Claims

1. Method for determining the presence or amount of a target nucleic acid molecule in a sample using loop mediated isothermal amplification (LAMP), the method comprising:

(a) combining a LAMP reaction mixture, a DNA polymerase with strand displacement properties, a sample containing the target nucleic acid molecule, at least one detection probe, and at least one cleaving agent;
wherein the LAMP reaction mixture comprises a LAMP primer set of 4 to 6 primers, comprising two inner primers (FIP and BIP), two outer primers (F3 primer and B3 primer), and optionally one or two loop primers (LF and/or LB); and
wherein the at least one detection probe is a single stranded probe comprising a DNA sequence complementary to a sequence in a loop region of the amplicons formed by the LAMP reaction that can hybridize to said amplicons under LAMP assay conditions and form a double-stranded probe:target complex, the at least one detection probe comprising a scissile linkage in the DNA sequence complementary to a sequence in a loop region of the amplicons formed by the LAMP reaction, wherein said scissile linkage in the probe:target complex but not the non-hybridized probe is cleavable under LAMP assay conditions;
wherein under LAMP assay conditions the at least one cleaving agent is capable of cleaving the at least one detection probe at said scissile linkage when said probe is hybridized to its target, wherein upon cleavage of said at least one detection probe at the scissile linkage the fragments of said at least one detection probe are released from the probe:target complex so that the cycle of detection probe binding, cleavage of the probe and release of the probe fragments can be repeated;

(b) amplifying the target nucleic acid molecule by LAMP under suitable assay conditions that allow generation of the target amplicons by the LAMP reaction and multiple cycles of: (i) hybridization of the at least one detection probe to the amplicons formed by the LAMP reaction, (ii) cleavage of the detection probe in the formed probe:target complex, and (iii) release of the cleaved probe fragments from the target; and

(c) detecting and optionally quantifying the released fragments of said at least one detection probe, and therefrom determining the presence or amount of the target nucleic acid molecule in the sample.

2. The method of claim 1, wherein

(1) the two inner primers each comprise a target complementary region on their 3' end (F2 and B2) and a target identical region on their 5' end (F1c and B1c), wherein in the target nucleic acid the sequence targeted by the target complementary region of the inner primers lies 3' to the sequence identical to the target identical sequence on the 5' end of the inner primers; and

(2) the two outer primers each comprise a target complementary region (F3 and B3), wherein in the target nucleic acid the sequence targeted by the target complementary region of

the outer primers is located 3' to the sequence of the target nucleic acid targeted by the target complementary region of the inner primers; and, optionally,

(3) the one or two loop primers each comprise a target complementary region that targets a sequence of the amplicons formed between the target complementary region on the 3' end of the inner primers and the sequence complementary to the target identical sequence on the 5' end of the inner primers.

3. The method of claim 1 or 2, wherein the at least one detection probe has the structure DNA1-R-DNA2,
wherein DNA1 and DNA2 are DNA sequences that are complementary to adjacent regions in the target nucleic acid molecule and R is the scissile linkage.

4. The method of any one of claims 1 to 3, wherein the scissile linkage comprises at least one ribonucleotide or modified ribonucleotide and the cleaving agent comprises a thermostable RNase H.

5. The method of any one of claims 1 to 4, wherein the scissile linkage comprises at least one apurinic/apyrimidinic site (AP site) and the cleaving agent comprises a thermostable AP endonuclease, spermine, or spermidine.

6. The method of any one of claims 1 to 4, wherein the scissile linkage comprises at least one deoxyribonucleotide with its base methylated or substituted and the cleaving agent comprises a specific endonuclease that recognizes the base modification.

7. The method of any one of claims 1 to 6, wherein the released fragments are labeled with a detectable marker and labeled fragments are detected in step (c).

8. The method of any one of claims 1 to 6, wherein the at least one detection probe comprises a fluorescent marker, preferably a fluorescence resonance energy transfer (FRET) pair consisting of two fluorophores, two chromophores or a fluorophore/chromophore quencher pair, wherein the first member of the pair is located on the 5' end of the probe or in the sequence 5' to the scissile linkage and the second member of the pair is located on the 3' end of the probe or in the sequence 3' to the scissile linkage, wherein the members of the pair are located such that they can interact in the intact non-cleaved probe and selected such that the fluorescence signal increases or decreases upon cleavage of the probe.

9. The method of any one of claims 1 to 6, wherein the detection method in step (c) is lateral flow detection.

10. The method of any one of claims 1 to 9, wherein the sequence in the target nucleic acid molecule targeted by the target complementary region of the at least one detection probe is located between the sequence targeted by the target complementary region on the 3' end of the inner primers and the sequence identical to the target identical sequence on the 5' end of the inner primers and, optionally, 5' or 3' ,preferably 3', to the sequence in the target nucleic acid molecule targeted by the target complementary region of the loop primer(s).

11. The method of any one of claims 1 to 10, wherein the sequences targeted by the target complementary regions of the LAMP primers and the at least one detection probe are non-overlapping.

12. The method of any one of claims 1 to 11, wherein the method uses more than one detection probe for a single target nucleic acid molecule.

13. The method of any one of claims 1 to 12, wherein the method is a multiplexing method and is for determining the presence or amount of two or more target nucleic acid molecules in a sample, wherein the method uses one or more LAMP primer sets and/or one or more detection probes for each target nucleic acid molecule or for multiple related target nucleic acid molecules.

14. The method of any one of claims 1 to 13, wherein the target nucleic acid molecule is a nucleic acid of a pathogen, optionally a human pathogen, preferably a bacterial, fungal, parasite or viral nucleic acid molecule or a cDNA reverse transcript of a bacterial, fungal, parasite or viral RNA, preferably a cDNA of a dengue virus RNA.

15. The method of claim 14, wherein
    (1) the LAMP inner forward primer comprises the nucleic acid sequence set forth in SEQ ID NO:1, 2 or 3 (GB_T1_3UTR_FIP and GB_T1T3_3UTR_FIP and GB_T3_3UTR_FIP) or a variant thereof having at least 90 % sequence identity over the entire length;
    (2) the LAMP inner backward primer comprises the nucleic acid sequences set forth in SEQ ID NO: 4 (GB_T1_3UTR_BIP) or a variant thereof having at least 90 % sequence identity over the entire length;
    (3) the LAMP outer forward primer comprises the nucleic acid sequence set forth in SEQ ID NO:5 (GB_T1_3UTR_F3) or a variant thereof having at least 90 % sequence identity over the entire length;
    (4) the LAMP outer backward primer comprises the nucleic acid sequence set forth in SEQ ID NO:6 (GB_T1_3UTR_B3) or a variant thereof having at least 90 % sequence identity over the entire length;

(5) the LAMP loop forward primer, if present, comprises the nucleic acid sequence set forth in SEQ ID NO:7 or 8 (GB_T1_3UTR_LPF or GB_T1T3_3UTR_LPF) or a variant thereof having at least 90 % sequence identity over the entire length; and

(6) the LAMP loop backward primer, if present, comprises the nucleic acid sequence set forth in SEQ ID NO:9 (GB_T1_3UTR_LPB) or a variant thereof having at least 90 % sequence identity over the entire length.

16. The method of claim 14 or 15, wherein the at least one detection probe comprises the nucleic acid sequence set forth in any one of SEQ ID Nos. 10-12 (T1 3UTR F Probe, T3 3UTR F Probe, T1_3UTR_B2LP_CP_rA) or a variant thereof having at least 90 % sequence identity over the entire length.

17. Kit for the determining of the presence or amount of a target nucleic acid molecule in a sample by loop mediated isothermal amplification (LAMP), the kit comprising:

A. a LAMP reaction mixture;

B. a DNA polymerase with strand displacement properties;

C. at least one detection probe; and

D. at least one cleaving agent for the at least one detection probe;

wherein the LAMP reaction mixture comprises a LAMP primer set of 4 to 6 primers, comprising two inner primers (FIP and BIP), two outer primers (F3 and B3), and optionally one or two loop primers (LF and/or LB), the primers being specific for the target nucleic acid molecule;

wherein the at least one detection probe is a single stranded probe comprising a DNA sequence complementary to a sequence in a loop region of the amplicons formed by the LAMP reaction that can hybridize to said amplicons under LAMP assay conditions and form a double-stranded probe:target complex, the at least one detection probe comprising a scissile linkage in the DNA sequence complementary to a sequence in a loop region of the amplicons formed by the LAMP reaction, wherein said scissile linkage in the probe:target complex but not the non-hybridized probe is cleavable under LAMP assay conditions and said cleavage leads to release of one or more fragments of said at least one detection probe adjacent to said scissile linkage from the probe:target complex; and

wherein under LAMP assay conditions the at least one cleaving agent is capable of cleaving the at least one detection probe at said scissile linkage when said probe is hybridized to its target, wherein upon cleavage of said at least one detection probe at the scissile linkage the fragments of said at least one detection probe are released from the probe:target complex so that the cycle of probe binding, cleavage and release of the probe fragments can be repeated.