

Claims

1. Method for detecting the presence or amount of a target nucleic acid in a sample, comprising:
 - (a) contacting the target nucleic acid and/or an amplicon thereof with a nucleic acid detection system, said nucleic acid detection system comprising (1) at least one Cas12a enzyme, (2) at least one gRNA, and (3) at least one detection reagent;

wherein the Cas12a enzyme is an engineered AsCas12a variant that retains Cas12a functionality and comprises the amino acid sequence set forth in SEQ ID NO:1;

wherein the at least one gRNA (2) comprises at least two different gRNAs that bind to two different, non-overlapping target sequences in the same target nucleic acid; and

wherein said at least two different gRNAs comprises a spacer sequence of at least 20 nucleotides in length that specifically recognizes and binds a target sequence in the target nucleic acid, under conditions that allow binding of the complex of the Cas12a enzyme and the at least two different gRNAs to the target sequence and resultant activation of the Cas12a enzyme;

wherein the activated Cas12a enzyme generates, by interaction with the at least one detection reagent (3), a detectable, and optionally quantifiable, signal; and

 - (b) detecting said detectable signal.
2. The method of claim 1, wherein the target nucleic acid is a viral nucleic acid.
3. The method of claim 1 or 2, wherein the target nucleic acid is DNA or RNA.
4. The method of any one of claims 1 to 3, wherein the amplicon is a DNA amplicon.
5. The method of any one of claims 1 to 4, wherein the method further comprises a step of amplifying the target nucleic acid to obtain an amplicon thereof and wherein in the contacting step the amplicons of the target nucleic acid are contacted with the nucleic acid detection system.
6. The method of claim 5, wherein the amplifying step is carried out using an isothermal amplification method.
7. The method of claim 6, wherein the isothermal amplification method is loop-mediated isothermal amplification (LAMP), preferably reverse transcription loop-mediated isothermal amplification (RT-LAMP).
8. The method of claim 7, wherein the LAMP method comprises the use of two internal primers (FIP and BIP), two displacement primers (F3 and B3) and optionally two loop primers (LF and LB).

9. The method of claim 8, wherein the LAMP method further comprises the use of at least one swarm primer set or at least one stem primer set, preferably a swarm primer set.
10. The method of any one of claims 5 to 9, wherein amplification is carried out in the presence of glycine, taurine or guanidine, preferably guanidine.
11. The method of any one of claims 5 to 10, wherein the amplification is carried out (1) at a temperature of 60 to 65 °C; and/or (2) for a time period of 10 to 60 minutes, preferably 12 to 22 minutes.
12. The method of claim 8 or 9, wherein the LAMP method further comprises the use of 3' or 5' truncated internal primers that differ from the internal primers by a truncation of one nucleotide at the 3' or 5' end of their target-complementary sequence.
13. The method of any one of claims 8-9 and 12, wherein the LAMP method further comprises the use of a high fidelity DNA polymerase with a proofreading capability, preferably with a 3'-to-5'-exonuclease activity.
14. The method of any one of claims 8, 9 and 12 to 13, wherein the target nucleic acid is SARS-CoV-2 RNA or a DNA amplicon thereof and a first internal primer (FIP) has the nucleotide sequence set forth in SEQ ID NO:4, a second internal primer (BIP) has the nucleotide sequence set forth in SEQ ID NO:5, a first displacement primer (F3) has the nucleotide sequence set forth in SEQ ID NO:6, and/or a second displacement primer (B3) has the nucleotide sequence set forth in SEQ ID NO:7.
15. The method of any one of claims 8, 9 and 12-14, wherein the target nucleic acid is SARS-CoV-2 RNA or a DNA amplicon thereof and the LAMP method comprises the use of loop primers, wherein a first loop primer (LF) has the nucleotide sequence set forth in SEQ ID NO:8 and/or a second loop primer (LB) has the nucleotide sequence set forth in SEQ ID NO:9.
16. The method of any one of claims 8, 9 and 12-15, wherein the target nucleic acid is SARS-CoV-2 RNA or a DNA amplicon thereof and the LAMP method comprises the use of swarm primers, wherein a first swarm primer has the nucleotide sequence set forth in SEQ ID NO:10 and/or a second swarm primer has the nucleotide sequence set forth in SEQ ID NO:11.
17. The method of any one of claims 1 to 16, wherein the Cas12a enzyme consists of the amino acid sequence set forth in SEQ ID NO:1 (enAsCas12a).

18. The method of any one of claims 1 to 17, wherein the target nucleic acid is SARS-CoV-2 RNA or a DNA amplicon thereof and the SARS-CoV-2 target sequence comprises or consists of the nucleotide sequence GAAGAAGAAUCACCAGGAGU (SEQ ID NO:14) and GUAUUAACAUCACUAGGUUU (SEQ ID NO:15) or a variant thereof that shares at least 85% sequence identity with SEQ ID NO:14 or 15.
19. The method of claim 1, wherein the target nucleic acid is SARS-CoV-2 RNA or a DNA amplicon thereof and the at least two different gRNAs comprise a first gRNA comprising a spacer sequence comprising or consisting of the nucleotide sequence ACUCCUGGUGAUUCUUCUUC (SEQ ID NO:12) or a variant thereof having at least 85% sequence identity to SEQ ID NO:12, and a second gRNA comprising a spacer sequence comprising or consisting of the nucleotide sequence AAACCUAGUGAUGUUAUAC (SEQ ID NO:13) or a variant thereof having at least 85% sequence identity to SEQ ID NO:13.
20. The method of any one of claims 1 to 19, wherein the at least two different gRNAs comprises a 5'-terminal extension of at least 2, preferably 4 to 9 nucleotides.
21. The method of any one of claims 1 to 20, wherein the at least two different gRNA sequence comprises at least one chemical modification of a nucleotide selected from 2'-O-methyl RNA, 2'-fluoro base nucleotide and phosphorothioate linkage.
22. The method of any one of claims 1 to 21, wherein the at least two different gRNAs are a DNA-RNA hybrid and comprise at least 1 DNA nucleotide, preferably 2 to 4 DNA nucleotides, in the spacer sequence, the rest being RNA nucleotides.
23. The method of claim 22, wherein the DNA nucleotides are located (1) at the 3' terminus of the spacer sequence, preferably the 3'-terminal and/or the 3'-penultimate nucleotide, and/or (2) at position 1 and/or (3) at any one of positions 2-12 of the spacer sequence.
24. The method of any one of claims 1 to 23, wherein the detection step (b) is conducted at a temperature of at least 37°C, preferably at a temperature in the range of from 37°C to 65°C.
25. The method of any one of claims 1 to 24, wherein the detection reagent is an oligonucleotide that upon cleavage by the activated Cas12a enzyme generates a detectable signal.
26. The method of claim 25, wherein the oligonucleotide
 - (1) is an ssDNA molecule; and/or
 - (2) comprises a donor fluorophore/acceptor or fluorophore/quencher pair, wherein upon cleavage of the oligonucleotide, the (donor) fluorophore generates a detectable signal.

27. Nucleic acid detection system comprising:
 - at least one Cas12a enzyme, at least one gRNA, and at least one detection reagent;
 - wherein said at least one Cas12a enzyme is an engineered variant of AsCas12a that retains Cas12a functionality and comprises the amino acid sequence set forth in SEQ ID NO:1;
 - wherein the at least one gRNA comprises at least two different gRNAs that bind to two different, non-overlapping target sequences in the same target nucleic acid; and
 - wherein said at least two different gRNAs comprises a spacer sequence of at least 20 nucleotides in length that specifically recognizes and binds a target sequence in the target nucleic acid, wherein said binding of the target nucleic acid results in activation of the Cas12a enzyme and the activated Cas12a generates, by interaction with the at least one detection reagent, a detectable, and optionally quantifiable, signal;
28. The nucleic acid detection system according to claim 27, wherein the at least two different gRNAs are a DNA-RNA hybrid and comprises at least 1 DNA nucleotide, preferably 2 to 4 DNA nucleotides, in the spacer sequence, the rest being RNA nucleotides.