Different platforms have been developed to exploit the collateral cleavage activity by these two proteins. Specific high sensitivity enzymatic reporter unlocking (SHERLOCK) was introduced in 2017, which exploits Cas13a for the detection of RNA molecules and a diagnostic platform based on this method was developed in 2018 (Gootenberg *et al.* (2017) *Science* 356:438-442; Gootenberg *et al.* (2018) *Science* 360:439). At the same time, a Cas-12a-based diagnostic tool called one-hour low-cost multipurpose highly efficient system (HOLMES) was introduced in 2017 and a diagnosis platform was soon described (Cheng (2017) CN107488710; Li *et al.* (2018) *Cell Disc.* 4:20). Chen *et al.* (2018) also describes a Cas12-based detection system, DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR) (*Science* 10:1126). Conveniently, these methods allow for direction the Cas enzyme to a target sequence using a guide RNA, cleavage of the target sequence, and collateral cleavage of nearby reporter nucleotides that are detectable upon cleavage. In these methods, a fluorophore-quencher (FQ) reporter oligonucleotide is used to measure the collateral cleavage activity, typically composed of a fluorophore (F) and a quencher (Q) connected by a short 5-8 nucleotide sequence.

Current CRISPR-Cas based methods involve several steps, expensive kits and equipment (e.g., extraction and amplification kits, magnetic bead separator or centrifuge, thermocycler), and risk carryover contamination. For example, the method can involve nucleic acid isolation from a sample, moving the nucleic acids to an amplification reaction, amplification involving high temperature (e.g., by PCR), either opening the amplification vessel to add the CRISPR-Cas reagents, or transferring the nucleic acids to a separate vessel for cleavage and detection.

It is an object of the present invention to streamline current CRISPR-Cas detection methods and avoid carryover contamination.

SUMMARY

Provided herein are methods, kits, and compositions for specific target nucleic acid detection using Cas12 collateral cleavage to cleave a dual labelled reporter oligonucleotide. In one aspect, the present disclosure provides a Cas12 reporter oligonucleotide comprising: a) at least 25 nucleotides; b) an arm-stem-loop-stem-arm structure, wherein: the arm regions are single-stranded, the stem regions hybridize to one another, with base pairing extending over 4 or more nucleotides, and the loop region is single-stranded and greater than 5 nucleotides in length; c) at least 2 Cas12 preferred dinucleotides, wherein the Cas12 preferred dinucleotides are in the loop and/or arm regions; and d) a fluorophore molecule on one end of the oligonucleotide and a quencher molecule on the other end; wherein the oligonucleotide is able to be cleaved by Cas12 enzyme in the presence of a guide RNA bound to a target sequence. In some examples, the oligonucleotide is cleaved by the Cas12 enzyme in the presence of the guide RNA bound to target sequence.

Cleavage of the reporter oligonucleotide by Cas12 occurs more quickly as the number of Cas12 preferred dinucleotides increases. In some examples, the reporter

oligonucleotide comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more Cas12 preferred dinucleotides. In some examples, the Cas12 preferred dinucleotides are CT and TC. In some examples the Cas12 preferred dinucleotides are AT and TA, GT and TG, and CA and AC. In some examples, the reporter oligonucleotide has no dinucleotide preference.

In some examples, one of the arm regions has 6-14 nucleotides, and the Cas12 preferred dinucleotides are in the arm. In some examples, the loop region has 8-14 nucleotides, and the Cas12 preferred dinucleotides are in the loop.

The structure of the reporter oligonucleotide is such that the dinucleotides preferred by the Cas12 are present in the available, single-stranded part of the oligonucleotide, ensuring that collateral cleavage can occur efficiently.

In some examples, the Cas12 enzyme is Cas12a, Cas12b, Cas12c, Cas12d, or Cas12e. In some examples, the Cas12 enzyme is selected from the group consisting of AapCas12a, AacCas12b, CmeCas12a, FnCas12a, Engen® LbaCas12a (NEB), or YmeCas12a (NEB). In some examples, the Cas12 enzyme is thermotolerant, e.g., AapCas12a, AacCas12b, YmeCas12a, CmeCas12a, and Engen® LbaCas12a (NEB).

In some examples, the Cas12 enzyme is CmeCas12a, YmeCas12a, or LbaCas12a. In some examples, the Cas12 enzyme is CmeCas12a and the Cas12 preferred dinucleotides are CT and TC. In some examples, the Cas12 enzyme is LbaCas12a.

Various combinations of arm, stem and loop region lengths are possible within the parameters described above, that result in formation of an oligonucleotide secondary structure that brings the fluorophore and quencher into sufficient proximity to quench fluorescence. In some examples, the fluorophore and quencher are within about 50 angstroms or less of each other. In some examples, the fluorophore and quencher are within about 40 angstroms or less of each other. For example, in some cases the fluorophore and quencher are within about 34 angstroms or less, about 30 angstroms or less, about 27 angstroms or less, about 24 angstroms or less, about 20 angstroms or less, about 17 angstroms or less, or about 15 angstroms or less of each other.

In some examples, the Cas12 reporter oligonucleotide further comprises an internal quencher. In some examples, the internal quencher is positioned close enough to the fluorophore molecule to effectively quench signal (in combination with the other non-internal quencher), whether the proximity is through linear or secondary structure. In some examples, at least 2 Cas12 preferred dinucleotides are present between the fluorophore and internal quencher, e.g., 2, 3, 4, 5, 6, 7, 8, or more Cas12 preferred dinucleotides. In some examples, the internal quencher is placed 5, 6, 7, 8, 9, 10, 11, 12, or more nucleotides (or about 17, about 20, about 24, about 27, about 30, about 34, about 37, or about 40 angstroms) away from the fluorophore molecule. In some examples, the internal quencher is ZEN and the quencher (non-internal) is Iowa Black FQ. In some examples, the internal ZEN quencher is incorporated

between the 9th and 10th nucleotides away from the fluorophore molecule. In some examples, the internal ZEN quencher is incorporated between 25-30 angstroms away from the fluorophore molecule.

In some examples, the Cas12 reporter oligonucleotide comprises a sequence that is at least 75, 80, 81, 82, 83, 84, 85, 86, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to a sequence selected from the group consisting of: SEQ ID NO:1-17 and 24-28. In some examples, the Cas12 reporter oligonucleotide comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

In some examples, the Cas12 reporter oligonucleotide comprises a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and

TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

In some examples, the Cas12 reporter oligonucleotide is a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

In another aspect, further provided are kits, wherein the kit comprises: a) a Cas12 reporter oligonucleotide as described herein; b) a nucleotide polymerase; and c) a thermotolerant Cas12 enzyme. In some examples, the nucleotide polymerase is a DNA and/or RNA polymerase. In some examples, the nucleotide polymerase is Bst2.0 and optionally reverse transcriptase. In some examples, the nucleotide polymerase is Q5U and optionally reverse transcriptase. In some examples, the kit further comprises primers specific for a target sequence. In some examples, the kit further comprises a guide RNA specific for a target sequence. In some examples, the kit further includes at least one buffer, dNTPs, or other reagents for amplification and/or Cas12 activity.

In yet another aspect, the present disclosure provides a method of detecting the presence or absence of a target sequence comprising

a) isolating target nucleic acid;

b) contacting the target nucleic acid with: a nucleotide polymerase, oligonucleotide primers specific for a target sequence on the target nucleic acid, a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide as described herein;

c) incubating at a temperature of 42C to 58C for at least 15 minutes; and

d) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence. Also provided are methods for detecting a target sequence. In some

examples, the method comprises: a) isolating target nucleic acid; b) contacting the target nucleic acid with: a nucleotide polymerase, oligonucleotide primers specific for a target sequence on the target nucleic acid, a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and a Cas12 reporter oligonucleotide as described herein; c) incubating at a temperature of about 35C to about 70C, for example about 37C to

about 60C, for at least 10 minutes; and d) detecting fluorescence when the oligonucleotide is cleaved by the Cas12 enzyme. In some examples, the method comprises a) isolating target nucleic acid; b) contacting the target nucleic acid with: a nucleotide polymerase, oligonucleotide primers specific for a target sequence on the target nucleic acid, a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide as described herein; c) incubating at a temperature of about 35C to about 70C, e.g., about 37C to about 60C, for at least 10 minutes; and d) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence. In some examples, the incubating is at a temperature of about 37C, about 42C, about 45C, about 48C, about 50C, about 52C, about 55C, about 58C, or about 60C. In some examples, the incubating is at least 15, 30, 45, or 60 minutes. In some examples, the nucleotide polymerase is Bst2.0. In some examples, reverse transcriptase is also included.

In a further aspect, the present disclosure provides a method of detecting the presence or absence of a target sequence comprising

a) isolating target nucleic acid;

b) contacting the target nucleic acid with a nucleotide polymerase and oligonucleotide primers specific for a target sequence on the target nucleic acid, and amplifying the target sequence to produce amplified target sequence;

c) contacting the amplified target sequence with a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide as described herein;

d) incubating at a temperature of 42C to 58C for at least 15 minutes; and

e) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence. In some examples, the method comprises a) isolating target nucleic acid; b) contacting the target nucleic acid with a nucleotide polymerase and oligonucleotide primers specific for a target sequence on the target nucleic acid, and amplifying the target sequence to produce amplified target sequence; c) contacting the amplified target sequence with a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide as described herein; d) incubating at a temperature of about 35C to about 70C, for example about 37C to about 60C, for at least 15 minutes; and e) detecting fluorescence when the oligonucleotide is cleaved by the Cas12 enzyme. In some examples, the method comprises a) isolating target nucleic acid; b) contacting the target nucleic acid with a nucleotide polymerase and oligonucleotide primers specific for a target sequence on the

target nucleic acid, and amplifying the target sequence to produce amplified target sequence; c) contacting the amplified target sequence with a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide as described herein; d) incubating at a temperature of about 35C to about 70C, e.g., about 37C to about 60C, for at least 10 minutes; and e) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence. In some examples, the incubating is at a temperature of about 37C, about 42C, about 45C, about 48C, about 50C, about 52C, about 55C, about 58C, or about 60C. In some examples, the incubating is at least 15, 30, 45, or 60 minutes. In some examples, the nucleotide polymerase is Q5U, Taq, or a Taq derivative. In some examples, reverse transcriptase is also included.

In some examples, the thermotolerant Cas12 enzyme is CmeCas12a, YmeCas12a, or LbaCas12a. In some examples, the isolating is carried out using enzymatic purification.

In some examples, the methods can be multiplexed to detect more than one target sequence. In that case, at least a first guide RNA that specifically hybridizes to a first target sequence and a second guide RNA that specifically hybridizes to a second target sequence are used. The level of multiplexing can be increased, e.g, to detect 2, 3, 4, 5, 6, 7, 8 or more target sequences. One Cas12 reporter oligonucleotide can be used

so that a single signal (e.g., a single wavelength) is detected upon cleavage, regardless of how many, or which, target sequence is present. Such multiplexing can be useful, e.g., to detect multiple target sequences from a single pathogen, or multiple mutations linked to a single disorder. Alternatively, more than one Cas12 reporter oligonucleotide can be used, e.g., with different fluorophore and quencher pairs. In such cases, each Cas12 reporter oligonucleotide can be designed to have the preferred dinucleotides of a different Cas12 enzyme. For example, a first Cas12 reporter oligonucleotide can be designed with a first fluorophore/ quencher pair and multiple CT or TC dinucleotides for cleavage by CmeCas12a, and a second Cas12 reporter oligonucleotide can be designed with a second fluorophore/ quencher pair and multiple alternative preferred dinucleotides for cleavage by a different Cas12a. The activity of each Cas12 enzyme can be targeted to the appropriate target sequence by having each guide RNA targeted to the preferred PAM site of each enzyme on the target sequence.

In some examples, the Cas12 reporter oligonucleotide comprises a sequence that is at least 75, 80, 81, 82, 83, 84, 85, 86, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identical to a sequence selected from the group consisting of: SEQ ID NO:1-17 and 24-28. In some examples, the Cas12 reporter oligonucleotide comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

In some examples, the Cas12 reporter oligonucleotide comprises a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),

TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

In some examples, the Cas12 reporter oligonucleotide is a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the mode of action for Cas9, Cas12a and the process of CRISPR-DX using Cas12a. Figure 1A depicts the dsDNA cleavage mechanism of Cas9. TracrRNA: trans-activating crispr RNA; sgRNA: single guide RNA; crRNA: crispr RNA; PAM: protospacer adjacent motif. The target sequence is targeted with the crRNA. The PAM sequence is typically 3-4 nucleotides downstream of the site cut by the Cas9 enzyme.

Figure 1B depicts the dsDNA cleavage mechanism of Cas12. In contrast to Cas9, Cas12 activity requires a 5’ PAM site and results in a staggered cut of the double stranded target DNA distal to the PAM site. It also results in non-specific collateral cleavage of ssDNA once a sequence specific dsDNA is cleaved.

CLAIMS

1. A Cas12 reporter oligonucleotide comprising:

a) at least 25 nucleotides;

b) an arm-stem-loop-stem-arm structure, wherein:

the arm regions are single-stranded,

the stem regions hybridize to one another, with base pairing extending over 4 or more nucleotides, and

the loop region is single-stranded and greater than 5 nucleotides in length;

c) 2-14 Cas12 preferred dinucleotides, wherein the Cas12 preferred dinucleotides are in the loop and/or arm regions; and

d) a fluorophore molecule on one end of the oligonucleotide and a quencher molecule on the other end;

wherein the oligonucleotide is able to be cleaved by Cas12 enzyme in the presence of a guide RNA bound to a target sequence.

2. The Cas12 reporter oligonucleotide of claim 1, wherein the Cas12 preferred dinucleotides are selected from CT and TC, AT and TA, CA and AC, and GT and TG.

3. The Cas12 reporter oligonucleotide of claim 1 or 2, wherein one of the arm regions has 6-14 nucleotides, and the Cas12 preferred dinucleotides are in the arm.

4. The Cas12 reporter oligonucleotide of claim 1 or 2, wherein the loop region has 8-14 nucleotides, and the Cas12 preferred dinucleotides are in the loop.

5. The Cas12 reporter oligonucleotide of any one of claims 1-4, further comprising an internal quencher placed 6-10 nucleotides away from the fluorophore molecule.

6. The Cas12 reporter oligonucleotide of claim 5, wherein the internal quencher is ZEN and the quencher is Iowa Black FQ.

7. The Cas12 reporter oligonucleotide of any one of claims 1-6, wherein the Cas12 enzyme is CmeCas12a, YmeCas12a, or LbaCas12a.

8. The Cas12 reporter oligonucleotide of any one of claims 1-7, wherein the Cas12 enzyme is CmeCas12a and the Cas12 preferred dinucleotides are CT and TC.

9. The Cas12 reporter oligonucleotide of any one of claims 1-7, wherein the Cas12 enzyme is LbaCas12a and the Cas12 preferred dinucleotides are AT and TA.

10. The Cas12 reporter oligonucleotide of any one of claims 1-9, wherein the oligonucleotide comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

11. The Cas12 reporter oligonucleotide of any one of claims 1 to 10, wherein the oligonucleotide comprises a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24),
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26),
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27),
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28),
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

12. A kit comprising:

a) the Cas12 reporter oligonucleotide of any one of claims 1 to 11;
b) a nucleotide polymerase; and

c) a thermotolerant Cas12 enzyme.

13. The kit of claim 12, further comprising:

d) oligonucleotide primers specific for a target sequence; and

e) a guide RNA specific for the target sequence.

14. The kit of claim 12 or 13, wherein the nucleotide polymerase is a DNA and/or RNA polymerase.

15. The kit of any one of claims 12-14, wherein the nucleotide polymerase is Bst2.0 and optionally reverse transcriptase.

16. The kit of any one of claims 12-14, wherein the nucleotide polymerase is Q5U and optionally reverse transcriptase.

17. A method of detecting the presence or absence of a target sequence comprising

a) isolating target nucleic acid;

b) contacting the target nucleic acid with: a nucleotide polymerase, oligonucleotide primers specific for a target sequence on the target nucleic acid, a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide of any one of claims 1 to 11;

c) incubating at a temperature of 42C to 58C for at least 15 minutes; and

d) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence.

18. The method of claim 17, wherein the nucleotide polymerase is Bst2.0 polymerase and optionally reverse transcriptase.

19. A method of detecting the presence or absence of a target sequence comprising

a) isolating target nucleic acid;

b) contacting the target nucleic acid with a nucleotide polymerase and oligonucleotide primers specific for a target sequence on the target nucleic acid, and amplifying the target sequence to produce amplified target sequence;

c) contacting the amplified target sequence with a guide RNA oligonucleotide specific for the target sequence, a thermotolerant Cas12 enzyme, and the Cas12 reporter oligonucleotide of any one of claims 1 to 11;

d) incubating at a temperature of 42C to 58C for at least 15 minutes; and

e) measuring fluorescence, wherein detecting fluorescence produced by cleavage of the oligonucleotide by the Cas12 enzyme is indicative of the presence of the target sequence, and wherein the absence of fluorescence is indicative of the absence of the target sequence.

20. The method of claim 19, wherein the nucleotide polymerase is Q5U and optionally reverse transcriptase.

21. The method of any one of claims 17-20, wherein the thermotolerant Cas12 enzyme is CmeCas12a, YmeCas12a, or LbaCas12a.

22. The method of any one of claims 17-21, wherein the oligonucleotide comprises a sequence that is at least 90% identical to a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24)
AACGTCACTCTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26)
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27)
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28)
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

23. The method of any one of claims 17-22, wherein the oligonucleotide comprises a sequence selected from the group consisting of:

ACTCCTCTCAGCTCTCATTCCTATCAAC (R3, SEQ ID NO:3),
CGTCCCTTCTTCTTTACCTTCTCCTGGCTC (R5, SEQ ID NO:5),
AAGCAGCTTGAAGACGGTCGAACTCTGG (R8, SEQ ID NO:8),
AAGAGTCACTCACTTTTGACAGTTATACG (R9, SEQ ID NO:9),
TGTCAACACTCTCCCAGGTAGTAACG (R12, SEQ ID NO:12),
TCCATCGCCACCTAATTCTCTTTCCT (R13, SEQ ID NO:13),
AACAGTCACTCAGCATTTTGACAATAG (R17, SEQ ID NO:17),
ATCTCGTCACTCTCTCTCTCTGACGTG (R19, SEQ ID NO:24)

AACGTCACTCTCTCTCTCTTGACGAG (R20, SEQ ID NO:25),
CTCTCTCTCTAGTCACTGAATGACTTTA (R21, SEQ ID NO:26)
CTCTCTCTCTAGTCACATGAGTGACTT (R22, SEQ ID NO:27)
ATATATATATCGTCAATGAATGACGTC (R23, SEQ ID NO:28)
TACTCGTCACACACACACACTGACGTG (R19-CA, SEQ ID NO:32),
ATGTCGTCAGTGTGTGTGTGTGACGTG (R19-GT, SEQ ID NO:33), and
TAGTCGTCAGAGAGAGAGAGTGACGTG (R19-GA, SEQ ID NO:34).

24. The method of any one of claims 17-23, wherein step a) is carried out using enzymatic purification.