

ASSAYS AND METHODS FOR DETECTION OF NUCLEIC ACIDS

CROSS-REFERENCE

[0001] The present application claims priority to and benefit from U.S. Provisional Application No.: 62/863,178, filed on June 18, 2019, U.S. Provisional Application No.: 62/879,325, filed on July 26, 2019, U.S. Provisional Application No.: 62/881,809, filed on August 1, 2019, U.S. Provisional Application No.: 62/944,926, filed on December 6, 2019, and U.S. Provisional Application No.: 62/985,850, filed on March 5, 2020, the entire contents of each of which are herein incorporated by reference.

SEQUENCE LISTING

[0001a] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy is named 53694-730.601 SL.txt and is 1,121,102 bytes in size.

BACKGROUND

[0002] Various communicable diseases can easily spread from an individual or environment to an individual. These diseases may include but are not limited to influenza. Individuals with influenza may have poor outcomes. The detection of the ailments, especially at the early stages of infection, may provide guidance on treatment or intervention to reduce the progression or transmission of the ailment.

SUMMARY

[0002a] In one aspect, provided herein is a composition comprising a programmable nuclease having at least 80% sequence identity to SEQ ID NO: 37 and a non-naturally occurring guide nucleic acid.

[0002b] In another aspect, provided herein is a method of assay for a segment of a target nucleic acid in a sample, the method comprising: (a) contacting the sample to: (i) a reporter comprising a detector nucleic acid; and (ii) the composition of any one of claims 1-15, wherein the non-naturally occurring guide nucleic acid hybridizes to a segment of the target nucleic acid; and (b) assaying for a signal produced by cleavage of the detector nucleic acid.

[0003] In various ~~aspect~~examples, the present disclosure provides a microfluidic cartridge for detecting a target nucleic acid comprising: an amplification chamber fluidically connected to a valve; a detection chamber fluidically connected to the valve, wherein the valve is connected to a sample metering channel; a detection reagent chamber fluidically connected to the detection chamber via a resistance channel, the detection reagent chamber comprising a programmable nuclease, a guide nucleic acid, and a labeled detector nucleic acid, wherein the labeled detector nucleic acid is capable of being cleaved upon binding of the guide nucleic acid to a segment of a target nucleic acid.

[0004] In some [aspectexamples](#), the sample metering channel controls volumes of liquids dispensed in a channel or chamber. In some [aspectexamples](#), the sample metering channel is fluidically connected to the detection chamber. In some [aspectexamples](#), the resistance channel has a serpentine path, an angular path, or a circuitous path. In some [aspectexamples](#), the valve is a rotary valve, pneumatic valve, a hydraulic valve, an elastomeric valve. In some [aspectexamples](#), the resistance channel is fluidically connected with the valve. In some [aspectexamples](#), the valve comprises casing comprising a “substrate” or an “over-mold.” In some [aspectexamples](#), the valve is actuated by a solenoid. In some [aspectexamples](#), the valve is controlled manually, magnetically, electrically, thermally, by a bistable circuit, with a piezoelectric material, electrochemically, with phase change, rheologically, pneumatically, with

a check valve, with capillarity, or any combination thereof. In some [aspectexamples](#), the rotary valve fluidically connects at least 3, at least, 4, or at least 5 chambers.

[0005] In some [aspectexamples](#), the microfluidic cartridge further comprises an amplification reagent chamber fluidically connected to the amplification chamber. In some [aspectexample](#), the microfluidic cartridge further comprises a sample chamber fluidically connected to the amplification reagent chamber. In some [aspectexamples](#), the microfluidic cartridge further comprises a sample inlet connected to the sample chamber. In some [aspectexamples](#), the sample inlet is sealable. In some [aspectexample](#), the sample inlet forms a seal around the sample.

[0006] In some [aspectexamples](#), the sample chamber comprises a lysis buffer. In some [aspectexample](#), the microfluidic cartridge further comprises a lysis buffer storage chamber fluidically connected to the sample chamber. In some [aspectexample](#), the lysis buffer storage chamber comprises a lysis buffer. In some [aspectexamples](#), the lysis buffer is a dual lysis/amplification buffer.

[0007] In some [aspectexamples](#), the lysis buffer storage chamber is fluidically connected to the sample chamber through a second valve. In some [aspectexamples](#), the sample chamber is fluidically connected to the amplification chamber through the amplification reagent chamber. In some [aspectexample](#), the sample chamber is fluidically connected to the amplification reagent chamber through the amplification chamber. In some [aspectexamples](#), the microfluidic cartridge is configured to direct fluid bidirectionally between the amplification reagent chamber and amplification chamber. In some [aspectexamples](#), the detection reagent chamber is fluidically connected to the amplification chamber. In some [aspectexample](#), the amplification chamber is fluidically connected to the detection chamber through the detection reagent chamber. In some [aspectexamples](#), comprising a reagent port above the detection chamber configured to deliver fluid from the detection reagent chamber to the detection chamber. In some [aspectexample](#), the amplification chamber is fluidically connected to the detection reagent chamber through the detection chamber.

[0008] In some [aspectexamples](#), the resistance channel is configured to reduce backflow into the detection chamber and the detection reagent chamber. In some [aspectexample](#), the sample metering channel is configured to direct a predetermined volume of fluid from the detection reagent chamber to the detection chamber. In some [aspectexamples](#), the amplification chamber and detection chamber are thermally isolated. In some [aspectexamples](#), the detection reagent chamber is fluidically connected to the detection chamber. In some [aspectexample](#), the detection reagent chamber is fluidically connected to the detection chamber via a second resistance channel. In some [aspectexample](#), the resistance channel or the second resistance channel is a serpentine resistance channel. In some [aspectexample](#), the resistance channel or the second resistance channel comprises at least two hairpins.

In some [aspectexamples](#), the resistance channel or the second resistance channel comprises at least one, at least 2, at least 3, or at least 4 right angles.

[0009] In some [aspectexamples](#), the amplification chamber comprises a sealable sample inlet. In some [aspectexamples](#), the sample inlet is configured to form a seal around a swab. In some [aspectexamples](#), microfluidic cartridge is configured to connect to a first pump to pump fluid from the amplification chamber to the detection chamber. In some [aspectexamples](#), microfluidic cartridge is configured to connect to a second pump to pump fluid from the detection reagent chamber to the detection chamber. In some [aspectexamples](#), first pump or the second pump is a pneumatic pump, a peristaltic pump, a hydraulic pump, or a syringe pump. In some [aspectexamples](#), the amplification chamber is fluidically connected to a port configured to receive pneumatic pressure. In some [aspectexamples](#), the amplification chamber is fluidically connected to the port through a channel. In some [aspectexamples](#), the amplification reagent chamber is connected to a second port configured to receive pneumatic pressure. In some [aspectexamples](#), the amplification reagent chamber is fluidically connected to the second port through a second channel.

[0010] In some [aspectexamples](#), the microfluidic cartridge is configured to connect to a third pump to pump fluid from the amplification reagent chamber to the amplification chamber. In some [aspectexamples](#), the third pump is a pneumatic pump, a peristaltic pump, a hydraulic pump, or a syringe pump. In some [aspectexamples](#), the detection reagent chamber is connected to a port configured to receive pneumatic pressure. In some [aspectexamples](#), the detection reagent chamber is fluidically connected to a third port through a third channel.

[0011] In some [aspectexamples](#), the microfluidic cartridge is configured to connect to a fourth pump to pump fluid from the detection reagent chamber to the detection chamber. In some [aspectexamples](#), the fourth pump is a pneumatic pump, a peristaltic pump, a hydraulic pump, or a syringe pump.

[0012] In some [aspectexamples](#), the microfluidic cartridge further comprises a plurality of ports configured to couple to a gas manifold, wherein the plurality of ports is configured to receive pneumatic pressure. In some [aspectexamples](#), any chamber of the microfluidic cartridge is connected to the plurality of ports. In some [aspectexamples](#), the valve is opened upon application of current electrical signal.

[0013] In some [aspectexamples](#), the detection reagent chamber is circular. In some [aspectexamples](#), the detection reagent chamber is elongated. In some [aspectexamples](#), the detection reagent chamber is hexagonal. In some [aspectexamples](#), a region of the resistance channel is molded to direct flow in a direction perpendicular to the net flow direction. In some [aspectexamples](#), a region of the resistance channel is molded to direct flow in a direction perpendicular to the axis defined by two ends of the resistance channel. In some [aspectexamples](#), a region of the resistance channel is molded to direct flow

along the z-axis of the microfluidic cartridge. In some [aspectexamples](#), the valve is fluidically connected to two detection chambers via an amplification mix splitter. In some [aspectexamples](#), the valve is fluidically connected to 3, 4, 5, 6, 7, 8, 9, or 10 detection chambers via an amplification mix splitter.

[0014] In some [aspectexamples](#), the microfluidic cartridge further comprises a second valve fluidically connected to the detection reagent chamber and the detection chamber. In some [aspectexample](#), the detection chamber is vented with a hydrophobic PTFE vent. In some [aspectexample](#), the detection chamber comprises an optically transparent surface.

[0015] In some [aspectexamples](#), the amplification chamber is configured to hold from 10 μL to 500 μL of fluid. In some [aspectexample](#), the amplification reagent chamber is configured to hold from 10 μL to 500 μL of fluid. In some [aspectexamples](#), the microfluidic cartridge is configured to accept from 2 μL to 100 μL of a sample comprising a nucleic acid. In some [aspectexamples](#), the amplification reagent chamber comprises between 5 and 200 μL an amplification buffer. In some [aspectexample](#), the amplification chamber comprises 45 μL amplification buffer. In some [aspectexample](#), the detection reagent chamber stores from 5 to 200 μL of fluid containing the programmable nuclease, the guide nucleic acid, and the labeled detector nucleic acid.

[0016] In some [aspectexamples](#), the microfluidic cartridge comprises 2, 3, 4, 5, 6, 7, or 8 detection chambers. In some [aspectexample](#), the 2, 3, 4, 5, 6, 7, or 8 detection chambers are fluidically connected to a single sample chamber. In some [aspectexamples](#), the detection chamber holds up to 100 μL , 200 μL , 300 μL , or 400 μL of fluid.

[0017] In some [aspectexample](#), the microfluidic cartridge comprises 5-7 layers. In some [aspectexamples](#), the microfluidic cartridge comprises layers as shown in **FIG. 130B**. In some [aspectexamples](#), the microfluidic cartridge further comprises a sample inlet configured to adapt with a slip luer tip. In some [aspectexamples](#), the slip luer tip is adapted to fit a syringe holding a sample. In some [aspectexamples](#), the sample inlet is capable of being hermetically sealed.

[0018] In some [aspectexamples](#), the microfluidic cartridge further comprises a sliding valve. In some [aspectexample](#), the sliding valve connects the amplification reagent chamber to the amplification chamber. In some [aspectexamples](#), the sliding valve connects the amplification chamber to the detection reagent chamber. In some [aspectexamples](#), the sliding valve connects the amplification reagent chamber to the detection chamber.

[0019] In various [aspectexamples](#), the present disclosure provides a manifold configured to accept the microfluidic cartridge. In some [aspectexamples](#), the manifold comprises a pump configured to pump fluid into the detection chamber, an illumination source configured to illuminate the detection chamber, a detector configured to detect a detectable signal produced by the labeled detector

nucleic acid, and a heater configured to heat the amplification chamber. In some [aspectexamples](#), the manifold further comprises a second heater configured to heat the detection chamber.

[0020] In some [aspectexamples](#), the illumination source is a broad spectrum light source. In some [aspectexample](#), the illumination source light produces an illumination with a bandwidth of less than 5 nm. In some [aspectexamples](#), the illumination source is a light emitting diode. In some [aspectexamples](#), the light emitting diode produces white light, blue light, or green light.

[0021] In some [aspectexamples](#), the detectable signal is light. In some [aspectexample](#), the detector is a camera or a photodiode. In some [aspectexamples](#), the detector has a detection bandwidth of less than 100 nm, less than 75 nm, less than 50 nm, less than 40 nm, less than 30 nm, less than 20 nm, less than 10 nm, or less than 5 nm.

[0022] In some [aspectexamples](#), the manifold further comprises an optical filter configured to be between the detection chamber and the detector. In some [aspectexamples](#), the amplification chamber comprises amplification reagents. In some [aspectexamples](#), the amplification reagent chamber comprises amplification reagents. In some [aspectexamples](#), the amplification reagents comprise a primer, a polymerase, dNTPs, an amplification buffer. In some [aspectexamples](#), the amplification chamber comprises a lysis buffer. In some [aspectexamples](#), the amplification reagent chamber comprises a lysis buffer. In some [aspectexamples](#), the amplification reagents comprise a reverse transcriptase. In some [aspectexamples](#), the amplification reagents comprise reagents for thermal cycling amplification. In some [aspectexamples](#), the amplification reagents comprise reagents for isothermal amplification. In some [aspectexamples](#), the amplification reagents comprise reagents for transcription mediated amplification (TMA), helicase dependent amplification (HAD), circular helicase dependent amplification (cHDA), strand displacement amplification (SDA), loop mediated amplification (LAMP), exponential amplification reaction (EXPAR), 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 some [aspectexamples](#), the amplification reagents comprise reagents for loop mediated amplification (LAMP).

[0023] In some [aspectexamples](#), the lysis buffer and the amplification buffer are a single buffer. In some [aspectexamples](#), the lysis buffer storage chamber comprises a lysis buffer. In some [aspectexamples](#), the lysis buffer has a pH of from pH 4 to pH 5.

[0024] In some [aspectexamples](#), the microfluidic cartridge further comprises reverse transcription reagents. In some [aspectexample](#), the reverse transcription reagents comprise a reverse transcriptase,

a

primer, and dNTPs. In some [aspectexamples](#), the programmable nuclease comprises an RuvC catalytic domain. In some [aspectexamples](#), the programmable nuclease is a type V CRISPR/Cas effector protein. In some [aspectexamples](#), the type V CRISPR/Cas effector protein is a Cas12 protein. In some [aspectexamples](#), the Cas12 protein comprises a Cas12a polypeptide, a Cas12b polypeptide, a Cas12c polypeptide, a Cas12d polypeptide, a Cas12e polypeptide, a C2c4 polypeptide, a C2c8 polypeptide, a C2c5 polypeptide, a C2c10 polypeptide, and a C2c9 polypeptide. In some [aspectexample](#), the Cas12 protein has 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 any one of SEQ ID NO: 27 – SEQ ID NO: 37. In some [aspectexamples](#), the Cas12 protein is selected from SEQ ID NO: 27 – SEQ ID NO: 37.

[0025] In some [aspectexamples](#), the type V CRISPR/Cas effector protein is a Cas14 protein. In some [aspectexamples](#), the Cas14 protein comprises a Cas14a polypeptide, a Cas14b polypeptide, a Cas14c polypeptide, a Cas14d polypeptide, a Cas14e polypeptide, a Cas14f polypeptide, a Cas14g polypeptide, a Cas14h polypeptide, a Cas14i polypeptide, a Cas14j polypeptide, or a Cas14k polypeptide. In some [aspectexamples](#), the Cas14 protein has 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 any one of SEQ ID NO: 38 – SEQ ID NO: 129. In some [aspectexamples](#), the Cas14 protein is selected from SEQ ID NO: 38 – SEQ ID NO: 129.

[0026] In some [aspectexample](#), the type V CRISPR/Cas effector protein is a Cas Φ protein. In some [aspectexamples](#), the Cas Φ protein has 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 any one of SEQ ID NO: 274 – SEQ ID NO: 321. In some [aspectexamples](#), the Cas Φ protein is selected from SEQ ID NO: 274 – SEQ ID NO: 321.

[0027] In some [aspectexamples](#), microfluidic cartridge further provides one or more chambers for in vitro transcribing amplified coronavirus target nucleic acid. In some [aspectexamples](#), the in vitro transcribing comprises contacting the amplified coronavirus target nucleic acid to reagents for in vitro transcription. In some [aspectexamples](#), the reagents for in vitro transcription comprise an RNA polymerase, NTPs, and a primer.

[0028] In some [aspectexamples](#), the programable nuclease comprises a HEPN cleaving domain. In some [aspectexamples](#), the programmable nuclease is a type VI CRISPR/Cas effector protein. In some [aspectexamples](#), the type VI CRISPR/Cas effector protein is a Cas13 protein. In some [aspectexample](#), the Cas13 protein comprises a Cas13a polypeptide, a Cas13b polypeptide, a Cas13c polypeptide, a Cas13d polypeptide, or a Cas13e polypeptide. In some [aspectexamples](#), the Cas13 protein has 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 any one of SEQ ID NOs: 130 – SEQ ID NO: 137. In some [aspectexamples](#), the Cas13 protein is selected from SEQ ID NOs: 130 – SEQ ID NO: 137.

[0029] In some [aspectexamples](#), the target nucleic acid is from a virus. In some [aspectexamples](#), the virus comprises a respiratory virus. In some [aspectexamples](#), the respiratory virus is an upper respiratory virus. In some [aspectexamples](#), the virus comprises an influenza virus. In some [aspectexamples](#), the virus comprises a coronavirus.

[0030] In some [aspectexamples](#), the coronavirus target nucleic acid is from SARS-CoV-2. In some [aspectexamples](#), the coronavirus target nucleic acid is from an N gene, an E gene, or a combination thereof. In some [aspectexamples](#), the coronavirus target nucleic acid has a sequence of any one of SEQ ID NO: 333 – SEQ ID NO: 338. In some [aspectexamples](#), the influenza virus comprises an influenza A virus, influenza B virus, or a combination thereof. In some [aspectexamples](#), the plurality of target sequences comprises sequences from influenza A virus, influenza B virus, and a third pathogen.

[0031] In some [aspectexamples](#), the guide nucleic acid is a guide RNA. In some [aspectexamples](#), the guide nucleic acid has at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% sequence identify to any one of SEQ ID NO: 323 – SEQ ID NO: 328. In some [aspectexamples](#), the guide nucleic acid is selected from any one of SEQ ID NO: 323 – SEQ ID NO: 328.

In some [aspectexamples](#), the microfluidic cartridge comprises a control nucleic acid. In some [aspectexamples](#), the control nucleic acid is in the detection chamber. In some [aspectexamples](#), the control nucleic acid is RNaseP. In some [aspectexamples](#), the control nucleic acid has a sequence of SEQ ID NO: 379.

[0032] In some [aspectexamples](#), the guide nucleic acid has at least 80%, at least 85%, at least 90%, at least 92%, at least 95%, at least 97%, or at least 99% sequence identify to any one of SEQ ID NO: 330 – SEQ ID NO: 332. In some [aspectexamples](#), the guide nucleic acid is selected from any one of SEQ ID NO: 330 – SEQ ID NO: 332. In some [aspectexamples](#), the guide nucleic acid targets a plurality of target sequences.

[0033] In some [aspectexamples](#), the microfluidic cartridge comprises a plurality of guide sequences tiled against a virus. In some [aspectexamples](#), the labeled detector nucleic acid comprises a single stranded reporter comprising a detection moiety. In some [aspectexamples](#), the detection moiety is a fluorophore, a FRET pair, a fluorophore/quencher pair, or an electrochemical reporter molecule. In some [aspectexamples](#), the electrochemical reporter molecule comprises a species shown in **FIG. 149**. In some [aspectexamples](#), the labeled detector produced a detectable signal upon cleavage of the detector nucleic acid. In some [aspectexamples](#), the detectable signal is a colorimetric signal, a fluorescence signal, an amperometric signal, or a potentiometric signal.

[0034] In various [aspectexamples](#), the present disclosure provides a method of detecting a target nucleic acid, the method comprising: providing a sample from a subject; adding the sample to a microfluidic cartridge; correlating a detectable signal to the presence or absence of a target

nucleic acid; and optionally quantifying the detectable signal, thereby quantifying an amount of the target nucleic acid present in the sample.

[0035] In some [aspectexamples](#), a microfluidic cartridge may be used in a method for detecting a target nucleic acid. In some [aspectexamples](#), a system may be used in a method for detecting a targeting nucleic acid. In some [aspectexamples](#), a programmable nuclease may be used in a method for detecting a target nucleic acid. In some [aspectexamples](#), a composition may be used in a method for detecting a target a nucleic acid. In some [aspectexamples](#), a DNA-activated programmable RNA nuclease may be used in a method for assaying for a target deoxyribonucleic acid from a virus in a sample. In some [aspectexamples](#), a DNA-activated programmable RNA nuclease may be used in a method of assaying for a target ribonucleic acid from a virus in a sample. In some [aspectexamples](#), a programmable nuclease may be used in a method for detecting a target nucleic acid in a sample.

[0036] In various [aspectexamples](#), the present disclosure provides a system for detecting a target nucleic acid, said system comprising: a guide nucleic acid targeting a target sequence from a virus; a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence; and a reporter, wherein the reporter is capable of being cleaved by the activated nuclease, thereby generating a detectable signal.

[0037] In some [aspectexamples](#), the reporter comprises a single stranded reporter comprising a detection moiety. In some [aspectexamples](#), the virus comprises an influenza virus. In some [aspectexamples](#), the influenza virus comprises an influenza A virus, influenza B virus, or a combination thereof. In some [aspectexamples](#), the virus comprises a respiratory virus. In some [aspectexample](#), the respiratory virus is an upper respiratory virus. In some [aspectexamples](#), the guide nucleic acid targets a plurality of target sequences.

[0038] In some [aspectexamples](#), the system comprises a plurality of guide sequences tiled against the virus. In some [aspectexamples](#), the plurality of target sequences comprises sequences from influenza A virus, influenza B virus, and a third pathogen. In some [aspectexamples](#), the single stranded reporter comprises the detection moiety at the 5' end. In some [aspectexamples](#), the single stranded reporter comprises a biotin-dT/FAM moiety or a biotin-dT/ROX moiety. In some [aspectexamples](#), the single stranded reporter comprises a chemical functional handle at the 3'end capable of being conjugated to a substrate.

[0039] In some [aspectexamples](#), the substrate is a magnetic bead. In some [aspectexamples](#), the substrate is a surface of a reaction chamber. In some [aspectexamples](#), downstream of the reaction chamber is a test line. In some [aspectexamples](#), the test line comprises a streptavidin. In some [aspectexamples](#), downstream of the test line is a flow control line. In some [aspectexamples](#), the flow control line comprises an anti-IgG antibody. In some [aspectexamples](#), the anti-IgG antibody comprises an anti-rabbit IgG antibody.

[0040] In some [aspectexamples](#), the activated nuclease is capable of cleaving the single stranded reporter and releases the biotin-dT/FAM moiety or the biotin-dT/ROX moiety. In some [aspectexamples](#), the biotin-dT/FAM moiety is capable of binding the streptavidin at the test line. In some [aspectexamples](#), the reporter is an electroactive reporter. In some [aspectexamples](#), the electroactive reporter comprises biotin and methylene blue. In some [aspectexamples](#), the reporter is an enzyme-nucleic acid. In some [aspectexamples](#), the enzyme-nucleic acid is an invertase enzyme. In some [aspectexamples](#), an enzyme of the enzyme-nucleic acid is a sterically hindered enzyme.

[0041] In some [aspectexamples](#), upon cleavage of a nucleic acid of the enzyme-nucleic acid, the enzyme is functional. In some [aspectexamples](#), the detectable signal is a colorimetric signal, a fluorescence signal, an amperometric signal, or a potentiometric signal.

[0042] In various [aspectexamples](#), the present disclosure provides a method of detecting a target nucleic acid in a sample comprising: contacting the sample with a guide nucleic acid targeting a target sequence; a programmable nuclease capable of being activated when complexed with the guide nucleic acid and the target sequence; and a reporter, wherein the reporter is capable of being cleaved by the activated nuclease, thereby generating a detectable signal.

[0043] In some [aspectexamples](#), the target nucleic acid is from an exogenous pathogen. In some [aspectexamples](#), the exogenous pathogen comprises a virus. In some [aspectexamples](#), the virus comprises an influenza virus. In some [aspectexamples](#), the influenza virus comprises an influenza A virus, influenza B virus, or a combination thereof. In some [aspectexamples](#), the virus comprises a respiratory virus. In some [aspectexamples](#), the respiratory virus is an upper respiratory virus.

[0044] In some [aspectexamples](#), the detectable signal indicates presence of the virus in the sample. In some [aspectexamples](#), the method further comprises diagnosing a subject from which the sample was taken with the virus. In some [aspectexamples](#), the subject is a human. In some [aspectexamples](#), the sample is a buccal swab, a nasal swab, or urine. In some [aspectexamples](#), the reporter comprises a single stranded reporter comprising a detection moiety. In some [aspectexamples](#), the guide nucleic acid targets a plurality of target sequences.

[0045] In some [aspectexamples](#), the system comprises a plurality of guide sequences tiled against the virus. In some [aspectexamples](#), the plurality of target sequences comprises sequences from influenza A virus, influenza B virus, and a third pathogen. In some [aspectexamples](#), the single stranded reporter comprises the detection moiety at the 5' end. In some [aspectexamples](#), the single stranded reporter comprises a biotin-dT/FAM moiety or a biotin-dT/ROX moiety. In some [aspectexamples](#), the single stranded reporter comprises a chemical functional handle at the 3' end capable of being conjugated to a substrate. In some [aspectexamples](#), the substrate is a magnetic bead.

[0046] In some [aspectexamples](#), the substrate is a surface of a reaction chamber. In some [aspectexamples](#), downstream of the reaction chamber is a test line. In some [aspectexamples](#), the test line comprises a streptavidin. In some [aspectexamples](#), downstream of the test line is a flow control line. In some [aspectexamples](#), the flow control line comprises an anti-IgG antibody. In some [aspectexamples](#), the anti-IgG antibody comprises an anti-rabbit IgG antibody.

[0047] In some [aspectexamples](#), the activated nuclease is capable of cleaving the single stranded reporter and releases the biotin-dT/FAM moiety or the biotin-dT/ROX moiety. In some [aspectexamples](#), the biotin-dT/FAM moiety is capable of binding the streptavidin at the test line. In some [aspectexamples](#), the reporter is an electroactive reporter. In some [aspectexamples](#), the electroactive reporter comprises biotin and methylene blue. In some [aspectexamples](#), the reporter is an enzyme-nucleic acid. In some [aspectexamples](#), the enzyme-nucleic acid is an invertase enzyme. In some [aspectexamples](#), an enzyme of the enzyme-nucleic acid is a sterically hindered enzyme. In some [aspectexamples](#), upon cleavage of a nucleic acid of the enzyme-nucleic acid, the enzyme is functional. In some [aspectexamples](#), the detectable signal is a colorimetric signal, a fluorescence signal, an amperometric signal, or a potentiometric signal. In some [aspectexamples](#), in any of the above systems, the respiratory virus is a lower respiratory virus. In some [aspectexamples](#), in any of the above methods, the respiratory virus is a lower respiratory virus.

[0048] In some [aspectexamples](#), a composition comprises a DNA-activated programmable RNA nuclease; and a guide nucleic acid comprising a segment that is reverse complementary to a segment of a target deoxyribonucleic acid, wherein the DNA-activated programmable RNA nuclease binds to the guide nucleic acid to form a complex. In some [aspectexamples](#), the composition further comprises an RNA reporter. In some [aspectexamples](#), the composition further comprises the target deoxyribonucleic acid from a virus. In some [aspectexamples](#), the target deoxyribonucleic acid is an amplicon of a nucleic acid. In some [aspectexamples](#), wherein the nucleic acid is a deoxyribonucleic acid or a ribonucleic acid. In some [aspectexamples](#), the DNA-activated programmable RNA nuclease is a Type VI CRISPR/Cas enzyme. In some [aspectexamples](#), the DNA-activated programmable RNA nuclease is a Cas13. In some [aspectexamples](#), the DNA-activated programmable RNA nuclease is a Cas13a. In some [aspectexamples](#), the Cas13a is Lbu-Cas13a or Lwa-Cas13a. In some [aspectexamples](#), the composition has a pH from pH 6.8 to pH 8.2. In some [aspectexamples](#), the target deoxyribonucleic acid lacks a guanine at the 3' end. In some [aspectexamples](#), the target deoxyribonucleic acid is a single-stranded deoxyribonucleic acid. In some [aspectexamples](#), the composition further comprises a support medium. In some [aspectexamples](#), the composition further comprises a lateral flow assay device. In some [aspectexamples](#), the composition further comprises a device configured for fluorescence detection. In some [aspectexamples](#), the composition further comprises a second guide nucleic acid and a DNA-activated programmable DNA nuclease, wherein the second guide nucleic acid comprises a

segment that is reverse complementary to a segment of a second target deoxyribonucleic acid comprising a guide nucleic acid. In some [aspectexamples](#), the composition further comprises a DNA reporter. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Type V CRISPR/Cas enzyme. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Cas12. In some [aspectexamples](#), the Cas12 is a Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Cas14. In some [aspectexample](#)s, the Cas14 is a Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h.

[0049] In some [aspectexamples](#), a method of assaying for a target deoxyribonucleic acid from a virus in a sample comprises contacting the sample to a complex comprising a guide nucleic acid and a DNA-activated programmable RNA nuclease, wherein the guide nucleic acid comprises a segment that is reverse complementary to a segment of the target deoxyribonucleic acid, and

[0050] assaying for a signal produced by cleavage of at least some RNA reporters of a plurality of RNA reporters. In some [aspectexample](#)s, a method of assaying for a target ribonucleic acid from a virus in a sample comprises: amplifying a nucleic acid in a sample to produce a target deoxyribonucleic acid; contacting the target deoxyribonucleic acid to a complex comprising a guide nucleic acid and a DNA-activated programmable RNA nuclease, wherein the guide nucleic acid comprises a segment that is reverse complementary to a segment of the target deoxyribonucleic acid, and assaying for a signal produced by cleavage of at least some RNA reporters of a plurality of RNA reporters. In some [aspectexamples](#), the DNA-activated programmable RNA nuclease is a Type VI CRISPR nuclease. In some [aspectexamples](#), the DNA-activated programmable RNA nuclease is a Cas13. In some [aspectexamples](#), the Cas13 is a Cas13a. In some [aspectexamples](#), the Cas13a is Lbu-Cas13a or Lwa-Cas13a. In some [aspectexamples](#), cleavage of the at least some RNA reporters of the plurality of reporters occurs from pH 6.8 to pH 8.2. In some [aspectexamples](#), the target deoxyribonucleic acid lacks a guanine at the 3' end. In some [aspectexamples](#), the target deoxyribonucleic acid is a single-stranded deoxyribonucleic acid. In some [aspectexamples](#), the target deoxyribonucleic acid is an amplicon of a ribonucleic acid. In some [aspectexamples](#), the target deoxyribonucleic acid or the ribonucleic acid is from an organism. In some [aspectexamples](#), the organism is a virus, bacteria, plant, or animal. In some [aspectexamples](#), the target deoxyribonucleic acid is produced by a nucleic acid amplification method. In some [aspectexamples](#), the nucleic acid amplification method is isothermal amplification. In some [aspectexamples](#), the nucleic acid amplification method is thermal amplification. In some [aspectexamples](#), the nucleic acid amplification method is 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), or improved multiple displacement amplification (IMDA), or nucleic acid sequence-based amplification (NASBA). In some [aspectexamples](#), the signal is fluorescence, luminescence, colorimetric, electrochemical, enzymatic, calorimetric, optical, amperometric, or potentiometric. In some [aspectexamples](#), the method further comprises contacting the sample to a second guide nucleic acid and a DNA-activated programmable DNA nuclease, wherein the second guide nucleic acid comprises a segment that is reverse complementary to a segment of a second target deoxyribonucleic acid comprising a guide nucleic acid. In some [aspectexamples](#), the method further comprises assaying for a signal produced by cleavage of at least some DNA reporters of a plurality of DNA reporters. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Type V CRISPR nuclease. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Cas12. In some [aspectexamples](#), the Cas12 is a Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. In some [aspectexamples](#), the DNA-activated programmable DNA nuclease is a Cas14. In some [aspectexamples](#), the Cas14 is a Cas14a, Cas14b, Cas14c, Cas14d, Cas14e, Cas14f, Cas14g, or Cas14h. In some [aspectexamples](#), the guide nucleic acid comprises a crRNA. In some [aspectexamples](#), the guide nucleic acid comprises a crRNA and a tracrRNA. In some [aspectexamples](#), the signal is present prior to cleavage of the at least some RNA reporters. In some [aspectexamples](#), the signal is absent prior to cleavage of the at least some RNA reporters. In some [aspectexamples](#), 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 [aspectexamples](#), the method is carried out on a support medium. In some [aspectexamples](#), the method is carried out on a lateral flow assay device. In some [aspectexamples](#), the method is carried out on a device configured for fluorescence detection.

[0051] In various [aspectexamples](#), 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.

[0052] In various [aspectexamples](#), 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

[0053] measuring a detectable signal produced by cleavage of the reporter, wherein the measuring provides for detection of the target nucleic acid in the sample.

[0054] In some [aspectexamples](#), 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 [aspectexamples](#), 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 [aspectexamples](#), the guide nucleic acid does not hybridize to the forward inner primer and the backward inner primer.

[0055] In some [aspectexamples](#), a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some [aspectexamples](#), 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 [aspectexamples](#), 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 [aspectexample](#), 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 [aspectexamples](#), 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.

[0056] In some [aspectexamples](#), 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 [aspectexamples](#), 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.

[0057] In some [aspectexamples](#), 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 [aspectexamples](#), 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.

[0058] In various [aspectexamples](#), 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.

[0059] In various [aspectexamples](#), 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.

[0060] In various [aspectexamples](#), 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.

[0061] In various [aspectexamples](#), 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.

[0062] In some [aspectexamples](#), 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 [aspectexamples](#), 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.

[0063] In some [aspectexamples](#), a protospacer adjacent motif (PAM) or a protospacer flanking site (PFS) is 3' of the target nucleic acid. In some [aspectexamples](#), 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.

[0064] In some [aspectexamples](#), 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 [aspectexamples](#), 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.

[0065] In some [aspectexamples](#), the plurality of primers further comprises a loop forward primer. In some [aspectexamples](#), the plurality of primers further comprises a loop backward primer. In some [aspectexamples](#), the loop forward primer is between an F1c region and an F2c region. In some [aspectexamples](#), the loop backward primer is between a B1c region and a B2c region.

[0066] In some [aspectexamples](#), the target nucleic acid comprises a single nucleotide polymorphism (SNP). In some [aspectexamples](#), the single nucleotide polymorphism (SNP) comprises a HERC2 SNP. In some [aspectexamples](#), the single nucleotide polymorphism (SNP) is associated with an increased risk or

decreased risk of cancer. In some [aspectexamples](#), 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).

[0067] In some [aspectexamples](#), the plurality of primers and the guide nucleic acid are present together in a sample comprising the target nucleic acid. In some [aspectexamples](#), the contacting the sample to the plurality of primers results in amplifying the target nucleic acid. In some [aspectexamples](#), the amplifying and the contacting the sample to the guide nucleic acid occurs at the same time. In other [aspectexamples](#), the amplifying and the contacting the sample to the guide nucleic acid occur at different times. In some [aspectexamples](#), the method further comprises providing a polymerase, a dATP, a dTTP, a dGTP, a dCTP, or any combination thereof.

[0068] In some [aspectexamples](#), the target nucleic acid is from a virus. In some [aspectexamples](#), the virus comprises an influenza virus, respiratory syncytial virus, or a combination thereof. In further [aspectexamples](#), the influenza virus comprises an influenza A virus, influenza B virus, or a combination thereof. In some [aspectexamples](#), the virus comprises a respiratory virus. In further [aspectexamples](#), the respiratory virus is an upper respiratory virus.

[0069] In some [aspectexamples](#), the system further comprises a forward inner primer, a backward inner primer, a forward outer primer, a backward outer primer, a loop forward primer, a loop backward primer, or any combination thereof. In some [aspectexamples](#), method further comprising contacting the sample with a forward inner primer, a backward inner primer, a forward outer primer, a backward outer primer, a loop forward primer, a loop backward primer, or any combination thereof. In some [aspectexamples](#), method further comprising amplifying the target deoxyribonucleic acid with a forward inner primer, a backward inner primer, a forward outer primer, a backward outer primer, a loop forward primer, a loop backward primer, or any combination thereof. In some [aspectexamples](#), the amplifying comprises contacting the sample to a forward inner primer, a backward inner primer, a forward outer primer, a backward outer primer, a loop forward primer, a loop backward primer, or any combination thereof.

INCORPORATION BY REFERENCE

[0070] 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.