

COMPOSITIONS AND METHODS OF ISOTHERMAL NUCLEIC ACID DETECTION

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PATENT STATUS

Country	Type	Number	Dated	Case
United States Of America	Published Application	20230313282	10/05/2023	2021-005

Additional Patents Pending

BRIEF DESCRIPTION

An improved method for isothermal nucleic acid detection based on a loop mediated isothermal amplification (LAMP) technique that can be broadly applied for nucleic acid diagnostics.

LAMP is an isothermal amplification method that amplifies DNA or RNA. This iteration of LAMP allows for the integration of any short DNA sequence, including tags, restriction enzyme sites, or promoters, into an isothermally amplified amplicon.

The technique presented by the inventors allows for the insertion of sequence tags up to 35 nt into the flanking regions of the LAMP amplicon using the forward and backward inner primers (FIP and BIP), and loop primers. The inventors have demonstrated insertion of sequence fragments into the 5' and middle regions of the FIP and BIP primers, and the 5' region of the loop primers. In some embodiments, the sequence tag comprises a T7 RNA polymerase promoter, which is then incorporated into the LAMP amplicon (termed RT-LAMP/T7). With the addition of T7 polymerase, the amplicon can be in vitro transcribed, leading to additional amplification of the target molecule into an RNA substrate. This improves the efficiency of the amplification reaction and enables substrate conversion into different nucleic acid types.

In other embodiments, the amplified RNA sequence can be detected by CRISPR enzymes, such as RNA-targeting Cas13 systems.

SUGGESTED USES

Nucleic acid diagnostics.

For example, RT-LAMP/T7 can be used to isothermally amplify small quantities (as low as 20 aM sensitivity) of RNA or DNA robustly in well under 30 minutes. The amplified RNA output can then be detected through various means, such as CRISPR-Cas detection, molecular beacons, FRET probes, split-fluorescent protein probes, and RNA aptamers. The pairing of RT-LAMP/T7 and an RNA detection method has applications in traditional and point-of-care diagnostics of viral RNA and DNA.

ADVANTAGES

This technology improves the efficiency of the amplification reaction and enables substrate conversion into different nucleic acid types.

ADDITIONAL TECHNOLOGIES BY THESE INVENTORS

- ▶ RNA Writing: Programmable Splicing for Transcriptome Engineering
- ▶ RECOMBINASES FOR INTEGRATING DNA & RECOMBINASE FUSIONS

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OTHER INFORMATION

CATEGORIZED AS

- » **Biotechnology**
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