[00444] A fluidic device may be used to carry out any one of, or any combination of, steps 2-4 discussed above (nucleic acid amplification, programmable nuclease reaction incubation, detection (readout)). An example fluidic device for a programmable nuclease reaction with a fluorescence or electrochemical readout that may be used in Step 2 to Step 4 can be carried out in different iterations. For example, one variation is a fluidic device that performs the programmable nuclease reaction incubation and detection (readout) steps, but not amplification. Another variation of a fluidic device comprises a one-chamber reaction with amplification. Another variation of the fluidic device comprises a two-chamber reaction with amplification. Fluorescence or electrochemical processes that may be used for detection of the reaction in a fluidic device as described above.

[00445] The chip (also referred to as fluidic device) may be manufactured from a variety of different materials. Exemplary materials that may be used include plastic polymers, such as poly-methacrylate (PMMA), cyclic olefin polymer (COP), cyclic olefin copolymer (COC), polyethylene (PE), high-density polyethylene (HDPE), polypropylene (PP); glass; and silicon. Features of the chip may be manufactured by various processes. For example, features may be (1) embossed using injection molding, (2) micro-milled or micro-engraved using computer numerical control (CNC) micromachining, or non-contact laser drilling (by means of a CO2 laser source); (3) additive manufacturing, and/or (4) photolithographic methods.

[00446] The design may include up to three (3) input ports operated by three (3) pumps, for example. The pumps may be operated by external syringe pumps using low pressure or high pressure. The pumps may be passive, and/or active (pneumatic, piezoelectric, Braille pin, electroosmotic, acoustic, gas permeation, or other).

[00447] The ports may be connected to pneumatic pressure pumps, air or gas may be pumped into the microfluidic channels to control the injection of fluids into the fluidic device. At least three reservoirs may be connected to the device, each containing buffered solutions of: (1) sample, which may be a solution containing purified nucleic acids processed in a separate fluidic device, or neat sample (blood, saliva, urine, stool, and/or sputum); (2) amplification mastermix, which varies depending on the method used, wherein the method may include any of loop-mediated isothermal amplification (LAMP), strand displacement amplification (SDA), recombinase polymerase amplification (RPA), helicase dependent amplification (HDA), multiple displacement amplification (MDA), rolling circle amplification (RCA), and nucleic acid sequence-based amplification (NASBA), transcription mediated amplification (TMA), circular helicase dependent amplification (cHDA), exponential amplification reaction (EXPAR), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), single primer isothermal amplification (SPIA), hinge-initiated primer-dependent amplification of nucleic acids (HIP), nicking enzyme amplification reaction (NEAR), or improved multiple displacement amplification (IMDA); and (3) pre-complexed programmable nuclease mix, which includes one or more programmable nuclease and guide oligonucleotides. The method of nucleic acid amplification may also be polymerase chain reaction (PCR), which includes cycling of the incubation temperature at different levels, hence is not defined as isothermal. Often, the reagents for nucleic acid amplification comprise a recombinase, a 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. The nucleic amplification can produce a PAM target nucleic acid as disclosed by the methods herein. 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. Complex formation of a nuclease with guides (a programmable nuclease) and reporter probes may occur off the chip. An additional port for output of the final reaction products is depicted at the end of the fluidic path, and is operated by a similar pump. The

reactions product can be, thus, collected for additional processing and/or characterization, e.g., sequencing.

[00448] The flow of liquid in this fluidic device may be controlled using up to four (4) microvalves. These valves can be electro-kinetic microvalves, pneumatic microvalves, vacuum microvalves, capillary microvalves, pinch microvalves, phase-change microvalves, burst microvalves.

[00449] The flow to and from the fluidic channel from each microvalve can be controlled by valves. The volume of liquids pumped into the ports can vary from nL to mL depending in the overall size of the device.

[00450] A fluidic device in which no amplification is needed can also be used. After addition of sample and pre-complexed programmable nuclease mix, these reagents may be mixed in a serpentine channel which then leads to a chamber where the mixture may be incubated at the required temperature and time. The readout can be done simultaneously in the chamber. Thermoregulation in the chamber may be carried out using a thin-film planar heater manufactured, from e.g. Kapton, or other similar materials, and controlled by a proportional integral derivative (PID).

[00451] A fluidic device may also allow for addition of sample, amplification mix, and pre-complexed programmable nuclease mix, the reagents to then be mixed in a serpentine channel which then leads to a chamber where the mixture is incubated at the required temperature and time needed to efficient amplification, using any of the amplification methods described herein. The readout may be done simultaneously in the chamber. Thermoregulation may be achieved as previously described.

[00452] A fluidic device can allow for amplification and programmable nuclease reactions occur in separate chambers. The pre-complexed programmable nuclease mix can be pumped into the amplified mixture from a first chamber using a pump. The liquid flow is controlled by a valve, and directed into a serpentine mixer, and subsequently in another chamber for incubation the required temperature, for example at 37°C for 90 minutes.

[00453] During the detection step (step 4, for example), the programmable nuclease complexed to a guide nucleic acid binds to its target nucleic acid from the amplified sample to initiate cleavage of a detector nucleic acid to generate a signal readout. In the absence of a target nucleic acid, the programmable nuclease complexed to a guide nucleic acid does not cleave the detector nucleic acid. Detection of the signal can be achieved by multiple methods, which can detect a signal that is calorimetric, potentiometric, amperometric, optical (e.g., fluorescent, colorimetric, etc.), or piezo-electric, as non-limiting examples.

Support medium

[00454] A number of support mediums are consistent with the methods disclosed herein. These support mediums are, for example, consistent with fluidic devices disclosed herein for detection of a target nucleic acid sequence within the sample, wherein the fluidic device may comprise multiple pumps, valves, reservoirs, and chambers for sample preparation, amplification of a target nucleic acid sequence within the sample, mixing with a programmable nuclease, and detection of a detectable signal arising from cleavage of detector nucleic acids by the programmable nuclease within the fluidic system itself. These support mediums are compatible with the DETECTR assay methods disclosed herein. The support mediums, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The support mediums, 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The support mediums, 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 embodiments, amplification of the target nucleic acid sequence within the sample comprises producing a PAM target nucleic acid. These support mediums are compatible with the samples, reagents, and fluidic devices 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 support medium described herein can provide a way to present the results from the activity between the reagents and the sample. The support medium provides a medium to present the detectable signal in a detectable format. Optionally, the support medium concentrates the detectable signal to a detection spot in a detection region to increase the sensitivity, specificity, or accuracy of the assay. The support mediums can present the results of the assay and indicate the presence or absence of the disease of interest targeted by the target nucleic acid. The result on the support medium can be read by eye or using a machine. The support medium helps to stabilize the detectable signal generated by the cleaved detector molecule on the surface of the support medium. In some instances, the support medium is a

lateral flow assay strip. In some instances, the support medium is a PCR plate. The PCR plate can have 96 wells or 384 wells. The PCR plate can have a subset number of wells of a 96 well plate or a 384 well plate. A subset number of wells of a 96 well PCR plate is, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95 wells. For example, a PCR subset plate can have 4 wells wherein a well is the size of a well from a 96 well PCR plate (e.g., a 4 well PCR subset plate wherein the wells are the size of a well from a 96 well PCR plate). A subset number of wells of a 384 well PCR plate is, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, or 380 wells. For example, a PCR subset plate can have 20 wells wherein a well is the size of a well from a 384 well PCR plate (e.g., a 20 well PCR subset plate wherein the wells are the size of a well from a 384 well PCR plate). The PCR plate or PCR subset plate can be paired with a fluorescent light reader, a visible light reader, or other imaging device. Often, the imaging device is a digital camera, such a digital camera on a mobile device. The mobile device may have a software program or a mobile application that can capture an image of the PCR plate or PCR subset plate, identify the assay being performed, detect the individual wells and the sample therein, provide image properties of the individuals wells comprising the assayed sample, analyze the image properties of the contents of the individual wells, and provide a result.

[00455] The support medium has at least one specialized zone or region to present the detectable signal. The regions comprise at least one of a sample pad region, a nucleic acid amplification region, a conjugate pad region, a detection region, and a collection pad region. In some instances, the regions are overlapping completely, overlapping partially, or in series and in contact only at the edges of the regions, where the regions are in fluid communication with its adjacent regions. In some instances, the support medium has a sample pad located upstream of the other regions; a conjugate pad region having a means for specifically labeling the detector moiety; a detection region located downstream from sample pad; and at least one matrix which defines a flow path in fluid connection with the sample pad. In some instances, the support medium has an extended base layer on top of which the various zones or regions are placed. The extended base layer may provide a mechanical support for the zones.

[00456] Described herein are sample pad that provide an area to apply the sample to the support medium. The sample may be applied to the support medium by a dropper or a pipette on top of the sample pad, by pouring or dispensing the sample on top of the sample pad region, or by dipping the sample pad into a reagent chamber holding the sample. The sample can be applied

to the sample pad prior to reaction with the reagents when the reagents are placed on the support medium or be reacted with the reagents prior to application on the sample pad. The sample pad region can transfer the reacted reagents and sample into the other zones of the support medium. Transfer of the reacted reagents and sample may be by capillary action, diffusion, convection or active transport aided by a pump. In some cases, the support medium is integrated with or overlayed by microfluidic channels to facilitate the fluid transport.

[00457] The dropper or the pipette may dispense a predetermined volume. In some cases, the predetermined volume may range from about 1 μl to about 1000 μl, about 1 μl to about 500 μl, about 1 μl to about 100 μl, or about 1 μl to about 50 μl. In some cases, the predetermined volume may be at least 1 μl, 2 μl, 3 μl, 4 μl, 5 μl, 6 μl, 7 μl, 8 μl, 9 μl, 10 μl, 25 μl, 50 μl, 75 μl, 100 μl, 250 μl, 500 μl, 750 μl, or 1000 μl. The predetermined volume may be no more than 5 μl, 10 μl, 25 μl, 50 μl, 75 μl, 100 μl, 250 μl, 500 μl, 750 μl, or 1000 μl. The dropper or the pipette may be disposable or be single-use.

[00458] Optionally, a buffer or a fluid may also be applied to the sample pad to help drive the movement of the sample along the support medium. In some cases, the volume of the buffer or the fluid may range from about 1 μl to about 1000 μl, about 1 μl to about 500 μl, about 1 μl to about 100 μl, or about 1 μl to about 50 μl. In some cases, the volume of the buffer or the fluid may be at least 1 μl, 2 μl, 3 μl, 4 μl, 5 μl, 6 μl, 7 μl, 8 μl, 9 μl, 10 μl, 25 μl, 50 μl, 75 μl, 100 μl, 250 μl, 500 μl, 750 μl, or 1000 μl. The volume of the buffer or the fluid may be no more than than 5 μl, 10 μl, 25 μl, 50 μl, 75 μl, 100 μl, 250 μl, 500 μl, 750 μl, or 1000 μl. In some cases, the buffer or fluid may have a ratio of the sample to the buffer or fluid of at least 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, or 1:10.

[00459] The sample pad can be made from various materials that transfer most of the applied reacted reagents and samples to the subsequent regions. The sample pad may comprise cellulose fiber filters, woven meshes, porous plastic membranes, glass fiber filters, aluminum oxide coated membranes, nitrocellulose, paper, polyester filter, or polymer-based matrices. The material for the sample pad region may be hydrophilic and have low non-specific binding. The material for the sample pad may range from about 50 μm to about 1000 μm, about 50 μm to about 750 μm, about 50 μm to about 500 μm, or about 100 μm to about 500 μm.

[00460] The sample pad can be treated with chemicals to improve the presentation of the reaction results on the support medium. The sample pad can be treated to enhance extraction of nucleic acid in the sample, to control the transport of the reacted reagents and sample or the conjugate to other regions of the support medium, or to enhance the binding of the cleaved detection moiety to the conjugate binding molecule on the surface of the conjugate or to the

capture molecule in the detection region. The chemicals may comprise detergents, surfactants, buffers, salts, viscosity enhancers, or polypeptides. In some instances, the chemical comprises bovine serum albumin.

[00461] Described herein are conjugate pads that provide a region on the support medium comprising conjugates coated on its surface by conjugate binding molecules that can bind to the detector moiety from the cleaved detector molecule or to the control molecule. The conjugate pad can be made from various materials that facilitate binding of the conjugate binding molecule to the detection moiety from cleaved detector molecule and transfer of most of the conjugate-bound detection moiety to the subsequent regions. The conjugate pad may comprise the same material as the sample pad or other zones or a different material than the sample pad. The conjugate pad may comprise glass fiber filters, porous plastic membranes, aluminum oxide coated membranes, paper, cellulose fiber filters, woven meshes, polyester filter, or polymer-based matrices. The material for the conjugate pad region may be hydrophilic, have low non-specific binding, or have consistent fluid flow properties across the conjugate pad. In some cases, the material for the conjugate pad may range from about 50 μm to about 1000 μm, about 50 μm to about 750 μm, about 50 μm to about 500 μm, or about 100 μm to about 500 μm.

[00462] Further described herein are conjugates that are placed on the conjugate pad and immobilized to the conjugate pad until the sample is applied to the support medium. The conjugates may comprise a nanoparticle, a gold nanoparticle, a latex nanoparticle, a quantum dot, a chemiluminescent nanoparticle, a carbon nanoparticle, a selenium nanoparticle, a fluorescent nanoparticle, a liposome, or a dendrimer. The surface of the conjugate may be coated by a conjugate binding molecule that binds to the detection moiety from the cleaved detector molecule.

[00463] The conjugate binding molecules described herein coat the surface of the conjugates and can bind to detection moiety. The conjugate binding molecule binds selectively to the detection moiety cleaved from the detector nucleic acid. Some suitable conjugate binding molecules comprise an antibody, a polypeptide, or a single stranded nucleic acid. In some cases, the conjugate binding molecule binds a dye and a fluorophore. Some such conjugate binding molecules that bind to a dye or a fluorophore can quench their signal. In some cases, the conjugate binding molecule is a monoclonal antibody. In some cases, an antibody, also referred to as an immunoglobulin, includes any isotype, variable regions, constant regions, Fc region, Fab fragments, F(ab')2 fragments, and Fab' fragments. Alternatively, the conjugate binding molecule is a non-antibody compound that specifically binds the detection moiety. Sometimes, the conjugate binding molecule is a polypeptide that can bind to the detection moiety. Sometimes,

the conjugate binding molecule is avidin or a polypeptide that binds biotin. Sometimes, the conjugate binding molecule is a detector moiety binding nucleic acid.

[00464] The diameter of the conjugate may be selected to provide a desired surface to volume ratio. In some instances, a high surface area to volume ratio may allow for more conjugate binding molecules that are available to bind to the detection moiety per total volume of the conjugates. In some cases, the diameter of the conjugate may range from about 1 nm to about 1000 nm, about 1 nm to about 500 nm, about 1 nm to about 100 nm, or about 1 nm to about 50 nm. In some cases, the diameter of the conjugate may be at least 1 nm, 2 nm, 3 nm, 4 nm, 5 nm, 6 nm, 7 nm, 8 nm, 9 nm, 10 nm, 15 nm, 20 nm, 25 nm, 30 nm, 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 200 nm, 300 nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, or 1000 nm. In some cases, the diameter of the conjugate may be no more than 1 nm, 2 nm, 3 nm, 4 nm, 5 nm, 6 nm, 7 nm, 8 nm, 9 nm, 10 nm, 15 nm, 20 nm, 25 nm, 30 nm, 30 nm, 35 nm, 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 200 nm, 300 nm, 400 nm, 500 nm, 600 nm, 700 nm, 800 nm, 900 nm, or 1000 nm.

[00465] The ratio of conjugate binding molecules to the conjugates can be tailored to achieve desired binding properties between the conjugate binding molecules and the detection moiety. In some instances, the molar ratio of conjugate binding molecules to the conjugates is at least 1:1, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:110, 1:120, 1:130, 1:140, 1:150, 1:160, 1:170, 1:180, 1:190, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, or 1:500. In some instances, the mass ratio of conjugate binding molecules to the conjugates is at least 1:1, 1:5, 1:10, 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:110, 1:120, 1:130, 1:140, 1:150, 1:160, 1:170, 1:180, 1:190, 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, or 1:500. In some instances, the number of conjugate binding molecules per conjugate is at least 1, 10, 50, 100, 500, 1000, 5000, or 10000.

[00466] The conjugate binding molecules can be bound to the conjugates by various approached. Sometimes, the conjugate binding molecule can be bound to the conjugate by passive binding. Some such passive binding comprise adsorption, absorption, hydrophobic interaction, electrostatic interaction, ionic binding, or surface interactions. In some cases, the conjugate binding molecule can be bound to the conjugate covalently. Sometimes, the covalent bonding of the conjugate binding molecule to the conjugate is facilitated by EDC/NHS chemistry or thiol chemistry.

[00467] Described herein are detection region on the support medium that provide a region for presenting the assay results. The detection region can be made from various materials

that facilitate binding of the conjugate-bound detection moiety from cleaved detector molecule to the capture molecule specific for the detection moiety. The detection pad may comprise the same material as other zones or a different material than the other zones. The detection region may comprise nitrocellulose, paper, cellulose, cellulose fiber filters, glass fiber filters, porous plastic membranes, aluminum oxide coated membranes, woven meshes, polyester filter, or polymer-based matrices. Often the detection region may comprise nitrocellulose. The material for the region pad region may be hydrophilic, have low non-specific binding, or have consistent fluid flow properties across the region pad. The material for the conjugate pad may range from about 10 μm to about 1000 μm, about 10 μm to about 750 μm, about 10 μm to about 500 μm, or about 10 μm to about 300 μm.

[00468] The detection region comprises at least one capture area with a high density of a capture molecule that can bind to the detection moiety from cleaved detection molecule and at least one area with a high density of a positive control capture molecule. The capture area with a high density of capture molecule or a positive control capture molecule may be a line, a circle, an oval, a rectangle, a triangle, a plus sign, or any other shapes. In some instances, the detection region comprise more than one capture area with high densities of more than one capture molecules, where each capture area comprises one type of capture molecule that specifically binds to one type of detection moiety from cleaved detection molecule and are different from the capture molecules in the other capture areas. The capture areas with different capture molecules may be overlapping completely, overlapping partially, or spatially separate from each other. In some instances, the capture areas may overlap and produce a combined detectable signal distinct from the detectable signals generated by the individual capture areas. Usually, the positive control spot is spatially distinct from any of the detection spot.

[00469] The capture molecule described herein bind to detection moiety and immobilized in the detection spot in the detect region. Some suitable capture molecules comprise an antibody, a polypeptide, or a single stranded nucleic acid. In some cases, the capture molecule binds a dye and a fluorophore. Some such capture molecules that bind to a dye or a fluorophore can quench their signal. Sometimes, the capture molecule is an antibody that that binds to a dye or a fluorophore can quench their signal. In some cases, the capture molecule is a monoclonal antibody. In some cases, an antibody, also referred to as an immunoglobulin, includes any isotype, variable regions, constant regions, Fc region, Fab fragments, F(ab')2 fragments, and Fab' fragments. Alternatively, the capture molecule is a non-antibody compound that specifically binds the detection moiety. Sometimes, the capture molecule is a polypeptide that can bind to the detection moiety. In some instances, the detection moiety from cleaved detection molecule has a

conjugate bound to the detection moiety, and the conjugate-detection moiety complex may bind to the capture molecule specific to the detection moiety on the detection region. Sometimes, the capture molecule is a polypeptide that can bind to the detection moiety. Sometimes, the capture molecule is avidin or a polypeptide that binds biotin. Sometimes, the capture molecule is a detector moiety binding nucleic acid.

[00470] The detection region described herein comprises at least one area with a high density of a positive control capture molecule. The positive control spot in the detection region provides a validation of the assay and a confirmation of completion of the assay. If the positive control spot is not detectable by the visualization methods described herein, the assay is not valid and should be performed again with a new system or kit. The positive control capture molecule binds at least one of the conjugate, the conjugate binding molecule, or detection moiety and is immobilized in the positive control spot in the detect region. Some suitable positive control capture molecules comprise an antibody, a polypeptide, or a single stranded nucleic acid. In some cases, the positive control capture molecule binds to the conjugate binding molecule. Some such positive control capture molecules that bind to a dye or a fluorophore can quench their signal. Sometimes, the positive control capture molecule is an antibody that that binds to a dye or a fluorophore can quench their signal. In some cases, the positive control capture molecule is a monoclonal antibody. In some cases, an antibody includes any isotype, variable regions, constant regions, Fc region, Fab fragments, F(ab')2 fragments, and Fab' fragments. Alternatively, the positive control capture molecule is a non-antibody compound that specifically binds the detection moiety. Sometimes, the positive control capture molecule is a polypeptide that can bind to at least one of the conjugate, the conjugate binding molecule, or detection moiety. In some instances, the conjugate unbound to the detection moiety binds to the positive control capture molecule specific to at least one of the conjugate or the conjugate binding molecule.

[00471] The kit or system described herein may also comprise a positive control sample to determine that the activity of at least one of programmable nuclease, a guide nucleic acid, or a single stranded detector nucleic acid. Often, the positive control sample comprises a target nucleic acid that binds to the guide nucleic acid. The positive control sample is contacted with the reagents in the same manner as the test sample and visualized using the support medium. The visualization of the positive control spot and the detection spot for the positive control sample provides a validation of the reagents and the assay.

[00472] 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. 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. 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. Sometimes the nucleic acid amplification uses dTTP, dATP, dCTP, and dGTP. Often, the nucleic acid amplification uses dUTP, dATP, dCTP, and dGTP.

[00473] Described herein are collection pad region that provide a region to collect the sample that flows down the support medium. Often the collection pads are placed downstream of the detection region and comprise an absorbent material. The collection pad can increase the total volume of sample that enters the support medium by collecting and removing the sample from other regions of the support medium. This increased volume can be used to wash unbound conjugates away from the detection region to lower the background and enhance assay

sensitivity. When the design of the support medium does not include a collection pad, the volume of sample analyzed in the support medium may be determined by the bed volume of the support medium. The collection pad may provide a reservoir for sample volume and may help to provide capillary force for the flow of the sample down the support medium.

[00474] The collection pad may be prepared from various materials that are highly absorbent and able to retain fluids. Often the collection pads comprise cellulose filters. In some instances, the collection pads comprise cellulose, cotton, woven meshes, polymer-based matrices. The dimension of the collection pad, usually the length of the collection pad, may be adjusted to change the overall volume absorbed by the support medium.

[00475] The support medium described herein may have a barrier around the edge of the support medium. Often the barrier is a hydrophobic barrier that facilitates the maintenance of the sample within the support medium or flow of the sample within the support medium. Usually, the transport rate of the sample in the hydrophobic barrier is much lower than through the regions of the support medium. In some cases, the hydrophobic barrier is prepared by contacting a hydrophobic material around the edge of the support medium. Sometimes, the hydrophobic barrier comprises at least one of wax, polydimethylsiloxane, rubber, or silicone.

[00476] Any of the regions on the support medium can be treated with chemicals to improve the visualization of the detection spot and positive control spot on the support medium. The regions can be treated to enhance extraction of nucleic acid in the sample, to control the transport of the reacted reagents and sample or the conjugate to other regions of the support medium, or to enhance the binding of the cleaved detection moiety to the conjugate binding molecule on the surface of the conjugate or to the capture molecule in the detection region. The chemicals may comprise detergents, surfactants, buffers, salts, viscosity enhancers, or polypeptides. In some instances, the chemical comprises bovine serum albumin. In some cases, the chemicals or physical agents enhance flow of the sample with a more even flow across the width of the region. In some cases, the chemicals or physical agents provide a more even mixing of the sample across the width of the region. In some cases, the chemicals or physical agents control flow rate to be faster or slower in order to improve performance of the assay. Sometimes, the performance of the assay is measured by at least one of shorter assay time, longer times during cleavage activity, longer or shorter binding time with the conjugate, sensitivity, specificity, or accuracy.

Housing

[00477] A support medium as described herein can be housed in a number of ways that are consistent with the methods disclosed herein. The housing for the support medium are, for

example, consistent with fluidic devices disclosed herein for detection of a target nucleic acid sequence within the sample, wherein the fluidic device may comprise multiple pumps, valves, reservoirs, and chambers for sample preparation, amplification of a target nucleic acid sequence within the sample, mixing with a programmable nuclease, and detection of a detectable signal arising from cleavage of detector nucleic acids by the programmable nuclease within the fluidic system itself. The housing, as described herein, are compatible with the DETECTR assay methods disclosed herein. The housing, as described herein, are compatible with any of the programmable nucleases disclosed herein (e.g., a programmable nuclease with at least 60% sequence identity to SEQ ID NO: 11) and use of said programmable nuclease in a method of detecting a target nucleic acid. The housing, 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 (e.g., a programmable nuclease and a buffer with low salt (about 110 mM or less) and a pH of 7 to 8) and use of said compositions in a method of detecting a target nucleic acid. The housing, 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. For example, the fluidic device may comprise support mediums to channel the flow of fluid from one chamber to another and wherein the entire fluidic device is encased within the housing described herein. Typically, the support medium described herein is encased in a housing to protect the support medium from contamination and from disassembly. The housing can be made of more than one part and assembled to encase the support medium. In some instances, a single housing can encase more than one support medium. The housing can be made from cardboard, plastics, polymers, or materials that provide mechanical protection for the support medium. Often, the material for the housing is inert or does not react with the support medium or the reagents placed on the support medium. The housing may have an upper part which when in place exposes the sample pad to receive the sample and has an opening or window above the detection region to allow the results of the lateral flow assay to be read. The housing may have guide pins on its inner surface that are placed around and on the support medium to help secure the compartments and the support medium in place within the housing. In some cases, the housing encases the entire support medium. Alternatively, the sample pad of the support medium is not encased and is left exposed

to facilitate the receiving of the sample while the rest of the support medium is encased in the housing.

[00478] The housing and the support medium encased within the housing may be sized to be small, portable, and hand held. The small size of the housing and the support medium would facilitate the transport and use of the assay in remote regions or low resource settings. In some cases, the housing has a length of no more than 30 cm, 25 cm, 20 cm, 15 cm, 10 cm, or 5 cm. In some cases, the housing has a length of at least 1 cm, 5 cm, 10 cm, 15 cm, 20 cm, 25 cm, or 30 cm. In some cases, the housing has a width of no more than 30 cm, 25 cm, 20 cm, 15 cm, 10 cm, 5 cm, 4 cm, 3 cm, 2 cm, or 1 cm. In some cases, the housing has a width of at least 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 10 cm, 15 cm, 20 cm, 25 cm, or 30 cm. In some cases, the housing has a height of no more than 10 cm, 9 cm, 8 cm, 7 cm, 6 cm, 5 cm, 4 cm, 3 cm, 2 cm, or 1 cm. In some cases, the housing has a height of at least 1 cm, 2 cm, 3 cm, 4 cm, 5 cm, 6 cm, 7 cm, 8 cm, 9 cm, or 10 cm. Typically, the housing is rectangular in shape.

[00479] In some instances, the housing provides additional information on the outer surface of the upper cover to facilitate the identification of the test type, visualization of the detection region, and analysis of the results. The upper outer housing may have identification label including but not limited to barcodes, QR codes, identification label, or other visually identifiable labels. In some instances, the identification label is imaged by a camera on a mobile device, and the image is analyzed to identify the disease, cancer, or genetic disorder that is being tested for. The correct identification of the test is important to accurately visualize and analyze the results. In some instances, the upper outer housing has fiduciary markers to orient the detection region to distinguish the positive control spot from the detection spots. In some instances, the upper outer housing has a color reference guide. When the detection region is imaged with the color reference guide, the detection spots, located using the fiduciary marker, can be compared with the positive control spot and the color reference guide to determine various image properties of the detection spot such as color, color intensity, and size of the spot. In some instances, the color reference guide has red, green, blue, black, and white colors. In some cases, the image of the detection spot can be normalized to at least one of the reference colors of the color reference guide, compared to at least two of the reference colors of the color reference guide, and generate a value for the detection spot. Sometimes, the comparison to at least two of the reference colors is comparison to a standard reference scale. In some instance, the image of the detection spot in some instance undergoes transformation or filtering prior to analysis. Analysis of the image properties of the detection spot can provide information regarding presence or absence of the target nucleic acid targeted by the assay and the disease,

cancer, or genetic disorder associated with the target nucleic acid. In some instances, the analysis provides a qualitative result of presence or absence of the target nucleic acid in the sample. In some instances, the analysis provides a semi-quantitative or quantitative result of the level of the target nucleic acid present in the sample. Quantification may be performed by having a set of standards in spots/wells and comparing the test sample to the range of standards. A more semi-quantitative approach may be performed by calculating the color intensity of 2 spots/well compared to each other and measuring if one spot/well is more intense than the other.

Manufacturing

[00480] The support medium may be assembled with a variety of materials and reagents. Reagents may be dispensed or coated on to the surface of the material for the support medium. The material for the support medium may be laminated to a backing card, and the backing card may be singulated or cut into individual test strips. The device may be manufactured by completely manual, batch-style processing; or a completely automated, in-line continuous process; or a hybrid of the two processing approaches. The batch process may start with sheets or rolls of each material for the support medium. Individual zones of the support medium may be processed independently for dispensing and drying, and the final support medium may be assembled with the independently prepared zones and cut. The batch processing scheme may have a lower cost of equipment, and a higher labor cost than more automated in-line processing, which may have higher equipment costs. In some instances, batch processing may be preferred for low volume production due to the reduced capital investment. In some instances, automated in-line processing may be preferred for high volume production due to reduced production time. Both approaches may be scalable to production level.

[00481] In some instances, the support mediums are prepared using various instruments, including an XYZ-direction motion system with dispensers, impregnation tanks, drying ovens, a manual or semi-automated laminator, and cutting methods for reducing roll or sheet stock to appropriate lengths and widths for lamination. For dispensing the conjugate binding molecules for the conjugate zone and capture molecules for the detection zones, an XYZ-direction motion system with dispensers may be used. In some embodiments, the dispenser may dispense by a contact method or a non-contact method.

[00482] In automated or semi-automated preparation of the support medium, the support medium may be prepared from rolls of membranes for each region that are ordered into the final assembled order and unfurled from the rolls. For example, the membranes can be ordered from sample pad region to collection pad region from left to right with one membrane corresponding to a region on the support medium, all onto an adhesive cardstock. The dispenser places the

reagents, conjugates, detection molecules, and other treatments for the membrane onto the membrane. The dispensed fluids are dried onto the membranes by heat, in a low humidity chamber, or by freeze drying to stabilize the dispensed molecules. The membranes are cut into strips and placed into the housing and packaged.

Kits

[00483] Disclosed herein are kits for use to detect a target nucleic acid 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.

[00484] 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, a 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.

[00485] 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 detector nucleic acid comprising a detection moiety, wherein the detector 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.

[00486] 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 detector nucleic acid comprising a detection moiety, wherein the detector 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 detector 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.

[00487] 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. 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.

[00488] 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.

[00489] 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.

[00490] 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.

[00491] 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.

[00492] 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.

[00493] 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.

[00494] As used herein the terms "individual," "subject," and "patient" are used interchangeably and include any member of the animal kingdom, including humans.

[00495] As used herein the term "antibody" refers to, but not limited to, a monoclonal antibody, a synthetic antibody, a polyclonal antibody, a multispecific antibody (including a bi-specific antibody), a human antibody, a humanized antibody, a chimeric antibody, a single-chain Fvs (scFv) (including bi-specific scFvs), a single chain antibody, a Fab fragment, a F(ab') fragment, a disulfide-linked Fvs (sdFv), or an epitope-binding fragment thereof. In some cases, the antibody is an immunoglobulin molecule or an immunologically active portion of an immunoglobulin molecule. In some instances, an antibody is animal in origin including birds and mammals. Alternately, an antibody is human or a humanized monoclonal antibody.

[00496] At **FIG. 1**, one sees an improved SNP detection enzyme and method. At left is shown ALDH2 E540K G-SNP, while at right one sees E540K A-SNP. The ALDH2 G-SNP was detected with a G-SNP gRNA (SEQ ID NO: 425), and the ALDH2 A-SNP was detected with an A-SNP gRNA (SEQ ID NO: 426). LbCas12a (SEQ ID NO: 1) is shown at top, while a representative improved enzyme, a Cas12 variant corresponding to (SEQ ID NO: 11), is shown at bottom. One sees that the improved enzyme exhibits at least a 50% improvement in reaching reporter saturation signal, and exhibits no more than 33% off target reporter signal. At right, one sees that the improvement in reaching reporter saturation signal is at least 2x, and the off target reporter signal is no greater than 10% of the target signal.

[00497] Various compositions and implementations of the methods herein achieve an improvement in reaching reporter signal saturation of at least 50%, 60%, 70%, 80%, 90%, 2x, 2.5x, 3x, 3.5x, 4x, or more than 4x, or any improvement spanned by or greater than the range of improvements listed herein. Similarly, off target signal strength is observed to be no greater than 33%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or less than 1%, or any value spanned by or less than the range of improvements listed herein.

[00498] At **FIG. 2**, one sees the first of a series of experiments to assess buffer contents for detection using a Cas12 variant (SEQ ID NO: 11). It is observed that BIS-TRIS at pH 7.0, Imidazole at pH of 7.0, 7.5 or 7.8, MOPS at 7.0, HEPES at pH 7.0 or 7.5, and DIPSO at pH 7.0 exhibit top performance.

[00499] Accordingly, disclosed herein are buffers comprising at least one of the components listed above, at a pH such as a neutral pH, for example a pH ranging from 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, 8.1. 8.2, 8.3, 8.4, or 8.5, or any number falling within or adjacent to the range defined thereby.

[00500] At **FIG. 3**, one sees improvements conveyed by inclusion of acetate at concentrations of about 0, 10, 20, 37, 75, 150, 300 and 600 mM, from left to right on detection using a Cas12 variant (SEQ ID NO: 11). The left bar at each concentration is Cl and right bar is acetate.

[00501] Accordingly, one sees benefits conveyed by modulation of salt concentration, for example by addition of acetate, as well as limiting salt concentration to no greater than 10, 20, 37, 75, 150, 300 and 600 mM. Particular improvements are seen at less than 75nM, at no greater than 40nM, and at about 10-20nM. Disclosed herein are compositions having a reduced salt concentration, such as a salt concentration in nM of no greater than 1, 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, 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, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68,669, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 18, 190, 200, 220, 230, 240, 250, 260, 270, 280, 290, or 300.

[00502] At **FIG. 4**, one sees an improvement in SNP specificity upon inclusion of heparin in a reaction buffer when detected with a Cas12 variant (SEQ ID NO: 11). Inclusion of heparin increases both SNP-specific detection and general enzyme performance.

[00503] At **FIG. 5**, one sees optimization for a number of buffer additives, such as heparin, DTT, NP-40, and BSA (from left to right) over a series of 8 iterative dilutions when detected with a Cas12 variant (SEQ ID NO: 11) and a gRNA of SEQ ID NO: 423.

[00504] Accordingly, buffers comprising at least one additive selected from the group consisting of heparin, DTT, NP-40, and BSA, and optionally including in addition or in the alternative triton X, are disclosed herein.

[00505] At **FIG. 6**, one sees base sensitivity for each SNP allele, A, C, G, or T, at a SNP position, for LbCas12a (SEQ ID NO: 1), top, and a representative improved enzyme, a Cas12 variant corresponding to (SEQ ID NO: 11), below. One sees substantial improvement over LbCas12a. Target sequences corresponding to SEQ ID NO: 431 – SEQ ID NO: 438, provided in **TABLE 7** were detected. The A SNP allele was detected using a gRNA of SEQ ID NO: 427. The C SNP allele was detected using a gRNA of SEQ ID NO: 428. The G SNP allele was detected using a gRNA of SEQ ID NO: 429. The T SNP allele was detected using a gRNA of SEQ ID NO: 430.

TABLE 7 – Target and Non-Target Strands for SNP Allele Sensitivity

SEQ ID NO:	Name	Sequence
SEQ ID NO: 431	T-SNP Target Strand	TGGTATTCTTTCTCTTCCGCACCCAGCAGTTTGGCCaG CCCAAAATCTGTGATCT

SEQ ID NO:	Name	Sequence
SEQ ID NO: 432	T-SNP Non-Target Strand	AGATCACAGATTTTGGGCtGGCCAAACTGCTGGGTGC GGAAGAGAAAGAATACCA
SEQ ID NO: 433	G-SNP Target Strand	TGGTATTCTTTCTCTTCCGCACCCAGCAGTTTGGCCcG CCCAAAATCTGTGATCT
SEQ ID NO: 434	G-SNP Non-Target Strand	AGATCACAGATTTTGGGCgGGCCAAACTGCTGGGTGC GGAAGAGAAAGAATACCA
SEQ ID NO: 435	C-SNP Target Strand	TGGTATTCTTTCTCTTCCGCACCCAGCAGTTTGGCCgG CCCAAAATCTGTGATCT
SEQ ID NO: 436	C-SNP Non-Target Strand	AGATCACAGATTTTGGGCcGGCCAAACTGCTGGGTGC GGAAGAGAAAGAATACCA
SEQ ID NO: 437	A-SNP Target Strand	TGGTATTCTTTCTCTTCCGCACCCAGCAGTTTGGCCtG CCCAAAATCTGTGATCT
SEQ ID NO: 438	A-SNP Non-Target Strand	AGATCACAGATTTTGGGCaGGCCAAACTGCTGGGTGC GGAAGAGAAAGAATACCA

[00506] At **FIG. 7**, one sees template optimization for an improved enzyme, a Cas12 variant corresponding to SEQ ID NO: 11, as disclosed herein. Templates comprising a C SNP allele (SEQ ID NO: 440) or a T SNP allele (SEQ ID NO: 441) were detected using gRNAs directed to the C SNP (SEQ ID NO: 423) or the T SNP allele (SEQ ID NO: 439). Primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397 were used to amplify the target sequence and insert a PAM sequence.

[00507] At **FIG. 8**, one sees base sensitivity of an improved enzyme, a Cas12 variant corresponding to SEQ ID NO: 11, for each SNP allele, A, C, G, or T, for an EGFR SNP as disclosed herein. EGFR target sequences corresponding to SEQ ID NO: 444 – SEQ ID NO: 447, provided in **TABLE 8**, were detected. Primers corresponding to SEQ ID NO: 442 and SEQ ID NO: 443 were used to amplify the target sequences. The A SNP allele was detected using a gRNA of SEQ ID NO: 427. The C SNP allele was detected using a gRNA of SEQ ID NO: 428. The G SNP allele was detected using a gRNA of SEQ ID NO: 429. The T SNP allele was detected using a gRNA of SEQ ID NO: 430.

TABLE 8 – EGFR Target Sequences

SEQ ID NO:	Name	Sequence
SEQ ID NO: 444	EGFR WT (G-SNP)	TGGTCCCCGCCACCCCCCACCCCCACTTTGCAGATAAACCACATG CAGGAAGGTCAGCCTGGCAAGTCCAGTAAGTTCAAGCCCAGGTC TCAACTGGGCAGCAGAGCTCCTGCTCTTCTTTGTCCTCATATACGA

SEQ ID NO:	Name	Sequence
		GCACCTCTGGACTTAAAACTTGAGGAACTGGATGGAGAAAAGTT AATGGTCAGCAGCGGGTTACATCTTCTTTCATGCGCCTTTCCATTC TTTGGATCAGTAGTCACTAACGTTCGCCAGCCATAAGTCCTCGAC GTGGAGAGGCTCAGAGCCTGGCATGAACATGACCCTGAATTCGG ATGCAGAGCTTCTTCCCATGATGATCTGTCCCTCACAGCAGGGTC TTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGT GCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGC AGCATGTCAAGATCACAGATTTTGGGCTGGCCAAACTGCTGGGTG CGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAG GTGGCTTTAGGTCAGCCAGCATTTTCCTGACACCAGGGACCAGGC TGCCTTCCCACTAGCTGTATTGTTTAACACATGCAGGGGAGGATG CTCTCCAGACATTCTGGGTGAGCTCGCAGCAGCTGCTGCTGGCAG CTGGGTCCAGCCAGGGTCTCCTGGTAGTGTGAGCCAGAGCTGCTT TGGGAACAGTACTTGCTGGGACAGTGAATGAGGATGTTATCCCCA GGTGATCATTAGCAAATGTTAGGTTTCAGTCTCTCCCTGCAGGAT ATATAAGTCCCCTTCAATAGCGCAATTGGGAAAGGTCACAGCTGC CTTGGTGGTCCACTGCTGTCAAGGACACCTAAGGAACAGGAAAG GCCCCATGCGGACCCGAGCTCCCAGGGCTGTCTGTGGCTCGTGGC TGGGACAGGCAGCAATGGAGTCCTTCTCTCCCTTCACTGGCTCGG TTTCT
SEQ ID NO: 445	EGFR A-SNP	TGGTCCCCGCCACCCCCCACCCCCACTTTGCAGATAAACCACATG CAGGAAGGTCAGCCTGGCAAGTCCAGTAAGTTCAAGCCCAGGTC TCAACTGGGCAGCAGAGCTCCTGCTCTTCTTTGTCCTCATATACGA GCACCTCTGGACTTAAAACTTGAGGAACTGGATGGAGAAAAGTT AATGGTCAGCAGCGGGTTACATCTTCTTTCATGCGCCTTTCCATTC TTTGGATCAGTAGTCACTAACGTTCGCCAGCCATAAGTCCTCGAC GTGGAGAGGCTCAGAGCCTGGCATGAACATGACCCTGAATTCGG ATGCAGAGCTTCTTCCCATGATGATCTGTCCCTCACAGCAGGGTC TTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGT GCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGC AGCATGTCAAGATCACAGATTTTGGGCAGGCCAAACTGCTGGGTG CGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAG GTGGCTTTAGGTCAGCCAGCATTTTCCTGACACCAGGGACCAGGC TGCCTTCCCACTAGCTGTATTGTTTAACACATGCAGGGGAGGATG CTCTCCAGACATTCTGGGTGAGCTCGCAGCAGCTGCTGCTGGCAG CTGGGTCCAGCCAGGGTCTCCTGGTAGTGTGAGCCAGAGCTGCTT

SEQ ID NO:	Name	Sequence
		TGGGAACAGTACTTGCTGGGACAGTGAATGAGGATGTTATCCCCA GGTGATCATTAGCAAATGTTAGGTTTCAGTCTCTCCCTGCAGGAT ATATAAGTCCCCTTCAATAGCGCAATTGGGAAAGGTCACAGCTGC CTTGGTGGTCCACTGCTGTCAAGGACACCTAAGGAACAGGAAAG GCCCCATGCGGACCCGAGCTCCCAGGGCTGTCTGTGGCTCGTGGC TGGGACAGGCAGCAATGGAGTCCTTCTCTCCCTTCACTGGCTCGG TTTCT
SEQ ID NO: 446	EGFR T-SNP	TGGTCCCCGCCACCCCCCACCCCCACTTTGCAGATAAACCACATG CAGGAAGGTCAGCCTGGCAAGTCCAGTAAGTTCAAGCCCAGGTC TCAACTGGGCAGCAGAGCTCCTGCTCTTCTTTGTCCTCATATACGA GCACCTCTGGACTTAAAACTTGAGGAACTGGATGGAGAAAAGTT AATGGTCAGCAGCGGGTTACATCTTCTTTCATGCGCCTTTCCATTC TTTGGATCAGTAGTCACTAACGTTCGCCAGCCATAAGTCCTCGAC GTGGAGAGGCTCAGAGCCTGGCATGAACATGACCCTGAATTCGG ATGCAGAGCTTCTTCCCATGATGATCTGTCCCTCACAGCAGGGTC TTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGT GCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGC AGCATGTCAAGATCACAGATTTTGGGCTGGCCAAACTGCTGGGTG CGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAG GTGGCTTTAGGTCAGCCAGCATTTTCCTGACACCAGGGACCAGGC TGCCTTCCCACTAGCTGTATTGTTTAACACATGCAGGGGAGGATG CTCTCCAGACATTCTGGGTGAGCTCGCAGCAGCTGCTGCTGGCAG CTGGGTCCAGCCAGGGTCTCCTGGTAGTGTGAGCCAGAGCTGCTT TGGGAACAGTACTTGCTGGGACAGTGAATGAGGATGTTATCCCCA GGTGATCATTAGCAAATGTTAGGTTTCAGTCTCTCCCTGCAGGAT ATATAAGTCCCCTTCAATAGCGCAATTGGGAAAGGTCACAGCTGC CTTGGTGGTCCACTGCTGTCAAGGACACCTAAGGAACAGGAAAG GCCCCATGCGGACCCGAGCTCCCAGGGCTGTCTGTGGCTCGTGGC TGGGACAGGCAGCAATGGAGTCCTTCTCTCCCTTCACTGGCTCGG TTTCT
SEQ ID NO: 447	EGFR C-SNP	TGGTCCCCGCCACCCCCCACCCCCACTTTGCAGATAAACCACATG CAGGAAGGTCAGCCTGGCAAGTCCAGTAAGTTCAAGCCCAGGTC TCAACTGGGCAGCAGAGCTCCTGCTCTTCTTTGTCCTCATATACGA GCACCTCTGGACTTAAAACTTGAGGAACTGGATGGAGAAAAGTT AATGGTCAGCAGCGGGTTACATCTTCTTTCATGCGCCTTTCCATTC TTTGGATCAGTAGTCACTAACGTTCGCCAGCCATAAGTCCTCGAC

SEQ ID NO:	Name	Sequence
		GTGGAGAGGCTCAGAGCCTGGCATGAACATGACCCTGAATTCGG ATGCAGAGCTTCTTCCCATGATGATCTGTCCCTCACAGCAGGGTC TTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGT GCACCGCGACCTGGCAGCCAGGAACGTACTGGTGAAAACACCGC AGCATGTCAAGATCACAGATTTTGGGCCGGCCAAACTGCTGGGTG CGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAG GTGGCTTTAGGTCAGCCAGCATTTTCCTGACACCAGGGACCAGGC TGCCTTCCCACTAGCTGTATTGTTTAACACATGCAGGGGAGGATG CTCTCCAGACATTCTGGGTGAGCTCGCAGCAGCTGCTGCTGGCAG CTGGGTCCAGCCAGGGTCTCCTGGTAGTGTGAGCCAGAGCTGCTT TGGGAACAGTACTTGCTGGGACAGTGAATGAGGATGTTATCCCCA GGTGATCATTAGCAAATGTTAGGTTTCAGTCTCTCCCTGCAGGAT ATATAAGTCCCCTTCAATAGCGCAATTGGGAAAGGTCACAGCTGC CTTGGTGGTCCACTGCTGTCAAGGACACCTAAGGAACAGGAAAG GCCCCATGCGGACCCGAGCTCCCAGGGCTGTCTGTGGCTCGTGGC TGGGACAGGCAGCAATGGAGTCCTTCTCTCCCTTCACTGGCTCGG TTTCT

[00508] At **FIG. 9**, one sees an assessment of buffer additives and their effect on detection using a Cas12 variant (SEQ ID NO: 11). Highest performing additives include 4 M DMSO, 1M Pyridine, 500mM polypropylene glycol 400, 100mM dithiothreitol, 2M Erythritol, 500mM, polyethylene glycol, 1% w/v polyvinyl alcohol type II, and 5% w/v polyvinylpyrrolidone k15.

[00509] Accordingly, disclosed here are buffers supplemented or comprising at least one component selected from the list comprising DMSO, Pyridine, polypropylene glycol 400, dithiothreitol, Erythritol, polyethylene glycol, polyvinyl alcohol type II, and 5 polyvinylpyrrolidone k15. Concentrations are contemplated to be about or exactly the values presented above, such as 0, 1, 2, 3, 4, 5, 6, 7, or 8 M, or any interceding value in the range defined thereby, or 0, 100, 200, 300, 400, 500, 600, 700, or 800mM or any interceding value in the range defined thereby, or 0, 1, 2, 3, 4, 5, 6, 7, or 8% w/v, or any interceding value in the range defined thereby.

[00510] At **FIG. 10**, one sees trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 586, SEQ ID NO: 581, SEQ ID NO: 576, SEQ ID NO: 587, SEQ ID NO: 578, SEQ ID NO: 572, SEQ ID NO: 575, SEQ ID NO: 11, SEQ ID NO: 573, SEQ ID NO: 589, and SEQ ID NO: 583, and of LbCas12a (SEQ ID NO: 1) on targets containing various PAMs, double and single mismatched substrates. Shading indicates the

background subtracted fluorescence signal. NTS, single-stranded non-target substrate, TS, single-stranded target substrate; OFF, an off-target substrate; MM, location of a base mismatch.

[00511] Accordingly, disclosed herein are improved enzymes and associated kits and methods relating to enzymes having tolerance for or sensitivity to a particular PAM sequence or to a particular location of a mismatch, or both a PAM sequence and a particular location for a mismatch.

[00512] At **FIG. 11**, one sees trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 2, SEQ ID NO: 1, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, and SEQ ID NO: 599 – SEQ ID NO: 602 on targets containing various PAMs, double and single mismatched substrates. Shading indicates the background subtracted fluorescence signal. JSC142, AsCas12a; JSC143, LbCas12a; pLBH835, MAD7.

[00513] At **FIG. 12**, one sees trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, and SEQ ID NO: 3 on targets containing various PAMs, double and single mismatched substrates. Shading indicates the background subtracted fluorescence normalized to the maximum value for each.

[00514] At **FIG. 13A**, **FIG. 13B**, and **FIG. 13C**, one sees trans cleavage activity of various Cas12 orthologs corresponding SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, and SEQ ID NO: 3 on PCR targets containing a TTTA PAM using various guide RNA repeat sequences. Shading indicates the background subtracted fluorescence normalized to the maximum value for each. Each plot represents an independent replicate. Activity was detected in the presence of different Cas12 variants and different pre-crRNAs corresponding to different Cas12 variants. Sequences of the pre-crRNAs are provided in **TABLE 30**.

[00515] At **FIG. 14**, one sees activity of various Cas12 orthologs and other improved enzymes corresponding to SEQ ID NO: 571 – SEQ ID NO: 577, SEQ ID NO: 11, SEQ ID NO: 578 – SEQ ID NO: 589, SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 on a target PCR product. The negative control ("(-) control") is PCR product with no Cas12 added. The positive control is cleavage with a BamHI restriction enzyme ("BamHI"). Numbers above each lane correspond to the time in minutes before the reaction was quenched with 10mM EDTA. Lanes marked with "-" under each Cas12 ortholog correspond to negative control conditions with protein but no crRNA.

[00516] At **FIG. 15**, one sees limit of detection (LOD) assay results indicating trans cleavage activity of various Cas12 orthologs or other improved enzymes corresponding to SEQ ID NO:

572, SEQ ID NO: 576, SEQ ID NO: 11, SEQ ID NO: 582, SEQ ID NO: 583, SEQ ID NO: 587, SEQ ID NO: 1, SEQ ID NO: 591, SEQ ID NO: 595, SEQ ID NO: 597, SEQ ID NO: 600, SEQ ID NO: 601, and SEQ ID NO: 2 in the presence of various activator concentrations (shown on the left). Shading indicates the background subtracted fluorescence value of after 90min.

[00517] Accordingly, one sees improved enzymes, kits and methods exhibiting sensitivity of as low as 1nM, 100pM, 10pM, 1pM, 100fM, 10fM, or 1fM, or any number spanned by the range define thereby.

[00518] At **FIG. 16A** and **FIG. 16B**, one sees trans cleavage activity of various Cas12 orthologs corresponding to SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence of various salt concentrations. The shading represents the background subtracted fluorescence normalized to the maximum value for that protein. NaCl concentrations are given for the amount of salt added to the reaction for the added water (eg 0mM=40mM final salt concentration).

[00519] At **FIG. 17A** and **FIG. 17B**, one sees trans cleavage activity of various Cas12 orthologs corresponding to SEQ ID NO: 590 – SEQ ID NO: 598, SEQ ID NO: 580, SEQ ID NO: 599 – SEQ ID NO: 602, and SEQ ID NO: 2 in the presence of various salt concentrations. The color represents the raw background subtracted fluorescence (no normalization). NaCl concentrations are given for the amount of salt added to the reaction for the added water (e.g., 0mM=40mM final salt concentration).

[00520] Accordingly, disclosed herein are compositions supporting Cas12 or other improved enzyme activity and having reduced salt concentrations, such as limiting salt concentration to no greater than 10, 20, 37, 75, 150, 300 and 600 mM. Particular improvements are seen at less than 75nM, at no greater than 40nM, and at about 10-20nM. Disclosed herein are compositions having a reduced salt concentration, such as a salt concentration in nM of no greater than 1, 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, 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, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68,669, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 18, 190, 200, 220, 230, 240, 250, 260, 270, 280, 290, or 300.

Numbered Embodiments

[00521] The following embodiments recite non-limiting permutations of combinations of features disclosed herein. Other permutations of combinations of features are also contemplated. In particular, each of these numbered embodiments is contemplated as depending from or relating to every previous or subsequent numbered embodiment, independent of their order as

listed. 1. A programmable nuclease that elicits maximal reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 100nM. 2. The programmable nuclease of embodiment 1, wherein the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 3. The Cas12 protein of any one of embodiments 1-2, wherein said protein elicits maximal reporter activity following contacting to a target template at least 50% faster than LbCas12 at a given target template concentration. 4. The Cas12 protein of any one of embodiments 1-3, wherein said protein elicits maximal reporter activity following contacting to a target template at least 2x faster than LbCas12 at a given target template concentration. 5. The Cas12 protein of any one of embodiments 1-4, wherein said protein elicits maximal reporter activity following contacting to a target template at least 4x faster than LbCas12 at a given target template concentration. 6. The Cas12 protein of any one of embodiments 1-5, wherein said protein elicits no greater than 33% of maximal reporter activity following contacting to a template differing from a target template by a single base at a template concentration of 100nM. 7. The Cas12 protein of any one of embodiments 1-6, wherein the protein elicits maximal reporter activity in a composition comprising at least one component selected from the list consisting of acetate, heparin, dithiothreitol (DTT), triton-X, TCEP, BSA, NP-40, imidazole, MOPS, HEPES and DIPSO. 8. The Cas12 protein of any one of embodiments 1-7, wherein the template is unamplified. 9. The Cas12 protein of any one of embodiments 1-8, wherein the template is amplified prior to contacting. 10. The Cas12 protein of any one of embodiments 1-9, wherein the contacting is performed in an activity buffer, wherein the activity buffer comprises 125mM NaCl, 5mM MgCl2, 20mM Tris pH 7.5, and 1% glycerol. 11. The Cas12 protein of any one of embodiments 1-10, wherein the contacting is performed at about 25 °C. 12. The Cas12 protein of any one of embodiments 1-11, wherein the contacting is performed at about 37 °C. 13. A programmable nuclease reaction buffer comprising at least one component selected from the list consisting of acetate, heparin, dithiothreitol (DTT), triton-X, TCEP, BSA, NP-40, imidazole, MOPS, HEPES and DIPSO. 14. The programmable nuclease of any one of embodiments 1-13, wherein the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 15. The reaction buffer of any one of embodiments 13-14, wherein the programmable nuclease in said reaction buffer elicits no greater than 33% of maximal reporter activity following contacting to a template differing from a target template by a single base. 16. The reaction buffer of any one of embodiments 13-15, wherein the reaction buffer comprises no greater than 150mM NaCl. 17. The reaction buffer of any one of embodiments 13-16, wherein the reaction buffer comprises no greater than 100mM NaCl. 18. The reaction buffer of any one of embodiments 13-17, wherein the reaction buffer comprises no greater than 50mM

NaCl. 19. The reaction buffer of any one of embodiments 13-18, wherein the reaction buffer comprises no greater than 25mM NaCl. 20. The reaction buffer of any one of embodiments 13-19, wherein the reaction buffer comprises from 0 μg/mL heparin to 100 μg/mL heparin. 21. The reaction buffer of any one of embodiments 13-20, wherein the reaction buffer comprises 0 μg/mL heparin. 22. The reaction buffer of any one of embodiments 13-21, wherein the reaction buffer comprises 50 μg/mL heparin. 23. The reaction buffer of any one of embodiments 13-22, wherein the reaction buffer comprises from 0 mM DTT to 5 mM DTT. 24. The reaction buffer of any one of embodiments 13-23, wherein the reaction buffer comprises 1 mM DTT. 25. The reaction buffer of any one of embodiments 13-24, wherein the reaction buffer comprises from 0 mM to 50 mM Imidazole. 26. The reaction buffer of any one of embodiments 13-25, wherein the reaction buffer comprises 20 mM Imidazole. 27. A programmable nuclease reaction buffer comprising at least one component selected from the list consisting of DMSO, polyvinyl alcohol, polyvinylpyrrolidone, and polypropylene glycol. 28. The programmable nuclease of any one of embodiments 1-27, wherein the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 29. The reaction buffer of any one of embodiments 13-28, wherein the programmable nuclease in said reaction buffer elicits no greater than 33% of maximal reporter activity following contacting to a no-template control. 30. The reaction buffer of any one of embodiments 13-29, wherein the reaction buffer comprises no greater than 150mM NaCl. 31. The reaction buffer of any one of embodiments 13-30, wherein the reaction buffer comprises no greater than 100mM NaCl. 32. The reaction buffer of any one of embodiments 13-31, wherein the reaction buffer comprises no greater than 50mM NaCl. 33. The reaction buffer of any one of embodiments 13-32, wherein the reaction buffer comprises no greater than 25mM NaCl. 34. A programmable nuclease that elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1nM in an activity buffer, wherein the activity buffer comprises 125mM NaCl, 5mM MgCl2, 20mM Tris pH 7.5, and 1% glycerol. 35. The programmable nuclease of any one of embodiments 1-34, wherein the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 36. The Cas12 protein of any one of embodiments 1-35, wherein the Cas12 protein elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1pM. 37. The Cas12 protein of any one of embodiments 1-36, wherein the Cas12 protein elicits reporter activity no more than 60 minutes following contacting to a target template at a target template concentration of 1fM. 38. A programmable nuclease that exhibits at least 90% target cleavage in no more than 60 minutes. 39. The programmable nuclease of any one of embodiments 1-38, wherein the programmable nuclease comprises a Cas12 protein, a

Cas13 protein, or a Cas14 protein. 40. The Cas12 protein of any one of embodiments 1-39, wherein the Cas12 protein exhibits at least 90% target cleavage in no more than 15 minutes. 41. The Cas12 protein of any one of embodiments 1-40, wherein an activity buffer (5X :600mM NaCl, 25mM MgCl2, 100mM Tris pH 7.5, 5% glycerol) exhibits said target cleavage. 42. The Cas12 protein of any one of embodiments 1-41, wherein said cleavage is effected at a Cas12 concentration of from 50nM to 200nM. 43. The Cas12 protein of any one of embodiments 1-42, wherein said target cleavage is effected at a Cas12 concentration of 100nM. 44. The Cas12 protein of any one of embodiments 1-42, wherein said cleavage is effected at a target concentration of from 5nm to 25nM. 45. The Cas12 protein of any one of embodiments 1-44, wherein said target cleavage is effected at a target concentration of 15nM. 46. The Cas12 protein of any one of embodiments 1-45, wherein said target cleavage is effected at a guide RNA concentration of from 50nM to 200nM. 47. The Cas12 protein of any one of embodiments 1-46, wherein said target cleavage is effected at a guide RNA concentration of 125nM. 48. The Cas12 protein of any one of embodiments 1-47, wherein said target cleavage is effected at a temperature of from about 20 °C to about 40 °C. 49. The Cas12 protein of any one of embodiments 1-48, wherein said target cleavage is effected at a temperature of about 25 °C. 50. The Cas12 protein of any one of embodiments 1-49, wherein said target cleavage is effected at a temperature of about 37 °C. 51. A programmable nuclease that exhibits no more than 10% target cleavage in 60 minutes. 52. The programmable nuclease of any one of embodiments 1-51, wherein the programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 53. The Cas12 protein of any one of embodiments 1-52, wherein the programmable nuclease exhibits said target cleavage in an activity buffer comprising 125mM NaCl, 5mM MgCl2, 20mM Tris pH 7.5, and 1% glycerol. 54. The Cas12 protein of any one of embodiments 1-53, wherein said target cleavage is effected at a Cas12 concentration of 100nM. 55. The Cas12 protein of any one of embodiments 1-54, wherein said target cleavage is effected at a target concentration of 15nM. 56. The Cas12 protein of any one of embodiments 1-55, wherein said target cleavage is effected at a guide RNA concentration of 125nM. 57. The Cas12 protein of any one of embodiments 1-56, wherein said target cleavage is effected at a temperature of about 25 °C. 58. The Cas12 protein of any one of embodiments 1-57, wherein said target cleavage is effected at a temperature of about 37 °C. 59. A composition comprising a first programmable nuclease population and a second programmable nuclease population, wherein the first programmable nuclease population and the second programmable nuclease population do not recognize a common PAM sequence. 60. The composition of embodiment 59, comprising a third programmable nuclease population, wherein none of the first programmable nuclease population,

the second programmable nuclease population, and the third programmable nuclease population recognize a common PAM sequence. 61. The composition of any one of embodiments 59-60, comprising a fourth programmable nuclease population, wherein none of the first programmable nuclease population, the second programmable nuclease population, the third programmable nuclease population, and the fourth programmable nuclease population recognize a common PAM sequence. 62. The composition of any one of embodiments 59-61, wherein the first programmable nuclease, the second programmable nuclease, or a combination thereof comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 63. The composition of any one of embodiments 59-62, wherein the third programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 64. The composition of any one of embodiments 59-63, wherein the fourth programmable nuclease comprises a Cas12 protein, a Cas13 protein, or a Cas14 protein. 65. A method for cleaving a unique site of a nucleic acid molecule, comprising designing a guide nucleic acid to cleave the unique site of the nucleic acid molecule and contacting the guide nucleic acid to a programmable nuclease and to the unique site of the nucleic acid molecule, thereby cleaving the unique site of the nucleic acid molecule. 66. The method of any one of embodiments 65-65, wherein a PAM sequence is not considered in the designing of the guide nucleic acid. 67. The method of any one of embodiments 65-66, wherein the programmable nuclease comprises a Cas protein. 68. The method of any one of embodiments 65-67, wherein the Cas protein is Cas14. 69. A method of sequence specific cleavage of a nucleic acid molecule in a sample comprising contacting to a first PAM independent nuclease to a flank on one side of a cleavage site the nucleic acid molecule and a second PAM independent nuclease to a flank on the other side of the cleavage site of the nucleic acid molecule. 70. The method of any one of embodiments 65-69, further comprising contacting the sample to a DNA fragment for sequence specific break repair. 71. The method of any one of embodiments 65-70, wherein the PAM independent nuclease is a Cas protein. 72. The method of any one of embodiments 65-71, wherein the Cas protein is a nickase. 73. The method of any one of embodiments 65-72, wherein the Cas protein is Cas14. 74. A method of detecting a presence or an absence of a target nucleic acid in a sample, the method comprising: contacting a first volume to a second volume, wherein the first volume comprises the sample and the second volume comprises: i) a guide nucleic acid having at least 10 nucleotides reverse complementary to a target nucleic acid in the sample; and ii) a programmable nuclease activated upon binding of the guide nucleic acid to the target nucleic acid; iii) a reporter comprising a nucleic acid and a detection moiety, wherein the second volume is at least 4-fold greater than the first volume; and detecting the presence or the absence of the target nucleic acid by measuring a signal produced

by cleavage of the nucleic acid of the reporter, wherein cleavage occurs when the programmable nuclease is activated. 75. The method of any one of embodiments 65-74, wherein the first volume comprises from 1 μL to 10 μL. 76. The method of any one of embodiments 65-75, wherein the first volume comprises from 1 μL to 5 μL. 77. The method of any one of embodiments 65-76, wherein the first volume comprises about 2 μL. 78. The method of any one of embodiments 65-77, wherein the first volume comprises about 4 μL. 79. The method of any one of embodiments 65-78, wherein the second volume comprises from 5 μL to 40 μL. 80. The method of any one of embodiments 65-79, wherein the second volume comprises from 10 μL to 30 μL. 81. The method of any one of embodiments 65-80, wherein the second volume comprises about 20 μL. 82. The method of any one of embodiments 65-81, wherein the second volume comprises about 30 μL. 83. The method of any one of embodiments 65-82, wherein the first volume comprises one or more of a buffer for cell lysis, a buffer for amplification, a primer, a polymerase, target nucleic acid, a non-target nucleic acid, a single-stranded DNA, a double-stranded DNA, a salt, a buffering agent, an NTP, a dNTP, or any combination thereof. 84. The method of any one of embodiments 65-83, wherein the sample is a biological sample comprising 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. 85. The method of any one of embodiments 65-84, wherein the programmable nuclease is a programmable Type V CRISPR/Cas enzyme. 86. The method of any one of embodiments 65-85, wherein the programmable Type V CRISPR/Cas enzyme is a programmable Cas12 nuclease. 87. The method of any one of embodiments 65-86, wherein the programmable Cas12 nuclease is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. 88. The method of any one of embodiments 65-87, wherein the programmable Type V CRISPR/Cas enzyme is a programmable Cas14 nuclease. 89. The method of any one of embodiments 65-88, wherein the programmable Cas14 nuclease is Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. 90. The method of any one of embodiments 65-89, wherein the programmable nuclease is a programmable Type VI CRISPR/Cas enzyme. 91. The method of any one of embodiments 65-90, wherein the programmable Type VI CRISPR/Cas enzyme is a programmable Cas13 nuclease. 92. The method of any one of embodiments 65-91, wherein the programmable Cas13 nuclease is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. 93. A method of designing a plurality of primers for amplification of a target nucleic acid, the method comprising: providing a target nucleic acid, wherein a guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between an F1c region and a B1 region or between an F1 and a B1c region; and designing the plurality of primers comprising: i) a forward

inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 94. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between the F1c region and a B1 region or between an F1 region and the B1c region; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 95. The method of any one of embodiments 65-94, wherein the sequence between the F1c region and the B1 region or the sequence between the B1c region and the F1 region is at least 50% reverse complementary to the guide nucleic acid sequence. 96. The method of any one of embodiments 65-95, wherein the guide nucleic acid sequence is reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, or a combination thereof. 97. The method of any one of embodiments 65-96, wherein the guide nucleic acid does not hybridize to the forward inner primer and the backward inner primer. 98. The method of any one of embodiments 65-97, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. 99. The method of any one of embodiments 65-98, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1 region and 5' of the F1c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1 region and 5' of the B1c region. 100. The method of any one of embodiments 65-99, wherein the 3' end of the target nucleic acid is 5' of the 5' end of the F3c region or the 3' end of the target nucleic acid is 5' of the 5' end of the B3c region. 101. The method of any one of embodiments 65-100, wherein the 3' end of the target nucleic acid is 5' of the 5' end of the F2c region or 3' end of the target nucleic acid is 5' of the 5' end of the B2c region. 102. The method of any one of embodiments 65-101, wherein the target nucleic acid is between the F1c region and the B1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the F2c region, or

wherein the target nucleic acid is between the B1c region and the F1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the B2c region. 103. The method of any one of embodiments 65-102, wherein the guide nucleic acid has a sequence reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 104. The method of any one of embodiments 65-103, wherein the guide nucleic acid sequence does not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 105. The method of any one of embodiments 65-104, wherein the guide nucleic acid sequence has a sequence reverse complementary to no more than 50% of a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. 106. The method of any one of embodiments 65-105, wherein the guide nucleic acid sequence does not hybridize to a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. 107. A method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a B2 region and a B1 region or between an F2 region and an F1 region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 108. A method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a F1c region and an F2c region or between a B1c region and a B2c region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 109. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target

nucleic acid comprises a sequence between a B2 region and a B1 region or between the F2 region and an F1 region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 110. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between the F1c region and an F2c region or between the B1c region and a B2c region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 111. The method of any one of embodiments 65-110, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B2 region and 5' of the B1 region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F2 region and 5' of the F1 region. 112. The method of any one of embodiments 65-111, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1c region and 5' of the B2c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1c region and 5' of the F2c region. 113. The method of any one of embodiments 65-112, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. 114. The method of any one of embodiments 65-113, wherein the PAM and the PFS are 5' of the 5' end of the F1c region, 5' of the 5' end of the B1c region, 3' of the 3' end of the F3 region, 3' of the 3' end of the B3 region, 3' of the 3' end of the F2 region, 3' of the 3' end of the B2 region, or any combination thereof. 115. The method of any one of embodiments 65-114, wherein the PAM and the PFS do not overlap the F2 region, the B3 region, the F1c region, the F2 region, the B1c region, the B2 region, or any combination thereof. 116. The method of any one of embodiments 65-115, wherein the PAM and the PFS do not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 117. The method of any one of embodiments 65-116,

wherein the plurality of primers further comprises a loop forward primer. 118. The method of any one of embodiments 65-117, wherein the plurality of primers further comprises a loop backward primer. 119. The method of any one of embodiments 65-118, wherein the loop forward primer is between an F1c region and an F2c region. 120. The method of any one of embodiments 65-119, wherein the loop backward primer is between a B1c region and a B2c region. 121. The method of any one of embodiments 65-120, wherein the target nucleic acid comprises a single nucleotide polymorphism (SNP). 122. The method of any one of embodiments 65-121, wherein the single nucleotide polymorphism (SNP) comprises a HERC2 SNP, an ALDH2 SNP, an EGFR SNP, a PNPLA3 SNP, a CYP2C19*2 SNP, a PAH SNP, a CFTR SNP, a ß-globin SNP, a DMD SNP, a APOB SNP, a LDLR SNP, a LDLRAP1 SNP, a PCSK9 SNP, a NF1 SNP, a PKD1 SNP, a DMPK SNP, a F9 SNP, a F8 SNP, a PKD1 SNP, a PHEX SNP, or a MECP SNP. 123. The method of any one of embodiments 65-122, wherein the single nucleotide polymorphism (SNP) is associated with an increased risk or decreased risk of cancer. 124. The method of any one of embodiments 65-123, wherein the target nucleic acid comprises a single nucleotide polymorphism (SNP), and wherein the detectable signal is higher in the presence of a guide nucleic acid that is 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP) than in the presence of a guide nucleic acid that is less than 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP). 125. The method of any one of embodiments 65-124, wherein the plurality of primers and the guide nucleic acid are present together in a sample comprising the target nucleic acid. 126. The method of any one of embodiments 65-125, wherein the amplifying and the contacting the sample to the guide nucleic acid occurs at the same time. 127. The method of any one of embodiments 65-126, wherein the amplifying and the contacting the sample to the guide nucleic acid occur at different times. 128. The method of any one of embodiments 65-127, wherein the method further comprises providing a polymerase, a dATP, a dTTP, a dGTP, a dCTP, or any combination thereof. 129. A method of assaying for a target nucleic acid in a sample, comprising: 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, 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 detector nucleic acids of a population of detector 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. 130. The method of embodiment 129, wherein the target nucleic acid is from 0.05% to 20% of total nucleic acids in the sample. 131. The method of any one of embodiments 129-130, wherein the target nucleic acid is from 0.1% to 10% of total nucleic acids in the sample. 132. The method of any one of embodiments 129-131, wherein the target nucleic acid is from 0.1% to 5% of total nucleic acids in the sample. 133. The method of any one of embodiments 129-132, wherein the contacting is performed in a buffer comprising heparin and NaCl. 134. The method of any one of embodiments 129-133, wherein the NaCl is from 50 mM NaCl to 200 mM NaCl. 135. The method of any one of embodiments 129-134, wherein the NaCl is 100 mM NaCl. 136. The method of any one of embodiments 129-135, wherein the heparin is from 20 μg/ml heparin to 100 μg/ml heparin. 137. The method any one of embodiments 129-136, wherein the heparin is 50 μg/ml heparin. 138. The method of any one of embodiments 129-137, wherein the sample comprises at least one nucleic acid comprising at least 80% sequence identity to the segment of the target nucleic acid. 139. The method of any one of embodiments 129-138, wherein the sample comprises at least one nucleic acid comprising at least 90% sequence identity to the segment of the target nucleic acid. 140. The method of any one of embodiments 129-139, wherein the sample comprises at least one nucleic acid comprising at least 99% sequence identity to the segment of the target nucleic acid. 141. The method of any one of embodiments 129-140, wherein the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 50% sequence identity to the segment of the target nucleic acid. 142. The method of any one of embodiments 129-141, wherein the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 80% sequence identity to the segment of the target nucleic acid. 143. The method of any one of embodiments 129-142, wherein the sample comprises at least one nucleic acid comprising less than 100% sequence identity to the segment of the target nucleic acid and no less than 90% sequence identity to the segment of the target nucleic acid. 144. The method of any one of embodiments 129-143, wherein the target nucleic acid comprises a single nucleotide mutation. 145. The method of any one of embodiments 129-144, wherein the segment of the target nucleic acid comprises a single nucleotide mutation. 146. The method of any one of embodiments 129-145, wherein the single nucleotide mutation is a synonymous substitution or a nonsynonymous substitution. 147. The method of any one of embodiments 129-146, wherein the synonymous substitution is a silent substitution. 148. The method of any one of embodiments 129-147, wherein the nonsynonymous substitution is a missense substitution or a nonsense point mutation. 149. The method of any one of embodiments 129-148, wherein the target nucleic acid

comprises a deletion. 150. The method of any one of embodiments 129-149, wherein the segment of the target nucleic acid comprises a deletion. 151. The method of any one of embodiments 129-150, wherein the deletion comprises a deletion of from 1 to 50 nucleotides. 152. The method of any one of embodiments 129-151, wherein the deletion comprises a deletion of from 9 to 21 nucleotides. 153. The method of any one of embodiments 129-152, further comprising amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to target sequence of an amplification product before the contacting. 154. The method of any one of embodiments 129-153, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 1-8 nucleotides from the 3' end of the sequence encoding the PAM. 155. The method of any one of embodiments 129-154, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 4 nucleotides from the 3' end of the sequence encoding the PAM. 156. The method of any one of embodiments 129-155, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 5 nucleotides from the 3' end of the sequence encoding the PAM. 157. The method of any one of embodiments 129-156, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 6 nucleotides from the 3' end of the sequence encoding the PAM. 158. The method of any one of embodiments 129-157, wherein the segment of the target nucleic acid comprises the single nucleotide mutation at 5-9 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 159. The method of any one of embodiments 129-158, wherein the segment of the target nucleic acid comprises the single nucleotide mutation at 6 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 160. The method of any one of embodiments 129-159, wherein the segment of the target nucleic acid comprises the single nucleotide mutation at 7 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 161. The method of any one of embodiments 129-160, wherein the segment of the target nucleic acid comprises the single nucleotide mutation at 8 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 162. The method of any one of embodiments 129-161, wherein the segment of the target nucleic acid comprises the deletion at 5-9 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 163. The method of any one of embodiments 129-162, wherein the segment of the target nucleic acid comprises the deletion at 6 nucleotides downstream of the

5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 164. The method of any one of embodiments 129-163, wherein the segment of the target nucleic acid comprises the deletion at 7 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 165. The method of any one of embodiments 129-164, wherein the segment of the target nucleic acid comprises the deletion at 8 nucleotides downstream of the 5' end the segment of the target nucleic acid comprising the sequence the encoding the PAM. 166. The method of any one of embodiments 129-165, further comprising amplifying the target nucleic acid before the contacting. 167. The method of any one of embodiments 129-166, wherein the amplifying the target nucleic acid before the contacting comprises using a blocking primer. 168. The method of any one of embodiments 129-167, wherein the blocking primer binds to a nucleic acid comprising encoding the wild type sequence of the target nucleic acid segment. 169. The method of any one of embodiments 129-168, wherein the amplifying comprises COLD-PCR. 170. The method of any one of embodiments 129-169, wherein the COLD-PCR comprises full COLD-PCR. 171. The method of any one of embodiments 129-170, wherein the COLD-PCR comprises fast COLD-PCR. 172. The method of any one of embodiments 129-171, wherein the amplifying comprises fast COLD-PCR. 173. The method of any one of embodiments 129-172, wherein the amplifying comprises allele-specific PCR. 174. The method of any one of embodiments 129-173, wherein the amplifying further comprises COLD-PCR. 175. The method of any one of embodiments 129-174, further comprising removing a nucleic acid comprising at least 50% sequence identity to the target nucleic acid by binding a protein to the nucleic acid before the contacting. 176. The method of any one of embodiments 129-175, wherein the protein is an antibody. 177. The method of any one of embodiments 129-176, wherein the protein is a programmable nuclease without endonuclease activity. 178. The method of any one of embodiments 129-177, further comprising binding a protein to the target nucleic acid to remove other nucleic acids of the sample. 179. The method of any one of embodiments 129-178, wherein the other nucleic acids comprise a nucleic acid comprising at least 50% sequence identity to the target nucleic acid. 180. The method of any one of embodiments 129-179, wherein the protein is attached to a surface. 181. The method of any one of embodiments 129-180, wherein the removing of the other nucleic acids comprises washing away nucleic acids that are not bound to the protein. 182. The method of any one of embodiments 129-181, wherein the protein is an antibody. 183. The method of any one of embodiments 129-182, wherein the protein is a programmable nuclease without endonuclease activity. 184. The method of any one of embodiments 129-183, wherein the programmable nuclease is a target nucleic acid activated effector protein that exhibits sequence independent

cleavage upon activation. 185. The method of any one of embodiments 129-184, wherein the programmable nuclease is an RNA guided nuclease. 186. The method of any one of embodiments 129-185, wherein the programmable nuclease comprises a Cas nuclease. 187. The method of any one of embodiments 129-186, wherein the Cas nuclease is Cas13. 188. The method of any one of embodiments 129-187, wherein the Cas13 is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e. 189. The method of any one of embodiments 129-188, wherein the Cas nuclease is Cas12. 190. The method of any one of embodiments 129-189, wherein the Cas12 is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. 191. The method of any one of embodiments 129-190, wherein the Cas nuclease is Cas14. 192. The method of any one of embodiments 129-191, wherein the Cas14 is Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. 193. The method of any one of embodiments 129-192, wherein the Cas nuclease is Csm1, Cas9, C2c4, C2c8, C2c5, C2c10, or C2c9. 194. The method of any one of embodiments 129-193, wherein the guide nucleic acid comprises a crRNA. 195. The method of any one of embodiments 129-194, wherein the guide nucleic acid comprises a crRNA and a tracrRNA. 196. The method of any one of embodiments 129-195, wherein cleavage of at least one detector nucleic acid yields a signal. 197. The method of any one of embodiments 129-196, wherein cleavage of at least one detector nucleic acid activates a photoexcitable fluorophore. 198. The method of any one of embodiments 129-197, wherein cleavage of at least one detector nucleic acid deactivates a photoexcitable fluorophore. 199. The method of any one of embodiments 129-198, wherein the signal is present prior to detector nucleic acid cleavage. 200. The method of any one of embodiments 129-199, wherein the signal is absent prior to detector nucleic acid cleavage. 201. The method of any one of embodiments 129-200, wherein the sample comprises 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. 202. The method of any one of embodiments 129-201, wherein the single nucleotide mutation is a single nucleotide polymorphism. 203. A method, comprising: contacting a programmable nuclease comprising a polypeptide having endonuclease activity and a guide nucleic acid to a target nucleic acid in a buffer comprising heparin. 204. The method of any one of embodiments 129-203, wherein the heparin is present at a concentration of from 1 to 100 μg/ml heparin. 205. The method of any one of embodiments 129-204, wherein the heparin is present at a concentration of from 40 to 60 μg/ml heparin. 206. The method of any one of embodiments 129-205, wherein the heparin is present at a concentration 50 μg/ml heparin. 207. The method of any one of embodiments 129-206, wherein the buffer further comprises NaCl. 208. The method of any one of embodiments 129-207, wherein the NaCl is present at a

concentration of from 1 to 200 mM NaCl. 209. The method of any one of embodiments 129-208, wherein the NaCl is present at a concentration of from 80 to 120 mM NaCl. 210. The method of any one of embodiments 129-209, wherein the NaCl is present at a concentration of 100 mM NaCl. 211. The method of any one of any one of embodiments 129-210, wherein the target nucleic acid is a substrate target nucleic acid. 212. The method of any one of embodiments 129-211, wherein the substrate nucleic acid comprises a cancer allele. 213. The method of any one of embodiments 129-212, wherein the cancer allele is present at a low concentration relative to a wild type allele. 214. The method of any one of embodiments 129-213, wherein the substrate target nucleic acid comprises a splice variant. 215. The method of any one of embodiments 129-214, wherein the substrate target nucleic acid comprises an edited base. 216. The method of any one of embodiments 129-215, wherein the substrate target nucleic acid comprises a bisulfite-treated base. 217. The method of any one of embodiments 129-216, wherein the substrate target nucleic acid comprises a segment that is reverse complementary to a segment of the guide nucleic acid. 218. A method of designing a plurality of primers for amplification of a target nucleic acid, the method comprising: providing a target nucleic acid, wherein a guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between an F1c region and a B1 region or between an F1 and a B1c region; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 219. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the guide nucleic acid hybridizes to the target nucleic acid and wherein at least 60% of a sequence of the target nucleic acid is between the F1c region and a B1 region or between an F1 region and the B1c region; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 220. The method of any one of embodiments

129-219, wherein the sequence between the F1c region and the B1 region or the sequence between the B1c region and the F1 region is at least 50% reverse complementary to the guide nucleic acid sequence. 221. The method of any one of embodiments 129-220, wherein the guide nucleic acid sequence is reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, or a combination thereof. 222. The method of any one of embodiments 129-221, wherein the guide nucleic acid does not hybridize to the forward inner primer and the backward inner primer. 223. The method of any one of embodiments 129-222, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. 224. The method of any one of embodiments 129-223, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1 region and 5' of the F1c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1 region and 5' of the B1c region. 225. The method of any one of embodiments 129-224, wherein the 3' end of the target nucleic acid is 5' of the 5' end of the F3c region or the 3' end of the target nucleic acid is 5' of the 5' end of the B3c region. 226. The method of any one of embodiments 129-225, wherein the 3' end of the target nucleic acid is 5' of the 5' end of the F2c region or 3' end of the target nucleic acid is 5' of the 5' end of the B2c region. 227. The method of any one of embodiments 129-226, wherein the target nucleic acid is between the F1c region and the B1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the F2c region, or wherein the target nucleic acid is between the B1c region and the F1 region and the 3' end of the target nucleic acid is 5' of the 3' end of the B2c region. 228. The method of any one of embodiments 129-227, wherein the guide nucleic acid has a sequence reverse complementary to no more than 50% of the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 229. The method of any one of embodiments 129-228, wherein the guide nucleic acid sequence does not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 230. The method of any one of embodiments 129-229, wherein the guide nucleic acid sequence has a sequence reverse complementary to no more than 50% of a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. 231. The method of any one of embodiments 129-230, wherein the guide nucleic acid sequence does not hybridize to a sequence of an F3c region, an F2c region, the F1c region, the B1c region, an B2c region, an B3c region, or any combination thereof. 232. A method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a B2 region and a B1 region or between an F2 region and an F1 region that

hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 233. A method of designing a plurality of primer for amplification of a target nucleic acid, the method comprising: providing the target nucleic acid comprising a sequence between a F1c region and an F2c region or between a B1c region and a B2c region that hybridizes to a guide nucleic acid; and designing the plurality of primers comprising: i) a forward inner primer comprising a sequence of the F1c region 5' of a sequence of an F2 region; ii) a backward inner primer comprising a sequence of the B1c region 5' of a sequence of a B2 region; iii) a forward outer primer comprising a sequence of an F3 region; and iv) a backward outer primer comprising a sequence of a B3 region. 234. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between a B2 region and a B1 region or between the F2 region and an F1 region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 235. A method of detecting a target nucleic acid in a sample, the method comprising: contacting the sample to: a plurality of primers comprising: i) a forward inner primer comprising a sequence corresponding to an F1c region 5' of a sequence corresponding to an F2 region; ii) a backward inner primer comprising a sequence corresponding to a B1c region 5' of a sequence corresponding to a B2 region; iii) a forward outer primer comprising a sequence corresponding to an F3 region; and iv) a backward outer primer comprising a sequence corresponding to a B3 region; a guide nucleic acid, wherein the target nucleic acid comprises a sequence between the F1c region and an F2c region or between the B1c region and a B2c region that hybridizes to the guide nucleic acid; a reporter; and a programmable nuclease that cleaves the reporter when complexed with the guide nucleic acid upon hybriziation of the guide nucleic acid to the target nucleic acid; and measuring a detectable signal produced

by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample. 236. The method of any one of embodiments 129-235, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B2 region and 5' of the B1 region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F2 region and 5' of the F1 region. 237. The method of any one of embodiments 129-236, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the B1c region and 5' of the B2c region or the protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the F1c region and 5' of the F2c region. 238. The method of any one of embodiments 129-237, wherein a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. 239. The method of any one of embodiments 129-238, wherein the PAM and the PFS are 5' of the 5' end of the F1c region, 5' of the 5' end of the B1c region, 3' of the 3' end of the F3 region, 3' of the 3' end of the B3 region, 3' of the 3' end of the F2 region, 3' of the 3' end of the B2 region, or any combination thereof. 240. The method of any one of embodiments 129-239, wherein the PAM and the PFS do not overlap the F2 region, the B3 region, the F1c region, the F2 region, the B1c region, the B2 region, or any combination thereof. 241. The method of any one of embodiments 129-240, wherein the PAM and the PFS do not hybridize to the forward inner primer, the backward inner primer, the forward outer primer, the backward outer primer, or any combination thereof. 242. The method of any one of embodiments 129-241, wherein the plurality of primers further comprises a loop forward primer. 243. The method of any one of embodiments 129-242, wherein the plurality of primers further comprises a loop backward primer. 244. The method of any one of embodiments 129-243, wherein the loop forward primer is between an F1c region and an F2c region. 245. The method of any one of embodiments 129-244, wherein the loop backward primer is between a B1c region and a B2c region. 246. The method of any one of embodiments 129-245, wherein the target nucleic acid comprises a single nucleotide polymorphism (SNP). 247. The method of any one of embodiments 129-246, wherein the single nucleotide polymorphism (SNP) comprises a HERC2 SNP. 248. The method of any one of embodiments 129-247, wherein the single nucleotide polymorphism (SNP) is associated with an increased risk or decreased risk of cancer. 249. The method of any one of embodiments 129-248, wherein the target nucleic acid comprises a single nucleotide polymorphism (SNP), and wherein the detectable signal is higher in the presence of a guide nucleic acid that is 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP) than in the presence of a guide nucleic acid that is less than 100% complementary to the target nucleic acid comprising the single nucleotide polymorphism (SNP). 250. The method of any one of embodiments 129-249, wherein the plurality of primers

and the guide nucleic acid are present together in a sample comprising the target nucleic acid. 251. The method of any one of embodiments 129-250, wherein the amplifying and the contacting the sample to the guide nucleic acid occurs at the same time. 252. The method of any one of embodiments 129-251, wherein the amplifying and the contacting the sample to the guide nucleic acid occur at different times. 253. The method of any one of embodiments 129-252, wherein the method further comprises providing a polymerase, a dATP, a dTTP, a dGTP, a dCTP, or any combination thereof. 254. A method of assaying for a target nucleic acid segment in a sample, wherein the target nucleic acid segment lacks a PAM sequence, comprising: amplifying the target nucleic acid segment using a primer having a region that is reverse complementary to the target nucleic acid segment and a region that has a PAM sequence reverse complement, thereby generating a PAM target nucleic acid having a PAM sequence adjacent to a target sequence of an amplification product; contacting the PAM target nucleic acid to a PAM-dependent sequence specific nuclease complex comprising a guide 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 PAM target nucleic acid; and assaying for cleavage of at least one detector nucleic acid of a population of detector nucleic acids, wherein the cleavage indicates a presence of the target nucleic acid in the sample and wherein the absence of the cleavage indicates an absence of the target nucleic acid in the sample. 255. The method of embodiment 254, wherein the sequence encoding the PAM comprises dUdUdUN. 256. The method any one of embodiments 254-255, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 3 nucleotides from the 3' end of the sequence encoding the PAM. 257. The method any one of embodiments 254-256, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 1-2 or 4-8 nucleotides from the 3' end of the sequence encoding the PAM. 258. The method of any one of embodiments 254-257, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 2 nucleotides from the 3' end of the sequence encoding the PAM. 259. The method of any one of embodiments 254-258, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 4 nucleotides from the 3' end of the sequence encoding the PAM. 260. The method of any one of embodiments 254-259, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 5 nucleotides from the 3' end of the sequence encoding the PAM. 261. The method of any one of embodiments 254-260, wherein the primer is a forward primer comprising the sequence encoding the PAM and has 6 nucleotides from the 3' end of the sequence encoding the PAM. 262. The method of any one of embodiments 254-261, wherein a mismatch for single nucleotide polymorphism

(SNP) detection is 3-10 nucleotides downstream of the PAM in PAM target nucleic acid. 263. The method of any one of embodiments 254-262, wherein a mismatch for single nucleotide polymorphism (SNP) detection is 6 nucleotides downstream of the PAM in PAM target nucleic acid. 264. The method of any one of embodiments 254-263, wherein a mismatch for single nucleotide polymorphism (SNP) detection is 7 nucleotides downstream of the PAM in PAM target nucleic acid. 265. The method of any one of embodiments 254-264, wherein a mismatch for single nucleotide polymorphism (SNP) detection is 8 nucleotides downstream of the PAM in PAM target nucleic acid. 266. The method of any one of embodiments 254-265, wherein the amplifying comprises thermal cycling amplification. 267. The method of any one of embodiments 254-266, wherein the amplifying comprises isothermal amplification. 268. The method of any one of embodiments 254-267, wherein the isothermal amplification is select from the group consisting of isothermal recombinase polymerase amplification (RPA), transcription mediated amplification (TMA), strand displacement amplification (SDA), helicase dependent amplification (HDA), loop mediated amplification (LAMP), rolling circle amplification (RCA), single primer isothermal amplification (SPIA), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), improved multiple displacement amplification (IMDA), and nucleic acid sequence-based amplification (NASBA). 269. The method of any one of embodiments 254-268, wherein the producing, the contacting, and the assaying are performed in a common reaction volume. 270. The method of any one of embodiments 254-269, wherein the programmable nuclease is a nucleic acid activated effector protein that exhibits sequence independent cleavage upon activation. 271. The method of any one of embodiments 254-270, wherein the programmable nuclease is an RNA guided nuclease. 272. The method of any one of embodiments 254-271, wherein the programmable nuclease comprises a Cas nuclease. 273. The method of any one of embodiments 254-272, wherein the Cas nuclease is Cas12. 274. The method of any one of embodiments 254-273, wherein the Cas12 is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. 275. The method of any one of embodiments 254-274, wherein the cas nuclease is Cas13. 276. The method of any one of embodiments 254-275, wherein the cas nuclease is Cas13a, Cas13b, Cas13c, or Cas13d. 277. The method of any one of embodiments 254-276, wherein the guide nucleic acid comprises a crRNA. 278. The method of any one of embodiments 254-277, wherein cleavage of at least one detector nucleic acid yields a signal. 279. The method of any one of embodiments 254-278, wherein cleavage of at least one detector nucleic acid activates a photoexcitable fluorophore. 280. The method of any one of embodiments 254-279, wherein cleavage of at least one detector nucleic acid deactivates a photoexcitable fluorophore. 281. The method of any one of embodiments 254-280, wherein the signal is present

prior to detector nucleic acid cleavage. 282. The method of any one of embodiments 254-281, wherein the signal is absent prior to detector nucleic acid cleavage. 283. The method of any one of embodiments 254-282, wherein the at least one detector nucleic acid comprises a nucleic acid comprising a detectable moiety. 284. The method of any one of embodiments 254-283, wherein the at least one detector nucleic acid comprises a nucleic acid comprising at least two nucleotides, a fluorophore, and a fluorescence quencher, wherein the fluorophore and the fluorescence quencher are linked by the nucleic acid. 285. The method of any one of embodiments 254-284, wherein the sample comprises 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. 286. The method of any one of embodiments 254-285, wherein the target nucleic acid comprises a sequence encoding a single nucleotide polymorphism (SNP). 287. The method of any one of embodiments 254-286, wherein the target nucleic acid comprises a sequence encoding a wild type sequence. 288. The method of any one of embodiments 254-287, wherein the SNP is in the EGFR gene. 289. The method of any one of embodiments 254-288, wherein the SNP is associated with a disease. 290. The method of any one of embodiments 254-289, wherein the SNP is a HERC2 SNP, an ALDH2 SNP, an EGFR SNP, a PNPLA3 SNP, a CYP2C19*2 SNP, a PAH SNP, a CFTR SNP, a ß-globin SNP, a DMD SNP, a APOB SNP, a LDLR SNP, a LDLRAP1 SNP, a PCSK9 SNP, a NF1 SNP, a PKD1 SNP, a DMPK SNP, a F9 SNP, a F8 SNP, a PKD1 SNP, a PHEX SNP, or a MECP SNP. 291. The method of any one of embodiments 254-290, wherein the disease is cancer. 292. The method of any one of embodiments 254-291, wherein the disease is a genetic disorder. 293. The method of any one of embodiments 254-292, wherein the SNP is associated with altered phenotype compared to a wild type sequence.

EXAMPLES

[00522] The following examples are illustrative and non-limiting to the scope of the devices, systems, fluidic devices, kits, and methods described herein.

EXAMPLE 1

Substrate screen (trans cleavage)

[00523] Trans cleavage assays were performed activity buffer (buffer: 120 mM NaCl, 5 mM $MgCl_2$, 20 mM Tris pH 7.5, 1% glycerol). A final concentration of 100nM and 50nM of different target dsDNA (varying in PAM and mismatches) and ssDNA-FQ reporter molecule were used in the assay respectively. Target dsDNA was obtained by annealing complementary ssDNA primers with 2:1 ratio of non-target strand to target strand in hybridization buffer (10X Hybridization

buffer: 500mM NaCl, 10mM Tris pH 8.0, 1mM EDTA) This ensures double-stranded DNA is being detected instead of single-stranded DNA.

[00524] crRNA was synthesized via in-vitro transcription using T7 RNA polymerase and a DNA template that consists of T7 binding site sequence followed by repeat region and targets sequence. Synthesized crRNA was then purified via bead purification and quantified using Quant-It miRNA kit.

[00525] To prepare the assay, a mastermix that consists of nuclease free water, ssDNA-FQ reporter, and 5X activity buffer was made and distributed to 12 different 1.5mL microcentrifuge tubes. Each tube is for each protein ortholog. Add the protein of interest and the guide RNA to each tube and proceed to incubation for 20 minutes at 37C. Transfer 16uL of each of the incubated mastermix per reaction.

[00526] To activate the assay, add 4uL of 500nM target dsDNA into the reaction. Place the plate to a fluorescence reader for 2 hours.

EXAMPLE 2

Cis (target) cleavage assays

[00527] Cis (target) cleavage assays were performed at 25°C or 37°C in activity buffer (120mM NaCl, 5mM $MgCl_2$, 20mM Tris pH 7.5, 1% glycerol). Cas12a–crRNA complex formation was performed in activity buffer, generally at a molar ratio of 1:1.25 protein to crRNA at 37 °C for 10 min, a target dsDNA. The target for cis-cleavage is a PCR product that is 1200bp long and contains the target sequence at the 700th position. A restriction site for BamHI was also introduced around the vicinity of the target sequence. Unless otherwise indicated, final concentrations of protein, guide and targets were 100nM, 125nM and 15nM, respectively, for all reactions. Reactions were quenched with 6X loading dye and resolved by prestained 2% agarose gel (1×TAE buffer). The cis cleavage reaction has the same conditions as the trans cleavage reaction but without a reporter molecule and a target dsDNA final concentration of 15nM.

EXAMPLE 3

Limit of Detection Assays

[00528] To monitor the limit of detection (LOD) of each chosen protein ortholog, DETECTR assay was used. The reaction cocktail is identical to that of the substrate screen assay. The only difference is the target concentration; target concentrations of 100nM, 10nM, 1nM, 100pM, 10pM, 1pM, 100fM, 10fM, and 1fM were prepared via rehybridization as mentioned above and serial dilution.

EXAMPLE 4

Guide Processing Assays.

[00529] Pre-crRNA cleavage assays are performed at 37°C in Activity Buffer based on previous buffer optimization experiments 100-fold molar excess of Cpf1 relative to synthesized crRNA (final concentrations of 100nM and <1nM, respectively). Unless otherwise indicated, the reaction is quenched after 1h with 2×RNA loading dye (100% formamide, 0.025% (w/v) bromophenol blue and 200μg /mL heparin). After quenching, reactions are denatured at 95°C for 2min before resolving by 15% denaturing PAGE (1×TBE buffer).

EXAMPLE 5

Temperature assays

[00530] Each protein is pre-complexed by adding its crRNA and preincubated at 25C for 1 hour. After the 1 hour period, each protein complex is incubated in different temperatures for 10 minutes. The temperatures are: 4, 22, 37, and 48. After incubation, the protein complex is tested via DETECTR.

EXAMPLE 6

Nickase assays

[00531] A pUC19 is treated with a Cas14 comprising a guide nucleic comprising a segment of nucleic acid that is reverse complementary with a segment of pUC19. After treatment, a band is produced when run on a gel that is higher than the linearized pUC19 fragment produced by digestion with EcoR1. A band that is higher than the linearized pUC19 is produced when no tracr nucleic acid is added to the treatment, and a band that is higher than the linearized pUC19 is produced when either a tracr nucleic acid comprising or lacking a PAM sequence is added to the treatment. This indicates that the Cas14 is a nickase and is PAM independent and tracr nucleic acid independent. However, a lower band than the linearized pUC19 is produced when no guide nucleic acid is added, indicating that the cleavage is guide nucleic acid directed.

EXAMPLE 7

Optimization of Temperature and Temperature Tolerance of CRISPR-Cas proteins in CRISPR Diagnostics

[00532] This example describes optimization of temperature and temperature tolerance of CRISPR-Cas proteins in CRISPR diagnostics. The CRISPR diagnostics of the present disclosure leverage the unique biochemical properties of Type V (*e.g.*, Cas12) and Type VI (*e.g.*, Cas13) CRISPR-Cas proteins to enable the specific detection of nucleic acids. These proteins are directed to their target nucleic acid by a CRISPR RNA (crRNA), which is also known as a guide

RNA (gRNA). Once bound to a complementary target sequence, the Cas protein initiates indiscriminate cleavage of surrounding single-strand DNA or single-strand RNA. When coupled to a quenched fluorescence reporter or other cleavage reporter, fluorescent or other signal can be generated by the Cas protein only in the presence of the target nucleic acid. CRISPR-Cas proteins have been isolated from a variety of natural contexts and therefore have different tolerances for elevated temperatures and optimal temperature ranges. These different tolerances for temperature can be used to activate or inhibit the proteins at different stages to allow for other molecular processes, such as target amplification, to occur.

[00533] A Cas12 variant (SEQ ID NO: 11), LbCas12a (SEQ ID NO: 1), and LbuCas13a (SEQ ID NO: 104) were incubated at 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C with a target nucleic acid sequence. Detection assays using the various Cas proteins were set up using 1 nM DNA target for Cas12 proteins and 25 pM RNA target for Cas13a. The max_rate (fluriescence units/2 min) was determined for evaluating the efficiency of the proteins at various temperatures. Darker squares indicate a higher max_rate and more efficient activity.

[00534] FIG. 18 shows activity of three programmable nucleases, a Cas12 variant (SEQ ID NO: 11), LbCas12a (SEQ ID NO: 1), and LbuCas13a (SEQ ID NO: 104, also referred to herein as Lbu C2C2). The results show that the functional range for the Cas12 variant (SEQ ID NO: 11) is between 25°C and 45°C, with maximal activity at 35°C. For the Type V Cas12 protein LbCas12a (SEQ ID NO: 1) the functional range is from 35°C to 50 °C with peak activity around 40°C. For the Type VI protein LbuCas13a (SEQ ID NO: 104) the functional range is between 25°C and 40°C with maximal activity between 30°C and 35°C. As suggested in **FIG. 18**, it appears that Type V proteins, such as the Cas12 variant (SEQ ID NO: 11) and LbCas12a (SEQ ID NO: 1), may be stable and functional at elevated temperatures. To test how stable each of these proteins are, proteins were incubated for 15 minutes at 45°C, 50°C, 55°C, 60°C, 65°C, or 70°C and then decreased the reaction temperature to 37°C.

[00535] FIG. 19 shows the results of incubating two Cas12 proteins, SEQ ID NO: 1 and SEQ ID NO: 11, for 15 minutes at 45°C, 50°C, 55°C, 60°C, 65°C, or 70°C and then decreasing the reaction temperature to 37°C. LbCas12a (SEQ ID NO: 1) was found to be functional even after incubation at 65°C. The Cas12 variant (SEQ ID NO: 11) was found to have no activity while at temperatures above 50°C, but after lowering the temperature to 37°C, the enzymatic activity of the protein returned. This temperature shifting may be exploitable for use in isothermal amplification methods, where the amplification occurs at a higher temperature, but after lowering the reaction temperature the Cas protein can be activated without compromising its functionality.

[00536] FIG. 20 shows that the stability of the Cas12 variant (SEQ ID NO: 11) at elevated temperatures is dependent on the buffer composition. Stability of the Cas12 variant was assessed after exposure to elevated temperatures for 30 minutes and then lowering the reaction temperature to 37°C. A variety of buffers were tested to determine their impact on the ability to turn the Cas12 variant on and off based on the reaction temperature. 0.5X NEBuffer4 (New England Biolabs, 1X: 50 mM Potassium Acetate; 20 mM Tris-acetate, pH 7.9; 10 mM Magnesium Acetate; 1 mM DTT) + 0.05% Tween gave the best results, followed by 1X MBuffer3 (20 mM HEPES pH 7.5; 2 mM Potassium Acetate; 5 mM Mg Acetate; 1% glycerol; 0.00016% Triton-X). 0.5X of Isothermal Amplification (IsoAmp) buffer (New England Biolabs) inhibited the Cas12 variant reaction completely.

EXAMPLE 8

Optimization of Assay Conditions for CRISPR DETECTR-based Diagnostic Assays

[00537] This example describes optimization of assay conditions for the CRISPR-Cas DETECTR-based diagnostic assays disclosed herein. The components of the DETECTR reaction, such as protein concentration, crRNA, and buffer components impact the rate and efficiency of the reaction. Optimization of the buffers allows for the development of an assay with increased sensitivity and specificity.

[00538] Improvements to buffers and assay conditions were identified for LbuCas13a (SEQ ID NO: 104) included 100 ng/μL of tRNA. The performance of a HEPES pH 6.8 buffer for Cas13a detection (20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 ng/μL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol) is shown on the graph is the middle-most line. Cas13a was incubated with 1 pM of target RNA at 37C with varying concentrations of tRNA in the reaction buffer. As a control, the assay was also performed with 0 pM of the target RNA. **FIG. 21** shows graphs of activity of a Cas13 (SEQ ID NO: 104), as measured by fluorescence, with (left graph) and without (right graph) activator over time. **FIG. 21** shows that increasing the amount of tRNA in the reaction decreases the efficiency of the Cas13a detection assay. Similarly, decreasing the amount of tRNA in the reaction or eliminating it completely, increases the efficiency of the Cas13a detection assay without dramatically changing the stability of the reaction in the absence of activator.

[00539] Urea is an additive that is used to increase the efficiency of some enzymatic reactions, such as proteinase K digestion, and is present in urine. To evaluate inhibition of Cas13a activity in the DETECTR assays, 1 pM of target RNA at 37° C was incubated with varying concentrations of urea. The activator, shown in the following graphs, is the target RNA. **FIG. 22** shows inhibition of Cas13a (SEQ ID NO: 104) activity by SDS and urea. **FIG. 22A** shows the

Cas13a (SEQ ID NO: 104) detection assay performed in the presence of 0-200 mM urea. Concentrations above 300 mM urea inhibited the assay (left graph shows with activator and right graph shows without activator). The orange line indicates the performance of the assay with 0 mM urea (a control showing uninhibited Cas13a activity). SDS is a common inhibitor of RNases and is used to eliminate RNase contamination and denature proteins. To evaluate inhibition of Cas13a activity in DETECTR assays, 1 pM target RNA at 37° C was incubated with varying amounts of SDS. **FIG. 22B** shows complete inhibition of Cas13a (SEQ ID NO: 104) upon addition of 0.1% or greater amounts of SDS to the reaction (left graph shows with activator and right graph shows without activator). The orange line indicates performance of Cas13a with 0% SDS (a control showing uninhibited Cas13a activity).

[00540] The importance of salt type and salt concentration on the performance of Cas13a in a DETECTR assay was evaluated. DETECTR assays were performed with 10 pM of target or 0 pM of target (control). **FIG. 23** shows the performance of Cas13a (SEQ ID NO: 104) in DETECTR reactions with varying concentrations of salt. **FIG. 23A** shows the results of varying the concentration of NaCl in a Cas13a (SEQ ID NO: 104) DETECTR reaction. **FIG. 23B** shows the results of varying the concentration of KCl in a Cas13a (SEQ ID NO: 104) DETECTR reaction. Cas13a performed comparably between NaCl and KCl salt types. Cas13a performance decreased at 30 mM salt and below, and was inhibited by salt concentrations above 80 mM.

[00541] The importance of DTT in different salt types and its impact on Cas13a (SEQ ID NO: 104) performance in a DETECTR assay was evaluated. DTT was used to stabilize proteins, such as RNase inhibitors, and increase the efficiency of some enzymes. DETECTR assays were carried out using Cas13a for detection of 10 pM of target or no target (control). **FIG. 24** shows optimization of DTT concentration in a Cas13a (SEQ ID NO: 104) DETECTR assay. **FIG. 24A** shows activity of a Cas13a (SEQ ID NO: 104) at varying DTT concentration in NaCl. **FIG. 24B** shows activity of a Cas13a (SEQ ID NO: 104) at varying DTT concentrations in KCl. The orange bar indicates buffer conditions with 50 mM KCl and no DTT. In addition to the indicated KCl and DTT concentration, each buffer condition also contained 20 mM HEPES pH 6.8, 5 mM $MgCl_2$, 10 μg/mL BSA, 100 μg/mL tRNA, 0.01% Igepal Ca-630 (NP-40), and 5% Glycerol). The results showed that the Cas13a DETECTR assay was not affected by DTT concentrations from 0 - 10 mM in buffers containing either NaCl or KCl.

[00542] Reporter choice for the Cas13a DETECTR assay was evaluated. The quenched fluorescent reporter generates the fluorescent signal that is used to monitor Cas13a detection performance in the DETECTR assays. A variety of different RNA reporter sequences was evaluated for their impact on assay performance. Cas13a detection assays were performed with

either 1 pM target RNA or no target RNA at 37°C. Reactions were performed in either a HEPES pH 6.8 Cas13a reaction buffer (HEPES pH 6.8 buffer with tRNA: 20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630; 5% glycerol) or in an identical buffer that lacked background tRNA "RNAlessPB". **FIG. 25** shows the activity of Cas13a (SEQ ID NO: 104) in the DETECTR assay, as measured by fluorescence, for each of the tested reporters. The "U5" reporter (/5-6FAM/rUrUrUrUrU/3IABkFQ/) and the "UU" reporter (/56-FAM/TArUrUGC/3IABkFQ/) exhibited the best performance. A reporter with the same nucleotide sequence as the "U5" reporter but with a different fluorophore and quencher, "TYE665U5" (/5- TYE665/rUrUrUrUrU/3IABkRQ/) also performed well. Increasing the length of the reporter generated higher background in processing buffers that did not contain background RNA.

[00543] The optimal buffer composition and pH for Cas13a DETECTR assays was identified. To determine the ideal buffer and pH for the Cas13a detection assay, 84 different combinations of buffers and pH were tested. The final buffer concentration used in each assay was 20 mM. Aside from the buffer itself, the remaining assay components included 50 mM KCl, 5 mM $MgCl_2$, 10 μg/mL BSA, 100 μg/ML tRNA, 0.01% Igepal Ca-630, and 5% Glycerol. Cas13a DETECTR assays were performed with 1 pM target RNA or no target RNA as a control. The dotted line indicates performance of a HEPES pH 6.8 Cas13a reaction buffer (also referred to as "HEPES pH 6.8 buffer"; HEPES pH 6.8 buffer with tRNA: 20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630; 5% glycerol). Dots indicate replicates. **FIG. 26** shows Cas13a activity in the DETECTR assay, as measured by fluorescence, for each of the tested conditions. These results demonstrated that the optimal pH is around 7.5 and that the imidazole, phosphate, tricine, and SPG buffers are all high performing buffers, in comparison to the HEPES pH 6.8 buffer (20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol). Cas13a detection was inhibited at pH values below 6.5.

[00544] Cas13a activity in DETECTR assays was assessed in a variety of commercially available buffers. Cas13a detection assays were carried out with either 1 pM target RNA or no target RNA at 37°C. Reactions were performed either in the presence or absence of 100 ng/μL tRNA. Buffers used included NEB1 (NEBuffer1, New England Biolabs (NEB)), NEB2 (NEBuffer2, NEB), NEB3 (NEBuffer3, NEB), Cutsmart (NEB), RNPB (RNA polymerase buffer, NEB), and the HEPES pH 6.8 buffer (20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 μg/mL BSA; 100 μg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol). These buffer compositions are as follows: NEBuffer 1.1 (1X Buffer Components, 10mM Bis-Tris-Propane-

HCl, 10mM $MgCl_2$, 100µg/ml BSA, pH 7.0@25°C); NEBuffer 2.1 (1X Buffer Components, 50mM NaCl, 10mM Tris-HCl, 10mM $MgCl_2$, 100µg/ml BSA, pH 7.9@25°C); NEBuffer 3.1 (1X Buffer Components, 100mM NaCl, 50mM Tris-HCl, 10mM $MgCl_2$, 100µg/ml BSA, pH 7.9@25°C); CutSmart Buffer (1X Buffer Components, 50mM Potassium Acetate, 20mM Tris-acetate, 10mM Magnesium Acetate, 100µg/ml BSA, pH 7.9@25°C); and 1X RNAPol Reaction Buffer (40 mM Tris-HCl, 6 mM $MgCl_2$, 1 mM DTT, 2 mM spermidine (pH 7.9 @ 25°C)). The results demonstrated that Cas13a performance improved in NEBuffer2 and Cutsmart in comparison to the HEPES pH 6.8 buffer (20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 µg/mL BSA; 100 µg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol). **FIG. 27** shows Cas13a (SEQ ID NO: 104) performance in the DETECTR assay, as measured by fluorescence, for each of the five commercially available buffers and a HEPES pH 6.8 buffer (20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 µg/mL BSA; 100 µg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol).

[00545] Combining the above described observations of buffer performance, an high performance Cas13a buffer called MBuffer1 was developed. 1X MBuffer1 include 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 µg/µL BSA, 0.01% Igepal Ca-630, and 5% glycerol. **FIG. 28** shows a comparison of a HEPES pH 6.8 buffer ("Original Buffer," 20 mM HEPES pH 6.8; 50 mM KCl; 5 mM $MgCl_2$; 10 µg/mL BSA; 100 µg/mL tRNA; 0.01% Igepal Ca-630 (NP-40); 5% Glycerol) to the high performance buffer ("MBuffer1," 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 µg/µL BSA, 0.01% Igepal Ca-630, and 5% glycerol) for a Cas13a DETECTR assay with serially diluted target RNAs and run at 37°C for 30 minutes. The limit of detection for the HEPES pH 6.8 buffer was around 1 pM, whereas the limit of detection for the high performance buffer was found to be between 100 fM and 10 fM. Thus, **FIG. 28** demonstrates that there is a 10X and 100X improvement in assay performance using the high performance buffer.

[00546] Cas13a performance in DETECTR assays was evaluated with and without glycerol. Glycerol is commonly used in many enzymatic buffers. Cas13a detection assays with varying concentrations of target RNA were run at 37°C for 30 minutes in either an high performance buffer with glycerol ("MBuffer1," 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 µg/µL BSA, 0.01% Igepal Ca-630, and 5% glycerol) or an high performance buffer without glycerol ("MBuffer1 – no glycerol," 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 10 µg/µL BSA, and 0.01% Igepal Ca-630). **FIG. 29** shows that 5% glycerol in the high performance buffer ("MBuffer1," left graph) increases performance of a Cas13a (SEQ ID NO: 104)

DETECTR assay in comparison to an identical buffer without glycerol ("MBuffer1 – no glycerol," right graph).

[00547] Cas13a performance in DETECTR assays was evaluated with varying concentrations of BSA and NP-40. BSA and NP-40 (Igecal-Ca 630) are used in many enzymatic buffers to increase assay performance and decrease binding of the protein to plastic surfaces. Cas13a DETECTR assays were run with 1 pM target RNA or no target RNA at 37°C for 30 minutes in an high performance buffer with varying concentrations and combinations of NP-40 (Igepal Ca-630) and BSA. In addition to the indicated concentrations of NP-40 and BSA, each buffer contained 20 mM imidazole pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, and 5% glycerol. **FIG. 30** shows a gradient chart of Cas13a (SEQ ID NO: 104) activity in the DETECTR assay, as measured by fluorescence, (darker squares indicate increased Cas13a activity) versus varying NP-40 concentration along the x-axis and varying BSA concentration along the y-axis. The results indicated that both BSA and NP-40 improve the assay. NP-40 (Igecal-Ca 630) was found to be important for the efficiency of the Cas13a detection assay. Small amounts of BSA also improved the performance of the assay. Concentrations of 0.05% to 0.0625% NP-40 were most optimal and concentrations of 2.5 to 0.625 μg/mL BSA were most optimal. BSA did not improve assay performance unless NP-40 was also present.

[00548] To determine which types of compounds may increase or inhibit the performance of Cas13a in DETECTR assays, assays were run with 96 different additives (JBScreen Plus HTS, Jena Biosciences). Additives from the Jena Biosciences plate were diluted 1:66 into the final Cas13a DETECTR assay with 100 pM of target. **FIG. 31** shows Cas13a performance in DETECTR assays, as measured by fluorescence, versus the different additives tested. Results showed that the specific compounds that inhibited the performance of the assay included: beryllium sulfate, manganese chloride, zinc chloride, tri-sodium citrate, copper chloride, yttrium chloride, 1-6- Diaminohexane, 1-8-diaminooctane, ammonium fluoride, ethanolamine, lithium salicylate, magnesium sulfate, potassium cyanate, and sodium fluoride.

[00549] A buffer developed for LbCas12a (SEQ ID NO: 1) used Tris pH 7.5. **FIG. 32** shows the results of screening 84 different buffer and pH combinations to determine the optimal buffer for LbCas12a activity in DETECTR assays, as measured by fluorescence. A final buffer concentration of 20 mM was used for each assay. The remaining assay components included 100 mM KCl, 5 mM $MgCl_2$, 50 μg/mL heparin, 1 mM DTT, and 5% Glycerol. LbCas12a DETECTR assays were performed at 37°C with 100 pM target DNA or no target DNA as a control. The dotted line indicates performance of LbCas12a in the Tris pH 7.5 buffer (20 mM Tris-HCl, pH 7.5; 100 mM KCl; 5 mM $MgCl_2$; 1 mM DTT; 5% glycerol; 50 μg/mL heparin). Dots indicate

replicates. Results of this experiment showed that LbCas12a prefers pH 8.0 and works well in AMPD, BIS-TRISpropane, DIPSO, HEPES, MOPS, TAPS, TRIS, and tricine buffers. LbCas12a was inhibited at pH 6.5 and below and was not functional in phosphate, succinate, malonate, citrate, MES, and ADA buffers.

[00550] The optimal salt type and salt concentration was determined for LbCas12a performance in DETECTR assays. LbCas12a DETECTR assays were run with 10 pM of target DNA or no target DNA at 37°C for 30 minutes with varying concentrations of KCl. **FIG. 33** shows LbCas12a performance in DETECTR assays, as measured by fluorescence, in each of the tested conditions. Results indicated that the LbCas12a performed best in assays with low KCl concentrations (0-40 mM or less than 20 mM salt and less KCl). Above 80 mM the assay was inhibited, with little to no activity above 160 mM.

[00551] The optimal buffer type and pH was determined for the Type V CRISPR-Cas Cas12 variant (SEQ ID NO: 11) performance in DETECTR assays. **FIG. 34** shows the performance of SEQ ID NO: 11 in DETECTR assays, as measured by fluorescence, for each of the tested conditions (buffer type and pH). The final concentration of buffer in each assay was 20 mM. The remaining assay components included 120 mM NaCl, 5 mM $MgCl_2$, and 1% Glycerol. SEQ ID NO: 11 DETECTR assays were performed at 37°C with 1 nM target DNA or no target DNA (0 nM) as a control. The dotted line indicates the performance of Cas12 variant in the Tris pH 7.5 buffer (20 mM Tris-HCl, pH 7.5; 100 mM KCl; 5 mM $MgCl_2$; 1 mM DTT; 5% glycerol; 50 µg/mL heparin). Results showed that SEQ ID NO: 11 performed optimally in a pH of 7.5. High performance buffers included DIPSO, HEPES, MOPS, TAPS, imidazole, and tricine. SEQ ID NO: 11 was inhibited in Tris buffers but was still functional. SEQ ID NO: 11 showed little or no functional activity in succincate, malonate, MES, ADA, citrate, SPG, and phosphate buffers.

[00552] Further investigation of the optimal buffer type and pH was carried out for SEQ ID NO: 11. Some proteins prefer buffers that have reduced numbers of chloride ions. To determine whether SEQ ID NO: 11 performed better in chloride- or acetate-based buffers, a screen of salt type and concentration was carried out. **FIG. 35** shows SEQ ID NO: 11 performance in DETECTR assays, as measured by fluorescence, for the various salt types and concentrations tested. Assay components included 20 mM HEPES pH 7.3, 1% Glycerol, and 5 mM of MgCl or MgOAc. Varying amounts of KCl or KOAc were screened with the corresponding magnesium type. SEQ ID NO: 11 detection assays were carried out at 37°C with 1 nM target DNA and 0 nM target DNA as a control for 30 minutes. SEQ ID NO: 11 performed best at low salt concentrations of around 4 mM (ranging from 2-10 nM) and showed increased activity in buffers with MgOAc and KOAc (acetate buffers), in comparison to buffers with MgCl and KCl.

[00553] The optimal concentrations of heparin and salt concentrations were determined for SEQ ID NO: 11, since a relationship was observed between salt and heparin for SNP sensitivity using LbCas12a (SEQ ID NO: 1). The base buffer included 20 mM HEPES pH 7.3, 5mM MgOAc, and 1% Glycerol. Varying amounts of KOAc and heparin were screened. SEQ ID NO: 11 DETECTR assays were performed at 37°C with 1 nM target DNA or no target DNA as a control for 30 minutes. For LbCas12a heparin and salt concentrations combined to affect the specificity of the enzyme. **FIG. 36** shows SEQ ID NO: 11 performance in DETECTR assays, as measured by fluorescence (darker squares indicate greater fluorescence and more activity), versus heparin concentration on the x-axis and KOAc buffer concentration on the y-axis. The results of this experiment indicated that SEQ ID NO: 11 trans-cleavage activity was inhibited by heparin and SEQ ID NO: 11 prefers low salt.

[00554] Inhibitors and enhancers of assay performance was evaluated for SEQ ID NO: 11 DETECTR assays. DETECTR assays were run with 96 different additives (JBScreen Plus HTS, Jena Biosciences). Additives from the Jena Biosciences plate were diluted 1:66 into a final SEQ ID NO: 11 detection assays with 1 nM of target. **FIG. 37** shows that specific compounds inhibited the performance of the Cas12 variant (SEQ ID NO: 11) DETECTR assay including: benzamidine hydrochloride, beryllium sulfate, manganese chloride, potassium bromide, sodium iodine, zinc chloride, di-ammonium hydrogen phosphate, tri-lithium citrate, tri-sodium citrate, cadmium chloride, copper chloride, yttrium chloride, 1-6 diaminohexane, 1-8-diaminooctane, ammonium fluoride, and ammonium sulfate. Compounds that increased assay performance included: polyvinyl alcohol type II, DTT, DMSO, polyvinylpyrrolidone K15, polyethylene glycol (PEG) 600, and polypropylene glycol 400. Concentrations in the legend are listed as the stock concentration. Buffer concentrations in the assay are 2% of the concentration listed in the figure legend. In addition to the buffers indicated on the x-axis, the remaining assay components included 120 mM NaCl, 5 mM $MgCl_2$, and 1% glycerol. The dotted line indicates the performance of the Cas12 variant in the Tris pH 7.5 buffer (20 mM Tris-HCl, pH 7.5; 100 mM KCl; 5 mM $MgCl_2$; 1 mM DTT; 5% glycerol; 50 μg/mL heparin).

[00555] The positions along a target sequence most sensitive to single mutations was identified by tiling all nucleotide possibility (A, T, C, G) at the 20 positions downstream of the PAM motif along a SEQ ID NO: 11 target site on HERC2 and ALDH. **FIG. 38** shows the results of evaluating SNP sensitivity along target sequences for SEQ ID NO: 11. Purple squares indicate the WT sequence that matched the crRNA was used to interrogate the sensitivity of SEQ ID NO: 11 to mutations along a target site on HERC2 and ALDH. Results indicated stronger SNP differentiation for SEQ ID NO: 11 along the 3' end of the crRNA (distal from the PAM). A

similar complementary experiment using LbCas12a using the same sets of target sites and crRNAs was carried out. **FIG. 39** shows the results of evaluating SNP sensitivity along target sequences for LbCas12a. LbCas12a displayed strong mutation sensitivity at all positions along HERC2, and sensitivity on the PAM proximal (complementary to the 5' end of the crRNA target sequence) on ALDH2. This suggested that LbCas12a was more sensitive to mutations in this region and that mutation sensitivity as target site dependent.

EXAMPLE 9

Volumes of Sample and the Detection Reaction

[00556] This example describes volumes of sample and the detection reaction of DETECTR assays provided herein. A first volume containing a sample is provided. The first volume is contacted to a second volume. The second volume contains a guide nucleic acid, a programmable nuclease (e.g., a Cas12 or a Cas13), and a reporter. The first volume contains a sample that is unlysed, a sample that has been lysed, or a sample that has been lysed and undergone: reverse transcription, amplification, in vitro transcription, or any combination thereof. The sample contains a buffer for cell lysis, a buffer for amplification, a primer, a polymerase, target nucleic acid, a non-target nucleic acid, a single-stranded DNA, a double-stranded DNA, a salt, a buffering agent, an NTP, a dNTP, or any combination thereof. The first volume is 1 to 5 μL. The second volume is 18 to 22 μL. The programmable nuclease is able to efficiently and rapidly cleave a nucleic acid of the reporter and the detectable signal produced in the presence of a target nucleic acid sequence in the first volume is not dampened.

EXAMPLE 10

Primer Design for Combined LAMP and DETECTR Reactions

[00557] This example describes primer design for combined LAMP and DETECTR reactions for amplification and detection of a target nucleic acid, as provided herein. Strategies for designing primers for use in combined LAMP and DETECTR reactions were tested and evaluated for multiple target nucleic acids. From these experiments, a set of design guidelines was determined to facilitate combined LAMP and DETECTR reactions for DNA nucleic acid targets or RT-LAMP and DETECTR reactions for RNA nucleic acid targets.

[00558] FIG. 40 shows a scheme for designing primers for loop mediated isothermal amplification (LAMP) of a target nucleic acid sequence. LAMP generates concatemer amplicons, comprising the target nucleic acid sequence, that form from nucleic acid loops during amplification. To generate the loops, LAMP may use from four to six primers, including the

forward outer primer, the backward outer primer, the forward inner primer, the backward inner primer, optionally a loop forward primer, and optionally a loop backward primer.

[00559] FIG. 41 shows schematics of exemplary configurations of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for amplification and detection by LAMP and DETECTR.

[00560] FIG. 41A shows a schematic of an exemplary arrangement of the guide RNA (gRNA) with respect to the various regions of nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA is reverse complementary to a sequence of the target nucleic acid, which is between an F1c region and a B1 region.

[00561] FIG. 41B shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA is partially reverse complementary to a sequence of the target nucleic acid, which is between an F1c region and a B1 region. For example, the target nucleic acid comprises a sequence between an F1c region and a B1 region that is reverse complementary to at least 60% of a guide nucleic acid. In this arrangement, the guide RNA is not reverse complementary to the forward inner primer or the backward inner primer shown in **FIG. 40**.

[00562] FIG. 41C shows a schematic of an exemplary arrangement of the guide RNA with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA hybridizes to a sequence of the target nucleic acid, which is within the loop region between the B1 region and the B2 region. The primer sequences do not contain and are not reverse complementary to the PAM or PFS.

[00563] FIG. 41D shows a schematic of an exemplary arrangement of the guide RNA with respect to the various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the guide RNA hybridizes to a sequence of the target nucleic acid, which is within the loop region between the F2c region and F1c region. The primer sequences do not contain and are not reverse complementary to the PAM or PFS.

[00564] Primer sets and guide RNAs for combined LAMP and DETECTR reactions were tested for their sensitivity and specificity to detect the presence of a target nucleic acid in a sample. DETECTR signal, measured as raw fluorescence, was measured for each LAMP primer set with each of three guide RNAs designed for the specific LAMP primer set. DETECTR signal was measured in a sample containing 10000 copies of a target nucleic acid sequence and a sample

containing zero copies of a target nucleic acid sequence (negative control) for each LAMP primer and guide RNA pair.

[00565] FIG. 42 shows schematics of exemplary configurations of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences for combined LAMP and DETECTR for amplification and detection, respectively. At the right, the schematics also show corresponding fluorescence data using guide RNA sequences to detect the presence of a target nucleic acid sequence following amplification of the target nucleic acid using the LAMP amplification, where a fluorescence signal is the output of the DETECTR reaction and indicates presence of the target nucleic acid. Sequences and arrangements of the regions that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences are illustrated in **FIG. 43A – FIG. 43C**. Three exemplary guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) were tested in each primer configuration. Fluorescence signal from the DETECTR reactions, indicative of detection of a target nucleic acid, measured for each of the three guide RNAs was compared for two samples, one containing the target nucleic acid sequence (1000 genome copies per reaction) and a negative control (0 genome copies per reaction) that does not contain the target nucleic acid sequence. Sequences of the gRNAs and the primers are shown below in **TABLE 9**.

TABLE 9 – Exemplary LAMP Primer and DETECTR gRNA Sets

SEQ ID NO:	Name	Sequence
SEQ ID NO: 201	IAVE-MP-set5-F3	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 249	IAVE-MP-set5-F2	ATGAGTCTTCTAACCGAGGT
SEQ ID NO: 205	IAVE-MP-set5-LF	TGACGGGACGATAGAGAGAA
SEQ ID NO: 250	IAVE-MP-set5-F1c	TTCAAGTCTCTGCGCGATCTC
SEQ ID NO: 251	IAVE-MP-set5-B1c	TTGAGGCTCTCATGGAATGGC
SEQ ID NO: 206	IAVE-MP-set5-LB	ACAAGACCAATCCTGTCACC
SEQ ID NO: 252	IAVE-MP-set5-B2	AGCGTGAACACAAATCCTAA
SEQ ID NO: 202	IAVE-MP-set5-B3	CATTCCCATTGAGGGCATT
SEQ ID NO: 210	IAVE-MP-set8-F3	TCTTCTAACCGAGGTCGAA
SEQ ID NO: 253	IAVE-MP-set8-F2	GAAGATGTCTTTGCAGGGAA
SEQ ID NO: 214	IAVE-MP-set8-LF	ATTCCATGAGAGCCTCAAGATC
SEQ ID NO: 254	IAVE-MP-set8-F1c	TCAGAGGTGACAGGATTGGTCT

SEQ ID NO:	Name	Sequence
SEQ ID NO: 255	IAVE-MP-set8-B1c	TTGTGTTCACGCTCACCGTG
SEQ ID NO: 215	IAVE-MP-set8-LB	GAGGACTGCAGCGTAGAC
SEQ ID NO: 202	IAVE-MP-set8-B2	CATTCCCATTGAGGGCATT
SEQ ID NO: 211	IAVE-MP-set8-B3	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 256	IAVE-MP-set1-F3	GACTTGAAGATGTCTTTGCA
SEQ ID NO: 257	IAVE-MP-set1-F2	CAGATCTTGAGGCTCTC
SEQ ID NO: 258	IAVE-MP-set1-LF	GTCTTGTCTTGTCTTTAGCCA
SEQ ID NO: 259	IAVE-MP-set1-F1c	TTAGTCAGAGGTGACAGGATTG
SEQ ID NO: 255	IAVE-MP-set1-B1c	TTGTGTTCACGCTCACCGTG
SEQ ID NO: 188	IAVE-MP-set1-LB	CAGTGAGCGAGGACTG
SEQ ID NO: 260	IAVE-MP-set1-B2	TTTGGACAAAGCGTCTACG
SEQ ID NO: 184	IAVE-MP-set1-B3	TGTTGTTTGGGTCCCCATT
SEQ ID NO: 261	gRNA1	UUUGUGUUCACGCUCACCGUGCCC
SEQ ID NO: 262	gRNA2	UUUAGCCAUUCCAUGAGAGCCUCA
SEQ ID NO: 263	gRNA3	UUUGGACAAAGCGUCUACGCUGCA

[00566] FIG. 42A shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 205, SEQ ID NO: 206, and SEQ ID NO: 249 – SEQ ID NO: 252) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 partially overlaps with the B2c region and is, thus, reverse complementary to a portion of to the B2 region. gRNA2 overlaps with the B1 region and is, thus, reverse complementary to the B1c region. gRNA3 partially overlaps with the B3 region and partially overlaps with the B2 region and is, thus, partially reverse complementary to the B3c region and partially reverse complementary to the B2c region. The complementary regions (B1, B2c, B3c, F1, F2c, and F3c) are not depicted, but correspond to the regions shown in **FIG. 40**. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000 genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid. DETECTR reactions with gRNA1 and gRNA3 exhibited low fluorescence intensity, indicating low to no detection of the target nucleic acid (right). gRNA2 produced a fluorescent signal independent of the presence of the target nucleic acid due to hybridization of gRNA2 with the B1c region of the BIP and self-activation of the guide RNA and Cas cleavage activity. Hybridization of gRNA2 with the BIP may further lead to amplification of a non-target

sequence due to the formation of a primer dimer. The sequences and arrangements of the regions that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences are shown in **FIG. 43A**.

[00567] FIG. 42B shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 202, SEQ ID NO: 210, SEQ ID NO: 211, SEQ ID NO: 214, SEQ ID NO: 215, SEQ ID NO: 253 – SEQ ID NO: 255) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 overlaps with the B1c region and is, thus, reverse complementary to the B1 region. gRNA2 overlaps with the LF region and is, thus, reverse complementary to the LFc region. gRNA 3 partially overlaps with the B2 region and partially overlaps with the LBc region and is, thus, partially reverse complementary to the B2c region and is partially reverse complementary to the LB region. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000 genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid. All three guide RNAs detected the presence of the target nucleic acid in DETECTR reactions, as evidenced by a high fluorescence signal in the presence of the target nucleic acid (right). gRNA1 also produced a non-specific fluorescent signal in the absence of the target nucleic acid due to primer-dimer formation with the BIP. gRNA2 and gRNA3 did not produce a substantial non-specific fluorescent signal. The sequences and arrangements of the regions that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences are shown in **FIG. 43B**.

[00568] FIG. 42C shows a schematic of an arrangement of various regions of the nucleic acid sequence that correspond to or anneal LAMP primers (SEQ ID NO: 184, SEQ ID NO: 188, SEQ ID NO: 255 – SEQ ID NO: 260) and positions of three guide RNAs (gRNA1 (SEQ ID NO: 261), gRNA2 (SEQ ID NO: 262), and gRNA3 (SEQ ID NO: 263)) relative to the LAMP primers (at left). gRNA1 overlaps with the B1c region and is, thus, reverse complementary to the B1 region. gRNA2 partially overlaps with the LF region and partially overlaps with the F2c region and is, thus, partially reverse complementary to the LFc region and partially reverse complementary to the F2 region. gRNA3 overlaps with the B2 and is, thus, reverse complementary to the B2c region. At right is a graph of fluorescence from the DETECTR reaction in the presence of 10,000 genome copies (before amplification) of the target nucleic acid or 0 genome copies of the target nucleic acid. gRNA2 and gRNA3 specifically detected the presence of the target nucleic acid in

DETECTR reactions, as evidenced by a high fluorescence signal in the presence of the target nucleic acid and low fluorescence signal in the absence of the target nucleic acid (right). gRNA1 detected the presence of the target nucleic acid in a DETECTR reaction but also non-specifically produced a fluorescence signal in the absence of the target nucleic acid due to primer-dimer formation with the BIP, as evidenced by a high fluorescence signal in the presence of the target nucleic acid and a moderate fluorescence signal in the absence of the target nucleic acid. The sequences and arrangements of the regions that correspond to or anneal LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acid sequences are shown in **FIG. 43C**.

EXAMPLE 11

Detection of a Target Nucleic Acid with Combined LAMP and DETECTR Reactions

[00569] This example describes detection of a target nucleic acid with combined LAMP and DETECTR reactions. Ten LAMP primer sets (#1 – #10) for use in RT-LAMP assays were tested for sensitivity and specificity for samples containing a target nucleic acid sequence. Detection following RT-LAMP amplification was performed using either SYTO 9 detection or DETECTR. The sequences of the LAMP primers in each primer set are provided in **TABLE 10**.

TABLE 10 – LAMP Primers for RT-LAMP Amplification and Detection

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 138	F3 RSV-A-set13	#1	TGGAACAAGTTGTGGAGG
SEQ ID NO: 139	B3 RSV-A-set13	#1	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 140	FIP RSV-A-set13	#1	TAGTGATGCTTTTGGGTTGTTCAAT TGTATGAGTATGCTCAAAAATTGG
SEQ ID NO: 141	BIP RSV-A-set13	#1	GTGTAGTATTGGGCAATGCTGCTCC TTGGTGTACCTCTGT
SEQ ID NO: 142	LF RSV-A-set13	#1	TATGGTAGAATCCTGCTTCTCC
SEQ ID NO: 143	LB RSV-A-set13	#1	TGGCCTAGGCATAATGGGAGA
SEQ ID NO: 144	F3 RSV-A-set14	#2	AACAAGTTGTGGAGGTGTA

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 145	B3 RSV-A-set14	#2	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 146	FIP RSV-A-set14	#2	TAGTGATGCTTTTGGGTTGTTCAAG AGTATGCTCAAAAATTGGGTG
SEQ ID NO: 147	BIP RSV-A-set14	#2	GTATTGGGCAATGCTGCTGGCATAT AGATCTTGATTCCTTGGTG
SEQ ID NO: 148	LF RSV-A-set14	#2	ATATGGTAGAATCCTGCTTCTC
SEQ ID NO: 149	LB RSV-A-set14	#2	CCTAGGCATAATGGGAGAATAC
SEQ ID NO: 144	F3 RSV-A-set15	#3	AACAAGTTGTGGAGGTGTA
SEQ ID NO: 145	B3 RSV-A-set15	#3	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 150	FIP RSV-A-set15	#3	ATAGTGATGCTTTTGGGTTGTTCAA GTATGCTCAAAAATTGGGTG
SEQ ID NO: 151	BIP RSV-A-set15	#3	GCTGCTGGCCTAGGCATAATGCATC ATATAGATCTTGATTCCTT
SEQ ID NO: 406	LF RSV-A-set15	#3	TATATGGTAGAATCCTGCTTCTC
SEQ ID NO: 152	LB RSV-A-set15	#3	GGGAGAATACAGAGGTACAC
SEQ ID NO: 153	F3 RSV-A-set16	#4	GGGTCTTAGCAAAATCAGTT
SEQ ID NO: 139	B3 RSV-A-set16	#4	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 154	FIP RSV-A-set16	#4	GAATCCTGCTTCTCCACCCAATTGA CACGCTAGTGTACAAGC
SEQ ID NO: 141	BIP RSV-A-set16	#4	GTGTAGTATTGGGCAATGCTGCTCC TTGGTGTACCTCTGT
SEQ ID NO: 155	LF RSV-A-set16	#4	CCTCCACAACTTGTTCCATTTCT

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 156	LB RSV-A-set16	#4	TGGCCTAGGCATAATGGGAG
SEQ ID NO: 157	F3 RSV-A-set17	#5	AAGCAGAAATGGAACAAGTT
SEQ ID NO: 145	B3 RSV-A-set17	#5	CCATTTTCTTTGAGTTGTTCAG
SEQ ID NO: 158	FIP RSV-A-set17	#5	TAGTGATGCTTTTGGGTTGTTCAGT GGAGGTGTATGAGTATGC
SEQ ID NO: 159	BIP RSV-A-set17	#5	GTAGTATTGGGCAATGCTGCTGATA TAGATCTTGATTCCTTGGTG
SEQ ID NO: 160	LF RSV-A-set17	#5	TGCTTCTCCACCCAATTTTTGA
SEQ ID NO: 161	LB RSV-A-set17	#5	GCCTAGGCATAATGGGAGAATAC
SEQ ID NO: 153	F3 RSV-A-set18	#6	GGGTCTTAGCAAAATCAGTT
SEQ ID NO: 139	B3 RSV-A-set18	#6	TGCAGCATCATATAGATCTTGA
SEQ ID NO: 162	FIP RSV-A-set18	#6	GAATCCTGCTTCTCCACCCAGACAC GCTAGTGTACAAGC
SEQ ID NO: 141	BIP RSV-A-set18	#6	GTGTAGTATTGGGCAATGCTGCTCC TTGGTGTACCTCTGT
SEQ ID NO: 155	LF RSV-A-set18	#6	CCTCCACAACTTGTTCCATTTCT
SEQ ID NO: 156	LB RSV-A-set18	#6	TGGCCTAGGCATAATGGGAG
SEQ ID NO: 163	F3 RSV-A-set19	#7	TACACAGCTGCTGTTCAA
SEQ ID NO: 164	B3 RSV-A-set19	#7	GGTAAATTTGCTGGGCATT
SEQ ID NO: 165	FIP RSV-A-set19	#7	TTGGAACATGGGCACCCATAAATG TCCTAGAAAAAGACGATG

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 166	BIP RSV-A-set19	#7	CTAGTGAAACAAATATCCACACCC AGCACTGCACTTCTTGAGTT
SEQ ID NO: 167	LF RSV-A-set19	#7	TTGTAAGTGATGCAGGAT
SEQ ID NO: 168	LB RSV-A-set19	#7	AGGGACCCTCATTAAGAGTCATG
SEQ ID NO: 169	F3 RSV-A-set20	#8	ATACACAGCTGCTGTTCA
SEQ ID NO: 164	B3 RSV-A-set20	#8	GGTAAATTTGCTGGGCATT
SEQ ID NO: 170	FIP RSV-A-set20	#8	TCTGCTGGCATGGATGATTGAATGT CCTAGAAAAAGACGATG
SEQ ID NO: 166	BIP RSV-A-set20	#8	CTAGTGAAACAAATATCCACACCC AGCACTGCACTTCTTGAGTT
SEQ ID NO: 171	LF RSV-A-set20	#8	CCCATATTGTAAGTGATGCAGGAT
SEQ ID NO: 172	LB RSV-A-set20	#8	AGGGACCCTCATTAAGAGTCAT
SEQ ID NO: 169	F3 RSV-A-set21	#9	ATACACAGCTGCTGTTCA
SEQ ID NO: 173	B3 RSV-A-set21	#9	TGGTAAATTTGCTGGGCAT
SEQ ID NO: 170	FIP RSV-A-set21	#9	TCTGCTGGCATGGATGATTGAATGT CCTAGAAAAAGACGATG
SEQ ID NO: 174	BIP RSV-A-set21	#9	TGAAACAAATATCCACACCCAAGG GCACTGCACTTCTTGAGTT
SEQ ID NO: 175	LF RSV-A-set21	#9	CCATATTGTAAGTGATGCAGGAT
SEQ ID NO: 176	LB RSV-A-set21	#9	GACCCTCATTAAGAGTCATGAT
SEQ ID NO: 177	F3 RSV-A-set22	#10	AACATACGTGAACAAACTTCA

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 178	B3 RSV-A-set22	#10	GCACATATGGTAAATTTGCTGG
SEQ ID NO: 179	FIP RSV-A-set22	#10	ACCCATATTGTAAGTGATGCAGGAT AGGGCTCCACATACACAG
SEQ ID NO: 180	BIP RSV-A-set22	#10	CTAGTGAAACAAATATCCACACCC AAGCACTGCACTTCTTGAG
SEQ ID NO: 181	LF RSV-A-set22	#10	TTTCTAGGACATTGTATTGAACAGC
SEQ ID NO: 182	LB RSV-A-set22	#10	GGGACCCTCATTAAGAGTCATG

[00570] FIG. 44 shows the times to result of a reverse-transcription LAMP (RT-LAMP) reaction detected using a DNA binding dye. Amplification was performed using primer sets #1 - #10. Sequences of the primer sets are provided in **TABLE 10** LAMP amplification, measured by an increase in SYTO 9 fluorescence, was observed over time, and time to result was determined as the time to reach half maximum SYTO 9 fluorescence intensity. Time to result was compared for ten LAMP primer sets in the presence (1000 genome copies) or absence (0 genome copies) of a target sequence from an RNA virus. Primer sets, namely #1 (SEQ ID NO: 138 – SEQ ID NO: 143), #7 (SEQ ID NO: 163 – SEQ ID NO: 168), #8 (SEQ ID NO: 164, SEQ ID NO: 166, and SEQ ID NO: 169 – SEQ ID NO: 172), and #10 (SEQ ID NO: 177 – SEQ ID NO: 182), showed clear differentiation between a sample containing the target sequence and a negative control lacking the target sequence. A decreased time to result is indicative of a sample positive for the target nucleic acid sequence.

[00571] FIG. 45 shows fluorescence signal from a DETECTR reaction using a Cas12 variant (SEQ ID NO: 11) following a five-minute incubation with products from RT-LAMP reactions. Amplification was performed using primer sets #1 - #10. LAMP primer sets #1-6 were designed for use with guide RNA #2 (SEQ ID NO: 240), and LAMP primer sets #7-10 were designed for use with guide RNA #1 (SEQ ID NO: 239). Sequences of primers in each primer set are provided in **TABLE 10**. DETECTR signal was compared for each LAMP primer set in the presence (1000 genome copies) or absence (0 genome copies) of a target sequence using either a guide RNA having a sequence corresponding to SEQ ID NO: 239 (guide RNA#1, top bar graph) or guide RNA having a sequence corresponding to SEQ ID NO: 240 (guide RNA #2, bottom bar graph). Data shows clean differentiation between reactions with the target sequence and no target

control reactions when using DETECTR to differentiate between specific and non-specific LAMP amplification. The sequences of the gRNAs used in the DETECTR reaction are provided in **TABLE 11**.

TABLE 11 – DETECTR gRNAs for RT-LAMP Amplification with DETECTR

SEQ ID NO:	gRNA Name	Sequence
SEQ ID NO: 239	gRNA #1 (R1118)	UAAUUUCUACUAAGUGUAGAUCUUAUAAA AGAACUAGCCAA
SEQ ID NO: 240	gRNA #2 (R288)	UAAUUUCUACUAAGUGUAGAUACUCAAUU UCCUCACUUCUC

EXAMPLE 12

Amplifying Influenza A and B Virus using RT-LAMP and SYTO 9

[00572] This example describes amplifying influenza A and B virus using LAMP and SYTO 9. Samples containing either 0, 100, 1000, 10,000, or 100,000 copies of an influenza A virus (IAV) or 0, 100, 1000, 10,000, or 100,000 copies of an influenza B virus (IBV) target nucleic acid sequence were subjected to RT-LAMP amplification using different sets of LAMP primers. Sets of LAMP primers (1, 2, 4, 5, 6, 7, 8, 9, 10, 11, or a negative control) were compared for their ability to specifically amplify the target nucleic acid sequence. Amplification was measured as a time to result using SYTO 9. A decreased time to result is indicative of a sample positive for the target nucleic acid sequence.

[00573] Each reaction RT-LAMP reaction was performed in the presence of 1x NEB IsoAmp Buffer, 4.5 mM $MgSO_4$, 6.4 U/μL Bst 2.0 (NEB), 0.75 μL Warmstart RTx reverse transcriptase, 1 μL 10x primer mix, and 0.2 μL SYTO 9 per 10 μL reaction in nuclease free water.

[00574] FIG. 46 shows detection of sequences from influenza A virus (IAV) using SYTO 9 (a DNA binding dye) following RT-LAMP amplification with LAMP primer sets 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, or a negative control. Ten reactions were performed per primer set and reactions were performed in duplicate. Individual plots depict fluorescence intensity over time during the LAMP amplification reaction. Fluorescence from SYTO 9 was measured over time as a function of an amount of target sequence present in the reaction. Plots in rows show amplification in the presence of, from top to bottom, 0, 100, 1000, 10,000, or 100,000 copies of the target nucleic acid. Plots in columns show amplification using, from left to right, primer sets 1, 2, IBV, 4, 5, 6, 7, 8, 9, 10, and 11. Primer set 1 (SEQ ID NO: 183 – SEQ ID NO: 188) shows a flat negative control curve, indicating suitability for use in LAMP amplification reactions. Primer set 2 (SEQ ID NO: 189 – SEQ ID NO: 194) is well-suited for use in amplifying a target nucleic acid using

LAMP. Primer set 8 (SEQ ID NO: 210 – SEQ ID NO: 215) and primer set 10 (SEQ ID NO: 211, SEQ ID NO: 213 – SEQ ID NO: 215, and SEQ ID NO: 219 – SEQ ID NO: 220) also work well in amplifying a target nucleic acid using LAMP. Primer set 8 produces a lower negative control amplification signal than primer set 10. **FIG. 48** shows the time to amplification of an IAV target sequence following LAMP amplification with different primer sets as determined from the SYTO 9 fluorescence traces shown in **FIG. 46**. Time to result was determined as the time to reach half maximum SYTO 9 fluorescence intensity. Amplification was detected using SYTO 9 in the presence of increasing concentrations of the target nucleic acid sequence (0, 100, 1000, 10,000, or 100,000 genome copies of the target sequence per reaction). The assay was capable of distinguishing between negative control reactions (no target sequence) and reactions containing 100,000 genome copies of the target sequence for all primer sets. The sequences of the LAMP primers in each primer set are provided in **TABLE 12**.

TABLE 12 – Primers for Amplification and Detection of IAV and IBV Virus using RT-LAMP

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 183	IAV-MP-F3	#1	GACTTGAAGATGTCTTTGC
SEQ ID NO: 184	IAV-MP B3	#1	TGTTGTTTGGGTCCCCATT
SEQ ID NO: 185	IAV-MP-FIP	#1	TTAGTCAGAGGTGACAGGATTGCA GATCTTGAGGCTCTC
SEQ ID NO: 186	IAV-MP-BIP	#1	TTGTGTTCACGCTCACCGTGTTTGG ACAAAGCGTCTACG
SEQ ID NO: 187	IAV-MP FL	#1	GTCTTGTCTTTAGCCA
SEQ ID NO: 188	IAV-MP BL	#1	CAGTGAGCGAGGACTG
SEQ ID NO: 189	IAV F3 v2	#2	ACCGAGGTCGAAACGT
SEQ ID NO: 190	IAV B3 v2	#2	GGTCCCCATTCCCATTG
SEQ ID NO: 191	IAV FIP v2	#2	CAAAGACATCTTCAAGTCTCTGCG TTTTTTCTCTCTATCGTCCCGTCA
SEQ ID NO: 192	IAV BIP v2	#2	AATGGCTAAAGACAAGACCAATCC TTTTTTGTCTACGCTGCAGTCC
SEQ ID NO: 193	IAV LF v2	#2	CGATCTCGGCTTTGAGGG

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 194	IAV LB v2	#2	TCACCGTGCCCAGTGAG
SEQ ID NO: 195	IAV F3 v3	#3	CGAAAGCAGGTAGATATTGAAAG
SEQ ID NO: 196	IAV B3 v3	#3	TCTACGCTGCAGTCCTC
SEQ ID NO: 197	IAV FIP v3	#3	TCAAGTCTCTGCGCGATCTCTTTTT TGAGTCTTCTAACCGAGGT
SEQ ID NO: 198	IAV BIP v3	#3	AGATGTCTTTGCAGGGAAAAACAC TTTTTTCACAAATCCTAAAATCCCC TTAG
SEQ ID NO: 199	IAV LF v3	#3	GACGATAGAGAGAACGTACGTTTC
SEQ ID NO: 200	IAV LB v3	#3	AAGACCAATCCTGTCACCTCT
SEQ ID NO: 201	IAV-set4-F3	#4	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set4-B3	#4	CATTCCCATTGAGGGCATT
SEQ ID NO: 203	IAV-set4-FIP	#4	CTTCAAGTCTCTGCGCGATCTATG AGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set4-BIP	#4	TTGAGGCTCTCATGGAATGGCAGC GTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set4-LF	#4	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set4-LB	#4	ACAAGACCAATCCTGTCACC
SEQ ID NO: 201	IAV-set5-F3	#5	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set5-B3	#5	CATTCCCATTGAGGGCATT
SEQ ID NO: 207	IAV-set5-FIP	#5	TTCAAGTCTCTGCGCGATCTCATG AGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set5-BIP	#5	TTGAGGCTCTCATGGAATGGCAGC GTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set5-LF	#5	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set5-LB	#5	ACAAGACCAATCCTGTCACC
SEQ ID NO: 201	IAV-set6-F3	#6	GCGAAAGCAGGTAGATATTGA

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 208	IAV-set6-B3	#6	TTGGACAAAGCGTCTACG
SEQ ID NO: 203	IAV-set6-FIP	#6	CTTCAAGTCTCTGCGCGATCTATG AGTCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set6-BIP	#6	TTGAGGCTCTCATGGAATGGCAGC GTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set6-LF	#6	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set6-LB	#6	ACAAGACCAATCCTGTCACC
SEQ ID NO: 201	IAV-set7-F3	#7	GCGAAAGCAGGTAGATATTGA
SEQ ID NO: 202	IAV-set7-B3	#7	CATTCCCATTGAGGGCATT
SEQ ID NO: 209	IAV-set7-FIP	#7	AAGTCTCTGCGCGATCTCGATGAG TCTTCTAACCGAGGT
SEQ ID NO: 204	IAV-set7-BIP	#7	TTGAGGCTCTCATGGAATGGCAGC GTGAACACAAATCCTAA
SEQ ID NO: 205	IAV-set7-LF	#7	TGACGGGACGATAGAGAGAA
SEQ ID NO: 206	IAV-set7-LB	#7	ACAAGACCAATCCTGTCACC
SEQ ID NO: 210	IAV-set8-F3	#8	TCTTCTAACCGAGGTCGAA
SEQ ID NO: 211	IAV-set8-B3	#8	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 212	IAV-set8-FIP	#8	TCAGAGGTGACAGGATTGGTCTGA AGATGTCTTTGCAGGGAA
SEQ ID NO: 213	IAV-set8-BIP	#8	TTGTGTTCACGCTCACCGTCATTCC CATTGAGGGCATT
SEQ ID NO: 214	IAV-set8-LF	#8	ATTCCATGAGAGCCTCAAGATC
SEQ ID NO: 215	IAV-set8-LB	#8	GAGGACTGCAGCGTAGAC
SEQ ID NO: 216	IAV-set9-F3	#9	TTCTCTCTATCGTCCCGTC
SEQ ID NO: 211	IAV-set9-B3	#9	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 217	IAV-set9-FIP	#9	CCCTTAGTCAGAGGTGACAGGAAC ACAGATCTTGAGGCTCT

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 213	IAV-set9-BIP	#9	TTGTGTTCACGCTCACCGTCATTCC CATTGAGGGCATT
SEQ ID NO: 218	IAV-set9-LF	#9	GGTCTTGTCTTTAGCCATTCCA
SEQ ID NO: 215	IAV-set9-LB	#9	GAGGACTGCAGCGTAGAC
SEQ ID NO: 219	IAV-set10-F3	#10	GTCTTCTAACCGAGGTCGA
SEQ ID NO: 211	IAV-set10-B3	#10	CTGCTCTGTCCATGTTGTT
SEQ ID NO: 220	IAV-set10-FIP	#10	GAGGTGACAGGATTGGTCTTGTTG AAGATGTCTTTGCAGGG
SEQ ID NO: 213	IAV-set10-BIP	#10	TTGTGTTCACGCTCACCGTCATTCC CATTGAGGGCATT
SEQ ID NO: 214	IAV-set10-LF	#10	ATTCCATGAGAGCCTCAAGATC
SEQ ID NO: 215	IAV-set10-LB	#10	GAGGACTGCAGCGTAGAC
SEQ ID NO: 221	IAV-set11-F3	#11	AAGAAGACAAGAGATATGGC
SEQ ID NO: 222	IAV-set11-B3	#11	CAATTCGACACTAATTGATGGC
SEQ ID NO: 223	IAV-set11-FIP	#11	GTCTCCTTGCCCAATTAGCAAGCA TCAATGAACTGAGCA
SEQ ID NO: 224	IAV-set11-BIP	#11	GTGGTGTTGGTAATGAAACGAAGC TGTCTGGCTGTCAGTA
SEQ ID NO: 225	IAV-set11-LF	#11	ACATTAGCCTTCTCTCCTTT
SEQ ID NO: 226	IAV-set11-LB	#11	AACGGGACTCTAGCATACT
SEQ ID NO: 227	M605 F3 IBV LAMP	IBV	AGGGACATGAACAACAAAGA
SEQ ID NO: 228	M606 B3 IBV LAMP	IBV	CAAGTTTAGCAACAAGCCT
SEQ ID NO: 229	M607 FIP IBV LAMP	IBV	TCAGGGACAATACATTACGCATAT CGATAAAGGAGGAAGTAAACACT CA
SEQ ID NO: 230	M608 BIP IBV LAMP	IBV	TAAACGGAACATTCCTCAAACACC ACTCTGGTCATATGCATTC

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 231	M609 LF IBV LAMP	IBV	TCAAACGGAACTTCCCTTCTTTC
SEQ ID NO: 232	M610 LB IBV LAMP	IBV	GGATACAAGTCCTTATCAACTCTGC

[00575] FIG. 47 shows the time to amplification of an influenza B virus (IBV) target sequence following RT-LAMP amplification. Amplification was detected using SYTO 9 in the presence of increasing concentrations of target sequence (0, 100, 1000, 10,000, or 100,000 copies of the target sequence per reaction). RT-LAMP amplification was performed using primer set #8 (SEQ ID NO: 210 – SEQ ID NO: 215), provided in **TABLE 12**.

EXAMPLE 13

Detection of Influenza A Virus using LAMP and DETECTR

[00576] This example describes detection of influenza A virus using LAMP and DETECTR. Samples containing an influenza A virus (IAV) target nucleic acid sequence or lacking the IAV target nucleic acid sequence were subjected to RT-LAMP amplification using different sets of LAMP primers. Sets of LAMP primers were compared for their ability to specifically amplify the target nucleic acid sequence. Presence or absence of the target nucleic acid in the sample was subsequently measured using DETECTR. DETECTR signal, measured by an increase in fluorescent signal upon activation of a programmable nuclease, was observed over time. An increase in fluorescence indicates the presence of the target nucleic acid sequence.

[00577] Each RT-LAMP reaction was performed in the presence of 1x NEB IsoAmp Buffer, 4.5 mM $MgSO_4$, 1.4 mM dNTPs (NEB), 6.4 U/µL Bst 2.0 (NEB), 1.5 µL Warmstart RTx, and 2 µL 10x primer mix per 20 µL reaction in nuclease-free water. Each DETECTR reaction was performed in the presence of 1x Processing Buffer, 250 nM crRNA, and 200 nM Sr-WT LbCas12a programmable nuclease in nuclease-free water.

[00578] FIG. 49 shows detection of target nucleic acid sequences from influenza A virus (IAV) using DETECTR following RT-LAMP amplification with LAMP primer sets 1, 2, 4, 5, 6, 7, 8, 9, 10, or a negative control. RT-LAMP amplification was performed using the primer sets provided in **TABLE 12**. Ten reactions were performed per primer set. DETECTR was performed with different gRNAs. The sequences of the gRNAs used in the DETECTR reaction are provided in **TABLE 13**. DETECTR signal was measured as a function of an amount of target sequence present in the reaction. Individual plots depict fluorescence intensity over time during

DETECTR reaction following LAMP amplification. Individual traces on each plot show amplification followed by DETECTR with a guide RNA corresponding to SEQ ID NO: 241 (R283 gRNA, blue), a guide RNA corresponding to SEQ ID NO: 242 (R781 gRNA, red), a guide RNA corresponding to SEQ ID NO: 243 (R782 gRNA, green), or a guide RNA corresponding to SEQ ID NO: 244 (IBV gRNA, purple). Plots in rows show DETECTR following LAMP amplification in the presence of, from top to bottom, 0, 100, 1000, 10,000, or 100,000 copies of the target nucleic acid. Plots in columns show DETECTR following LAMP amplification using, from left to right, primer sets 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, or IBV. Using primer set 1 resulted in robust amplification of the target nucleic acid by RT-LAMP. Primer set 2 was also found to be well-suited for use in combined methods of amplifying a target nucleic acid sequence by RT-LAMP and detecting the target nucleic acid sequence by DETECTR. Primer set 8 (SEQ ID NO: 210 – SEQ ID NO: 215) and primer set 10 (SEQ ID NO: 211, SEQ ID NO: 213 – SEQ ID NO: 215, and SEQ ID NO: 219 – SEQ ID NO: 220) were well suited for use in combined RT-LAMP and DETECTR reactions when detected using the guide RNA corresponding to SEQ ID NO: 243 (gRNA R782), as indicated by robust amplification and detection of the target nucleic acid without non-specific amplification or detection in the absence of the target nucleic acid. Target nucleic acid sequences from IBV were also detected by DETECTR after RT-LAMP amplification of the target.

TABLE 13 – DETECTR gRNAs for RT-LAMP Amplification with DETECTR of IAV or IBV

SEQ ID NO:	gRNA Name	Sequence
SEQ ID NO: 241	R283	UAAUUUCUACUAAGUGUAGAUUGUUCACG CUCACCGUGCCC
SEQ ID NO: 242	R781	UAAUUUCUACUAAGUGUAGAUGCCAUUCC AUGAGAGCCUCA
SEQ ID NO: 243	R782	UAAUUUCUACUAAGUGUAGAUGACAAAGC GUCUACGCUGCA
SEQ ID NO: 244	IBV (R778)	UAAUUUCUACUAAGUGUAGAUCUAACACU CUCAGGGACAAU

EXAMPLE 14

Detection of a SNP using LAMP and DETECTR

[00579] This example describes detection of a SNP using LAMP and DETECTR. Strategies for designing primers for use in combined LAMP and DETECTR reactions to detect SNPs were tested and evaluated for multiple target SNPs. From these experiments, a set of design guidelines

was determined to facilitate combined LAMP and DETECTR reactions for DNA nucleic acid targets or RT-LAMP and DETECTR reactions for RNA nucleic acid targets.

[00580] FIG. 50 shows a scheme for designing primers for LAMP amplification of a target nucleic acid sequence and detection of a single nucleotide polymorphism (SNP) in the target nucleic acid sequence. In an exemplary arrangement, the SNP of the target nucleic acid is positioned between the F1c region and the B1 region.

[00581] FIG. 51 shows schematics of exemplary arrangements of LAMP primers, guide RNA sequences, protospacer-adjacent motif (PAM) or protospacer flanking site (PFS), and target nucleic acids with a SNP for methods of LAMP amplification of a target nucleic acid and detection of the target nucleic acid using DETECTR.

[00582] FIG. 51A shows a schematic of an exemplary arrangement of the guide RNA with respect to various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between an F1c region and a B1 region. The entirety of the guide RNA sequence may be between the F1c region and the B1c region. The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00583] FIG. 51B shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to various regions of nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between an F1c region and a B1 region and the target nucleic acid comprises a sequence between an F1c region and a B1 region that is reverse complementary to at least 60% of a guide nucleic acid. In this example, the guide RNA is not reverse complementary to the forward inner primer or the backward inner primer . The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00584] FIG. 51C shows a schematic of an exemplary arrangement of the guide RNA sequence with respect to various regions of the nucleic acid sequence that correspond to or anneal LAMP primers. In this arrangement, the PAM or PFS of the target nucleic acid is positioned between the F1c region and the B1 region and the entirety of the guide RNA sequence is between the F1c region and the B1 region. The SNP is shown as positioned within a sequence of the target nucleic acid that hybridizes to the guide RNA.

[00585] FIG. 52 shows an exemplary sequence of a nucleic acid comprising two PAM sites and a HERC2 SNP. The positions of two gRNAs targeting the HERC2 A SNP allele at either position 9 with respect to a first PAM site (SEQ ID NO: 245) or at position 14 with respect to a second PAM site (SEQ ID NO: 247) are shown. The position of a SNP is indicated with a

triangle. The SNP is positioned at position 9 relative to a first PAM site or position 14 relative to a second PAM site. The target sequence is shown in the figure. The top strand has a sequence of 5'- CCAGTTTCATTTGAGCATTAAGTGTCAAGTTCTG-3' (SEQ ID NO: 750) and the bottom strand has a sequence of 5'- CAGAACTTGACACTTAATGCTCAAATGAAACTGG-3' (SEQ ID NO: 751).

[00586] FIG. 53 shows results from DETECTR reactions to detect a HERC2 SNP at position 9 relative to a first PAM site or position 14 relative to a second PAM site following LAMP amplification. The SNP position is indicated by a triangle. Fluorescence signal, indicative of detection of the target sequence, was measured over time in the presence of a target sequence comprising either a G SNP allele or an A SNP allele in HERC2. The target nucleic acid comprising the SNP was amplified using the primers presented in **TABLE 14**.

TABLE 14 –LAMP Primers for Amplification and Detection of a HERC2 SNP

SEQ ID NO:	Primer Name	Primer Set	Sequence
SEQ ID NO: 233	M948 F3 HERC2 set3	HERC2	CTTGTAATCAACATCAGGGTAA
SEQ ID NO: 234	M949 B3 HERC2 set3	HERC2	AGAAACGACAAGTAGACCATT
SEQ ID NO: 235	M950 FIP HERC2 set3	HERC2	CGCCTCTTGGATCAGACACATGTG TTAATACAAAGGTACAGGA
SEQ ID NO: 236	M951 BIP HERC2 set3	HERC2	CACGCTATCATCATCAGGGGCTGC TTCAAGTGTATATAAACTCAC
SEQ ID NO: 237	M952 LF HERC2 set3	HERC2	GAGAGCCATGAAGAACAAATTCT
SEQ ID NO: 238	M953 LB HERC2 set3	HERC2	CGAGGCTTCTCTTTGTTTTTAAT

[00587] The target sequence was detected using a guide RNA (crRNA only) to detect either the A allele with the first PAM site (SNP Position 9, "A SNP"), the G allele with the first PAM site (SNP Position 9, "G SNP"), the A allele with the second PAM site (SNP Position 14, "A SNP")or the G allele with the second PAM site (SNP Position 14, "G SNP"). Four guide RNAs designed for each condition were used. The guide RNAs used for the detection of the two SNP alleles relative to the two PAM sites are presented in **TABLE 15**. The guide RNA corresponding to SEQ ID NO: 245 was designed to detect the A allele at position 9, the guide RNA

corresponding to SEQ ID NO: 246 was designed to detect the G allele at position 9, the guide RNA corresponding to SEQ ID NO: 247 was designed to detect the A allele at position 14, and the guide RNA corresponding to SEQ ID NO: 248 was designed to detect the G allele at position 14. A high fluorescence signal was detected for the G allele in the presence of the position 9 G SNP guide RNA (SEQ ID NO: 246, top left) and the A allele in the presence of the position 9 A SNP guide RNA (SEQ ID NO: 245, bottom right). Minimal fluorescence signal was detected for the G allele in the presence of the position 9 A SNP guide RNA (SEQ ID NO: 245, top right) and the position 9 A allele in the presence of the G SNP guide RNA (SEQ ID NO: 246, bottom left).This indicates that the position 9 G SNP and position 9 A SNP guide RNAs show specificity for the G allele and A allele, respectively. The position 14 A SNP guide RNA (SEQ ID NO: 247) and the position 14 G SNP guide RNA (SEQ ID NO: 248) detected both alleles, as shown by high fluorescence signal when detecting the SNP with the position 14 A SNP or G SNP guide RNAs, independent of the target sequence present.

[00588] FIG. 54 shows a heatmap of fluorescence from a DETECTR reaction following LAMP amplification of the target nucleic acid sequence. The DETECTR reaction differentiated between two HERC2 SNP alleles at position 9 with respect to the PAM, using guide RNAs (crRNA only) specific for the A allele (SEQ ID NO: 245) or the G allele (SEQ ID NO: 246). Positive detection is indicated by a high fluorescence value in the DETECTR reaction. Guide RNA corresponding to SEQ ID NO: 245 was specific for A allele, as indicated by (i) a high fluorescence signal in the A SNP positive control, the HeLa sample, and Sample 2, and (ii) low fluorescence signal in the G SNP positive control, the negative control, and Sample 1. Guide RNA corresponding to SEQ ID NO: 246 was specific for G allele, as indicated by (i) a high fluorescence signal in the G SNP positive control, the HeLa sample, and Sample 1, and (ii) low fluorescence signal in the A SNP positive control, the negative control, and Sample 2. Sample 1 was homozygous for the G allele and Sample 2 was homozygous for the A allele.

TABLE 15 – DETECTR Guide RNAs for Amplification and Detection of a HERC2 SNP

SEQ ID NO:	gRNA Name	Sequence
SEQ ID NO: 245	A SNP Position 9 (R570)	UAAUUUCUACUAAGUGUAGAUAGCAUUAAAUGUCAAGUUCU
SEQ ID NO: 246	G SNP Position 9 (R571)	UAAUUUCUACUAAGUGUAGAUAGCAUUAAGUGUCAAGUUCU
SEQ ID NO: 247	A SNP Position 14 (R1138)	UAAUUUCUACUAAGUGUAGAUAUUUGAGCAUUAAAUGUCAA
SEQ ID NO: 248	G SNP Position 14 (R1139)	UAAUUUCUACUAAGUGUAGAUAUUUGAGCAUUAAGUGUCAA

[00589] **FIG. 55** shows combined LAMP amplification of a target nucleic acid by LAMP and detection of the target nucleic acid by DETECTR. Detection was carried out visually with DETECTR by illuminating the samples with a red LED. Each reaction contained a target nucleic acid sequence comprising a SNP allele for either a blue eye phenotype ("Blue Eye") or a brown eye phenotype ("Brown Eye"). Samples "Brown *" and "Blue *" were an A allele positive control and a G allele positive control, respectively. A position 9 guide RNA for either the brown eye phenotype (SEQ ID NO: 245, "Br") or the blue eye phenotype (SEQ ID NO: 246, "Bl") was used for each LAMP DETECTR reaction. The presence of either the blue eye allele or the brown eye allele was visually detected by eye, as shown by an increase in fluorescence in each tube containing a target nucleic acid sequence and a corresponding guide RNA. The guide RNA for the brown eye allele phenotype (SEQ ID NO: 245) was specific for the A allele, as shown by a high fluorescence signal (brighter tubes) in tubes containing the brown eye guide RNA and either the brown eye target nucleic acid or the A SNP positive control, and low fluorescence signal (darker tubes) in tubes containing the brown eye guide RNA and either the blue eye target nucleic acid or the G SNP positive control. The guide RNA for the blue eye allele (SEQ ID NO: 246) was specific for the G allele, as shown by a high fluorescence signal (brighter tubes) in tubes containing the blue eye guide RNA and either the blue eye target nucleic acid or the G SNP positive control, and low fluorescence signal (darker tubes) in tubes containing the blue eye guide RNA and either the brown eye target nucleic acid or the A SNP positive control.

EXAMPLE 15

High Specificity Buffer

[00590] This example shows a high specificity buffer comprising 100 mM NaCl and 50 μg/ml heparin enhances the targeting specificity and enhanced SNP discrimination capabilities of LbCas12. **FIG. 56A – FIG. 56H** shows high sensitivity and high specificity buffers for LbCas12a (SEQ ID NO: 1). In the presence of 50 μg/ml heparin and 100 mM salt, Cas12a has improved targeting specificity and enhanced SNP discrimination capabilities. Target sequences were detected using a crRNA directed to the EGFR wild type sequence (SEQ ID NO: 448) or a crRNA directed to the EGFR mutant sequence (G SNP, SEQ ID NO: 449). In the absence of heparin and salt, Cas12a has improved sensitivity. For all SNP-related studies, high specificity buffer was used.

EXAMPLE 16

Detection of the EGFR SNP T790M (c.2369C>T)

[00591] This example shows that Cas12a can be used to detect a single nucleotide polymorphism (SNP) versus wild-type (WT) of EGFR. The EGFR SNP detected was the SNP T790M (c.2369C>T). The sample comprised both the C SNP (WT) and the T SNP (T790M) cell free DNA EGFR DNA standards. Guide RNA sequences for Cas12a detection of the SNPs described in this example are listed in **TABLE 17.**

[00592] **FIG. 57** shows a schematic of PCR primers and guide RNA targeting sequence for EGFR T790M SNP. The forward primer represents a PAM primer (SEQ ID NO: 396), also referred to as a PAMplification primer, which embeds a PAM sequence ('TTTV') upstream of the targeting sequence and includes a 6nt 3' extension for priming. The PAM sequence is required for Cas12a-guide RNA to recognize the matching DNA target. In this schematic, the guide RNA was designed to target the mismatch located 7 nt downstream of the 5' end of the target sequence (SEQ ID NO: 400). This guide RNA/primer design is used for **FIG. 59 – FIG. 61**.

[00593] **FIG. 58A – FIG. 58C** shows PAMplification F primers (PAM F primers) with varying 3' extensions (4 nt in **FIG. 58A**, 5nt in **FIG. 58B**, 6 nt in **FIG. 58C**, SEQ ID NO: 394, SEQ ID NO: 395, and SEQ ID NO: 396, respectively) tested with guide RNA targeting T790M with a mismatch at the 7th position (SEQ ID NO: 400). The PAMplification F primer with 6nt extension demonstrated optimal detection with the guide RNA. This PAMplification F primer was used for **FIG. 59 – FIG. 63**.

[00594] **FIG. 59A – FIG. 59C** illustrates that Cas12 guide RNAs designed to target a wild type sequence ("WT" C SNP allele) and sequence comprising a T790M T SNP allele show specific Cas12-based detection in the presence of cognate single nucleotide polymorphism (SNP). Targets were detected with a crRNA directed to the wild type sequence (SEQ ID NO: 423) or a crRNA directed to the T SNP allele sequence (SEQ ID NO: 439). Time courses show activation of the WT or mutant crRNA only in the presence of the matching target (**FIG. 59A** and **FIG. 59B**). A heatmap represents time course data at t = 60 min (**FIG. 59C**) n = 3 technical replicates; synthetic oligo targets; bars represent mean ± SD.

[00595] **FIG. 60A – FIG. 60D** show that Cas12a can detect down to 0.1-1% minor allele frequency (MAF) of EGFR T790M (T SNP allele) in mock cfDNA samples (Horizon Discovery), with 2 ng of total DNA input and a PCR pre-amplification step. Targets were detected with a crRNA directed to the wild type sequence (SEQ ID NO: 423) or a crRNA directed to the T SNP allele sequence (SEQ ID NO: 439). Detection of WT (C SNP allele) and

mutant allele at t = 90 min with low frequency EGFR standards is shown in **FIG. 60A**. Bar graphs of mutant allele detection only is shown in **FIG. 60B**. A heat map representation of WT and mutant allele detection is shown in **FIG. 60C**. Samples were run with n = 3 replicates and statistical significance was determined by a two-tailed Student's t-test, with *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and bars representing mean plus SD. **FIG. 60D** shows the different percentages of the WT and mutant allele in sample in a single test tube as pictorial representation of the percentage of MAF in the samples tested. The turnaround time was 90 minutes and the assay volume was 20 μL.

[00596] **FIG. 61** shows limit of detection studies illustrating that 2 ng total DNA is the minimum input allowed for detection of 0.1-1% minor allele frequency (MAF) of EGFR T790M (T SNP allele) in mock cfDNA samples (Horizon Discovery) with a PCR pre-amplification step. Samples were run with n = 3 replicates and statistical significance was determined by a two-tailed Student's t-test, with *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and bars representing mean plus SD. Targets were detected using 7mm guide RNA directed to T SNP allele (SEQ ID NO: 403). Targets were amplified using primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397. **FIG. 62** shows a table of **FIG. 61** assay parameters.

TABLE 16. DNA sequences used in this study

Description	**Sequence (5' → 3')**
EGFR T790M PAMplification F primer (4nt extension) (SEQ ID NO: 394)	TCACCTCCACCGTG**TTTC**TCAT
EGFR T790M PAMplification F primer (5nt extension) (SEQ ID NO: 395)	TCACCTCCACCGTG**TTTC**TCATC
EGFR T790M PAMplification F primer (6nt extension) (SEQ ID NO: 396)	TCACCTCCACCGTG**TTTC**TCATCA
EGFR T790M R primer (SEQ ID NO: 397)	GGAGCCAATATTGTCTTTGTGTTCCC
EGFR T790M Blocking primer (SEQ ID NO: 398)	TCATCACGCAGCTCATGC/3Phos/

TABLE 17. RNA sequences used in this study with LbCas12a.

Description	**Sequence**
EGFR T790M C-SNP 6mm guide RNA (SEQ ID NO: 399)	TAATTTCTACTAAGTGTAGAT**CATCACGCAGCTCATGCCCT**
EGFR T790M C-SNP 7mm guide RNA (SEQ ID NO: 400)	TAATTTCTACTAAGTGTAGAT**TCATCACGCAGCTCATGCCC**
EGFR T790M C-SNP 8mm guide RNA (SEQ ID NO: 401)	TAATTTCTACTAAGTGTAGAT**CTCATCACGCAGCTCATGCC**

Description	Sequence
EGFR T790M T-SNP 6mm guide RNA (SEQ ID NO: 402)	TAATTTCTACTAAGTGTAGAT**CATCATGCAGCTCATGCCCT**
EGFR T790M T-SNP 7mm guide RNA (SEQ ID NO: 403)	TAATTTCTACTAAGTGTAGAT**TCATCATGCAGCTCATGCCC**
EGFR T790M T-SNP 8mm guide RNA (SEQ ID NO: 404)	TAATTTCTACTAAGTGTAGAT**CTCATCATGCAGCTCATGCC**

EXAMPLE 17

Amplification of EGFR SNP T790M (c.2369C>T) using a Blocking Primer

[00597] This example shows that a blocking primer enriches a sample for a single nucleotide polymorphism (SNP) versus wild-type (WT) of EGFR. The enriched EGFR SNP was the SNP T790M (c.2369C>T). The sample comprised both the C SNP (WT) and the T SNP (T790M) cell free DNA EGFR DNA standards.

[00598] **FIG. 63A** shows how the blocking primer blocks the forward primer from binding to the WT nucleic acid for amplification.

[00599] **FIG. 63B** shows how the mutation in SNP does not result in the binding of the blocking primer, and therefore allowing the forward primer to bind to the SNP nucleic acid for amplification.

[00600] **FIG. 63C and FIG. 63D** show the detection of the EGFR C SNP using an input of 6 ng and the detection of the EGFR T SNP using an input of 6 ng, respectively, after amplification using the blocking primer strategy of **FIG. 64A** and **FIG. 64B**. PAMplification and blocking primers are provided in **TABLE 18**.

TABLE 18. Primers used in this Example

Description	Sequence (5' → 3')
EGFR T790M PAMplification F primer (6nt extension) (SEQ ID NO: 396)	TCACCTCCACCGTG**TTTC**TCATCA
EGFR T790M R primer (SEQ ID NO: 397)	GGAGCCAATATTGTCTTTGTGTTCCC
EGFR T790M Blocking primer (SEQ ID NO: 398)	TCATCACGCAGCTCATGC/3Phos/

EXAMPLE 18

Amplification of EGFR SNP T790M (c.2369C>T) using COLD-PCR

[00601] This example shows that a COLD-PCR enriches a sample for a single nucleotide polymorphism (SNP) versus wild-type (WT) of EGFR. The enriched EGFR SNP was the SNP

T790M (c.2369C>T). The sample comprised both the C SNP (WT) and the T SNP (T790M) cell free DNA EGFR DNA standards.

[00602] **FIG. 64A** shows an exemplary full COLD-PCR strategy for enriching for a mutation, such as an EGFR SNP T790M (c.2369C>T).

[00603] **FIG. 64B** shows an exemplary full COLD-PCR strategy for enriching for a mutation, such as an EGFR SNP T790M (c.2369C>T).

[00604] **FIG. 65A** shows the detection of the EGFR C SNP using an input of 6 ng and a crRNA corresponding to SEQ ID NO: 423 and LbCas12a (SEQ ID NO: 1) after amplification using COLD-PCR. **FIG. 65B** shows the detection of the EGFR C SNP using an input of 6 ng the detection of the EGFR T SNP using an input of 6 ng and a crRNA corresponding to SEQ ID NO: 439 and LbCas12a (SEQ ID NO: 1) after amplification using COLD-PCR. COLD-PCR was performed using primers corresponding to SEQ ID NO: 396 and SEQ ID NO: 397.

EXAMPLE 19

Detection of the EGFR SNP L858R (c. 573T>G)

[00605] This example shows that that Cas12a can be used to detect a single nucleotide polymorphism (SNP) versus wild-type (WT) of EGFR. The EGFR SNP detected was the SNP L858R (c.2573T>G). The sample comprised synthetic EGFR DNA standards for both WT EGFR allele with a T at position 2573 and the G SNP (a T to G missense substitution at position 2573 in the L858R locus).

[00606] **FIG. 66A – FIG. 66B** shows Cas12a (SEQ ID NO: 1) can detect down to 0.1-1% minor allele frequency (MAF) of EGFR L858R G SNP allele in mock cfDNA samples (Horizon Discovery), with 1 ng total DNA input and a COLD-PCR pre-amplification step. Detection of mutant (**FIG. 66A**) and WT (**FIG. 66B**) alleles at t = 40 min with low frequency EGFR standards. **FIG. 66A** shows detection of the mutant allele using a gRNA corresponding to SEQ ID NO: 430 and **FIG. 66B** shows detection of the WT allele using a gRNA corresponding to SEQ ID NO: 429. n = 3 replicates, two-tailed Student's t-test; *$p < 0.05$, **$p < 0.01$; bars represent mean plus SD. Target sequences were amplified using primers corresponding to SEQ ID NO: 450 and SEQ ID NO: 451. **TABLE 19** shows the amino acid mutations (AA mutation), the change that has occurred in the nucleotide sequence (CDS mutation), the type of mutation, and the guide nucleic acid CRISPR RNA (crRNA) sequence used to detect the mutation.

TABLE 19 – Guide for Detecting Various Mutations

Exon	EGFR Mutation Group	CDS mutation	AA mutation	Type	Start	End	COSMIC ID	Notes	Spacer sequence (RC)	Spacer sequence	LbCas12a crRNA	LbCas12a crRNA handle:
Exon 19	Exon19 (WT)	WT							TTAAGAGAAGCAACATCTCC (SEQ ID NO: 661)	GGAGATGTTGCTTCTCTTAA (SEQ ID NO: 688)	TAATTTCTACTAAGTGTAGATGGAGATGTTGCTTCTCTTAA (SEQ ID NO: 718)	R0292 EGFR Ex19 WT
	Ex19Del (28 targets from Roche cobas test)	c.2240_2251del12	p.L747_T751>S	Complex_deletion_inframe	55174777	55174788	6210		GCTATCAAGGAATCATCTCC (SEQ ID NO: 662)	GGAGATGATTCCTTGATAGC (SEQ ID NO: 689)	TAATTTCTACTAAGTGTAGATGGAGATGATTCCTTGATAGC (SEQ ID NO: 719)	R0293 EGFR Ex19 var1
		c.2239_2247delTTAAGAGAA	p.L747_E749delLRE	Deletion_In_Frame	55174776	55174784	6218		ATCAAGGAAGCAACATCTCC (SEQ ID NO: 663)	GGAGATGTTGCTTCCTTGAT (SEQ ID NO: 690)	TAATTTCTACTAAGTGTAGATGGAGATGTTGCTTCCTTGAT (SEQ ID NO: 720)	R0294 EGFR Ex19 var2
		c.2238_2255del18	p.E746_S752>D	Complex_deletion_inframe	55174775	55174792	6220		CCCGTCGCTATCAAGGATCC (SEQ ID NO: 664)	GGATCCTTGATAGCGACGGG (SEQ ID NO: 691)	TAATTTCTACTAAGTGTAGATGGATCCTTGATAGCGACGGG (SEQ ID NO: 721)	R0295 EGFR Ex19 var3
		c.2235_2249del15	p.E746_A750delELREA	Deletion_In_Frame	55174772	55174786	6223		GTCGCTATCAAAACATCTCC (SEQ ID NO: 665)	GGAGATGTTTTGATAGCGAC (SEQ ID NO: 692)	TAATTTCTACTAAGTGTAGATGGAGATGTTTTGATAGCGAC (SEQ ID NO: 722)	R0296 EGFR Ex19 var4
		c.2236_2250del15	p.E746_A750delELREA	Deletion_In_Frame	55174773	55174787	6225		GTCGCTATCAAGACATCTCC (SEQ ID NO: 666)	GGAGATGTCTTGATAGCGAC (SEQ ID NO: 693)	TAATTTCTACTAAGTGTAGATGGAGATGTCTTGATAGCGAC (SEQ ID NO: 723)	R0297 EGFR Ex19 var5
		c.2239_2256del18	p.L747_S752delLREATS	Deletion_In_Frame	55174776	55174793	6255		CCCGTCGCTATCAAGGAACC (SEQ ID NO: 667)	GGTTCCTTGATAGCGACGGG (SEQ ID NO: 694)	TAATTTCTACTAAGTGTAGATGGTTCCTTGATAGCGACGGG (SEQ ID NO: 724)	R0298 EGFR Ex19 var6

Exon	EGFR Mutation Group	CDS mutation	AA mutation	Type	Start	End	COSMIC ID	Notes	Spacer sequence (RC)	Spacer sequence	LbCas12a crRNA	LbCas12a crRNA handle:
		c.2237_2254del18	p.E746_S752>A	Complex_deletion_inframe	55174774	55174791	12367		CCCGTCGCTATCAAGGCTCC (SEQ ID NO: 668)	GGAGCCTTGATAGCGACGGG (SEQ ID NO: 695)	TAATTTCTACTAAGTGTAGATGGAGCCTTGATAGCGACGGG (SEQ ID NO: 725)	R0299 EGFR Ex19 var7
		c.2240_2254del15	p.L747_T751delLREAT	Deletion_In_frame	55174777	55174791	12369		GTCGCTATCAAGGAATCTCC (SEQ ID NO: 669)	GGAGATTCCTTGATAGCGAC (SEQ ID NO: 696)	TAATTTCTACTAAGTGTAGATGGAGATTCCTTGATAGCGAC (SEQ ID NO: 726)	R0300 EGFR Ex19 var8
		c.2240_2257del18	p.L747_P753>S	Complex_deletion_inframe	55174777	55174794	12370		CCCGTCGCTATCAAGGAATC (SEQ ID NO: 670)	GATTCCTTGATAGCGACGGG (SEQ ID NO: 697)	TAATTTCTACTAAGTGTAGATGATTCCTTGATAGCGACGGG (SEQ ID NO: 727)	R0301 EGFR Ex19 var9
		c.2239_2248TTAAGAGAAG>C	p.L747_A750>P	Complex_deletion_inframe	55174776	55174785	12382		ATCAAGGAACCAACATCTCC (SEQ ID NO: 671)	GGAGATGTTGGTTCCTTGAT (SEQ ID NO: 698)	TAATTTCTACTAAGTGTAGATGGAGATGTTGGTTCCTTGAT (SEQ ID NO: 728)	R0302 EGFR Ex19 var10
		c.2239_2251>C	p.L747_T751>P	Complex_deletion_inframe	55174776	55174788	12383		GCTATCAAGGAACCATCTCC (SEQ ID NO: 672)	GGAGATGGTTCCTTGATAGC (SEQ ID NO: 699)	TAATTTCTACTAAGTGTAGATGGAGATGGTTCCTTGATAGC (SEQ ID NO: 729)	R0303 EGFR Ex19 var11
		c.2237_2255>T	p.E746_S752>V	Complex_deletion_inframe	55174774	55174792	12384		CCCGTCGCTATCAAGGTTCC (SEQ ID NO: 673)	GGAACCTTGATAGCGACGGG (SEQ ID NO: 700)	TAATTTCTACTAAGTGTAGATGGAACCTTGATAGCGACGGG (SEQ ID NO: 730)	R0304 EGFR Ex19 var12
		c.2235_2255>AAT	p.E746_S752>I	Complex_deletion_inframe	55174772	55174792	12385		CCCGTCGCTATCAAAATTCC (SEQ ID NO: 674)	GGAATTTTGATAGCGACGGG (SEQ ID NO: 701)	TAATTTCTACTAAGTGTAGATGGAATTTTGATAGCGACGGG (SEQ ID NO: 731)	R0305 EGFR Ex19 var13
		c.2237_2252>T	p.E746_T751>V	Complex_del	55174774	55174789	12386		GTCGCTATCAAGGTAT	GGAGATACCTTGATAGCG	TAATTTCTACTAAGTGTAGATGGAGATACCTTGA	R0306 EGFR Ex19 var14

Exon	EGFR Mutation Group	CDS mutation	AA mutation	Type	Start	End	COSMIC ID	Notes	Spacer sequence (RC)	Spacer sequence	LbCas12a crRNA	LbCas12a crRNA handle:
				etion_inframe					CTCC (SEQ ID NO: 675)	AC (SEQ ID NO: 702)	TAGCGAC (SEQ ID NO: 732)	
		c.2239_2258>CA	p.L747_P753>Q	Complex_deletion_inframe	55174776	55174795	12387		CCCGTCGCTATCAAGGAACA (SEQ ID NO: 676)	TGTTCCTTGATAGCGACGGG (SEQ ID NO: 703)	TAATTTCTACTAAGTGTAGATTGTTCCTTGATAGCGACGGG (SEQ ID NO: 733)	R0307 EGFR Ex19 var15
		c.2239_2256>CAA	p.L747_S752>Q	Complex_deletion_inframe	55174776	55174793	12403		GTCGCTATCAAGGAACAACC (SEQ ID NO: 677)	GGTTGTTCCTTGATAGCGAC (SEQ ID NO: 704)	TAATTTCTACTAAGTGTAGATGGTTGTTCCTTGATAGCGAC (SEQ ID NO: 734)	R0308 EGFR Ex19 var16
		c.2237_2253>TTGCT	p.E746_T751>VA	Complex_deletion_inframe	55174774	55174790	12416		GCTATCAAGGTTGCTTCTCC (SEQ ID NO: 678)	GGAGAAGCAACCTTGATAGC (SEQ ID NO: 705)	TAATTTCTACTAAGTGTAGATGGAGAAGCAACCTTGATAGC (SEQ ID NO: 735)	R0309 EGFR Ex19 var17
		c.2238_2252>GCA	p.L747_T751>Q	Complex_deletion_inframe	55174775	55174789	12419		GCTATCAAGGAGCAATCTCC (SEQ ID NO: 679)	GGAGATTGCTCCTTGATAGC (SEQ ID NO: 706)	TAATTTCTACTAAGTGTAGATGGAGATTGCTCCTTGATAGC (SEQ ID NO: 736)	R0310 EGFR Ex19 var18
		c.2238_2248>GC	p.L747_A750>P	Complex_deletion_inframe	55174775	55174785	12422		ATCAAGGAGCCAACATCTCC (SEQ ID NO: 680)	GGAGATGTTGGCTCCTTGAT (SEQ ID NO: 707)	TAATTTCTACTAAGTGTAGATGGAGATGTTGGCTCCTTGAT (SEQ ID NO: 737)	R0311 EGFR Ex19 var19
		c.2237_2251del15	p.E746_T751>A	Complex_deletion_inframe	55174774	55174788	12678		GTCGCTATCAAGGCATCTCC (SEQ ID NO: 681)	GGAGATGCCTTGATAGCGAC (SEQ ID NO: 708)	TAATTTCTACTAAGTGTAGATGGAGATGCCTTGATAGCGAC (SEQ ID NO: 738)	R0312 EGFR Ex19 var20
		c.2236_2253del18	p.E746_T751delELREAT	Deletion_In_frame	55174773	55174790	12728		CCCGTCGCTATCAAGTCTCC (SEQ ID NO: 682)	GGAGACTTGATAGCGACGGG (SEQ ID NO: 709)	TAATTTCTACTAAGTGTAGATGGAGACTTGATAGCGACGGG (SEQ ID NO: 739)	R0313 EGFR Ex19 var21

Exon	EGFR Mutation Group	CDS mutation	AA mutation	Type	Start	End	COSMIC ID	Notes	Spacer sequence (RC)	Spacer sequence	LbCas12a crRNA	LbCas12a crRNA handle:
		c.2235_2248>AATTC	p.E746_A750>IP	Complex_deletion_inframe	55174772	55174785	13550		ATCAAAATTCCAACATCTCC (SEQ ID NO: 683)	GGAGATGTTGGAATTTTGAT (SEQ ID NO: 710)	TAATTTCTACTAAGTGTAGATGGAGATGTTGGAATTTTGAT (SEQ ID NO: 740)	R0314 EGFR Ex19 var22
		c.2235_2252>AAT	p.E746_T751>I	Complex_deletion_inframe	55174772	55174789	13551		GTCGCTATCAAAATATCTCC (SEQ ID NO: 684)	GGAGATATTTTGATAGCGAC (SEQ ID NO: 711)	TAATTTCTACTAAGTGTAGATGGAGATATTTTGATAGCGAC (SEQ ID NO: 741)	R0315 EGFR Ex19 var23
		c.2235_2251>AATTC	p.E746_T751>IP	Complex_deletion_inframe	55174772	55174788	13552		GCTATCAAAATTCCATCTCC (SEQ ID NO: 685)	GGAGATGGAATTTTGATAGC (SEQ ID NO: 712)	TAATTTCTACTAAGTGTAGATGGAGATGGAATTTTGATAGC (SEQ ID NO: 742)	R0316 EGFR Ex19 var24
		c.2237_2257>TCT	p.E746_P753>VS	Complex_deletion_inframe	55174774	55174794	18427		CCCGTCGCTATCAAGGTCTC (SEQ ID NO: 686)	GAGACCTTGATAGCGACGGG (SEQ ID NO: 713)	TAATTTCTACTAAGTGTAGATGAGACCTTGATAGCGACGGG (SEQ ID NO: 743)	R0317 EGFR Ex19 var25
		c.2233_2247del15	p.K745_E749delKELRE	Deletion_In_frame	55174770	55174784	26038		GTCGCTATCGCAACATCTCC (SEQ ID NO: 687)	GGAGATGTTGCGATAGCGAC (SEQ ID NO: 714)	TAATTTCTACTAAGTGTAGATGGAGATGTTGCGATAGCGAC (SEQ ID NO: 744)	R0319 EGFR Ex19 var27
Exon 20	T790 (WT)	WT						Requires PAM plification				
	T790M	c.2369C>T	p.T790M	Substitution_Missense	55181378	55181378	6240	Requires PAM plification				
Exon 21	L858 (WT)	WT								GGCTGGCCAAACTGCTGG	TAATTTCTACTAAGTGTAGATGGCTGGCCAAACT	R0435 EGFR L858 WT

Exon	EGFR Mutation Group	CDS mutation	AA mutation	Type	Start	End	COSMIC ID	Notes	Spacer sequence (RC)	Spacer sequence	LbCas12a crRNA	LbCas12a crRNA handle:
										GT (SEQ ID NO: 715)	GCTGGGT (SEQ ID NO: 745)	
	L858R	c.2573T>G	p.L858R	Substitution_Missense	55191822	55191822	6224			GGCGGGCCAAACTGCTGGGT (SEQ ID NO: 716)	TAATTTCTACTAAGTGTAGATGGCGGGCCAAACTGCTGGGT (SEQ ID NO: 746)	R0436 EGFR L858R (G-SNP)
		c.2573_2574TG>GT	p.L858R	Substitution_Missense	55191822	55191823	12429			GGCGTGCCAAACTGCTGGGT (SEQ ID NO: 717)	TAATTTCTACTAAGTGTAGATGGCGTGCCAAACTGCTGGGT (SEQ ID NO: 747)	R0437 EGFR L858R (GT-SNP)

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EXAMPLE 20

Assessment of Guide RNAs for Detection of EGFR-Exon 19 Deletions

[00607] This example shows that Cas12a can be used to detect deletions in EGFR. The deletions detected were located in exon 19.Twenty-six guide RNAs were designed to detect deletions in exon 19 of the EGFR DNA sequence.

[00608] 26 guides (SEQ ID NO: 481 – SEQ ID NO: 506, shown in **FIG. 67** as "Var") were designed and compared to a wild-type guide (SEQ ID NO: 480). The remaining 26 guides were used to screen 1 nM synthetic DNA twist fragments. Guide sequence are provided in **TABLE 20**.

TABLE 20 – Wild Type and Variant gRNAs for Detection of Exon 19 Deletions

SEQ ID NO:	Variant	gRNA Sequence
SEQ ID NO: 480	EGFR Exon19 WT	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGCUUCUCUUAA
SEQ ID NO: 481	EGFR Exon19 var01	UAAUUUCUACUAAGUGUAGAUGGAGAUGAUUCCUUGAUAGC
SEQ ID NO: 482	EGFR Exon19 var02	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGCUUCCUUGAU
SEQ ID NO: 483	EGFR Exon19 var03	UAAUUUCUACUAAGUGUAGAUGGAUCCUUGAUAGCGACGGG
SEQ ID NO: 484	EGFR Exon19 var04	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUUUGAUAGCGAC
SEQ ID NO: 485	EGFR Exon19 var05	UAAUUUCUACUAAGUGUAGAUGGAGAUGUCUUGAUAGCGAC
SEQ ID NO: 486	EGFR Exon19 var06	UAAUUUCUACUAAGUGUAGAUGGUUCCUUGAUAGCGACGGG
SEQ ID NO: 487	EGFR Exon19 var07	UAAUUUCUACUAAGUGUAGAUGGAGCCUUGAUAGCGACGGG
SEQ ID NO: 488	EGFR Exon19 var08	UAAUUUCUACUAAGUGUAGAUGGAGAUUCCUUGAUAGCGAC
SEQ ID NO: 489	EGFR Exon19 var09	UAAUUUCUACUAAGUGUAGAUGAUUCCUUGAUAGCGACGGG

SEQ ID NO:	Variant	gRNA Sequence
SEQ ID NO: 490	EGFR Exon19 var10	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGGUUCCUUGAU
SEQ ID NO: 491	EGFR Exon19 var11	UAAUUUCUACUAAGUGUAGAUGGAGAUGGUUCCUUGAUAGC
SEQ ID NO: 492	EGFR Exon19 var12	UAAUUUCUACUAAGUGUAGAUGGAACCUUGAUAGCGACGGG
SEQ ID NO: 493	EGFR Exon19 var13	UAAUUUCUACUAAGUGUAGAUGGAAUUUUGAUAGCGACGGG
SEQ ID NO: 494	R306 EGFR Exon19 var14	UAAUUUCUACUAAGUGUAGAUGGAGAUACCUUGAUAGCGAC
SEQ ID NO: 495	EGFR Exon19 var15	UAAUUUCUACUAAGUGUAGAUUGUUCCUUGAUAGCGACGGG
SEQ ID NO: 496	EGFR Exon19 var16	UAAUUUCUACUAAGUGUAGAUGGUUGUUCCUUGAUAGCGAC
SEQ ID NO: 497	EGFR Exon19 var17	UAAUUUCUACUAAGUGUAGAUGGAGAAGCAACCUUGAUAGC
SEQ ID NO: 498	EGFR Exon19 var18	UAAUUUCUACUAAGUGUAGAUGGAGAUUGCUCCUUGAUAGC
SEQ ID NO: 499	EGFR Exon19 var19	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGGCUCCUUGAU
SEQ ID NO: 500	EGFR Exon19 var20	UAAUUUCUACUAAGUGUAGAUGGAGAUGCCUUGAUAGCGAC
SEQ ID NO: 501	EGFR Exon19 var21	UAAUUUCUACUAAGUGUAGAUGGAGACUUGAUAGCGACGGG
SEQ ID NO: 502	EGFR Exon19 var22	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGGAAUUUUGAU

SEQ ID NO:	Variant	gRNA Sequence
SEQ ID NO: 503	EGFR Exon19 var23	UAAUUUCUACUAAGUGUAGAUGGAGAUAUUUUGAUAGCGAC
SEQ ID NO: 504	EGFR Exon19 var24	UAAUUUCUACUAAGUGUAGAUGGAGAUGGAAUUUUGAUAGC
SEQ ID NO: 505	EGFR Exon19 var25	UAAUUUCUACUAAGUGUAGAUGAGACCUUGAUAGCGACGGG
SEQ ID NO: 488	EGFR Exon19 var26	UAAUUUCUACUAAGUGUAGAUGGAGAUUCCUUGAUAGCGAC
SEQ ID NO: 506	EGFR Exon19 var27	UAAUUUCUACUAAGUGUAGAUGGAGAUGUUGCGAUAGCGAC

[00609] Resulting signals were measured using DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) techniques. Two guide sequences (SEQ ID NO: 493 and SEQ ID NO: 499) showed similar detection sensitivity to wild-type (**FIG. 67**). The other 24 guides showed activity greater than wild-type, with three variants (SEQ ID NO: 485, SEQ ID NO: 488, and SEQ ID NO: 490) showing the highest detection sensitivity. Targets corresponding to SEQ ID NO: 452 – SEQ ID NO: 477 and SEQ ID NO: 479, provided in **TABLE 21**, were detected.

TABLE 21 –EGFR Exon 19 Deletions Wild Type and Variant Sequences

SEQ ID NO:	Variant	gRNA Sequence
SEQ ID NO: 452	EGFR-Exon19-var01	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAATCATCTCCGAAAGCCAACAAGGAAATCCTCGATGTG AGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCC CACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCAT CTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 453	EGFR-Exon19-var02	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAAGCAACATCTCCGAAAGCCAACAAGGAAATCCTCGA TGTGAGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCA GGCCCACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGC

SEQ ID NO:	Variant	gRNA Sequence
		TCATCTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGT G
SEQ ID NO: 454	EGFR-Exon19-var03	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGATCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 455	EGFR-Exon19-var04	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAAACATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 456	EGFR-Exon19-var05	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGACATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 457	EGFR-Exon19-var06	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAACCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 458	EGFR-Exon19-var07	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 459	EGFR-Exon19-var08	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC

SEQ ID NO:	Variant	gRNA Sequence
		CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 460	EGFR-Exon19-var09	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAATCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 461	EGFR-Exon19-var10	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAACCAACATCTCCGAAAGCCAACAAGGAAATCCTCGA TGTGAGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCA GGCCCACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGC TCATCTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGT G
SEQ ID NO: 462	EGFR-Exon19-var11	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAACCATCTCCGAAAGCCAACAAGGAAATCCTCGATGTG AGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCC CACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCAT CTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 463	EGFR-Exon19-var12	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGTTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 464	EGFR-Exon19-var13	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAAATTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 465	EGFR-Exon19-var14	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGTATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC

SEQ ID NO:	Variant	gRNA Sequence
		CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 466	EGFR-Exon19-var15	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAACAGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 467	EGFR-Exon19-var16	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAACAACCGAAAGCCAACAAGGAAATCCTCGATGTGAG TTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCA CCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCT CCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 468	EGFR-Exon19-var17	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGTTGCTTCTCCGAAAGCCAACAAGGAAATCCTCGATGTG AGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCC CACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCAT CTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 469	EGFR-Exon19-var18	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAGCAATCTCCGAAAGCCAACAAGGAAATCCTCGATGTG AGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCC CACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCAT CTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 470	EGFR-Exon19-var19	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAGCCAACATCTCCGAAAGCCAACAAGGAAATCCTCGA TGTGAGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCA GGCCCACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGC TCATCTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGT G
SEQ ID NO: 471	EGFR-Exon19-var20	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGCATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC

SEQ ID NO:	Variant	gRNA Sequence
		CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 472	EGFR-Exon19-var21	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGTCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 473	EGFR-Exon19-var22	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAAATTCCAACATCTCCGAAAGCCAACAAGGAAATCCTCGAT GTGAGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAG GCCCACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCT CATCTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 474	EGFR-Exon19-var23	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAAATATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 475	EGFR-Exon19-var24	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAAATTCCATCTCCGAAAGCCAACAAGGAAATCCTCGATGTG AGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCC CACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCAT CTCCACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 476	EGFR-Exon19-var25	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGTCTCGAAAGCCAACAAGGAAATCCTCGATGTGAGTTTC TGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTT TTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCAC ATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 477	EGFR-Exon19-var27	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCGCAACATCTCCGAAAGCCAACAAGGAAATCCTCGATGTGAGT TTCTGCTTTGCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCAC

SEQ ID NO:	Variant	gRNA Sequence
		CTTTTCTCATGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTC CACATCCTAAATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 478	EGFR-Exon19-var28	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAATTAAGAGAAGCAACCCTCGATGTGAGTTTCTGCTTT GCTGTGTGGGGGTCCATGGCTCTGAACCTCAGGCCCACCTTTTCTCA TGTCTGGCAGCTGCTCTGCTCTAGACCCTGCTCATCTCCACATCCTA AATGTTCACTAGGCTAGGTGGAGGCTCAGTG
SEQ ID NO: 479	EGFR-Exon19-WT	GAAGTGCCATTCCGCCTGACCTAGCCCCAGTGTCCCTCACCTTCGG GGTGCATCGCTGGTAACATCCACCCAGATCACTGGGCAGCATGTGG CACCATCTCACAATTGCCAGTTAACGTCTTCCTTCTCTCTCTGTCAT AGGGACTCTGGATCCCAGAAGGTGAGAAAGTTAAAATTCCCGTCGC TATCAAGGAATTAAGAGAAGCAACATCTCCGAAAGCCAACAAGGA AATCCTCGATGTGAGTTTCTGCTTTGCTGTGTGGGGGTCCATGGCTC TGAACCTCAGGCCCACCTTTTCTCATGTCTGGCAGCTGCTCTGCTCT AGACCCTGCTCATCTCCACATCCTAAATGTTCACTAGGCTAGGTGG AGGCTCAGTG

[00610] **FIG. 67** shows a heat map of the DETECTR assays for each of the 26 guide variants ("Var") and a wild type ("WT") control tested. Fluorescence is the output of the DETECTR assay, and indicates that a Cas12a programmable nuclease (SEQ ID NO: 1) was activated by a target DNA to collaterally cleave a fluorescently labeled reporter, and is, thus, a measure of variant detection sensitivity. Guide variants detected the EGFR sequence comprising the deletion, while the WT control detected the EGFR wild type sequence. Screening was performed in the presence of 1 nM synthetic DNA twist fragments.

[00611] **TABLE 19** shows the amino acid mutations (AA mutation), the change that has occurred in the nucleotide sequence (CDS mutation), the type of mutation, the start and end nucleotide positions of the deletion on the corresponding chromosome, and the guide nucleic acid CRISPR RNA (crRNA) sequence used to detect the mutation.

EXAMPLE 21

PAM Forward Primer (PAMplification primer)

[00612] This example shows the optimal PAM forward primer (also referred to as a PAMplification primer) for use in amplifying a target nucleic acid to comprise a sequence encoding a PAM.

[00613] **FIG. 68A – FIG. 68B** and **FIG. 69A – FIG. 69B** shows the PAM forward primer (also referred to as a PAMplification primer). The single nucleotide mismatch was anchored at positions 3-8 or 5-8 nt downstream of the PAM. PAMplification primers with 2nt or 4nt

extensions at the 3'end were tested for their ability to discriminate the non-cognate target containing a single nucleotide mismatch/polymorphism (SNP). Here, a 4nt PAMplification 3'extension is better at SNP detection compared to the 2nt extension. The mismatch position is optimal around positions 6, 7 or 8. Primers used in this assay are provided in **TABLE 22**.

EXAMPLE 22

Cas12 Recognizes dU-containing PAM and Target Nucleic Acids

[00614] This example shows that Cas12 recognizes dU-containing PAM. Furthermore, a Cas12 recognizes target nucleic acids comprising dU.

[00615] FIG. 70A – FIG. 70B shows that Cas12 recognizes dU-containing PAM and target sequences from 100 nM to 10 pM. **FIG. 70A**: WT SNP-targeting guide RNA; **FIG. 70B**: mutant SNP-targeting guide RNA. Left to right for both **FIG. 70A** and **FIG. 70B**: (top left) WT sequence with dT-containing target, (top middle) mutant sequence with dT-containing target, (top right) mutant sequence with dU-containing PAM and target, (bottom left) no target, (bottom right) mutant sequence with dT-containing PAM and dU-containing target. Cas12 is capable of SNP detection with dU-containing sequences (both PAM and target) without compromising sensitivity. Primers used in this assay are provided in **TABLE 22**.

EXAMPLE 23

Cas12 Recognizes dU-containing Amplicons of theALDH2 WT Allele

[00616] This example shows that Cas 12 recognizes a dU-containing amplicons of the ALDH2 WT allele. Additionally, the Cas12 was able to distinguish dU-containing amplicons of the ALDH2 WT allele and dU-containing amplicons of the ALDH2 SNP allele.

[00617] FIG. 71A – FIG. 71B shows the detection of ALDH2 WT allele from human genomic DNA (SEQ ID NO: 417) with dU-containing amplicons with Cas12. The sequence of the target is shown in **FIG. 71A** - the top strand has a sequence of 5'-CACACTCACAGTTTTCACTTCAGTGTATGCCTGCAGCCCGTACTCGCCCAACTCC-3' (SEQ ID NO: 752) and the bottom strand has a sequence of 5'-GGAGTTGGGCGAGTACGGGCTGCAGGCATACACTGAAGTGAAAACTGTGAGTGTG-3' (SEQ ID NO: 753). The ALDH2 gene was amplified from human saliva containing the WT allele using Taq master mix containing dUTP in place of dTTP, such that all T nucleotides with the above annotated ALDH2 target sequence has been replaced by U nucleotides. The amplicon was added directly to a Cas12 DETECTR assay. Cas12 guide RNAs targeting the ALDH2 WT allele detected only the cognate WT sequence and not the mutant allele, demonstrating that Cas12 is capable of SNP detection with dU-containing targets.

EXAMPLE 24

Cas12 Recognizes dU-containing Amplicons of at a Low Frequency

[00618] This example shows that Cas 12 recognizes a dU-containing amplicons at a low frequency.

[00619] **FIG. 58A – FIG. 58C** show the PAMplification primer produces dU-containing amplicons for detection of mutant sequences at low frequency. Cas12 guide RNAs were designed to target the T790M mutant allele (c.2369C>T, at guide mismatch position 7) in Horizon Discovery EGFR cfDNA standards at 0-5% minor allele frequencies (MAF) with 2ng input DNA. PAMplification primers include 4-6 nt extensions at the 3'end downstream of the embedded PAM. n = 3 technical replicates; bars represent mean ± SD.

FIG. 60A – FIG. 60C show the detection of low frequency SNPs using PAMplification with 6nt extension and dU-containing amplicons. Cas12a can detect down to 0.1-1% minor allele frequency (MAF) of EGFR T790M in mock cfDNA samples (Horizon Discovery), with 2 ng total DNA input. n = 3 replicates, two-tailed Student's t-test; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; bars represent mean plus SD.

TABLE 22 – Primers used in Examples 21, 22, and 24

Description	**Sequence (5' → 3')**
T790M dUTP PAM 6mm NTS (SEQ ID NO: 408)	CTCCACCGTGC/ideoxyU//ideoxyU//ideoxyU/GCA/ideoxyU/ CACGCAGC/ideoxyU/CA/ideoxyU/GCCC/ideoxyU//ideoxyU/ TCGGCGCCTCCTGGACTAT
T790M dUTP PAM 6mm TS (SEQ ID NO: 409)	ATAGTCCAGGAGGCAGCCGAAGGGCA/ideoxyU/GAGC /ideoxyU/GCG/ideoxyU/GA/ideoxyU/GCAAAGCACGGTGGAG
T790M dUTP 6mm NTS (SEQ ID NO: 410)	CTCCACCGTGCTTTGCA/ideoxyU/CACGCAGC /ideoxyU/CA/ideoxyU/GCCC/ideoxyU//ideoxyU/ TCGGCGCCTCCTGGACTAT
T790M dUTP 6mm TS (SEQ ID NO: 411)	ATAGTCCAGGAGGCAGCCGAAGGGCA/ideoxyU/ GAGC/ideoxyU/GCG/ideoxyU/GA/ideoxyU/ GCAAAGCACGGTGGAG
EGFR T790M 7mm PAM 4nt_F (SEQ ID NO: 394)	TCACCTCCACCGTG**TTTC**TCAT
EGFR T790M 7mm PAM 5nt_F (SEQ ID NO: 395)	TCACCTCCACCGTG**TTTC**TCATC
EGFR T790M 7mm PAM 6nt_F (SEQ ID NO: 396)	TCACCTCCACCGTG**TTTC**TCATCA
EGFR T790M std R (SEQ ID NO: 397)	GGAGCCAATATTGTCTTTGTGTTCCC

EXAMPLE 25

Ratio of LAMP Amplicon in Cas12 Detection Reaction

[00620] This example describes ratios of LAMP amplicon used in Cas12 detection reactions provided herein. A detection assay using a Cas12 variant (SEQ ID NO: 11) was performed in the presence of increasing amounts of LAMP amplified genomic DNA target nucleic acid sequence. The target nucleic acid sequence was amplified for 30 minutes at 60° C using LAMP amplification. Increasing volumes of the amplified nucleic acid sequence were combined in a 20 µL Cas12 detection reaction. The Cas12 detection assay was run for 30 minutes at 37° C.

[00621] FIG. 72 shows detection of amplified HERC2 genomic DNA using a Cas12 variant (SEQ ID NO: 11) in the presence of increasing amounts of LAMP amplified DNA ("LAMP.Amplicon"). The HERC2 target was amplified from HeLa genomic DNA using LAMP amplification with the HERC2 LAMP primers shown in **TABLE 14** (SEQ ID NO: 233 – SEQ ID NO: 238). Each detection reaction was performed in the presence of 1 µL to 14 µL LAMP amplified DNA in 20 µL reactions. A negative control reaction was performed without LAMP amplified DNA (0 µL). Detection of the LAMP amplified DNA was quantified by fluorescence upon cleavage of a reporter (SEQ ID NO: 119 with N-terminal /5Alex594N/ and C-terminal /3IAbRQSp/) by an activated Cas12 programmable nuclease upon binding of a guide RNA to the target LAMP amplified DNA.

[00622] The results indicated that the performance of the Cas12 detection assay was stable at 1 µL of LAMP amplified DNA in 20 µL reaction volumes up to 1 µL LAMP amplified DNA in 20 µL reaction volumes. Increasing the ratio to 12 µL, 13 µL, or 14 µL of LAMP amplicon in 20 µL reaction volumes, led to a decrease in assay performance.

EXAMPLE 26

Addition of an Artificial PAM to LAMP FIP or BIP Primers

[00623] This example describes addition of an artificial PAM to LAMP FIP or BIP primers as described herein. An artificial PAM was added to a target nucleic sequence by LAMP amplifying the target nucleic acid using a FIP or BIP primer with the artificial PAM sequence. The PAM was inserted at different positions using different FIP primers shown in **TABLE 23**, with the PAM indicated by bold and underlining. This method of PAM introduction using LAMP amplification (referred to herein as PAMplification) was used to generate a target site for a CRISPR/Cas system that would not have otherwise been accessible.

TABLE 23 – Artificial PAM Position within LAMP Amplification FIP Primers

SEQ ID NO:	PAM Position	Sequence
SEQ ID NO: 235	No PAM	CGCCTCTTGGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 265	Position 1	**CAAA**TCTTGGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 266	Position 2	C**CAAA**CTTGGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 267	Position 3	CG**CAAA**TTGGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 268	Position 4	CGC**CAAA**TGGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 269	Position 5	CGCC**CAAA**GGATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 270	Position 6	CGCCT**CAAA**GATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 271	Position 7	CGCCTC**CAAA**ATCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 272	Position 8	CGCCTCT**CAAA**TCAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 273	Position 9	CGCCTCTT**CAAA**CAGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 274	Position 10	CGCCTCTTG**CAAA**AGACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 275	Position 11	CGCCTCTTGG**CAAA**GACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 276	Position 12	CGCCTCTTGGA**CAAA**ACACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 277	Position 13	CGCCTCTTGGAT**CAAA**CACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 278	Position 14	CGCCTCTTGGATC**CAAA**ACATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 279	Position 15	CGCCTCTTGGATCA**CAAA**CATGTGTTAATACAAAGGTACAGGA
SEQ ID NO: 280	Position 16	CGCCTCTTGGATCAG**CAAA**ATGTGTTAATACAAAGGTACAGGA

SEQ ID NO:	PAM Position	Sequence
SEQ ID NO: 281	Position 17	CGCCTCTTGGATCAGA**CAAA**TGTGTTAATACAAAGGTACAGGA

[00624] FIG. 73 shows a schematic of addition of an artificial PAM to LAMP FIP or BIP primers. PAMs were introduced at different positions within the LAMP primer, and gRNAs were designed relative to each PAM for use in CRISPR-based detection assays of target nucleic acids. The PAM was introduced at different positions within the LAMP FIP primer, and the target nucleic acid was detected with gRNAs for each PAM position to assess the impact of PAM placement in an FIP primer on (1) the efficiency of LAMP amplification and (2) non-specific activation of trans cleavage by the primer binding to the gRNA-Cas protein complex.

[00625] FIG. 74 shows LAMP amplification of a target human genomic DNA (HERC2, SEQ ID NO: 416) with an FIP primer having PAM sequences at varying positions to introduce an artificial PAM in the HERC2 target nucleic acid. PAM introduction with the FIP primer and gRNA binding sites for each corresponding PAM containing FIP primer are shown in **FIG. 73**. For example, an FIP primer having the PAM sequence at position 17 (17th nucleotide from the 5' end of the FIP primer; depicted as "PAM Pos 17") is used with a gRNA sequence for Pos 17 (5' end of gRNA is adjacent to the 5' end of the PAM sequence in the primer; depicted as "gRNA seq for Pos 17"). The target was amplified using primers corresponding to SEQ ID NO: 233 – SEQ ID NO: 234 and SEQ ID NO: 236 – SEQ ID NO: 238 with a variable FIP depending on the position of the artificially introduced PAM. FIPs corresponding to SEQ ID NO: 265 – SEQ ID NO: 281 were used to insert artificial PAMs at position 1 – position 17, respectively. The FIP corresponding to SEQ ID NO: 235 was used to amplify the target without introducing a PAM. Amplification was monitored using a SYTO9 DNA binding dye. Rate of amplification was quantified by the time to result, which was determined by the time to reach half maximum SYTO9 fluorescence intensity. Time to result was indicative of the time to reach exponential amplification. A lower time to result value indicated faster amplification. The results demonstrated that positioning the PAM sequence near the 5' end of the LAMP FIP primer led to slower amplification compared to the control FIP primer lacking a PAM. Most added PAM sequences positioned near the center of the FIP primer (from about position 6 to about position 15) showed similar amplification times compared to the control.

[00626] A HERC2 target nucleic acid with artificially inserted PAM sequences at various positions (position 1 to position 17) within the target nucleic acid were detected using a Cas12 detection assay. The HERC2 target was amplified using LAMP primers SEQ ID NO: 233, SEQ

ID NO: 234, and SEQ ID NO: 235 – SEQ ID NO: 238. FIP primers corresponding to SEQ ID NO: 265 – SEQ ID NO: 281 were used to introduce artificial PAMs at position 1 – postion 17, respectively. The FIP primer corresponding to SEQ ID NO: 235 was used to amplify the target without inserting an artificial PAM. gRNAs were designed to hybridize to the target nucleic acid sequence with the PAM sequence inserted at various positions. **FIG. 75** shows detection of a target nucleic acid with an artificially introduced PAM using a Cas12 variant (SEQ ID NO: 11). gRNAs corresponding to SEQ ID NO: 283 – SEQ ID NO: 299 were used to detect target nucleic acids with artificially introduced PAMs at position 1 – position 17, respectively. Sequences of the gRNAs are provided in **TABLE 24**. Artificial PAMs were introduced at different positions of a FIP primer, as illustrated in **FIG. 73**. Upon hybridization of the gRNA to the target, SEQ ID NO: 11 was activated and cleaved reporters (SEQ ID NO: 119 with N-terminal /5Alex594N/ and C-terminal /3IAbRQSp/), releasing a fluorescent detectable signal. Thus, target nucleic acids were detected by measuring fluorescence. Fluorescence was measured following LAMP with genomic DNA, LAMP with no target (negative control), or a water negative control ("water control for detection assay"). The detection assay was performed at 37° C for 90 minutes using 1 μL of the LAMP amplicon per 20 μL reaction.

TABLE 24 – gRNAs for Detection of Target Sequences with Artifically Introdiced PAMs

SEQ ID NO:	PAM Position	gRNA Sequence
SEQ ID NO: 283	Position 1	UAAUUUCUACUAAGUGUAGAUTGCTCAAATGAAACTGGCCT
SEQ ID NO: 284	Position 2	UAAUUUCUACUAAGUGUAGAUGCTCAAATGAAACTGGCCTC
SEQ ID NO: 285	Position 3	UAAUUUCUACUAAGUGUAGAUCTCAAATGAAACTGGCCTCG
SEQ ID NO: 286	Position 4	UAAUUUCUACUAAGUGUAGAUTCAAATGAAACTGGCCTCGC
SEQ ID NO: 287	Position 5	UAAUUUCUACUAAGUGUAGAUCAAATGAAACTGGCCTCGCC
SEQ ID NO: 288	Position 6	UAAUUUCUACUAAGUGUAGAUAAATGAAACTGGCCTCGCCT
SEQ ID NO: 289	Position 7	UAAUUUCUACUAAGUGUAGAUAATGAAACTGGCCTCGCCTC
SEQ ID NO: 290	Position 8	UAAUUUCUACUAAGUGUAGAUATGAAACTGGCCTCGCCTCT

SEQ ID NO:	PAM Position	gRNA Sequence
SEQ ID NO: 291	Position 9	UAAUUUCUACUAAGUGUAGAUTGAAACTGGCCTCGCCTCTT
SEQ ID NO: 292	Position 10	UAAUUUCUACUAAGUGUAGAUGAAACTGGCCTCGCCTCTTG
SEQ ID NO: 293	Position 11	UAAUUUCUACUAAGUGUAGAUAAACTGGCCTCGCCTCTTGG
SEQ ID NO: 294	Position 12	UAAUUUCUACUAAGUGUAGAUAACTGGCCTCGCCTCTTGGA
SEQ ID NO: 295	Position 13	UAAUUUCUACUAAGUGUAGAUACTGGCCTCGCCTCTTGGAT
SEQ ID NO: 296	Position 14	UAAUUUCUACUAAGUGUAGAUCTGGCCTCGCCTCTTGGATC
SEQ ID NO: 297	Position 15	UAAUUUCUACUAAGUGUAGAUTGGCCTCGCCTCTTGGATCA
SEQ ID NO: 298	Position 16	UAAUUUCUACUAAGUGUAGAUGGCCTCGCCTCTTGGATCAG
SEQ ID NO: 299	Position 17	UAAUUUCUACUAAGUGUAGAUGCCTCGCCTCTTGGATCAGA

[00627] Adding an artificial PAM at various positions within a LAMP FIP primer led to non-specific activation of SEQ ID NO: 11 trans cleavage activity when at least 12 nucleotides overlapped between the gRNA and the LAMP FIP primer. The degree of non-specific trans cleavage activity is expected to be impacted by the melting temperature of the overlapping gRNA and LAMP primer sequence, with a higher melting temperature leading to more non-specific trans cleavage activity.

[00628] Based on the time to amplification shown in **FIG. 74** and the Cas12 detection shown in **FIG. 75**, the results demonstrated that artificial PAM sequences were preferably positioned away from the 5' end of the FIP (F1c region) or BIP primer (B1c region) and towards the center (position 6 to position 15) of the FIP or BIP primers. Additionally, positioning the artificial PAM such that less than 50% of the primer overlapped with the gRNA sequence decreased non-specific trans cleavage activation. The assay also showed better detection sensitivity and specificity at PAM insertion positions where fewer mutations were made in the primer to insert the artificial PAM sequence (e.g., PAMs inserted at positions 13, 15, or 17 having 1 or 2 changes relative to the wild type sequence). In contrast, detection sensitivity was lower at PAM insertion positions where more mutations were made in the primer ot inset the artificial PAM sequence

(e.g., PAMs inserted at posotions 12 or 14 having 3 or 4 changes relative to the wild type sequence).

EXAMPLE 27

SEQ ID NO: 11 Programmable Nuclease SNP Sensitivity Along a Target Sequence

[00629] This example describes sensitivity of a Cas12 variant programmable nuclease (SEQ ID NO: 11) to SNPs positioned along a target sequence.

[00630] In a first assay, sensitivity to point mutations in a target sequence with a native PAM site was tested. To determine which positions along a target nucleic acid sequence were most sensitive to single point mutations, all four nucleotide possibilities (A, T, C, or G) at each position were tiled along a target of a target nucleic acid sequence. The assay was performed for two target nucleic acid sequences, a HERC2 target nucleic acid sequence and an ALDH target nucleic acid sequence. Both target nucleic acid sequences comprised a native PAM site. The target nucleic acids comprising the PAM site with each of all possible point mutations were detected using a SEQ ID NO: 11 programmable nuclease. **FIG. 76** shows detection of single point mutations at different positions along a nucleic acid sequence using a SEQ ID NO: 11 programmable nuclease. Point mutations to each nucleic acid (A, T, C, or G, "SNP Base (target)") were made along a target nucleic acid sequence at different positions relative to a native PAM. To determine the sensitivity of the detection assay to single point mutations (e.g., a SNP), the target nucleic acid was detected using a gRNA directed to hybridize to the wild type sequence. Black circles label with "WT" indicate the nucleotide at each position of the wild type sequence that is reverse complementary to the gRNA sequence. The assay was performed with a HERC2 target sequence (top panel, wild type sequence TCGTAATTCACAGTTCAAGA, SEQ ID NO: 416) or an ALDH target sequence (bottom panel, wild type sequence TGAAGTCACATACGGACGTC, SEQ ID NO: 417). The HERC2 sequence was detected using a gRNA corresponding to SEQ ID NO: 246 (top plot) and the ALDH sequence was detected using a gRNA corresponding to SEQ ID NO: 425 (bottom plot). Upon hybridization of gRNA to the target nucleic acid, SEQ ID NO: 11 is activated and trans cleaves a reporter (SEQ ID NO: 119 with N-terminal /56-FAM/ and C-terminal /3IABkFQ/), releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label, and the maximum rate ("Average Max Rate") was calculated as fluorescence units per minute and averaged between four replicates. Results indicated that SEQ ID NO: 11 was sensitive to point mutations along the entire length of the gRNA target site. The specificity for individual point mutations depended on sequence context of the target nucleic acid.

[00631] In a second assay, a programmable nuclease of SEQ ID NO: 11 was used to detect variants at two SNP sites in a target nucleic acid sequence without a native PAM. The detection assay was run for 90 minutes at 37° C with either a wild type DNA ("WT"), a target DNA with a mutation at a first SNP ("rs738408"), or a target DNA with a mutation at a second SNP ("rs738409"). **FIG. 77** shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native "TTTN" PAM sequence using a programmable nuclease of SEQ ID NO: 11. Target nucleic acids tested contained the wild type sequence ("WT"), a sequence with a mutation at a first SNP ("rs738408"), a sequence with a mutation at a second SNP ("rs738409"), or a sequence with mutations at the first SNP and the second SNP ("rs738409/408"). Target sequences are provided in **TABLE 25**.

TABLE 25 – PNPLA3 Target Sequences

SEQ ID NO:	Target Name	Target Sequence
SEQ ID NO: 412	PNPLA3 rs738409+rs738408	TGCCTGCTGACTGCTCTGTAGCACAGTGCTTCGCAAAGTGTG ATCCTGGGACCAGCAGAGCAGCAGCTCCTTTGAGCTTATTGG AATGGCAGACCCTCAGGTCCCACCTCTGACCTGCTGCATGGG AATTCTGGGGAGGGACGCAGAATCTCTGGTTCCACAGGCTCT CCGGTGATGCTAATGAATACCGGCATTTGAACAGCACCGATC TAGCCCCTTTCAGTCCATGAGCCAACAACCCTTGGTCCTGTCT GTGGTGACCCAGTGTGACTCTCATGGGGAGCAAGGAGAGGA AGTTGAAGTTCACTGACAGGGTTGTTAAGGGGATTATGCAAT AGATGAGACCCATGGGCCTGAAGTCCGAGGGTGTATGTTAGT TCCCCGTTCTTTTGACCCATGGATTAACCTACTCTGTGCAAAG GGCATTTTCAAGTTTGTTGCCCTGCTCACTTGGAGAAAGCTT ATGAAGGATCAGGAAAATTAAAAGGGTGCTCTCGCCTATAA CTTCTCTCTCCTTTGCTTTCACAGGCCTTGGTATGTTCCTGCTT CATGCCTTTCTACAGTGGCCTTATCCCTCCTTCCTTCAGAGGC GTGGTAAGTCGGCTTTCTCTGCTAGCGCTGAGTCCTGGGGGC CTCTGAAGTGTGCTCACACATCTCCTGCCTGCAGGGCACTGG TGTCGGGCACCTCAGGGTCTGTCCCATGGTGGAGCCCCATGC CTCACTGCCTTTCAGACAGAGTAGCCACAGCTGGCCCTATTT CCAGGCTACCCGGGCAGCAAAACTTACTGCATGTGTAATTAA TTATTTGGCTATCTGTAAGGTAAACTGGCTGGTTCACTTAATC TGCACCTTAAGCATCAGATAGCTTCTCAGTGATCTAGTTAAA CTATATGATGTTGGCCAGGCGCGGTGGCTCATGTCTGTAATC CCAGCACTTTGGGAGCCTGAAGCAGGCAGATCACTTGAGGTC AGGAGTTCGAGACCAGCCTGGCCAACAGTGTGAAACTCTGTC TCTCCTAAAAATACAAAAATTAGCTGGGCATGGTGGTGTGCA CCTGTAATCCCAGCTGCTCGGGAGGCTGAGGCAGGAGAATT GCTTGAACTTGGGA
SEQ ID NO: 413	PNPLA3 rs738408	TGCCTGCTGACTGCTCTGTAGCACAGTGCTTCGCAAAGTGTG ATCCTGGGACCAGCAGAGCAGCAGCTCCTTTGAGCTTATTGG AATGGCAGACCCTCAGGTCCCACCTCTGACCTGCTGCATGGG AATTCTGGGGAGGGACGCAGAATCTCTGGTTCCACAGGCTCT CCGGTGATGCTAATGAATACCGGCATTTGAACAGCACCGATC TAGCCCCTTTCAGTCCATGAGCCAACAACCCTTGGTCCTGTCT GTGGTGACCCAGTGTGACTCTCATGGGGAGCAAGGAGAGGA AGTTGAAGTTCACTGACAGGGTTGTTAAGGGGATTATGCAAT

SEQ ID NO:	Target Name	Target Sequence
		AGATGAGACCCATGGGCCTGAAGTCCGAGGGTGTATGTTAGT TCCCCGTTCTTTTGACCCATGGATTAACCTACTCTGTGCAAAG GGCATTTTCAAGTTTGTTGCCCTGCTCACTTGGAGAAAGCTT ATGAAGGATCAGGAAAATTAAAAGGGTGCTCTCGCCTATAA CTTCTCTCTCCTTTGCTTTCACAGGCCTTGGTATGTTCCTGCTT CATCCCTTTCTACAGTGGCCTTATCCCTCCTTCCTTCAGAGGC GTGGTAAGTCGGCTTTCTCTGCTAGCGCTGAGTCCTGGGGGC CTCTGAAGTGTGCTCACACATCTCCTGCCTGCAGGGCACTGG TGTCGGGCACCTCAGGGTCTGTCCCATGGTGGAGCCCCATGC CTCACTGCCTTTCAGACAGAGTAGCCACAGCTGGCCCTATTT CCAGGCTACCCGGGCAGCAAAACTTACTGCATGTGTAATTAA TTATTTGGCTATCTGTAAGGTAAACTGGCTGGTTCACTTAATC TGCACCTTAAGCATCAGATAGCTTCTCAGTGATCTAGTTAAA CTATATGATGTTGGCCAGGCGCGGTGGCTCATGTCTGTAATC CCAGCACTTTGGGAGCCTGAAGCAGGCAGATCACTTGAGGTC AGGAGTTCGAGACCAGCCTGGCCAACAGTGTGAAACTCTGTC TCTCCTAAAAATACAAAAATTAGCTGGGCATGGTGGTGTGCA CCTGTAATCCCAGCTGCTCGGGAGGCTGAGGCAGGAGAATT GCTTGAACTTGGGA
SEQ ID NO: 414	PNPLA3 rs738409	TGCCTGCTGACTGCTCTGTAGCACAGTGCTTCGCAAAGTGTG ATCCTGGGACCAGCAGAGCAGCAGCTCCTTTGAGCTTATTGG AATGGCAGACCCTCAGGTCCCACCTCTGACCTGCTGCATGGG AATTCTGGGGAGGGACGCAGAATCTCTGGTTCCACAGGCTCT CCGGTGATGCTAATGAATACCGGCATTTGAACAGCACCGATC TAGCCCCTTTCAGTCCATGAGCCAACAACCCTTGGTCCTGTCT GTGGTGACCCAGTGTGACTCTCATGGGGAGCAAGGAGAGGA AGTTGAAGTTCACTGACAGGGTTGTTAAGGGGATTATGCAAT AGATGAGACCCATGGGCCTGAAGTCCGAGGGTGTATGTTAGT TCCCCGTTCTTTTGACCCATGGATTAACCTACTCTGTGCAAAG GGCATTTTCAAGTTTGTTGCCCTGCTCACTTGGAGAAAGCTT ATGAAGGATCAGGAAAATTAAAAGGGTGCTCTCGCCTATAA CTTCTCTCTCCTTTGCTTTCACAGGCCTTGGTATGTTCCTGCTT CATGCCCTTCTACAGTGGCCTTATCCCTCCTTCCTTCAGAGGC GTGGTAAGTCGGCTTTCTCTGCTAGCGCTGAGTCCTGGGGGC CTCTGAAGTGTGCTCACACATCTCCTGCCTGCAGGGCACTGG TGTCGGGCACCTCAGGGTCTGTCCCATGGTGGAGCCCCATGC CTCACTGCCTTTCAGACAGAGTAGCCACAGCTGGCCCTATTT CCAGGCTACCCGGGCAGCAAAACTTACTGCATGTGTAATTAA TTATTTGGCTATCTGTAAGGTAAACTGGCTGGTTCACTTAATC TGCACCTTAAGCATCAGATAGCTTCTCAGTGATCTAGTTAAA CTATATGATGTTGGCCAGGCGCGGTGGCTCATGTCTGTAATC CCAGCACTTTGGGAGCCTGAAGCAGGCAGATCACTTGAGGTC AGGAGTTCGAGACCAGCCTGGCCAACAGTGTGAAACTCTGTC TCTCCTAAAAATACAAAAATTAGCTGGGCATGGTGGTGTGCA CCTGTAATCCCAGCTGCTCGGGAGGCTGAGGCAGGAGAATT GCTTGAACTTGGGA
SEQ ID NO: 415	PNPLA3 WT	TGCCTGCTGACTGCTCTGTAGCACAGTGCTTCGCAAAGTGTG ATCCTGGGACCAGCAGAGCAGCAGCTCCTTTGAGCTTATTGG AATGGCAGACCCTCAGGTCCCACCTCTGACCTGCTGCATGGG AATTCTGGGGAGGGACGCAGAATCTCTGGTTCCACAGGCTCT CCGGTGATGCTAATGAATACCGGCATTTGAACAGCACCGATC TAGCCCCTTTCAGTCCATGAGCCAACAACCCTTGGTCCTGTCT GTGGTGACCCAGTGTGACTCTCATGGGGAGCAAGGAGAGGA

SEQ ID NO:	Target Name	Target Sequence
		AGTTGAAGTTCACTGACAGGGTTGTTAAGGGGATTATGCAAT AGATGAGACCCATGGGCCTGAAGTCCGAGGGTGTATGTTAGT TCCCCGTTCTTTTGACCCATGGATTAACCTACTCTGTGCAAAG GGCATTTTCAAGTTTGTTGCCCTGCTCACTTGGAGAAAGCTT ATGAAGGATCAGGAAAATTAAAAGGGTGCTCTCGCCTATAA CTTCTCTCTCCTTTGCTTTCACAGGCCTTGGTATGTTCCTGCTT CATCCCCTTCTACAGTGGCCTTATCCCTCCTTCCTTCAGAGGC GTGGTAAGTCGGCTTTCTCTGCTAGCGCTGAGTCCTGGGGGC CTCTGAAGTGTGCTCACACATCTCCTGCCTGCAGGGCACTGG TGTCGGGCACCTCAGGGTCTGTCCCATGGTGGAGCCCCATGC CTCACTGCCTTTCAGACAGAGTAGCCACAGCTGGCCCTATTT CCAGGCTACCCGGGCAGCAAAACTTACTGCATGTGTAATTAA TTATTTGGCTATCTGTAAGGTAAACTGGCTGGTTCACTTAATC TGCACCTTAAGCATCAGATAGCTTCTCAGTGATCTAGTTAAA CTATATGATGTTGGCCAGGCGCGGTGGCTCATGTCTGTAATC CCAGCACTTTGGGAGCCTGAAGCAGGCAGATCACTTGAGGTC AGGAGTTCGAGACCAGCCTGGCCAACAGTGTGAAACTCTGTC TCTCCTAAAAATACAAAAATTAGCTGGGCATGGTGGTGTGCA CCTGTAATCCCAGCTGCTCGGGAGGCTGAGGCAGGAGAATT GCTTGAACTTGGGA

[00632] Guide RNAs were designed to detect the wild type sequence or the sequence with a mutation at the second SNP ("rs738409"), but ignore the sequence with the mutation at the first SNP ("rs738408"). Guide RNAs corresponding to SEQ ID NO: 300 – SEQ ID NO: 319 were directed to the wild type (SEQ ID NO: 415, "WT") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 320 – SEQ ID NO: 339 were directed to the wild type ("WT") sequence on the reverse strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 340 – SEQ ID NO: 359 were directed to the mutant (SEQ ID NO: 414, "rs738409") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 360 – SEQ ID NO: 379 were directed to the mutant ("rs738409") sequence on the reverse strand at position 1 – position 20, respectively. Each gRNA was used to detect four different target sequences corresponding to the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a point mutation at a first site (SEQ ID NO: 413, "rs738408"), a sequence with a point mutation at a second site (SEQ ID NO: 414, "rs738409"), or a sequence with point mutations at both the first site and the second site (SEQ ID NO: 412, "rs738409+rs738408").

[00633] Detection reactions were carried out using gRNAs designed to target different positions on the target nucleic acid relative to the position of the SNP on either the forward or reverse strand (shown on the x-axis is the position relative to the SNP on either the forward or reverse strand). Upon hybridization of gRNA to the target nucleic acid, SEQ ID NO: 11 is activated and trans cleaves a reporter (SEQ ID NO: 119 with N-terminal /56-FAM/ and C-terminal

/3IABkFQ/), releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label, and the maximum rate ("Max Rate (AU/min)") was calculated as fluorescence units per minute. gRNAs that exhibited specificity for the wild type sequence ("WT") or the sequence with the mutation at the second SNP ("rs738409"), but did not non-specifically detect the sequence with the mutation at the first SNP ("rs738408"), are indicated by black arrows. Sequences of the gRNAs used are provided in **TABLE 26**.

TABLE 26 – gRNAs for Detection of Target Sequences with Artifically Introduced PAMs

SEQ ID NO:	Target	gRNA Sequence
SEQ ID NO: 300	WT-FWD-1	UAAUUUCUACUAAGUGUAGAUCCCCUUCUACAGUGGCCUUA
SEQ ID NO: 301	WT-FWD-2	UAAUUUCUACUAAGUGUAGAUUCCCCUUCUACAGUGGCCUU
SEQ ID NO: 302	WT-FWD-3	UAAUUUCUACUAAGUGUAGAUAUCCCCUUCUACAGUGGCCU
SEQ ID NO: 303	WT-FWD-4	UAAUUUCUACUAAGUGUAGAUCAUCCCCUUCUACAGUGGCC
SEQ ID NO: 304	WT-FWD-5	UAAUUUCUACUAAGUGUAGAUUCAUCCCCUUCUACAGUGGC
SEQ ID NO: 305	WT-FWD-6	UAAUUUCUACUAAGUGUAGAUUUCAUCCCCUUCUACAGUGG
SEQ ID NO: 306	WT-FWD-7	UAAUUUCUACUAAGUGUAGAUCUUCAUCCCCUUCUACAGUG
SEQ ID NO: 307	WT-FWD-8	UAAUUUCUACUAAGUGUAGAUGCUUCAUCCCCUUCUACAGU
SEQ ID NO: 308	WT-FWD-9	UAAUUUCUACUAAGUGUAGAUUGCUUCAUCCCCUUCUACAG
SEQ ID NO: 309	WT-FWD-10	UAAUUUCUACUAAGUGUAGAUCUGCUUCAUCCCCUUCUACA
SEQ ID NO: 310	WT-FWD-11	UAAUUUCUACUAAGUGUAGAUCCUGCUUCAUCCCCUUCUAC
SEQ ID NO: 311	WT-FWD-12	UAAUUUCUACUAAGUGUAGAUUCCUGCUUCAUCCCCUUCUA
SEQ ID NO: 312	WT-FWD-13	UAAUUUCUACUAAGUGUAGAUUUCCUGCUUCAUCCCCUUCU

SEQ ID NO:	Target	gRNA Sequence
SEQ ID NO: 313	WT-FWD-14	UAAUUUCUACUAAGUGUAGAUGUUCCUGCUUCAUCCCCUUC
SEQ ID NO: 314	WT-FWD-15	UAAUUUCUACUAAGUGUAGAUUGUUCCUGCUUCAUCCCCUU
SEQ ID NO: 315	WT-FWD-16	UAAUUUCUACUAAGUGUAGAUAUGUUCCUGCUUCAUCCCCU
SEQ ID NO: 316	WT-FWD-17	UAAUUUCUACUAAGUGUAGAUUAUGUUCCUGCUUCAUCCCC
SEQ ID NO: 317	WT-FWD-18	UAAUUUCUACUAAGUGUAGAUGUAUGUUCCUGCUUCAUCCC
SEQ ID NO: 318	WT-FWD-19	UAAUUUCUACUAAGUGUAGAUGGUAUGUUCCUGCUUCAUCC
SEQ ID NO: 319	WT-FWD-20	UAAUUUCUACUAAGUGUAGAUUGGUAUGUUCCUGCUUCAUC
SEQ ID NO: 320	WT-REV-1	UAAUUUCUACUAAGUGUAGAUGAUGAAGCAGGAACAUACCA
SEQ ID NO: 321	WT-REV-2	UAAUUUCUACUAAGUGUAGAUGGAUGAAGCAGGAACAUACC
SEQ ID NO: 322	WT-REV-3	UAAUUUCUACUAAGUGUAGAUGGGAUGAAGCAGGAACAUAC
SEQ ID NO: 323	WT-REV-4	UAAUUUCUACUAAGUGUAGAUGGGGAUGAAGCAGGAACAUA
SEQ ID NO: 324	WT-REV-5	UAAUUUCUACUAAGUGUAGAUAGGGGAUGAAGCAGGAACAU
SEQ ID NO: 325	WT-REV-6	UAAUUUCUACUAAGUGUAGAUAAGGGGAUGAAGCAGGAACA
SEQ ID NO: 326	WT-REV-7	UAAUUUCUACUAAGUGUAGAUGAAGGGGAUGAAGCAGGAAC
SEQ ID NO: 327	WT-REV-8	UAAUUUCUACUAAGUGUAGAUAGAAGGGGAUGAAGCAGGAA
SEQ ID NO: 328	WT-REV-9	UAAUUUCUACUAAGUGUAGAUUAGAAGGGGAUGAAGCAGGA
SEQ ID NO: 329	WT-REV-10	UAAUUUCUACUAAGUGUAGAUGUAGAAGGGGAUGAAGCAGG
SEQ ID NO: 330	WT-REV-11	UAAUUUCUACUAAGUGUAGAUUGUAGAAGGGGAUGAAGCAG

SEQ ID NO:	Target	gRNA Sequence
SEQ ID NO: 331	WT-REV-12	UAAUUUCUACUAAGUGUAGAUCUGUAGAAGGGGAUGAAGCA
SEQ ID NO: 332	WT-REV-13	UAAUUUCUACUAAGUGUAGAUACUGUAGAAGGGGAUGAAGC
SEQ ID NO: 333	WT-REV-14	UAAUUUCUACUAAGUGUAGAUCACUGUAGAAGGGGAUGAAG
SEQ ID NO: 334	WT-REV-15	UAAUUUCUACUAAGUGUAGAUCCACUGUAGAAGGGGAUGAA
SEQ ID NO: 335	WT-REV-16	UAAUUUCUACUAAGUGUAGAUGCCACUGUAGAAGGGGAUGA
SEQ ID NO: 336	WT-REV-17	UAAUUUCUACUAAGUGUAGAUGGCCACUGUAGAAGGGGAUG
SEQ ID NO: 337	WT-REV-18	UAAUUUCUACUAAGUGUAGAUAGGCCACUGUAGAAGGGGAU
SEQ ID NO: 338	WT-REV-19	UAAUUUCUACUAAGUGUAGAUAAGGCCACUGUAGAAGGGGA
SEQ ID NO: 339	WT-REV-20	UAAUUUCUACUAAGUGUAGAUUAAGGCCACUGUAGAAGGGG
SEQ ID NO: 340	rs738409-FWD-1	UAAUUUCUACUAAGUGUAGAUGCCCUUCUACAGUGGCCUUA
SEQ ID NO: 341	rs738409-FWD-2	UAAUUUCUACUAAGUGUAGAUUGCCCUUCUACAGUGGCCUU
SEQ ID NO: 342	rs738409-FWD-3	UAAUUUCUACUAAGUGUAGAUAUGCCCUUCUACAGUGGCCU
SEQ ID NO: 343	rs738409-FWD-4	UAAUUUCUACUAAGUGUAGAUCAUGCCCUUCUACAGUGGCC
SEQ ID NO: 344	rs738409-FWD-5	UAAUUUCUACUAAGUGUAGAUUCAUGCCCUUCUACAGUGGC
SEQ ID NO: 345	rs738409-FWD-6	UAAUUUCUACUAAGUGUAGAUUUCAUGCCCUUCUACAGUGG
SEQ ID NO: 346	rs738409-FWD-7	UAAUUUCUACUAAGUGUAGAUCUUCAUGCCCUUCUACAGUG
SEQ ID NO: 347	rs738409-FWD-8	UAAUUUCUACUAAGUGUAGAUGCUUCAUGCCCUUCUACAGU
SEQ ID NO: 348	rs738409-FWD-9	UAAUUUCUACUAAGUGUAGAUUGCUUCAUGCCCUUCUACAG

SEQ ID NO:	Target	gRNA Sequence
SEQ ID NO: 349	rs738409-FWD-10	UAAUUUCUACUAAGUGUAGAUCUGCUUCAUGCCCUUCUACA
SEQ ID NO: 350	rs738409-FWD-11	UAAUUUCUACUAAGUGUAGAUCCUGCUUCAUGCCCUUCUAC
SEQ ID NO: 351	rs738409-FWD-12	UAAUUUCUACUAAGUGUAGAUUCCUGCUUCAUGCCCUUCUA
SEQ ID NO: 352	rs738409-FWD-13	UAAUUUCUACUAAGUGUAGAUUUCCUGCUUCAUGCCCUUCU
SEQ ID NO: 353	rs738409-FWD-14	UAAUUUCUACUAAGUGUAGAUGUUCCUGCUUCAUGCCCUUC
SEQ ID NO: 354	rs738409-FWD-15	UAAUUUCUACUAAGUGUAGAUUGUUCCUGCUUCAUGCCCUU
SEQ ID NO: 355	rs738409-FWD-16	UAAUUUCUACUAAGUGUAGAUAUGUUCCUGCUUCAUGCCCU
SEQ ID NO: 356	rs738409-FWD-17	UAAUUUCUACUAAGUGUAGAUUAUGUUCCUGCUUCAUGCCC
SEQ ID NO: 357	rs738409-FWD-18	UAAUUUCUACUAAGUGUAGAUGUAUGUUCCUGCUUCAUGCC
SEQ ID NO: 358	rs738409-FWD-19	UAAUUUCUACUAAGUGUAGAUGGUAUGUUCCUGCUUCAUGC
SEQ ID NO: 359	rs738409-FWD-20	UAAUUUCUACUAAGUGUAGAUUGGUAUGUUCCUGCUUCAUG
SEQ ID NO: 360	rs738409-REV-1	UAAUUUCUACUAAGUGUAGAUCAUGAAGCAGGAACAUACCA
SEQ ID NO: 361	rs738409-REV-2	UAAUUUCUACUAAGUGUAGAUGCAUGAAGCAGGAACAUACC
SEQ ID NO: 362	rs738409-REV-3	UAAUUUCUACUAAGUGUAGAUGGCAUGAAGCAGGAACAUAC
SEQ ID NO: 363	rs738409-REV-4	UAAUUUCUACUAAGUGUAGAUGGGCAUGAAGCAGGAACAUA
SEQ ID NO: 364	rs738409-REV-5	UAAUUUCUACUAAGUGUAGAUAGGGCAUGAAGCAGGAACAU
SEQ ID NO: 365	rs738409-REV-6	UAAUUUCUACUAAGUGUAGAUAAGGGCAUGAAGCAGGAACA
SEQ ID NO: 366	rs738409-REV-7	UAAUUUCUACUAAGUGUAGAUGAAGGGCAUGAAGCAGGAAC

SEQ ID NO:	Target	gRNA Sequence
SEQ ID NO: 367	rs738409-REV-8	UAAUUUCUACUAAGUGUAGAUAGAAGGGCAUGAAGCAGGAA
SEQ ID NO: 368	rs738409-REV-9	UAAUUUCUACUAAGUGUAGAUUAGAAGGGCAUGAAGCAGGA
SEQ ID NO: 369	rs738409-REV-10	UAAUUUCUACUAAGUGUAGAUGUAGAAGGGCAUGAAGCAGG
SEQ ID NO: 370	rs738409-REV-11	UAAUUUCUACUAAGUGUAGAUUGUAGAAGGGCAUGAAGCAG
SEQ ID NO: 371	rs738409-REV-12	UAAUUUCUACUAAGUGUAGAUCUGUAGAAGGGCAUGAAGCA
SEQ ID NO: 372	rs738409-REV-13	UAAUUUCUACUAAGUGUAGAUACUGUAGAAGGGCAUGAAGC
SEQ ID NO: 373	rs738409-REV-14	UAAUUUCUACUAAGUGUAGAUCACUGUAGAAGGGCAUGAAG
SEQ ID NO: 374	rs738409-REV-15	UAAUUUCUACUAAGUGUAGAUCCACUGUAGAAGGGCAUGAA
SEQ ID NO: 375	rs738409-REV-16	UAAUUUCUACUAAGUGUAGAUGCCACUGUAGAAGGGCAUGA
SEQ ID NO: 376	rs738409-REV-17	UAAUUUCUACUAAGUGUAGAUGGCCACUGUAGAAGGGCAUG
SEQ ID NO: 377	rs738409-REV-18	UAAUUUCUACUAAGUGUAGAUAGGCCACUGUAGAAGGGCAU
SEQ ID NO: 378	rs738409-REV-19	UAAUUUCUACUAAGUGUAGAUAAGGCCACUGUAGAAGGGCA
SEQ ID NO: 379	rs738409-REV-20	UAAUUUCUACUAAGUGUAGAUUAAGGCCACUGUAGAAGGGC

[00634] In a third assay, sensitivity of the detection assay to single- and double-point mutations was tested. As used in the assay shown in **FIG. 78**, the PNPLA3 target sequence with two SNP sites was capable of being detected by certain gRNAs. Sensitivity of a programmable nuclease of SEQ ID NO: 11 to SNP mutations was tested individually and in combination. **FIG. 78** shows detection of single and double mutations in a target nucleic acid sequence using a programmable nuclease of SEQ ID NO: 11. Target sequences corresponding to SEQ ID NO: 412 – SEQ ID NO: 415 were detected. Samples containing a target nucleic acid ("target") with either a wild type nucleic acid sequence (SEQ ID NO: 415, "WT"), a sequence with a mutation at a first SNP (SEQ ID NO: 413, "rs738408"), a sequence with a mutation at a second SNP (SEQ ID NO: 414,

"rs738409"), or a sequence with mutations at the first SNP and the second SNP (SEQ ID NO: 412, "rs738409/408") were detected using a gRNA directed to the wild type sequence (SEQ ID NO: 301, "R1287 – WT"), a gRNA directed to the sequence with a mutation at a second SNP (SEQ ID NO: 341, "R1327 - rs738409"), a gRNA directed to the sequence with a mutation at a first SNP (SEQ ID NO: 421, "R1434 - rs738408"), or a gRNA directed to the sequence with mutations at the first SNP and the second SNP (SEQ ID NO: 422, "R1435 – rs738409/408"). Detection was measured using fluorescence, and the maximum rate ("Max Rate (AU/min)") was calculated as fluorescence units per minute. The results showed that gRNAs could be designed that were specific to each of the wild type sequence (SEQ ID NO: 415, "WT"), the sequence with a mutation at a first SNP (SEQ ID NO: 413, "rs738408"), the sequence with a mutation at a second SNP (SEQ ID NO: 414, "rs738409"), or the sequence with mutations at both the first SNP and the second SNP (SEQ ID NO: 412, "rs738409/408"). Furthermore, a programmable nuclease of SEQ ID NO: 11 was sensitive to single and double mutations and gRNAs were designed to detect all allele possibilities. NTC shows control experiments without the target nucleic acid.

[00635] In a fourth assay, the functionality of gRNAs targeted to specific point mutations in combined pools was tested. The wild type, single-, and double-point mutants tested in the assay shown in **FIG. 78** were tested with pooled gRNAs. **FIG. 79** shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native PAM using a programmable nuclease of SEQ ID NO: 11. Target nucleic acids containing the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a mutation at a first SNP (SEQ ID NO: 413, "rs738408"), a sequence with a mutation at a second SNP (SEQ ID NO: 414, "rs738409"), or a sequence with mutations at the first SNP allele and the second SNP (SEQ ID NO: 412, "rs738409/408"). A sample without a target sequence (non-target control, "NTC") was used as a negative control. Guide RNAs designed to detect the wild type sequence or the sequence with a mutation at the first SNP (SEQ ID NO: 301 and SEQ ID NO: 421, "WT DETECTR") were pooled to detect the target nucleic acid in each sample type. Guide RNAs designed to detect the sequence with a mutation at the second SNP (SEQ ID NO: 341 and SEQ ID NO: 422, "rs738409 DETECTR") were pooled to detect the target nucleic acid in each sample type. Detection was measured using fluorescence, and the maximum rate ("Max Rate (AU/min)") was calculated as fluorescence units per minute. The results showed that gRNAs could be pooled to selectively detect SNPs of interest and not other nearby genetic variation. A first gRNA pool was able to detect both the wild type sequence (SEQ ID NO: 415, "WT") and the sequence with a mutation at a first SNP (SEQ ID NO: 413, "rs738408"), while a second gRNA pool was able to detect the sequence with a mutation at a second SNP (SEQ ID NO: 414, "rs738409") and the sequence with mutations at both the first

SNP and the second SNP (SEQ ID NO: 412, "rs738409/408"). Guide RNA sequences used in each pool are provided in **TABLE 27**.

TABLE 27 – gRNAs Pools for Detection of two PNPLA3 SNP Alleles

SEQ ID NO:	gRNA Pool	gRNA Sequence
SEQ ID NO: 301	WT DETECTR gRNAs	UAAUUUCUACUAAGUGUAGAUUCCCCUUCUACAGUG GCCUU
SEQ ID NO: 421	WT DETECTR gRNAs	UAAUUUCUACUAAGUGUAGAUUCCCUUUCUACAGUG GCCUU
SEQ ID NO: 341	Rs738409 DETECTR gRNAs	UAAUUUCUACUAAGUGUAGAUUGCCCUUCUACAGUG GCCUU
SEQ ID NO: 422	Rs738409 DETECTR gRNAs	UAAUUUCUACUAAGUGUAGAUUGCCUUUCUACAGUG GCCUU

EXAMPLE 28

LbCas12a SNP Sensitivity Along a Target Sequence

[00636] This example describes sensitivity of LbCas12a to SNPs positioned along a target sequence. In a first assay, sensitivity to point mutations in a target sequence with a native PAM site was tested. To determine which positions along a target nucleic acid sequence were most sensitive to single point mutations, all four nucleotide possibilities (A, T, C, or G) at each position were tiled along a target of a target nucleic acid sequence. The assay was performed for two target nucleic acid sequences, a HERC2 target nucleic acid sequence and an ALDH target nucleic acid sequence. Both target nucleic acid sequences comprised a native PAM site. The target nucleic acids comprising the PAM site with each of all possible point mutations were detected with using LbCas12a (SEQ ID NO: 1).

[00637] FIG. 80 shows detection of single point mutations at different positions along a nucleic acid sequence using LbCas12a (SEQ ID NO: 1). Point mutations to each nucleic acid (A, T, C, or G, "SNP Base (target)") were made along a target nucleic acid sequence at different positions relative to a native PAM. To determine the sensitivity of the detection assay to single point mutations (e.g., a SNP), the target nucleic acid was detected using a gRNA directed to hybridize to the wild type sequence. Black circles label with "WT" indicate nucleotide at each position of the wild type sequence that is reverse complementary to the gRNA sequence. The assay was performed with a HERC2 target sequence (top panel, wild type sequence corresponding to SEQ ID NO: 416) or an ALDH target sequence (bottom panel, wild type sequence corresponding to SEQ ID NO: 417). Upon hybridization of gRNA to the target nucleic acid, SEQ ID NO: 11 is

activated and trans cleaves a reporter, releasing a fluorescent detectable label. The HERC2 sequence was detected using a gRNA corresponding to SEQ ID NO: 246 (top plot) and the ALDH sequence was detected using a gRNA corresponding to SEQ ID NO: 425 (bottom plot). Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label, and the maximum rate ("Average Max Rate") was calculated as fluorescence units per minute and averaged between four replicates. Results indicated that LbCas12a was sensitive to point mutations along the entire length of the gRNA target site. The specificity for individual point mutations depended on sequence context of the target nucleic acid.

[00638] In a second assay, LbCas12a (SEQ ID NO: 1) was used to detect variants at two SNP sites in a target nucleic acid sequence without a native PAM. The detection assay was run for 90 minutes at 37° C with either a wild type DNA ("WT"), a target DNA with a mutation at a first SNP ("rs738408"), or a target DNA with a mutation at a second SNP ("rs738409").

[00639] FIG. 81 shows detection of two PNPLA3 SNPs in a target nucleic acid sequence without a native "TTTN" PAM sequence using LbCas12a (SEQ ID NO: 1). Target nucleic acids tested contained the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a mutation at a first SNP (SEQ ID NO: 413, "rs738408"), a sequence with a mutation at a second SNP (SEQ ID NO: 414, "rs738409"), or a sequence with mutations at the first SNP allele and the second SNP (SEQ ID NO: 412, "rs738409/408"). Guide RNAs were designed to detect the wild type sequence ("WT specific") or the sequence with a mutation at the second SNP ("rs738409 specific"). Guide RNAs corresponding to SEQ ID NO: 300 – SEQ ID NO: 319 were directed to the wild type (SEQ ID NO: 415, "WT") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 320 – SEQ ID NO: 339 were directed to the wild type ("WT") sequence on the reverse strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 340 – SEQ ID NO: 359 were directed to the mutant (SEQ ID NO: 414, "rs738409") sequence on the forward strand at position 1 – position 20, respectively. gRNAs corresponding to SEQ ID NO: 360 – SEQ ID NO: 379 were directed to the mutant ("rs738409") sequence on the reverse strand at position 1 – position 20, respectively. Each gRNA was used to detect four different target sequences corresponding to the wild type sequence (SEQ ID NO: 415, "WT"), a sequence with a point mutation at a first site (SEQ ID NO: 413, "rs738408"), a sequence with a point mutation at a second site (SEQ ID NO: 414, "rs738409"), or a sequence with point mutations at both the first site and the second site (SEQ ID NO: 412, "rs738409+rs738408"). Sequences of the gRNAs used in this experiment are provided in **TABLE 26**.

[00640] Detection reactions were carried out using gRNAs designed to target different positions on the target nucleic acid relative to the position of the SNP on either the forward or reverse strand (shown on the x-axis is the position relative to the SNP on either the forward or reverse strand). Upon hybridization of gRNA to the target nucleic acid, LbCas12a was activated and trans cleaved a reporter (SEQ ID NO: 119 with N-terminal /56-FAM/ and C-terminal /3IABkFQ/), releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label, and the maximum rate ("Max Rate (AU/min)") was calculated as fluorescence units per minute. gRNAs that exhibited specificity for the wild type sequence or the sequence with the mutation at the second SNP are indicated by arrows. The results demonstrated that certain gRNAs were specific for the wild type sequence ("WT") or the sequence with the mutation at the second SNP ("rs738409"), but did not non-specifically detect the sequence with the mutation at the first SNP ("rs738408").

EXAMPLE 29

CasY3 SNP Sensitivity Along a CYP2C19*2 SNP Target Sequence

[00641] This example describes sensitivity of CasY3 to the CYP2C19*2 SNP positioned along a target sequence. The ability for CasY3 to discriminate single point mutations is tested. The SNP sensitivity of CasY3 for the CYP2C19*2 SNP on sequences with and without native TR PAMs is tested. Target nucleic acids having either a wild type sequence ("WT") or a sequence with a single point mutation ("mt") are detected using CasY3. Target DNA is detected at a concentration of 1 nM in the detection assay, which is run for 90 minutes at 37°C. Target nucleic acid sequences with or without a native TR PAM using CasY3 (SEQ ID NO: 282) are detected. Samples comprise a wild type target nucleic acid sequence or a sequence with a mutation at a SNP are detected with gRNAs comprising a crRNA and a scout RNA (sctRNA) designed to target different positions relative on the target nucleic acid relative to the position of the SNP on either the forward strand or the reverse strand.

[00642] crRNAs are directed to the wild type sequence on the forward strand at position 1 – position 18 on a target nucleic acid, respectively. crRNAs corresponding to are directed to the wild type sequence on the reverse strand at position 1 – position 18 on a target nucleic acid, respectively. Upon hybridization of gRNA to the target nucleic acid, CasY3 is activated and trans cleaves a reporter, releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs is carried out by measuring fluorescence from the cleaved detectable label. The maximum rate ("Max Rate (AU/min)") is calculated as fluorescence units per minute. gRNAs are identified that are specific for the wild type sequence or the sequence with the mutation at the second SNP.

EXAMPLE 30

LbuCas13a SNP Sensitivity Along a Target Sequence

[00643] This example describes sensitivity of LbuCas13a to SNPs positioned along a target sequence. RNA and ssDNA target nucleic acid sequences were tested. In a first assay, sensitivity to point mutations in an RNA target nucleic acid was tested. To determine which positions along an RNA target nucleic acid sequence were most sensitive to single point mutations, all four nucleotide possibilities (A, T, C, or G) at each position were tiled along a target of a target nucleic acid sequence. The assay was performed for a target RNA sequence from influenza A virus. The RNA target nucleic acid with each of all possible point mutations was detected with using LbuCas13a (SEQ ID NO: 104). The RNA target nucleic acid was detected at 0.25 nM target, and the assay was run for 20 minutes.

[00644] **FIG. 82** shows detection of single point mutations at different positions along target RNA sequence (SEQ ID NO: 748) using LbuCas13a (SEQ ID NO: 104). Point mutations to each nucleic acid (A, T, C, or G, "SNP Base (target)") were made along a target nucleic acid sequence at different positions relative to a native PAM. To determine the sensitivity of the detection assay to single point mutations (e.g., a SNP), the target nucleic acid was detected using a gRNA directed to hybridize to the wild type sequence. Black circles label with "WT" indicate nucleotide at each position of the wild type sequence that is reverse complementary to the gRNA sequence. Data is not shown for wild type positions (black circles labeled with "WT"). Detection of the wild type sequence is shown in the square marked "WT" at SNP position 1. Detection of a negative control (water) is shown in the sqare marked "None" at position "None." The targets were detected using a gRNA corresponding to SEQ ID NO: 507. Upon hybridization of gRNA to the target nucleic acid, LbuCas13a was activated and trans cleaved a reporter, releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label. Results showed that LbuCas13a was able to differentiate certain single point mutations at some positions along the RNA target sequence. Sites at which LbuCas13a was able to distinguish all four nucleotide positions are indicated by arrows.

[00645] In a second assay, sensitivity to point mutations in a ssDNA target nucleic acid was tested. To determine which positions along a ssDNA target nucleic acid sequence were most sensitive to single point mutations, all four nucleotide possibilities (A, T, C, or G) were tiled along a target of a target nucleic acid sequence. The assay was performed for a target ssDNA sequence from influenza A virus. The RNA target nucleic acid with each of all possible point mutations was detected with using LbuCas13a (SEQ ID NO: 104). The RNA target nucleic acid was detected at 2.5 nM target, and the assay was run for 20 minutes at 37°C. **FIG. 83** shows

detection of single point mutations at different positions along target ssDNA (SEQ ID NO: 749)sequence using LbuCas13a (SEQ ID NO: 104). Point mutations to each nucleic acid (A, T, C, or G, "SNP Base (target)") were made along a target nucleic acid sequence with a wild type sequence corresponding to SEQ ID NO: 407 (TCTACGCTGCAGTCCTCGCT) at different positions relative to a native PAM. To determine the sensitivity of the detection assay to single point mutations (e.g., a SNP), the target nucleic acid was detected using a gRNA directed to the wild type sequence. Black circles label with "WT" indicate the nucleotide at each position of the wild type sequence that is reverse complementary to the gRNA sequence. Data is not shown for wild type positions (black circles labeled with "WT"). Detection of the wild type sequence is shown in the square marked "WT" at SNP position 1. Detection of a negative control (water) is shown in the sqare marked "None" at position "None." The targets were detected using a gRNA corresponding to SEQ ID NO: 507. Upon hybridization of gRNAs to the target nucleic acid, LbuCas31a was activated and trans cleaved a reporter, releasing a fluorescent detectable label. Detection of target nucleic acids with SNPs was carried out by measuring fluorescence from the cleaved detectable label. Results showed that LbuCas13a was able to differentiate certain single point mutations at some positions along the ssDNA target sequence. Sites at which LbuCas13a was able to distinguish all four nucleotide mutations are indicated by black arrows. Sites at which LbuCas13a was able to distinguish at least one nucleotide mutation are indicated by gray arrows.

EXAMPLE 31

Detection of a Nucleic Acid Amplified with dUTPs using DETECTR

[00646] This example describes detection of a nucleic acid that had been amplified with dUTPs using a DETECTR reaction. Two target nucleic acid sequences, ALDH2 wild type sequence and ALDH2 with a single point mutation at T790M ("T790M"), were amplified in a PCR reaction performed with dUTP nucleotide bases in place of dTTP nucleotide bases. Each PCR amplification reaction included 1 μM forward primer, 1 μM reverse primer, Taq polymerase (in Taq master mix), UDG enzyme, and a template sequence with either the ALDH2 wild type sequence or ALDH2 T790M mutant sequence. The UDG enzyme was heat-activated at 50°C prior to amplification to degrade nucleic acid contaminants containing dU bases. The UDG enzyme was subsequently inactivated at 90°C before initiating PCR amplification of the target nucleic acid. Amplification of the target sequence was verified by gel electrophoresis. The ALDH2 wild type sequence was successfully amplified. In an alternate assay configuration, a thermolabile UDG enzyme was used in place of the UDG enzyme. The thermolabile UDG enzyme was activated at 25°C for 10 minutes prior to amplification to degrade nucleic acid

contaminants containing dU bases. The thermolabile UDG enzyme was subsequently inactivated at a temperature of at least 50°C before initiating PCR amplification of the target nucleic acid.

[00647] The amplified ALDH2 wild type sequence with dUTPs was detected using an LbCas12a detection assay. The ALDH2 wild type sequence was detected with either a gRNA directed to hybridize to the wild type sequence ("ALDH2 (WT SNP")) or a gRNA directed to hybridize to the T790M mutant sequence ("ALDH2 (Mutant SNP)"). Each detection reaction included 1.25 μM gRNA, 200 nM LbCas12a (SEQ ID NO: 1), 100 nM ssDNA-FQ reporter substrate, and the target sequence containing dUTPs.

[00648] FIG. 71A – FIG. 71B show the detection of ALDH2 WT allele from human genomic DNA (SEQ ID NO: 417) with dU-containing amplicons with Cas12. The ALDH2 gene was amplified from human saliva containing the WT allele using Taq master mix containing dUTP in place of dTTP, such that all T nucleotides with the annotated ALDH2 target sequence shown in **FIG. 71A** have been replaced by U nucleotides. The amplicon was added directly to a Cas12 DETECTR assay. Cas12 guide RNAs targeting the ALDH2 WT allele detected only the cognate WT sequence and not the mutant allele, demonstrating that Cas12 is capable of SNP detection with dU-containing targets. **FIG. 71B** shows a DETECTR reaction of an ALDH2 target nucleic acid sequence amplified with dUTPs using LbCas12a (SEQ ID NO: 1). Fluorescence was measured over time in the presence of the wild type nucleic acid sequence ("WT SNP", top most line), a sequence with a point mutation ("Mutant SNP", middle line), or a negative control without the target nucleic acid sequence (bottom line). Samples were detected with gRNAs directed to hybridize to the wild type sequence. Results showed that the LbCas12a detection assay detected target nucleic acid sequences amplified with dUTPs. The detection assay was specific for the gRNA directed to the wild type sequence and did not non-specifically detect the wild type sequence with the gRNA directed to hybridize to the T790M mutant sequence. Primers used in this assay are provided in **TABLE 22**.

[00649] To verify that the amplified product detected in the LbCas12a detection reaction contained dUTPs, the target nucleic acid was contacted with UDG enzyme which degrades nucleic acid sequences with dUTPs. The amplified target sequence was successfully degraded by UDG. Degradation of the target sequence by UDG was verified by gel electrophoresis.

EXAMPLE 32

In vitro Transcription of a Nucleic Acid Reverse Transcribed and Amplified with dUTPs

[00650] This example describes *in vitro* transcription of a nucleic acid that had been reverse transcribed and amplified with dUTPs. Target RNA nucleic acid sequences were amplified using primers directed to sites with the prostate cancer biomarkers PCA3, PSA, and T2ERG. The target

RNA sequences were reverse transcribed using a reverse transcriptase enzyme. The reverse transcribed DNA was then amplified using PCR with dUTP nucleic acid bases in place of dTTP nucleic acid bases. Amplification was verified by gel electrophoresis. The amplified DNA was then transcribed into RNA using an *in vitro* transcription reaction with a T7 RNA polymerase.

EXAMPLE 33

Detection of Chlamydia using PCR, IVT, and DETECTR

[00651] This example describes detection of chlamydia nucleic acids in a sample using PCR, *in vitro* transcription (IVT) and DETECTR. Detection sensitivity for chlamydia nucleic acids was improved by amplifying a chlamydia target nucleic acid sequence and reverse transcribing the amplified sequence by *in vitro* transcription. The target sequence was PCR amplified using dUTPs in place of dTTPs, as described in **EXAMPLE 31**. The amplified PCR product was used as a template for the *in vitro* transcription reaction. *In vitro* transcription reaction was performed with a T7 RNA polymerase. The amplified and transcribed nucleic acid sequence was detected using an LbuCas13a detection assay. The detection assay was performed with 40 nM gRNA and 40 nM LbuCas13a (SEQ ID NO: 104). The reaction was run for 30 minutes at 37° C. Following incubation, FAM-U5 reporter and RNase inhibitor were added to the reaction mixture and fluorescence was measured.

[00652] FIG. 84 shows detection of a *Chlamydia trachomatis*target nucleic acid sequence with LbuCas13a (SEQ ID NO: 104) following polymerase chain reaction (PCR) amplification and *in vitro* transcription (IVT) of samples that were either positive or negative for Chlamydia. Targets were detected with either a gRNA targeted to Chlamydia 5S rRNA (SEQ ID NO: 418), a gRNA targeted to Chlamydia 16S rRNA (SEQ ID NO: 419), or an off-target gRNA (SEQ ID NO: 420). Fluorescence was measured over time. An increase in fluorescence indicated detection of the target nucleic acid sequence. Thirty-one samples either positive or negative for Chlamydia or a negative control with no target nucleic acid were detected. Fluorescence over time detected in each sample is shown in individual plots. The top left plot shows the negative control. Each sample was detected with each of three different gRNAs (SEQ ID NO: 418, "CT001-33;" SEQ ID NO: 419, "SSU-1368;" or SEQ ID NO: 410, "C"), as shown by individual traces in each plot. Sequences for the gRNAs are provided in **TABLE 28**.

TABLE 28 – gRNAs for Detection of a Chlamhydia Target Nucleic Acid

SEQ ID NO:	gRNA Name	gRNA Sequence
SEQ ID NO: 418	CT001-33	GGCCACCCCAAAAAUGAAGGGGACUAAAACACUUCUGAG UUCGGAAUGGUG

SEQ ID NO:	gRNA Name	gRNA Sequence
SEQ ID NO: 419	SSU-1368	GGCCACCCCAAAAAUGAAGGGGACUAAAACAACGUAUUC ACGGCGUUAUGG
SEQ ID NO: 420	R003	GGCCACCCCAAAAAUGAAGGGGACUAAAACAGUGAUAAG UGGAAUGCCAUG

[00653] FIG. 85 shows heatmaps of the fluorescence detected in **FIG. 84** (right). Panels on the right indicate the maximum fluorescent rate detected with either a gRNA targeting a Chlamydia 16S RNA sequence (SEQ ID NO: 419, "16S gRNA"), a gRNA targeting a Chlamydia 5S RNA sequence (SEQ ID NO: 418, "5S gRNA"), or a gRNA not directed to a Chlamydia target sequence (SEQ ID NO: 420, "off-target gRNA"). Shaded boxes in the left column ("Ct") indicate that the sample was positive for Chlamydia. Results showed that SSU-1368 (SEQ ID NO: 419) is more specific for the chlamydia target sequence than CT001-33 (SEQ ID NO: 418).

EXAMPLE 34

Identification of Cas12 Variants with Trans Cleavage Activity

[00654] This example describes the identification of Cas12 variants with trans cleavage activity. Different Cas12 variants corresponding to SEQ ID NO: 11, SEQ ID NO: 3, and SEQ ID NO: 571 – SEQ ID NO: 589 were tested for trans cleavage activity as well as sensitivity and specificity for target sequences.

[00655] In a first assay, each Cas12 variant was tested for sensitivity and ability to detect a target nucleic acid sequence with different PAM sequences. DETECTR trans cleavage assays were performed in the presence of activity buffer (5mM $MgCl_2$, 20 mM, Tris pH 7.5, 120 mM NaCl, and 1% glycerol). Different dsDNA target nucleic acids with different PAM sequences were detected at a final concentration of 100 nM. ssDNA reporters were present in each reaction at a concentration of 50 nM. Target dsDNA was obtained by annealing complementary ssDNA primers at a ratio of 2:1 of non-target strand to target strand in hybridization buffer (50mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA), to ensure double-stranded DNA is being detected instead of single-stranded DNA. Pre-crRNA was ordered from Synthego. The protein of interest and the guide RNA were added to each tube and incubated for 20 minutes at 37°C. Each reaction contained 16 μL of the incubated mastermix. **FIG. 85** shows trans cleavage rates of different Cas12 variants upon complex formation with a gRNA and a target sequence comprising different PAM sequences. PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.Individual plots show trans cleavage rates for each Cas12 variant, and each part illustrates the cleavage rate for target sequences comprising different PAM sequences. The pre-crRNA used in each reaction was

GUUUCAAAGAUUAAAUAAUUUCUACUAAGUGUAGAUUCCUGCAGCAGAAAAUCA AAGACAAUGAAUAUUUCGGCGC (SEQ ID NO: 380). Trans cleavage activity was measured as a function of cleavage rate. Variants including SEQ ID NO: 11, SEQ ID NO: 575, SEQ ID NO: 581, SEQ ID NO: 587, and SEQ ID NO: 3 exhibit transcleavage activity in the presence of a variety of PAM sequences.

[00656] In a second assay, each Cas12 variant was tested for sensitivity of trans cleavage activity to base pair mismatches between the target nucleic acid and the gRNA. To measure the tolerance of each Cas12 variant to mismatches, single or double mismatches were introduced in the first ("1MM"), fifth ("5MM"), tenth ("10MM"), fifteenth ("15MM"), and twentieth ("20MM") nucleotide position after the PAM (TTTA). Sequences of the mismatched strands are listed in **TABLE 29**. DETECTR trans cleavage assays were performed in the presence of activity buffer (5mM $MgCl_2$, 20 mM, Tris pH 7.5). Different dsDNA target nucleic acids with different base pair mismatched sequences were detected at a final concentration of 100 nM. ssDNA reporters were present in each reaction at a concentration of 50 nM. Target dsDNA was obtained by annealing complementary ssDNA primers at a ratio of 2:1 of non-target strand to target strand in hybridization buffer (50mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA), to ensure double-stranded DNA is being detected instead of single-stranded DNA. Pre-crRNA was ordered from Synthego. The protein of interest and the guide RNA were added to each tube and incubated for 20 minutes at 37°C. Each reaction contained 16 µL of the incubated mastermix. **FIG. 87A** shows a schematic of a Cas protein, gRNA, and target sequence complex comprising either a single base pair mismatch (top) or a double base pair mismatch (bottom) between the gRNA and the target sequence. **FIG. 87B** shows trans cleavage activity of different Cas12 variants upon complex formation with a gRNA and a target sequence having either a single base pair mismatch (top) or a double base pair mismatch (bottom). The gRNA used in each reaction was GUUUCAAAGAUUAAAUAAUUUCUACUAAGUGUAGAUUCCUGCAGCAGAAAAUCA AAGACAAUGAAUAUUUCGGCGC (SEQ ID NO: 380). Trans cleavage activity was measured as a function of fluorescence. Most Cas12 variants were able to tolerate both single and double mismatches starting from 15th and 20th position with respect to the PAM sequence, but trans cleavage rate decreased when a mismatch was introduced within the seed region (nucleotides at positions 1-10 of the spacer region at the PAM-proximal end). PAM sequences and the sequences of the target and non-target strands are provided in **TABLE 29**.

TABLE 29 – Substrate nucleic acid sequences for the target and non-target strands

Substrates	Non Target Strand	Target Strand
TTTT (SEQ ID NO: 381)	GCCCGCGGGATTTTTTCCTGCAGC AGAAAATCAAAGACAATGAATAT	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGAAAAAATCC

Substrates	Non Target Strand	Target Strand
	TTCGGCGC (SEQ ID NO: 603)	CGCGGGC (SEQ ID NO: 604)
TTTG (SEQ ID NO: 382)	GCCCGCGGGATTTTGTCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 605)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGACAAAATCC CGCGGGC (SEQ ID NO: 606)
TTTC (SEQ ID NO: 383)	GCCCGCGGGATTTTCTCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 607)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGAGAAAATCC CGCGGGC (SEQ ID NO: 608)
TTTA (SEQ ID NO: 384)	GCCCGCGGGATTTTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 609)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 610)
TTGA (SEQ ID NO: 385)	GCCCGCGGGATTTGATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 611)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATCAAATCC CGCGGGC (SEQ ID NO: 612)
TTCA (SEQ ID NO: 386)	GCCCGCGGGATTTCATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 613)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATGAAATCC CGCGGGC (SEQ ID NO: 614)
TTAA (SEQ ID NO: 387)	GCCCGCGGGATTTAATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 615)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATTAAATCC CGCGGGC (SEQ ID NO: 616)
TGTA (SEQ ID NO: 388)	GCCCGCGGGATTGTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 617)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATACAATCC CGCGGGC (SEQ ID NO: 618)
TCTA (SEQ ID NO: 389)	GCCCGCGGGATTCTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 619)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAGAATCC CGCGGGC (SEQ ID NO: 620)
TATA (SEQ ID NO: 390)	GCCCGCGGGATTATATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 621)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATATAATCC CGCGGGC (SEQ ID NO: 622)
GTTA (SEQ ID NO: 391)	GCCCGCGGGATGTTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 623)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAACATCC CGCGGGC (SEQ ID NO: 624)
CTTA (SEQ ID NO: 392)	GCCCGCGGGATCTTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 625)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAAGATCC CGCGGGC (SEQ ID NO: 626)
ATTA (SEQ ID NO: 393)	GCCCGCGGGATATTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 627)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAATATCC CGCGGGC (SEQ ID NO: 628)
1MM	GCCCGCGGGATTTTACCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 629)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGGTAAAATCC CGCGGGC (SEQ ID NO: 630)
5MM	GCCCGCGGGATTTTATCCTACAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 631)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGTAGGATAAAATCC CGCGGGC (SEQ ID NO: 632)

Substrates	Non Target Strand	Target Strand
10MM	GCCCGCGGGATTTTATCCTGCAGC GGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 633)	GCGCCGAAATATTCATTGTCTTTG ATTTTCCGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 634)
15MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAGTCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 635)	GCGCCGAAATATTCATTGTCTTTG ACTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 636)
20MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 609)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 610)
1-2MM	GCCCGCGGGATTTTACTCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 637)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGAGTAAAATCC CGCGGGC (SEQ ID NO: 638)
3-4MM	GCCCGCGGGATTTTATCTCGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 639)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCGAGATAAAATCC CGCGGGC (SEQ ID NO: 640)
5-6MM	GCCCGCGGGATTTTATCCTATAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 641)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTATAGGATAAAATCC CGCGGGC (SEQ ID NO: 642)
7-8MM	GCCCGCGGGATTTTATCCTGCGAC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 643)	GCGCCGAAATATTCATTGTCTTTG ATTTTCTGTCGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 644)
9-10MM	GCCCGCGGGATTTTATCCTGCAGT GGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 645)	GCGCCGAAATATTCATTGTCTTTG ATTTTCCACTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 646)
11-12MM	GCCCGCGGGATTTTATCCTGCAGC AAGAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 647)	GCGCCGAAATATTCATTGTCTTTG ATTTCTTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 648)
13-14MM	GCCCGCGGGATTTTATCCTGCAGC AGAGGATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 649)	GCGCCGAAATATTCATTGTCTTTG ATCCTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 650)
15-16MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAGCCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 651)	GCGCCGAAATATTCATTGTCTTTG GCTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 652)
17-18MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAATTGAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 653)	GCGCCGAAATATTCATTGTCTTCA ATTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 654)
19-20MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAATCAGGGACAATGAATAT TTCGGCGC (SEQ ID NO: 655)	GCGCCGAAATATTCATTGTCCCTG ATTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 656)
21-22MM	GCCCGCGGGATTTTATCCTGCAGC AGAAAATCAAAAGCAATGAATAT TTCGGCGC (SEQ ID NO: 657)	GCGCCGAAATATTCATTGCTTTTG ATTTTCTGCTGCAGGATAAAATCC CGCGGGC (SEQ ID NO: 658)
dsDNA off	GGCCAGTTTCATTTGAGCATTAAA TGTCAAGTTCTGCACGCTATCATC	CCTGATGATGATAGCGTGCAGAA CTTGACATTTAATGCTCAAATGAA

Substrates	Non Target Strand	Target Strand
	ATCAGG (SEQ ID NO: 659)	ACTGGCC (SEQ ID NO: 660)
ssDNA off	GCCCGCGGGATTTTCTCCTGCAGC AGAAAATCAAAGACAATGAATAT TTCGGCGC (SEQ ID NO: 607)	
ssDNA on		GCGCCGAAATATTCATTGTCTTTG ATTTTCTGCTGCAGGAGAAAATCC CGCGGGC (SEQ ID NO: 608)

[00657] In a third assay, each Cas12 variant was tested for sensitivity of trans cleavage activity to salt concentration. DETECTR trans cleavage assays were performed in the presence of activity buffer (5mM $MgCl_2$, 20 mM, Tris pH 7.5, 120 mM NaCl, and 1% glycerol). dsDNA target nucleic acid was detected at a final concentration of 100 nM. ssDNA reporters were present in each reaction at a concentration of 50 nM. Target dsDNA was obtained by annealing complementary ssDNA primers at a ratio of 2:1 of non-target strand to target strand in hybridization buffer (50mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA), to ensure double-stranded DNA is being detected instead of single-stranded DNA. Pre-crRNA was ordered from Synthego. The protein of interest and the guide RNA were added to each tube and incubated for 20 minutes at 37°C. Each reaction contained 16 µL of the incubated mastermix. **FIG. 88** shows trans cleavage activity of different Cas12 variants at different concentrations of NaCl. Most Cas12 variants showed increased trans cleavage activity at low salt concentrations. The gRNA used in each reaction was GUUUCAAAGAUUAAAUAAUUUCUACUAAGUGUAGAUUCCUGCAGCAGAAAAUCA AAGACAAUGAAUAUUUCGGCGC (SEQ ID NO: 380). Trans cleavage activity was measured as a function of fluorescence.

EXAMPLE 35

Identification of Cas12 Variants with pre-crRNA Processing Activity

[00658] This example describes the identification of Cas12 variants with pre-crRNA processing activity. Each Cas12 variant was tested for pre-crRNA processing activity. Processing of pre-crRNA was performed in the presence of activity buffer (5mM $MgCl_2$, 20 mM, Tris pH 7.5). Pre-crRNA cleavage assays were performed at 37°C with 4-fold molar excess of the Cas12 variant relative to synthesized crRNA (final concentrations of 100nM and 50nM, respectively). Unless otherwise indicated, the reaction was quenched after 1h with 2×RNA loading dye. Following quenching, reactions were denatured at 95°C for 5 minutes before resolving by 15% denaturing PAGE in 0.5×TBE buffer. **FIG. 89** shows urea PAGE gels of pre-crRNA processing activity of different Cas12 variants in the presence ("+") or absence ("-") of a Cas protein. Bands shown are

RNA bands. Pre-crRNA processing activity was observed for most Cas12 variants, with different Cas12 variants processing at different rates.

EXAMPLE 36

Trans Cleavage Activity of Cas12 Variants in the Presence of crRNAs for Native Cas Proteins

[00659] This example describes trans cleavage activity of Cas12 variants in the presence of crRNAs for native Cas proteins. Each Cas12 variant was tested for orthogonality of the corresponding crRNA to native Cas12 proteins. Each Cas12 variant was incubated with different synthetic Synthego pre-crRNAs and trans cleavage activity was measured. Each pre-crRNA differed in the repeat sequence. The repeat sequence in each crRNA was based on the repeat sequence found in each CRISPR locus of Cas12 proteins. Cas12 variants showed different trans cleavage activity when paired with different pre-crRNAs from different native Cas12 proteins. DETECTR trans cleavage assays were performed in the presence of activity buffer (5mM $MgCl_2$, 20 mM, Tris pH 7.5). dsDNA target nucleic acid was detected at a final concentration of 100 nM. ssDNA reporters were present in each reaction at a concentration of 50 nM. Target dsDNA was obtained by annealing complementary ssDNA primers at a ratio of 2:1 of non-target strand to target strand in hybridization buffer (50mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA), to ensure double-stranded DNA is being detected instead of single-stranded DNA. Pre-crRNA was ordered from Synthego. The protein of interest and the guide RNA were added to each tube and incubated for 20 minutes at 37°C. Each reaction contained 16 μL of the incubated mastermix. **FIG. 90** shows trans cleavage activity of different Cas12 variants in the presence of different crRNAs based on the native crRNAs found in the CRISPR locus for native Cas12 proteins. Trans cleavage activity was measured using fluorescence. Using crRNAs with different variants showed different transcleavage activity, indicating that some Cas12 variants are more promiscuous with respect to crRNA than others. Pre-crRNA sequences are provided in **TABLE 30**. Sequence alignments of the repeat regions of different Cas12 variants aligned to the repeat sequence of LbCas12a (SEQ ID NO: 1) are shown in **FIG. 92**. Repeat sequences of the Cas12 variants correspond to SEQ ID NO: 508 – SEQ ID NO: 520 and SEQ ID NO: 522 – SEQ ID NO: 536. The repeat sequence of LbCas12a corresponds to SEQ ID NO: 521. The target sequence is set forth in SEQ ID NO: 610.

TABLE 30 – Cas12 Ortholog Pre-crRNA Sequences

Cas12 Ortholog SEQ ID NO	Repeat Sequence	Spacer Sequence	Pre-crRNA Sequence
SEQ ID NO: 571	GUCUAAACCUCAAUGAAAAUUUCUACUGUU (SEQ ID NO: 508)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUCUAAACCUCAAUGAAAAUUUCUACUGUUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 540)
SEQ ID NO: 572	CCUAAUAAUUUCUACUGUUGUAGAU (SEQ ID NO: 509)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	CCUAAUAAUUUCUACUGUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 541)
SEQ ID NO: 573	CUCGAAUACCUAUAUUAAAUUUCUACUUUUGUAGAU (SEQ ID NO: 510)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	CUCGAAUACCUAUAUUAAAUUUCUACUUUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 542)
SEQ ID NO: 575	GUUUAAUAAACACUUAUAAUUUCUACUGUUGUAGAU (SEQ ID NO: 511)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUAAUAAACACUUAUAAUUUCUACUGUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 543)
SEQ ID NO: 11	GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAU (SEQ ID NO: 512)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 544)
SEQ ID NO: 579	GUUGAGUAACCAUAAGAAAAUUUCUACUGUGUAGAU (SEQ ID NO: 513)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUGAGUAACCAUAAGAAAAUUUCUACUGUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 545)
SEQ ID NO: 580	GUUUAAUAAGUAAUAAAUGUCUACUGUAGUGUAGAU (SEQ ID NO: 514)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUAAUAAGUAAUAAAUGUCUACUGUAGUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 546)
SEQ ID NO: 581	AGUUAAAUAAUAAGAAAGAAUUUCUACUAGUGUAGAU (SEQ ID NO: 515)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	AGUUAAAUAAUAAGAAAGAAUUUCUACUAGUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 547)
SEQ ID NO: 582	GUUAAGUAAUAUAAAAGAAUUUCUACUAUUGUAGAU (SEQ ID NO: 516)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUAAGUAAUAUAAAAGAAUUUCUACUAUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 548)
SEQ ID NO: 583	GUUUGACCUACUAAUUAAAUUUCUACUGUUGUAGAU (SEQ ID NO: 517)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUGACCUACUAAUUAAAUUUCUACUGUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 549)
SEQ ID NO: 584 and SEQ ID NO: 588	GGCUAAAAGUAAUAAACAAUUUCUACUUUCGUAGAU (SEQ ID NO: 518)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GGCUAAAAGUAAUAAACAAUUUCUACUUUCGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 550)
SEQ ID NO: 587	GUCAAAUAGUAACUAACAAUUUCUACUUCGGUAGAU (SEQ ID NO: 519)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUCAAAUAGUAACUAACAAUUUCUACUUCGGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 551)
SEQ ID NO: 589	AUCUACACAAAGUAGAGAUUCGAAUGAGUUUUGAC (SEQ ID NO: 520)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	AUCUACACAAAGUAGAGAUUCGAAUGAGUUUUGACUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 552)

Cas12 Ortholog SEQ ID NO	Repeat Sequence	Spacer Sequence	Pre-crRNA Sequence
LbCas12a (SEQ ID NO: 1)	GUUUCAAAGAUU AAAUAAUUUCUA CUAAGUGUAGAU (SEQ ID NO: 521)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUUUCAAAGAUUAAAUAAUUUC UACUAAGUGUAGAUUCCUGCAG CAGAAAAUCAAAGACA (SEQ ID NO: 553)
SEQ ID NO: 3	GUCUAAGAACUU UAAAUAAUUUCU ACUGUUGUAGAU (SEQ ID NO: 522)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUCUAAGAACUUUAAAUAAUUU CUACUGUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 554)
SEQ ID NO: 590	AUUUGAAAGCAU CUUUUAAUUUCU ACU-AUUGUAGAU (SEQ ID NO: 523)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	AUUUGAAAGCAUCUUUUAAUUU CUACU-AUUGUAGAUUCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 555)
SEQ ID NO: 591	CUCUAAUAAGAG AUAUGAAUUUCU ACUGUUGUAGAU (SEQ ID NO: 524)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	CUCUAAUAAGAGAUAUGAAUUU CUACUGUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 556)
Cas12 Variant	CUCUACAACUGAU AAAGAAUUUCUA CUUUUGUAGAU (SEQ ID NO: 525)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	CUCUACAACUGAUAAAGAAUUU CUACUUUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 557)
Cas12 Variant	CUCUAGCAGGCCU GGCAAAUUUCUAC U-GUUGUAGAU (SEQ ID NO: 526)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	CUCUAGCAGGCCUGGCAAAUUUC UACU-GUUGUAGAUUCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 558)
Cas12 Variant	GCCAAAUACCUCU AUAAAAUUUCUA CUUUUGUAGAU (SEQ ID NO: 527)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GCCAAAUACCUCUAUAAAAUUUC UACUUUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 559)
Cas12 Variant	GCCAAGAACCUAU AGAUAAUUUCUA CUGUUGUAGAU (SEQ ID NO: 528)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GCCAAGAACCUAUAGAUAAUUU CUACUGUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 560)
Cas12 Variant	GGCUAUAAAGCU UAUUUAAUUUCU ACUAUUGUAGAU (SEQ ID NO: 529)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GGCUAUAAAGCUUAUUUAAUUU CUACUAUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 561)
SEQ ID NO: 2	GUCAAAAGACCUU UUUAAUUUCUAC UCUUGUAGAU (SEQ ID NO: 530)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUCAAAAGACCUUUUUAAUUUC UACUCUUGUAGAUUCCUGCAGCA GAAAAUCAAAGACA (SEQ ID NO: 562)
Cas12 Variant	GUCUAAAACUCAU UCAGAAUUUCUAC UAGUGUAGAU (SEQ ID NO: 531)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUCUAAAACUCAUUCAGAAUUU CUACUAGUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 563)
Cas12 Variant	GUCUAACUACCUU UUAAUUUCUACU GUUUGUAGAU (SEQ ID NO: 532)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUCUAACUACCUUUUAAUUUCU ACUGUUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 564)
Cas12 Variant	GUCUAUAAGACA UUUAUAAUUUCU ACUAUUGUAGAU (SEQ ID NO: 533)	UCCUGCAGCAGAA AAUCAAAGACA (SEQ ID NO: 539)	GUCUAUAAGACAUUUAUAAUUU CUACUAUUGUAGAUUCCUGCAGC AGAAAAUCAAAGACA (SEQ ID NO: 565)

Cas12 Ortholog SEQ ID NO	Repeat Sequence	Spacer Sequence	Pre-crRNA Sequence
Cas12 Variant	GUUUAAAACCACUUUAAAAUUUCUACUAUUGUAGAU (SEQ ID NO: 534)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUAAAACCACUUUAAAAUUUCUACUAUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 566)
Cas12 Variant	GUUUAAAAGUCCUAUUGGAUUUCACUUUUGUAGAU (SEQ ID NO: 535)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUUAAAAGUCCUAUUGGAUUUCUACUUUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 567)
Cas12 Variant	UGCUUAGAACAUUUAAAGAAUUUACUACUAUUGUAGAU (SEQ ID NO: 536)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	UGCUUAGAACAUUUAAAGAAUUUCUACUACUAUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 568)
Cas12 Variant	UGCUUAGUACUUAUAAAGAAUUUACUACUAUUGUAGAU (SEQ ID NO: 537)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	UGCUUAGUACUUAUAAAGAAUUUCUACUACUAUUGUAGAUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 569)
MAD7	GUUAAGUUAUAUAGAAUAAUUUCACU (SEQ ID NO: 538)	UCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 539)	GUUAAGUUAUAUAGAAUAAUUUCUACUUCCUGCAGCAGAAAAUCAAAGACA (SEQ ID NO: 570)

EXAMPLE 37

Cis Cleavage Activity of Cas12 Variants

[00660] This example describes the cis cleavage activity of Cas12 variants. Cis (target) cleavage assays were performed at 25°C or 37°C in activity buffer (120 mM NaCl, 5 mM $MgCl_2$, 20mM Tris pH 7.5, 1% glycerol). Cas12 variant–crRNA complex formation was performed in activity buffer at a molar ratio of 1:1.25 protein to crRNA at 37 °C for 10 min. The cis cleavage target was a 1200 bp PCR product that contained the target sequence at the 700th position. A restriction site for BamHI was introduced near the target sequence. Unless otherwise indicated, the final concentrations of protein, guide and targets were 100nM, 125nM and 15nM, respectively, for all reactions. Cis cleavage assays were performed in the presence of activity buffer. Target dsDNA was detected at a final concentration of 15nM Target dsDNA was obtained by annealing complementary ssDNA primers at a ratio of 2:1 of non-target strand to target strand in hybridization buffer (50mM NaCl, 1 mM Tris pH 8.0, 0.1 mM EDTA), to ensure double-stranded DNA is being detected instead of single-stranded DNA. The protein of interest and the guide RNA were added to each tube and incubated for 20 minutes at 37°C. Each reaction contained 16 μL of the incubated mastermix. Reactions were quenched with 6X loading dye and resolved by pre-stained 2% agarose gel in 1×TAE buffer. **FIG. 91** shows cis cleavage activity of different Cas12 variants after incubation with a target nucleic acid sequence for 10 minutes.

Cleavage with BamHI is shown as a cleavage positive control. Different Cas12 variants demonstrate different rates of cis cleavage activity.

EXAMPLE 38

Trans Cleavage Activity of a Cas12 Variant with Different gRNAs

[00661] This example describes the trans cleavage activity of a Cas12 variant of SEQ ID NO: 11 with different gRNAs. A detection assay was performed using gRNAs with either the repeat sequence of LbCas12a (SEQ ID NO: 1) or the repeat sequence of the Cas12 variant of SEQ ID NO: 11. Target nucleic acid was detected at a final concentration of 1 nM or 0 nM (negative control).

[00662] FIG. 93 shows the results of an assay comparing DETECTR assay efficiency for a Cas12 variant of SEQ ID NO: 11 with two different gRNAs. The gRNA contains either the LbCas12a repeat sequence ("gRNA #1," SEQ ID NO: 423, UAAUUUCUACUAAGUGUAGAUUCAUCACGCAGCUCAUGCCC) or the Cas12 variant repeat sequence ("gRNA #2," SEQ ID NO: 424, GUUUGGUACCUUUAUUAAUUUCUACUAAGUGUAGAUUCAUCACGCAGCUCAUGCCC). The detection reaction was performed at 37° C for 30 minutes with 1 nM target DNA. A sample with 0 nM target DNA was tested as a negative control. The results indicated that the Cas12 variant is compatible with the gRNA corresponding to the repeat sequence of LbCas12a ("gRNA #1," SEQ ID NO: 423). Results further indicated that the Cas12 variant showed increased trans cleavage activity in the presence of the shorter gRNA ("gRNA #1," SEQ ID NO: 423).

[00663] While preferred embodiments of the present invention have been shown and described herein, it will be obvious 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 programmable nuclease having at least 60% sequence identity to SEQ ID NO: 11 and a non-naturally occurring guide nucleic acid

2. The composition of claim 1, wherein the programmable nuclease comprises a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute.

3. The composition of claim 1 or 2, wherein the programmable nuclease recognizes a protospacer adjacent motif of YYN.

4. A composition comprising programmable nuclease having a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute and a non-naturally occurring guide nucleic acid.

5. The composition of claim 6, wherein the programmable nuclease recognizes a protospacer adjacent motif of YYN.

6. A composition comprising a non-naturally occurring guide nucleic acid and a programmable nuclease, wherein the programmable nuclease comprises a turnover rate of at least about 0.1 cleaved detector nucleic acid molecules per minute and recognizes a protospacer adjacent motif of YYN.

7. The composition of any one of claims 1-6, wherein the programmable nuclease is a Type V programmable nuclease.

8. The composition any one of claims 1-7, wherein the programmable nuclease is a Cas12 nuclease.

9. The composition of any one of claims 1-8, wherein the programmable nuclease comprises three partial RuvC domains.

10. The composition of any one of claims 1-9, wherein the programmable nuclease comprises a RuvC-I subdomain, a RuvC-II subdomain, and a RuvC-III subdomain.

11. The composition any one of claims 4-10, wherein the programmable nuclease has at least 60% sequence identity to SEQ ID NO: 11.

12. The composition of any one of claims 1-11, wherein the programmable nuclease has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% sequence identity to SEQ ID NO: 11.

13. The method of any one of claims 1-12, wherein the programmable nuclease is SEQ ID NO: 11.

14. The composition of any one of claims 3 or 5-13, wherein the Y is a C or T nucleotide.

15. The composition of any one of claims 3 or 5-14, wherein the N is any nucleotide.

16. The composition of any one of claims 1-15, wherein the composition further comprises a buffer.

17. The composition of claim 16, wherein the buffer comprises a buffering agent, a salt, a crowding agent, a detergent, or any combination thereof.

18. The composition of claim 17, wherein the buffering agent is at a concentration of from 5 mM to 100 mM.

19. The composition of any one of claims 17-18, wherein the buffering agent is at a concentration of from 10 mM to 40 mM.

20. The composition of any one of claims 17-19, wherein the buffering agent is at a concentration of about 20 mM.

21. The composition of any one of claims 17-20, wherein the salt is from 5 mM to 100 mM.

22. The composition of any one of claims 17-21, wherein the salt is from 5 mM to 10 mM.

23. The composition of any one of claims 17-22, wherein the crowding agent is from 0.5% (v/v) to 2% (v/v).

24. The composition of any one of claims 17-23, wherein the crowding agent is about 1%.

25. The composition of any one of claims 17-24, wherein the detergent is about 2% (v/v) or less

26. The composition of any one of claims 17-25, wherein the detergent is about 0.00016% (v/v).

27. The composition of any one of claims 17-26, wherein the buffering agent is HEPES.

28. The composition of any one of claims 17-27, wherein the salt is potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof.

29. The composition of any one of claims 17-28, wherein the crowding agent is glycerol.

30. The composition of any one of claims 17-29, wherein the detergent is Tween, Triton-X, or any combination thereof.

31. The composition of any one of claims 1-30, wherein a pH of the composition is from 7 to 8.

32. The composition of any one of claims 1-31, wherein a pH of the composition is 7.5.

33. The composition of any one of claims 1-32, wherein the composition is at a temperature of from 25°C to 45°C.

34. The composition of any one of claims 1-33, wherein the programmable nuclease exhibits catalytic activity at a temperature of from 25°C to 45°C.

35. The composition of any one of claims 1-34, wherein the programmable nuclease exhibits catalytic activity after heating the composition to a temperature of greater than 45°C and restoring the temperature to from 25°C to 45°C.

36. A method of assaying for a segment of a target nucleic acid in a sample, the method comprising:

contacting the sample to:

a detector nucleic acid; and

the composition of any one of claims 1-35, wherein the guide nucleic acid hybridizes to a segment of the target nucleic acid; and

assaying for a signal produced by cleavage of the detector nucleic acid.

37. A method of assaying for a segment of a target nucleic acid in a sample from a subject comprising:

contacting the sample comprising a population of nucleic acids to:

a guide nucleic acid that hybridizes to the segment of the target nucleic acid;

a detector nucleic acid; and

a Cas12 nuclease that cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid; and

assaying for a signal produced by cleavage of the detector nucleic acid, wherein the signal is at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid and wherein the subject has a disease when the segment of the target nucleic acid is present.

38. The method of claim 37, the method further comprising administering a treatment for the disease.

39. A method of assaying for a segment of a target nucleic acid comprising:

contacting a sample comprising a population of nucleic acids, wherein the population comprises at least one nucleic acid comprising a segment having less than 100% sequence identity to the segment of the target nucleic acid and having no less than 50% sequence identity to the segment of the target nucleic acid to:

a guide nucleic acid that hybridizes to the segment of the target nucleic acid;

a detector nucleic acid; and

a Cas12 nuclease that cleaves the detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid; and

assaying for a signal produced by cleavage of the detector nucleic acid, wherein the signal is at least two-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

40. The method of any one of claims 37-39, wherein the segment of the at least one nucleic acid comprises at least two base mutations compared to the segment of the target nucleic acid.

41. The method of any one of claims 39-40, wherein the segment of the at least one nucleic acid comprises from one to ten base mutations compared to the segment of the target nucleic acid.

42. The method of any one of claims 39-40, wherein the segment of the at least one nucleic acid comprises one base mutation compared to the segment of the target nucleic acid.

43. The method of any one of claims 39-42, wherein the signal is from two-fold to 20-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

44. The method of any one of claims 39-43, wherein the signal is from two-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

45. The method of any one of claims 39-44, wherein the signal is from five-fold to 10-fold greater when the segment of the target nucleic acid is present in the sample than the signal when the sample lacks the segment of the target nucleic acid.

46. The method of claim 45, wherein the guide nucleic acid is reverse complementary to the segment of the target nucleic acid.

47. The method of claim 46, wherein the guide nucleic acid and the second guide nucleic acid lack synthetic mismatches.

48. The method of any one of claims 39-47, wherein the guide nucleic acid is at least 10 bases.

49. The method of any one of claims 39-48, wherein the guide nucleic acid is from 10 to 50 bases.

50. The method of any one of claims 39-49, wherein the guide nucleic acid is at least 25 bases.

51. The method of any one of claims 39-50, wherein the target nucleic acid is in the population of nucleic acids at a minor allele frequency of 10% or less.

52. The method of any one of claims 39-51, wherein the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 10%.

53. The method of any one of claims 39-52, wherein the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 5%.

54. The method of any one of claims 39-53, wherein the target nucleic acid is in the population of nucleic acids at a minor allele frequency of from 0.1% to 1%.

55. The method of any one of claims 39-54, wherein the Cas12 nuclease is Cas12a, Cas12b, Cas12c, CasY, or Cas12e.

56. The method of any one of claims 39-55, wherein the Cas12 nuclease is Cas12a.

57. The method of any one of claims 39-56, wherein the Cas12 nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602.

58. The method of any one of claims 39-57, wherein the Cas12 nuclease has at least 60% sequence identity to SEQ ID NO: 11.

59. The method of any one of claims 39-58, wherein the contacting is carried out in a buffer comprising a buffering agent, a salt, a crowding agent, a detergent, a reducing agent, a competitor, or any combination thereof.

60. The method of claim 59, wherein the buffering agent is at a concentration of from 5 mM to 100 mM.

61. The method of any one of claims 59-60, wherein the buffering agent is at a concentration of from 10 mM to 30 mM.

62. The method of any one of claims 59-61, wherein the salt is from 5 mM to 100 mM.

63. The method of any one of claims 59-62, wherein the salt is from 5 mM to 10 mM.

64. The method of any one of claims 59-63, wherein the crowding agent is from 0.5% (v/v) to 10% (v/v).

65. The method of any one of claims 59-64, wherein the crowding agent is from 1% (v/v) to 5% (v/v).

66. The method of any one of claims 59-65, wherein the detergent is at 2% (v/v) or less

67. The method of any one of claims 59-66, wherein the reducing agent is from 0.01 mM to 100 mM.

68. The method of any one of claims 59-67, wherein the reducing agent is from 0.1 mM to 10 mM.

69. The method of any one of claims 59-68, wherein the reducing agent is from 0.5 mM to 2 mM.

70. The method of any one of claims 59-69, wherein the competitor is from 1 ug/ml to 100 ug/ml.

71. The method of any one of claims 59-70, wherein the competitor is from 40 ug/ml to 60 ug/ml.

72. The method of any one of claims 59-71, wherein the buffering agent is HEPES, Tris, or any combination thereof.

73. The method of any one of claims 59-72, wherein the salt is potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, or any combination thereof.

74. The method of any one of claims 59-72, wherein the crowding agent is glycerol.

75. The method of any one of claims 59-74, wherein the detergent is Tween, Triton-X, or any combination thereof.

76. The method of any one of claims 59-75, wherein the reducing agent is DTT.

77. The method of any one of claims 59-76, wherein the competitor is heparin.

78. The method of any one of claims 59-77, wherein a pH of the composition is from 7 to 8.

79. The method of any one of claims 37-78, further comprising amplifying the target nucleic acid before the contacting.

80. The method of claim 79, wherein the amplifying the target nucleic acid before the contacting comprises using a blocking primer.

81. The method of claim 80, wherein the target nucleic acid segment comprises a single nucleotide mutation.

82. The method of claim 81, wherein the blocking primer binds to a nucleic acid comprising encoding the wild type sequence of the target nucleic acid segment.

83. The method of claim 79, wherein the amplifying comprises COLD-PCR.

84. The method of claim 83, wherein the COLD-PCR comprises full COLD-PCR.

85. The method of claim 83, wherein the COLD-PCR comprises fast COLD-PCR.

86. The method of claim 79, wherein the amplifying comprises fast COLD-PCR.

87. The method of claim 79, wherein the amplifying comprises allele-specific PCR.

88. The method of claim 87, wherein the amplifying further comprises COLD-PCR.

89. A composition comprising a programmable nuclease and a buffer, wherein the buffer comprises a salt at less than about 110 mM and wherein the buffer comprises a pH of from 7 to 8.

90. The composition of claim 89, wherein the salt is from 1 mM to 110 mM.

91. The composition of claim 89 or 90, wherein the salt is from 1 mM to 60 mM.

92. The composition of any one of claims 89-91, wherein the salt is from 1 mM to 10 mM.

93. The composition of any one of claims 89-92, wherein the salt is at about 105 mM.

94. The composition of any one of claims 89-93, wherein the salt is at about 55 mM.

95. The composition of any one of claims 89-94, wherein the salt is at about 7 mM.

96. The composition of any one of claims 89-95, wherein the salt comprises potassium acetate, magnesium acetate, sodium chloride, magnesium chloride, potassium chloride, or any combination thereof.

97. The composition of any one of claims 89-96, wherein the salt comprises potassium acetate and magnesium acetate.

98. The composition of any one of claims 89-96, wherein the salt comprises sodium chloride and magnesium chloride.

99. The composition of any one of claims 89-96, wherein the salt comprises potassium chloride and magnesium chloride.

100. The composition of any one of claims 89-99, wherein the pH comprises about 7.5.

101. The composition of any one of claims 89-99, wherein the pH comprises about 8.

102. The composition of any one of claims 89-101, wherein the buffer comprises a crowding agent or a competitor.

103. The composition of claim 102, wherein the crowding agent is present from 1% (v/v) to 10% (v/v).

104. The composition of any one of claims 102-103, wherein the crowding agent or the competitor is present from 1% (v/v) to 5% (v/v).

105. The composition of any one of claims 102-103, wherein the crowding agent or the competitor is present at about 5% (v/v).

106. The composition of any one of claims 102-103, wherein the crowding agent or the competitor is present at about 1% (v/v).

107. The composition of any one of claims 102-106, wherein the crowding agent or the competitor is present from 1 ug/mL to 100 ug/ml.

108. The composition of any one of claims 102-107, wherein the crowding agent or the competitor is present from 30 ug/ml to 70 ug/ml.

109. The composition of any one of claims 102-108, wherein the crowding agent or the competitor is present at about 50 ug/ml.

110. The composition of any one of claims 102-109, wherein the crowding agent or the competitor is present from 1 mM to 50 mM.

111. The composition of any one of claims 102-110, wherein the crowding agent or the competitor is present from 10 mM to 30 mM.

112. The composition of any one of claims 102-111, wherein the crowding agent or the competitor is present at about 20 mM.

113. The composition of any one of claims 102-112, wherein the crowding agent or the competitor is selected from the group consisting of: glycerol, heparin, bovine serum albumin, imidazole, and any combination thereof.

114. The composition of any one of claims 102-113, wherein the crowding agent or the competitor comprises glycerol.

115. The composition of any one of claims 102-114, wherein the crowding agent or the competitor comprises glycerol and heparin.

116. The composition of any one of claims 102-115, wherein the crowding agent or the competitor comprises glycerol, bovine serum albumin, and imidazole.

117. The composition of any one of claims 88-116, wherein the buffer comprises a buffering agent.

118. The composition of claim 117, wherein the buffering agent is present from 1 mM to 50 mM.

119. The composition of any one of claims 116-118, wherein the buffering agent is present from 1 mM to 30 mM.

120. The composition of any one of claims 116-119, wherein the buffering agent is present at about 20 mM.

121. The composition of any one of claims 116-120, wherein the buffering agent is HEPES.

122. The composition of any one of claims 116-120, wherein the buffering agent is Tris.

123. The composition of any one of claims 89-122, wherein the buffer comprises a detergent.

124. The composition of claim 123, wherein the detergent is present from 0.00001% (v/v) to 0.1% (v/v).

125. The composition of any one of claims 123-124, wherein the detergent is present from 0.00001% (v/v) to 0.01% (v/v).

126. The composition of any one of claims 123-125, wherein the detergent is at about 0.00016% (v/v).

127. The composition of any one of claims 123-125, wherein the detergent is at about 0.01% (v/v).

128. The composition of any one of claims 123-127, wherein the detergent is Triton-X.

129. The composition of any one of claims 123-127, wherein the detergent is IGEPAL CA-630.

130. The composition of any one of claims 89-129, wherein the buffer comprises a reducing agent.

131. The composition of claim 130, wherein the reducing agent is present from 0.01 mM to 100 mM.

132. The composition of any one of claims 130-131, wherein the reducing agent is present from 0.1 mM to 10 mM.

133. The composition of any one of claims 130-132, wherein the reducing agent is present at about 1 mM.

134. The composition of any one of claims 130-133, wherein the reducing agent is DTT.

135. The composition of any one of claims 89-134, wherein the programmable nuclease comprises a RuvC domain.

136. The composition of any one of claims 89-135, wherein the programmable nuclease comprises a Type V Cas protein.

137. The composition of any one of claims 89-136, wherein the programmable nuclease is a Cas12 protein.

138. The composition of claim 137, wherein the Cas12 protein is Cas12a, Cas12b, Cas12c, CasY, or Cas12e.

139. The composition of any one of claims 89-138, wherein the programmable nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, or SEQ ID NO: 571 – SEQ ID NO: 602.

140. The composition of any one of claims 89-139, wherein the programmable nuclease has at least 60%, at least 70%, 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: 1.

141. The composition of any one of claims 89-139, wherein the programmable nuclease has at least 60%, at least 70%, 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.

142. The composition of any one of claims 89-134, wherein the programmable nuclease comprises at least two HEPN domains.

143. The composition of any one of claims 89-134, wherein the programmable nuclease is a Type VI Cas protein.

144. The composition of any one of claims 89-134, wherein the programmable nuclease is a Cas13 protein.

145. The composition of claim 144, wherein the Cas13 protein is Cas13a, Cas13b, Cas13c, Cas13d, or Cas13e.

146. The composition of any one of claims 89-145, wherein the programmable nuclease has at least 60%, at least 70%, 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: 103 – SEQ ID NO: 137.

147. The composition of any one of claims 89-146, wherein the programmable nuclease has at least 60%, at least 70%, 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: 104.

148. The composition of any one of claims 89-92, 95-97, 100, 102-105, 113-114, 117-121, 123-126, 128, 135-139, or 141, wherein the programmable nuclease has at least 60%, at least 70%, 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 and the buffer comprises about 20 mM HEPES, about 2 mM potassium acetate, about 5 mM magnesium acetate, about 1% (v/v) glycerol, about 0.00016% (v/v) Triton-X, and a pH of about 7.5.

149. The composition of any one of claims 89-90, 93, 96, 98, 91-105, 107-109, 113, 115, 117-120, 122, or 130-140, wherein the programmable nuclease has at least 60%, at least 70%, 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: 1 and the buffer comprises about 20 mM Tris, about 100 mM sodium chloride, about 5 mM magnesium chloride, about 5% (v/v) glycerol, about 50 ug/mL heparin, about 1 mM DTT, and a pH of about 8.

150. The composition of any one of claims 89-92, 94, 96, 99-100, 102-103, 110-113, 116, 123-125, 127, 129, or 142-147, wherein the programmable nuclease has at least 60%, at least 70%, 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: 104 and the buffer comprises about 50 mM potassium chloride, about 5 mM magnesium chloride, about 10 ug/ml bovine serum albumin, about 5% glycerol, about 20 mM imidazole, about 0.01% (v/v) IGEPAL CA-630, and a pH of about 7.5.

151. The composition of any one of claims 89-150, further comprising a guide nucleic acid.

152. The composition of any one of claims 89-151, further comprising a detector nucleic acid.

153. A composition comprising:

a nucleic acid from a sample, wherein the nucleic acid comprises a PAM and a segment that hybridizes to a guide nucleic acid, wherein the PAM has a sequence of dUdUdUN;

a guide nucleic acid that hybridizes to the segment of the nucleic acid; and

a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to the segment of the target nucleic acid.

154. The composition of claim 153, further comprising a primer, wherein the primer comprises a first region that is reverse complementary to the PAM and a second region that is reverse complementary to a first segment of the nucleic acid.

155. A method of assaying for a target nucleic acid in a sample, wherein the target nucleic acid lacks a PAM, the method comprising:

amplifying the target nucleic acid from a sample using a primer comprising a first region that is reverse complementary to a PAM and a second region that is reverse complementary to a first segment of the target nucleic acid, wherein the PAM is dUdUdUN, thereby producing a PAM target nucleic acid;

contacting the PAM target nucleic acid to:

a guide nucleic acid that hybridizes to a segment of the PAM target nucleic acid;

a programmable nuclease that exhibits sequence independent cleavage of a detector nucleic acid upon hybridization of the guide nucleic acid to a segment of the PAM target nucleic acid; and

a detector nucleic acid; and

assaying for a signal produced by cleavage of the detector nucleic acid.

156. The composition or method of any one of claims 153-155, wherein the second region comprises from 4 to 12 bases.

157. The composition or method of any one of claims 152-156, wherein the second region comprises from 4 to 10 bases.

158. The composition or method of any one of claims 152-157, wherein the second region comprises from 4 to 7 bases.

159. The method of any one of claims 152-158, wherein the amplifying comprises thermal cycling amplification.

160. The method of any one of claims 152-159, wherein the amplifying comprises isothermal amplification.

161. The method of claim 160, wherein the isothermal amplification comprises isothermal recombinase polymerase amplification (RPA), transcription mediated amplification (TMA), strand displacement amplification (SDA), helicase dependent amplification (HDA), loop mediated amplification (LAMP), rolling circle amplification (RCA), single primer isothermal amplification (SPIA), ligase chain reaction (LCR), simple method amplifying RNA targets (SMART), improved multiple displacement amplification (IMDA), or nucleic acid sequence-based amplification (NASBA).

162. The method of any one of claims 160-161, wherein the isothermal amplification comprises loop mediated amplification (LAMP).

163. The composition or method of any one of claims 152-162, wherein a sequence of the primer and a sequence of the guide nucleic acid overlap by 50% or less.

164. The composition or method of any one of claims 152-162, wherein a sequence of the primer and a sequence of the guide nucleic acid do not overlap.

165. The composition or method of any one of claims 152-164, wherein the primer is a forward primer, a reverse primer, a forward inner primer, or a reverse inner primer.

166. The composition or method of any one of claims 152-165, wherein the segment of the nucleic acid or the segment of the target nucleic acid comprises at least one base mutation compared to at least one other segment of a nucleic acid in the sample.

167. The composition or method of claim 166, wherein the at least one base mutation is no more than 13 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid.

168. The method of any one of claims 166-167, wherein the at least one base mutation is no more than 10 nucleotides 3' of the PAM in the nucleic acid or the PAM target nucleic acid.

169. The method of any one of claims 166-168, wherein the at least one base mutation is no more than 9 nucleotides 3' of the PAM in the nucleic acid or in the PAM target nucleic acid.

170. The method of any one of claims 166-169, wherein the at least one base mutation is no more than 8 nucleotides 3' of the PAM in the nucleic acid or in the PAM target nucleic acid.

171. The composition or method of any one of claims 166-170, wherein the at least one base mutation is a single nucleotide polymorphism.

172. The composition or method of any one of claims 152-171, wherein the programmable nuclease comprises a RuvC domain.

173. The composition or method of any one of claims 152-172, wherein the programmable nuclease comprises three partial RuvC domains.

174. The composition or method of any one of claims 152-173, wherein the programmable nuclease comprises a RuvC-I subdomain, a RuvC-II subdomain, and a RuvC-III subdomain.

175. The composition or method of any one of claims 152-174, wherein the programmable nuclease comprises a Type V Cas protein.

176. The composition or method of any one of claims 152-175, wherein the programmable nuclease is a Cas12 protein.

177. The composition or method of claim 176, wherein the Cas12 protein is Cas12a, Cas12b, Cas12c, CasY, or Cas12e.

178. The composition or method of any one of claims 152-177, wherein the programmable nuclease has at least 60%, at least 70%, 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: 11, SEQ ID NO: 282, SEQ ID NO: 571 – SEQ ID NO: 602.

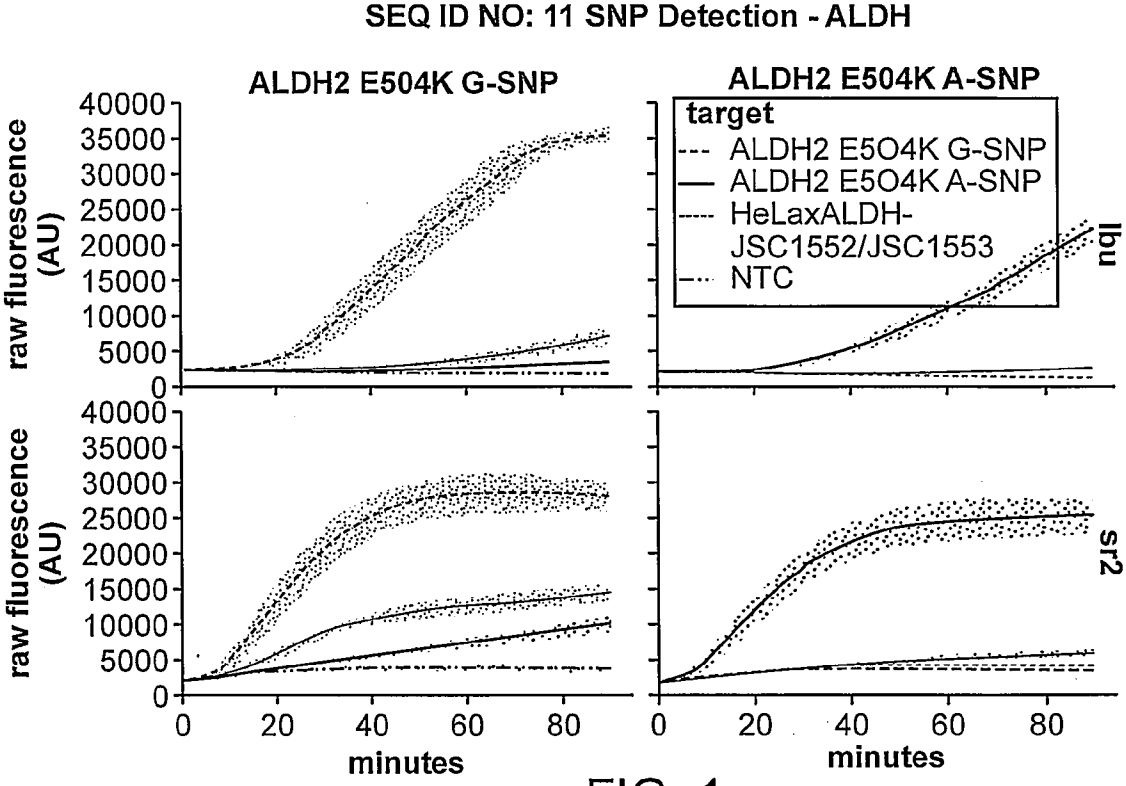
179. A Cas12 nuclease for use in diagnosis, wherein the Cas12 nuclease detects the segment of the target nucleic acid according to the method of any one of claims 37 to 88.

180. The composition according to any one of claims 1 to 35 for use in diagnosis.

181. The composition according to any one of claims 89 to 152 for use in diagnosis.

182. The composition according to any one of claims 153, 154, 156-158, 163-167 or 171-178 for use in diagnosis.

183. A programmable nuclease for use in diagnosis, wherein the programmable nuclease detects the target nucleic acid according to any one of claims 155 or 156-178.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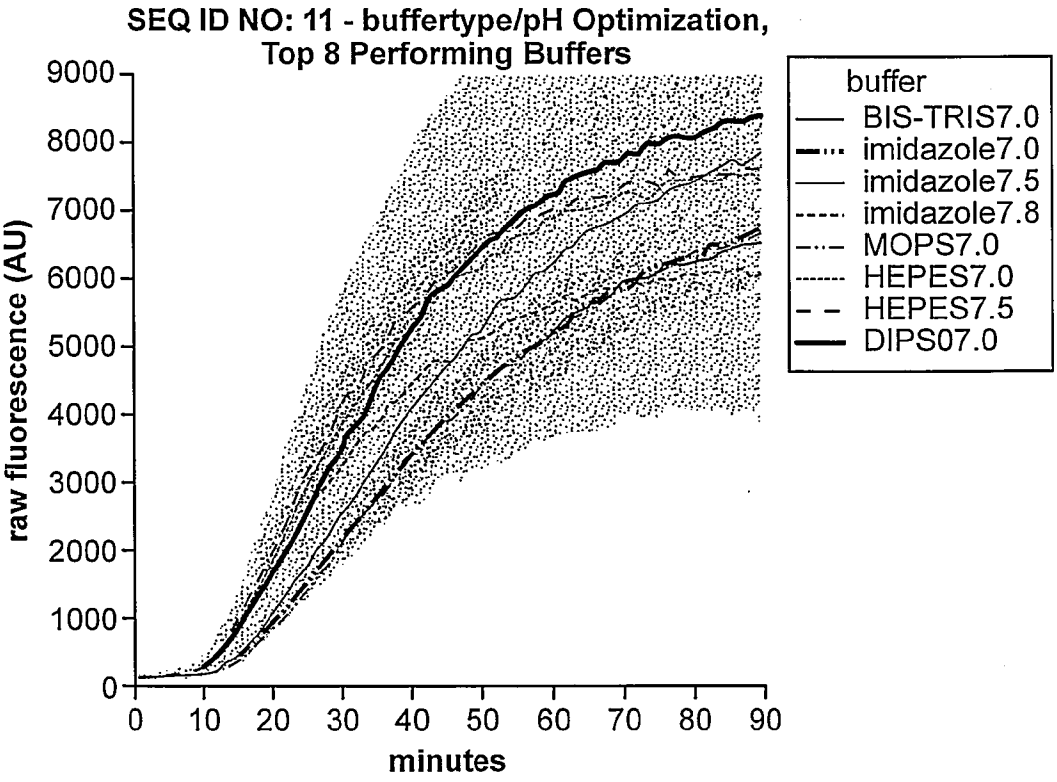
lbu
sr2
minutes

FIG. 1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imidazole7.5
imidazole7.8
MOPS7.0
HEPES7.0
HEPES7.5
DIPS07.0
raw fluorescence (AU)
minutes

FIG. 2

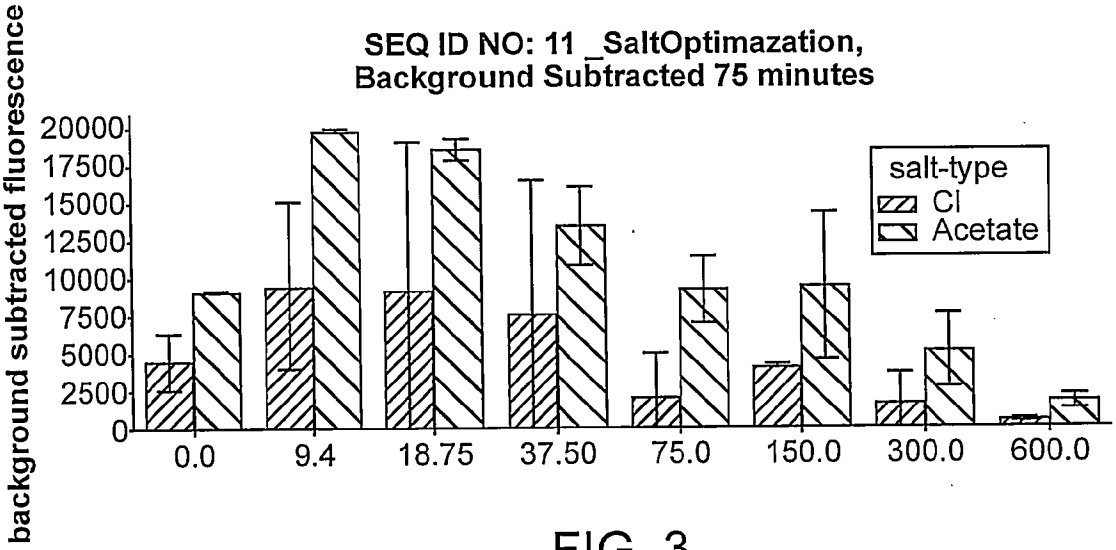

SEQ ID NO: 11 _SaltOptimazation,
Background Subtracted 75 minutes
background subtracted fluorescence
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
9.4
18.75
37.50
75.0
150.0
300.0
600.0
salt-type
Cl
Acetate

FIG. 3

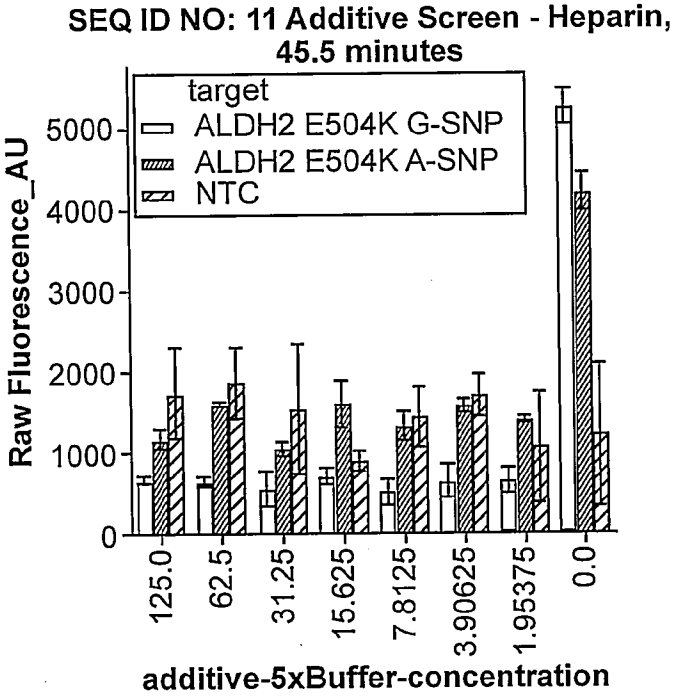

SEQ ID NO: 11 Additive Screen - Heparin,
45.5 minutes
target
ALDH2 E504K G-SNP
ALDH2 E504K A-SNP
NTC
Raw Fluorescence_AU
5000
4000
3000
2000
1000
0
125.0
62.5
31.25
15.625
7.8125
3.90625
1.95375
0.0
additive-5xBuffer-concentration

FIG. 4

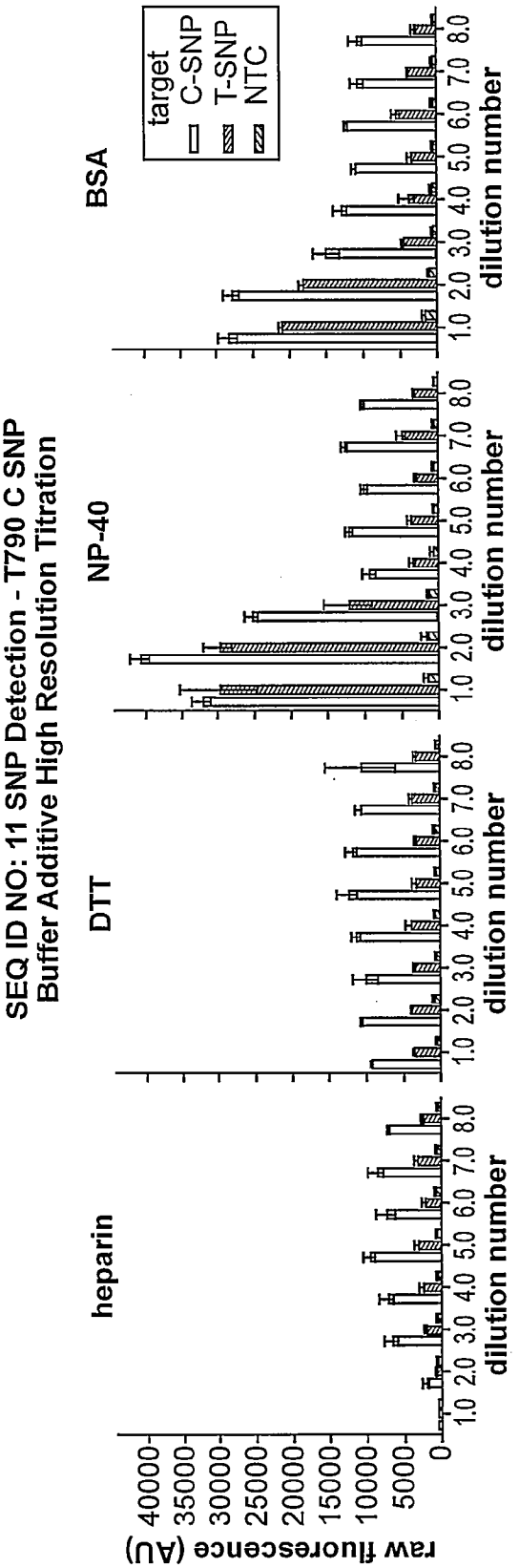

SEQ ID NO: 11 SNP Detection - T790 C SNP
Buffer Additive High Resolution Titration
heparin
DTT
NP-40
BSA
target
C-SNP
T-SNP
NTC
raw fluorescence (AU)
0
5000
10000
15000
20000
25000
30000
35000
40000
1.0 2.0 3.0 4.0 5.0 6.0 7.0 8.0
dilution number

FIG. 5

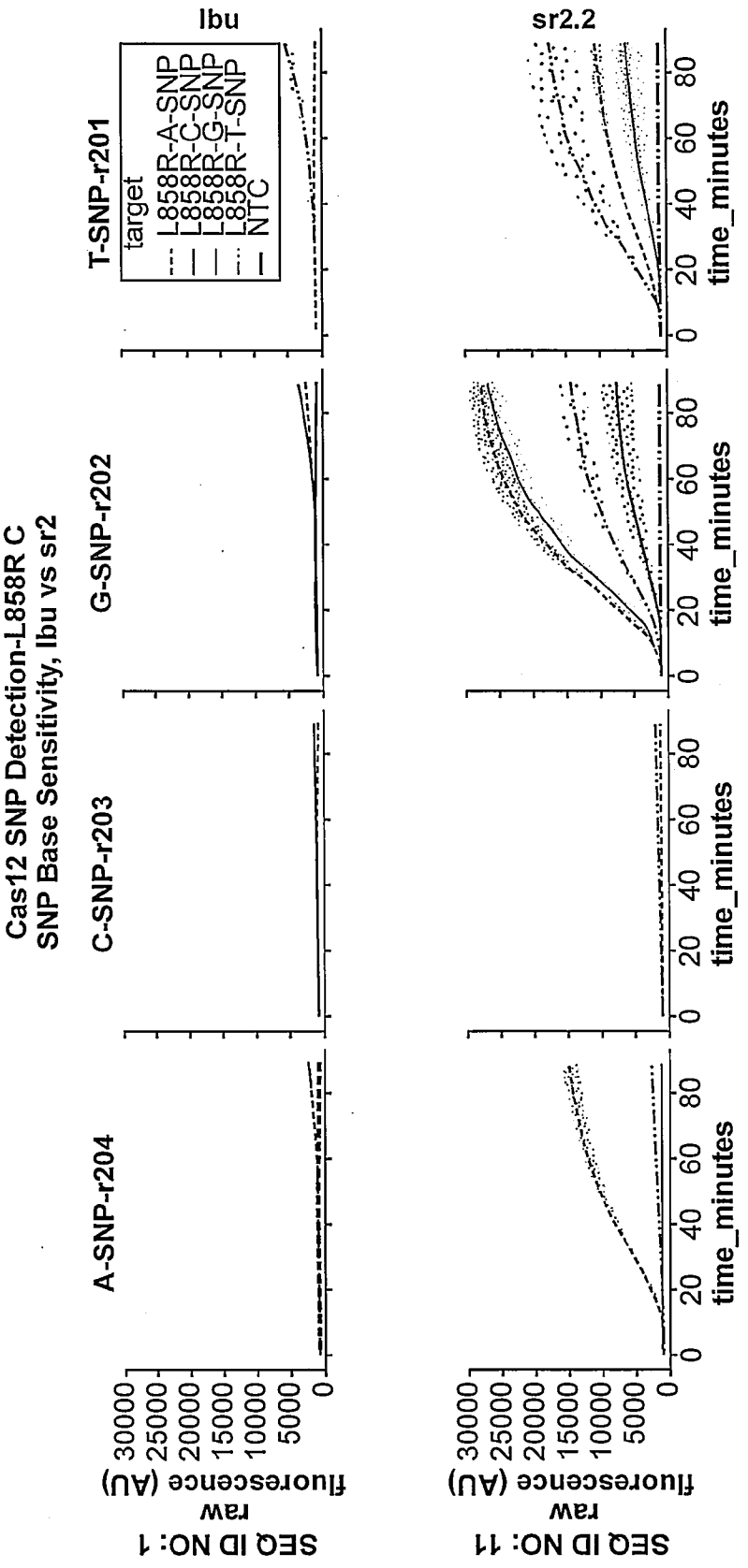

Cas12 SNP Detection-L858R C
SNP Base Sensitivity, lbu vs sr2
A-SNP-r204
C-SNP-r203
G-SNP-r202
T-SNP-r201
lbu
sr2.2
target
L858R-A-SNP
L858R-C-SNP
L858R-G-SNP
L858R-T-SNP
NTC
SEQ ID NO: 1
SEQ ID NO: 11
raw fluorescence (AU)
time_minutes
0
5000
10000
15000
20000
25000
30000
0
20
40
60
80

FIG. 6

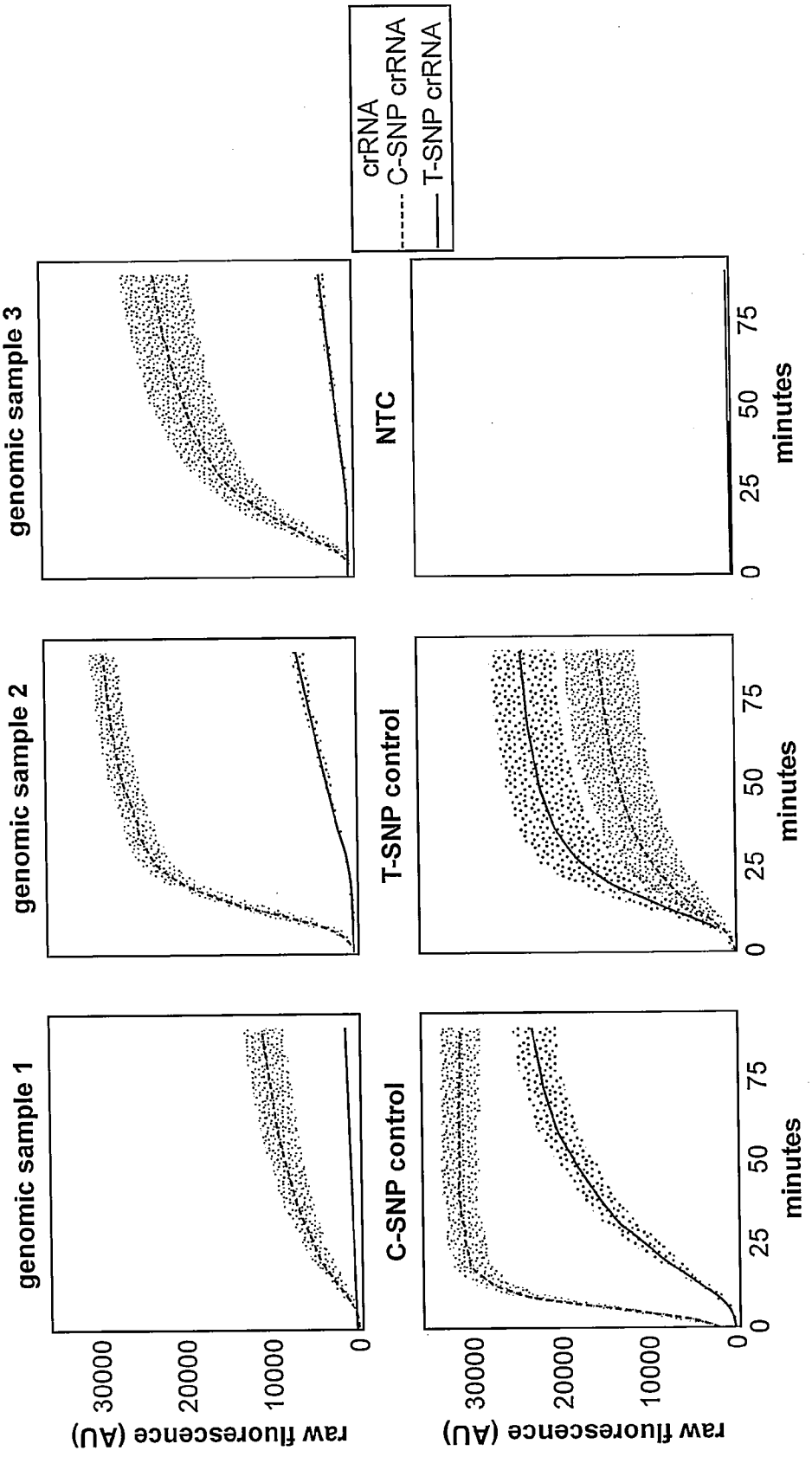

genomic sample 1
genomic sample 2
genomic sample 3
C-SNP control
T-SNP control
NTC
crRNA
C-SNP crRNA
T-SNP crRNA
raw fluorescence (AU)
30000
20000
10000
0
0
25
50
75
minutes

FIG. 7

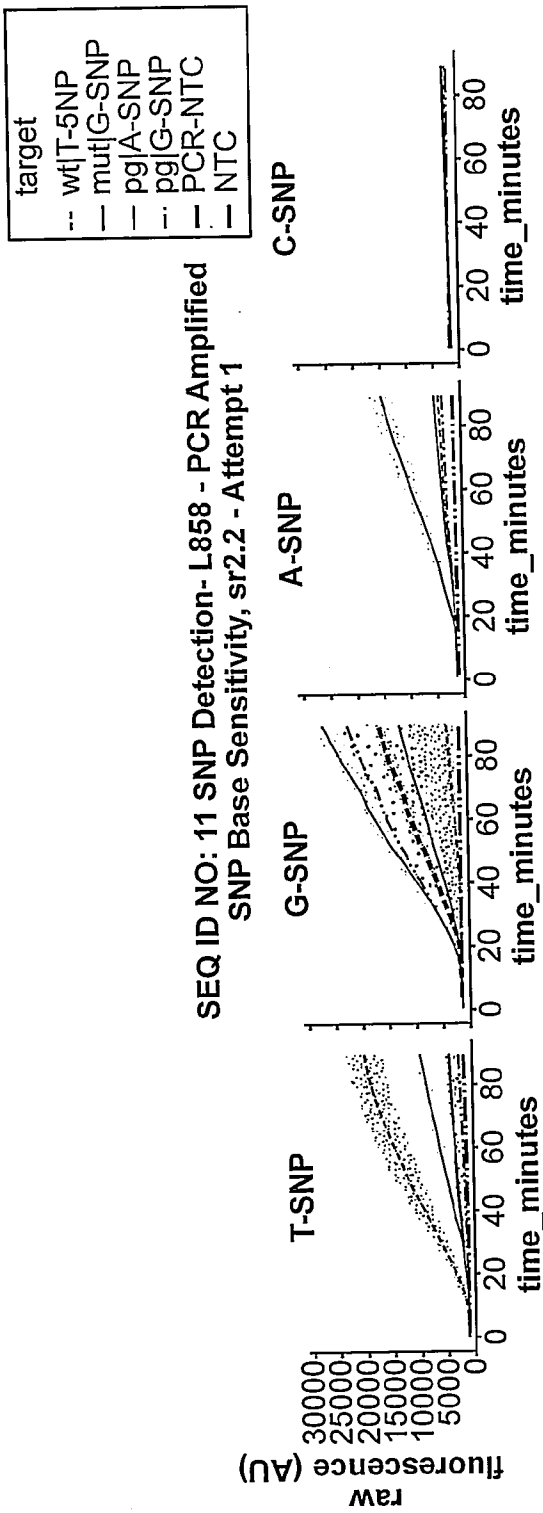

SEQ ID NO: 11 SNP Detection- L858 - PCR Amplified
SNP Base Sensitivity, sr2.2 - Attempt 1
target
wt|T-5NP
mut|G-SNP
pg|A-SNP
pg|G-SNP
PCR-NTC
NTC
T-SNP
G-SNP
A-SNP
C-SNP
raw fluorescence (AU)
time_minutes

FIG. 8

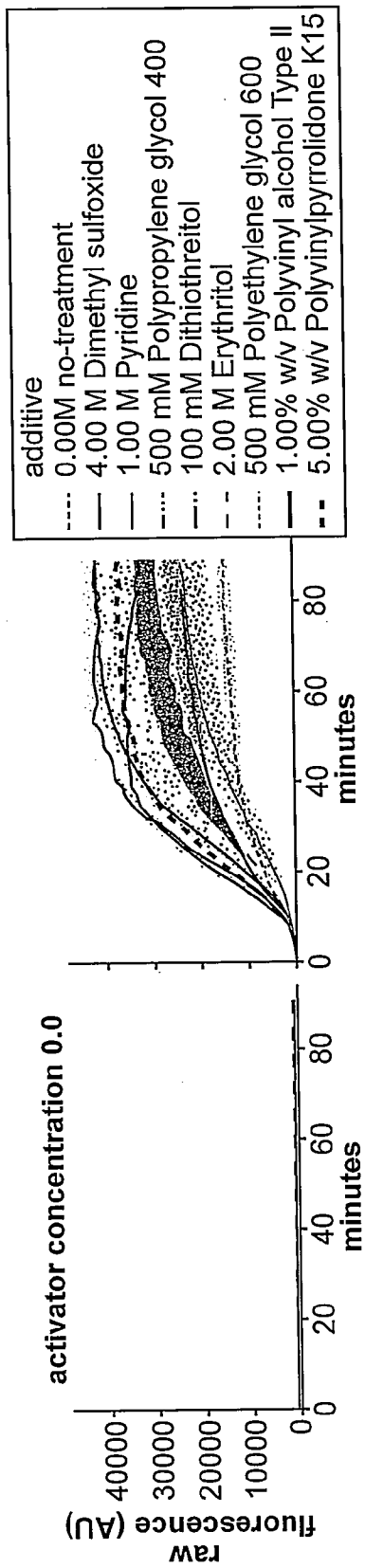

raw fluorescence (AU)
40000
30000
20000
10000
0
activator concentration 0.0
0
20
40
60
80
minutes
0
20
40
60
80
minutes
additive
0.00M no-treatment
4.00 M Dimethyl sulfoxide
1.00 M Pyridine
500 mM Polypropylene glycol 400
100 mM Dithiothreitol
2.00 M Erythritol
500 mM Polyethylene glycol 600
1.00% w/v Polyvinyl alcohol Type II
5.00% w/v Polyvinylpyrrolidone K15

FIG. 9

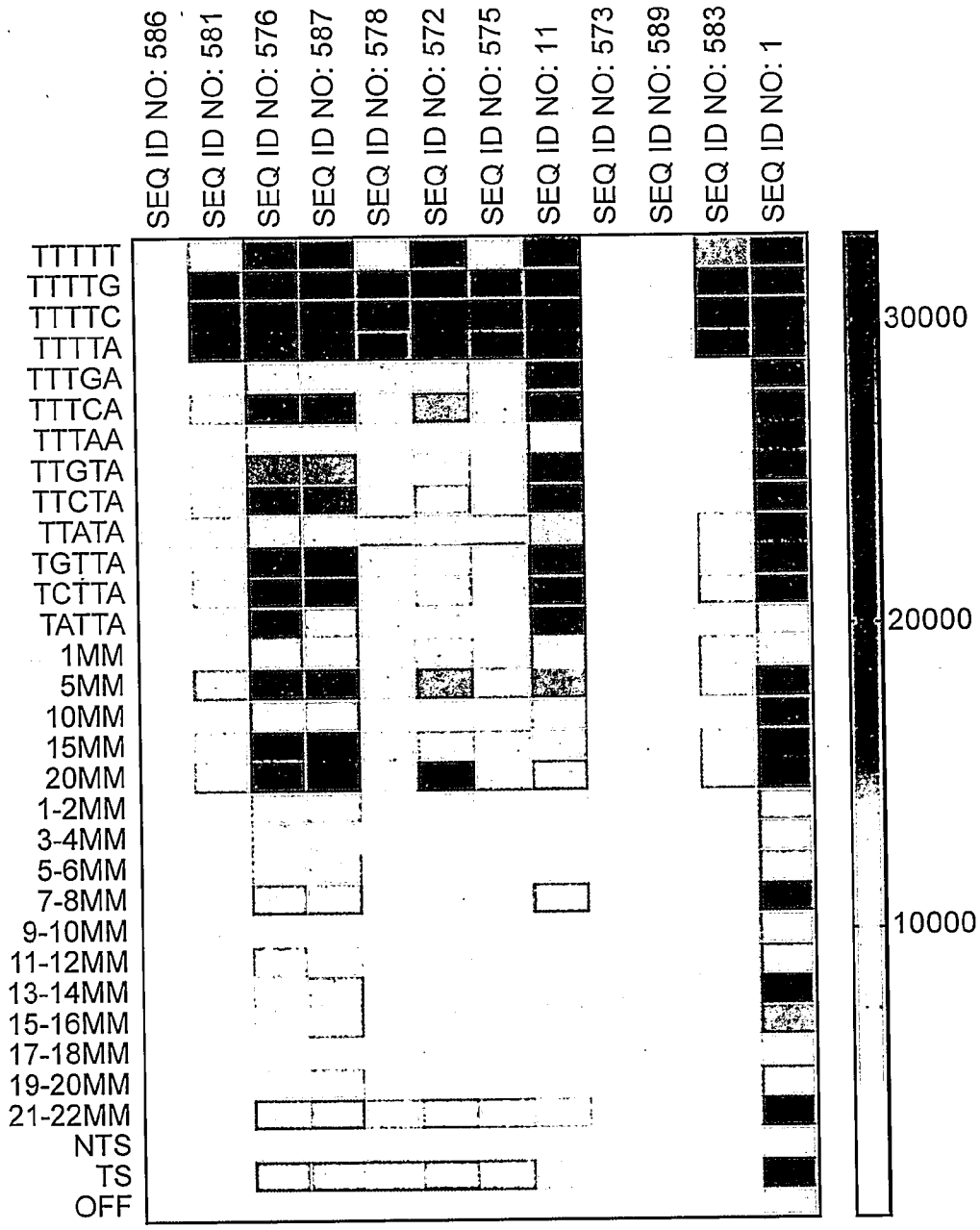

SEQ ID NO: 586
SEQ ID NO: 581
SEQ ID NO: 576
SEQ ID NO: 587
SEQ ID NO: 578
SEQ ID NO: 572
SEQ ID NO: 575
SEQ ID NO: 11
SEQ ID NO: 573
SEQ ID NO: 589
SEQ ID NO: 583
SEQ ID NO: 1
TTTTT
TTTTG
TTTTC
TTTTA
TTTGA
TTTCA
TTTAA
TTGTA
TTCTA
TTATA
TGTTA
TCTTA
TATTA
1MM
5MM
10MM
15MM
20MM
1-2MM
3-4MM
5-6MM
7-8MM
9-10MM
11-12MM
13-14MM
15-16MM
17-18MM
19-20MM
21-22MM
NTS
TS
OFF
30000
20000
10000

FIG. 10

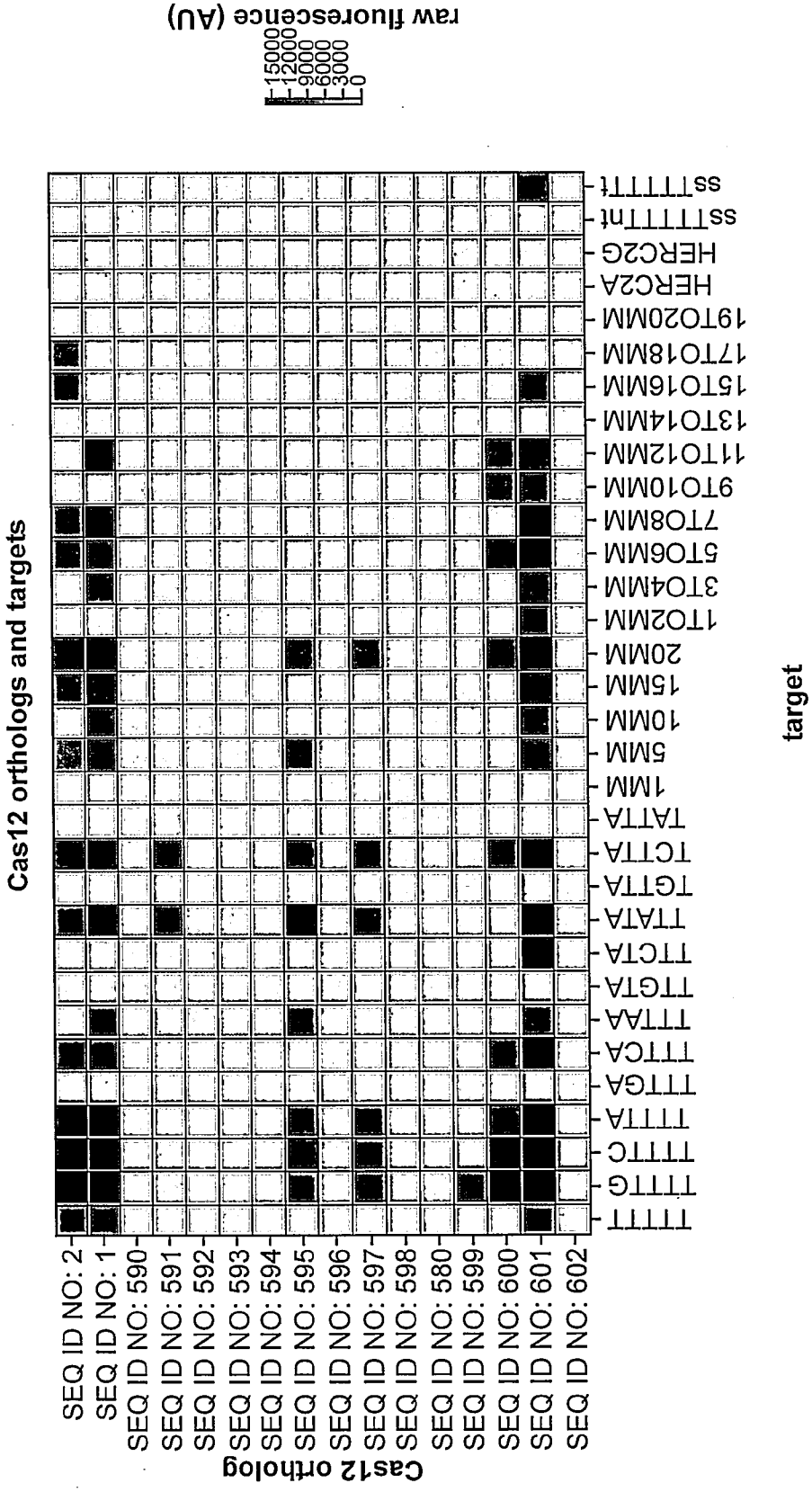

raw fluorescence (AU)
15000
12000
9000
6000
3000
0
Cas12 orthologs and targets
Cas12 ortholog
SEQ ID NO: 2
SEQ ID NO: 1
SEQ ID NO: 590
SEQ ID NO: 591
SEQ ID NO: 592
SEQ ID NO: 593
SEQ ID NO: 594
SEQ ID NO: 595
SEQ ID NO: 596
SEQ ID NO: 597
SEQ ID NO: 598
SEQ ID NO: 580
SEQ ID NO: 599
SEQ ID NO: 600
SEQ ID NO: 601
SEQ ID NO: 602
TTTTT
TTTTG
TTTTC
TTTTA
TTTGA
TTTCA
TTTAA
TTGTA
TTCTA
TTATA
TGTTA
TCTTA
TATTA
1MM
5MM
10MM
15MM
20MM
1TO2MM
3TO4MM
5TO6MM
7TO8MM
9TO10MM
11TO12MM
13TO14MM
15TO16MM
17TO18MM
19TO20MM
HERC2A
HERC2G
ssTTTTTnt
ssTTTTTt
target

FIG. 11

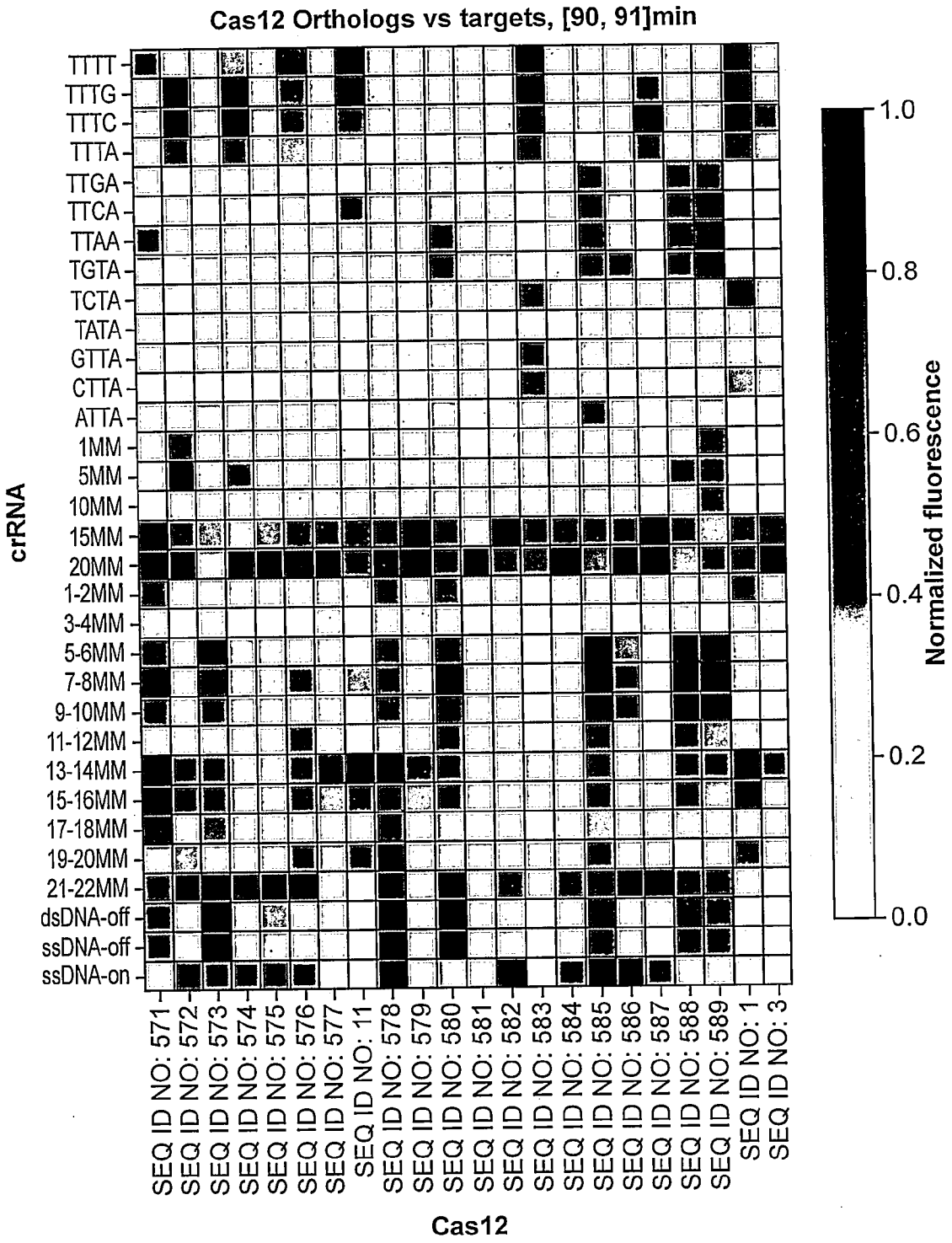

Cas12 Orthologs vs targets, [90, 91]min
crRNA
TTTT
TTTG
TTTC
TTTA
TTGA
TTCA
TTAA
TGTA
TCTA
TATA
GTTA
CTTA
ATTA
1MM
5MM
10MM
15MM
20MM
1-2MM
3-4MM
5-6MM
7-8MM
9-10MM
11-12MM
13-14MM
15-16MM
17-18MM
19-20MM
21-22MM
dsDNA-off
ssDNA-off
ssDNA-on
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3
Cas12
1.0
0.8
0.6
0.4
0.2
0.0
Normalized fluorescence

FIG. 12

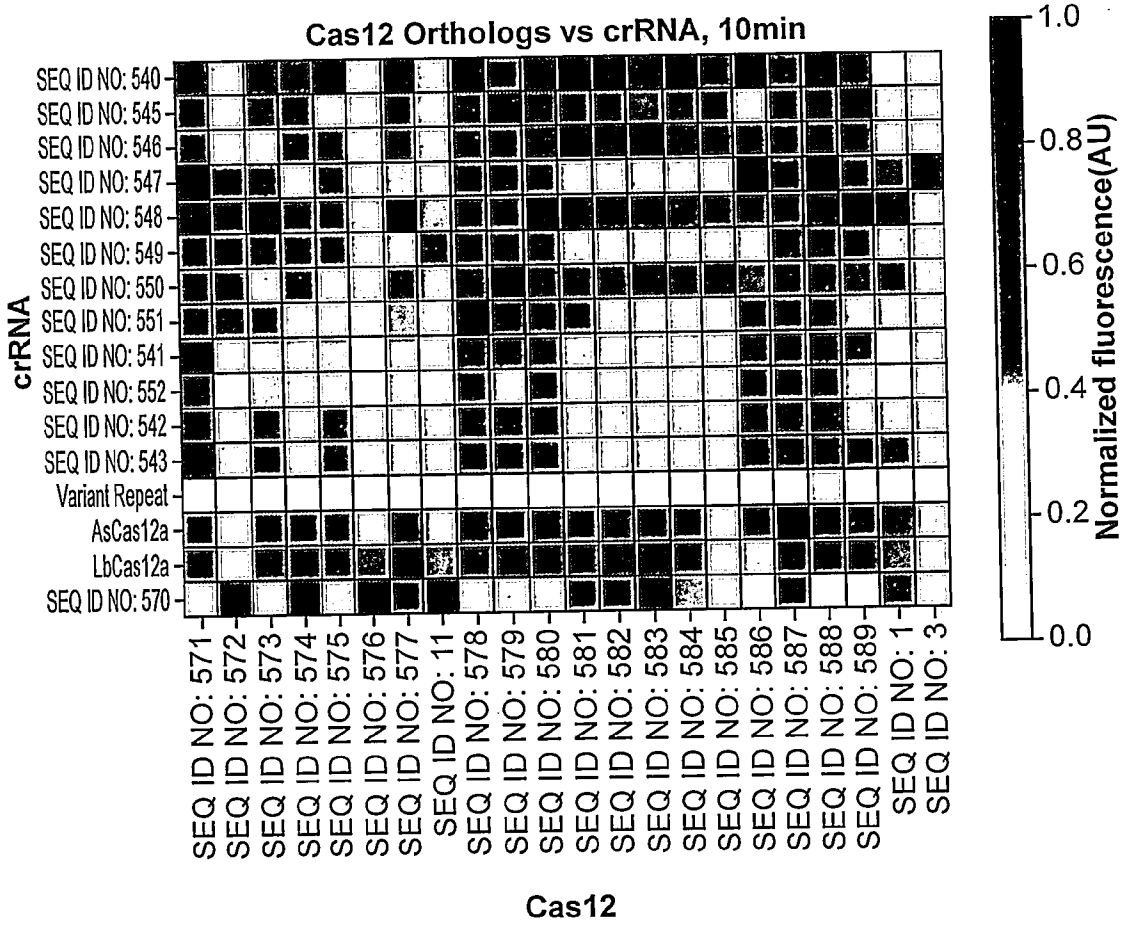

Cas12 Orthologs vs crRNA, 10min
crRNA
SEQ ID NO: 540
SEQ ID NO: 545
SEQ ID NO: 546
SEQ ID NO: 547
SEQ ID NO: 548
SEQ ID NO: 549
SEQ ID NO: 550
SEQ ID NO: 551
SEQ ID NO: 541
SEQ ID NO: 552
SEQ ID NO: 542
SEQ ID NO: 543
Variant Repeat
AsCas12a
LbCas12a
SEQ ID NO: 570
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3
Cas12
1.0
0.8
0.6
0.4
0.2
0.0
Normalized fluorescence(AU)

FIG. 13A

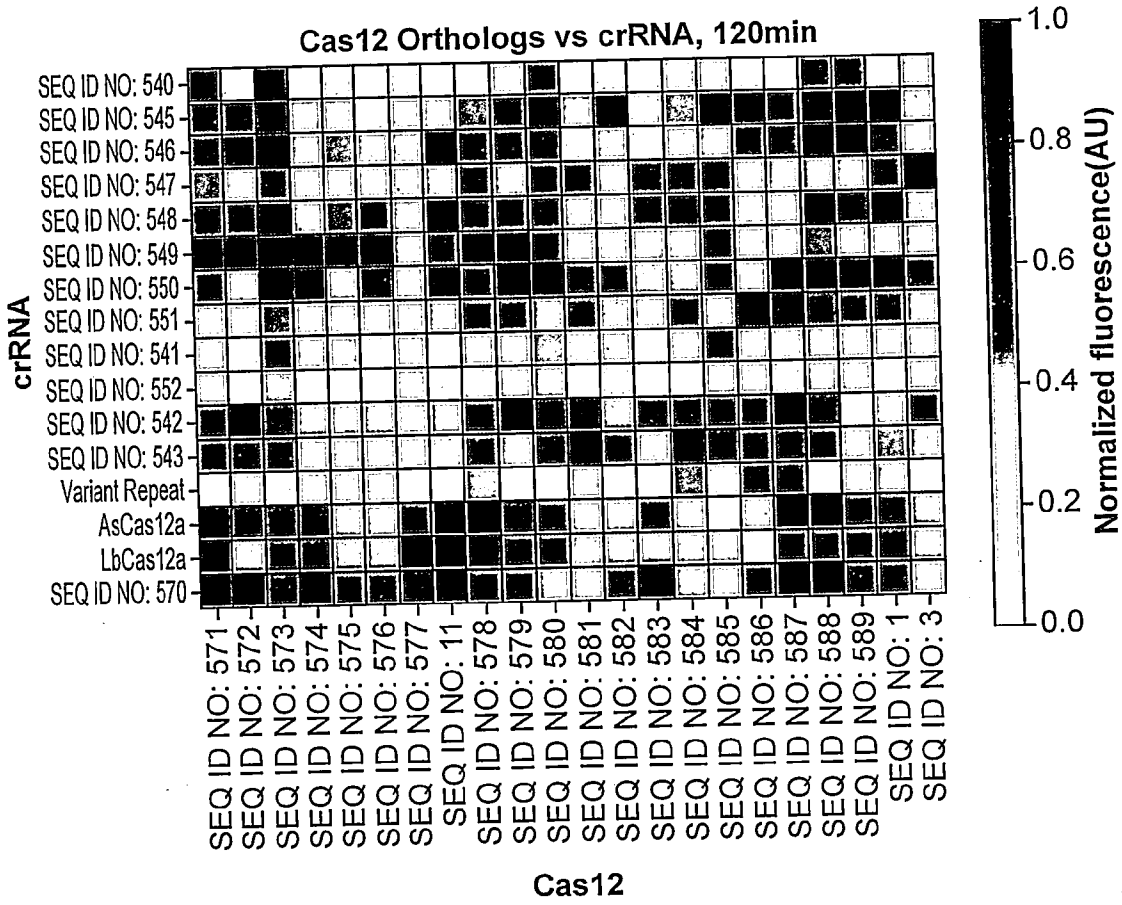

Cas12 Orthologs vs crRNA, 120min
crRNA
SEQ ID NO: 540
SEQ ID NO: 545
SEQ ID NO: 546
SEQ ID NO: 547
SEQ ID NO: 548
SEQ ID NO: 549
SEQ ID NO: 550
SEQ ID NO: 551
SEQ ID NO: 541
SEQ ID NO: 552
SEQ ID NO: 542
SEQ ID NO: 543
Variant Repeat
AsCas12a
LbCas12a
SEQ ID NO: 570
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3
Cas12
1.0
0.8
0.6
0.4
0.2
0.0
Normalized fluorescence(AU)

FIG. 13B

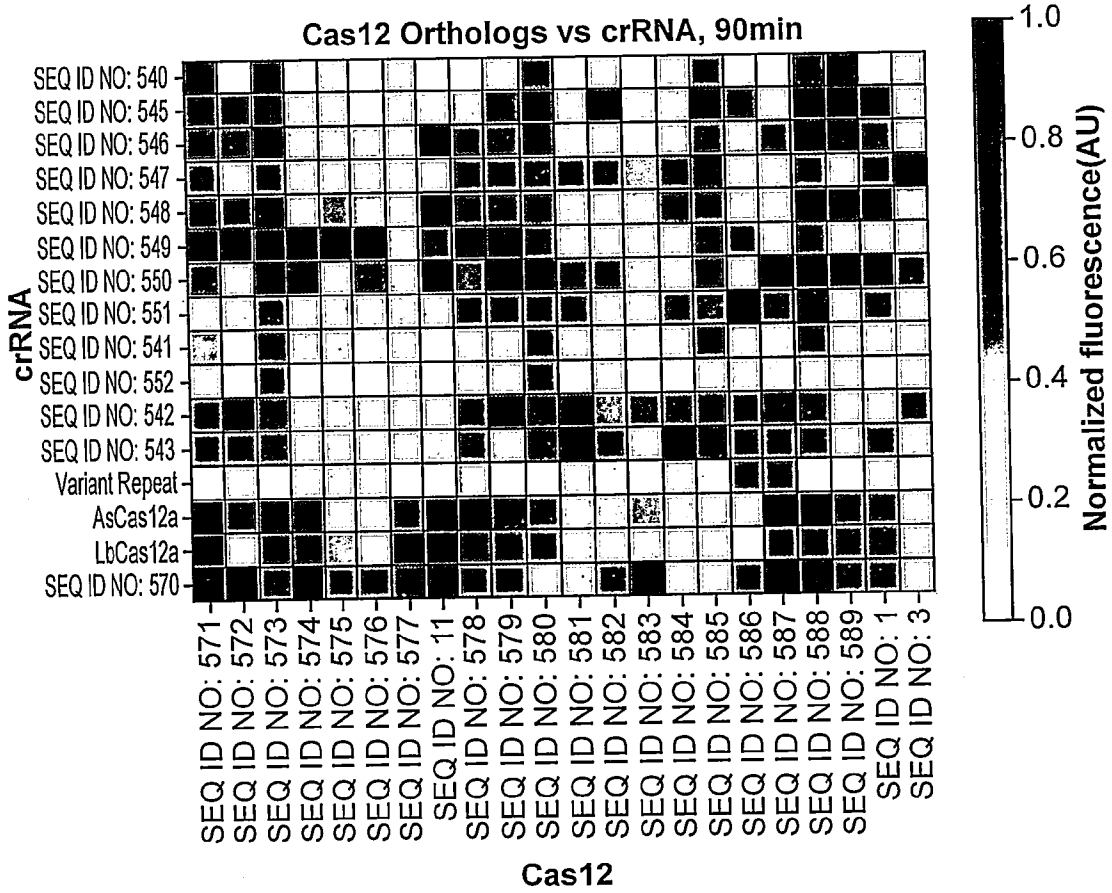

Cas12 Orthologs vs crRNA, 90min
crRNA
SEQ ID NO: 540
SEQ ID NO: 545
SEQ ID NO: 546
SEQ ID NO: 547
SEQ ID NO: 548
SEQ ID NO: 549
SEQ ID NO: 550
SEQ ID NO: 551
SEQ ID NO: 541
SEQ ID NO: 552
SEQ ID NO: 542
SEQ ID NO: 543
Variant Repeat
AsCas12a
LbCas12a
SEQ ID NO: 570
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3
Cas12
1.0
0.8
0.6
0.4
0.2
0.0
Normalized fluorescence(AU)

FIG. 13C

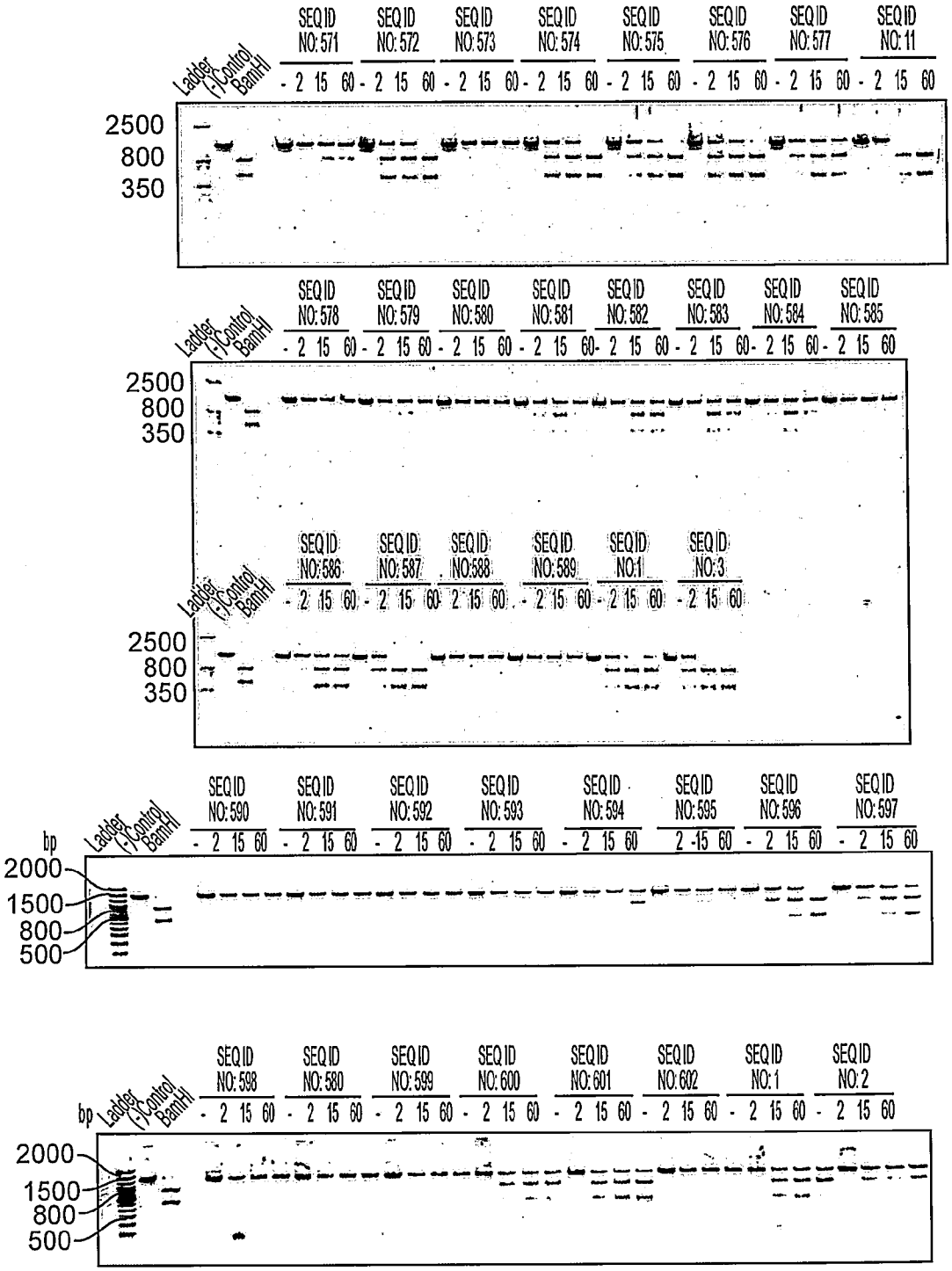

SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
Ladder
(-) Control
BamHI
- 2 15 60
2500
800
350
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3
bp
2000
1500
800
500
SEQ ID NO: 590
SEQ ID NO: 591
SEQ ID NO: 592
SEQ ID NO: 593
SEQ ID NO: 594
SEQ ID NO: 595
SEQ ID NO: 596
SEQ ID NO: 597
SEQ ID NO: 598
SEQ ID NO: 580
SEQ ID NO: 599
SEQ ID NO: 600
SEQ ID NO: 601
SEQ ID NO: 602
SEQ ID NO: 1
SEQ ID NO: 2

FIG. 14

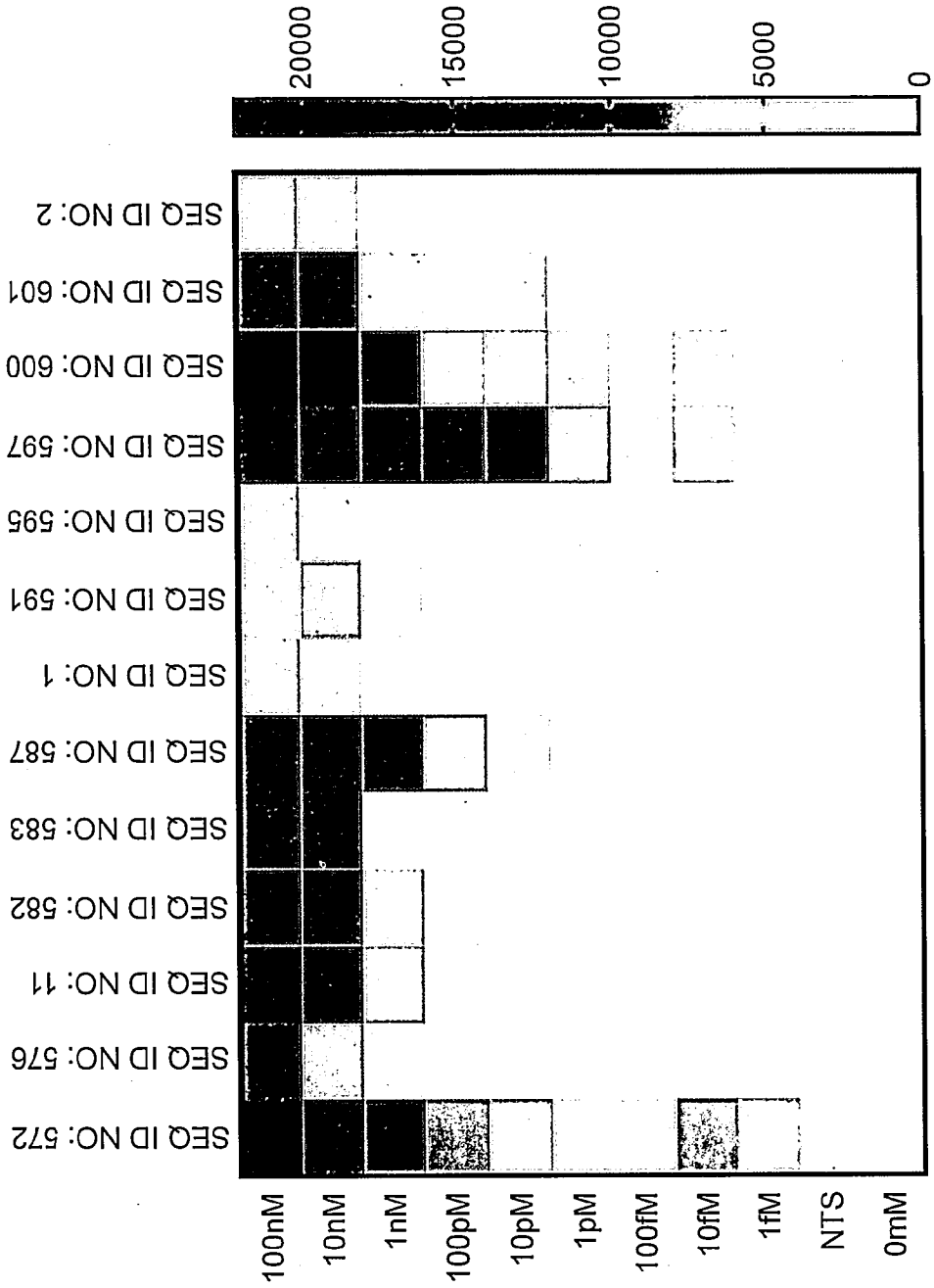

20000
15000
10000
5000
0
SEQ ID NO: 2
SEQ ID NO: 601
SEQ ID NO: 600
SEQ ID NO: 597
SEQ ID NO: 595
SEQ ID NO: 591
SEQ ID NO: 1
SEQ ID NO: 587
SEQ ID NO: 583
SEQ ID NO: 582
SEQ ID NO: 11
SEQ ID NO: 576
SEQ ID NO: 572
100nM
10nM
1nM
100pM
10pM
1pM
100fM
10fM
1fM
NTS
0mM

FIG. 15

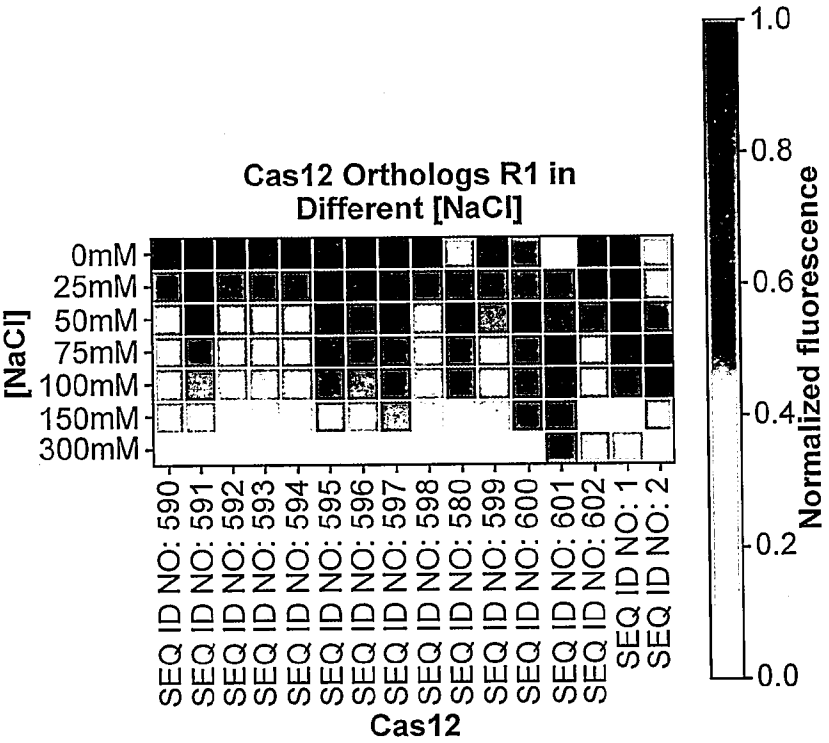


FIG. 16A

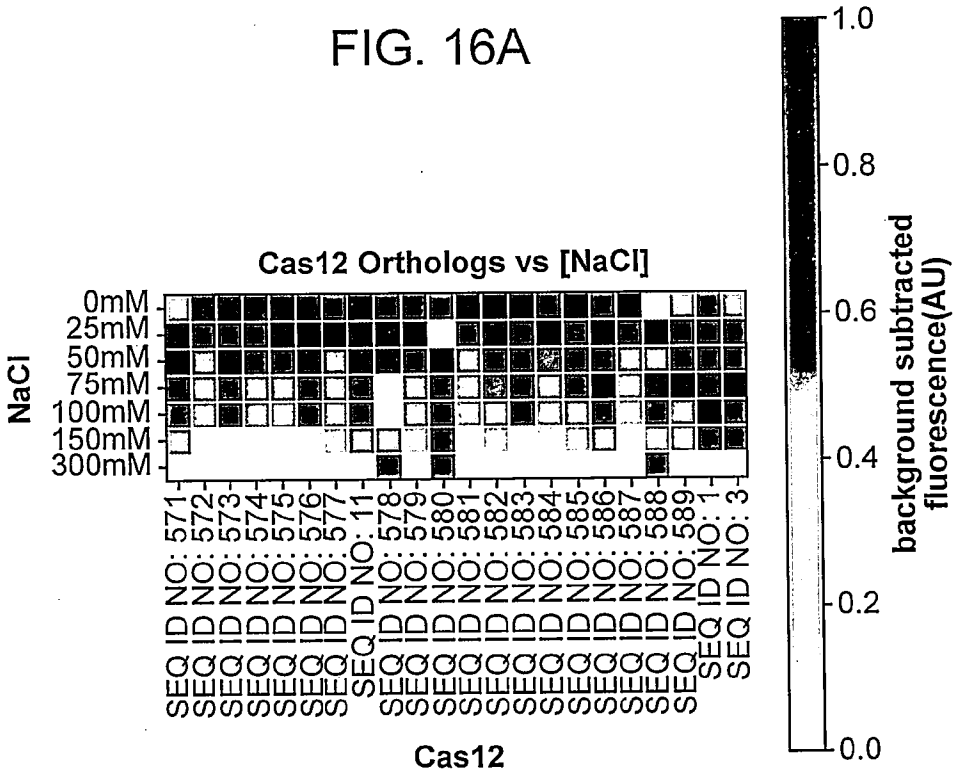


FIG. 16B

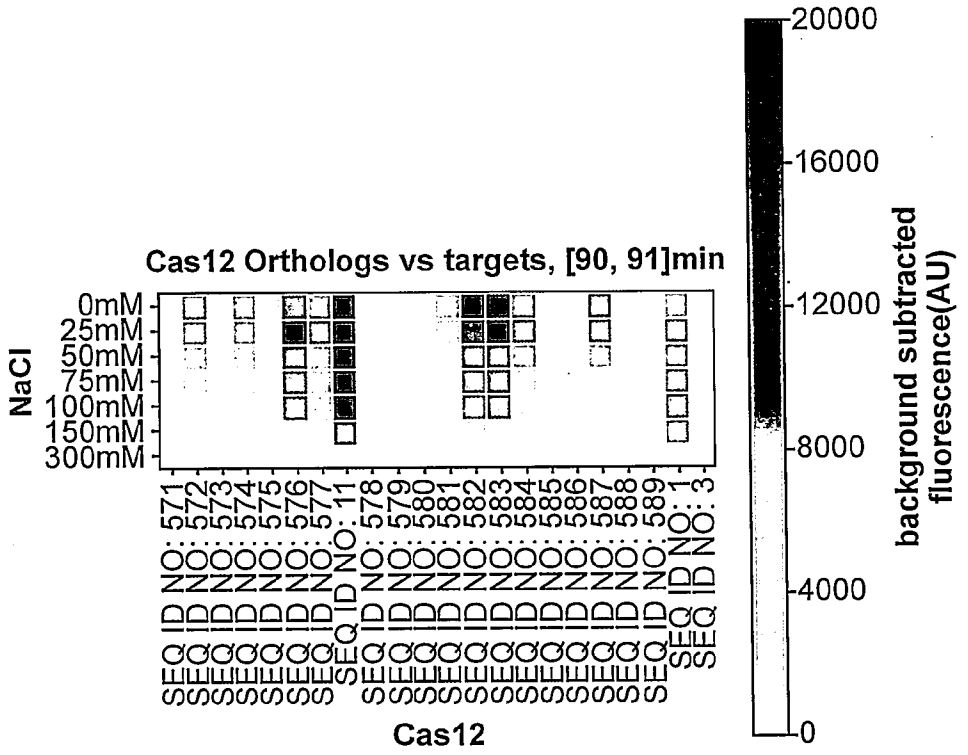


FIG. 17A

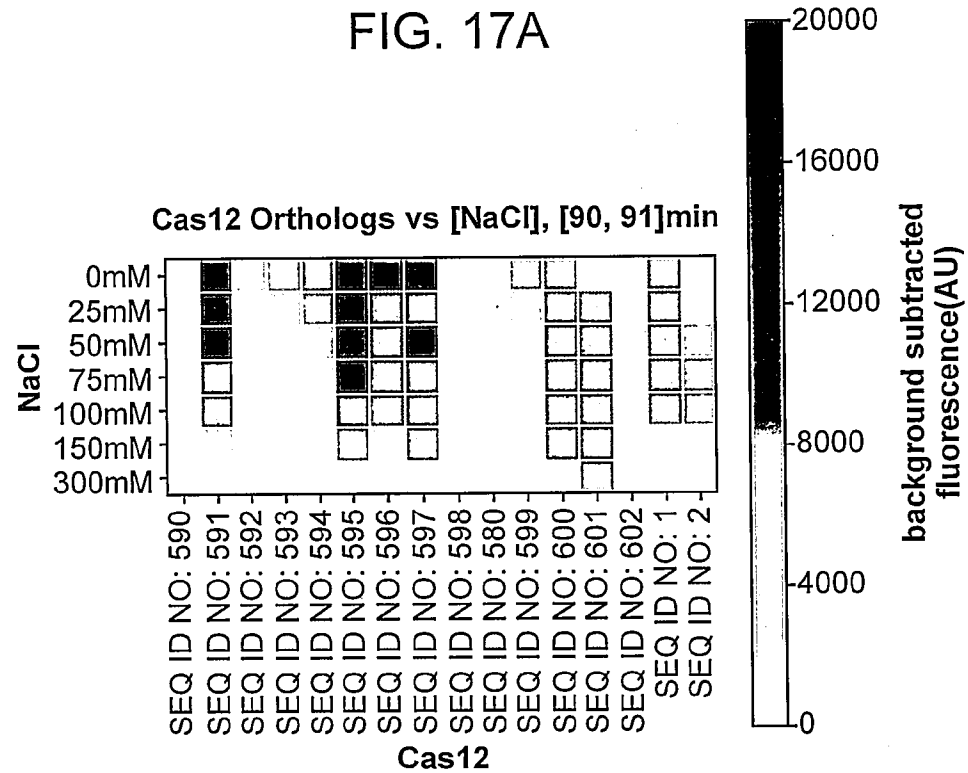


FIG. 17B

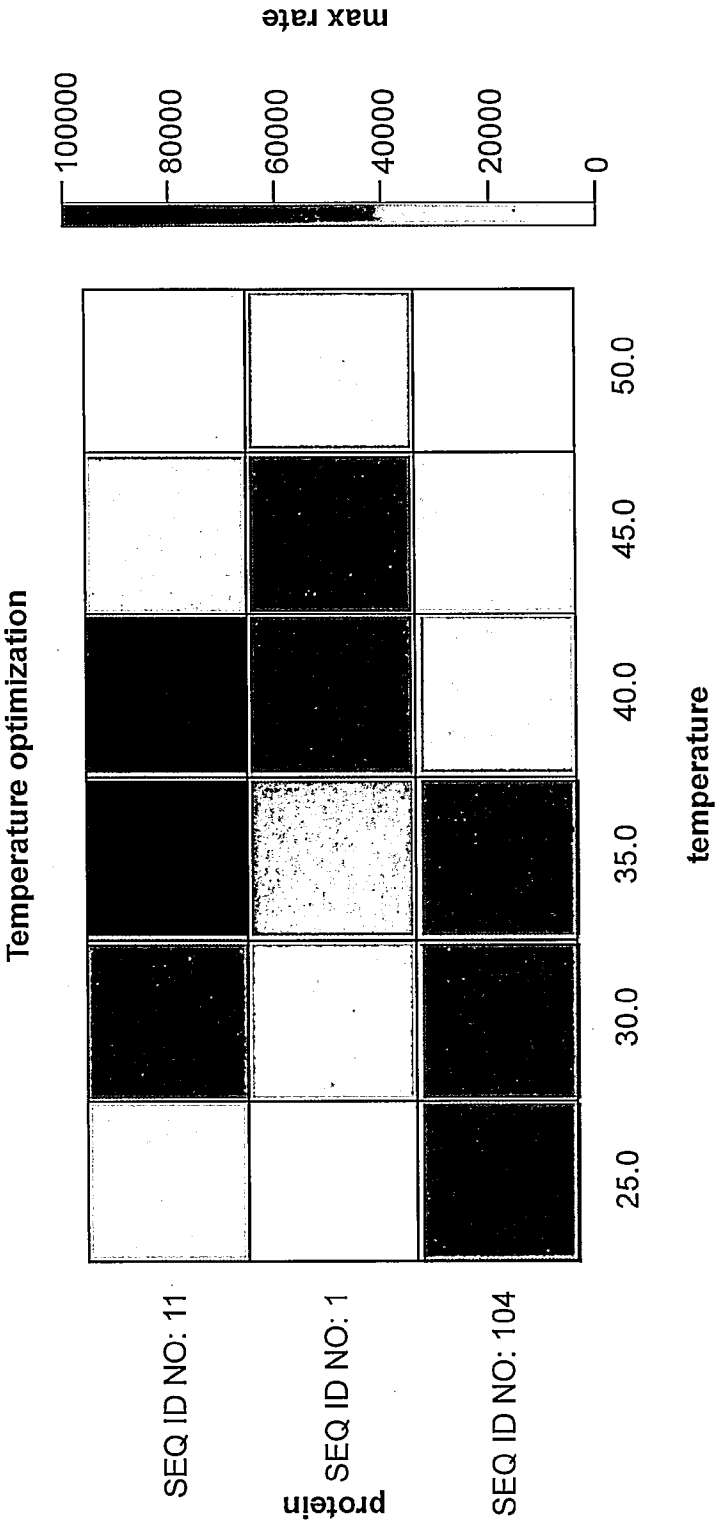

max rate
100000
80000
60000
40000
20000
0
Temperature optimization
protein
SEQ ID NO: 11
SEQ ID NO: 1
SEQ ID NO: 104
25.0
30.0
35.0
40.0
45.0
50.0
temperature

FIG. 18

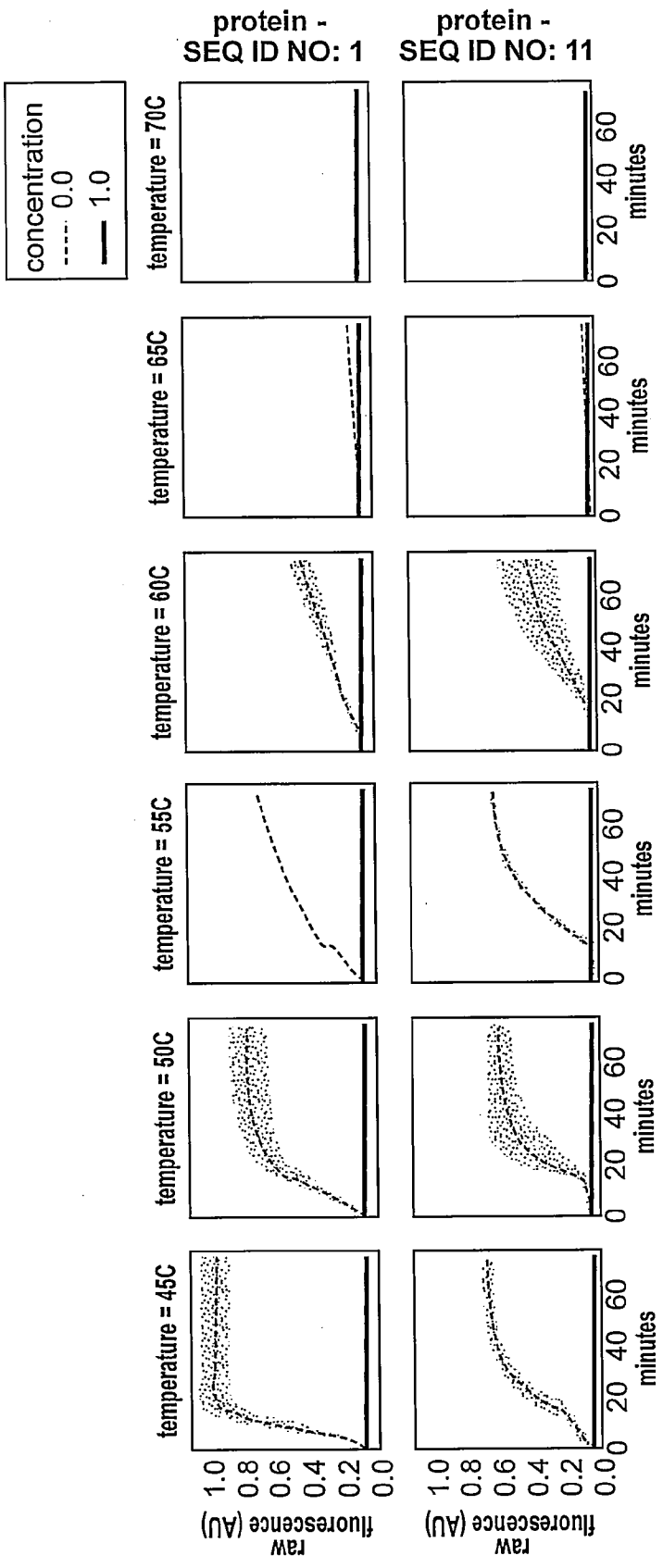

protein - SEQ ID NO: 1
protein - SEQ ID NO: 11
concentration
0.0
1.0
temperature = 70C
temperature = 65C
temperature = 60C
temperature = 55C
temperature = 50C
temperature = 45C
raw fluorescence (AU)
minutes
0
20
40
60
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 19

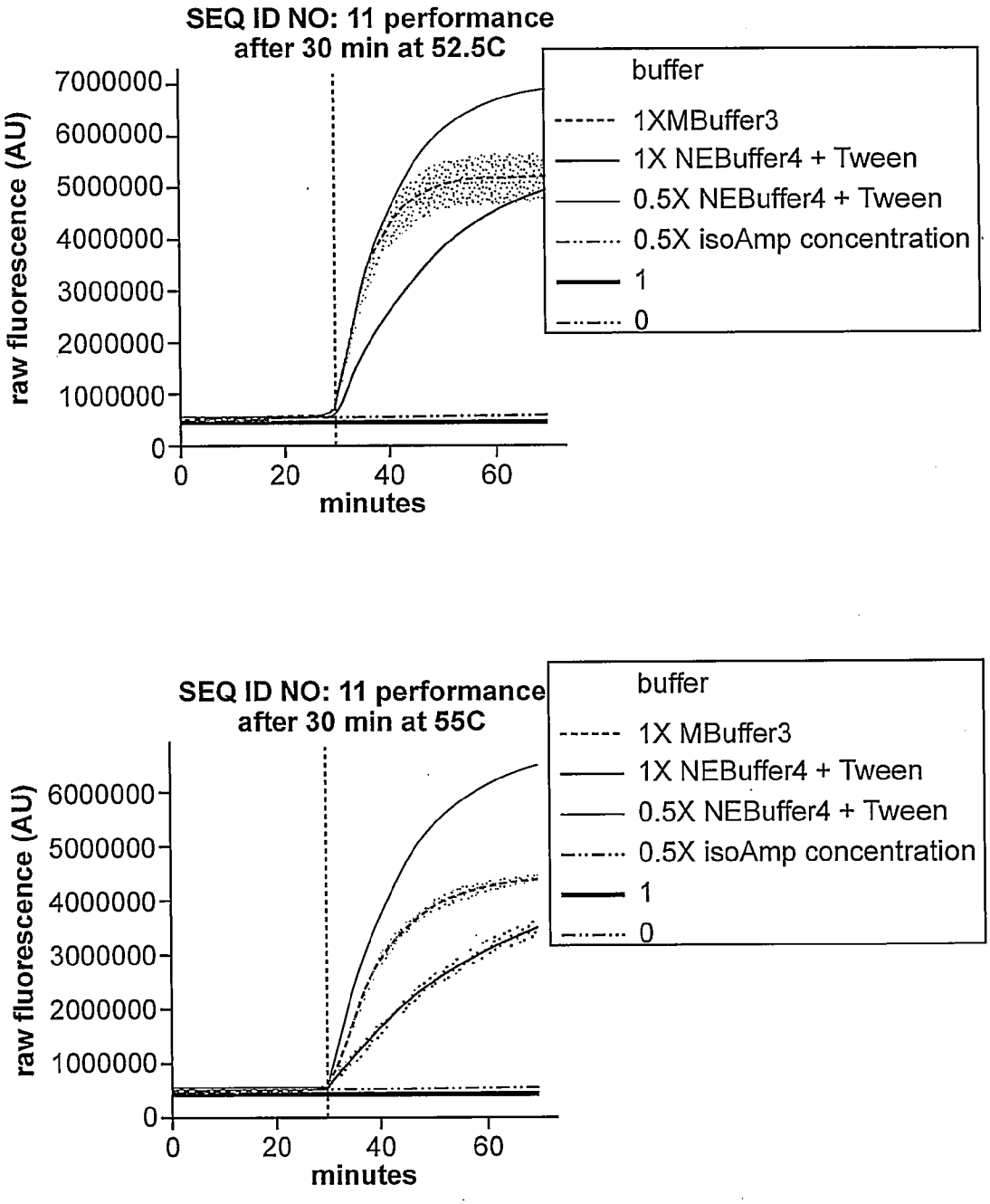

SEQ ID NO: 11 performance
after 30 min at 52.5C
raw fluorescence (AU)
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
0
20
40
60
minutes
buffer
1XMBuffer3
1X NEBuffer4 + Tween
0.5X NEBuffer4 + Tween
0.5X isoAmp concentration
1
0
SEQ ID NO: 11 performance
after 30 min at 55C
raw fluorescence (AU)
6000000
5000000
4000000
3000000
2000000
1000000
0
0
20
40
60
minutes
buffer
1X MBuffer3
1X NEBuffer4 + Tween
0.5X NEBuffer4 + Tween
0.5X isoAmp concentration
1
0

FIG. 20

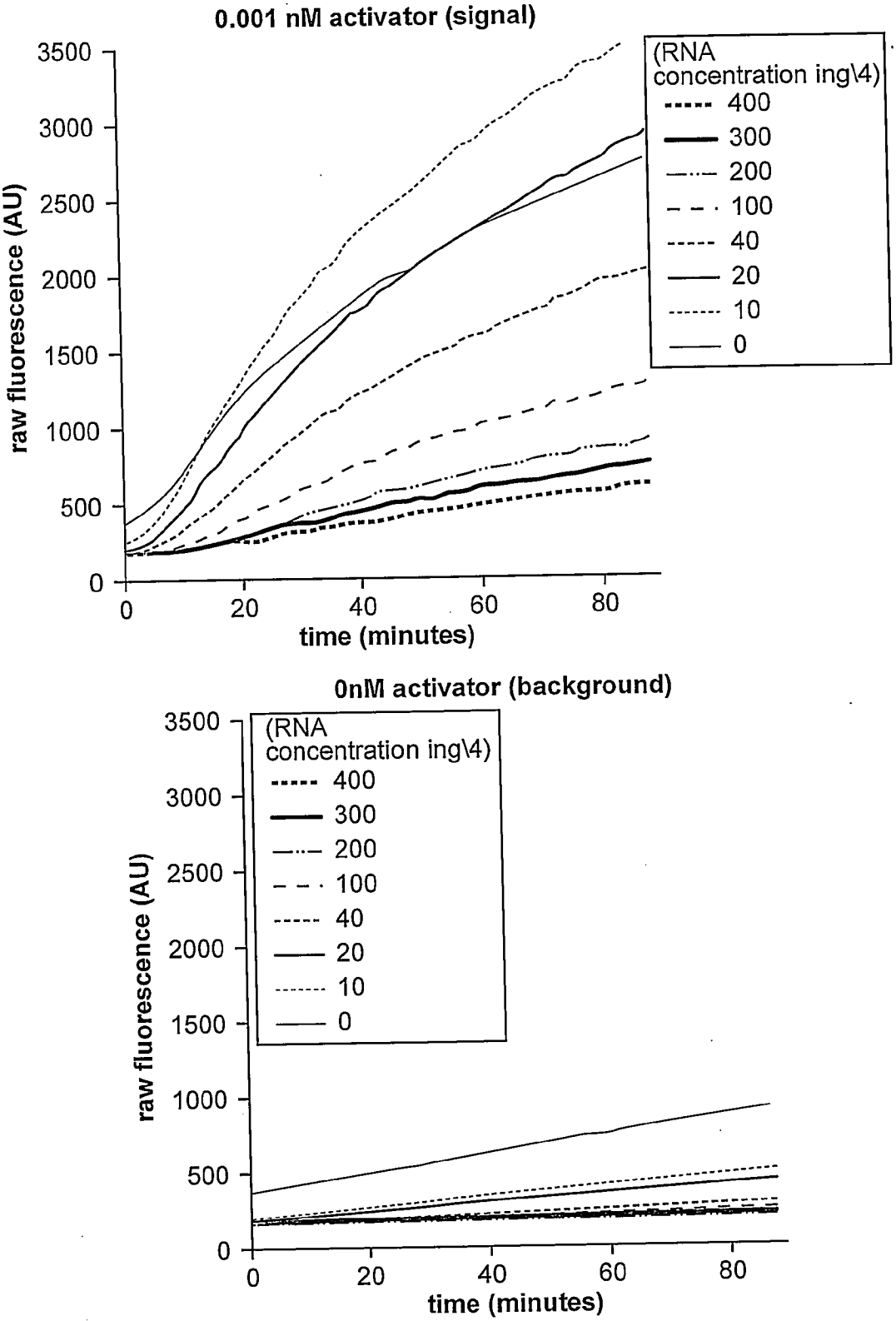

0.001 nM activator (signal)
raw fluorescence (AU)
time (minutes)
(RNA concentration ing\4)
400
300
200
100
40
20
10
0
0nM activator (background)
raw fluorescence (AU)
time (minutes)
(RNA concentration ing\4)
400
300
200
100
40
20
10
0

FIG. 21

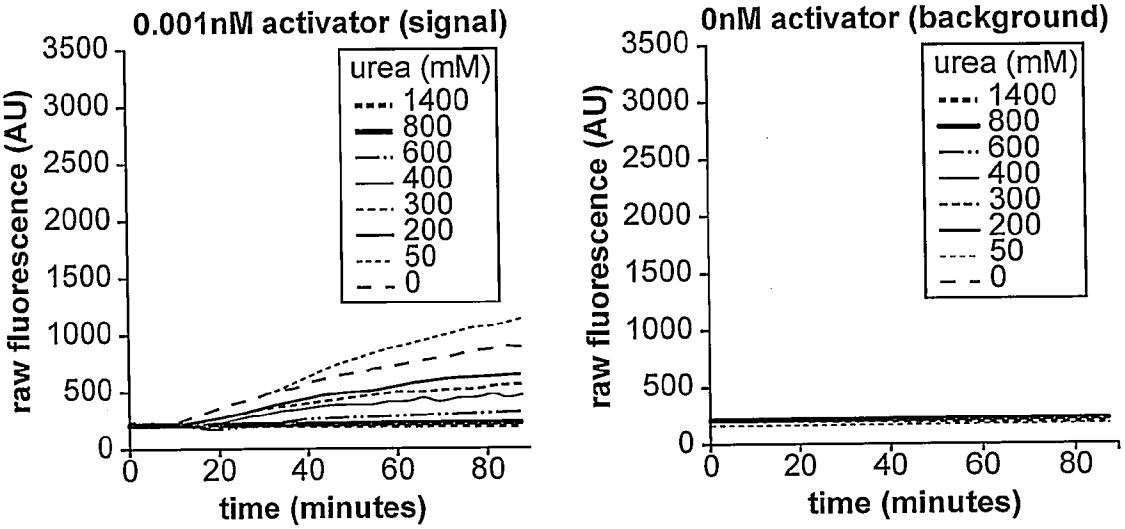


FIG. 22A

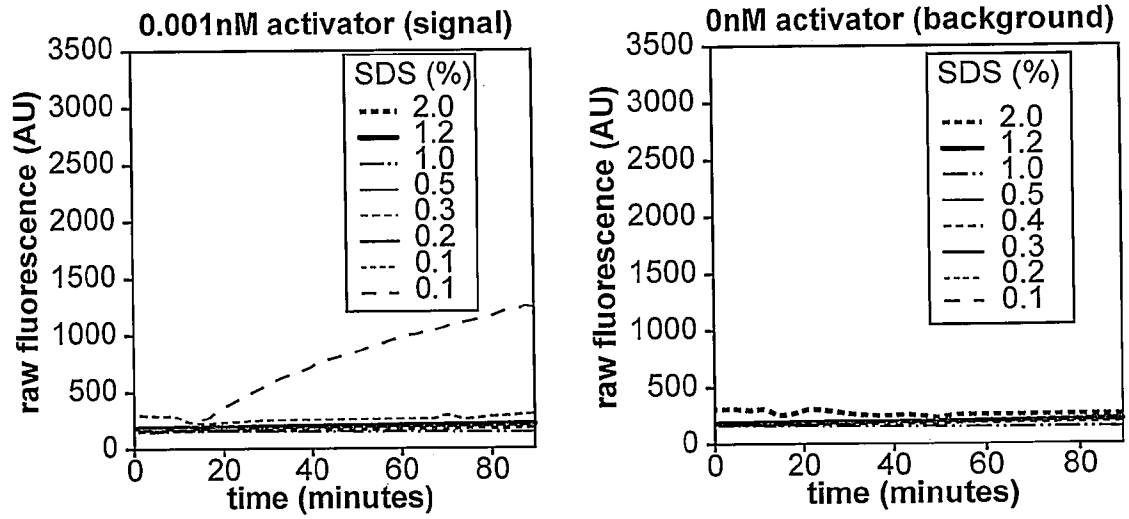


FIG. 22B

FIG. 22

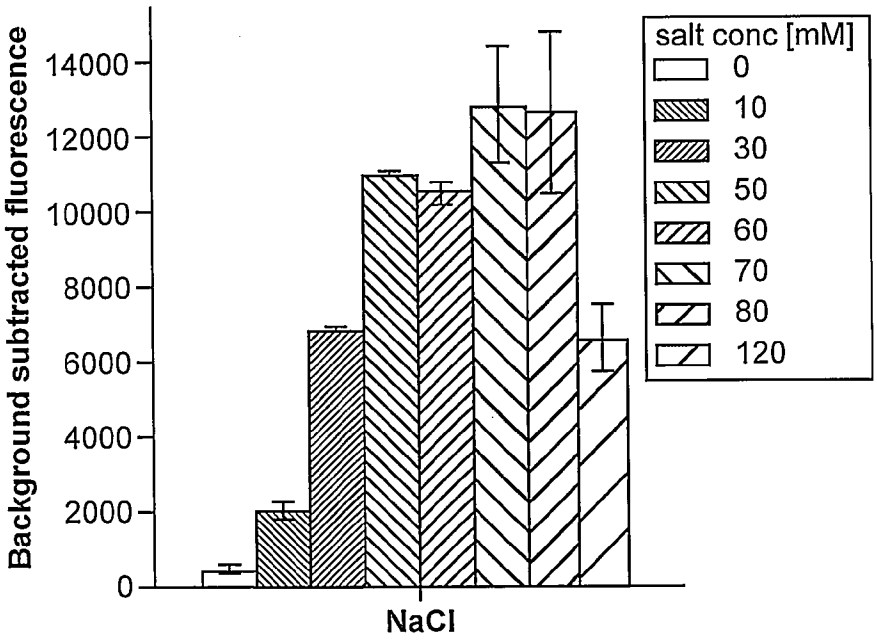


FIG. 23A

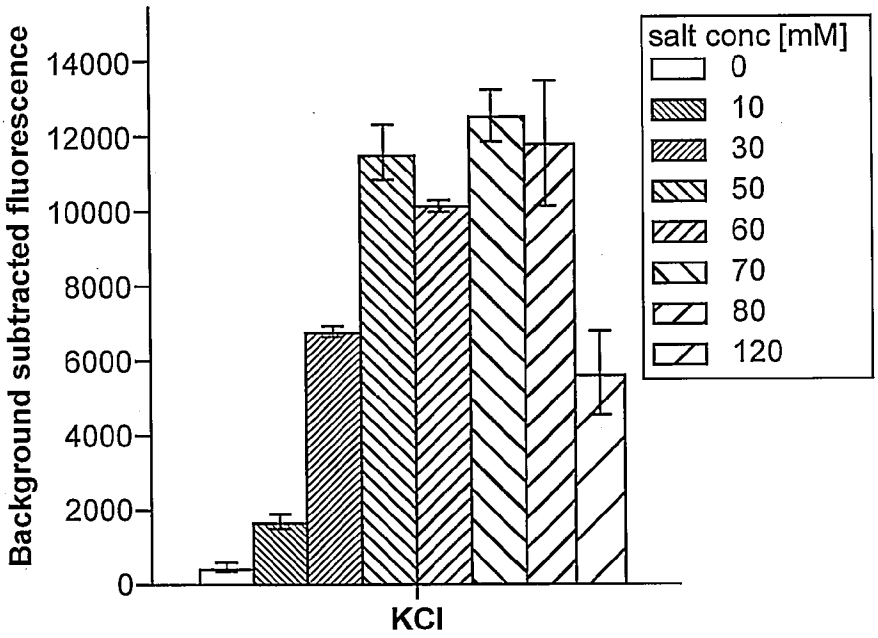


FIG. 23B

FIG. 23

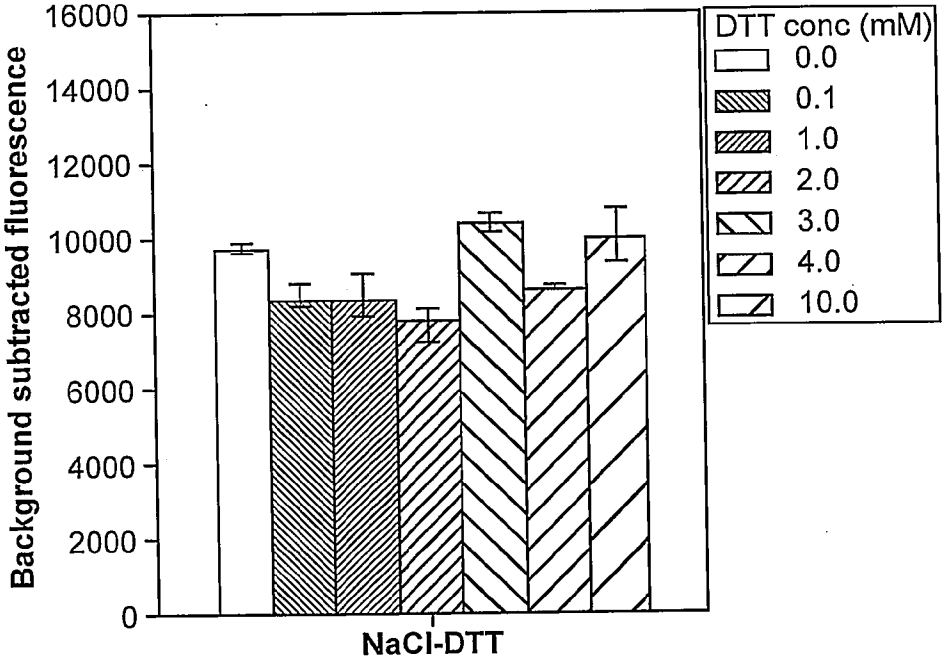


FIG. 24A

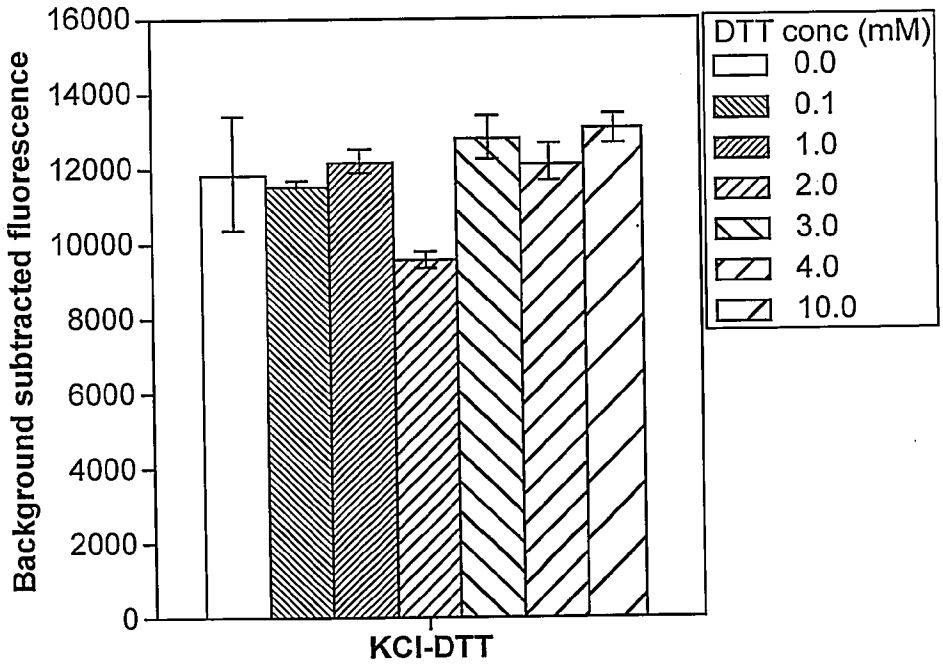


FIG. 24B

FIG. 24

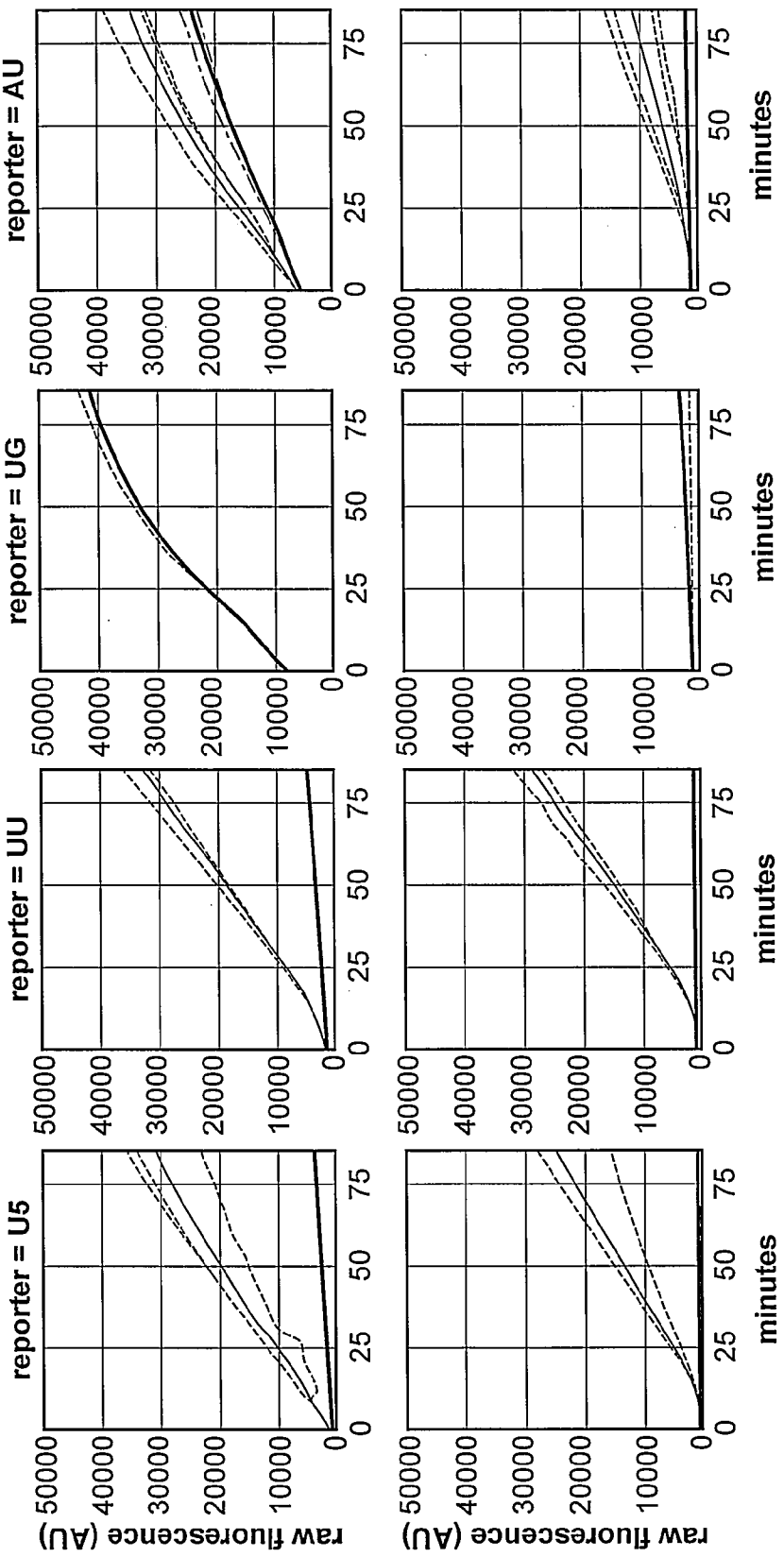

reporter = U5
reporter = UU
reporter = UG
reporter = AU
raw fluorescence (AU)
minutes
0
10000
20000
30000
40000
50000
0
25
50
75

FIG. 25

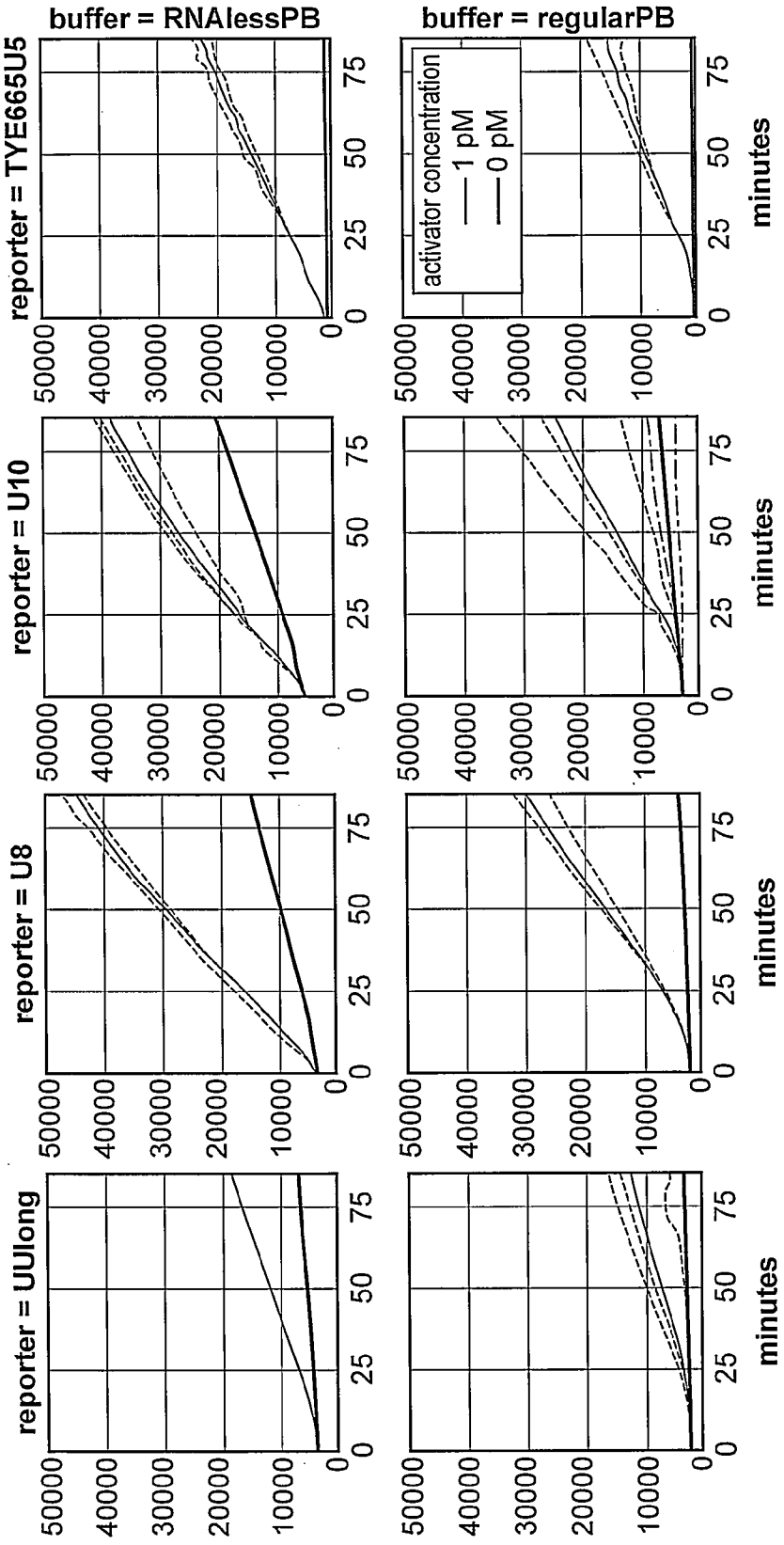

buffer = RNAlessPB
buffer = regularPB
reporter = TYE665U5
reporter = U10
reporter = U8
reporter = UUlong
activator concentration
1 pM
0 pM
50000
40000
30000
20000
10000
0
0
25
50
75
minutes

FIG. 25 (Cont.)

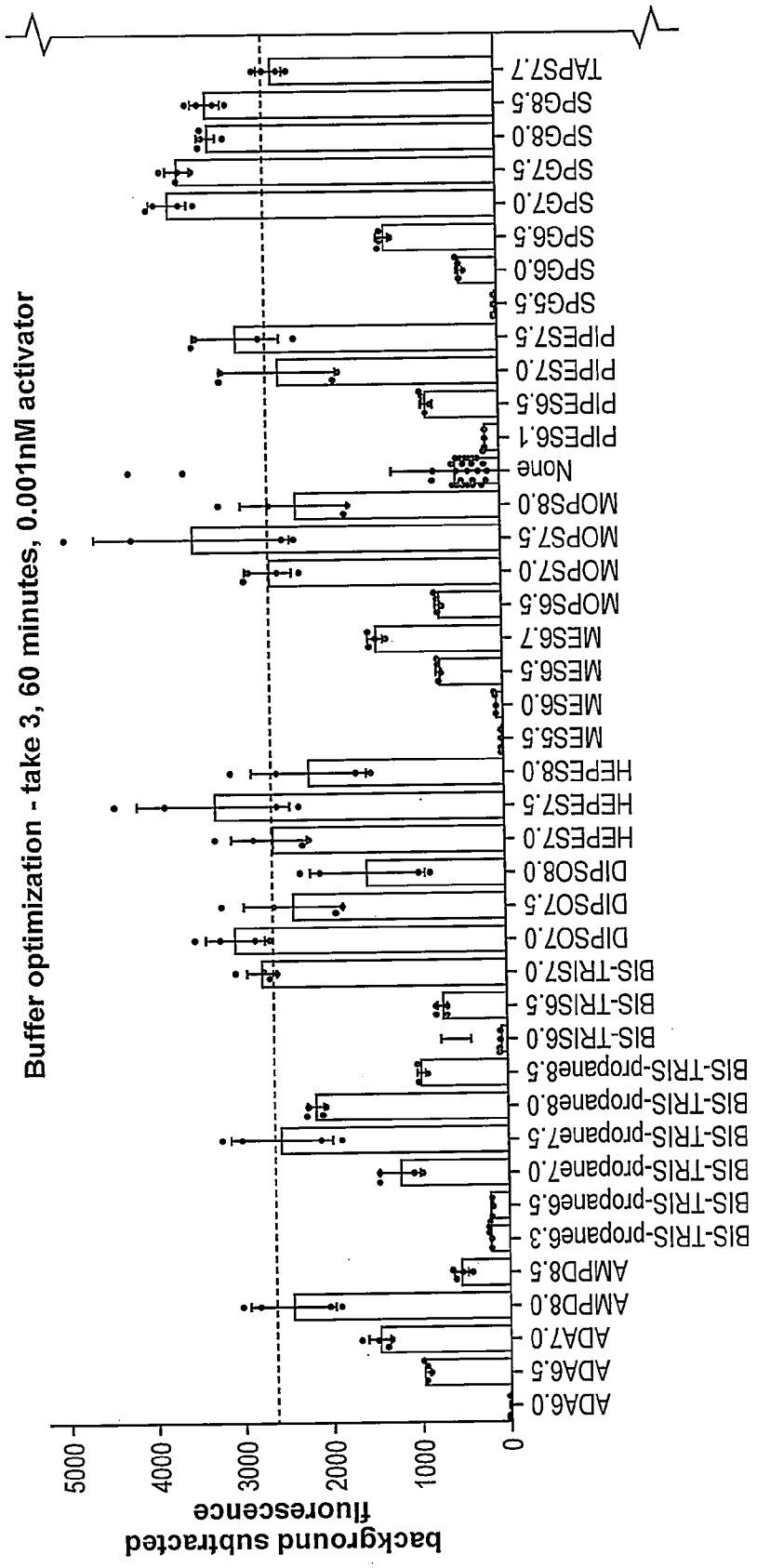

Buffer optimization - take 3, 60 minutes, 0.001nM activator
background subtracted fluorescence
0
1000
2000
3000
4000
5000
ADA6.0
ADA6.5
ADA7.0
AMPD8.0
AMPD8.5
BIS-TRIS-propane6.3
BIS-TRIS-propane6.5
BIS-TRIS-propane7.0
BIS-TRIS-propane7.5
BIS-TRIS-propane8.0
BIS-TRIS-propane8.5
BIS-TRIS6.0
BIS-TRIS6.5
BIS-TRIS7.0
DIPSO7.0
DIPSO7.5
DIPSO8.0
HEPES7.0
HEPES7.5
HEPES8.0
MES5.5
MES6.0
MES6.5
MES6.7
MOPS6.5
MOPS7.0
MOPS7.5
MOPS8.0
None
PIPES6.1
PIPES6.5
PIPES7.0
PIPES7.5
SPG5.5
SPG6.0
SPG6.5
SPG7.0
SPG7.5
SPG8.0
SPG8.5
TAPS7.7

FIG. 26

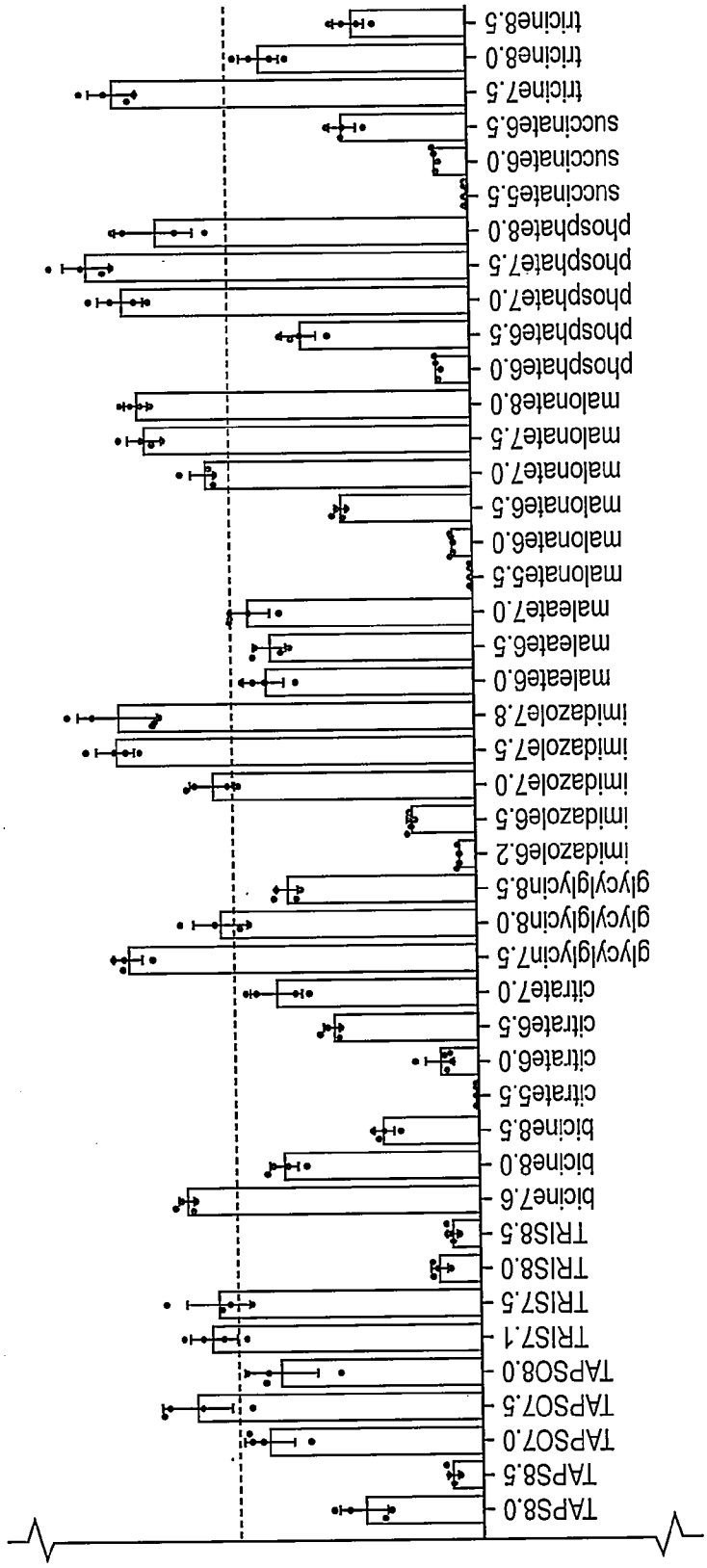

TAPS8.0
TAPS8.5
TAPSO7.0
TAPSO7.5
TAPSO8.0
TRIS7.1
TRIS7.5
TRIS8.0
TRIS8.5
bicine7.6
bicine8.0
bicine8.5
citrate5.5
citrate6.0
citrate6.5
citrate7.0
glycylglycin7.5
glycylglycine8.0
glycylglycine8.5
imidazole6.2
imidazole6.5
imidazole7.0
imidazole7.5
imidazole7.8
maleate6.0
maleate6.5
maleate7.0
malonate5.5
malonate6.0
malonate6.5
malonate7.0
malonate7.5
malonate8.0
phosphate6.0
phosphate6.5
phosphate7.0
phosphate7.5
phosphate8.0
succinate5.5
succinate6.0
succinate6.5
tricine7.5
tricine8.0
tricine8.5

FIG. 26 (Cont.)

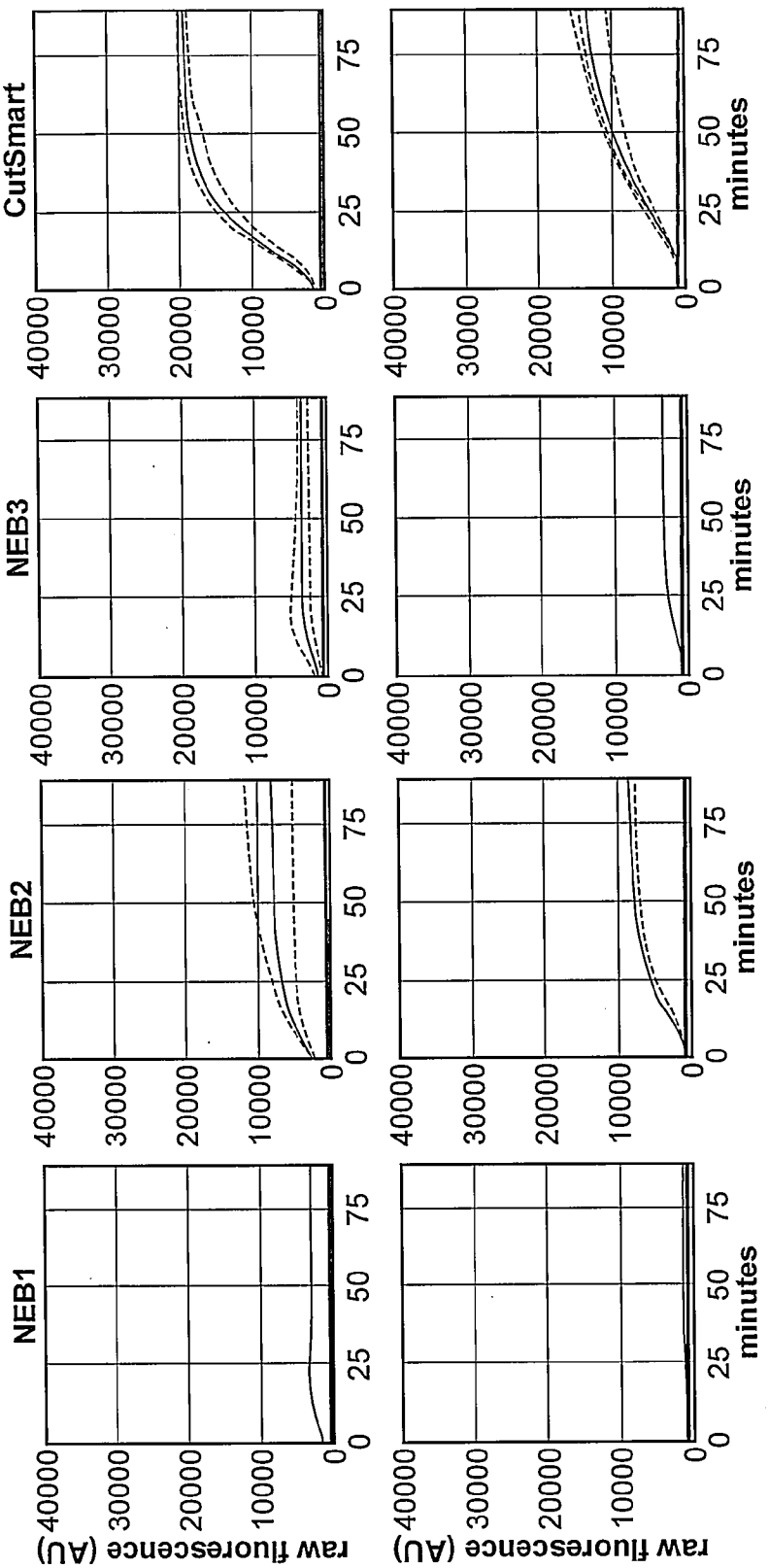

NEB1
NEB2
NEB3
CutSmart
raw fluorescence (AU)
minutes
0
10000
20000
30000
40000
0
25
50
75

FIG. 27

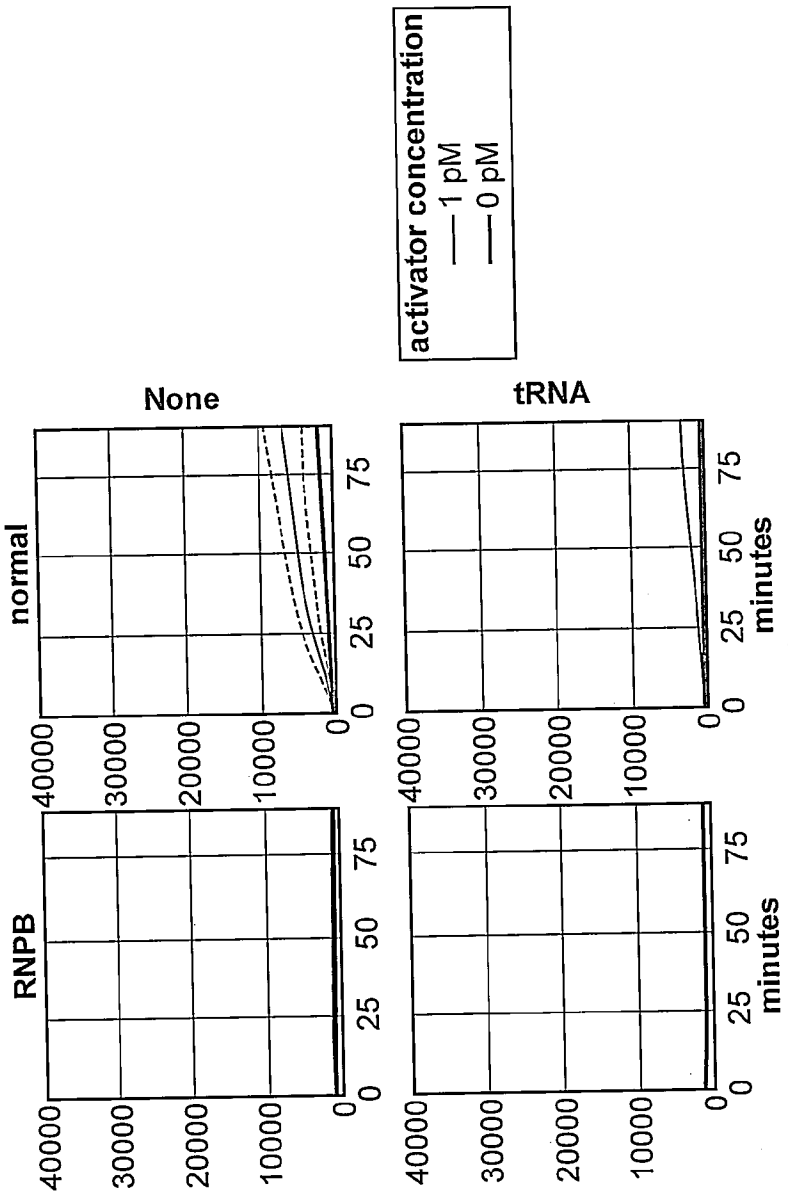

activator concentration
1 pM
0 pM
None
tRNA
normal
RNPB
40000
30000
20000
10000
0
0
25
50
75
minutes

FIG. 27 (Cont.)

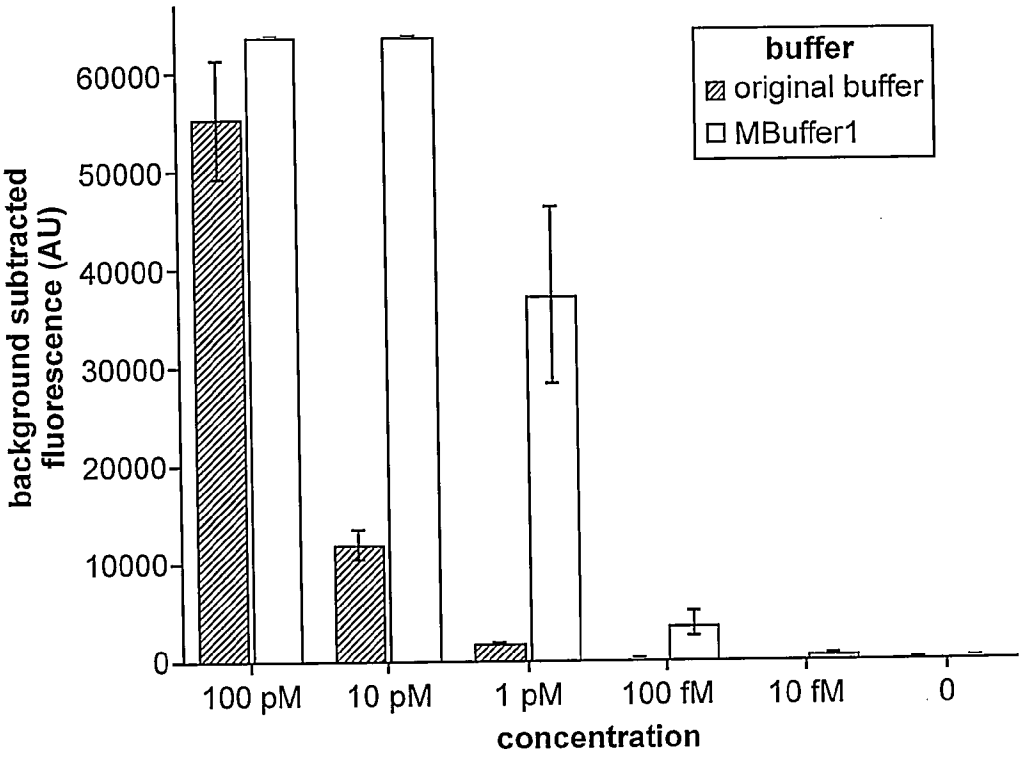

buffer
original buffer
MBuffer1
background subtracted fluorescence (AU)
60000
50000
40000
30000
20000
10000
0
100 pM
10 pM
1 pM
100 fM
10 fM
0
concentration

FIG. 28

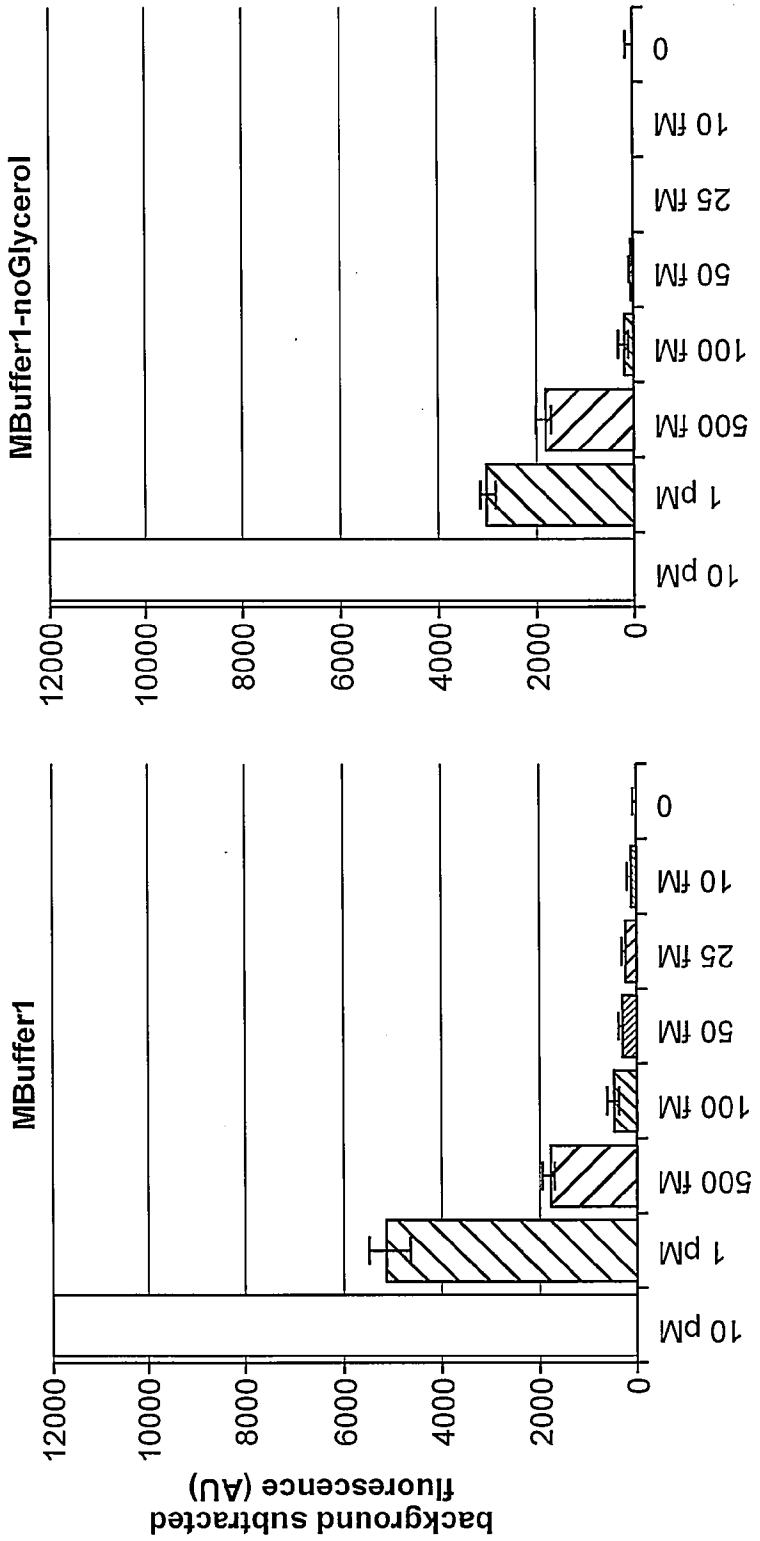

MBuffer1
MBuffer1-noGlycerol
background subtracted fluorescence (AU)
12000
10000
8000
6000
4000
2000
0
10 pM
1 pM
500 fM
100 fM
50 fM
25 fM
10 fM
0

FIG. 29

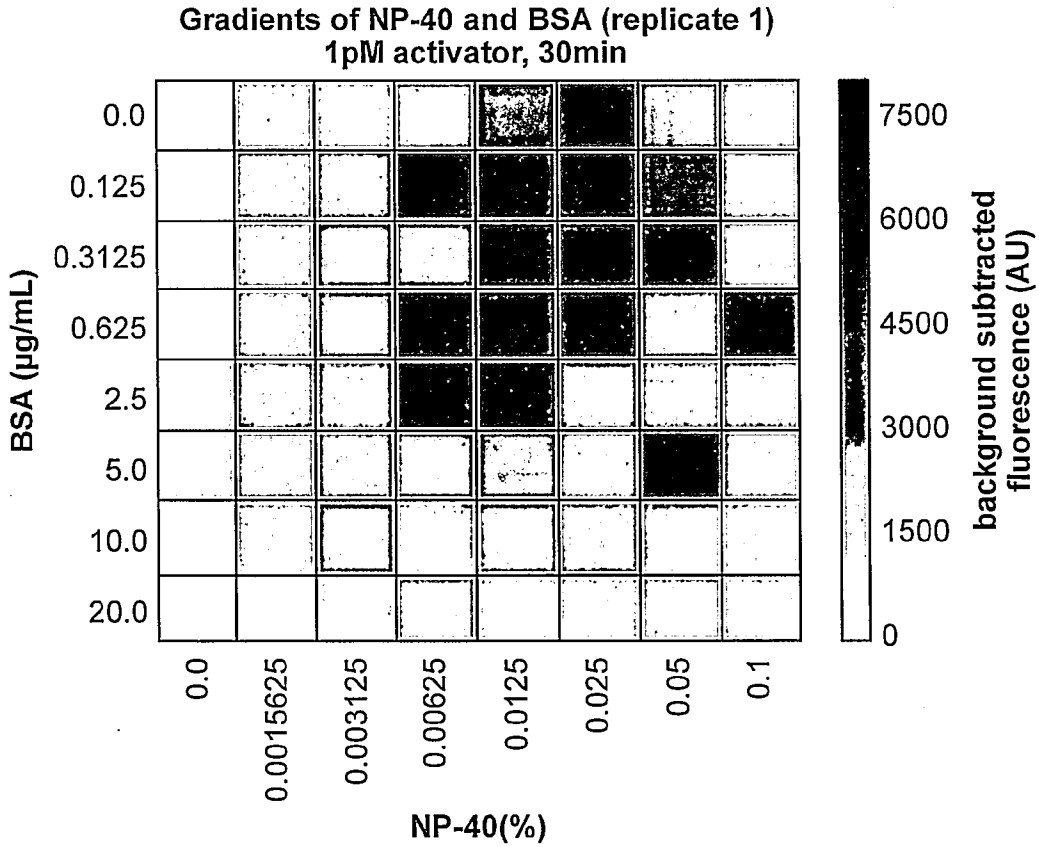

Gradients of NP-40 and BSA (replicate 1)
1pM activator, 30min
BSA (µg/mL)
0.0
0.125
0.3125
0.625
2.5
5.0
10.0
20.0
0.0
0.0015625
0.003125
0.00625
0.0125
0.025
0.05
0.1
NP-40(%)
7500
6000
4500
3000
1500
0
background subtracted fluorescence (AU)

FIG. 30

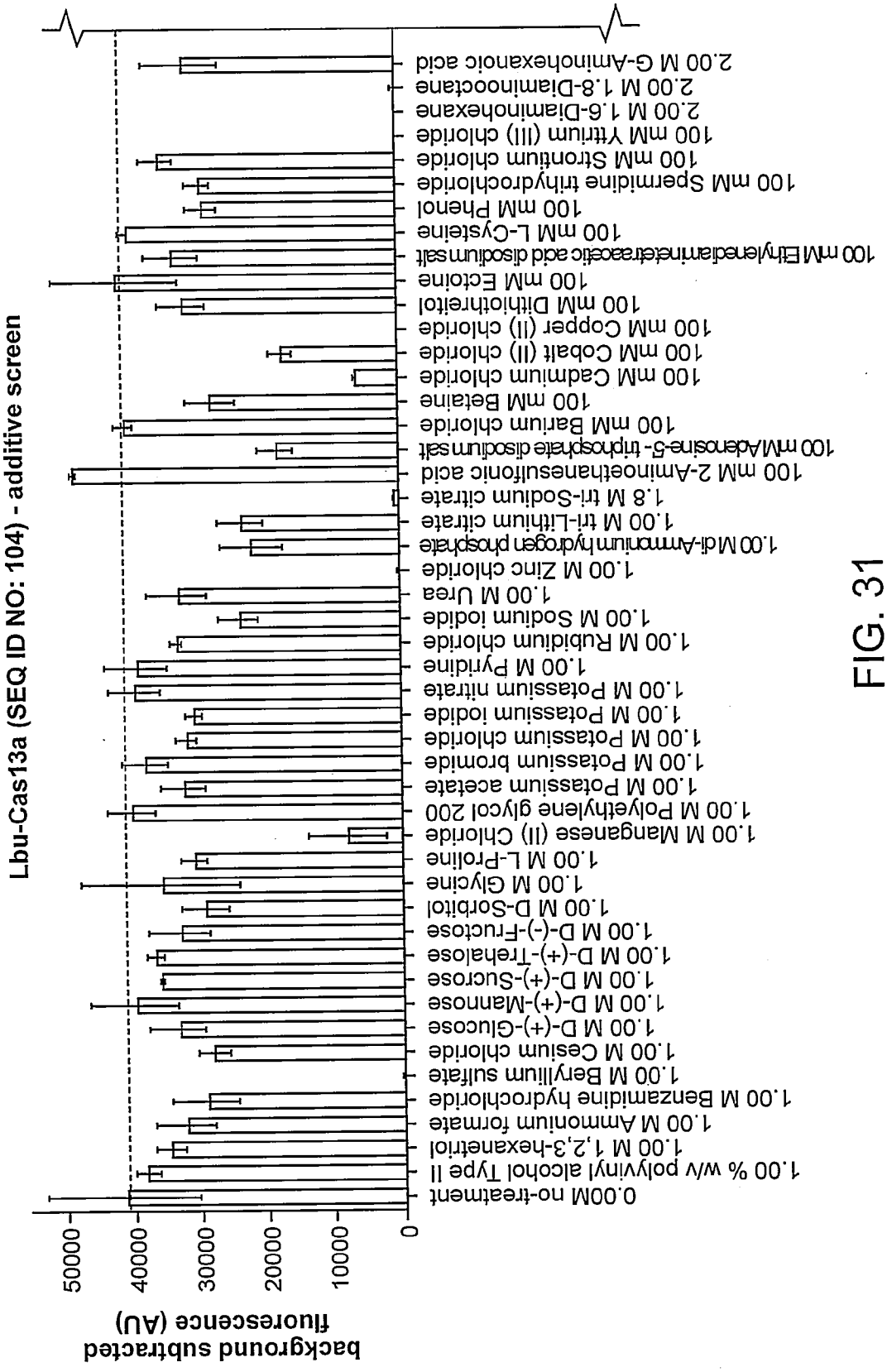

Lbu-Cas13a (SEQ ID NO: 104) - additive screen
background subtracted fluorescence (AU)
50000
40000
30000
20000
10000
0
0.00M no-treatment
1.00 % w/v polyvinyl alcohol Type II
1.00 M 1,2,3-hexanetriol
1.00 M Ammonium formate
1.00 M Benzamidine hydrochloride
1.00 M Beryllium sulfate
1.00 M Cesium chloride
1.00 M D-(+)-Glucose
1.00 M D-(+)-Mannose
1.00 M D-(+)-Sucrose
1.00 M D-(+)-Trehalose
1.00 M D-(-)-Fructose
1.00 M D-Sorbitol
1.00 M Glycine
1.00 M L-Proline
1.00 M Manganese (II) Chloride
1.00 M Polyethylene glycol 200
1.00 M Potassium acetate
1.00 M Potassium bromide
1.00 M Potassium chloride
1.00 M Potassium iodide
1.00 M Potassium nitrate
1.00 M Pyridine
1.00 M Rubidium chloride
1.00 M Sodium iodide
1.00 M Urea
1.00 M Zinc chloride
1.00 M di-Ammonium hydrogen phosphate
1.00 M tri-Lithium citrate
1.8 M tri-Sodium citrate
100 mM 2-Aminoethanesulfonic acid
100 mM Adenosine-5- triphosphate disodium salt
100 mM Barium chloride
100 mM Betaine
100 mM Cadmium chloride
100 mM Cobalt (II) chloride
100 mM Copper (II) chloride
100 mM Dithiothreitol
100 mM Ectoine
100 mM Ethylenediaminetetraacetic acid disodium salt
100 mM L-Cysteine
100 mM Phenol
100 mM Spermidine trihydrochloride
100 mM Strontium chloride
100 mM Yttrium (III) chloride
2.00 M 1.6-Diaminohexane
2.00 M 1.8-Diaminooctane
2.00 M G-Aminohexanoic acid

FIG. 31

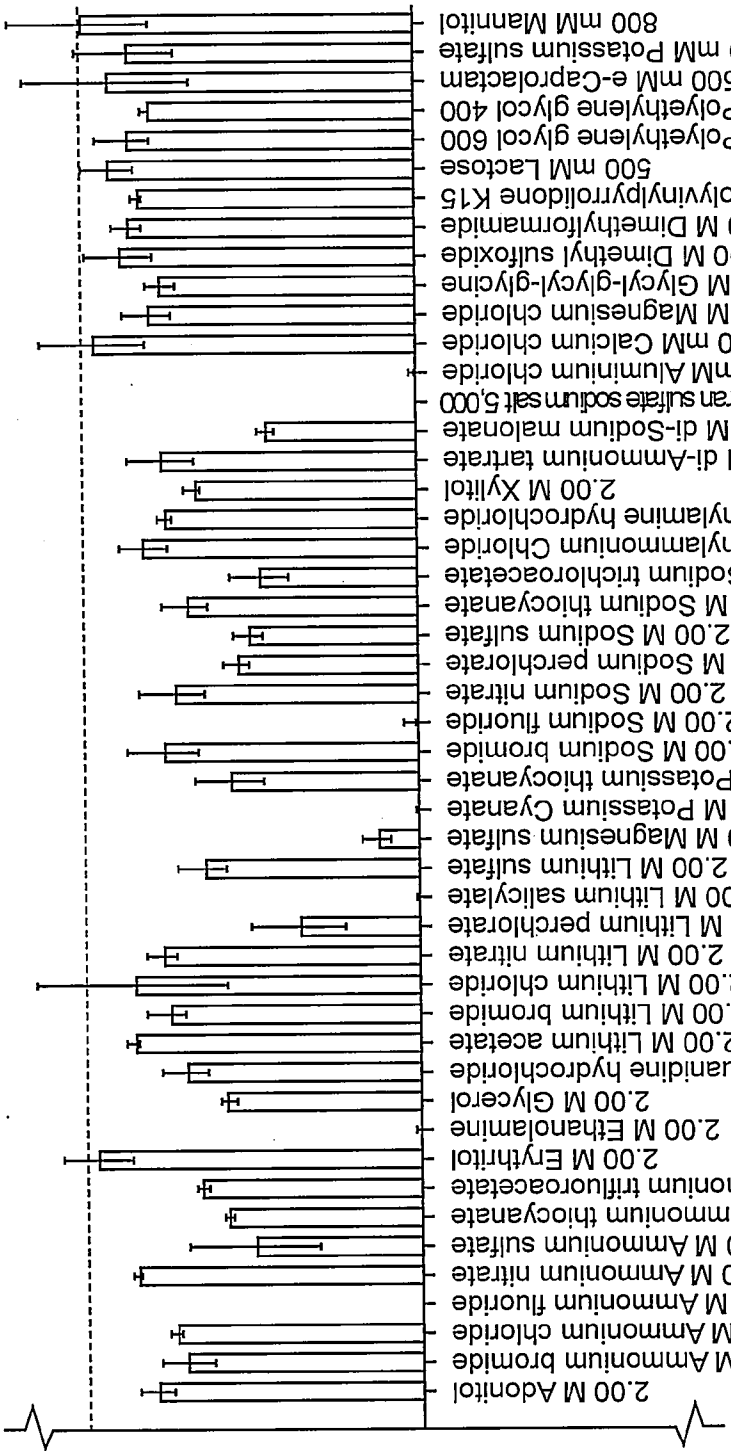

2.00 M Adonitol
2.00 M Ammonium bromide
2.00 M Ammonium chloride
2.00 M Ammonium fluoride
2.00 M Ammonium nitrate
2.00 M Ammonium sulfate
2.00 M Ammonium thiocyanate
2.00 M Ammonium trifluoroacetate
2.00 M Erythritol
2.00 M Ethanolamine
2.00 M Glycerol
2.00 M Guanidine hydrochloride
2.00 M Lithium acetate
2.00 M Lithium bromide
2.00 M Lithium chloride
2.00 M Lithium nitrate
2.00 M Lithium perchlorate
2.00 M Lithium salicylate
2.00 M Lithium sulfate
2.00 M Magnesium sulfate
2.00 M Potassium Cyanate
2.00 M Potassium thiocyanate
2.00 M Sodium bromide
2.00 M Sodium fluoride
2.00 M Sodium nitrate
2.00 M Sodium perchlorate
2.00 M Sodium sulfate
2.00 M Sodium thiocyanate
2.00 M Sodium trichloroacetate
2.00 M Tetramethylammonium Chloride
2.00 M Trimethylamine hydrochloride
2.00 M Xylitol
2.00 M di-Ammonium tartrate
2.00 M di-Sodium malonate
20.00 mM Dextran sulfate sodium salt 5,000
200 mM Aluminium chloride
200 mM Calcium chloride
200 mM Magnesium chloride
300 mM Glycyl-glycyl-glycine
4.00 M Dimethyl sulfoxide
4.00 M Dimethylformamide
5.00 % w/v Polyvinylpyrrolidone K15
500 mM Lactose
500 mM Polyethylene glycol 600
500 mM Polyethylene glycol 400
500 mM e-Caprolactam
600 mM Potassium sulfate
800 mM Mannitol

FIG. 31 (Cont.)

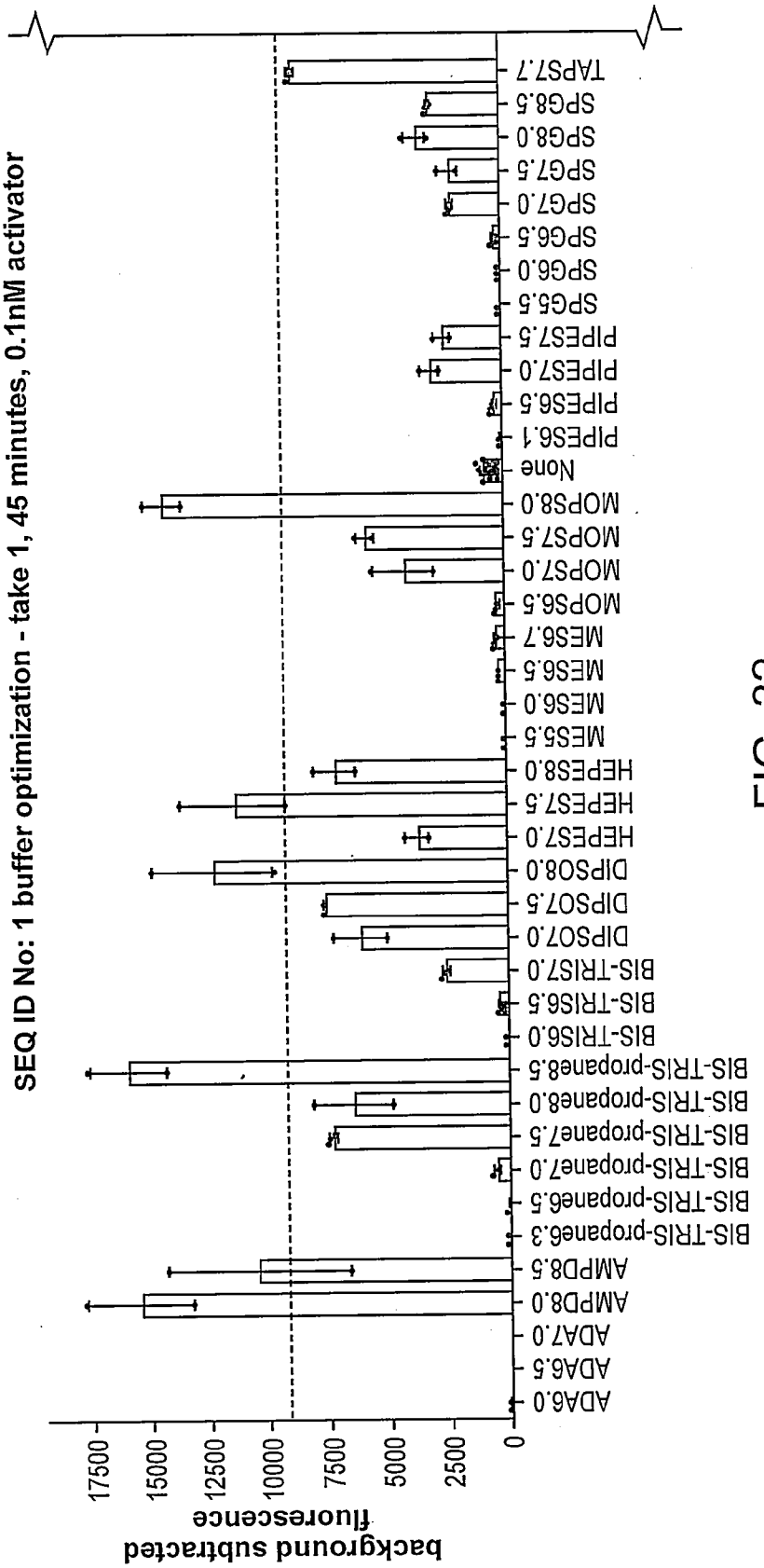

SEQ ID No: 1 buffer optimization - take 1, 45 minutes, 0.1nM activator
background subtracted fluorescence
0
2500
5000
7500
10000
12500
15000
17500
ADA6.0
ADA6.5
ADA7.0
AMPD8.0
AMPD8.5
BIS-TRIS-propane6.3
BIS-TRIS-propane6.5
BIS-TRIS-propane7.0
BIS-TRIS-propane7.5
BIS-TRIS-propane8.0
BIS-TRIS-propane8.5
BIS-TRIS6.0
BIS-TRIS6.5
BIS-TRIS7.0
DIPSO7.0
DIPSO7.5
DIPSO8.0
HEPES7.0
HEPES7.5
HEPES8.0
MES5.5
MES6.0
MES6.5
MES6.7
MOPS6.5
MOPS7.0
MOPS7.5
MOPS8.0
None
PIPES6.1
PIPES6.5
PIPES7.0
PIPES7.5
SPG5.5
SPG6.0
SPG6.5
SPG7.0
SPG7.5
SPG8.0
SPG8.5
TAPS7.7

FIG. 32

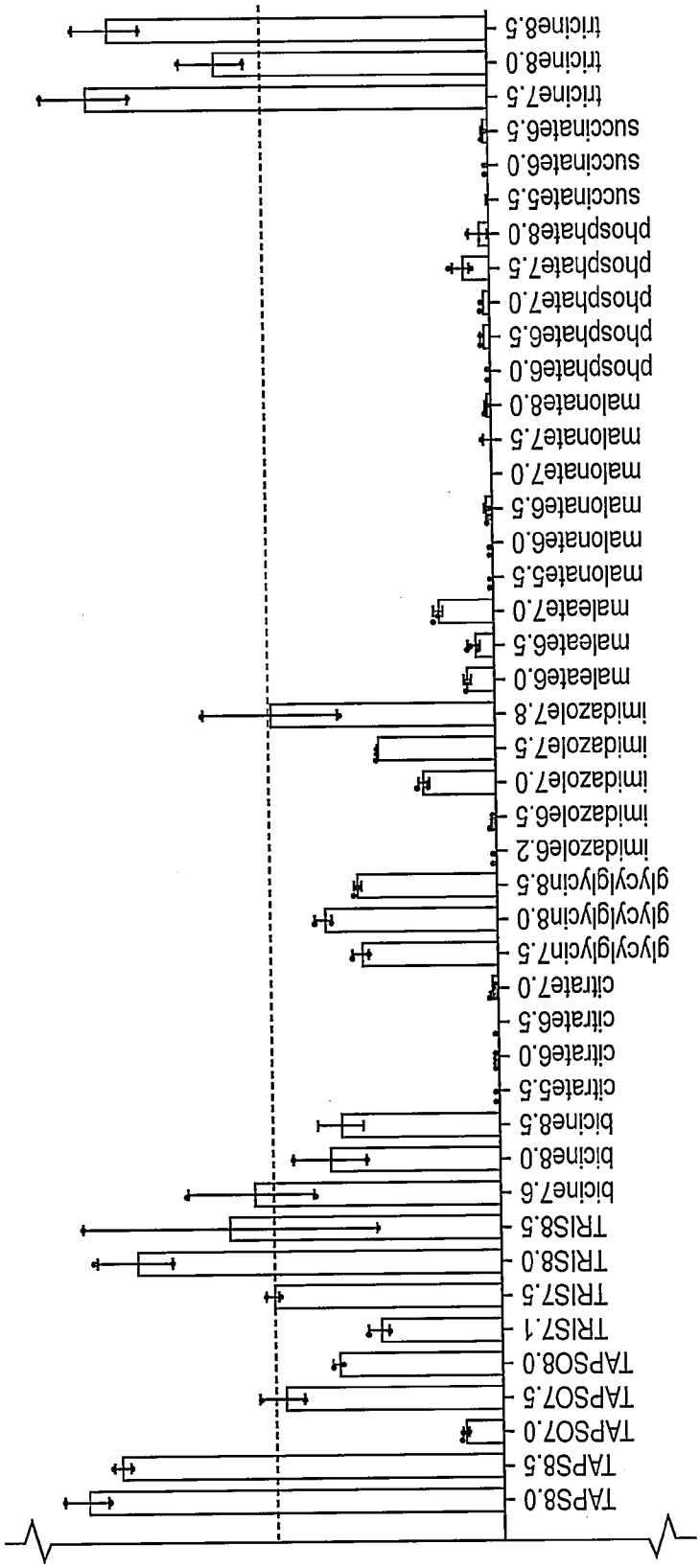

TAPS8.0
TAPS8.5
TAPSO7.0
TAPSO7.5
TAPSO8.0
TRIS7.1
TRIS7.5
TRIS8.0
TRIS8.5
bicine7.5
bicine8.0
bicine8.5
citrate5.5
citrate6.0
citrate6.5
citrate7.0
glycylglycin7.5
glycylglycin8.0
glycylglycin8.5
imidazole6.2
imidazole6.5
imidazole7.0
imidazole7.5
imidazole7.8
maleate6.0
maleate6.5
maleate7.0
malonate5.5
malonate6.0
malonate6.5
malonate7.0
malonate7.5
malonate8.0
phosphate6.0
phosphate6.5
phosphate7.0
phosphate7.5
phosphate8.0
succinate5.5
succinate6.0
succinate6.5
tricine7.5
tricine8.0
tricine8.5

FIG. 32 (Cont.)

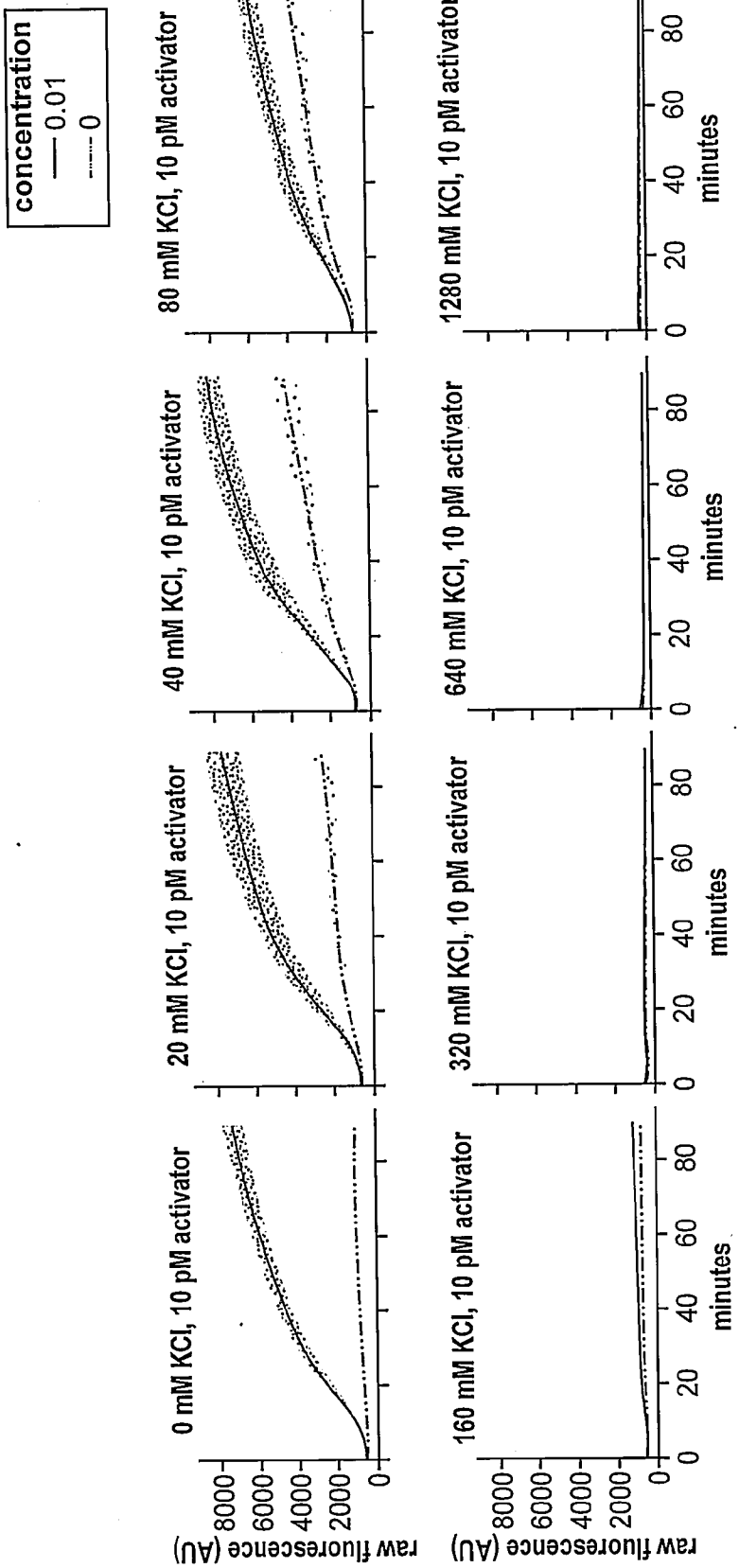

concentration
0.01
0
0 mM KCl, 10 pM activator
20 mM KCl, 10 pM activator
40 mM KCl, 10 pM activator
80 mM KCl, 10 pM activator
160 mM KCl, 10 pM activator
320 mM KCl, 10 pM activator
640 mM KCl, 10 pM activator
1280 mM KCl, 10 pM activator
raw fluorescence (AU)
minutes
0
2000
4000
6000
8000
0
20
40
60
80

FIG. 33

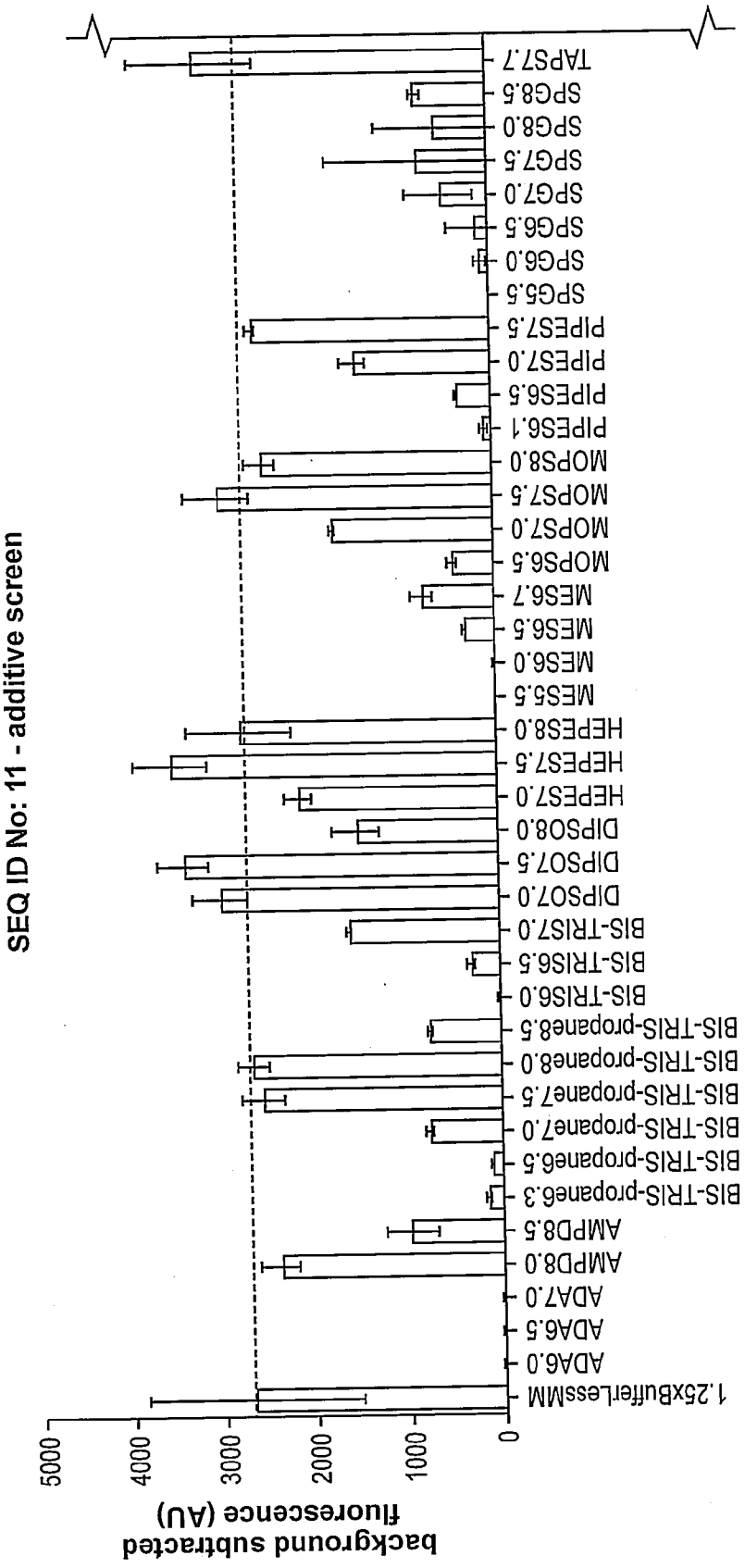

SEQ ID No: 11 - additive screen
background subtracted fluorescence (AU)
5000
4000
3000
2000
1000
0
1.25xBufferLessMM
ADA6.0
ADA6.5
ADA7.0
AMPD8.0
AMPD8.5
BIS-TRIS-propane6.3
BIS-TRIS-propane6.5
BIS-TRIS-propane7.0
BIS-TRIS-propane7.5
BIS-TRIS-propane8.0
BIS-TRIS-propane8.5
BIS-TRIS6.0
BIS-TRIS6.5
BIS-TRIS7.0
DIPSO7.0
DIPSO7.5
DIPSO8.0
HEPES7.0
HEPES7.5
HEPES8.0
MES5.5
MES6.0
MES6.5
MES6.7
MOPS6.5
MOPS7.0
MOPS7.5
MOPS8.0
PIPES6.1
PIPES6.5
PIPES7.0
PIPES7.5
SPG5.5
SPG6.0
SPG6.5
SPG7.0
SPG7.5
SPG8.0
SPG8.5
TAPS7.7

FIG. 34

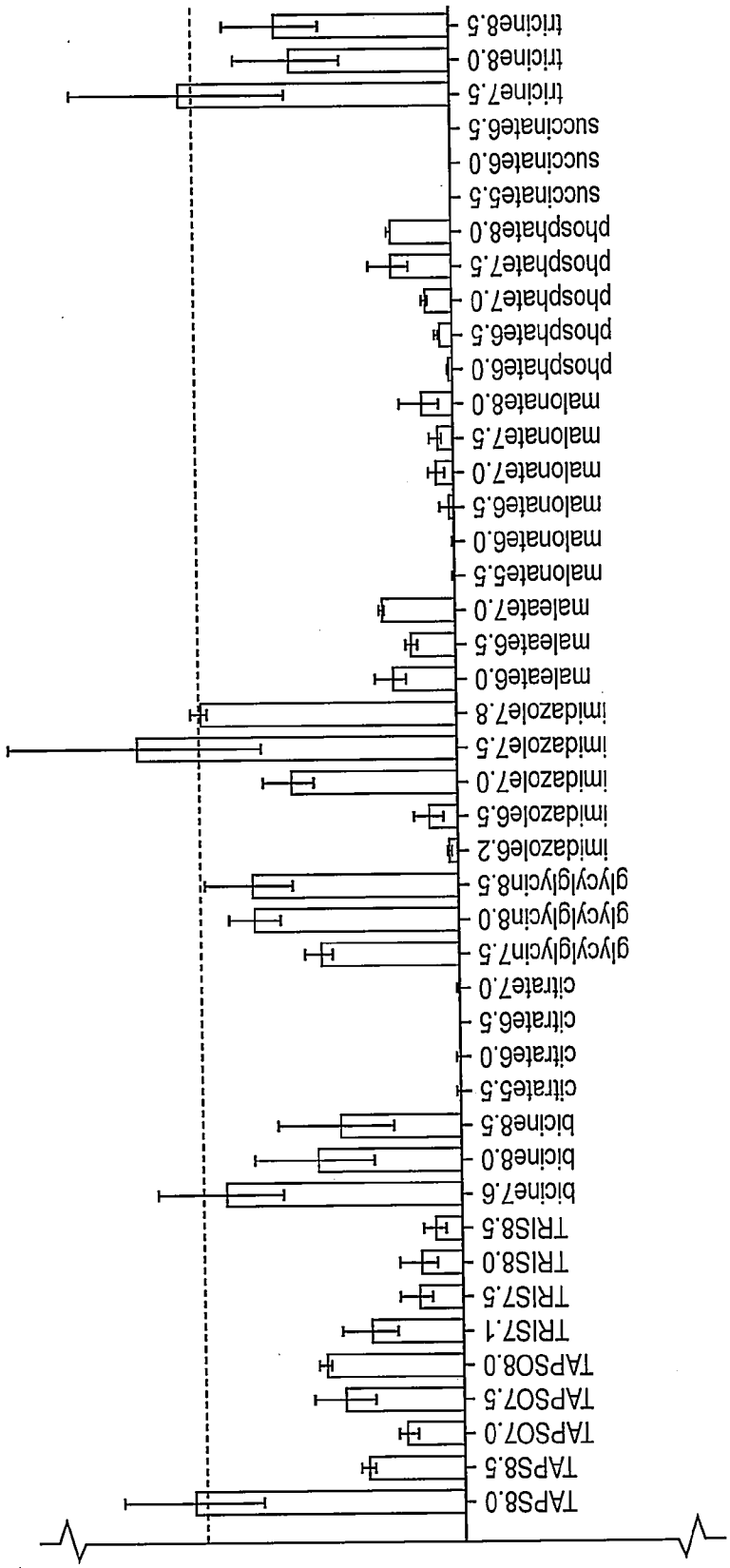

TAPS8.0
TAPS8.5
TAPSO7.0
TAPSO7.5
TAPSO8.0
TRIS7.1
TRIS7.5
TRIS8.0
TRIS8.5
bicine7.6
bicine8.0
bicine8.5
citrate5.5
citrate6.0
citrate6.5
citrate7.0
glycylglycin7.5
glycylglycin8.0
glycylglycin8.5
imidazole6.2
imidazole6.5
imidazole7.0
imidazole7.5
imidazole7.8
maleate6.0
maleate6.5
maleate7.0
malonate5.5
malonate6.0
malonate6.5
malonate7.0
malonate7.5
malonate8.0
phosphate6.0
phosphate6.5
phosphate7.0
phosphate7.5
phosphate8.0
succinate5.5
succinate6.0
succinate6.5
tricine7.5
tricine8.0
tricine8.5

FIG. 34 (Cont.)

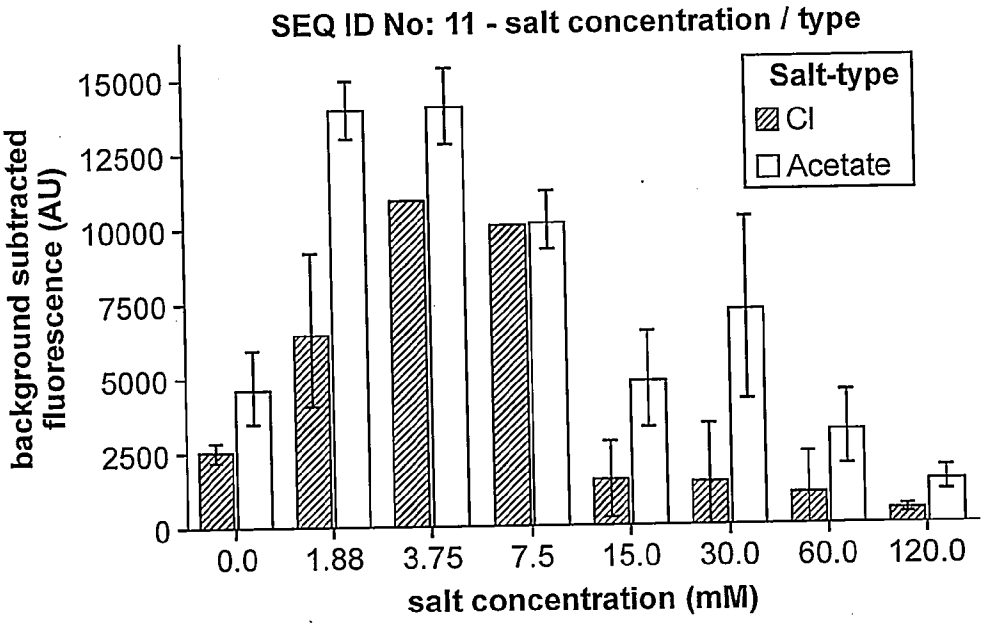

SEQ ID No: 11 - salt concentration / type
Salt-type
Cl
Acetate
background subtracted fluorescence (AU)
15000
12500
10000
7500
5000
2500
0
0.0
1.88
3.75
7.5
15.0
30.0
60.0
120.0
salt concentration (mM)

FIG. 35

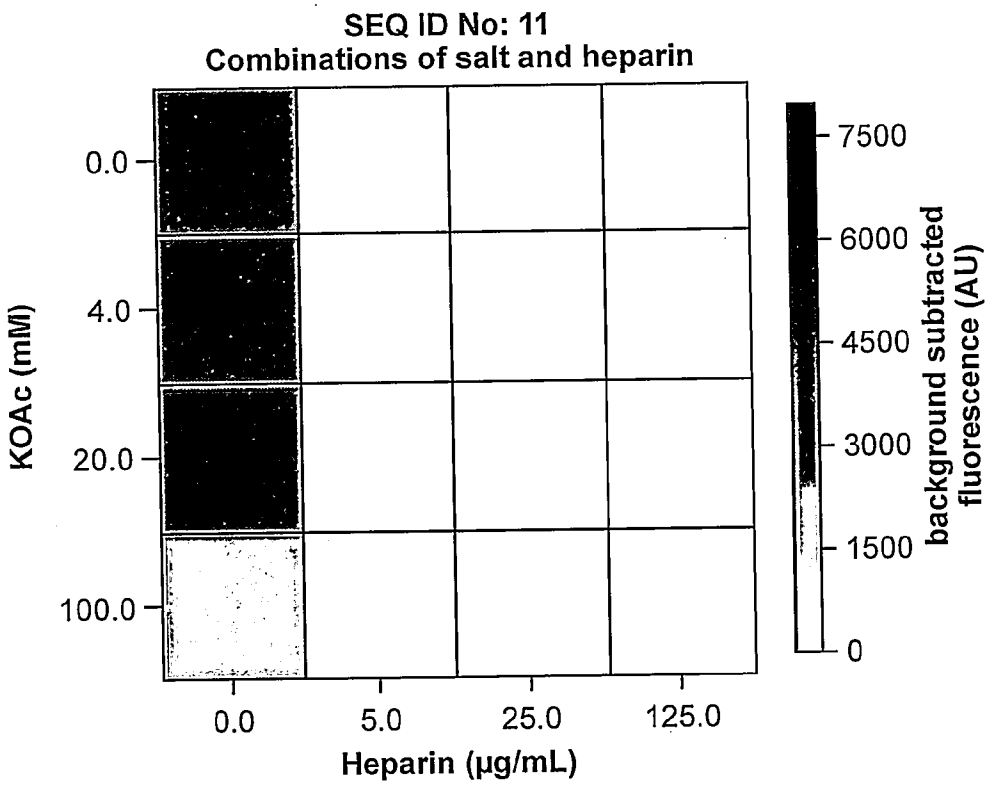

SEQ ID No: 11
Combinations of salt and heparin
KOAc (mM)
0.0
4.0
20.0
100.0
0.0
5.0
25.0
125.0
Heparin (µg/mL)
7500
6000
4500
3000
1500
0
background subtracted fluorescence (AU)

FIG. 36

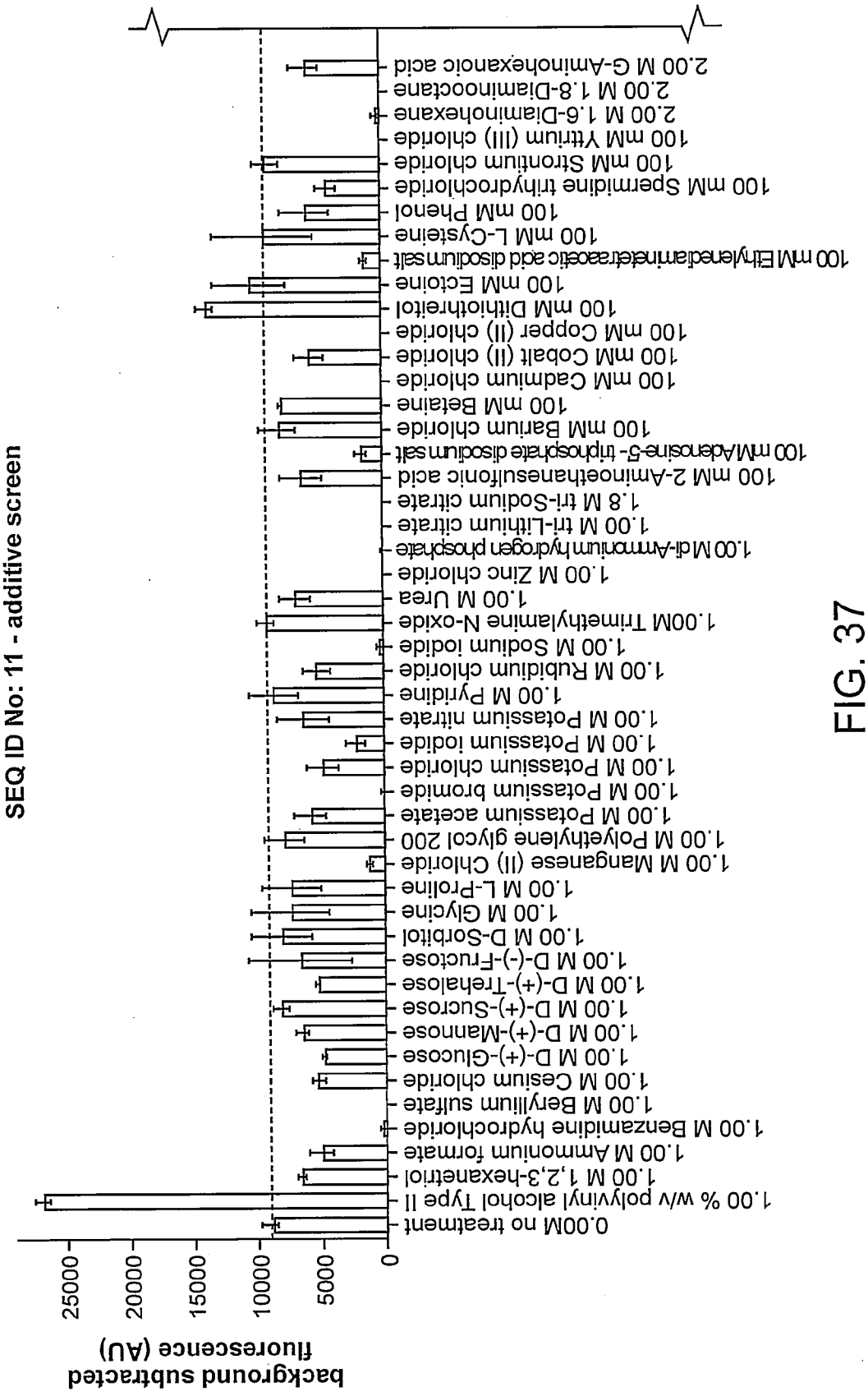

SEQ ID No: 11 - additive screen
background subtracted fluorescence (AU)
25000
20000
15000
10000
5000
0
0.00M no treatment
1.00 % w/v polyvinyl alcohol Type II
1.00 M 1,2,3-hexanetriol
1.00 M Ammonium formate
1.00 M Benzamidine hydrochloride
1.00 M Beryllium sulfate
1.00 M Cesium chloride
1.00 M D-(+)-Glucose
1.00 M D-(+)-Mannose
1.00 M D-(+)-Sucrose
1.00 M D-(+)-Trehalose
1.00 M D-(-)-Fructose
1.00 M D-Sorbitol
1.00 M Glycine
1.00 M L-Proline
1.00 M Manganese (II) Chloride
1.00 M Polyethylene glycol 200
1.00 M Potassium acetate
1.00 M Potassium bromide
1.00 M Potassium chloride
1.00 M Potassium iodide
1.00 M Potassium nitrate
1.00 M Pyridine
1.00 M Rubidium chloride
1.00 M Sodium iodide
1.00M Trimethylamine N-oxide
1.00 M Urea
1.00 M Zinc chloride
1.00 M di-Ammonium hydrogen phosphate
1.00 M tri-Lithium citrate
1.8 M tri-Sodium citrate
100 mM 2-Aminoethanesulfonic acid
100 mM Adenosine-5- triphosphate disodium salt
100 mM Barium chloride
100 mM Betaine
100 mM Cadmium chloride
100 mM Cobalt (II) chloride
100 mM Copper (II) chloride
100 mM Dithiothreitol
100 mM Ectoine
100 mM Ethylenediaminetetraacetic acid disodium salt
100 mM L-Cysteine
100 mM Phenol
100 mM Spermidine trihydrochloride
100 mM Strontium chloride
100 mM Yttrium (III) chloride
2.00 M 1.6-Diaminohexane
2.00 M 1.8-Diaminooctane
2.00 M G-Aminohexanoic acid

FIG. 37

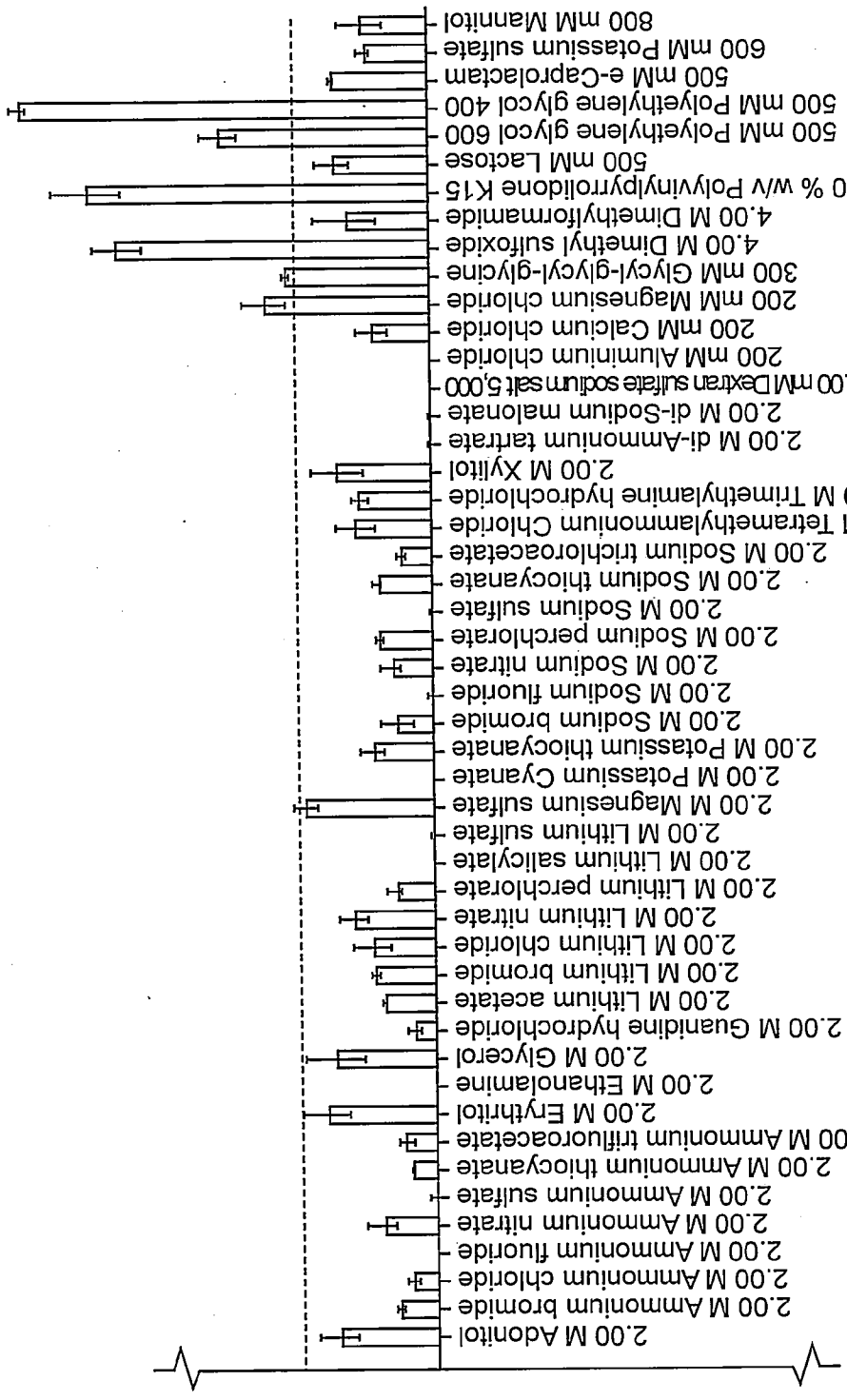

2.00 M Adonitol
2.00 M Ammonium bromide
2.00 M Ammonium chloride
2.00 M Ammonium fluoride
2.00 M Ammonium nitrate
2.00 M Ammonium sulfate
2.00 M Ammonium thiocyanate
2.00 M Ammonium trifluoroacetate
2.00 M Erythritol
2.00 M Ethanolamine
2.00 M Glycerol
2.00 M Guanidine hydrochloride
2.00 M Lithium acetate
2.00 M Lithium bromide
2.00 M Lithium chloride
2.00 M Lithium nitrate
2.00 M Lithium perchlorate
2.00 M Lithium salicylate
2.00 M Lithium sulfate
2.00 M Magnesium sulfate
2.00 M Potassium Cyanate
2.00 M Potassium thiocyanate
2.00 M Sodium bromide
2.00 M Sodium fluoride
2.00 M Sodium nitrate
2.00 M Sodium perchlorate
2.00 M Sodium sulfate
2.00 M Sodium thiocyanate
2.00 M Sodium trichloroacetate
2.00 M Tetramethylammonium Chloride
2.00 M Trimethylamine hydrochloride
2.00 M Xylitol
2.00 M di-Ammonium tartrate
2.00 M di-Sodium malonate
20.00 mM Dextran sulfate sodium salt 5,000
200 mM Aluminium chloride
200 mM Calcium chloride
200 mM Magnesium chloride
300 mM Glycyl-glycyl-glycine
4.00 M Dimethyl sulfoxide
4.00 M Dimethylformamide
5.00 % w/v Polyvinylpyrrolidone K15
500 mM Lactose
500 mM Polyethylene glycol 600
500 mM Polyethylene glycol 400
500 mM e-Caprolactam
600 mM Potassium sulfate
800 mM Mannitol

FIG. 37 (Cont.)

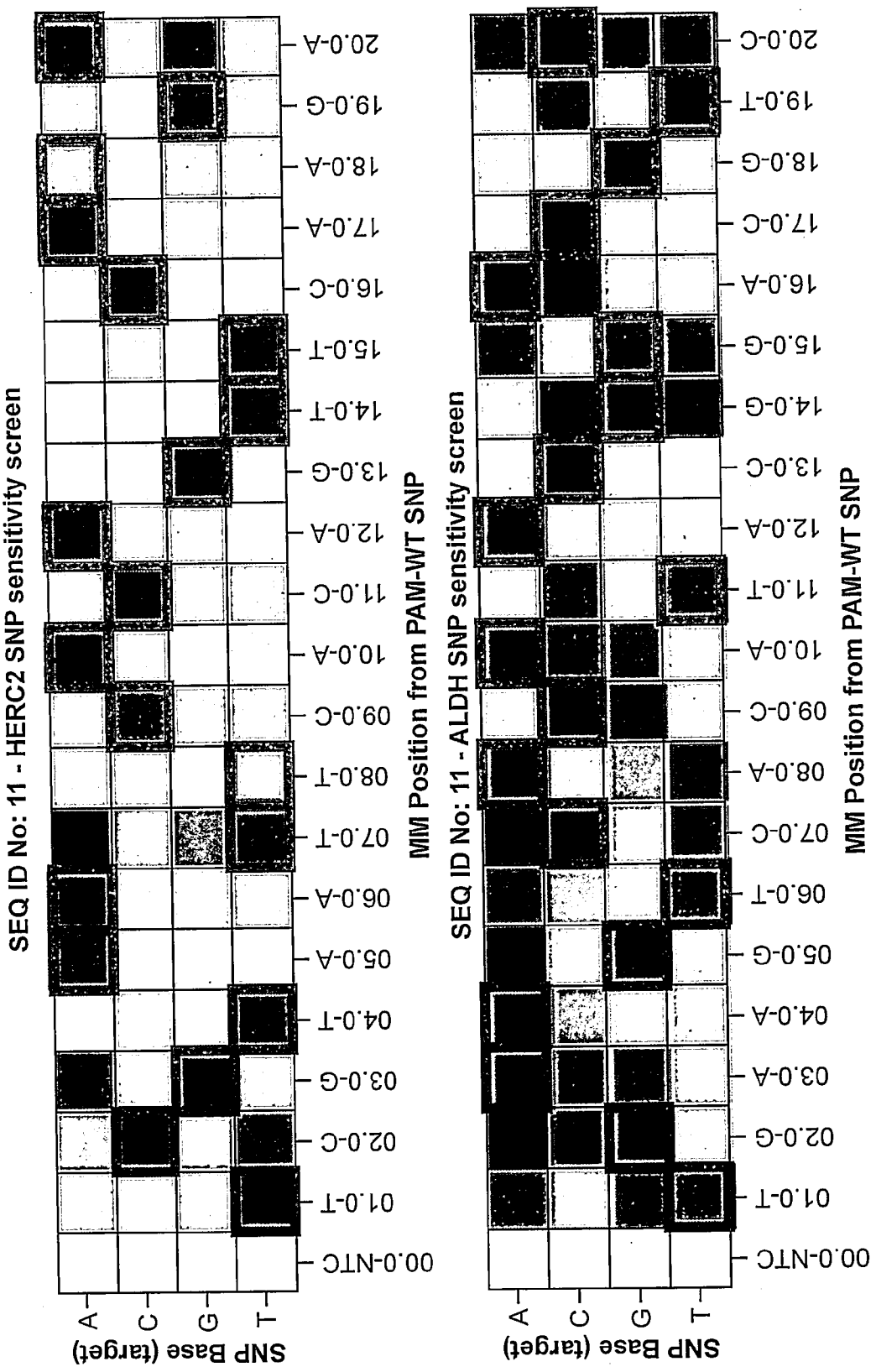

SEQ ID No: 11 - HERC2 SNP sensitivity screen
MM Position from PAM-WT SNP
SNP Base (target)
A
C
G
T
00.0-NTC
01.0-T
02.0-C
03.0-G
04.0-T
05.0-A
06.0-A
07.0-T
08.0-T
09.0-C
10.0-A
11.0-C
12.0-A
13.0-G
14.0-T
15.0-T
16.0-C
17.0-A
18.0-A
19.0-G
20.0-A
SEQ ID No: 11 - ALDH SNP sensitivity screen
MM Position from PAM-WT SNP
SNP Base (target)
A
C
G
T
00.0-NTC
01.0-T
02.0-G
03.0-A
04.0-A
05.0-G
06.0-T
07.0-C
08.0-A
09.0-C
10.0-A
11.0-T
12.0-A
13.0-C
14.0-G
15.0-G
16.0-A
17.0-C
18.0-G
19.0-T
20.0-C

FIG. 38

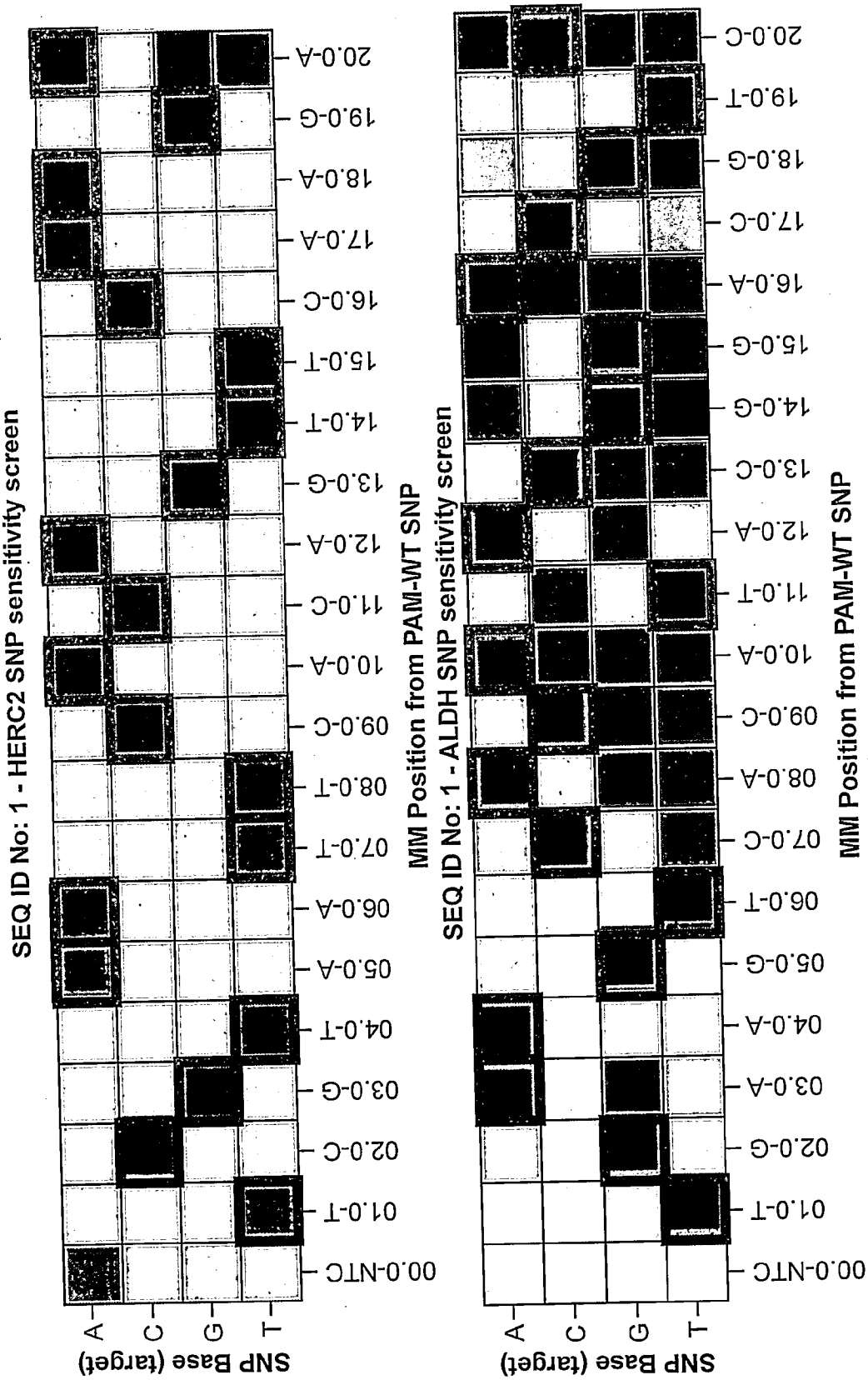

SEQ ID No: 1 - HERC2 SNP sensitivity screen
MM Position from PAM-WT SNP
SNP Base (target)
A
C
G
T
00.0-NTC
01.0-T
02.0-C
03.0-G
04.0-T
05.0-A
06.0-A
07.0-T
08.0-T
09.0-C
10.0-A
11.0-C
12.0-A
13.0-G
14.0-T
15.0-T
16.0-C
17.0-A
18.0-A
19.0-G
20.0-A
SEQ ID No: 1 - ALDH SNP sensitivity screen
MM Position from PAM-WT SNP
SNP Base (target)
A
C
G
T
00.0-NTC
01.0-T
02.0-G
03.0-A
04.0-A
05.0-G
06.0-T
07.0-C
08.0-A
09.0-C
10.0-A
11.0-T
12.0-A
13.0-C
14.0-G
15.0-G
16.0-A
17.0-C
18.0-G
19.0-T
20.0-C

FIG. 39

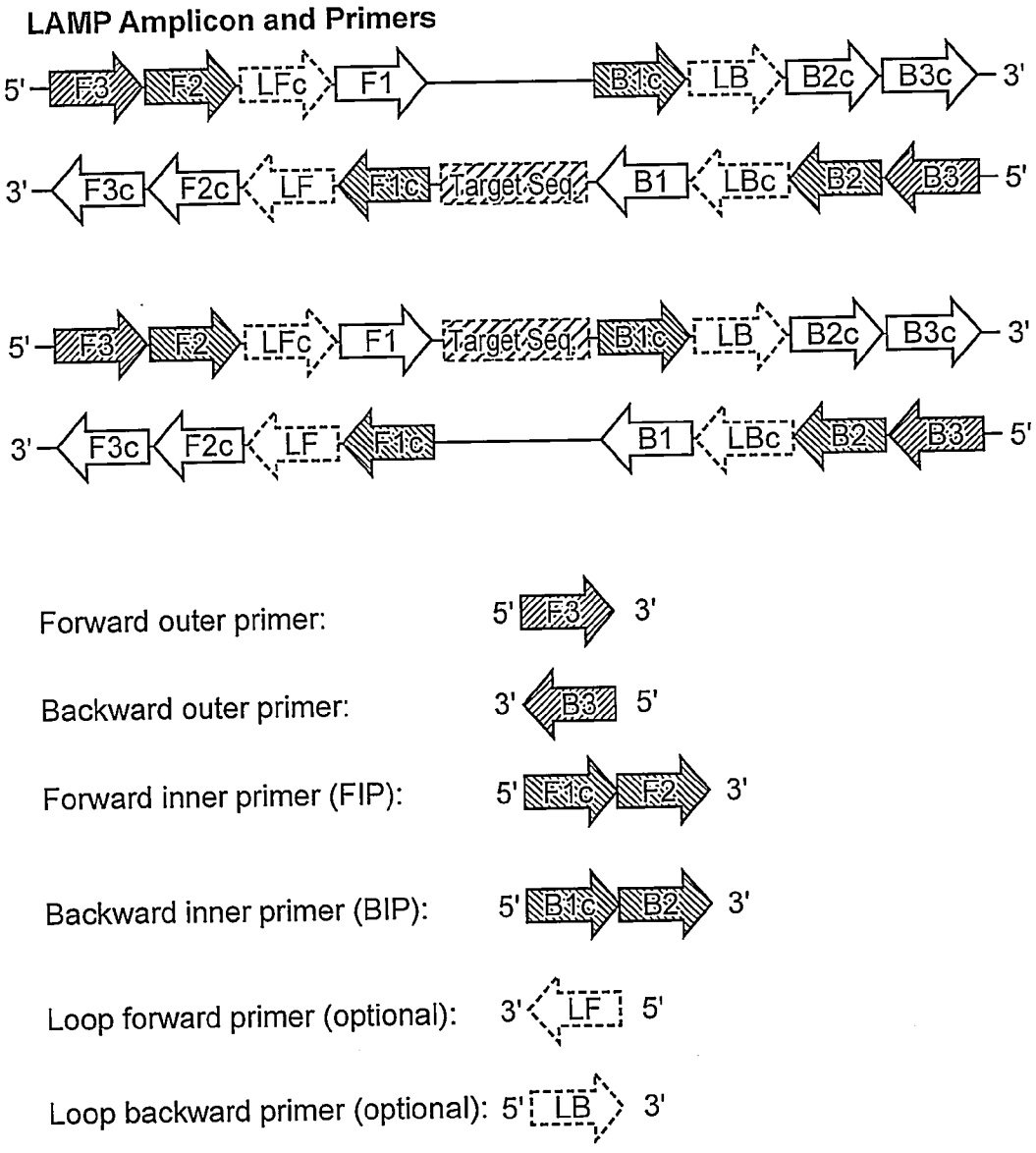

LAMP Amplicon and Primers
5'
F3
F2
LFc
F1
B1c
LB
B2c
B3c
3'
3'
F3c
F2c
LF
F1c
Target Seq
B1
LBc
B2
B3
5'
5'
F3
F2
LFc
F1
Target Seq
B1c
LB
B2c
B3c
3'
3'
F3c
F2c
LF
F1c
B1
LBc
B2
B3
5'
Forward outer primer:
5'
F3
3'
Backward outer primer:
3'
B3
5'
Forward inner primer (FIP):
5'
F1c
F2
3'
Backward inner primer (BIP):
5'
B1c
B2
3'
Loop forward primer (optional):
3'
LF
5'
Loop backward primer (optional):
5'
LB
3'

FIG. 40

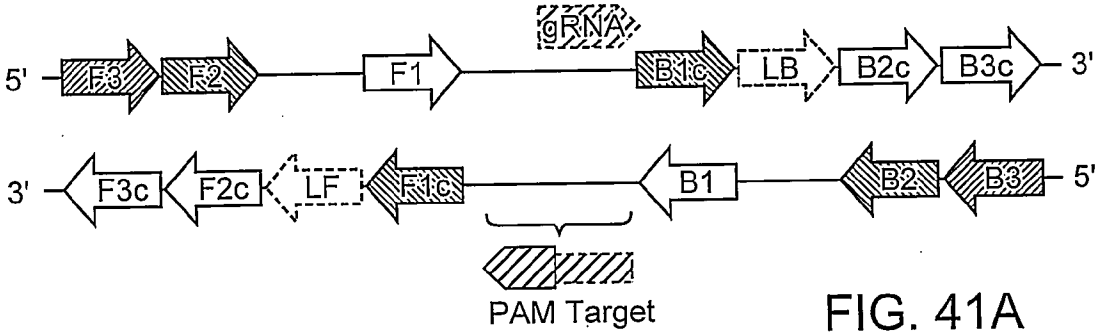


FIG. 41A

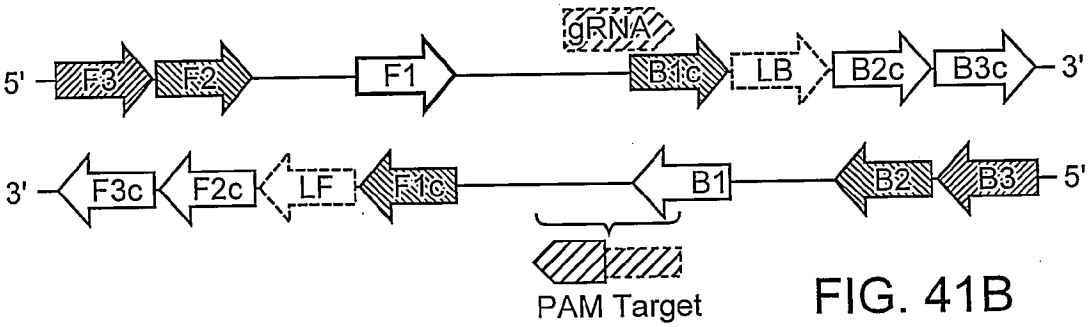


FIG. 41B

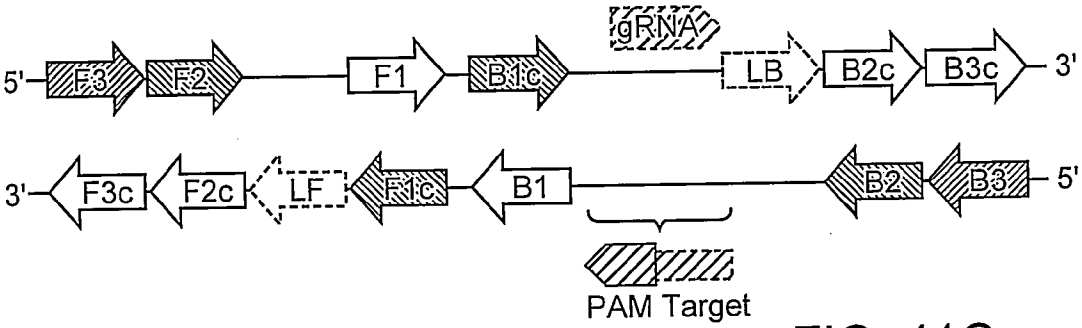


FIG. 41C

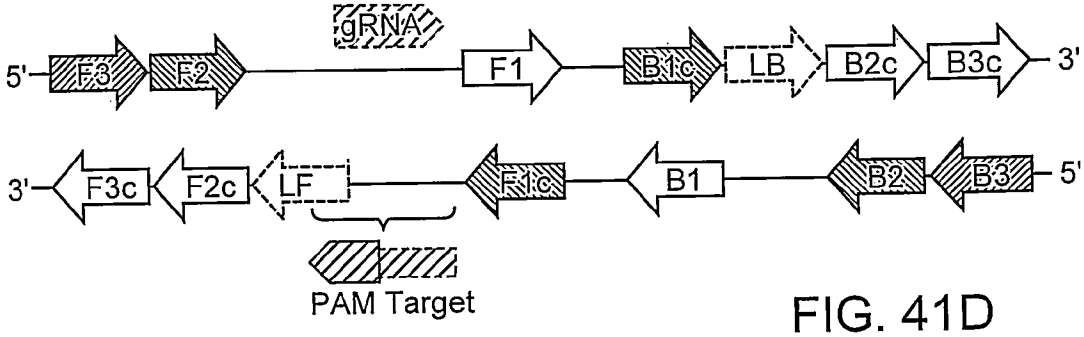


FIG. 41D

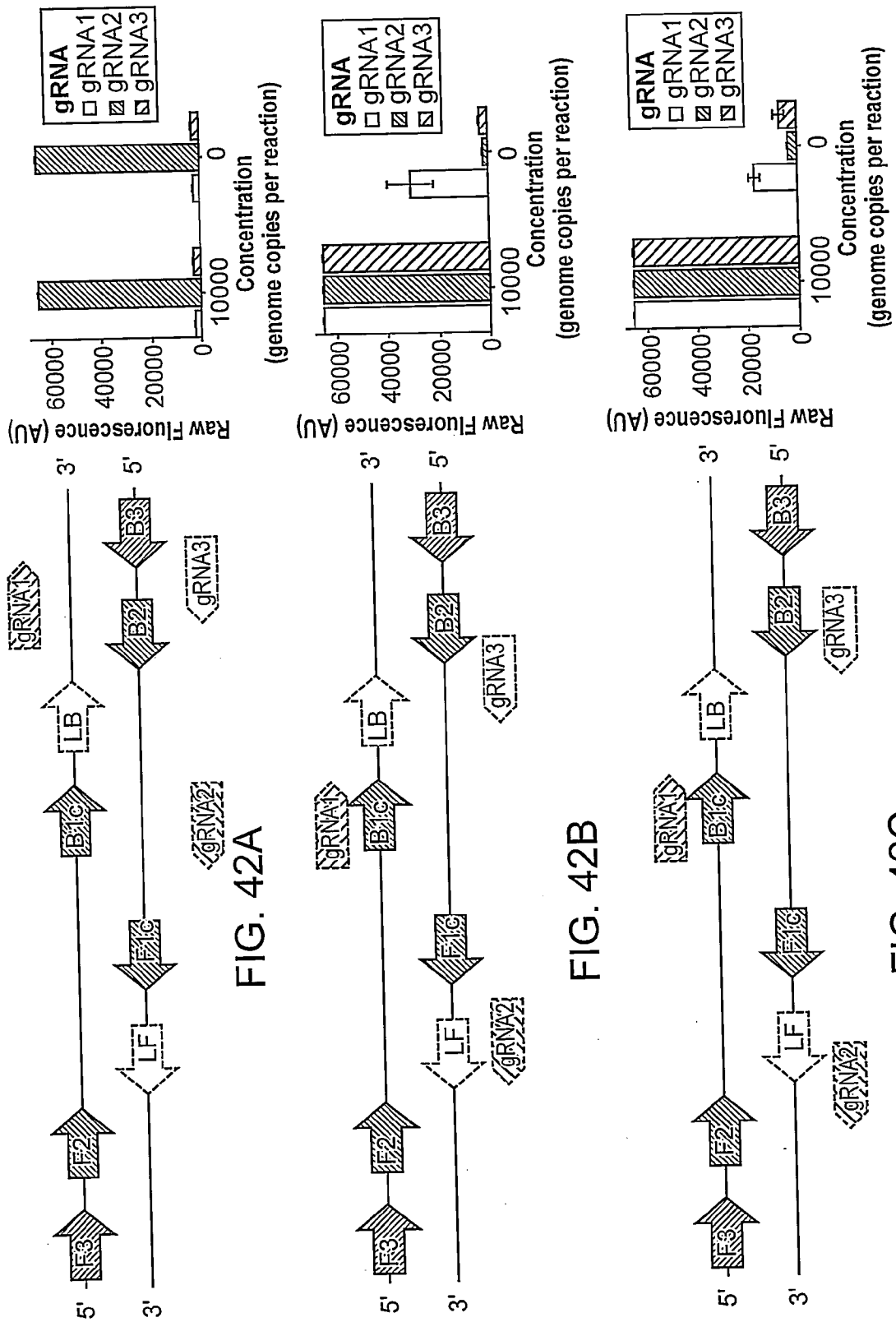


FIG. 42A

FIG. 42B

FIG. 42C

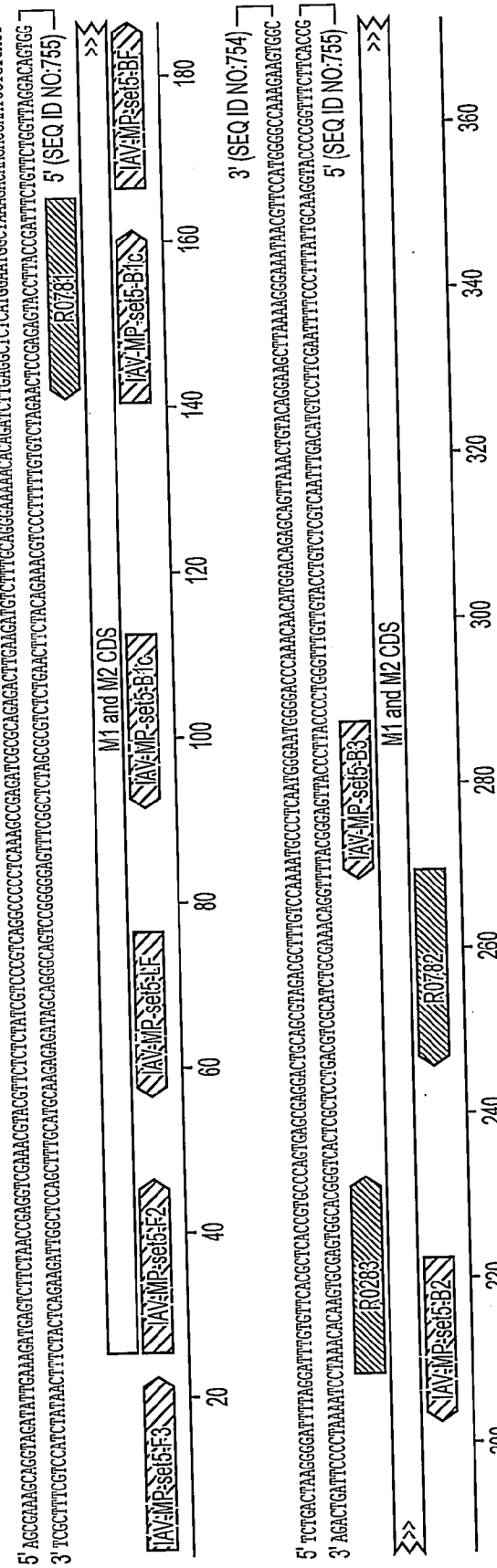

3' (SEQ ID NO:754)
5' AGCGAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGAGATCGCGCAGAGACTTGAAGATGTCTTTGCAGGCAAAAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAATCCTGTCACC
3' TCGCTTTCGTCCATCTATAACTTTCTACTCAGAAGATTGGCTCCAGCTTTGCATGCCAGCAGATAGCAGGCAGTCCGGGGGAGTTTCGGCTCTAGCCGCGTCTCTGAACTTCTACAGAAACGTCCCTTTTTGTGTCTACAGAACTCCGAGAGTACCTTACCGATTTCTGTTCTGGTTAGGACAGTGG
5' (SEQ ID NO:755)
R07681
>>
M1 and M2 CDS
IAV-MP-Sel6-F3
IAV-MP-Sel6-F2
IAV-MP-Sel5-F1
IAV-MP-Sel5-B1c
IAV-MP-Sel6-B1c
IAV-MP-Sel5-BF
20
40
60
80
100
120
140
160
180
3' (SEQ ID NO:754)
5' TCTGACTAAGGGGATTTTAGCATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTCAATGGGAATGGGGACCCAAACAACATGGACAGAGCAGCAGTTAAACTGTACAGGAAGCTTAAAAGGGAAATTAACGTTCCATGGGCCAAAGAAGTGGC
3' AGACTGATTCCCCTAAAATCCTAAACACAAGTGCGAGTGGCACGGGTCACTCGCTCCTGACGTCGCATCTGCCAAACAGGTTTACGGGAGTTACCCTACCCCTGGGTTTGTTGTACCTGTCGTCTCGTCAATTTGACATCTCCTTCGAATTTCCCTTTATTGCAAGGTACCCCGGTTTCTTCACCG
5' (SEQ ID NO:755)
R02683
R07682
>>
IAV-MP-Sel6-B2
IAV-MP-Sel5-B3
M1 and M2 CDS
200
220
240
260
280
300
320
340
360

FIG. 43A

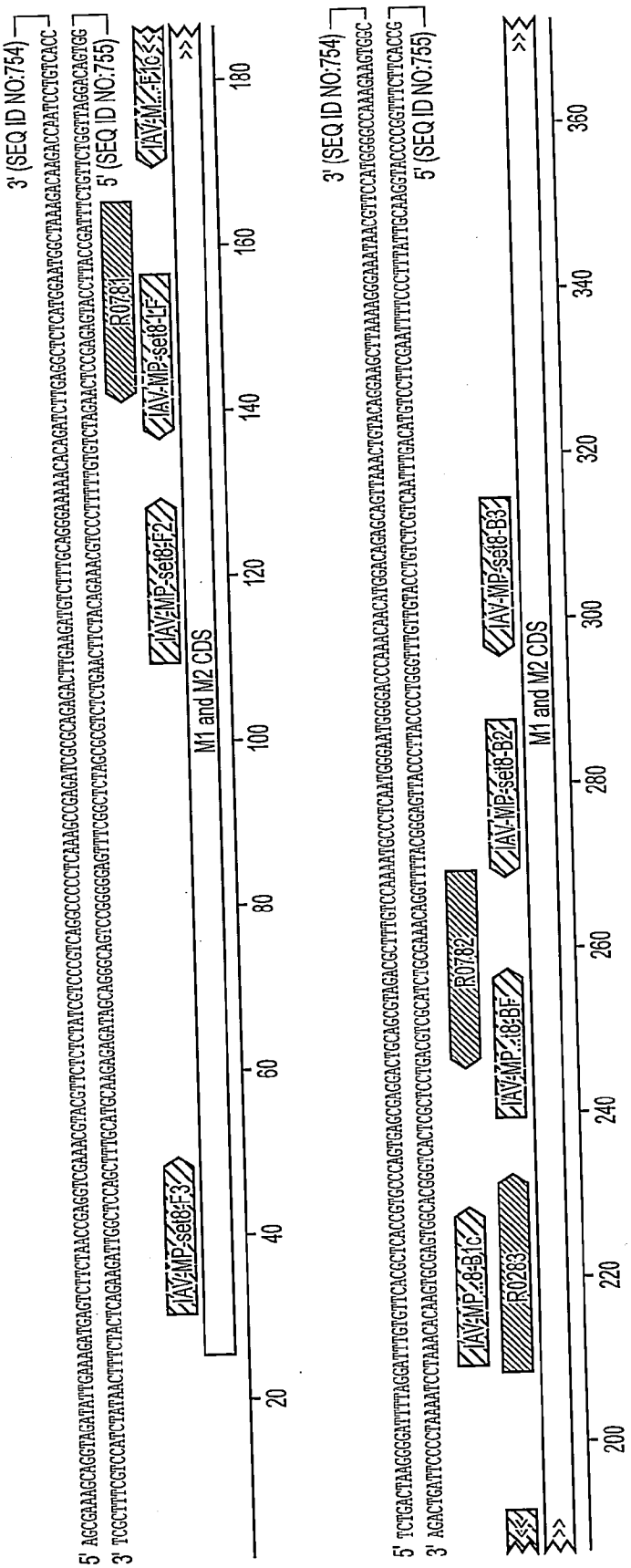

3' (SEQ ID NO:754)
5' (SEQ ID NO:755)
IAV-MP-set8-F3
IAV-MP-set8-F2
IAV-MP-set8-1-F
R0781
IAV-M...-F16
M1 and M2 CDS
20
40
60
80
100
120
140
160
180
3' (SEQ ID NO:754)
5' (SEQ ID NO:755)
IAV-MP...8-B1c
R0283
IAV-MP...t8-BF
R0782
IAV-MP-set8-B2
IAV-MP-set8-B3
M1 and M2 CDS
200
220
240
260
280
300
320
340
360

FIG. 43B

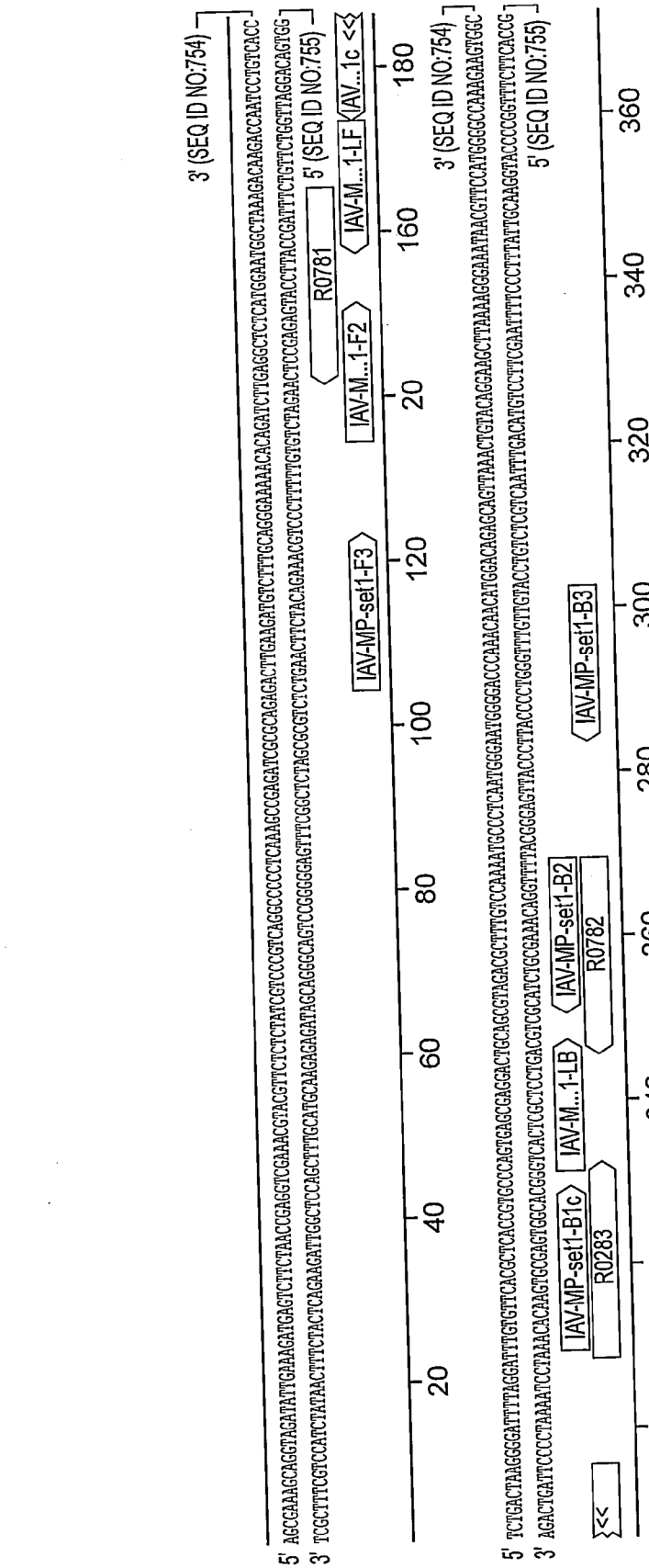

3' (SEQ ID NO:754)
5' AGCCAAAGCAGGTAGATATTGAAAGATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTCCCGTCAGGCCCCCTCAAAGCCGAGATCGCGCCAGACTTGAAGATCTCTTTGCAGGAAAAACACAGATCTTGAGGCTCTCATGGAATGGCTAAAGACAAGACCAATCCTGTCACC
3' TCGGTTTCGTCCATCTATAACTTTCTACTCAGAAGATTGGCTCCAGCTTTGCATGCAAGAGATAGCAGGCCAGTCCGGGGGAGTTTCGGCTCTAGCGCGTCTCTGAACTTCTACAGAAAGCGTCCCTTTTTGTGTCTAGAACTTCCAGAGTACCTTACCGATTTCTGTTCTGGTTAGGACAGTGG
R0781
5' (SEQ ID NO:755)
IAV-MP-set1-F3
IAV-M...1-F2
IAV-M...1-LF
IAV...1c <<
20
40
60
80
100
120
140
160
180
3' (SEQ ID NO:754)
5' TCTGACTAAGGGGATTTTAGGATTTGTGTTCACGCTCACCGTGCCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTCAAATTGGGAATGGGGACCCAAACAACATGGACAGCAGAGCAGCTTAAACTGTACAGGAAGCTTAAAAGGGAAATAACGTTCCATGGGGCCAAAGAAGTGGC
3' AGACTGATTCCCCTAAAATCCTAAAACACAAGTGCGAGTGGCACGGGTCACTCGCTCCTGACGTCGCATCTGCGAAACAGGTTTTACGGCAGTTACCCTTACCCTGGGTTTGTTGTACCTGTCTCGTCAATTTGACATGTCCTTCGAATTTTCCCTTTATTGCAAGGTACCCGGTTTCTTCACCG
5' (SEQ ID NO:755)
<<
IAV-MP-set1-B1c
IAV-M...1-LB
IAV-MP-set1-B2
IAV-MP-set1-B3
R0283
R0782
200
220
240
260
280
300
320
340
360

FIG. 43C

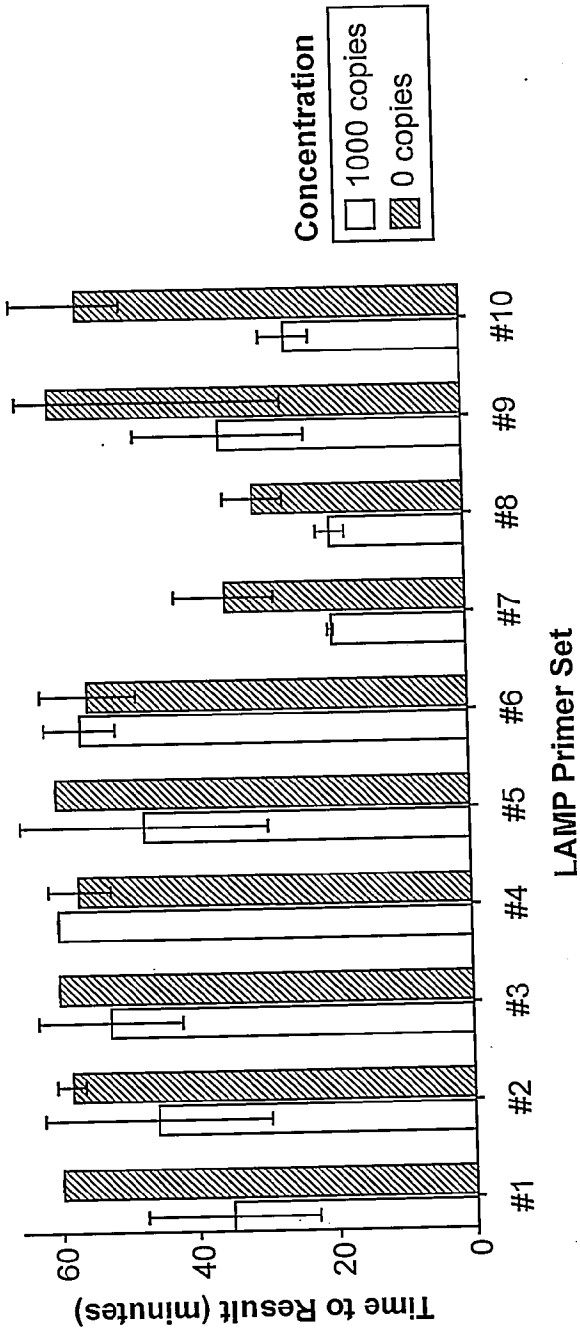

Concentration
1000 copies
0 copies
Time to Result (minutes)
0
20
40
60
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
LAMP Primer Set

FIG. 44

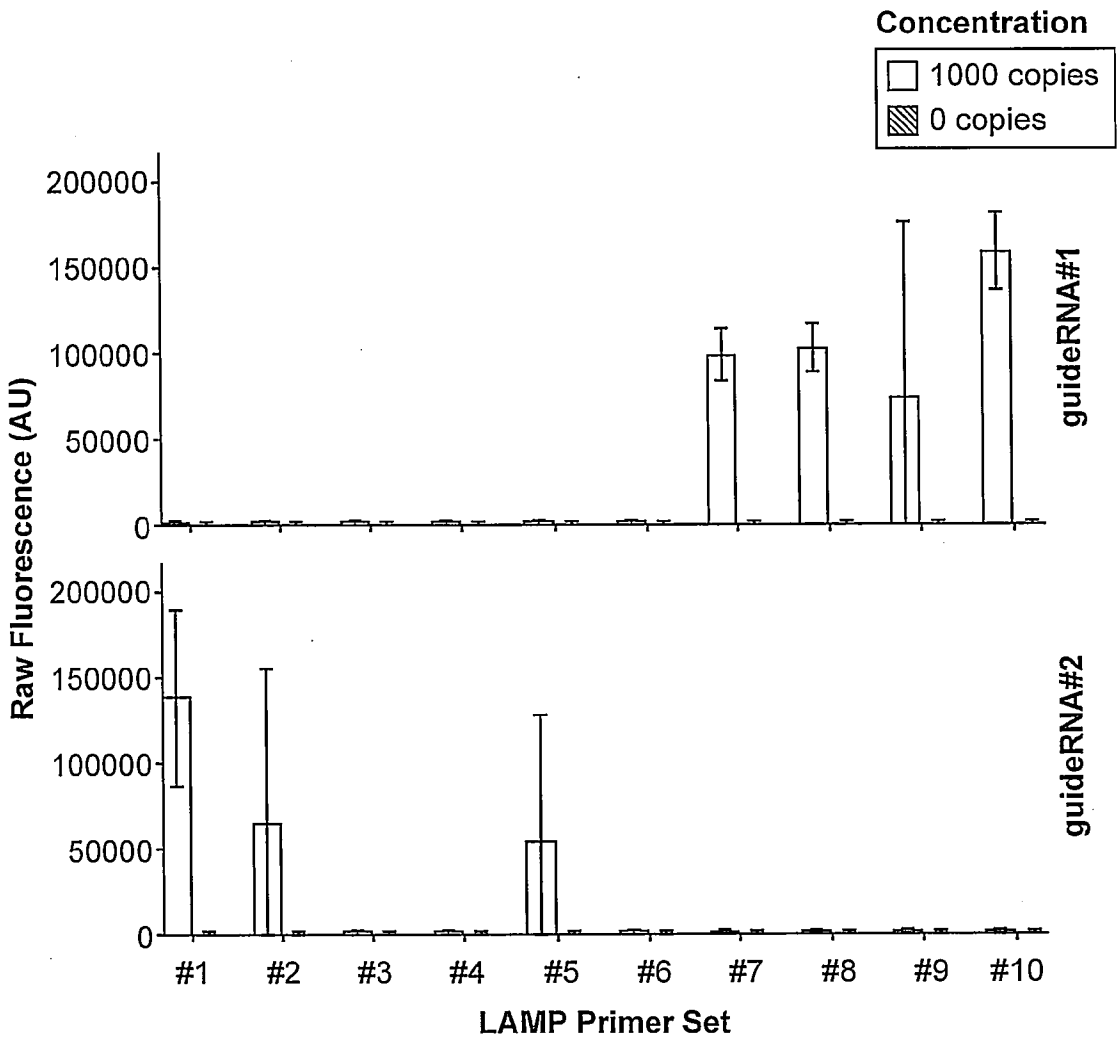

Concentration
1000 copies
0 copies
200000
150000
100000
50000
0
Raw Fluorescence (AU)
guideRNA#1
guideRNA#2
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
LAMP Primer Set

FIG. 45

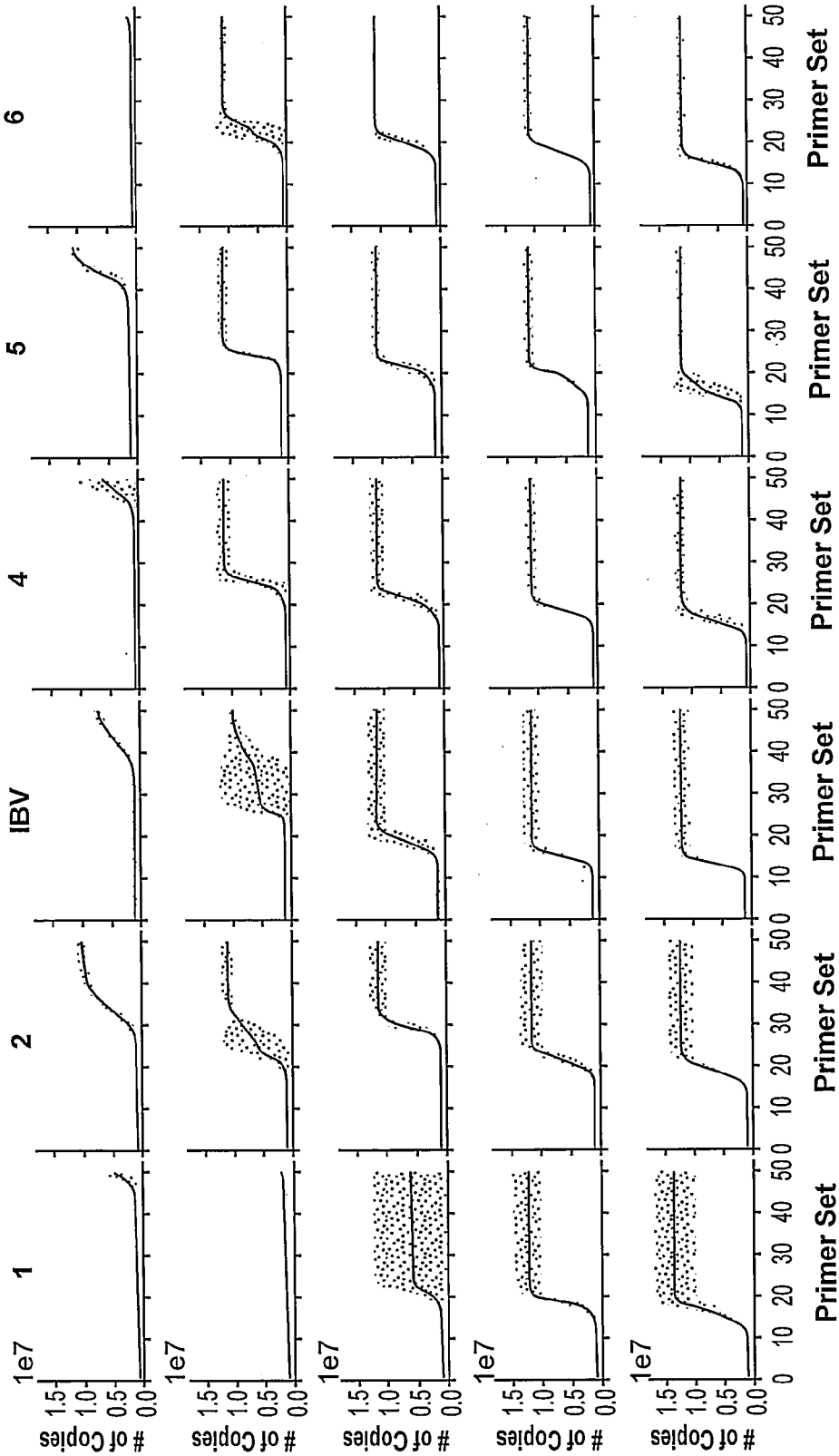

1
2
IBV
4
5
6
1e7
# of Copies
1.5
1.0
0.5
0.0
0 10 20 30 40 50 0 10 20 30 40 50 0 10 20 30 40 50 0 10 20 30 40 50
Primer Set

FIG. 46

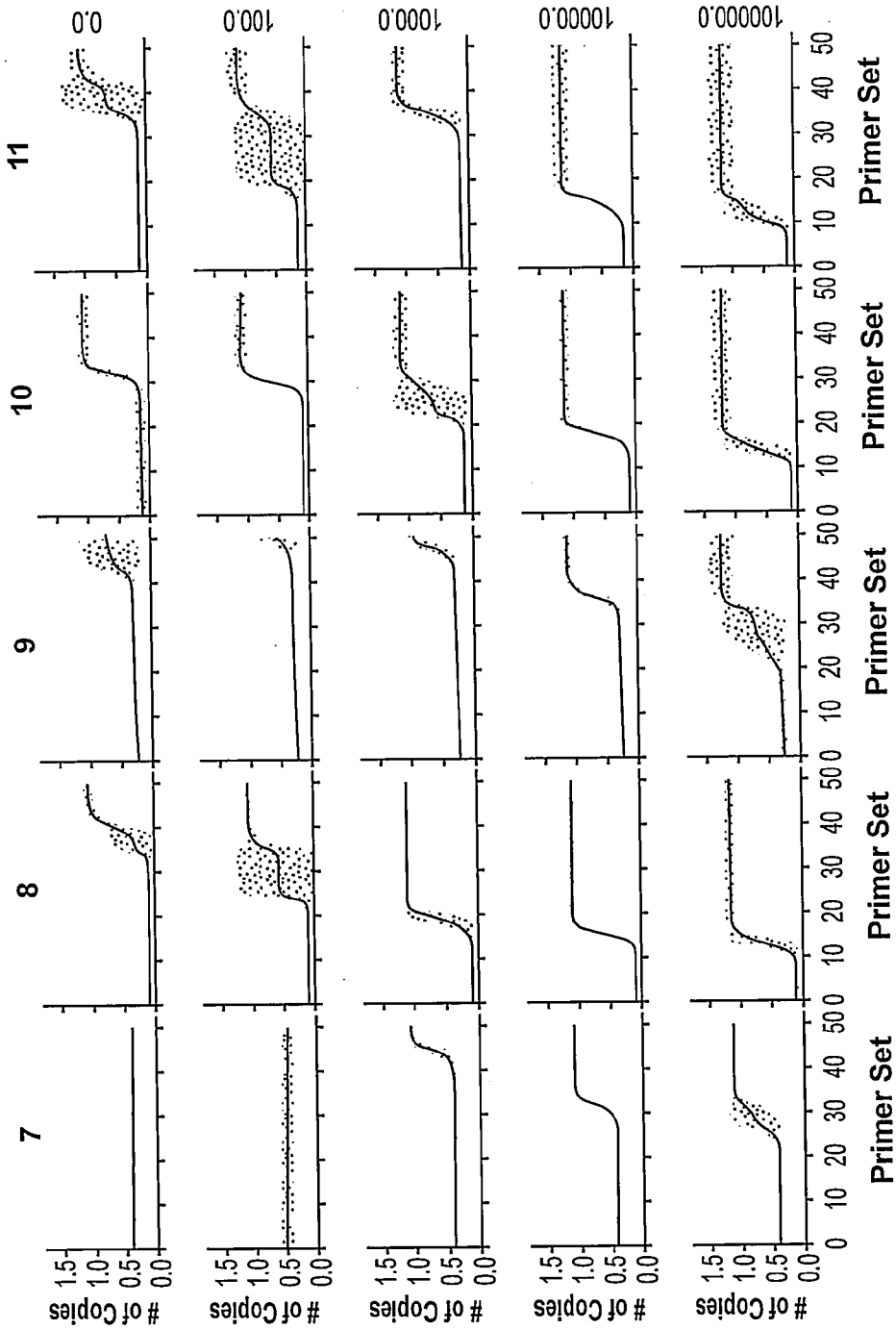

7
8
9
10
11
0.0
100.0
1000.0
10000.0
100000.0
# of Copies
1.5
1.0
0.5
0.0
0 10 20 30 40 50
Primer Set

FIG. 46 (Cont.)

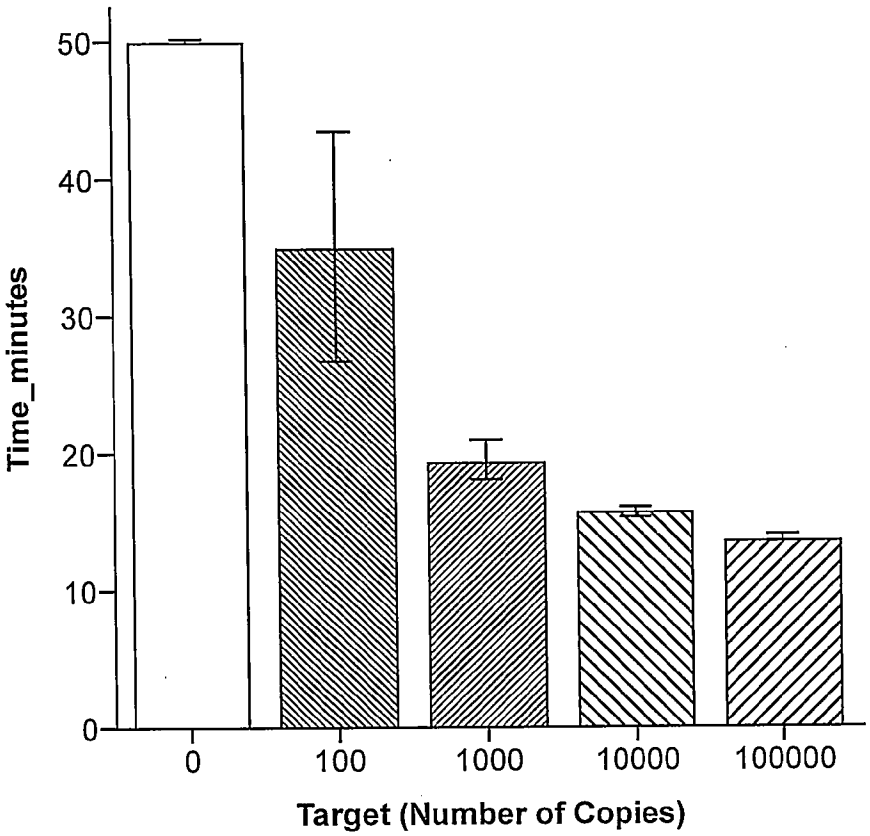

50
40
30
20
10
0
Time_minutes
0
100
1000
10000
100000
Target (Number of Copies)

FIG. 47

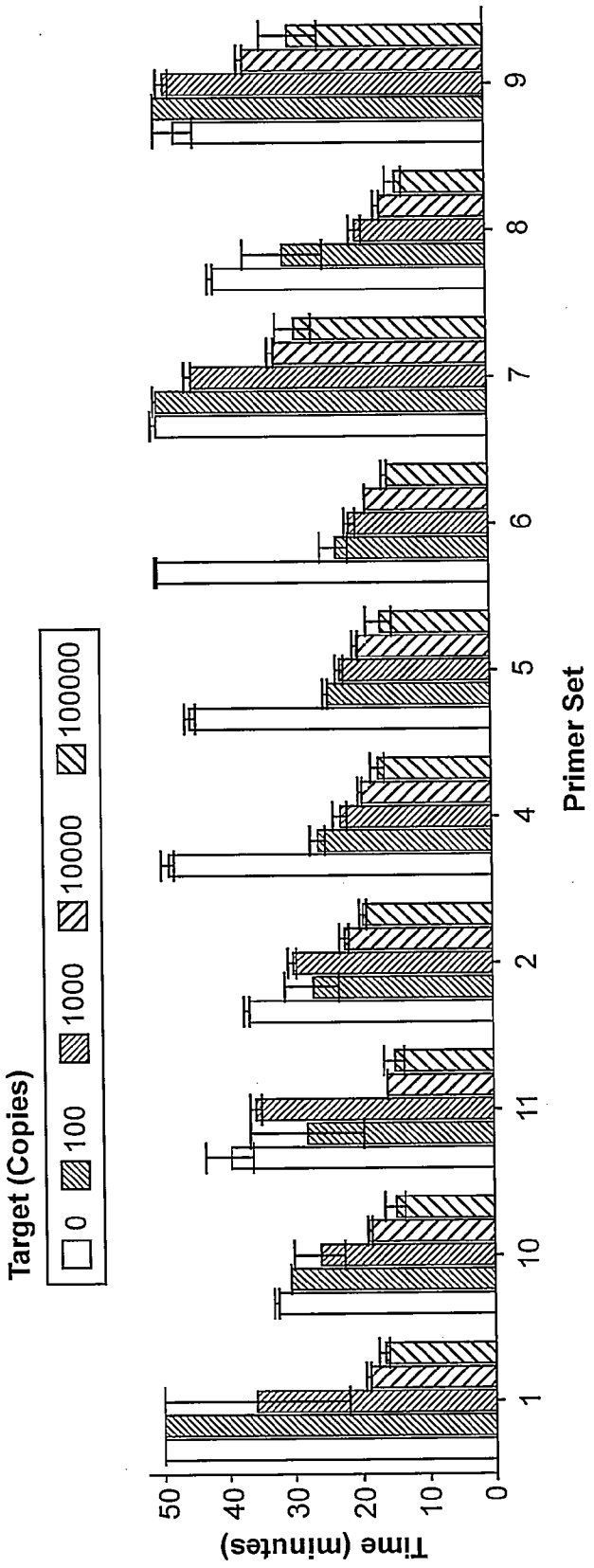

Target (Copies)
0
100
1000
10000
100000
Time (minutes)
0
10
20
30
40
50
1
10
11
2
4
5
6
7
8
9
Primer Set

FIG. 48

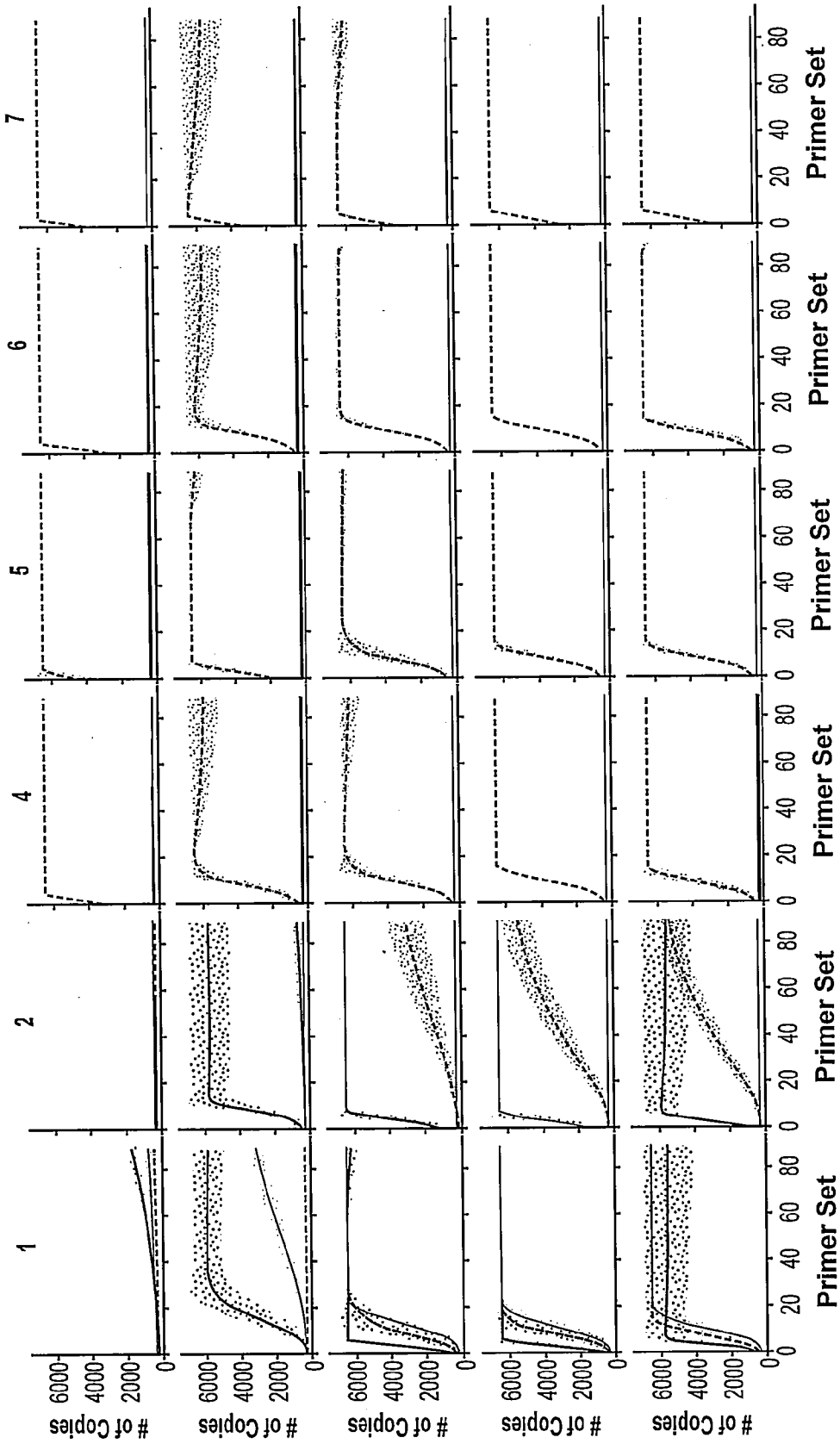


FIG. 49

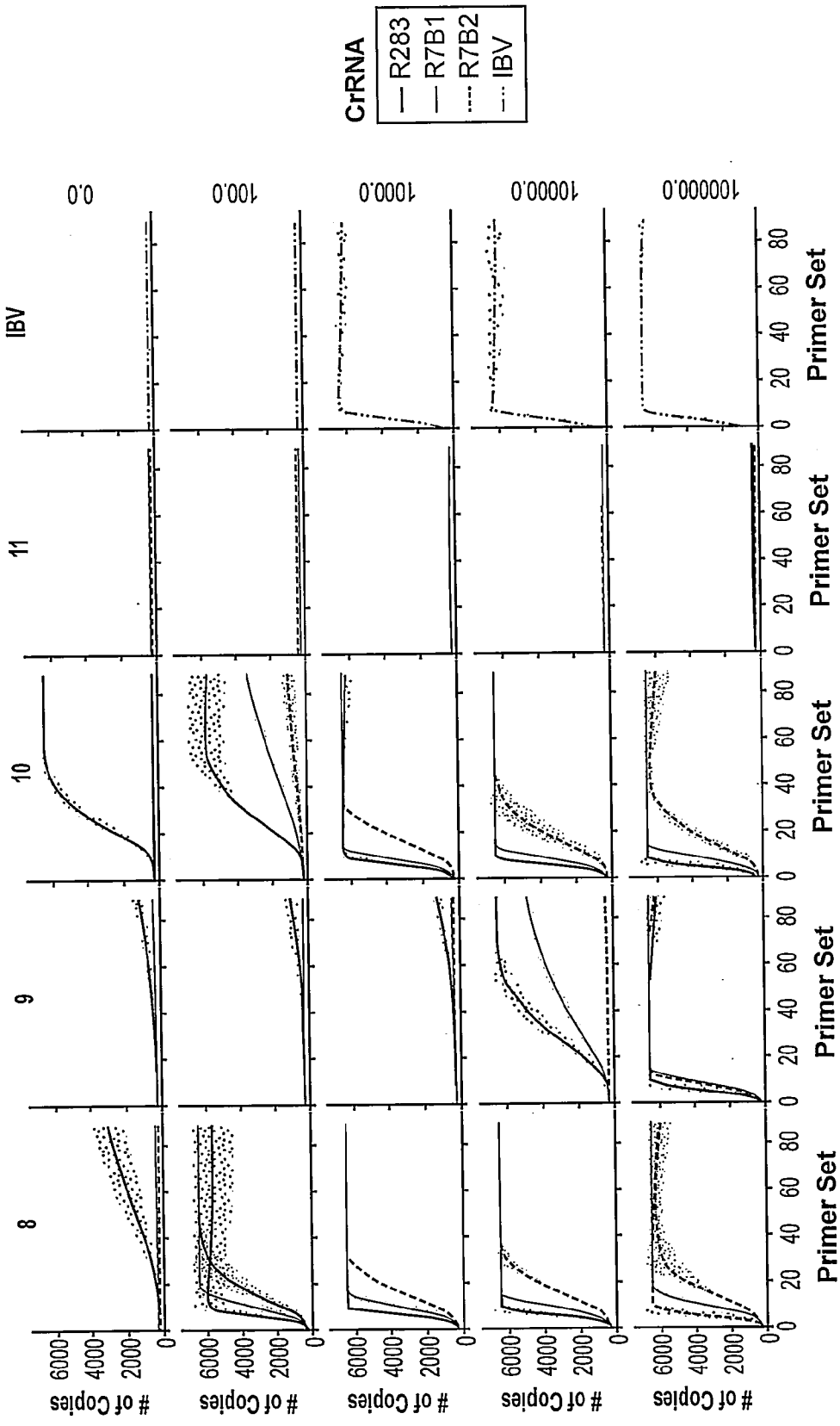

CrRNA
R283
R7B1
R7B2
IBV
0.0
100.0
1000.0
10000.0
100000.0
IBV
11
10
9
8
# of Copies
6000
4000
2000
0
Primer Set
0
20
40
60
80

FIG. 49 (Cont.)

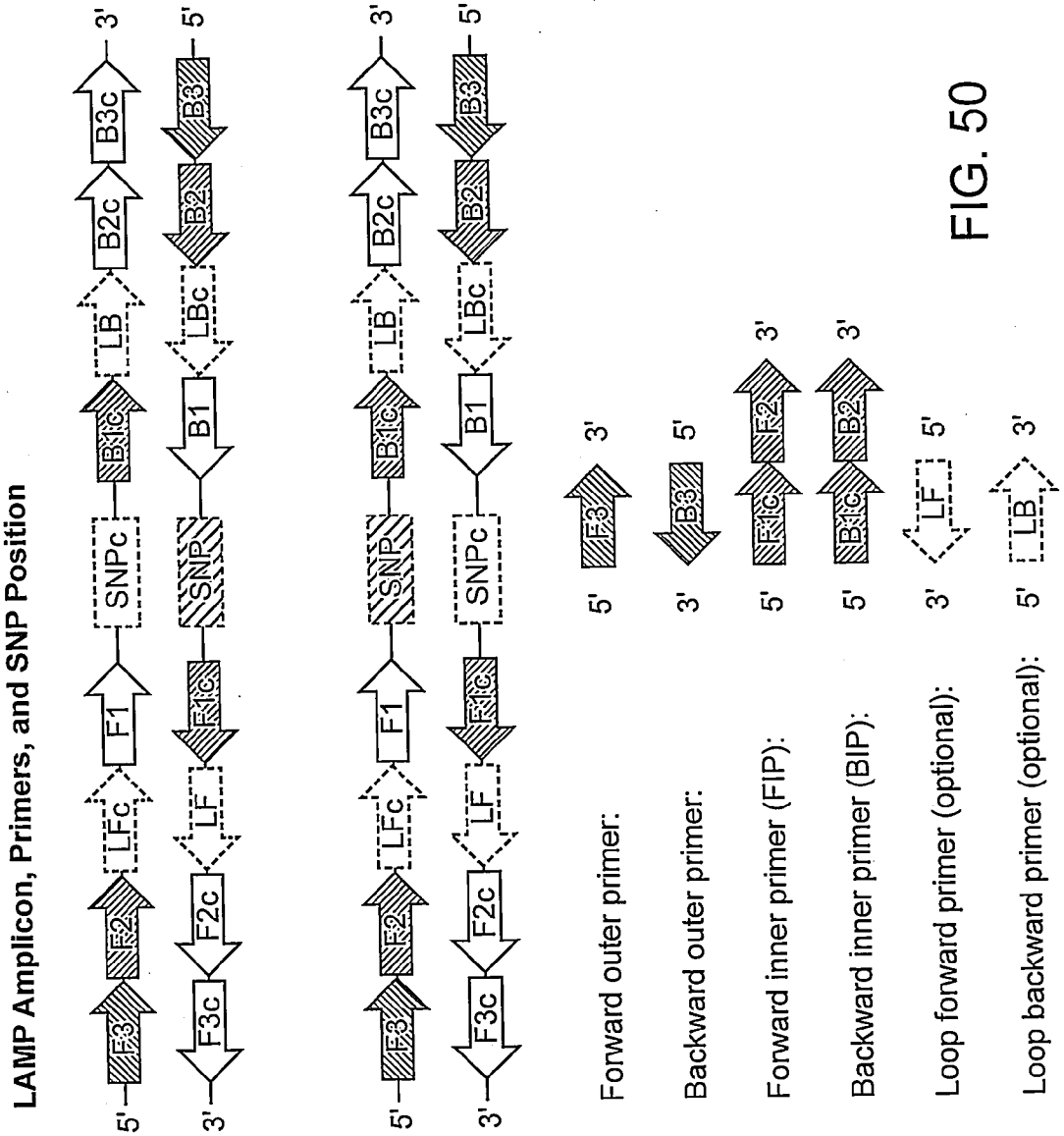

LAMP Amplicon, Primers, and SNP Position
5'
3'
F3
F2
LFc
F1
SNPc
B1c
LB
B2c
B3c
F3c
F2c
LF
F1c
SNP
B1
LBc
B2
B3
Forward outer primer:
Backward outer primer:
Forward inner primer (FIP):
Backward inner primer (BIP):
Loop forward primer (optional):
Loop backward primer (optional):

FIG. 50

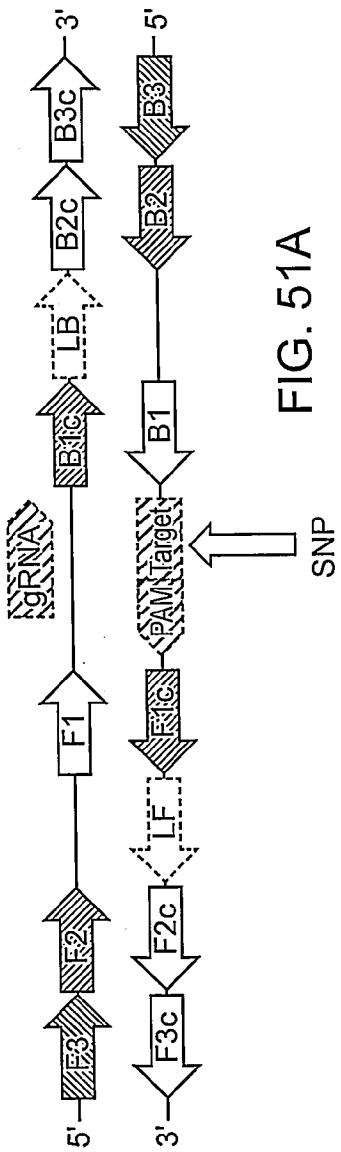


FIG. 51A

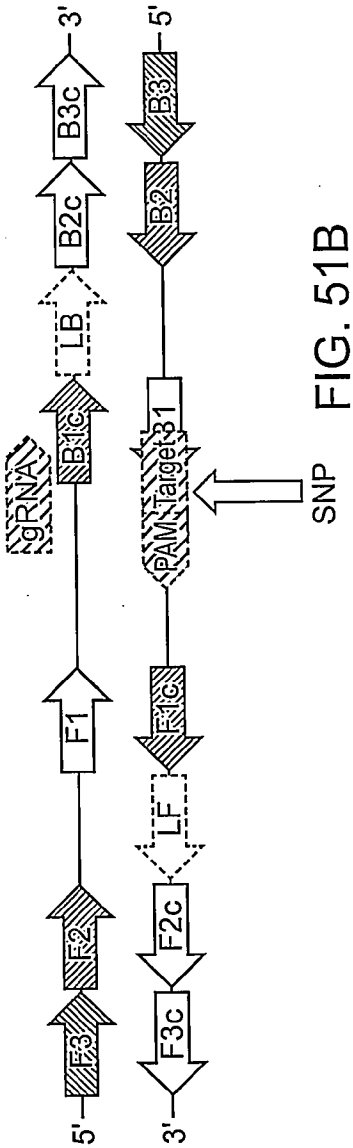


FIG. 51B

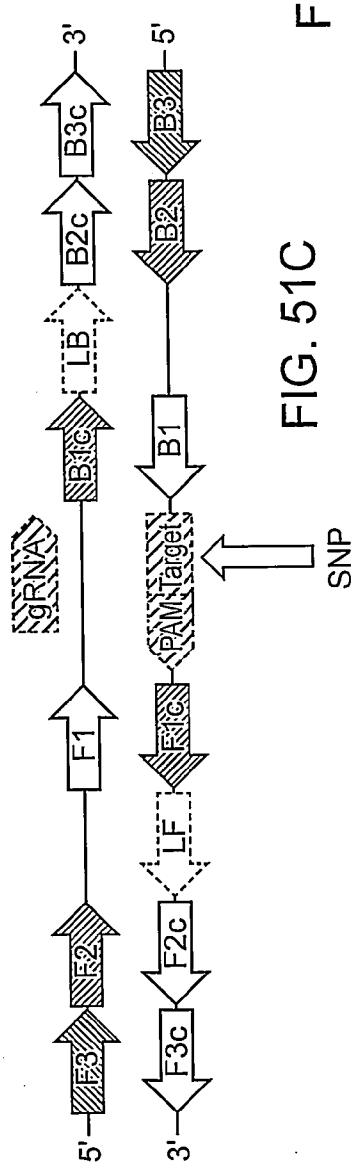


FIG. 51C

FIG. 51

5' CCAGTTTCATTTGAGCATTAAGTGTCAAGTTCTG 3' (SEQ ID NO:750)

3' GGTCAAAGTAAACTCGTAATTCACAGTTCAAGAC 5' (SEQ ID NO:751)

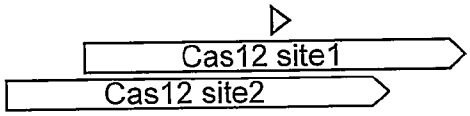


FIG. 52

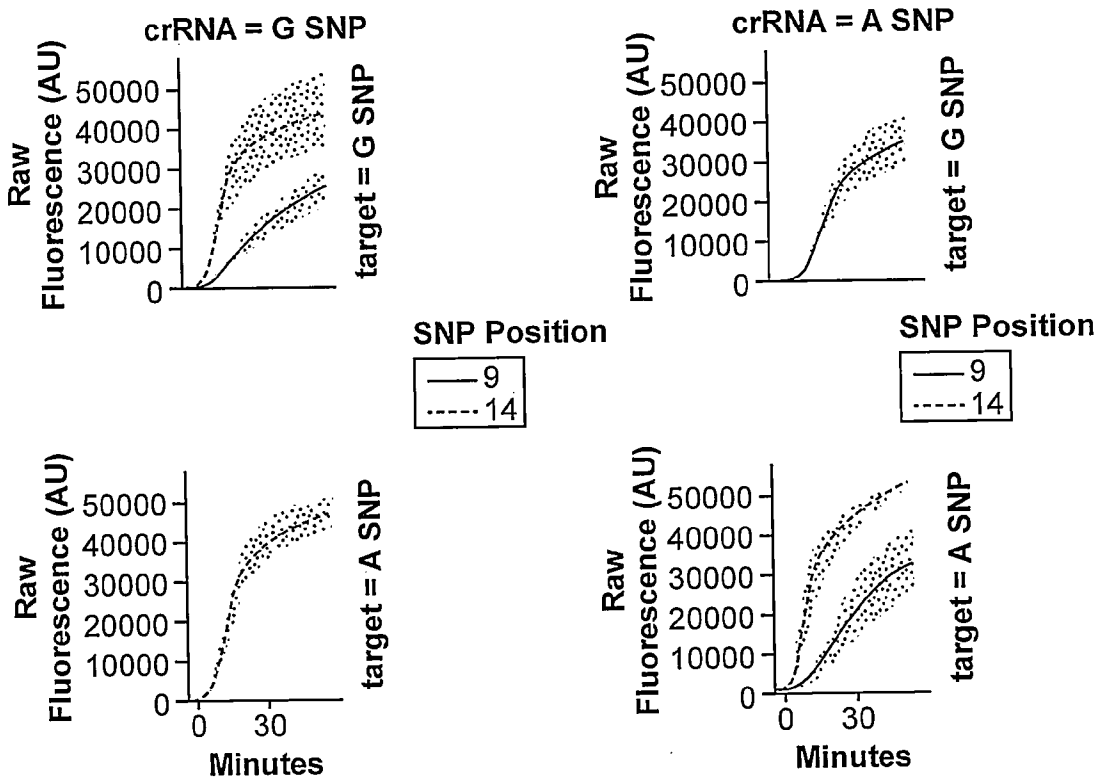


FIG. 53

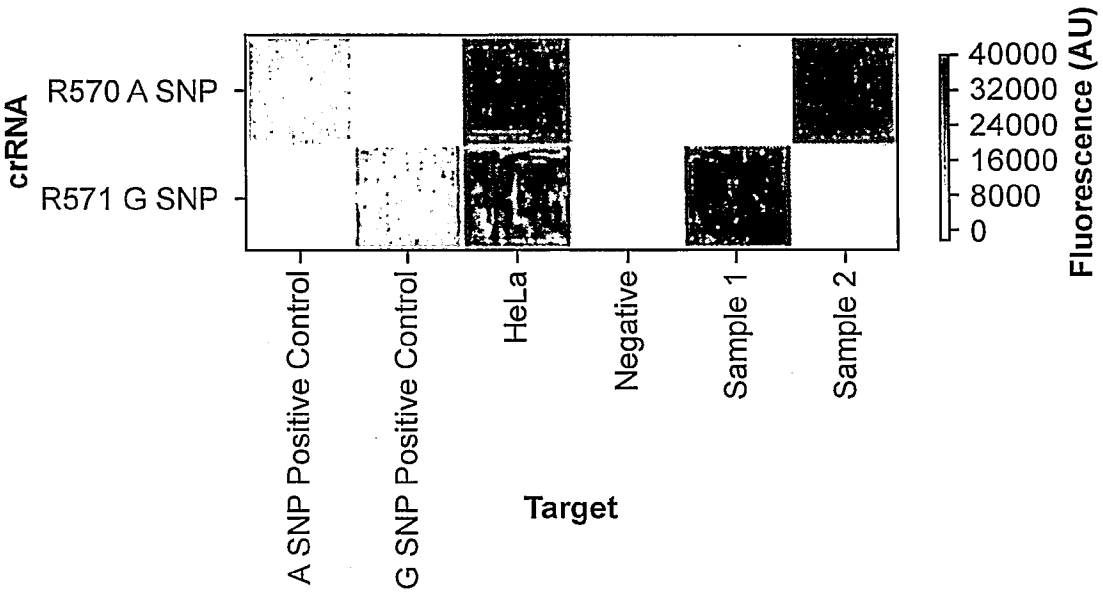

crRNA
R570 A SNP
R571 G SNP
A SNP Positive Control
G SNP Positive Control
HeLa
Negative
Sample 1
Sample 2
Target
40000
32000
24000
16000
8000
0
Fluorescence (AU)

FIG. 54

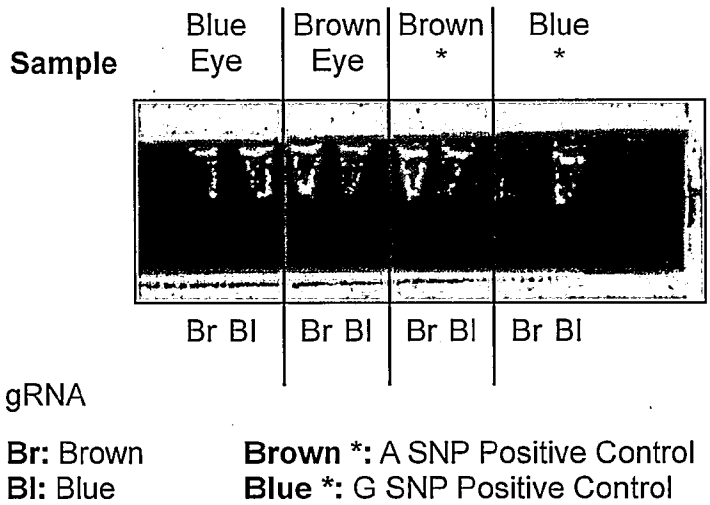

Sample
Blue Eye
Brown Eye
Brown *
Blue *
Br Bl
Br Bl
Br Bl
Br Bl
gRNA
Br: Brown
Bl: Blue
Brown *: A SNP Positive Control
Blue *: G SNP Positive Control

FIG. 55

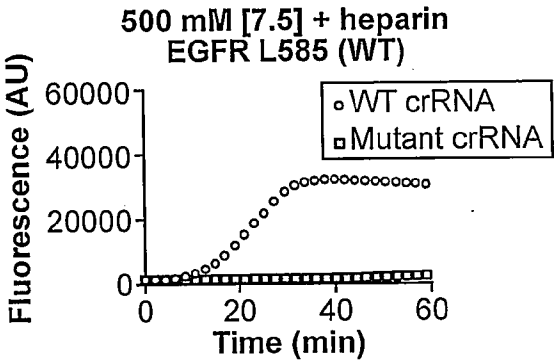


FIG. 56A

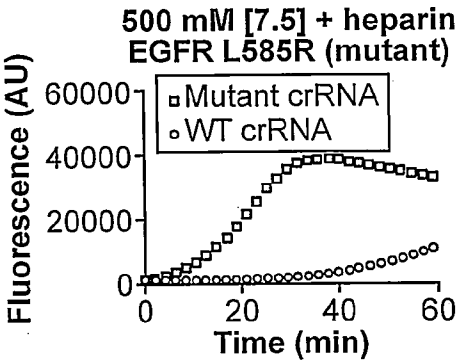


FIG. 56B

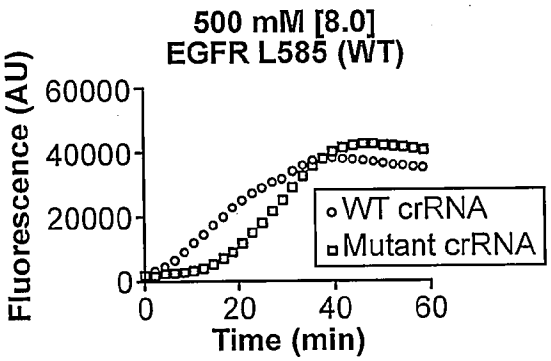


FIG. 56C

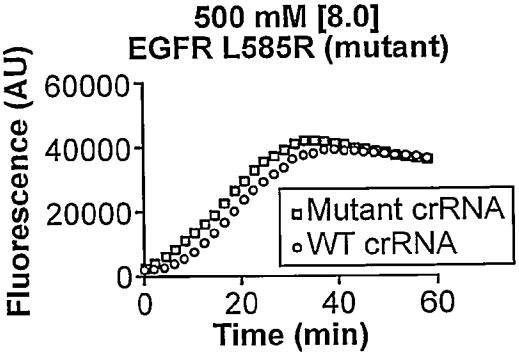


FIG. 56D

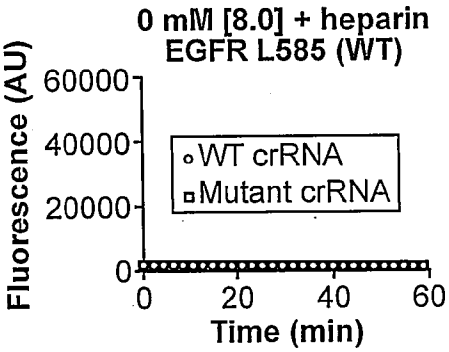


FIG. 56E

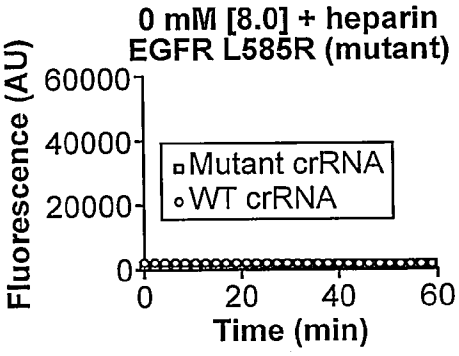


FIG. 56F

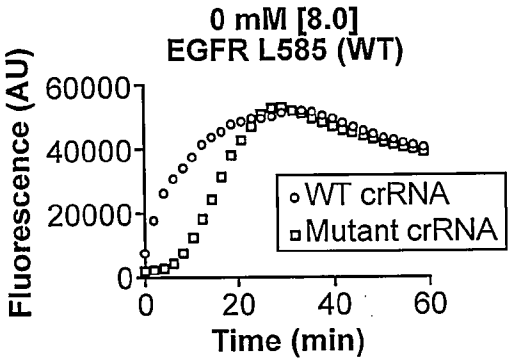


FIG. 56G

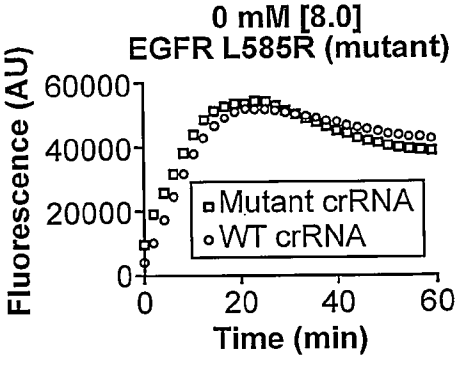


FIG. 56H

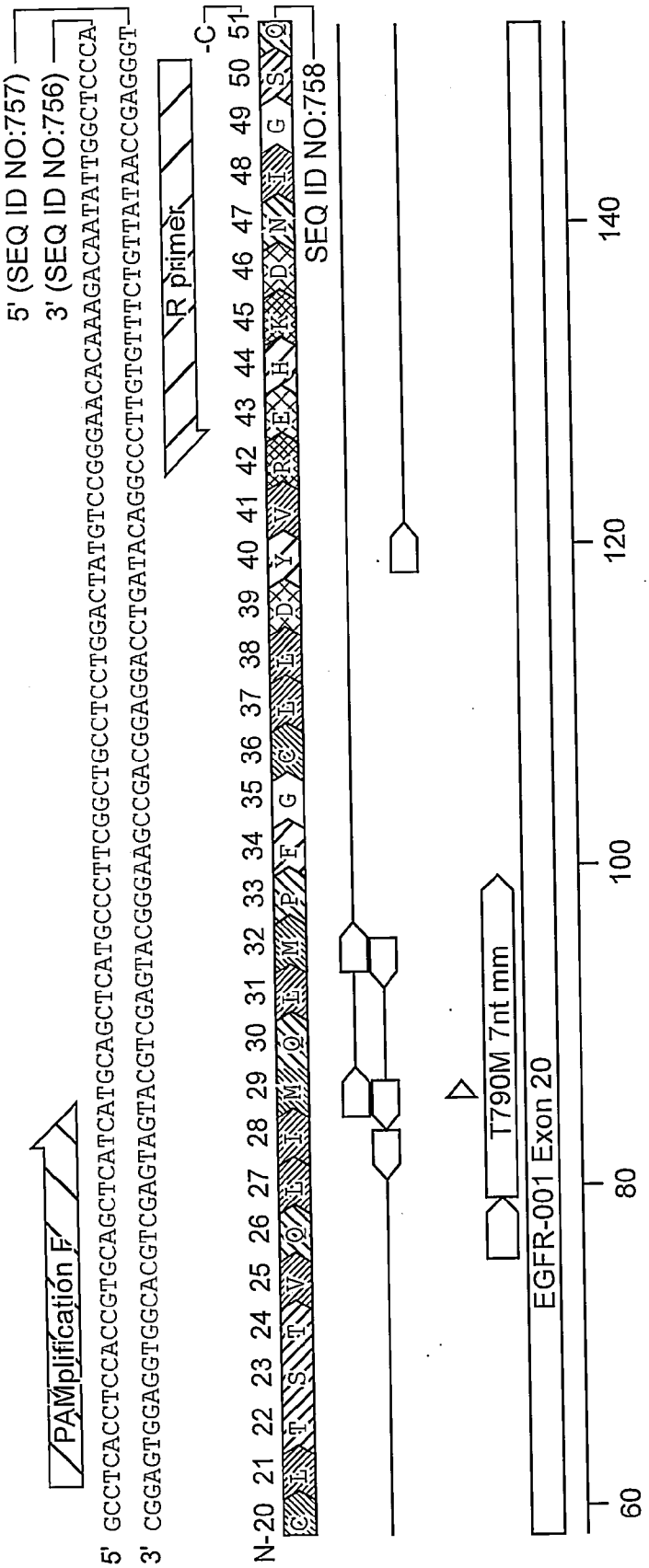
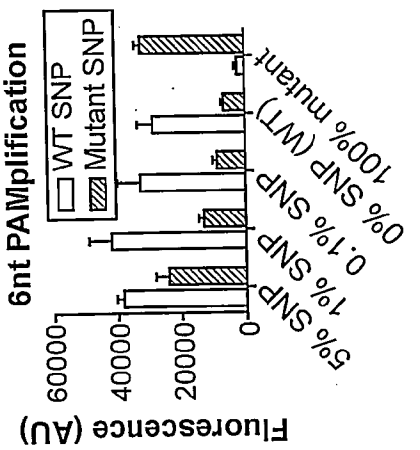
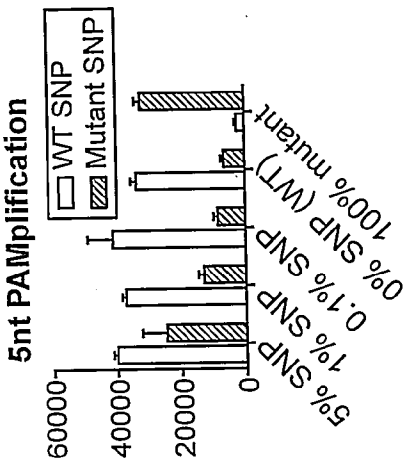

5' (SEQ ID NO:757)
3' (SEQ ID NO:756)
PAMplification F
5' GCCTCACCTCCACCGTGCAGCTCATCATGCAGCTCATGCCCTTCGGCTGCCTCCTGGACTATGTCCGGGAACACAAAGACAATATTGGCTCCCA
3' CGGAGTGGAGGTGGCACGTCGAGTAGTACGTCGAGTACGGGAAGCCGACGGAGGACCTGATACAGGCCCTTGTGTTTCTGTTATAACCGAGGGT
R primer
-C
N-20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51
SEQ ID NO:758
T790M 7nt mm
EGFR-001 Exon 20
60
80
100
120
140

FIG. 57





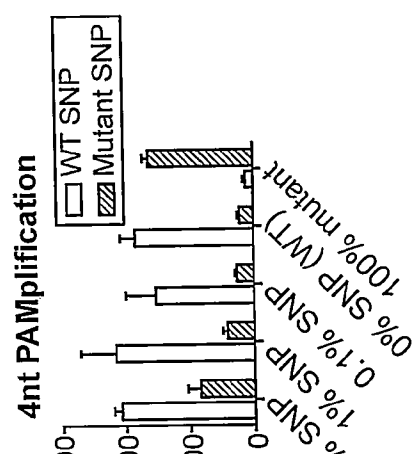


FIG. 58A

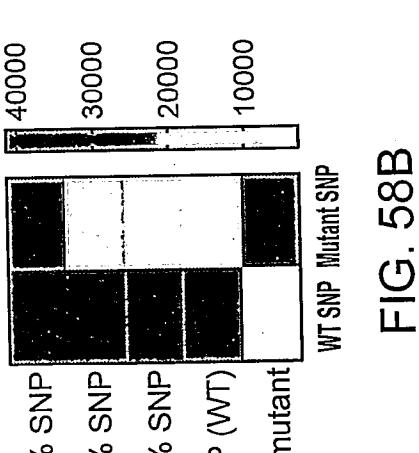


FIG. 58B

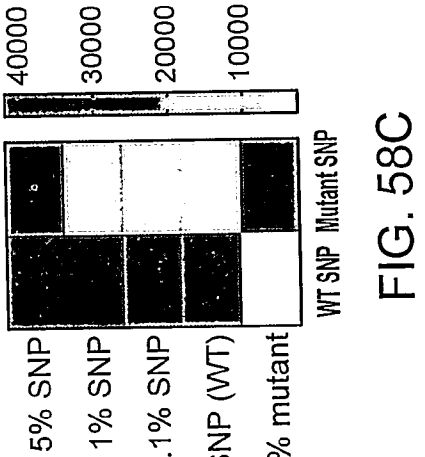


FIG. 58C

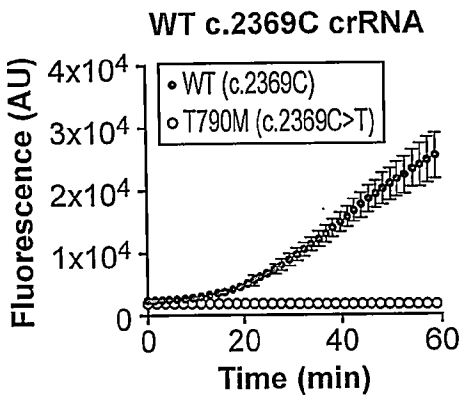


FIG. 59A

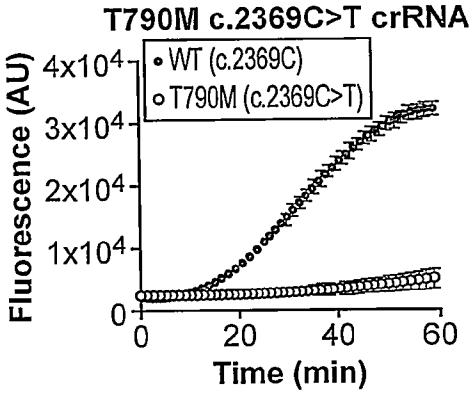


FIG. 59B

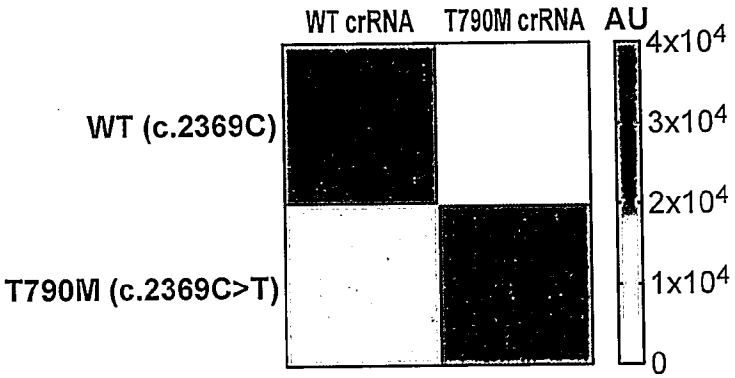


FIG. 59C

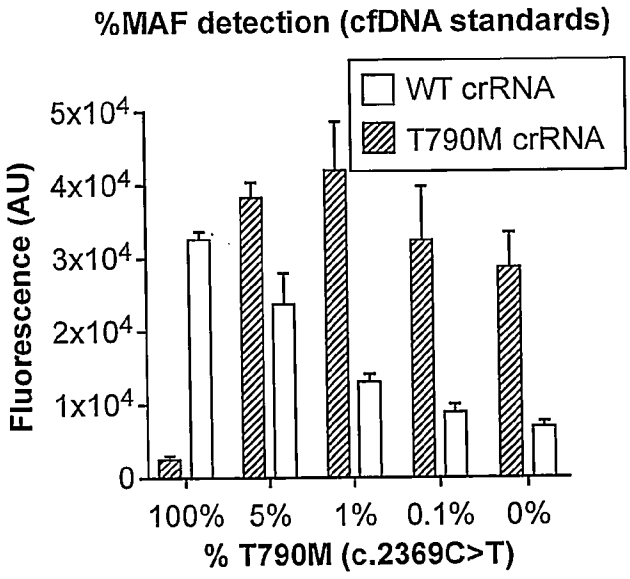


FIG. 60A

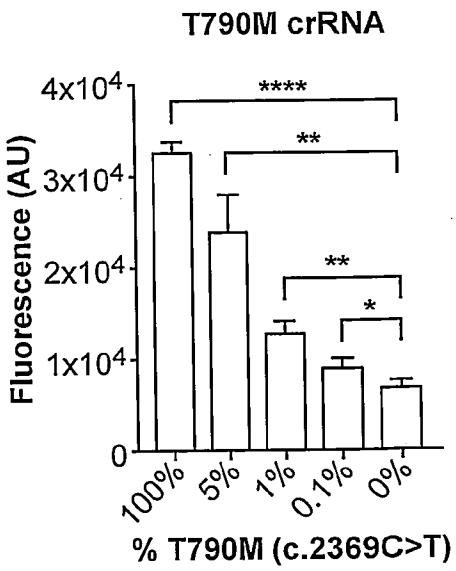


FIG. 60B

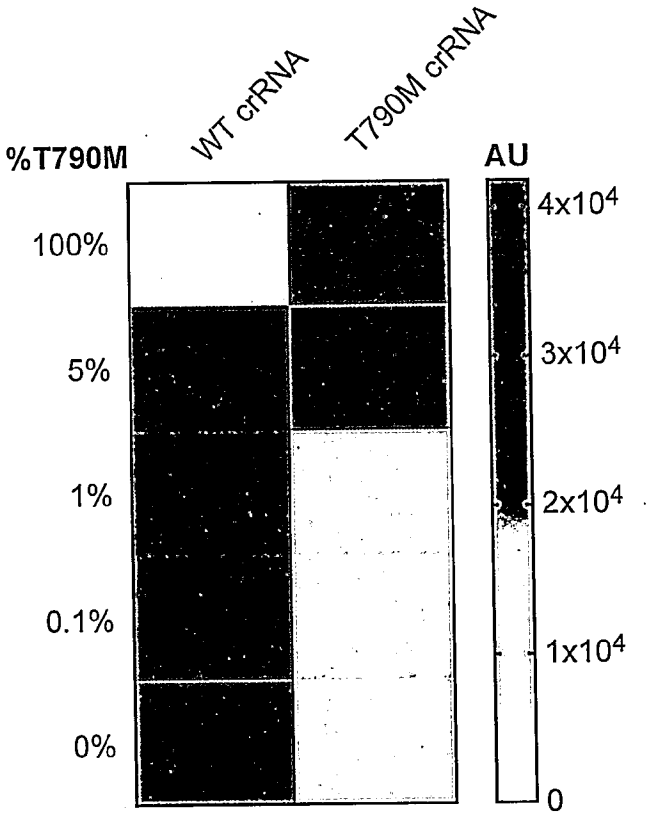


FIG. 60C

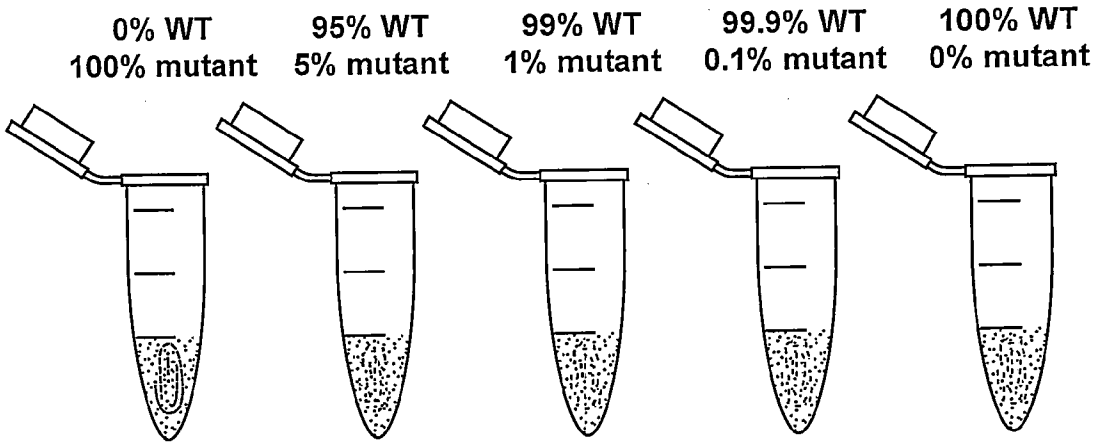


FIG. 60D

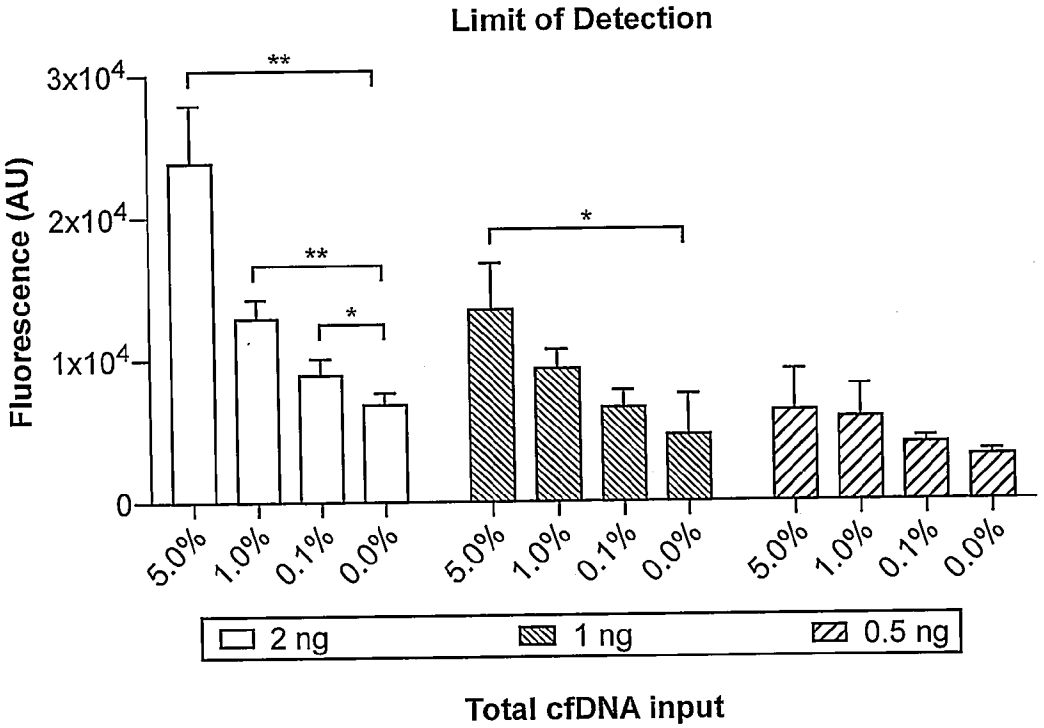


FIG. 61

	Roche cobas EGFR Mutation test v2	PCR + CRISPR
Sample	Tissue	cfDNA standards
Input	50 ng purified DNA	2 ng purified DNA
Detection sensitivity	≤ 5%	≤ 1%
Sensitivity	94.0%	N/A
Specificity	97.7%	N/A
TAT	1 day	2 hours
Hands-on time	4 hours (including sample prep)	<30 minutes (not including sample prep)

FIG. 62

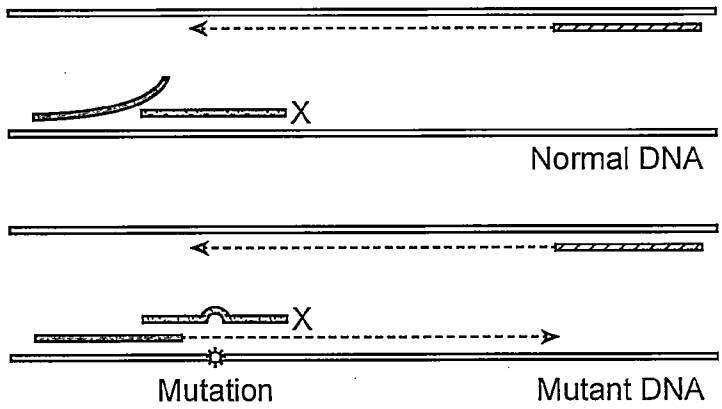


FIG. 63A

FIG. 63B

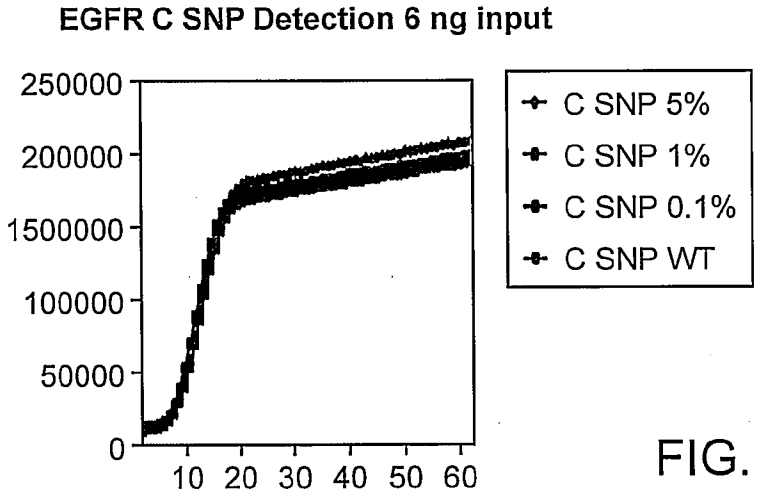


FIG. 63C

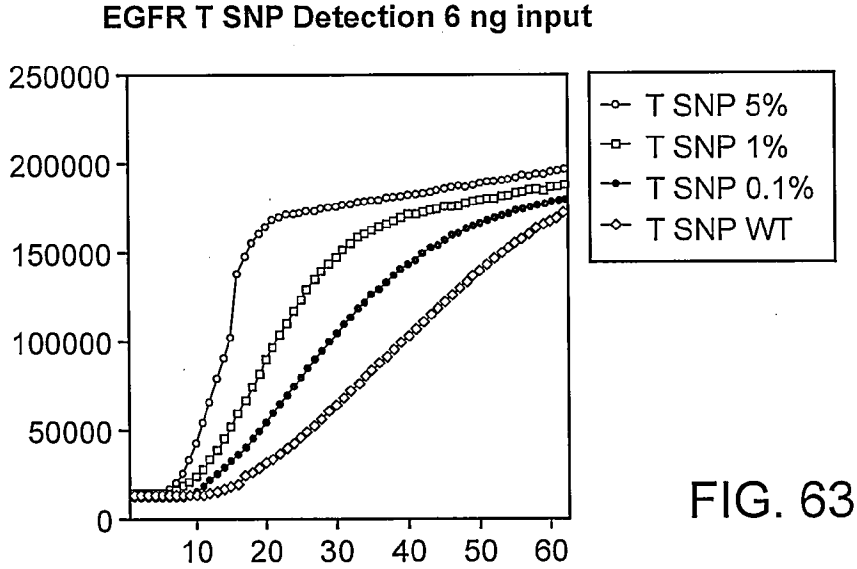


FIG. 63D

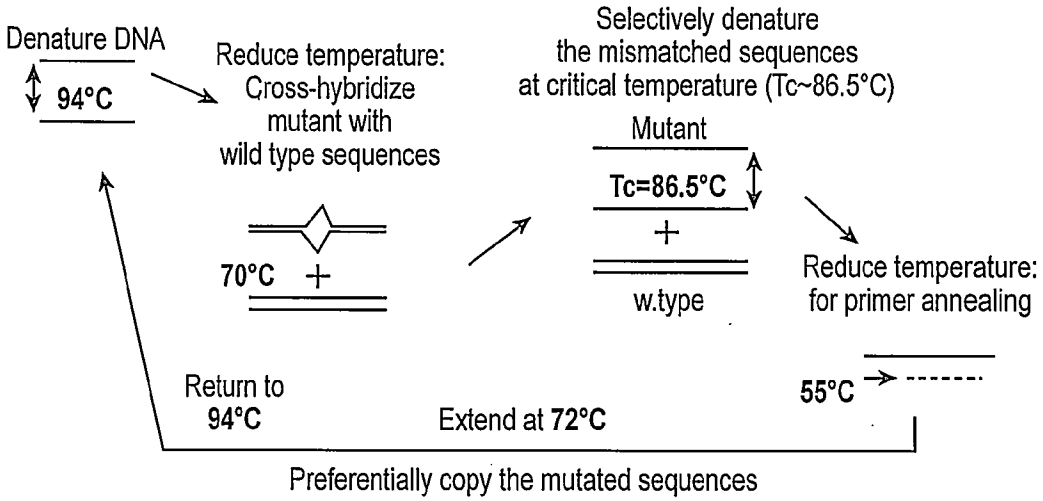


FIG. 64A

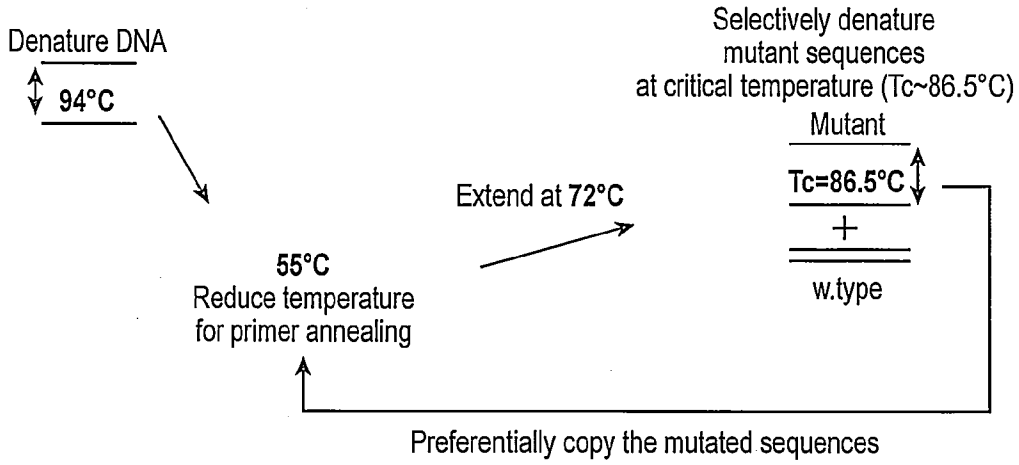


FIG. 64B

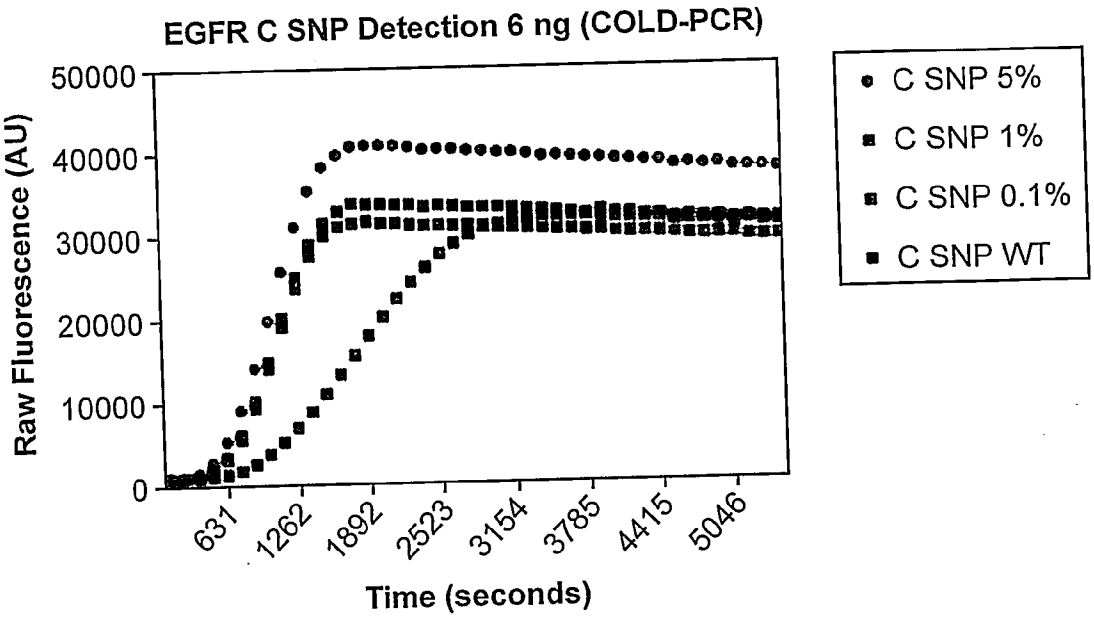


FIG. 65A

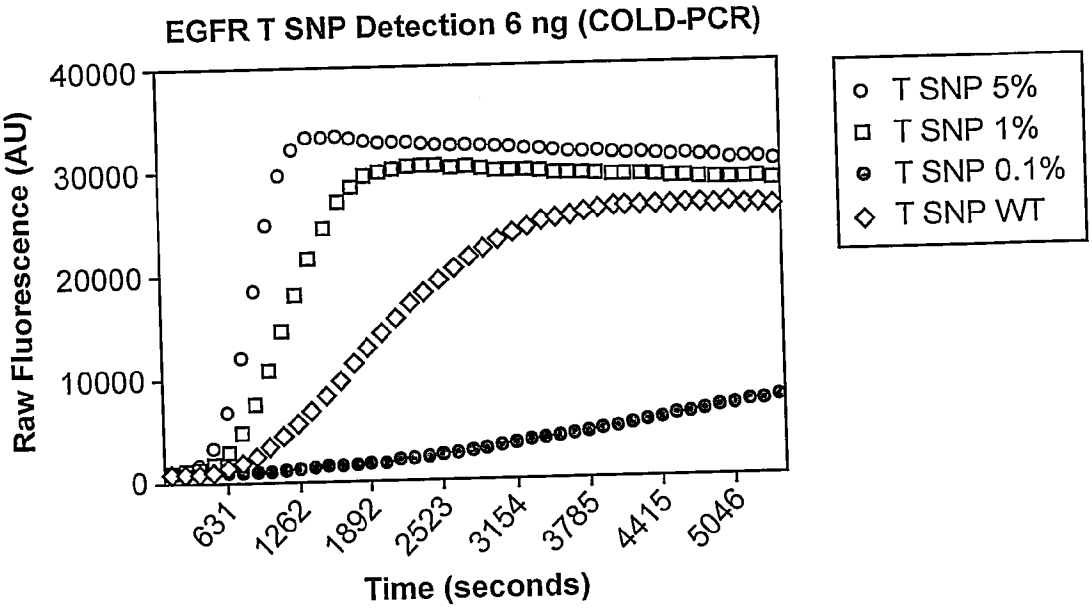


FIG. 65B

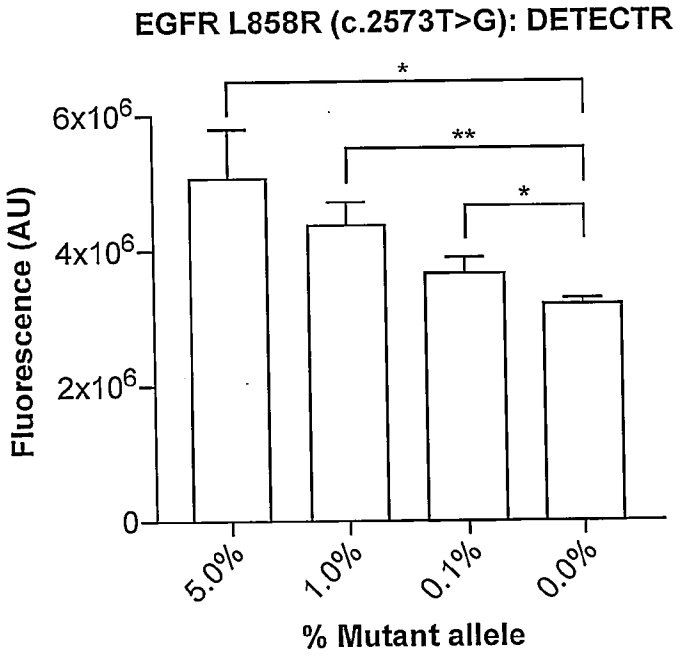


FIG. 66A

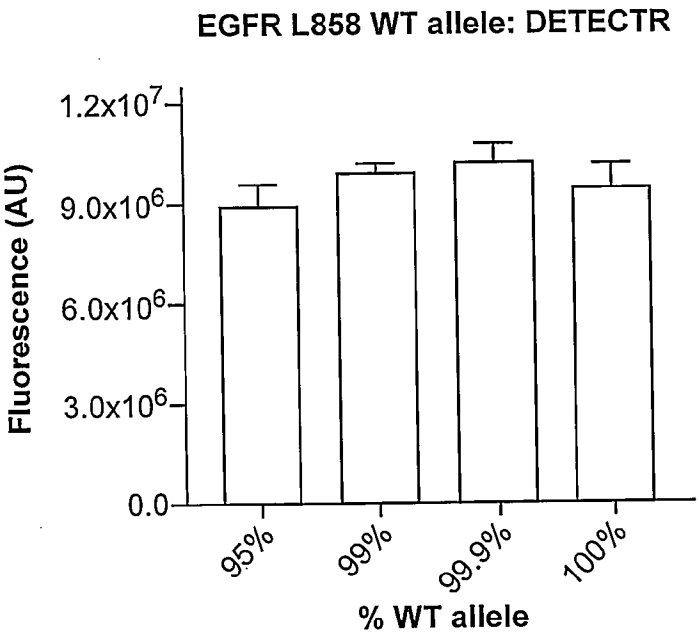


FIG. 66B

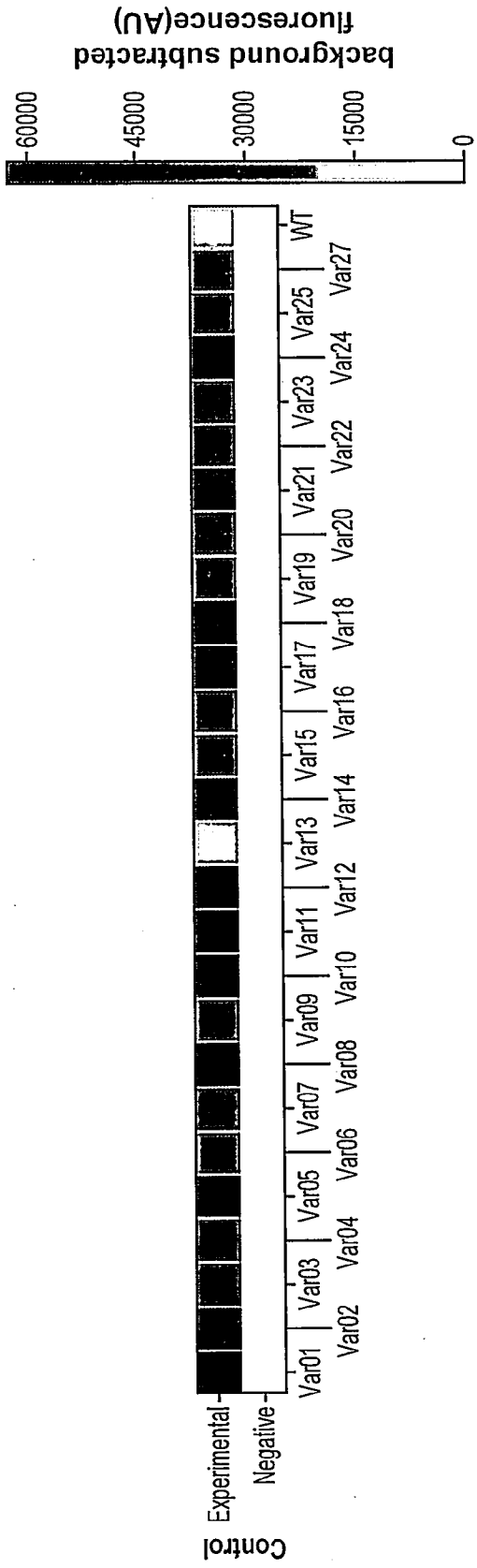

background subtracted fluorescence(AU)
60000
45000
30000
15000
0
Control
Experimental
Negative
Var01
Var02
Var03
Var04
Var05
Var06
Var07
Var08
Var09
Var10
Var11
Var12
Var13
Var14
Var15
Var16
Var17
Var18
Var19
Var20
Var21
Var22
Var23
Var24
Var25
Var27
WT

FIG. 67

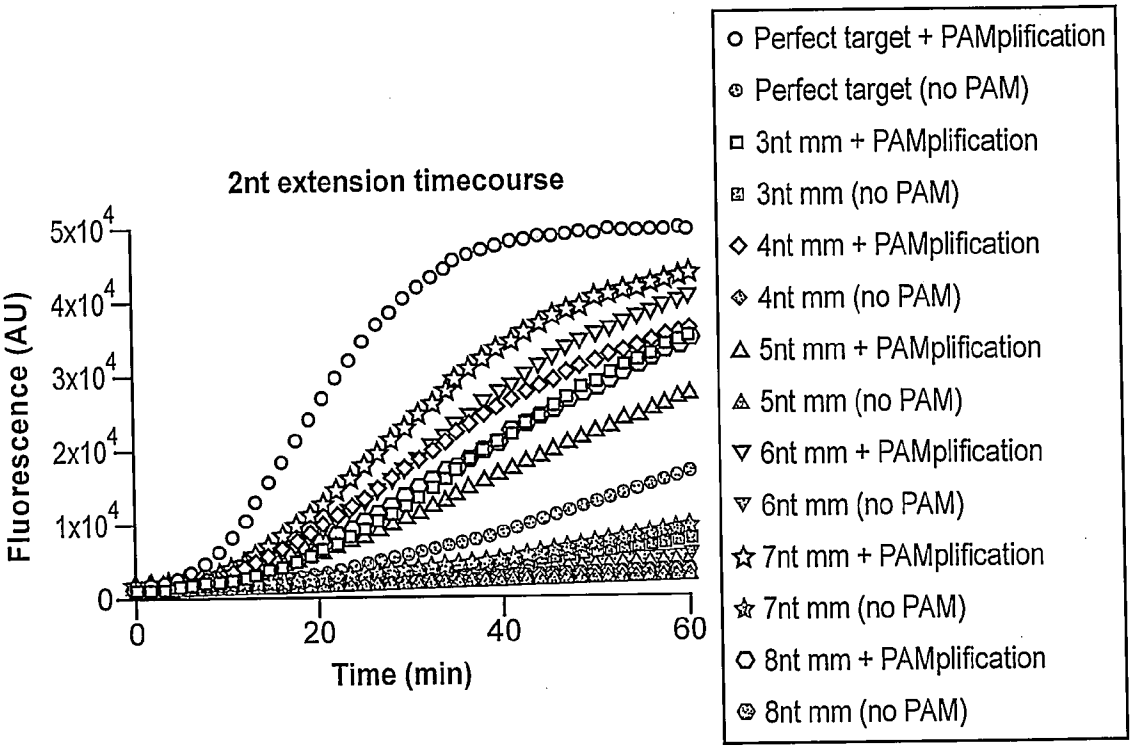


FIG. 68A

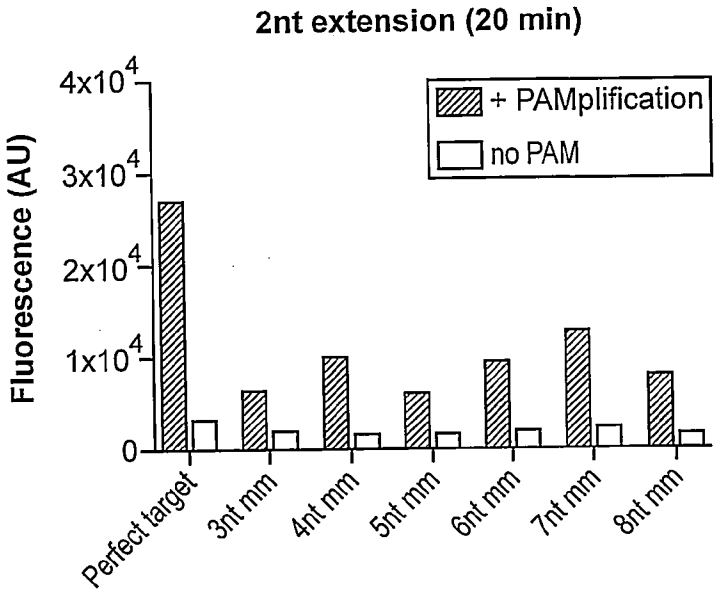


FIG. 68B

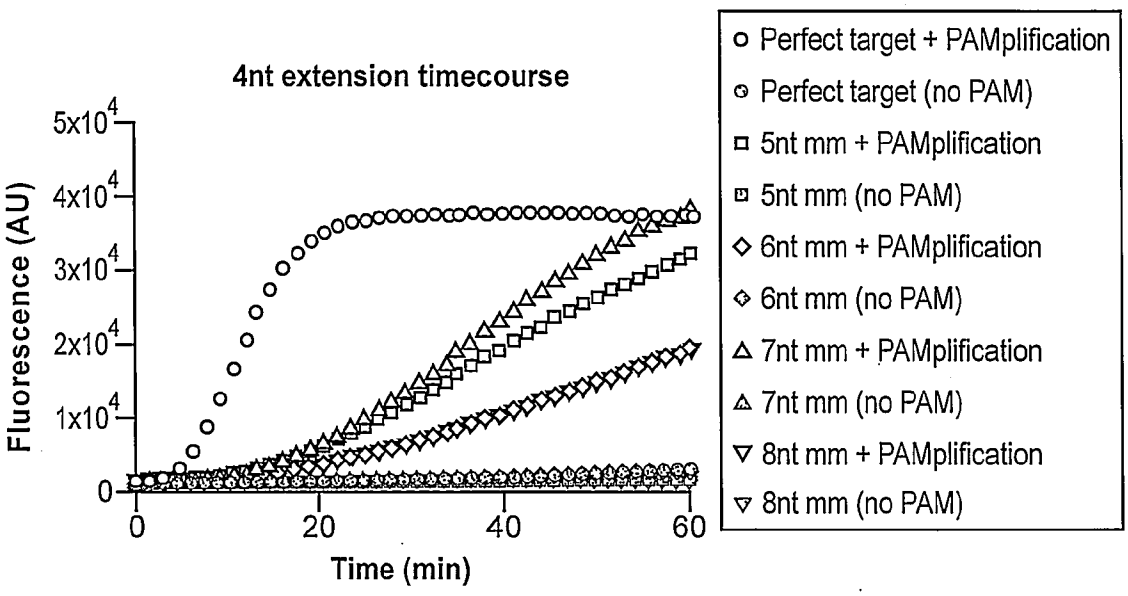


FIG. 69A

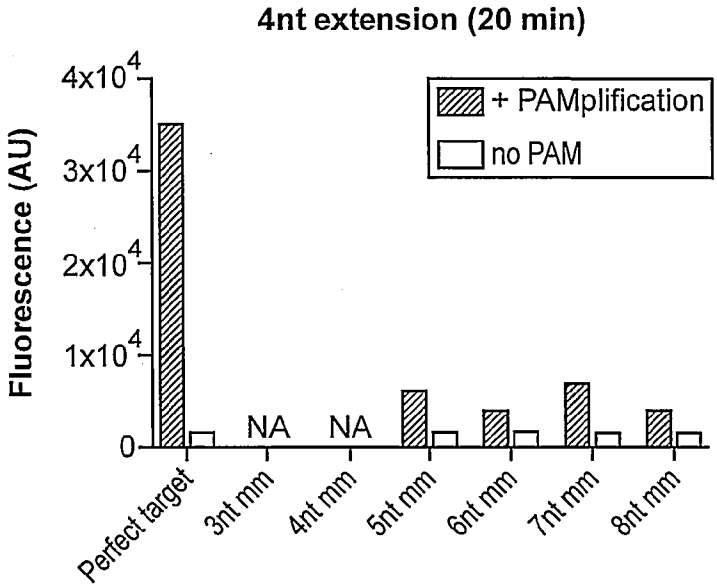


FIG. 69B

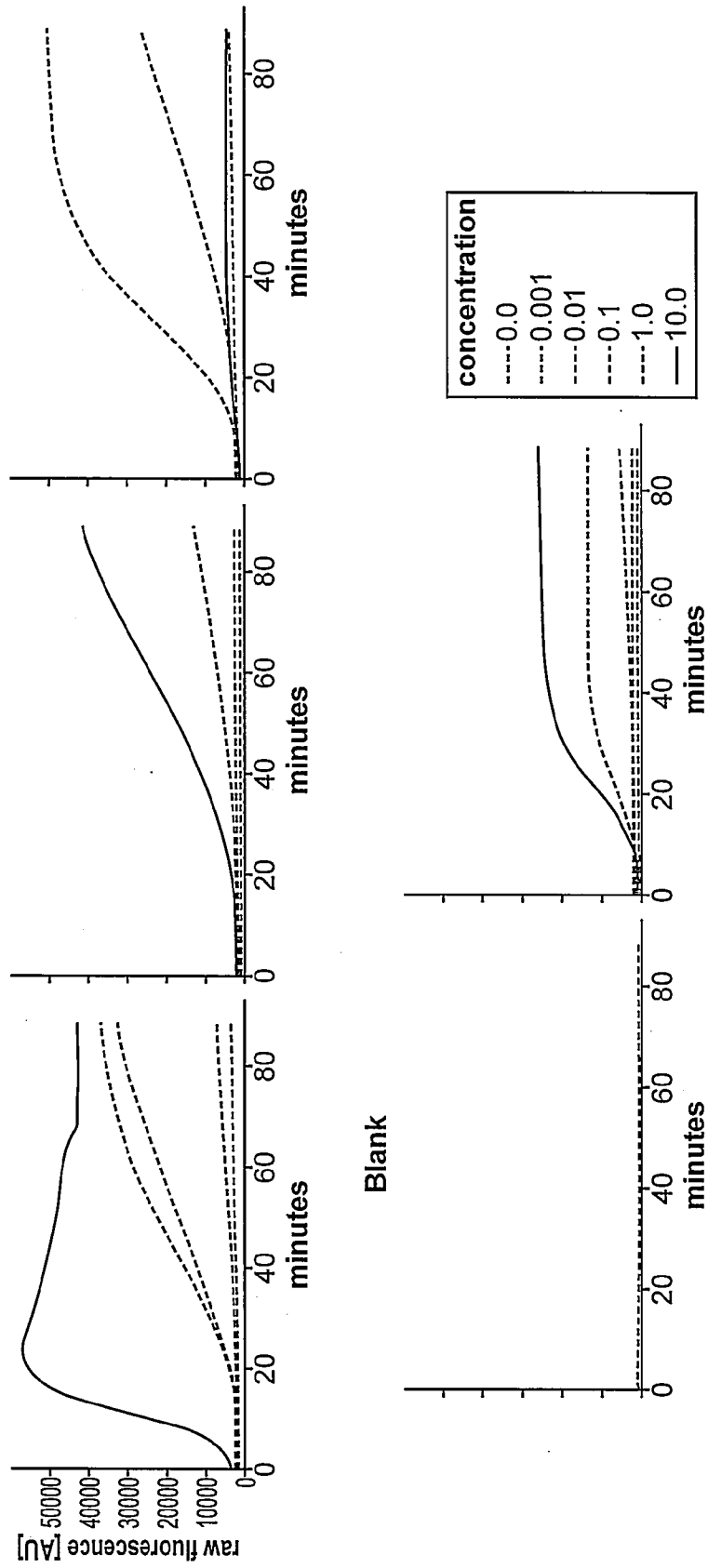

raw fluorescence [AU]
50000
40000
30000
20000
10000
0
0
20
40
60
80
minutes
Blank
concentration
0.0
0.001
0.01
0.1
1.0
10.0

FIG. 70A

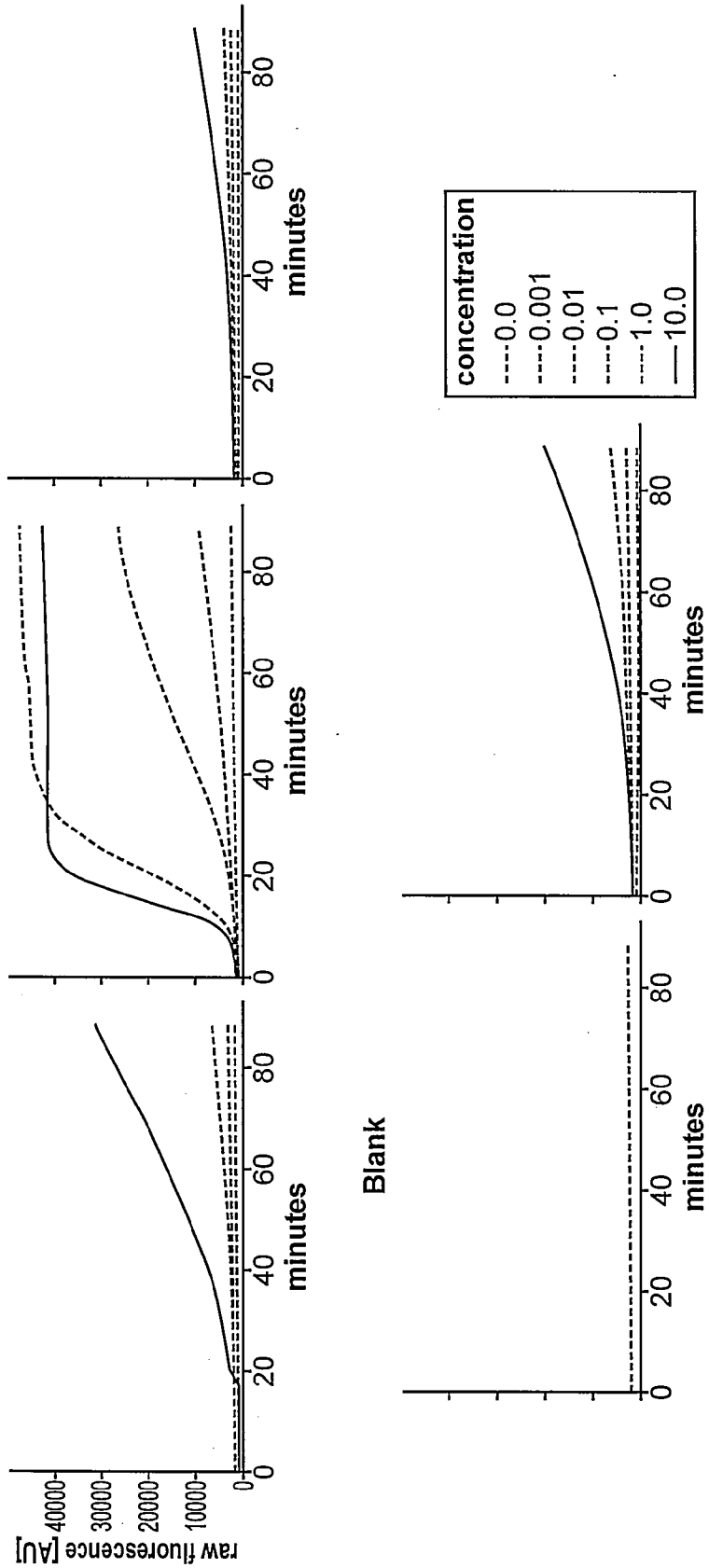

raw fluorescence [AU]
40000
30000
20000
10000
0
0
20
40
60
80
minutes
Blank
concentration
0.0
0.001
0.01
0.1
1.0
10.0

FIG. 70B

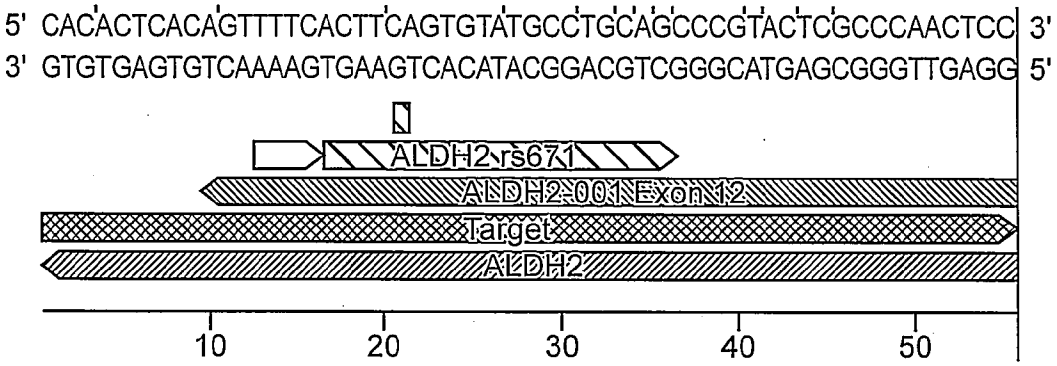


FIG. 71A

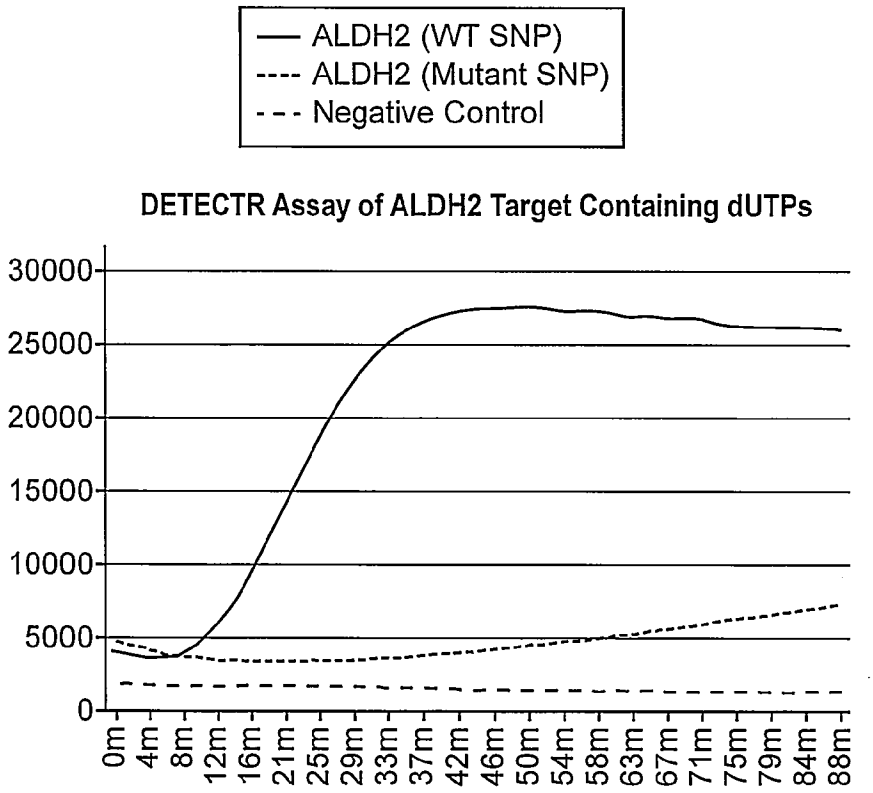


FIG. 71B

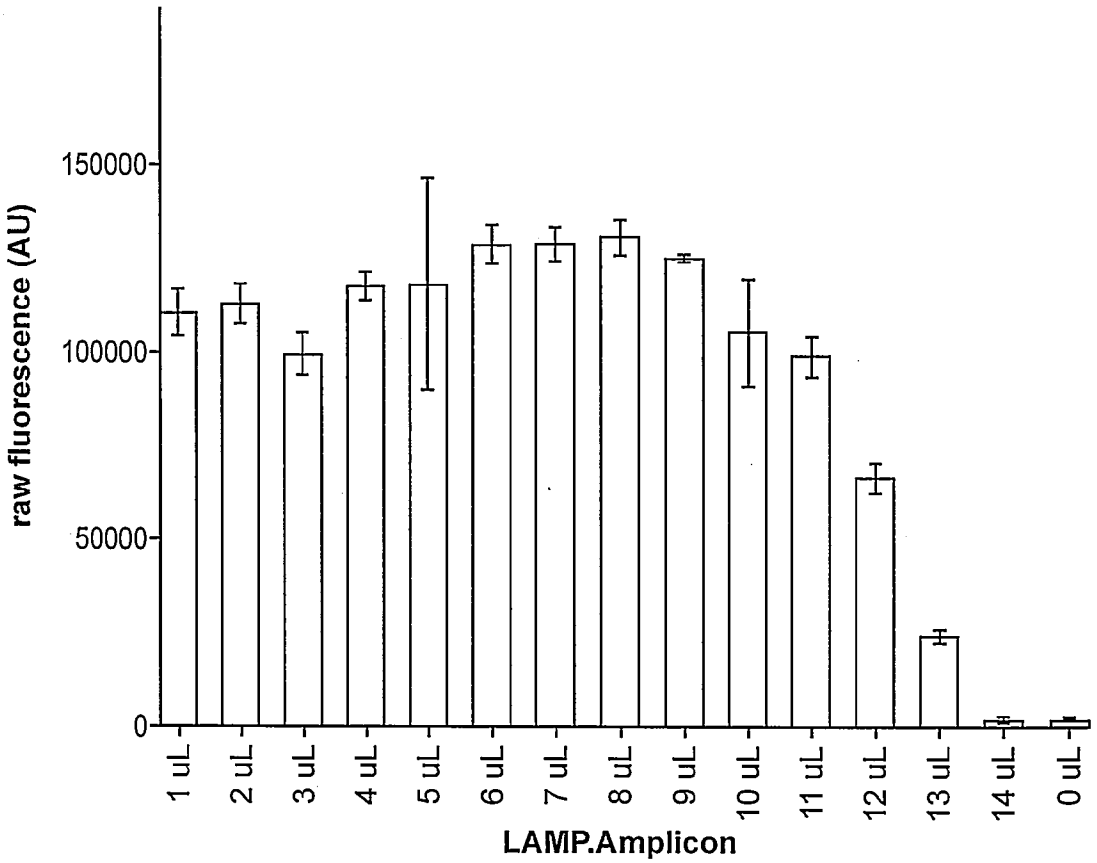

raw fluorescence (AU)
150000
100000
50000
0
1 uL
2 uL
3 uL
4 uL
5 uL
6 uL
7 uL
8 uL
9 uL
10 uL
11 uL
12 uL
13 uL
14 uL
0 uL
LAMP.Amplicon

FIG. 72

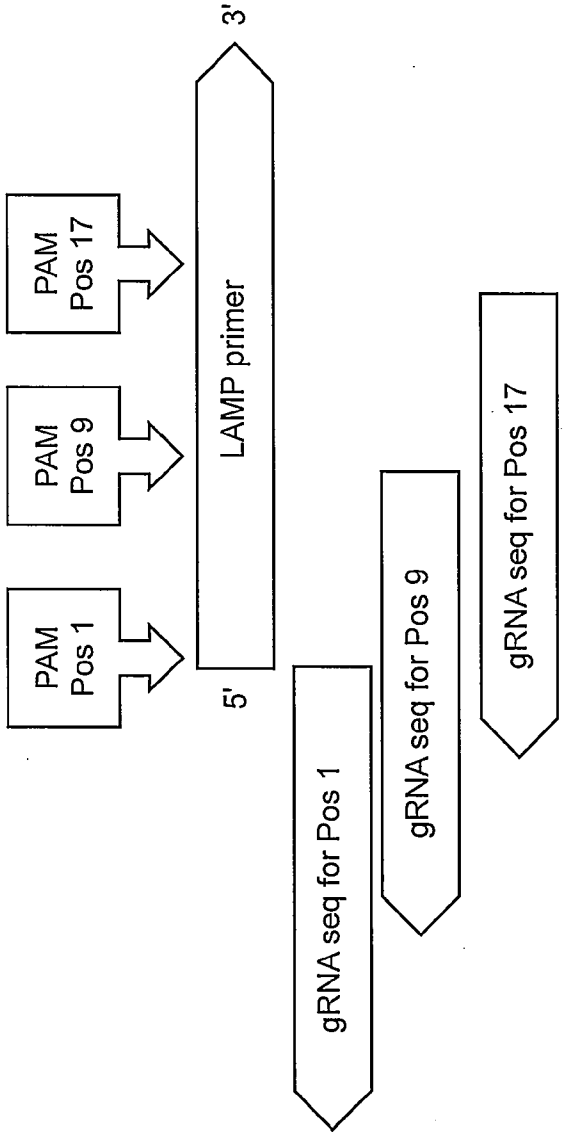

PAM Pos 1
PAM Pos 9
PAM Pos 17
LAMP primer
5'
3'
gRNA seq for Pos 1
gRNA seq for Pos 9
gRNA seq for Pos 17

FIG. 73

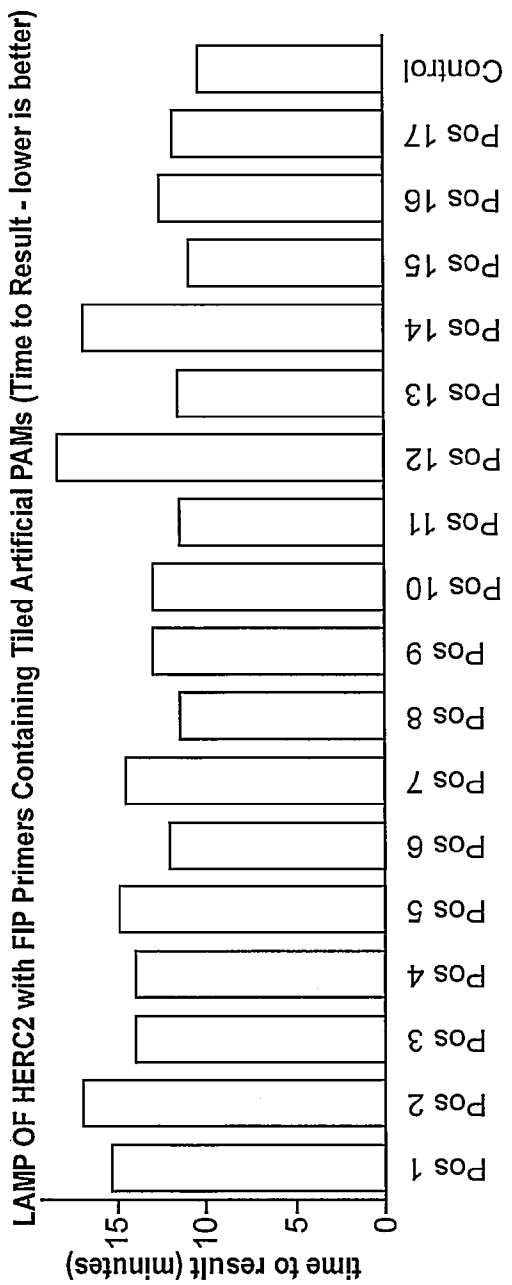

LAMP OF HERC2 with FIP Primers Containing Tiled Artificial PAMs (Time to Result - lower is better)
time to result (minutes)
0
5
10
15
Pos 1
Pos 2
Pos 3
Pos 4
Pos 5
Pos 6
Pos 7
Pos 8
Pos 9
Pos 10
Pos 11
Pos 12
Pos 13
Pos 14
Pos 15
Pos 16
Pos 17
Control
PAM

FIG. 74

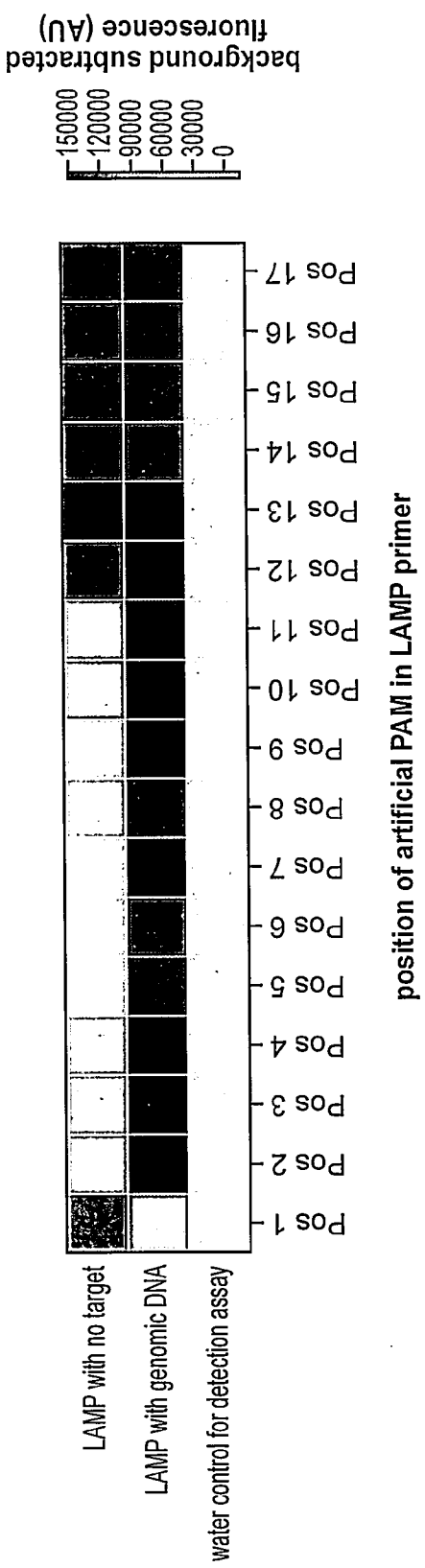

background subtracted fluorescence (AU)
150000
120000
90000
60000
30000
0
Pos 17
Pos 16
Pos 15
Pos 14
Pos 13
Pos 12
Pos 11
Pos 10
Pos 9
Pos 8
Pos 7
Pos 6
Pos 5
Pos 4
Pos 3
Pos 2
Pos 1
position of artificial PAM in LAMP primer
LAMP with no target
LAMP with genomic DNA
water control for detection assay

FIG. 75

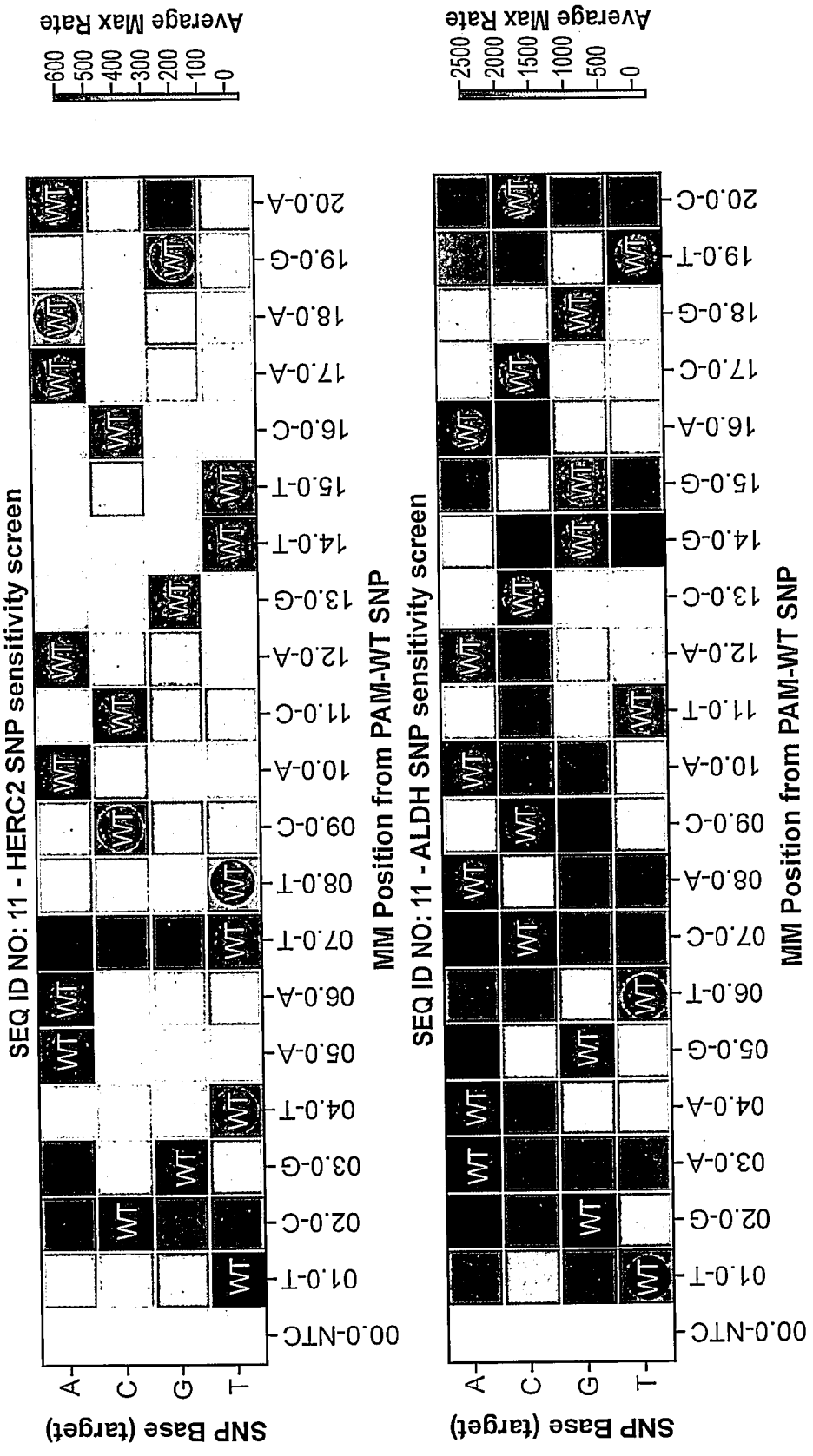

SEQ ID NO: 11 - HERC2 SNP sensitivity screen
Average Max Rate
600
500
400
300
200
100
0
SNP Base (target)
A
C
G
T
MM Position from PAM-WT SNP
00.0-NTC
01.0-T
02.0-C
03.0-G
04.0-T
05.0-A
06.0-A
07.0-T
08.0-T
09.0-C
10.0-A
11.0-C
12.0-A
13.0-G
14.0-T
15.0-T
16.0-C
17.0-A
18.0-A
19.0-G
20.0-A
WT
SEQ ID NO: 11 - ALDH SNP sensitivity screen
Average Max Rate
2500
2000
1500
1000
500
0
SNP Base (target)
A
C
G
T
MM Position from PAM-WT SNP
00.0-NTC
01.0-T
02.0-G
03.0-A
04.0-A
05.0-G
06.0-T
07.0-C
08.0-A
09.0-C
10.0-A
11.0-T
12.0-A
13.0-C
14.0-G
15.0-G
16.0-A
17.0-C
18.0-G
19.0-T
20.0-C
WT

FIG. 76

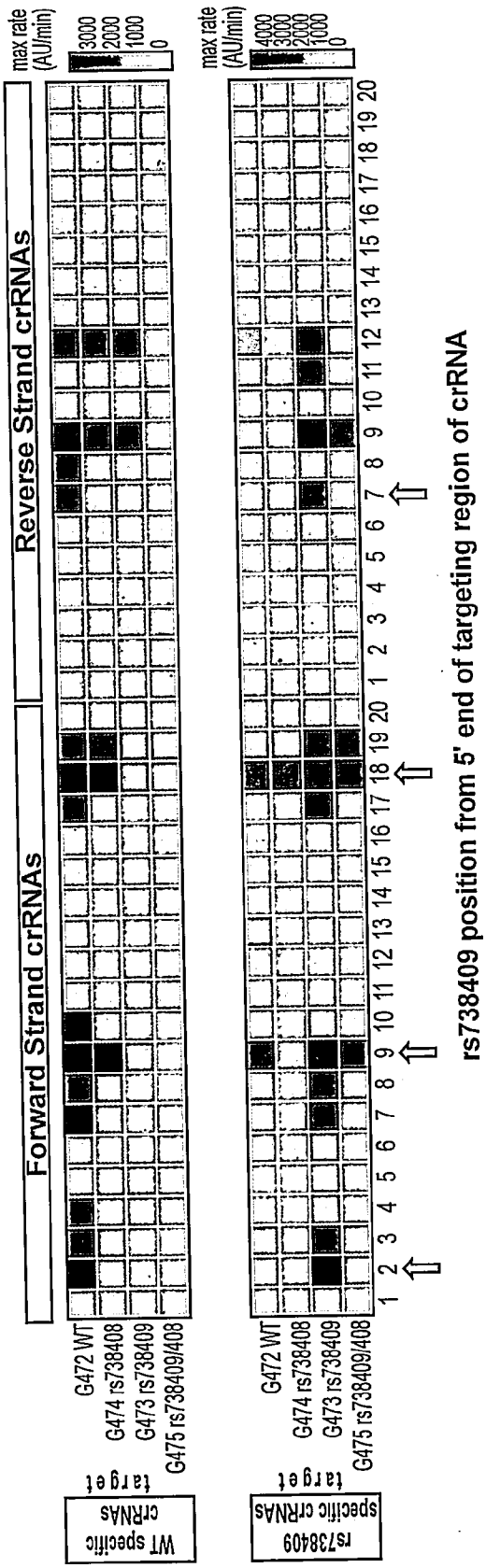

WT specific crRNAs
rs738409 specific crRNAs
target
G472 WT
G474 rs738408
G473 rs738409
G475 rs738409/408
Forward Strand crRNAs
Reverse Strand crRNAs
max rate (AU/min)
3000
2000
1000
0
4000
3000
2000
1000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
rs738409 position from 5' end of targeting region of crRNA

FIG. 77

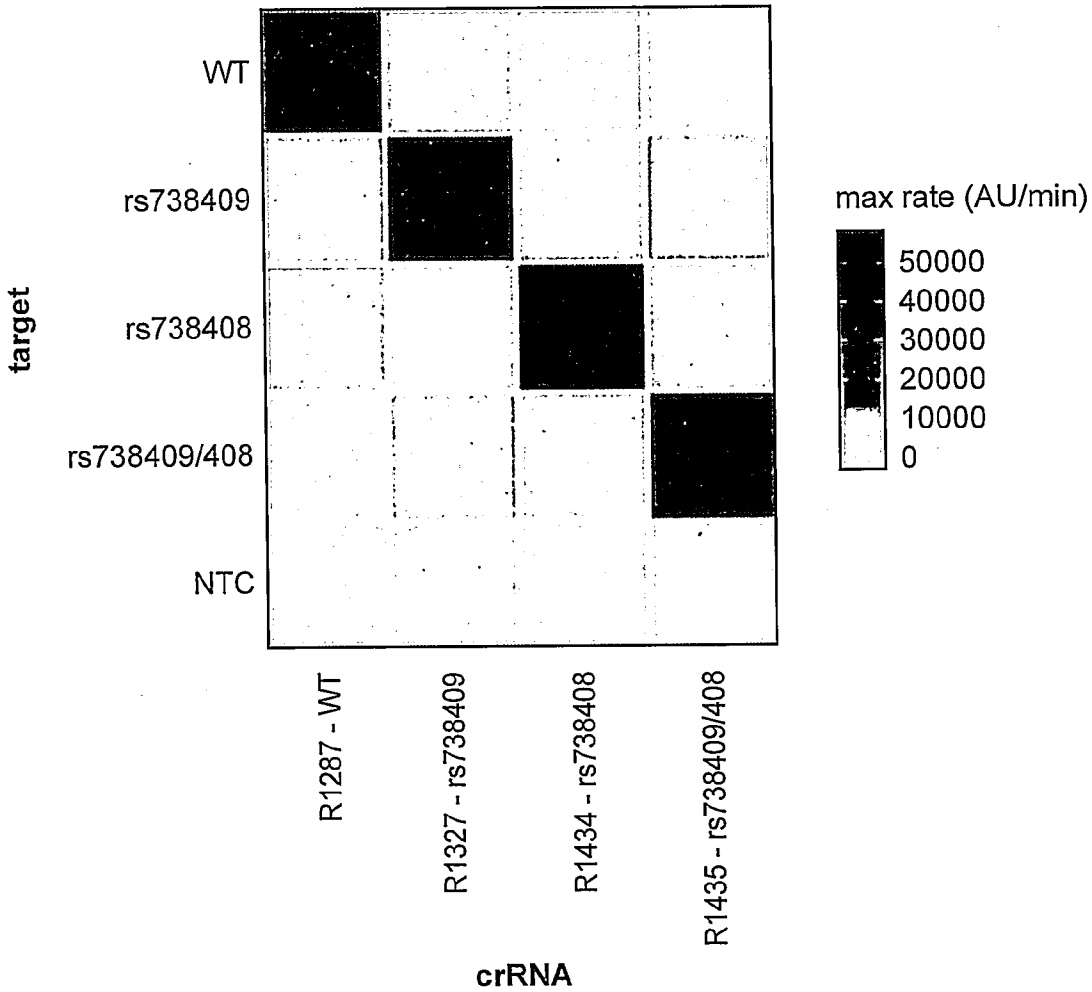

target
WT
rs738409
rs738408
rs738409/408
NTC
R1287 - WT
R1327 - rs738409
R1434 - rs738408
R1435 - rs738409/408
crRNA
max rate (AU/min)
50000
40000
30000
20000
10000
0

FIG. 78

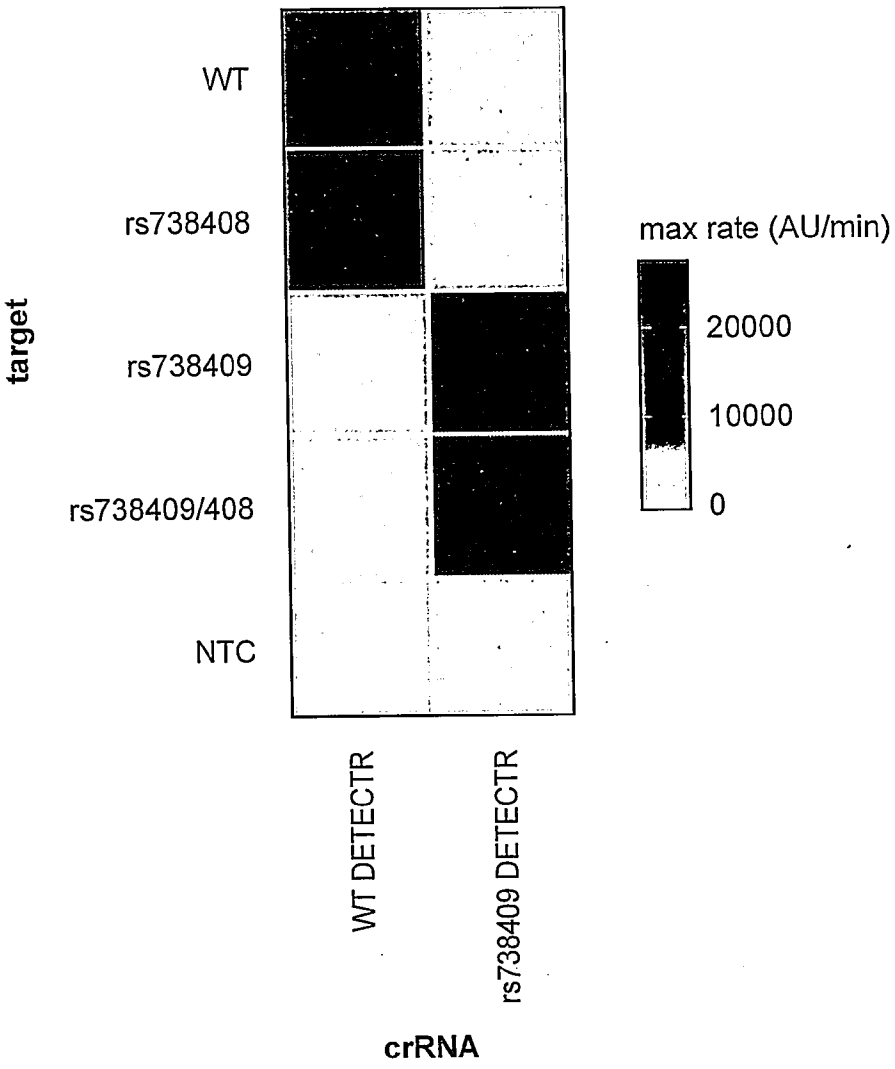

WT
rs738408
rs738409
rs738409/408
NTC
target
WT DETECTR
rs738409 DETECTR
crRNA
max rate (AU/min)
20000
10000
0

FIG. 79

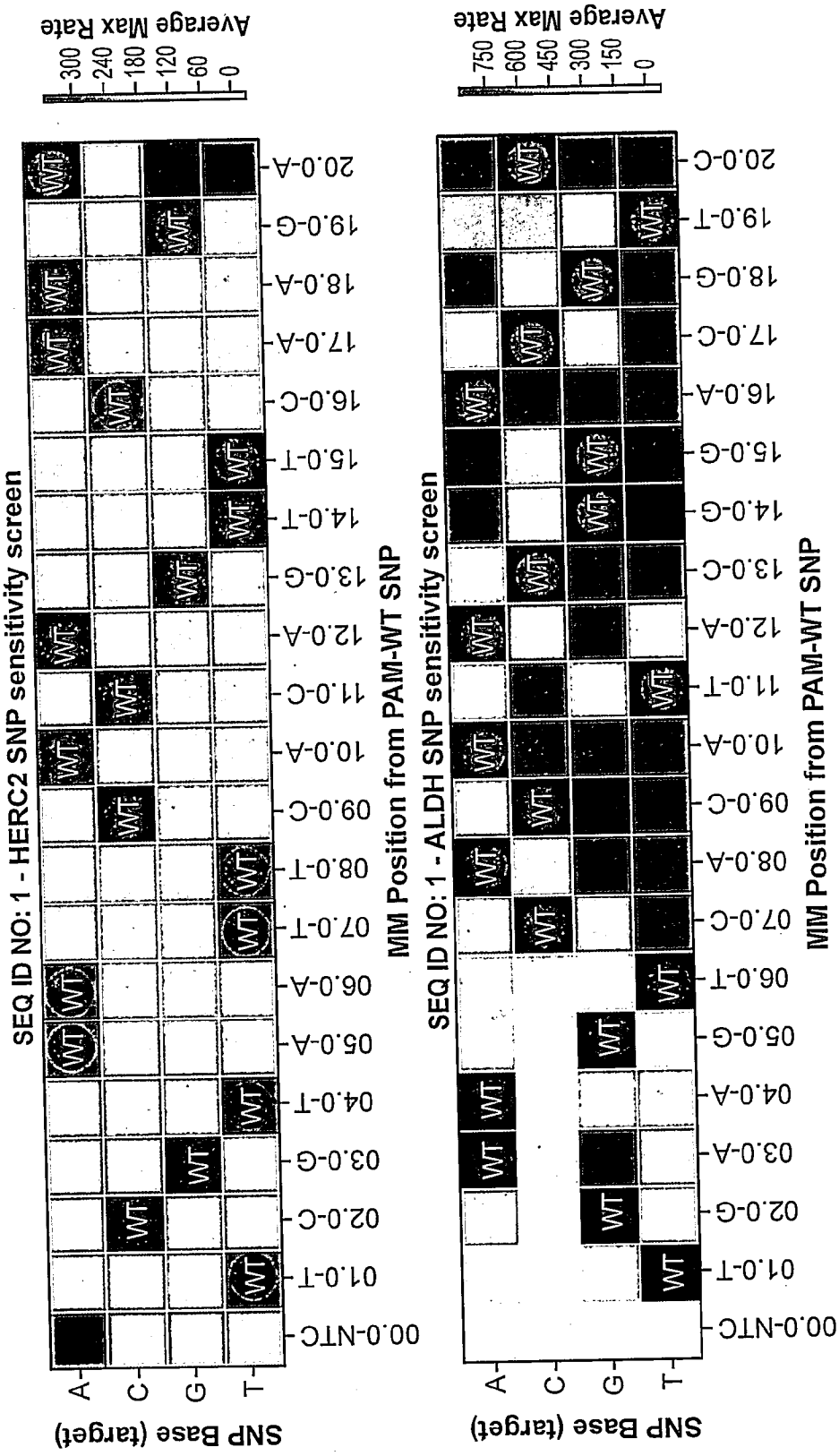

SEQ ID NO: 1 - HERC2 SNP sensitivity screen
Average Max Rate
300
240
180
120
60
0
MM Position from PAM-WT SNP
00.0-NTC
01.0-T
02.0-C
03.0-G
04.0-T
05.0-A
06.0-A
07.0-T
08.0-T
09.0-C
10.0-A
11.0-C
12.0-A
13.0-G
14.0-T
15.0-T
16.0-C
17.0-A
18.0-A
19.0-G
20.0-A
SNP Base (target)
A
C
G
T
SEQ ID NO: 1 - ALDH SNP sensitivity screen
Average Max Rate
750
600
450
300
150
0
MM Position from PAM-WT SNP
00.0-NTC
01.0-T
02.0-G
03.0-A
04.0-A
05.0-G
06.0-T
07.0-C
08.0-A
09.0-C
10.0-A
11.0-T
12.0-A
13.0-C
14.0-G
15.0-G
16.0-A
17.0-C
18.0-G
19.0-T
20.0-C
SNP Base (target)
A
C
G
T

FIG. 80

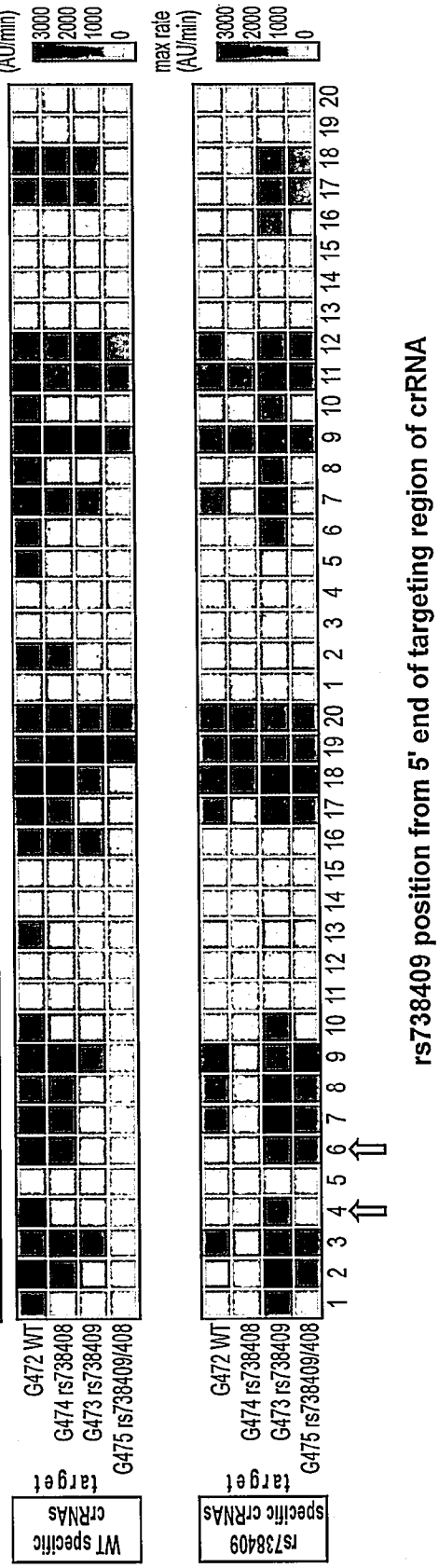

WT specific crRNAs
rs738409 specific crRNAs
target
G472 WT
G474 rs738408
G473 rs738409
G475 rs738409/408
Forward Strand crRNAs
Reverse Strand crRNAs
max rate (AU/min)
3000
2000
1000
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
rs738409 position from 5' end of targeting region of crRNA

FIG. 81

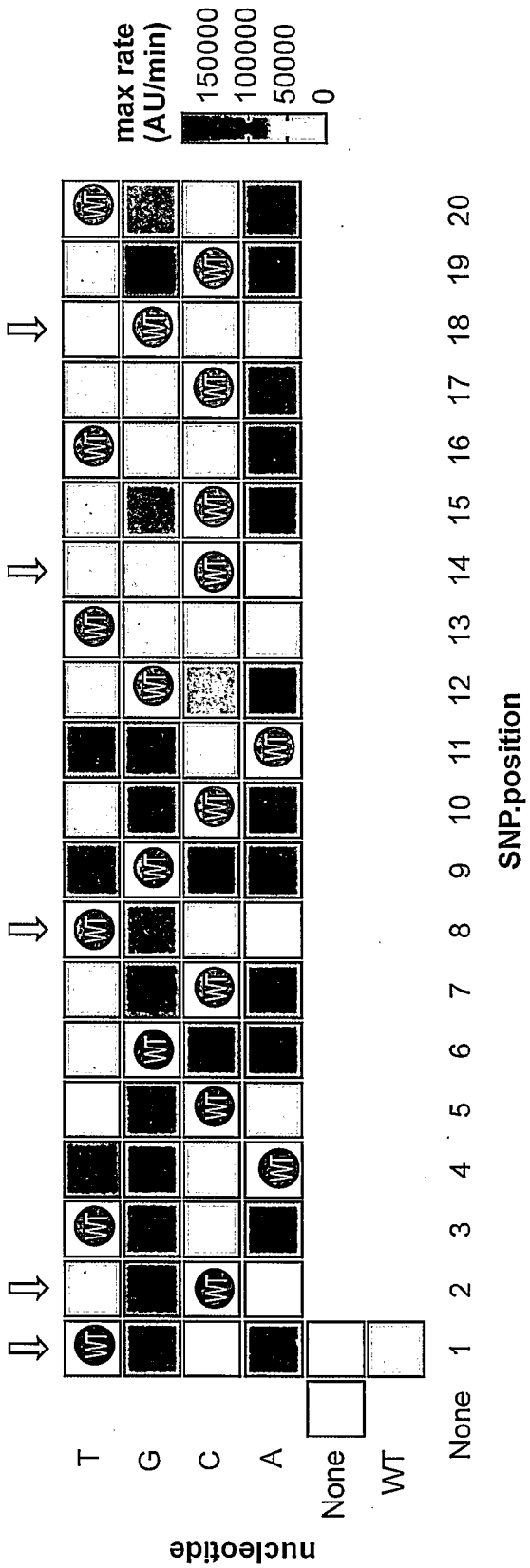

max rate (AU/min)
150000
100000
50000
0
nucleotide
T
G
C
A
None
WT
None 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
SNP.position

FIG. 82

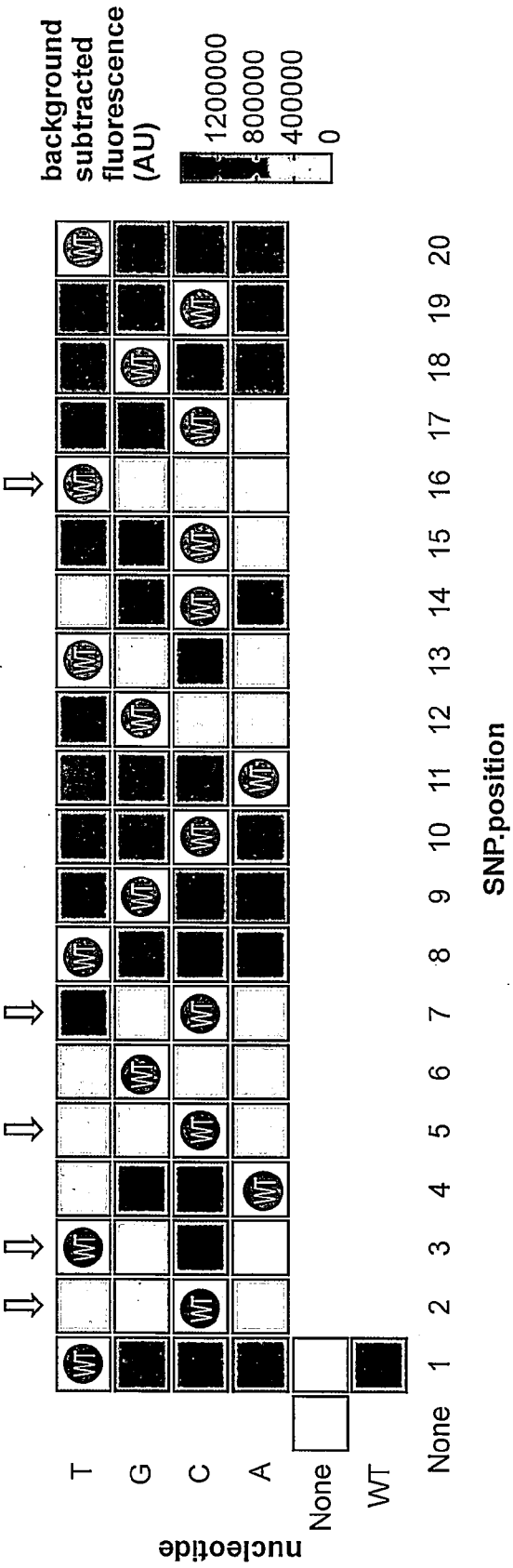

background subtracted fluorescence (AU)
1200000
800000
400000
0
nucleotide
T
G
C
A
None
WT
None 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
SNP.position

FIG. 83

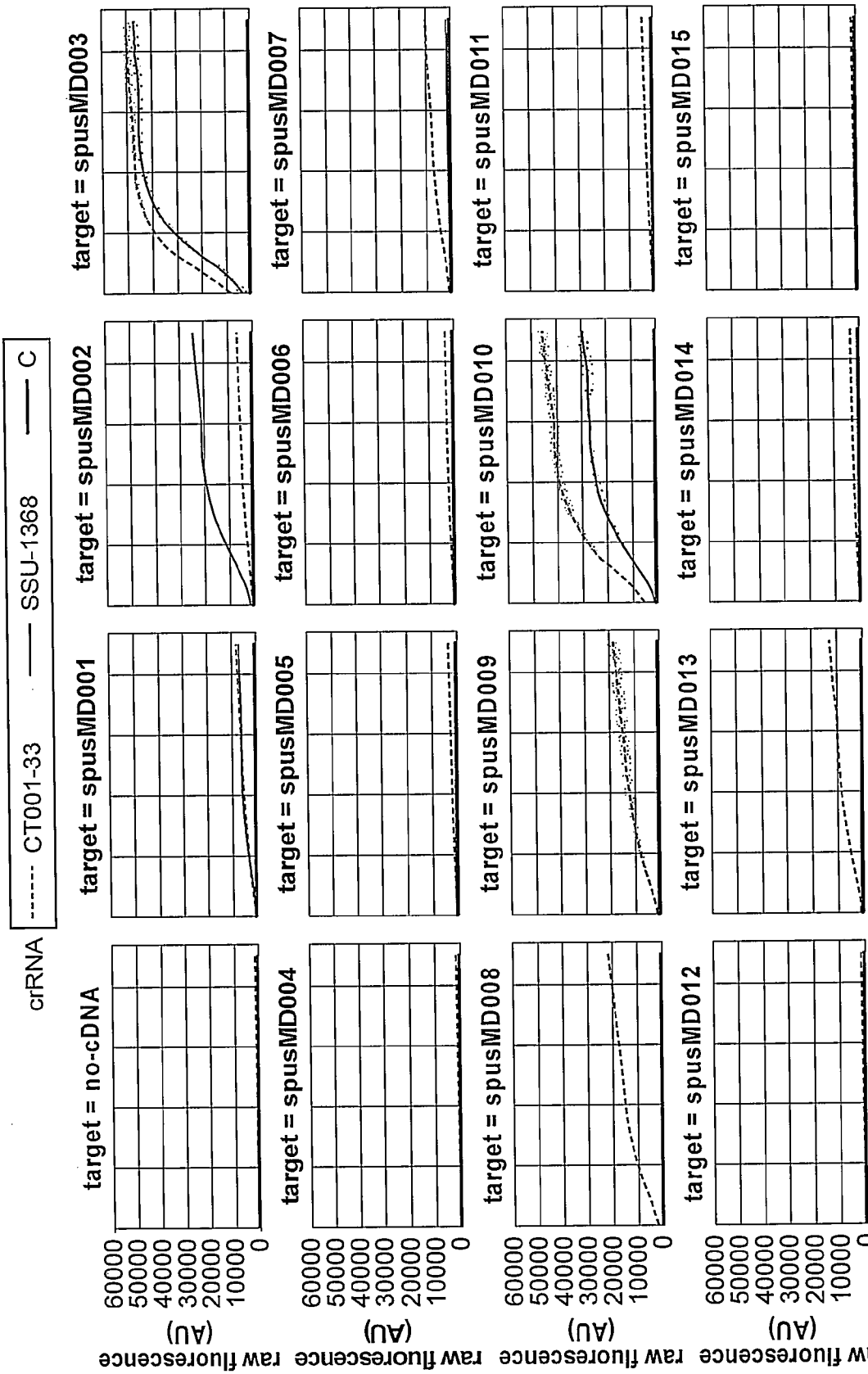

crRNA
CT001-33
SSU-1368
C
target = no-cDNA
target = spusMD001
target = spusMD002
target = spusMD003
target = spusMD004
target = spusMD005
target = spusMD006
target = spusMD007
target = spusMD008
target = spusMD009
target = spusMD010
target = spusMD011
target = spusMD012
target = spusMD013
target = spusMD014
target = spusMD015
raw fluorescence (AU)
60000
50000
40000
30000
20000
10000
0

FIG. 84

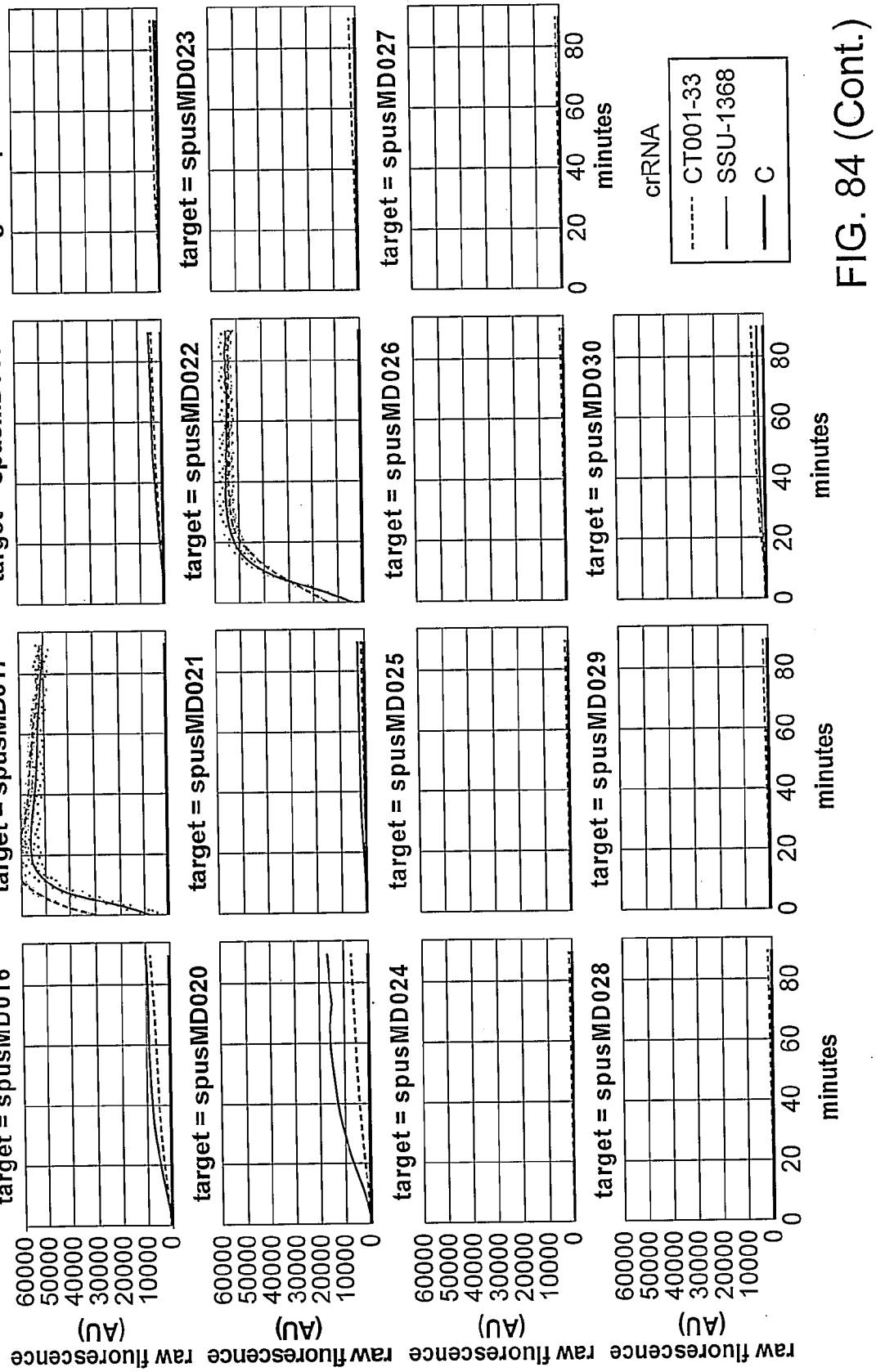

target = spusMD016
target = spusMD017
target = spusMD018
target = spusMD019
target = spusMD020
target = spusMD021
target = spusMD022
target = spusMD023
target = spusMD024
target = spusMD025
target = spusMD026
target = spusMD027
target = spusMD028
target = spusMD029
target = spusMD030
raw fluorescence (AU)
60000
50000
40000
30000
20000
10000
0
0
20
40
60
80
minutes
crRNA
CT001-33
SSU-1368
C

FIG. 84 (Cont.)

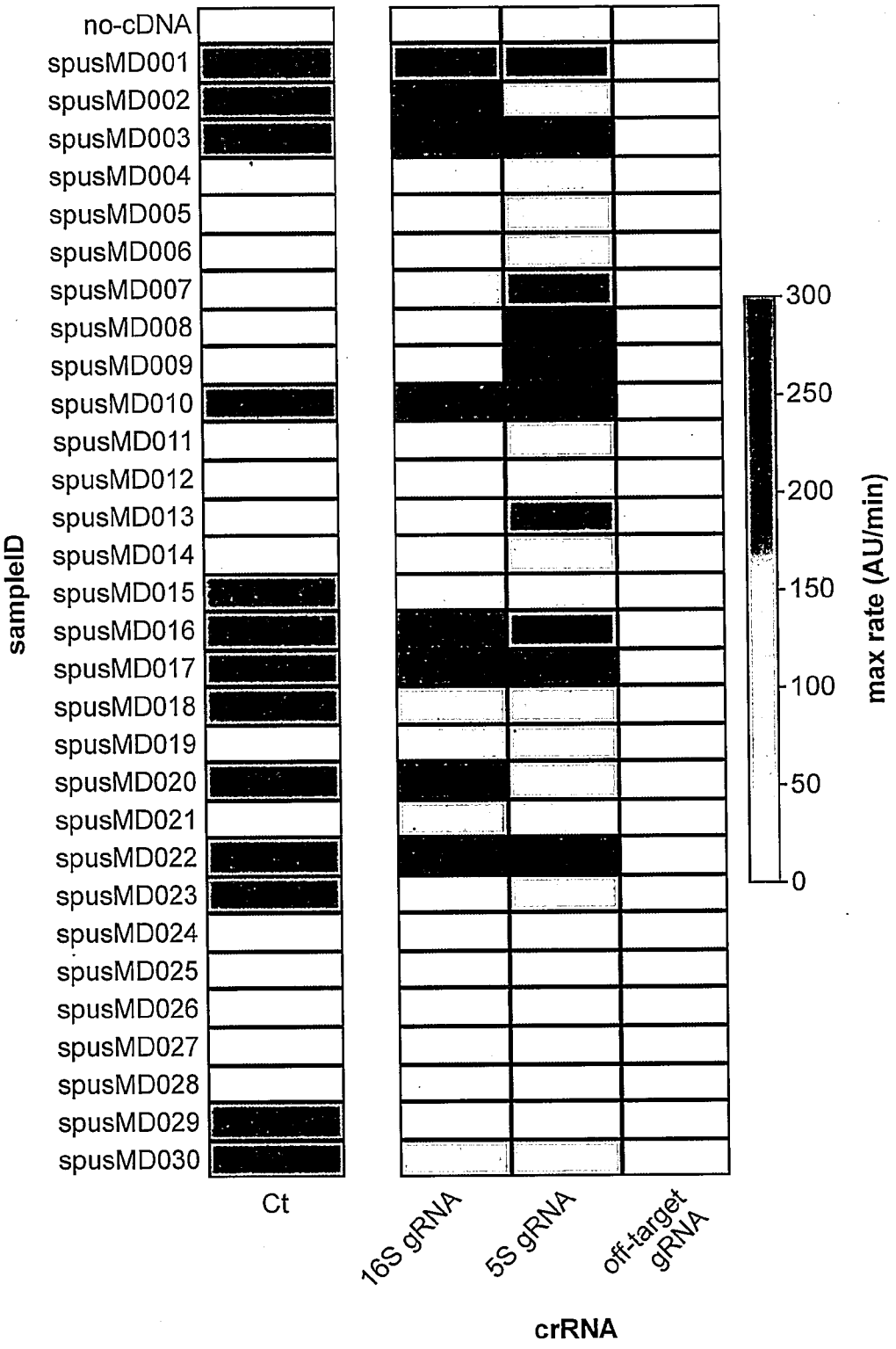

no-cDNA
spusMD001
spusMD002
spusMD003
spusMD004
spusMD005
spusMD006
spusMD007
spusMD008
spusMD009
spusMD010
spusMD011
spusMD012
spusMD013
spusMD014
spusMD015
spusMD016
spusMD017
spusMD018
spusMD019
spusMD020
spusMD021
spusMD022
spusMD023
spusMD024
spusMD025
spusMD026
spusMD027
spusMD028
spusMD029
spusMD030
sampleID
Ct
16S gRNA
5S gRNA
off-target gRNA
crRNA
300
250
200
150
100
50
0
max rate (AU/min)

FIG. 85

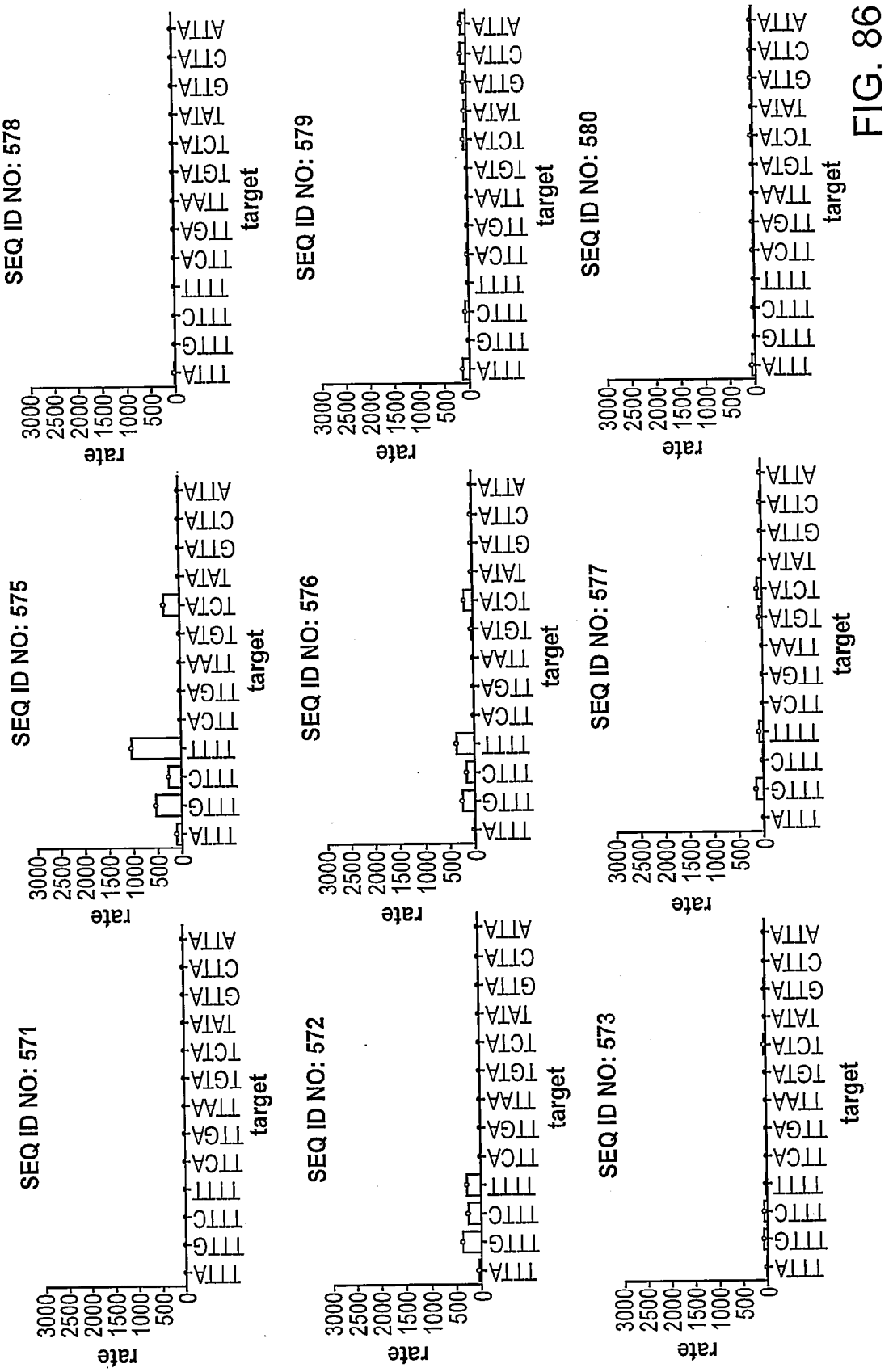

SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
rate
target
TTTA
TTTG
TTTC
TTTT
TTCA
TTGA
TTAA
TGTA
TCTA
TATA
GTTA
CTTA
ATTA
0
500
1000
1500
2000
2500
3000

FIG. 86

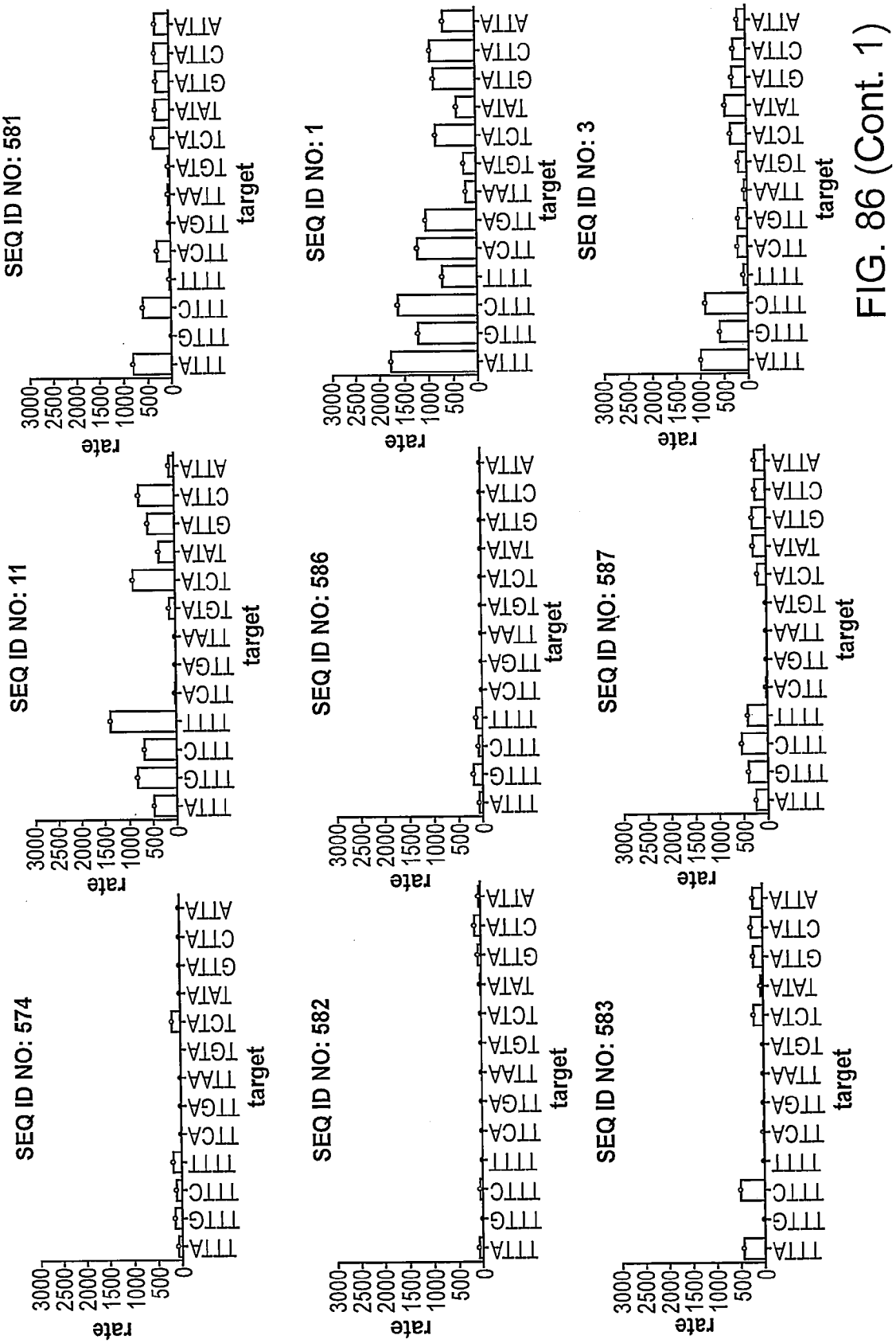

SEQ ID NO: 581
SEQ ID NO: 1
SEQ ID NO: 3
SEQ ID NO: 11
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 574
SEQ ID NO: 582
SEQ ID NO: 583
rate
target
TTTA
TTTG
TTTC
TTTT
TTCA
TTGA
TTAA
TGTA
TCTA
TATA
GTTA
CTTA
ATTA
0
500
1000
1500
2000
2500
3000

FIG. 86 (Cont. 1)

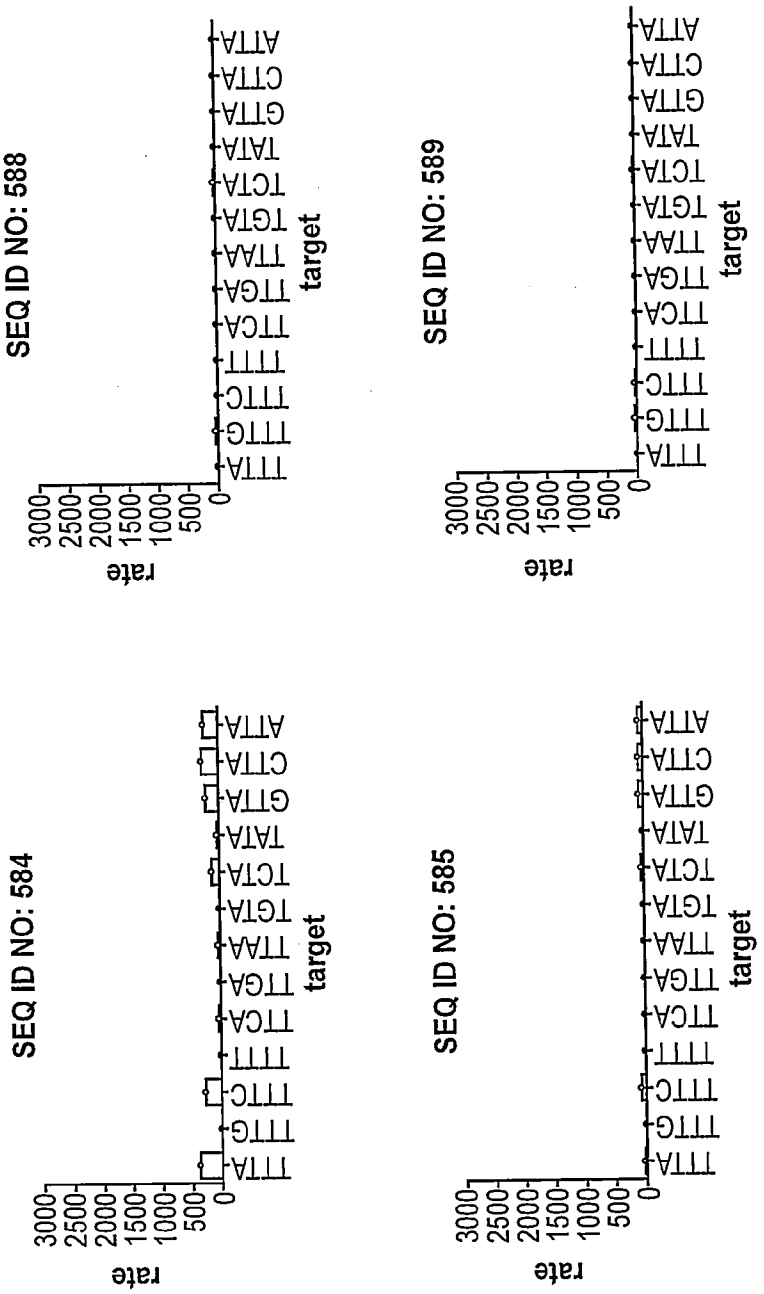

SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 584
SEQ ID NO: 585
rate
target
3000
2500
2000
1500
1000
500
0
TTTA
TTTG
TTTC
TTTT
TTCA
TTGA
TTAA
TGTA
TCTA
TATA
GTTA
CTTA
ATTA

FIG. 86 (Cont. 2)

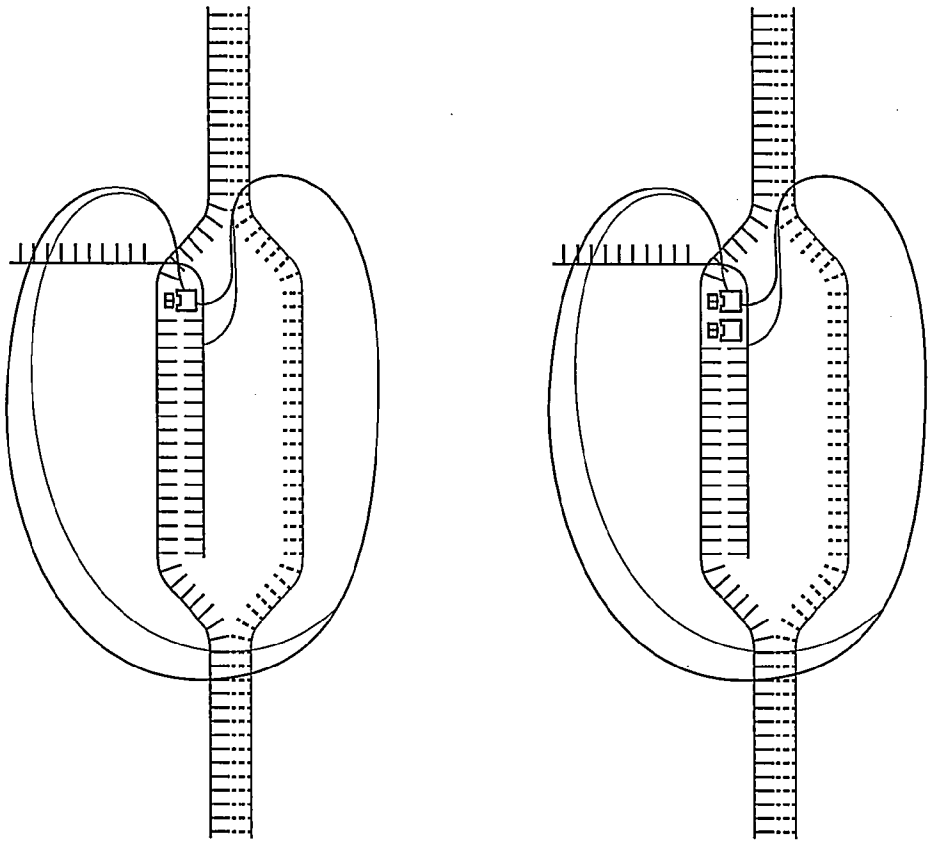


FIG. 87A

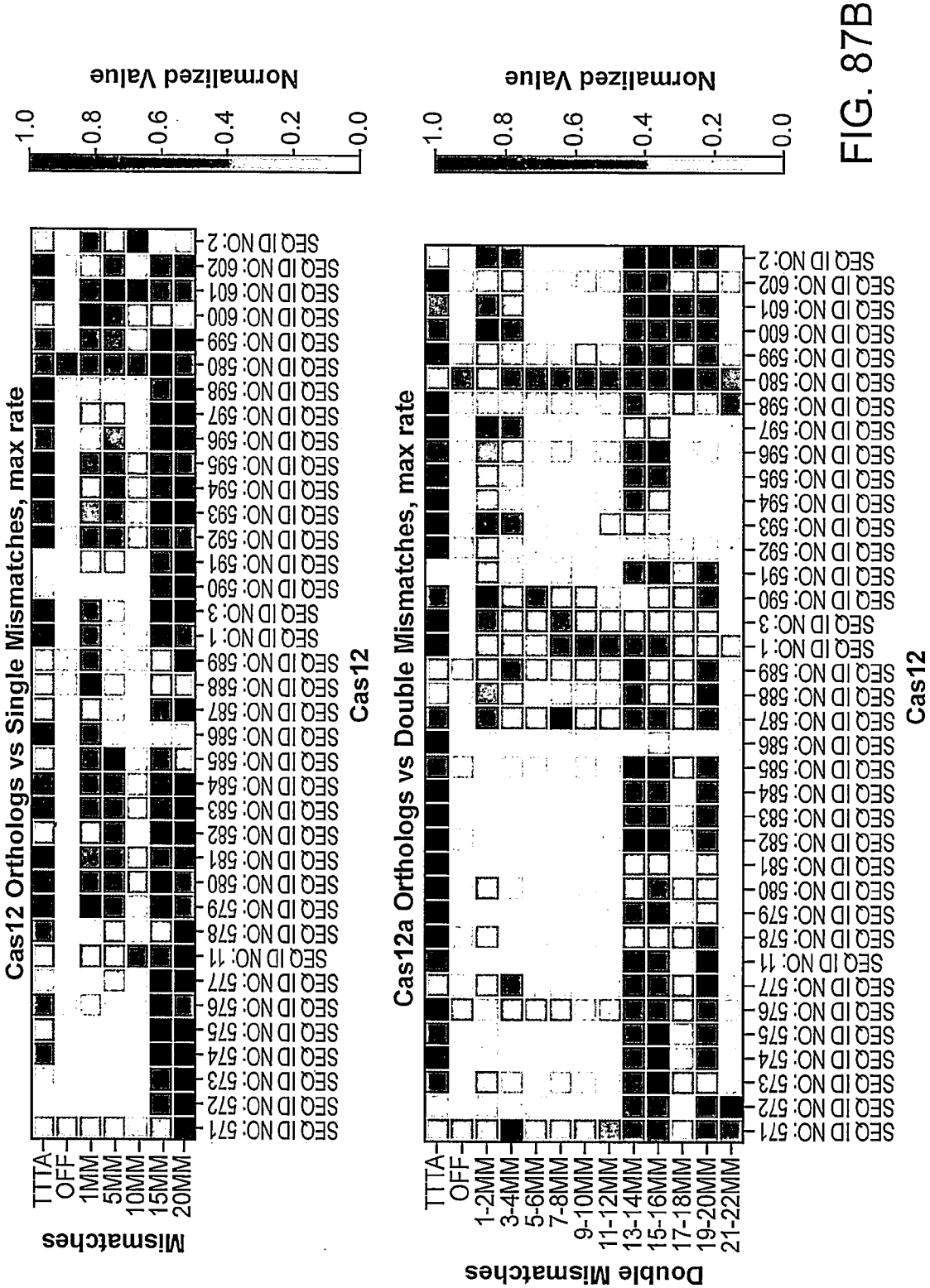

Cas12a Orthologs vs Double Mismatches, max rate
Double Mismatches
TTTA
OFF
1-2MM
3-4MM
5-6MM
7-8MM
9-10MM
11-12MM
13-14MM
15-16MM
17-18MM
19-20MM
21-22MM
Cas12
Normalized Value
1.0
0.8
0.6
0.4
0.2
0.0
Cas12 Orthologs vs Single Mismatches, max rate
Mismatches
TTTA
OFF
1MM
5MM
10MM
15MM
20MM
Cas12
Normalized Value
1.0
0.8
0.6
0.4
0.2
0.0

FIG. 87B

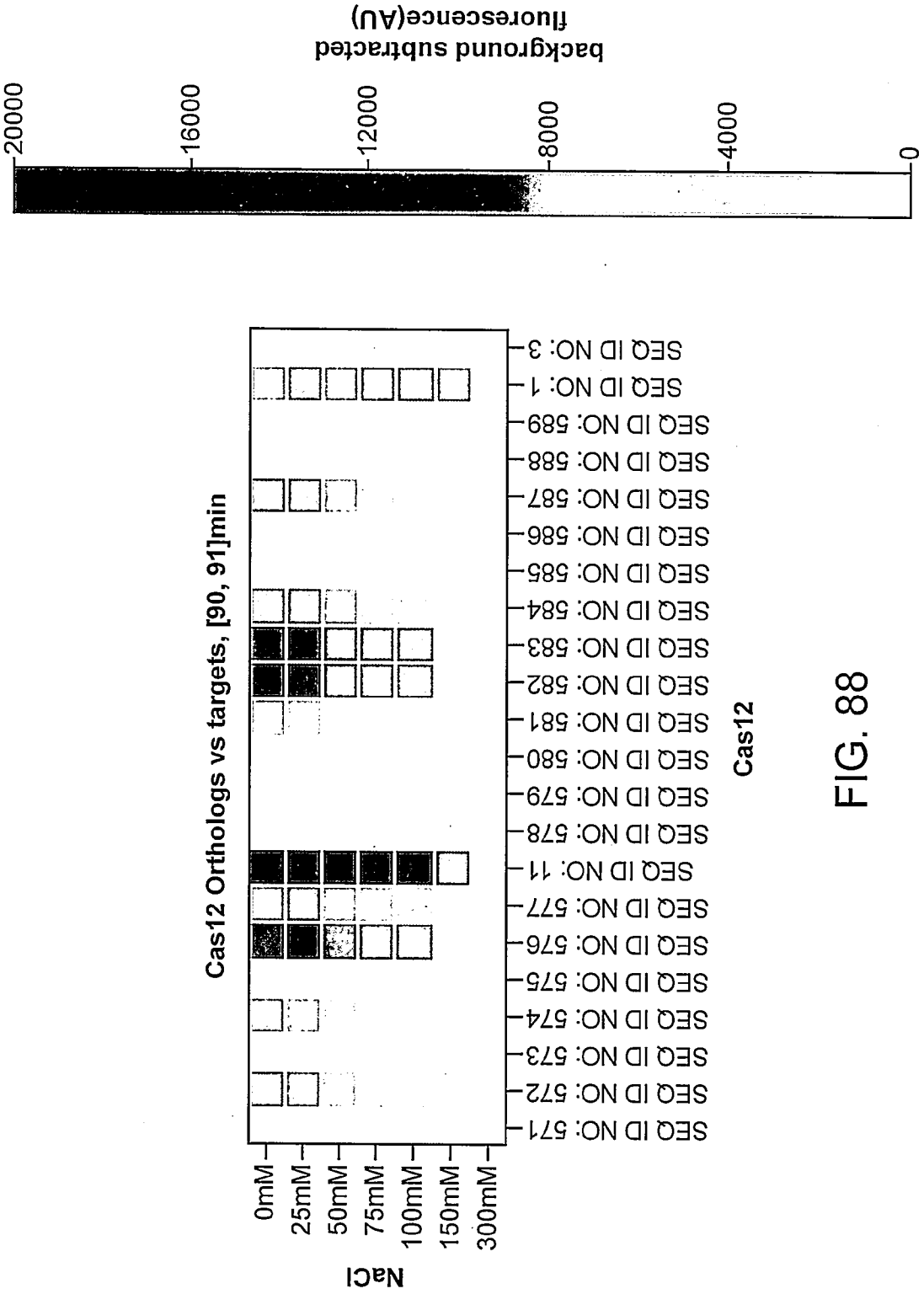

background subtracted
fluorescence(AU)
20000
16000
12000
8000
4000
0
Cas12 Orthologs vs targets, [90, 91]min
NaCl
0mM
25mM
50mM
75mM
100mM
150mM
300mM
Cas12
SEQ ID NO: 571
SEQ ID NO: 572
SEQ ID NO: 573
SEQ ID NO: 574
SEQ ID NO: 575
SEQ ID NO: 576
SEQ ID NO: 577
SEQ ID NO: 11
SEQ ID NO: 578
SEQ ID NO: 579
SEQ ID NO: 580
SEQ ID NO: 581
SEQ ID NO: 582
SEQ ID NO: 583
SEQ ID NO: 584
SEQ ID NO: 585
SEQ ID NO: 586
SEQ ID NO: 587
SEQ ID NO: 588
SEQ ID NO: 589
SEQ ID NO: 1
SEQ ID NO: 3

FIG. 88

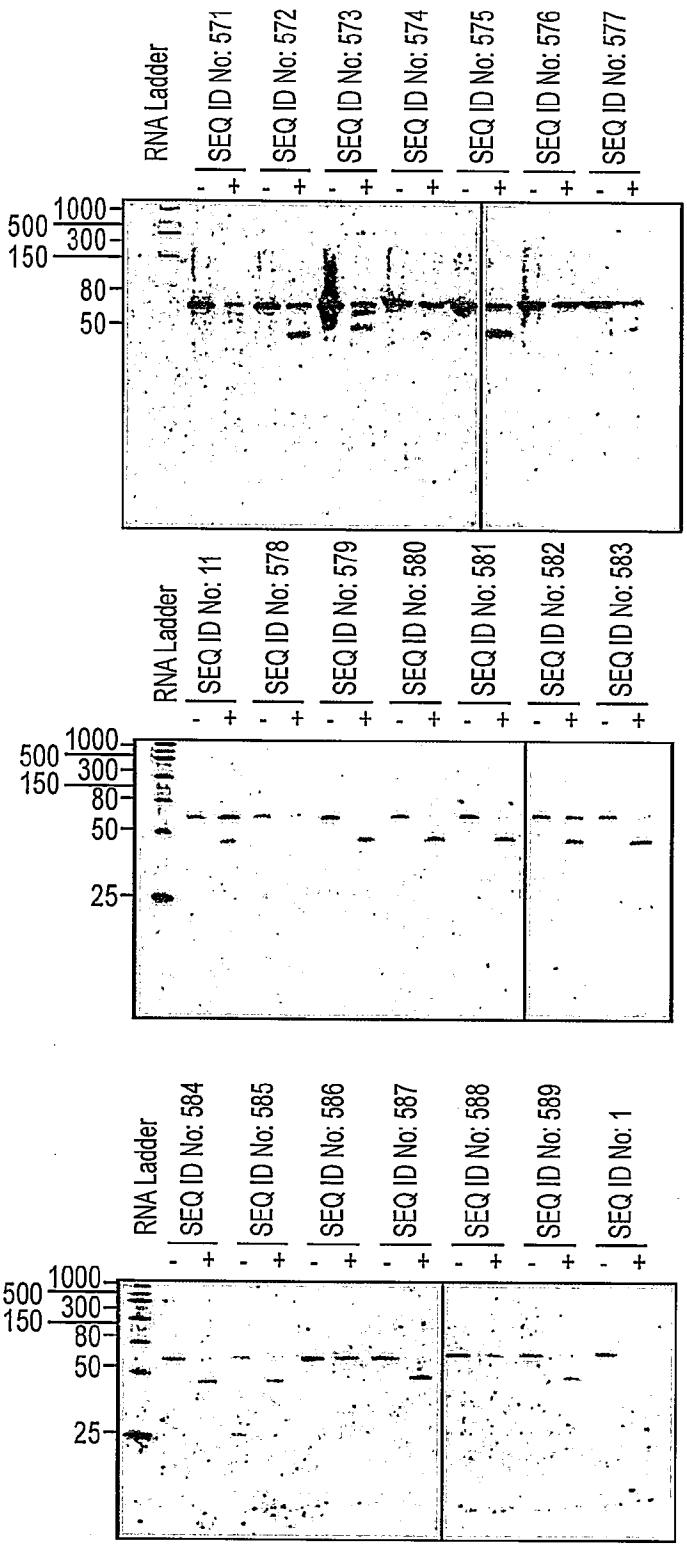

RNA Ladder
SEQ ID No: 571
SEQ ID No: 572
SEQ ID No: 573
SEQ ID No: 574
SEQ ID No: 575
SEQ ID No: 576
SEQ ID No: 577
1000
500
300
150
80
50
RNA Ladder
SEQ ID No: 11
SEQ ID No: 578
SEQ ID No: 579
SEQ ID No: 580
SEQ ID No: 581
SEQ ID No: 582
SEQ ID No: 583
1000
500
300
150
80
50
25
RNA Ladder
SEQ ID No: 584
SEQ ID No: 585
SEQ ID No: 586
SEQ ID No: 587
SEQ ID No: 588
SEQ ID No: 589
SEQ ID No: 1
1000
500
300
150
80
50
25

FIG. 89

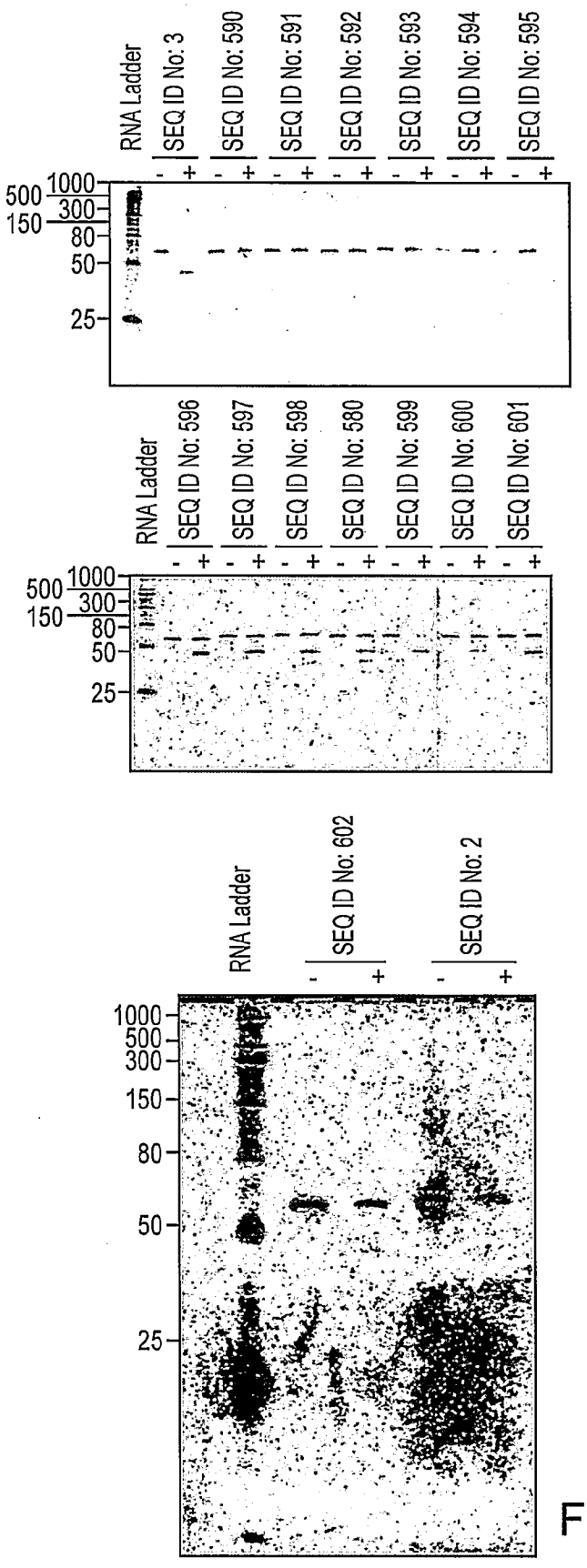

RNA Ladder
SEQ ID No: 3
SEQ ID No: 590
SEQ ID No: 591
SEQ ID No: 592
SEQ ID No: 593
SEQ ID No: 594
SEQ ID No: 595
1000
500
300
150
80
50
25
RNA Ladder
SEQ ID No: 596
SEQ ID No: 597
SEQ ID No: 598
SEQ ID No: 580
SEQ ID No: 599
SEQ ID No: 600
SEQ ID No: 601
RNA Ladder
SEQ ID No: 602
SEQ ID No: 2

FIG. 89 (Cont.)

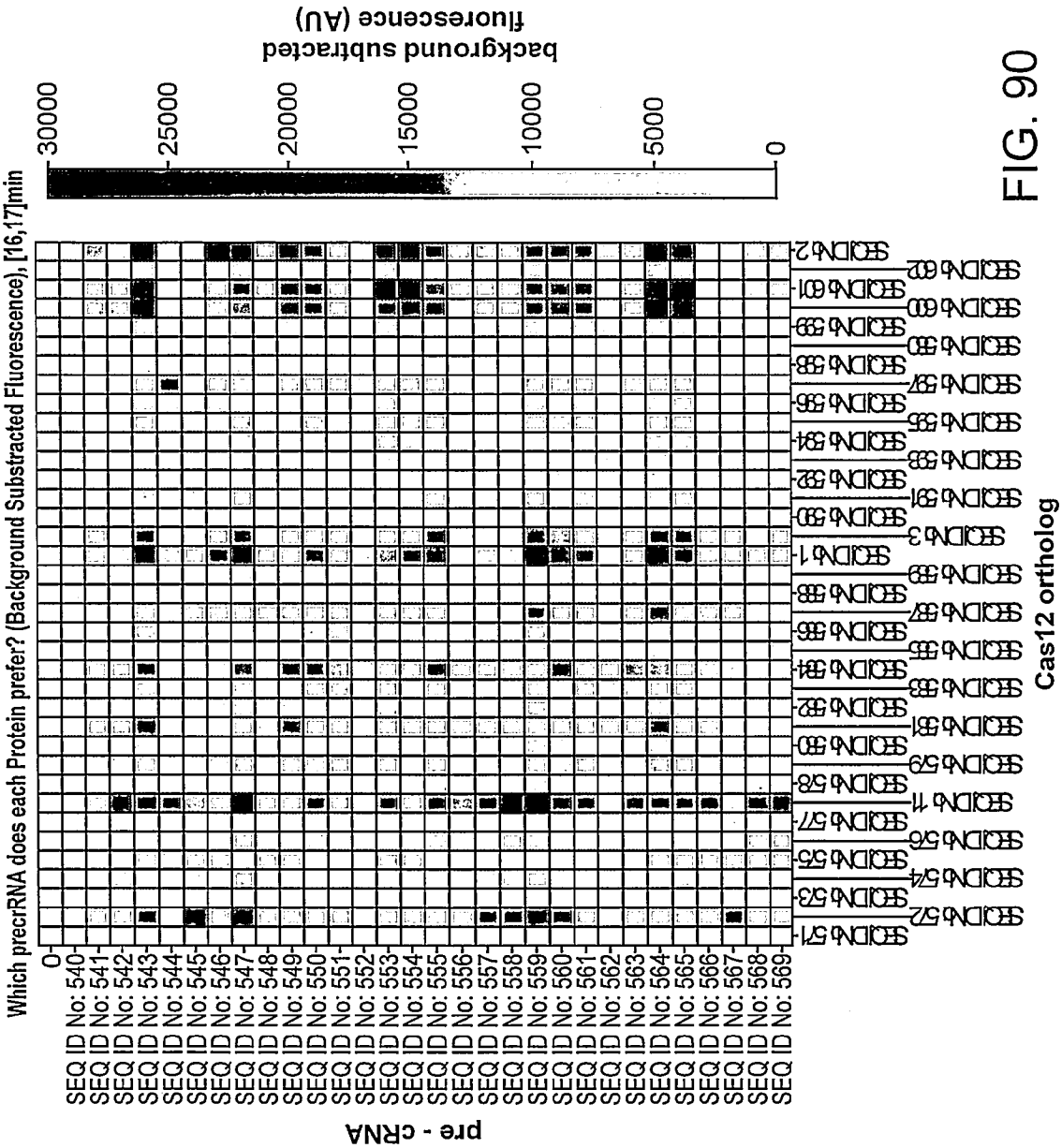

Which precrRNA does each Protein prefer? (Background Substracted Fluorescence), [16,17]min
background subtracted fluorescence (AU)
30000
25000
20000
15000
10000
5000
0
pre - crRNA
SEQ ID No: 540
SEQ ID No: 541
SEQ ID No: 542
SEQ ID No: 543
SEQ ID No: 544
SEQ ID No: 545
SEQ ID No: 546
SEQ ID No: 547
SEQ ID No: 548
SEQ ID No: 549
SEQ ID No: 550
SEQ ID No: 551
SEQ ID No: 552
SEQ ID No: 553
SEQ ID No: 554
SEQ ID No: 555
SEQ ID No: 556
SEQ ID No: 557
SEQ ID No: 558
SEQ ID No: 559
SEQ ID No: 560
SEQ ID No: 561
SEQ ID No: 562
SEQ ID No: 563
SEQ ID No: 564
SEQ ID No: 565
SEQ ID No: 566
SEQ ID No: 567
SEQ ID No: 568
SEQ ID No: 569
SEQ ID No: 571
SEQ ID No: 572
SEQ ID No: 573
SEQ ID No: 574
SEQ ID No: 575
SEQ ID No: 576
SEQ ID No: 577
SEQ ID No: 578
SEQ ID No: 579
SEQ ID No: 580
SEQ ID No: 581
SEQ ID No: 582
SEQ ID No: 583
SEQ ID No: 584
SEQ ID No: 585
SEQ ID No: 586
SEQ ID No: 587
SEQ ID No: 588
SEQ ID No: 589
SEQ ID No: 1
SEQ ID No: 3
SEQ ID No: 590
SEQ ID No: 591
SEQ ID No: 592
SEQ ID No: 593
SEQ ID No: 594
SEQ ID No: 595
SEQ ID No: 596
SEQ ID No: 597
SEQ ID No: 598
SEQ ID No: 599
SEQ ID No: 600
SEQ ID No: 601
SEQ ID No: 602
SEQ ID No: 2
Cas12 ortholog

FIG. 90

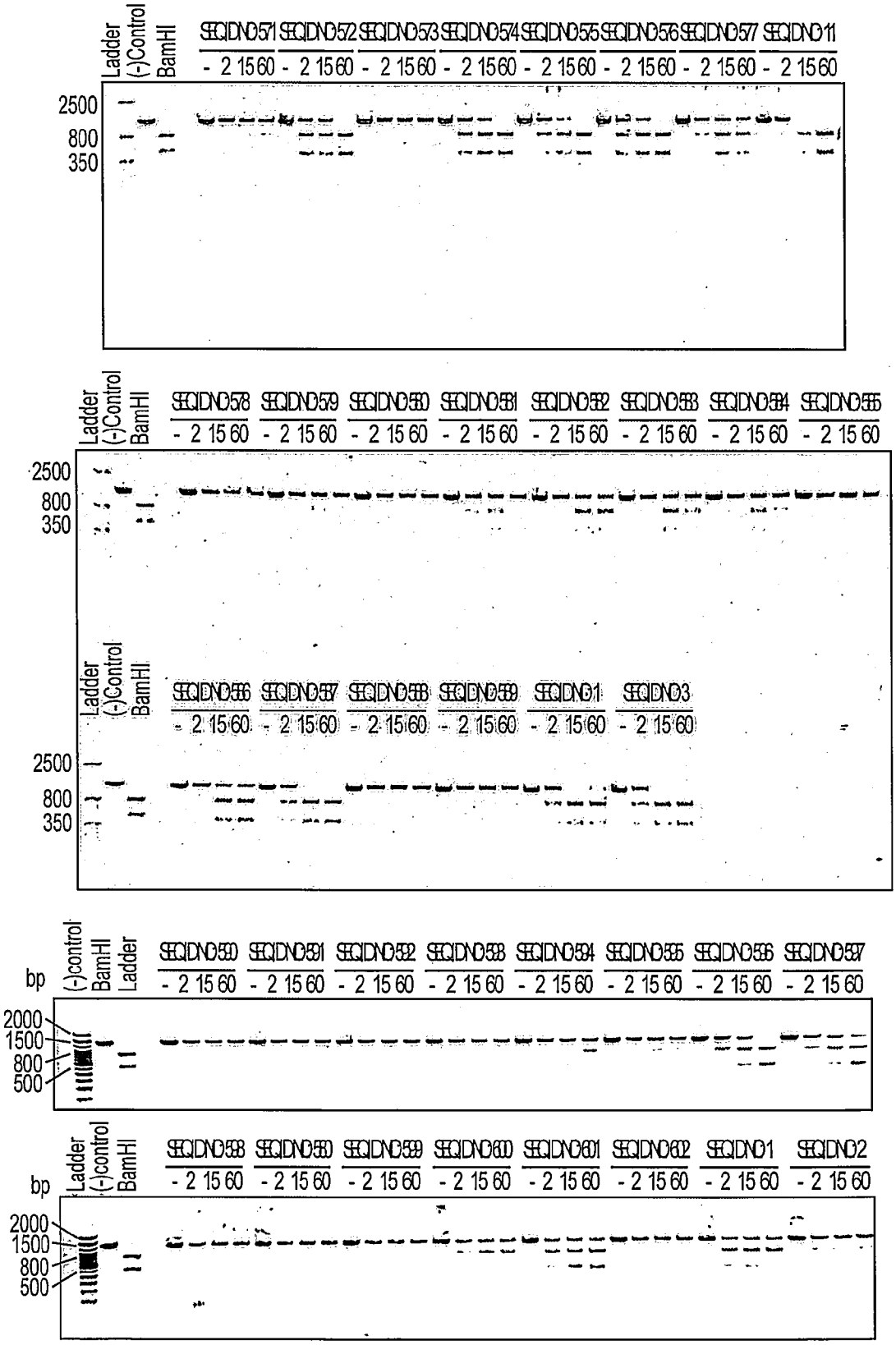

Ladder
(-)Control
BamHI
SEQ ID NO:571
SEQ ID NO:572
SEQ ID NO:573
SEQ ID NO:574
SEQ ID NO:575
SEQ ID NO:576
SEQ ID NO:577
SEQ ID NO:11
- 2 15 60
2500
800
350
SEQ ID NO:578
SEQ ID NO:579
SEQ ID NO:580
SEQ ID NO:581
SEQ ID NO:582
SEQ ID NO:583
SEQ ID NO:584
SEQ ID NO:585
SEQ ID NO:586
SEQ ID NO:587
SEQ ID NO:588
SEQ ID NO:589
SEQ ID NO:1
SEQ ID NO:3
bp
(-)control
2000
1500
800
500
SEQ ID NO:590
SEQ ID NO:591
SEQ ID NO:592
SEQ ID NO:593
SEQ ID NO:594
SEQ ID NO:595
SEQ ID NO:596
SEQ ID NO:597
SEQ ID NO:598
SEQ ID NO:599
SEQ ID NO:600
SEQ ID NO:601
SEQ ID NO:602
SEQ ID NO:1
SEQ ID NO:2

FIG. 91

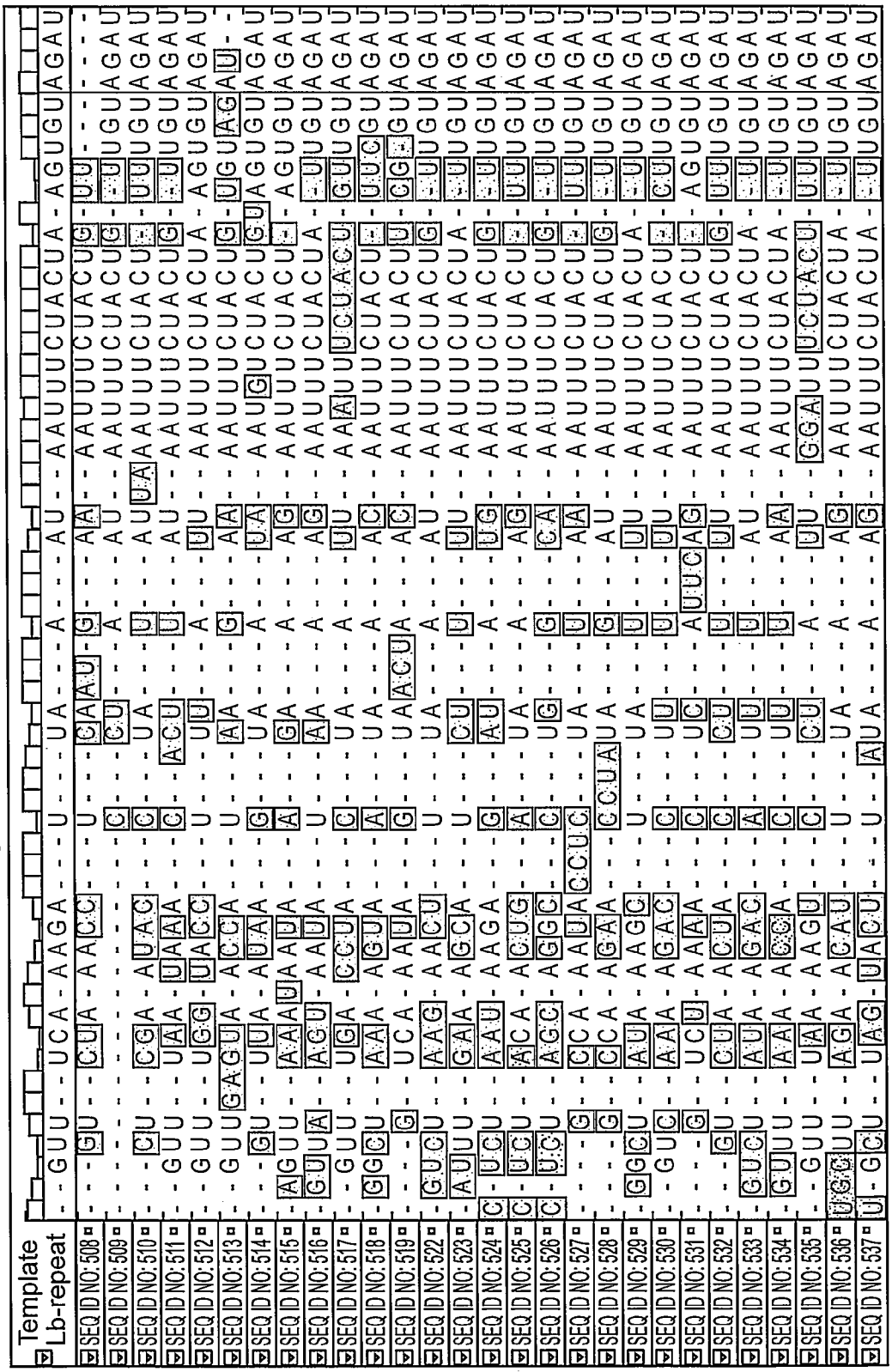

crRNA Repeat Sequences of Cas12 Orthologs
Template
Lb-repeat
SEQ ID NO: 508
SEQ ID NO: 509
SEQ ID NO: 510
SEQ ID NO: 511
SEQ ID NO: 512
SEQ ID NO: 513
SEQ ID NO: 514
SEQ ID NO: 515
SEQ ID NO: 516
SEQ ID NO: 517
SEQ ID NO: 518
SEQ ID NO: 519
SEQ ID NO: 522
SEQ ID NO: 523
SEQ ID NO: 524
SEQ ID NO: 525
SEQ ID NO: 526
SEQ ID NO: 527
SEQ ID NO: 528
SEQ ID NO: 529
SEQ ID NO: 530
SEQ ID NO: 531
SEQ ID NO: 532
SEQ ID NO: 533
SEQ ID NO: 534
SEQ ID NO: 535
SEQ ID NO: 536
SEQ ID NO: 537

FIG. 92

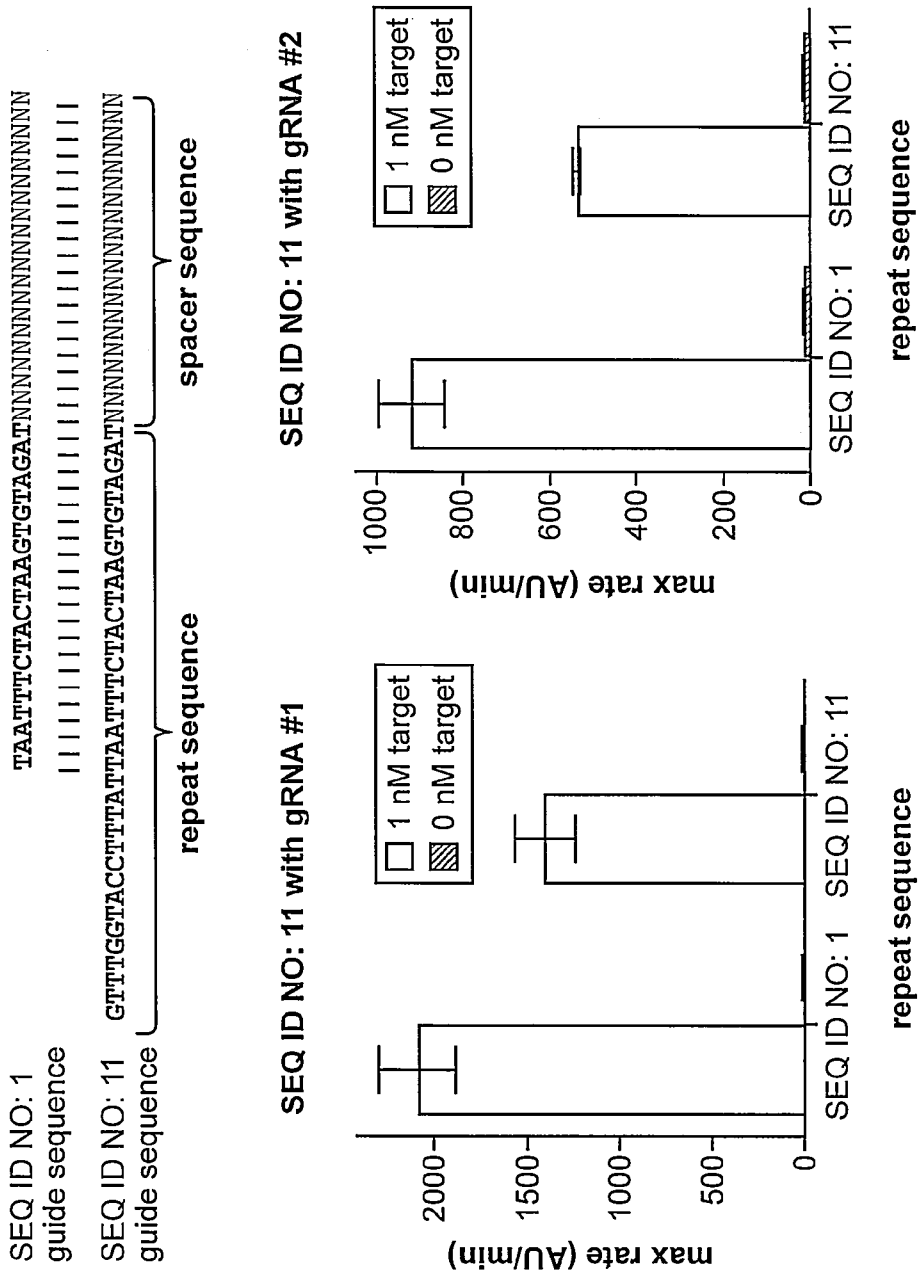

SEQ ID NO: 1
guide sequence
TAATTTCTACTAAGTGTAGATNNNNNNNNNNNNNNNNNNNN
SEQ ID NO: 11
guide sequence
GTTTGGTACCTTTATTAATTTCTACTAAGTGTAGATNNNNNNNNNNNNNNNNNNNN
repeat sequence
spacer sequence
SEQ ID NO: 11 with gRNA #1
SEQ ID NO: 11 with gRNA #2
1 nM target
0 nM target
max rate (ΔU/min)
2000
1500
1000
500
0
1000
800
600
400
200
SEQ ID NO: 1
SEQ ID NO: 11
repeat sequence

FIG. 93