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(54) Title: UNIVERSAL LAMP FOR CAPTURING NON-COGNATE NUCLEIC ACIDS IN LAMP CONCATEMERS

(57) Abstract: Universal LAMP can be used to capture target nucleic acid sequences within LAMP concatemers without having to design new LAMP primers for each target. These universal LAMP primers can be used in a variety of amplification techniques and can be included in a kit.

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UNIVERSAL LAMP FOR CAPTURING NON-COGNATE NUCLEIC ACIDS IN LAMP CONCATEMERS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims benefit of U.S. Provisional Application No. 63/508,633, filed June 16, 2023, incorporated herein by reference in its entirety.

SEQUENCE LISTING STATEMENT

A Sequence Listing conforming to the rules of WIPO Standard ST.26 is hereby incorporated by reference. Said Sequence Listing has been filed as an electronic document via PatentCenter encoded as XML in UTF-8 text. The electronic document, created on June 14, 2024, is entitled "10046-528WO1_ST26.xml", and is 39,367 bytes in size.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with government support under Grant No. R01 EB027202 awarded by the National Institutes of Health. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Rapid amplification of a target nucleic acid sequence finds increasing importance in a number of different applications, such as medical diagnostics, disease detection, and disease discovery. Among the conventionally used techniques, loop-mediated isothermal amplification (LAMP) has emerged as a powerful alternative to PCR due to its ability to operate under isothermal conditions, providing cheaper and more rapid amplification. LAMP employs a DNA polymerase with strand displacement activity and a set of usually four to six primers to recognize distinct regions within a target sequence.

Although LAMP offers several advantages over PCR, many LAMP assays are limited to specific target sequences. Thus, the design of new LAMP assays for detection of nucleic acid sequences of interest relies on tedious, time consuming, and expensive experiments for validation of potential primer sets. Moreover, the resulting concatemers often do not contain significant sections of the target sequence beyond those serving as primer binding sites, thereby limiting the amount of sequence information that may be obtained from the amplicons.

Therefore, there exists a need for a universal LAMP assay that overcomes the limitations of existing methods and enables rapid, cost-effective, and sensitive nucleic acid amplification.

SUMMARY OF THE INVENTION

Disclosed herein are compounds, compositions, methods for making and using such compounds and compositions.

In various aspects, disclosed herein are methods of amplifying a target nucleic acid sequence using a universal loop-mediated isothermal amplification (LAMP) target sequence, the method comprising: a) providing a target nucleic acid sequence, wherein said target nucleic acid sequence comprises a target-specific hybridization region; b) carrying out a nucleic acid amplification step using a forward primer and a reverse primer, wherein both primers comprise a universal LAMP handle, wherein said universal LAMP handles comprise a universal amplification support region comprising one or more regions for hybridization of universal priming sequences, thereby producing a universal LAMP target sequence, wherein said universal LAMP target sequence comprises a target sequence flanked by universal handles on both a 3' and 5' terminus of the target sequence; and c) carrying out amplification using LAMP with universal priming sequences which recognize the universal support region, thereby producing concatameric amplicons comprising amplified copies of the target nucleic acid sequence.

Also disclosed is a kit comprising: a) a forward universal LAMP handle comprising a forward nucleic acid extension sequence (F-strand), wherein the F-strand comprises in 3' to 5' order, a hybridization region to a target nucleic acid sequence, an F1 region, a loop region, an F2 region, and an F3 region, and further wherein the hybridization region hybridizes with the target sequence, and the F1, F2, and F3 regions are primer-interacting sequences; and b) a reverse universal LAMP handle comprising a reverse nucleic acid extension sequence (B-strand), wherein the B-strand comprises in 3' to 5' order, a hybridization region, a B1 region, a loop region, a B2 region, and a B3 region, and further wherein the hybridization region hybridizes with the target sequence and the B1, B2, and B3 regions are primer-interacting sequences.

Additional advantages of the disclosed subject matter will be set forth in part in the description that follows and the Figures, and in part will be obvious from the description, or can be learned by practice of the aspects described below. The advantages described below will be realized and attained by means of the elements and combinations particularly

pointed out in the appended claims. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows an example of the capture of target sequences in universal loop-mediated isothermal amplification (LAMP) concatemers for nanopore sequencing. The described universal LAMP can be used to capture almost any target nucleic acid sequence (few tens to hundreds of base pairs) within LAMP concatemers without having to design new LAMP primers. It is noted that a universal handle primer with a LAMP handle can be broken into two shorter primers with overlaps, instead of one long primer. In this case, nucleic acid amplification uses all four primers to generate the universal LAMP template.

Figure 2 shows PCR to LAMPSeq for *Chlamydia trachomatis* using SRB sequences as the universal LAMP amplicon.

Figure 3 shows BLAST results of PCR to LAMPSeq for *Chlamydia trachomatis* using SRB sequences as the universal LAMP amplicon.

Figure 4 shows alignment reads from nanopore sequencing of the PCR to LAMPSeq for *Chlamydia trachomatis* using SRB sequence as the universal LAMP amplicon (SEQ ID NOS 1 and 2).

Figure 5 shows alignment reads from nanopore sequencing of the PCR to LAMPSeq for *Chlamydia trachomatis* using SRB sequence as the universal LAMP amplicon (SEQ ID NOS: 1 and 2).

Figure 6 shows BLAST query of concatemer hits *C. trachomatis*.

Figure 7 shows PCR to LAMPSeq for *Fusobacterium nucleatum* FN1868.

Figure 8 shows BLAST results of PCR to LAMPSeq for *Fusobacterium nucleatum* FN1868.

Figure 9 shows alignment reads from nanopore sequencing of the PCR to LAMPSeq for *Fusobacterium nucleatum* FN1868 (SEQ ID NOS: 3 and 4).

Figure 10 shows alignment reads from nanopore sequencing of the PCR to LAMPSeq for *Fusobacterium nucleatum* FN1868 (SEQ ID NOS: 3 and 4).

Figure 11 shows BLAST query of concatemer hits *F. nucleatum.*

Figure 12 shows PCR to LAMPSeq for MERS-CoV upE using Ebolavirus LAMP amplicon-based universal handles.

Figure 13 shows BLAST results of PCR to LAMPSeq for MERS-CoV upE using Ebolavirus LAMP amplicon-based universal handles.

Figure 14 shows BLAST query of concatemer hits MERS-CoV.

Figure 15 shows additional universal handles for PCR to LAMPSeq were successfully tested using the two-step process (SEQ ID NOS: 5 and 6).

Figure 16 shows one pot isothermal universal Long LAMP using NRP2 sequences as the universal LAMP amplicons (SEQ ID NO: 7 and 8).

Figure 17 shows one pot isothermal universal LAMP can capture and amplify target sequences.

Figure 18A-18G show one pot isothermal universal LAMP detection limit. Picograms of target can be detected. Negative controls are clean. Figure 18B - 3 ng template + NRP2 primers + Fla PU primers; Figure 18C - 30 pg template + NRP2 primers + Fla PU primers; Figure 18D - 300 fg template + NRP2 primers + Fla PU primers; Figure 18E - 3 ng template + NRP2 primers only; Figure 18F - ; Figure 18F - 3 ng template + Fla PU primers only; Figure 18G - No template + NRP2 primers + Fla PU primers.

Figure 19 shows long LAMP can generate concatemeric targets for use as novel biomaterials in immobilization.

Figure 20 shows long LAMP can generate concatemeric targets for use as novel biomaterials in restriction enzyme use. By incorporating restriction enzyme sites in F1 and/or B1 regions of the universal LAMP amplicon the concatemer units of the captured target(s) may be released and recombined into long concatemers without the intervening universal LAMP.

Figure 21 shows long LAMP can generate concatemeric targets for use as novel biomaterials in nicking enzyme use. By incorporating nicking enzyme sites in F1 or B1 regions of the universal LAMP amplicon the concatemer units of the captured target(s) can be released as single stranded DNA upon denaturation. These ssDNA in turn can fold into hairpins and intermolecular double stranded DNA networks upon cooling. Reamplification via self-priming 3'-end hairpins (or primer addition) is feasible.

Figure 22 shows long LAMP can generate concatemeric targets for use as novel biomaterials in concatemer transcription and translation. Long LAMP can be used to capture and concatemerize transcription units designed to yield unit transcripts or very long concatemeric RNA. These RNA can have various uses, such as preparation of RNA hydrogels, probing single molecules, preparation of double stranded RNA, and translation of novel concatemeric proteins. They can also have various shapes due to hybridization between inverted repeats.

Figure 23A-D shows alternate methods of using the long LAMP method described herein. For example, shown is the use of hairpin primers for isothermal capture of longer targets. Chimeric target-specific primers whose 5'-ends are extended with universal LAMP or DCA hairpin primers can enable capture of longer targets. In each case outer primers, similar to outer primers in LAMP, can be used to displace strands synthesized by the hairpin primers to kickstart self-priming hairpin amplicon formation. (A) shows chimeric hairpin LAMP primers. (B) shows chimeric hairpin phosphorothioated DCA primers. (C) shows non-chimeric target-specific toehold hairpin primers. (D) shows various conformations.

DETAILED DESCRIPTION

The materials, compounds, compositions, articles, and methods described herein may be understood more readily by reference to the following detailed description of specific aspects of the disclosed subject matter and the Examples and Figures included therein.

Before the present materials, compounds, compositions, and methods are disclosed and described, it is to be understood that the aspects described below are not limited to specific synthetic methods or specific reagents, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

Also, throughout this specification, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which the disclosed matter pertains. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

Definitions

In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

Throughout the description and claims of this specification the word "comprise" and other forms of the word, such as "comprising" and "comprises," means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

It is understood that throughout this specification the identifiers "first" and "second" are used solely to aid in distinguishing the various components and steps of the disclosed subject matter. The identifiers "first" and "second" are not intended to imply any particular order, amount, preference, or importance to the components or steps modified by these terms.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed the "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed. It is also understood that the throughout the application, data is provided in a number of different formats, and that this data, represents endpoints and starting points, and ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point 15 are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to 10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

In this specification and in the claims which follow, reference will be made to a number of terms which shall be defined to have the following meanings:

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

A "self-assembly pathway" is a series of reactions autonomously executed by nucleic acid sequences in the execution of hybridized, detectable nucleic acid sequences. The self-assembly pathway comprises assembly, or hybridization, of nucleic acid sequences. In some embodiments, the self-assembly pathway can also comprise one or more disassembly reactions.

The term "nucleic acid" refers to natural nucleic acids, artificial nucleic acids, analogs thereof, or combinations thereof. Nucleic acids may also include analogs of DNA or RNA having modifications to either the bases or the backbone. For example, nucleic acid, as used herein, includes the use of peptide nucleic acids (PNA). The term "nucleic acids" also includes chimeric molecules.

The term "hairpin" as used herein refers to a structure formed by intramolecular base pairing in a single-stranded polynucleotide ending in an unpaired loop (the "hairpin loop"). In various embodiments, hairpins comprise a hairpin loop protected by stems. For example, a hairpin can comprise a first stem region, a hairpin loop region, and a second stem region. The first and second stem regions can hybridize to each other and together form a duplex region. Thus, a stem region of a hairpin nucleic acid is a region that hybridizes to a complementary portion of the same nucleic acid to form the duplex stem of a hairpin.

the term "hairpin loop" refers to a single stranded region that loops back on itself and is closed by a single base pair.

"Interior loop" and "internal loop," are used interchangeably and refer to a loop closed by two base pairs. The closing base pairs are separate by single stranded regions of zero or more bases. A "bulge loop" is an interior loop where one of the separated single-stranded regions is zero bases in length and the other is greater than zero bases in length.

An "initiator" is a molecule that is able to initiate the hybridization of two other nucleic acid sequences. The initiator is also referred to herein as the third nucleic acid sequence, while it facilitates the hybridization of what is referred to herein as the first and second nucleic acid sequences.

"Monomers" as used herein refers to individual nucleic acid sequences. For example, monomers are referred to herein as a first nucleic acid sequence, a second nucleic acid sequence, or a third nucleic acid sequence, etc.

By "nucleic acid sequence" is meant a nucleic acid which comprises an individual sequence. When a first, second, or third nucleic acid sequence is referred to, this is meant that the individual nucleotides of each of the first, second, third, etc., nucleic acid sequence are unique and differ from each other. In other words, the first nucleic acid sequence will differ in nucleotide sequences from the second and third, etc. There can be multiple nucleic acid sequences with the same sequence. For instance, when a "first nucleic acid sequence" is referred to, this can include multiple copies of the same sequence, all of which are referred to as a "first nucleic acid sequence."

As used herein, the term "target nucleic acid sequence" or "target sequence" refers to specific nucleic acid sequences of interest to be amplified and/or detected. This also includes the complementary second strand of the nucleic acid sequences to be amplified and either strand of a copy of the nucleic acid sequence which is produced by amplification. The target nucleic acid can originate from a variety of sources. For example, target nucleic acids can be naturally occurring DNA or RNA isolated from any source, recombinant molecules, cDNA, or synthetic analogs, as known in the art. In some embodiments, the target nucleic acid sequence may comprise one or more single-nucleotide polymorphisms (SNPs), allelic variants, and other mutations such as deletion mutations, insertion mutations, point mutations. In other embodiments, the target nucleic acid sequence may comprise a junction sequence of a fusion gene, possibly associated with cancer. In yet another embodiment, the target nucleic acid sequence may originate from a microorganism, including specific clones or strains of microorganisms, possibly involved in inducing diseases in human beings and animals.

Typically, at least two different nucleic acid sequences are used in self-assembly pathways, although three, four, five, six or more may be used. Typically each nucleic acid sequence comprises at least one domain that is complementary to at least a portion of one other sequence being used for the self-assembly pathway. Individual nucleic acid sequences are discussed in more detail below.

The term "domain" refers to a portion of a nucleic acid sequence. An "input domain" of a nucleic acid sequence refers to a domain that is configured to receive a signal which initiates a physical and/or chemical change, such as, a for example, a conformational change, of the nucleic acid sequence. In some embodiments, an input domain can be an initiator binding domain, an assembly complement domain, or a disassembly complement domain. An "output domain" of a nucleic acid sequence refers to a domain that is configured to confer a signal. For example, the signal can bind a complementary sequence

to an input domain. In some embodiments, an output domain is configured to confer a signal to an input domain of another nucleic acid sequence. In some embodiments, an output domain can be, for example, an assembly domain, or a disassembly domain. In some embodiments, an output domain can be present in an initiator.

The term "nucleate" as used herein means to begin a process of, for example, a physical and/or chemical change at a discrete point in a system. The term "nucleation" refers to the beginning of physical and/or chemical changes at discrete points in a system.

The term "toehold" refers to nucleation site of a domain comprising a nucleic acid sequence designed to initiate hybridization of the domain with a complementary nucleic acid sequence. The secondary structure of a nucleic acid sequence may be such that the toehold is exposed or sequestered. For example, in some embodiments, the secondary structure of the toehold is such that the toehold is available to hybridize to a complementary nucleic acid (the toehold is "exposed," or "accessible"), and in other embodiments, the secondary structure of the toehold is such that the toehold is not available to hybridize to a complementary nucleic acid (the toehold is "sequestered," or "inaccessible"). If the toehold is sequestered or otherwise unavailable, the toehold can be made available by some event such as, for example, the opening of the hairpin of which it is a part of. When exposed, a toehold is configured such that a complementary nucleic acid sequence can nucleate at the toehold.

A "propagation region" as used herein refers to a portion of a domain of a first nucleic acid sequence that is configured to hybridize to a complementary second nucleic acid sequence once the toehold of the domain nucleates at an exposed toehold of the second nucleic acid sequence. The propagation region is configured such that an available secondary nucleic acid sequence does not nucleate at the propagation region; rather, the propagation region hybridizes to the second nucleic acid sequence only after nucleation at the toehold of the same domain.

In some embodiments, nucleic acid sequences can be "metastable." That is, in the absence of an initiator they are kinetically disfavored from associating with other nucleic acid sequences comprising complementary regions.

As used herein, the terms "polymerization" and "assembly" are used interchangeably and refer to the association of two or more nucleic acid sequence, or one or more nucleic acid sequences and an initiator, to form a polymer. The "polymer" may comprise covalent bonds, non-covalent bonds or both. For example, in some embodiments a

first, second, and third nucleic acid sequence can hybridize sequentially to form a polymer comprising a three-arm branched junction.

As used herein term "disassembly" refers to the disassociation of an initiator or at least one nucleic acid sequence.

As used herein "reaction graph" refers to a representation of assembly (and, optionally, disassembly) pathways that can be translated into molecular executables.

As used herein the terms "flip" and "switch" are used interchangeably and refer to a change from one state (e.g., accessible) to another state (e.g., inaccessible).

"Kinetically trapped" means that the nucleic acid sequences are inaccessible. In other words, a nucleic acid sequence which is "kinetically trapped" is not available for hybridization. For example, a nucleic acid sequence which has formed a hairpin is considered to be kinetically trapped.

As used herein, an "aptamer" is an oligonucleotide that is able to specifically bind an analyte of interest other than by base pair hybridization. Aptamers typically comprise DNA or RNA or a mixture of DNA and RNA. Aptamers may be naturally occurring or made by synthetic or recombinant means. The aptamers are typically single stranded, but may also be double stranded or triple stranded. They may comprise naturally occurring nucleotides, nucleotides that have been modified in some way, such as by chemical modification, and unnatural bases, for example 2-aminopurine. See, for example, U.S. Pat. No. 5,840,867. The aptamers may be chemically modified, for example, by the addition of a label, such as a fluorophore, or by the addition of a molecule that allows the aptamer to be crosslinked to a molecule to which it is bound. Aptamers are of the same "type" if they have the same sequence or are capable of specific binding to the same molecule. The length of the aptamer will vary, but is typically less than about 100 nucleotides.

The term "oligonucleotides," or "oligos" as used herein refers to oligomers of natural (RNA or DNA) or modified nucleic acid sequences or linkages, including natural and unnatural deoxyribonucleotides, ribonucleotides, anomeric forms thereof, peptide nucleic acid monomers (PNAs), locked nucleotide acids monomers (LNA), and the like and/or combinations thereof, capable of specifically binding to a single-stranded polynucleotide by way of a regular pattern of sequence-to-sequence interactions, such as Watson-Crick type of base pairing, base stacking, Hoogsteen or reverse Hoogsteen types of base pairing, or the like. Usually nucleic acid sequences are linked by phosphodiester bonds or analogs thereof to form oligonucleotides ranging in size from a few base units, e.g., 8-12, to several tens of base units, e.g., 100-200. Suitable oligonucleotides may be prepared by

the phosphoramidite method described by Beaucage and Carruthers (Tetrahedron Lett., 22, 1859-1862, 1981), or by the triester method according to Matteucci, et al. (J. Am. Chem. Soc., 103, 3185, 1981), both incorporated herein by reference, or by other chemical methods such as using a commercial automated oligonucleotide synthesizer.

Oligonucleotides (both DNA and RNA) may also be synthesized enzymatically for instance by transcription or strand displacement amplification. Typically, oligonucleotides are single-stranded, but double-stranded or partially double-stranded oligos may also be used in certain embodiments of the invention. An "oligo pair" is a pair of oligos that specifically bind to one another (i.e., are complementary (e.g., perfectly complementary) to one another).

The terms "complementary" and "complementarity" refer to oligonucleotides related by base-pairing rules. Complementary nucleotides are, generally, A and T (or A and U), or C and G. For example, for the sequence "5′-AGT-3′," the perfectly complementary sequence is "3′-TCA-5′." Methods for calculating the level of complementarity between two nucleic acids are widely known to those of ordinary skill in the art. For example, complementarity may be computed using online resources, such as, e.g., the NCBI BLAST website (ncbi.nlm.nih.gov/blast/producttable.shtml) and the Oligonucleotides Properties Calculator on the Northwestern University website (basic.northwestem.edu/biotools/oligocalc.html). Two single-stranded RNA or DNA molecules may be considered substantially complementary when the nucleotides of one strand, optimally aligned and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the nucleotides of the other strand, usually at least about 90% to 95%, and more preferably from about 98 to 100%. Two single-stranded oligonucleotides are considered perfectly complementary when the nucleotides of one strand, optimally aligned and with appropriate nucleotide insertions or deletions, pair with 100% of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first oligonucleotide will hybridize under selective hybridization conditions to a second oligonucleotide. Selective hybridization conditions include, but are not limited to, stringent hybridization conditions. Selective hybridization, or substantially complementary hybridization, occurs when at least about 65% of the nucleic acid sequences within a first oligonucleotide over a stretch of at least 14 to 25 sequences pair with a perfectly complementary sequences within a second oligonucleotide, preferably at least about 75%, more preferably at least about 90%. Preferably, the two nucleic acid sequences have at least 95%, 96%, 97%, 98%, 99% or 100% of sequence identity. See, M. Kanehisa, Nucleic Acids

Res. 12, 203 (1984), incorporated herein by reference. For shorter nucleotide sequences selective hybridization occurs when at least about 65% of the nucleic acid sequences within a first oligonucleotide over a stretch of at least 8 to 12 nucleotides pair with a perfectly complementary nucleic acid sequence within a second oligonucleotide, preferably at least about 75%, more preferably at least about 90%. Stringent hybridization conditions will typically include salt concentrations of less than about 1 M, more usually less than about 500 mM and preferably less than about 200 mM. Hybridization temperatures can be as low as 5° C., and are preferably lower than about 30° C. However, longer fragments may require higher hybridization temperatures for specific hybridization. Hybridization temperatures are generally at least about 2° C. to 6° C. lower than melting temperatures (T_m), which are defined below.

As used herein, "two perfectly matched nucleotide sequences" refers to a nucleic acid duplex wherein the two nucleotide strands match according to the Watson-Crick basepair principle, i.e., A-T and C-G pairs in DNA:DNA duplex and A-U and C-G pairs in DNA:RNA or RNA:RNA duplex, and there is no deletion or addition in each of the two strands.

The term, "mismatch" refers to a nucleic acid duplex wherein at least one of the nucleotide base pairs do not form a match according to the Watson-Crick basepair principle. For example, A-C or U-G "pairs" are lined up, which are not capable of forming a basepair. The mismatch can be in a single set of bases, or in two, three, four, five, or more basepairs of the nucleic acid duplex.

As used herein, "complementary to each other over at least a portion of their sequence" means that at least two or more consecutive nucleotide base pairs are complementary to each other. For example, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more consecutive nucleotide base pairs can be complementary to each other over the length of the nucleic acid sequence. .

As used herein, "substantially hybridized" refers to the conditions under which a stable duplex is formed between two nucleic acid sequences, and can be detected. This is discussed in more detail below.

As used herein, "melting temperature" ("Tm") refers to the midpoint of the temperature range over which nucleic acid duplex, i.e., DNA:DNA, DNA:RNA and RNA:RNA, is denatured.

As used herein: "stringency of hybridization" in determining percentage mismatch is as follows:

1) high stringency: 0.1 X SSPE, 0.1% SDS, 65°C;

2) medium stringency: 0.2 X SSPE, 0.1% SDS, 50°C (also referred to as moderate stringency); and

3) low stringency: 1.0 X SSPE, 0.1% SDS, 50°C

It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures (See generally, Ausubel (Ed.) Current Protocols in Molecular Biology, 2.9A. Southern Blotting, 2.9B. Dot and Slot Blotting of DNA and 2.10. Hybridization Analysis of DNA Blots, John Wiley & Sons, Inc. (2000)).

As used herein, a "significant reduction in background hybridization" means that non-specific hybridization, or hybridization between unintended nucleic acid sequences, is reduced by at least 80%, more preferably by at least 90%, even more preferably by at least 95%, still more preferably by at least 99%.

By "preferentially binds" it is meant that a specific binding event between a first and second molecule occurs at least 20 times or more, preferably 50 times or more, more preferably 100 times or more, and even 1000 times or more often than a nonspecific binding event between the first molecule and a molecule that is not the second molecule. For example, a capture moiety can be designed to preferentially bind to a given target agent at least 20 times or more, preferably 50 times or more, more preferably 100 times or more, and even 1000 times or more often than to other molecules in a biological solution. Also, an immobilized binding partner, in certain embodiments, will preferentially bind to a target agent, capture moiety, or capture moiety/target agent complex. While not wishing to be limited by applicants present understanding of the invention, it is believed binding will be recognized as existing when the K_a is at 10^7 l/mole or greater, preferably 10^8 l/mole or greater. In the embodiment where the capture moiety is comprised of antibody, the binding affinity of 10^7 l/mole or more may be due to (1) a single monoclonal antibody (e.g., large numbers of one kind of antibody) or (2) a plurality of different monoclonal antibodies (e.g., large numbers of each of several different monoclonal antibodies) or (3) large numbers of polyclonal antibodies. It is also possible to use combinations of (1)-(3). The differential in binding affinity may be accomplished by using several different antibodies as per (1)-(3) above and as such some of the antibodies in a mixture could have less than a four-fold difference. For purposes of most embodiments of the invention an indication that no binding occurs means that the equilibrium or affinity constant K_a is 10^6 l/mole or less. Antibodies may be designed to maximize binding to the intended antigen by designing peptides to

specific epitopes that are more accessible to binding, as can be predicted by one skilled in the art.

The term "sample" in the present specification and claims is used in its broadest sense and can be, by non-limiting example, any sample that is suspected of containing a target agent(s) to be detected. It is meant to include specimens or cultures (e.g., microbiological cultures), and biological and environmental specimens as well as non-biological specimens. Biological samples may comprise animal-derived materials, including fluid (e.g., blood, saliva, urine, lymph, etc.), solid (e.g., stool) or tissue (e.g., buccal, organ-specific, skin, etc.), as well as liquid and solid food and feed products and ingredients such as dairy items, vegetables, meat and meat by-products, and waste. Biological samples may be obtained from, e.g., humans, any domestic or wild animals, plants, bacteria or other microorganisms, etc. Environmental samples can include environmental material such as surface matter, soil, water (e.g., contaminated water), air and industrial samples, as well as samples obtained from food and dairy processing instruments, apparatus, equipment, utensils, disposable and non-disposable items. These examples are not to be construed as limiting the sample types applicable to the present invention. Those of skill in the art would appreciate and understand the particular type of sample required for the detection of particular target agents (Pawliszyn, J., *Sampling and Sample Preparation for Field and Laboratory*, (2002); Venkatesh Iyengar, G., et al., *Element Analysis of Biological Samples: Principles and Practices* (1998); Drielak, S., *Hot Zone Forensics: Chemical, Biological, and Radiological Evidence Collection* (2004); and Nielsen, D. M., *Practical Handbook of Environmental Site Characterization and Ground-Water Monitoring* (2005)).

A substance is commonly said to be present in "excess" or "molar excess" relative to another component if that component is present at a higher molar concentration than the other component. Often, when present in excess, the component will be present in at least a 10-fold molar excess and commonly at 100-1,000,000 fold molar excess. Those of skill in the art would appreciate and understand the particular degree or amount of excess preferred for any particular reaction or reaction conditions. Such excess is often empirically determined and/or optimized for a particular reaction or reaction conditions.

As used herein, "a promoter, a promoter region or promoter element" refers to a segment of DNA or RNA that controls transcription of the DNA or RNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. This portion of the promoter region is referred to as the promoter. In addition, the promoter region includes

sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be cis acting or may be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated.

As used herein, "operatively linked or operationally associated" refers to the functional relationship of nucleic acids with regulatory and effector sequences of nucleotides, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA. In order to optimize expression and/or in vitro transcription, it may be necessary to remove, add or alter 5' untranslated portions of the clones to eliminate extra, potential inappropriate alternative translation initiation (i.e., start) codons or other sequences that may interfere with or reduce expression, either at the level of transcription or translation. Alternatively, consensus ribosome binding sites (see, e.g., Kozak, J. Biol. Chem., 266:19867-19870 (1991)) can be inserted immediately 5' of the start codon and may enhance expression. The desirability of (or need for) such modification may be empirically determined.

As used herein, "RNA polymerase" refers to an enzyme that synthesizes RNA using a DNA or RNA as the template. It is intended to encompass any RNA polymerase with conservative amino acid substitutions that do not substantially alter its activity.

As used herein, "reverse transcriptase" refers to an enzyme that synthesizes DNA using a RNA as the template. It is intended to encompass any reverse transcriptase with conservative amino acid substitutions that do not substantially alter its activity.

"Enzymatically produced" refers to the production or secondary or tertiary folding of a nucleic acid by an enzyme rather than by chemical synthesis. Enzymatically produced nucleic acids can be made *in vitro* or *in vivo*. For example, ribozyme-containing transcription template scaffolds can be engineered to enable enzymatic co-transcriptional synthesis of RNA circuits that can operate without any post-synthetic separation and re-folding of individual circuit components.

The term "primer," as used herein, generally refers to an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3′ end along the template so that an extended duplex is formed. The sequence of nucleotides added

during the extension reaction may be determined by the sequence of the template polynucleotide. Usually primers are extended by a DNA polymerase. Primers are generally of a length compatible with their use in synthesis of primer extension products, and usually are in the range of between 8 to 100 nucleotides in length, such as 10 to 75, 15 to 60, 15 to 40, 18 to 30, 20 to 40, 21 to 50, 22 to 45, 25 to 40, and so on, more typically in the range of between 18-40, 20-35, 21-30 nucleotides long, and any length between the stated ranges. Typical primers can be in the range of between 10-50 nucleotides long, such as 15-45, 18-40, 20-30, 21-25 and so on, and any length between the stated ranges. In some embodiments, the primers are usually not more than about 10, 12, 15, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, or 70 nucleotides in length. The term "primer-interacting sequences" refers to the region of a target nucleic acid sequence where a primer binds.

Primers are usually single-stranded for maximum efficiency in amplification, but may alternatively be double-stranded. If double-stranded, the primers can first be treated to separate its strands before being used to prepare primer extension products, or simply referred to as extension products. This denaturation step can be effectuated by heat, but may alternatively be carried out using alkali, followed by neutralization. Thus, a "primer" is complementary to a polynucleotide template, and complexes by hydrogen bonding or hybridization with the template to give a primer/template complex for initiation of synthesis by a polymerase, which is extended by the addition of covalently bonded bases linked at its 3′ end complementary to the template in the process of DNA synthesis.

The term "extension product," as used herein, generally refers to a product of a reaction in which a nucleotide primer is extended by the covalent addition of nucleotides. In some cases, the nucleotide incorporation can be guided by a template. In some cases, the nucleotide incorporation can occur without a template (e.g., "template-independent"). In some cases, an extension product is an amplification product, such as from PCR amplification, rolling circle amplification (RCA), or isothermal amplification.

A "barcode" is a label, or identifier, that conveys or is capable of conveying information (e.g., information about an analyte in a sample, a bead, and/or a capture probe). A barcode can be part of an analyte, or independent of an analyte. A barcode can be attached to an analyte. A particular barcode can be unique relative to other barcodes.

Reference will now be made in detail to specific aspects of the disclosed materials, compounds, compositions, articles, and methods, examples of which are illustrated in the accompanying Examples and Figures.

Methods

Previously, target sequences have been concatemerized by first converting them into circular DNA via DNA ligation and then performing rolling circle amplification. Unlike this circularization dependent process that requires several enzymatic steps and purification, the methods disclosed herein make use of "long LAMP" based concatemerization, which is a much simpler process (Volden 2018). LAMP primer sets where there is a long (~100 base pairs) gap between the F1 and B1 binding sites have been described (Gandelman 2011). This is not a universal LAMP process, however, but rather a process to include a longer target-derived region in a LAMP amplicon. Disclosed herein are successfully developed target-specific long LAMP primer sets where greater than100 base pairs of target sequence separate the F1 and B1 sites. Furthermore, the universal long LAMP method enables concatemerization of longer stretches of target DNA.

As a corollary application, target-specific primers that are designed to incorporate a larger target sequence between their F1 and B1 regions have been used. For example, it has been designed to amplify SARS-CoV-2 spike gene. 5-10 of such primer sets can be incorporated in a multiplex reaction that include some OSD probes to provide an immediate yes/no indication of sample positivity. Amplicons in the positive reactions can then be directly sequenced to obtain genomic information about almost the entire viral Spike gene, for example.

By labeling the 5'-end of the FIP or BIP LAMP primer, immobilized concatemers can be generated. By using long LAMP, desired concatemerized targets can be immobilized on surfaces. Such immobilized amplicons can be useful for various applications such as sequencing. It can also be used for capturing proteins or other molecules that interact with the chosen target sequence, such as DNA, RNA, or aptamer binding proteins and molecules for detection, purification, or assembling hybrid nanostructures and nanomaterials; single molecule detection, for instance by directing the single stranded loop region to bind the desired nucleic acid sequence and using DNA binding domain-FP fusion proteins, FRET probes, or other DNA readout methods to bind the concatemeric target for imaging.

Referring to Figure 1, disclosed herein are methods of amplifying a target nucleic acid sequence using a universal loop-mediated isothermal amplification (LAMP) target sequence, the method comprising: a) providing a target nucleic acid sequence, wherein said target nucleic acid sequence comprises a target-specific hybridization region; b) carrying out a nucleic acid amplification step using a forward primer and a reverse primer, wherein both primers comprise a universal LAMP handle, wherein said universal LAMP handles

comprise a universal amplification support region comprising one or more regions for hybridization of universal priming sequences, thereby producing a universal LAMP target sequence, wherein said universal LAMP target sequence comprises a target sequence flanked by universal handles on both a 3' and 5' terminus of the target sequence; and c) carrying out amplification using LAMP with universal priming sequences which recognize the universal support region, thereby producing concatameric amplicons comprising amplified copies of the target nucleic acid sequence.

More specifically, disclosed herein are methods of amplifying a target nucleic acid sequence using a universal loop-mediated isothermal amplification (LAMP) target sequence, the method comprising: a) providing a target nucleic acid sequence; b) hybridizing a forward universal handle (F-strand) to the target sequence, wherein the F-strand comprises in 3' to 5' order, a hybridization region, an F1 region, a loop region, an F2 region, and an F3 region, and further wherein the hybridization region hybridizes with the target sequence, and the F1, F2, and F3 regions are primer-interacting sequences; c) hybridizing a reverse universal handle (B-strand) to the target sequence, wherein the B-strand comprises in 3' to 5' order, a hybridization region, a B1 region, a loop region, a B2 region, and a B3 region, and further wherein the hybridization region hybridizes with the target sequence and the B1, B2, and B3 regions are primer-interacting sequences; d) carrying out nucleic acid amplification, thereby producing a target sequence with universal LAMP handles on both the 3' and 5' terminus, thereby producing a universal LAMP target sequence; and e) carrying out LAMP, thereby producing concatemeric amplicons.

The described method can be used to form concatemeric amplicons. As used herein, the terms "concatemeric amplicons" or "concatemer" are used interchangeably and generally refer to a polynucleic acid having substantially similar nucleotide sequences linked alternately in a single-stranded chain. These arrayed sequences may be simple repeats of each other, inverted repeats or combinations thereof.

In some aspects, the target sequence is 10-1000 nucleotides in length. In some aspects, the target sequence is 50-1000 nucleotides in length. In some aspects, the target sequence is 100-1000 nucleotides in length. In some aspects, the target sequence is 150-1000 nucleotides in length. In some aspects, the target sequence is 200-1000 nucleotides in length. In some aspects, the target sequence is 250-1000 nucleotides in length. In some aspects, the target sequence is 300-1000 nucleotides in length. In some aspects, the target sequence is 350-1000 nucleotides in length. In some aspects, the target sequence is 400-1000 nucleotides in length. In some aspects, the target sequence is 450-1000 nucleotides in

length. In some aspects, the target sequence is 500-1000 nucleotides in length. In some aspects, the target sequence is 550-1000 nucleotides in length. In some aspects, the target sequence is 600-1000 nucleotides in length. In some aspects, the target sequence is 650-1000 nucleotides in length. In some aspects, the target sequence is 700-1000 nucleotides in length. In some aspects, the target sequence is 750-1000 nucleotides in length.

In some aspects, the target sequence is 20-350 nucleotides in length. In some aspects, the target sequence is 50-350 nucleotides in length. In some aspects, the target sequence is 75-350 nucleotides in length. In some aspects, the target sequence is 100-350 nucleotides in length. In some aspects, the target sequence is 125-350 nucleotides in length. In some aspects, the target sequence is 150-350 nucleotides in length. In some aspects, the target sequence is 175-350 nucleotides in length. In some aspects, the target sequence is 200-350 nucleotides in length. In some aspects, the target sequence is 225-350 nucleotides in length. In some aspects, the target sequence is 250-350 nucleotides in length. In some aspects, the target sequence is 275-350 nucleotides in length. In some aspects, the target sequence is 300-350 nucleotides in length.

In some aspects, the F-strand and the B-strand bind the target sequence at the 3' and 5' ends of the target, respectively.

In some aspects, amplification is carried out using polymerase chain reaction (PCR) or an isothermal reaction. The term "polymerase chain reaction" (PCR) refers to an enzymatic nucleic acid amplification process that involves multiple cycles of denaturing template nucleic acid, annealing primers, and synthesizing a nucleic acid strand complimentary to the template strand. Each cycle typically involves raising and lowering the reaction temperature to provide the proper thermal environment for each step of the cycle. Denaturing template nucleic acid is usually accomplished using high temperature, while annealing primers requires a lower temperature. Synthesis of the nucleic acid complementary to the template strand can typically occur at a temperature between the temperatures used for denaturing and annealing. PCRs include multiplex polymerase chain reactions and single-plex polymerase chain reactions. Suitable PCR techniques are known to one skilled in the art. Isothermal reactions refer to a reaction that provides for the amplification of a nucleic acid using substantially isothermal conditions.

As used herein, the term "substantially isothermal" describes reaction conditions that do not require thermocycling. A substantially isothermal reaction may have temperature changes at the beginning and end of an amplification reaction. For example, substantially isothermal reactions include reactions that employ a "hot start" mechanism, in which the

reaction mixture is heated to a temperature necessary to activate a component of the reaction mixture and then optionally cooled to a temperature at which a nucleic acid polymerase catalyzes nucleic acid synthesis. Similarly, substantially isothermal reactions may employ a temperature to deactivate the amplification reaction, a temperature suitable for storage of the amplification products, a temperature for the release of stored reagents, or combination thereof. Thermocycling equipment can be employed to provide reaction conditions comprising a "hot start," the reaction temperature, a deactivating temperature, or a storage temperature. Examples of isothermal reactions include rolling circle amplification (RCA), multiple displacement amplification (MDA), loop-mediated isothermal amplification (LAMP), which involves specific primers to generate quasi circular DNA molecules, helicase-dependent amplification (HDA), and nicking enzyme amplification reaction (NEAR), which involve the generation of nicks in the DNA molecule that are used to prime replication.

In some aspects, "one-pot synthesis" is used to carry out the LAMP method. One-pot synthesis, as used herein, refers to the amplification of nucleic acid in a single reaction vessel or tube. In various aspects, the one-pot synthesis is carried out without the need for intermediate purification or transfer steps. One-pot synthesis approaches can provide a variety of advantages, including, for example, reduced contamination, time and cost efficiency, improved yield, and simplicity. For example, both the step of carrying out nucleic acid amplification, thereby producing a target sequence with universal LAMP handles on both the 3' and 5' terminus, thereby producing a universal LAMP target sequence; and the step of carrying out LAMP, thereby producing concatameric amplicons can be carried out in "one pot." These steps can be carried out at a temperature from 50°C to 90°C; from 55°C to 90°C; from 60°C to 90°C; from 50°C to 85°C; from 50°C to 80°C; from 50°C to 75°C; or at about 65°C. In some aspects, both steps are carried out at the same temperature. In some aspects, the steps are carried out at different temperatures.

In some aspects, the hybridization region of the F-strand is at a 3' terminus of the F-strand. In some aspects, the hybridization region of the B-strand is at a 3' terminus of the B-strand. In some aspects, both the loop region of both the F-strand and the B-strand comprises a self- complementary hairpin structure which is generated during LAMP.

In some aspects, the LAMP reaction step further comprises the steps of: i) providing a forward inner primer (FIP) which complements the F2 region of the target sequence at a 5' terminus of the FIP, and further wherein said FIP comprises a sequence which complements the F1 region of the universal LAMP target sequence at a 5' end of the

FIP; ii) amplifying a universal LAMP target sequence using FIP as a primer; iii) providing an F3 primer, wherein said F3 primer complements the F3 region of the universal LAMP target sequence, wherein said F3 complementary region is at a 5' end of the F3 primer; iv) initiating amplification using the F3 primer and producing a product therefrom, which displaces the product of step ii); v) providing a backward inner primer (BIP) which complements the B2 region of the target sequence at a 3' terminus of the BIP, and further wherein said BIP comprises a sequence which complements the B1 region of the universal LAMP target sequence; vi) amplifying a universal LAMP target sequence using BIP as a primer; vii) providing a B3 primer, wherein said B3 primer complements the B3 region of the universal LAMP target sequence, wherein said B3 complementary region is at a 5' end of the B3 primer; viii) initiating amplification of the B3 primer and producing a product therefrom, which displaces the product of step vi); ix) allowing hybridization of the F1 region and its complement and the B1 region and its complement; thereby forming a double-loop barbell product; xi) allowing binding of the FIP primer at the F2 complementary region and carrying out amplification thereof; and xii) allowing binding of the BIP primer at the B2 complementary region and carrying out amplification thereof.

In some aspects, loop primers are used during LAMP amplification. "Loop primers" can be added in conjunction with the other primers used in LAMP to produce significantly faster assays. Currently, several implementations of LAMP use a total of six primers: two loop-generating primers, two displacement primers and two loop primers. Loop primers are typically positioned between the B2 and B1 sites and the F2 and F1 sites, respectively, and orientated in a particular direction. Some suitable examples of loop primers can be found in WO2000/028082A1, which is hereby incorporated by reference.

In some aspects, the target sequence is not circularized prior to amplification.

In some aspects, two or more target sequences or sequence variants are detected concurrently, and wherein two or more sets of universal handle sequences are used.

In some aspects, the two or more target sequences or sequence variants are present in the same contiguous nucleotide sequence or on two different nucleotide sequences.

In some aspects, the two or more target sequences or sequence variants are derived from one organism or from two or more different organisms.

In some aspects, target sequences are synthetic or artificial.

In some aspects, after amplification, at least one extension product is detected.

In some aspects, one or more extension products are detected in real time.

In some aspects, the product is detected using a hybridization probe. Examples of hybridization probes include TaqMan hydrolysis probes (U.S. Pat. Nos. 5,210,015, 5,538,848, 5,487,972, and U.S. Pat. No. 5,804,375 (incorporated herein by reference in their entirety)), molecular beacons (U.S. Pat. No. 5,118,801 (incorporated herein by reference in its entirety)), and FRET hybridization probes (WO1997/046707, WO1997/046712, and WO1997/046714 (incorporated herein by reference in their entirety)).

In some aspects, the product is detected using fluorescence. Some suitable methods for detecting the product using fluorescence include the use of fluorescent labels, such as mutually quenching fluorescent labels, fluorescent label linking agents, enzymes, quenching agents, nucleic acid binding dyes, etc. In some aspects, a signal primer is used to detect amplification.

In some aspects, the product is detected by binding of or alterations in the state of nanomaterials or nanoparticles or formation of hydrogels or origami structures via endogenous or exogenous staples or connectors.

In some aspects, the product is detected by sequencing.

In some aspects, the product is used as template for amplification or transcription and the resulting amplicons or subsequent translation products are detected.

In some aspects, the product displays enzyme activity, which is used for detection

Kits

In various other aspects, disclosed herein are kits comprising: a) a forward universal LAMP handle comprising a forward nucleic acid extension sequence (F-strand), wherein the F-strand comprises in 3' to 5' order, a hybridization region to a target nucleic acid sequence, an F1 region, a loop region, an F2 region, and an F3 region, and further wherein the hybridization region hybridizes with the target sequence, and the F1, F2, and F3 regions are primer-interacting sequences; and b) a reverse universal LAMP handle comprising a reverse nucleic acid extension sequence (B-strand), wherein the B-strand comprises in 3' to 5' order, a hybridization region, a B1 region, a loop region, a B2 region, and a B3 region, and further wherein the hybridization region hybridizes with the target sequence and the B1, B2, and B3 regions are primer-interacting sequences.

In some aspects, the kit further comprises primers needed for LAMP reaction.

In some aspects, the kit further comprises loop primers. As described above, "loop primers" can be added in conjunction with the other primers used in LAMP to produce significantly faster assays. Loop primers are typically positioned between the B2 and B1 sites and the F2 and F1 sites, respectively, and orientated in a particular direction. Non-

limiting examples of loop primers suitable for the present kit can be found in WO2000/028082A1, which is hereby incorporated by reference.

In some aspects, the kit further comprises reagents for LAMP reaction. Some examples of LAMP reagents include LAMP primers for one or more target nucleic acids, DNA polymerase with high strand displacement activity (e.g., a DNA polymerase long fragment (LF) of a thermophilic bacterium, such as *Bacillus stearothermophilus* (Bst), *Bacillus Smithii* (Bsm), *Geobacillus sp. M* (GspM), or *Thermodesulfatator indicus* (Tin), or a Taq DNA polymerase including, for example, Bst large fragment polymerase, Bst 2.0, Bst 3.0, Bca (exo-), Vent, Vent (exo-), Deep Vent, Deep Vent (exo-), Φ29 phage, MS-2 phage, Z-Taq, KOD, Klenow fragment, GspSSD, GspF, OmniAmp Polimerase, SD Polimerase and any combination thereof), deoxyribonucleotide triphosphates ("dNTPs") (e.g., deoxyadenosine triphosphate ("dATP"), deoxyguanosine triphosphate ("dGTP"), deoxycytidine triphosphate ("dCTP"), and deoxythymidine triphosphate ("dTTP") and any combination thereof), buffers, magnesium sulfate or magnesium chloride ($MgSO_4$ or $MgCl_2$), loop primers, and/or betaine.

The kit may comprise a plurality of primers for amplification and/or for sequencing nucleic acids isolated from a collected specimen. The kit may provide at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, 100, 200, 500, 1000, or more primers. The kit may provide between about 1-3, 1-10, 5-20, 1-1000, 10-500, 20-200, or 50-100 primers. The primers may have 5, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200 or more nucleotides. The primers may have between about 1-8, 5-10, 6-20, 15-30, 20-50, 30-60, 40-80, 50-100, or 10-200 nucleotides.

The term "kit" refers to any delivery system for delivering materials or reagents for carrying out the method described above. In the context of reaction assays, such delivery systems can include systems and/or compounds (such as dilutants, surfactants, carriers, or the like) that allow for the storage, transport, or delivery of reaction reagents (e.g., fluorescent labels, such as mutually quenching fluorescent labels, fluorescent label linking agents, enzymes, quenching agents, etc. in the appropriate containers) and/or supporting materials (e.g., buffers, written instructions for performing the assay etc.) from one location to another. For example, kits can include one or more enclosures (e.g., boxes) containing the relevant reaction reagents and/or supporting materials. Such contents may be delivered to the intended recipient together or separately. For example, a first container may contain an enzyme for use in an assay, while a second or more containers contain mutually quenching fluorescent labels and/or quenching agents.

The kit may be provided to users, for example clinical pathology laboratories, a healthcare personnel, a physician, a nurse, a medical care assistance, or a home healthcare assistance. The kit may be intended as a stand-alone solution. Alternatively, the kit may be combined with other kits and instruments.

The sample collection device may be a glass slide coated with a functionalized surface. Analyte-specific reagents may be nucleic acid primers and/or probes to detect the panel of target and control nucleic acids. The kit may contain instructions to perform a test using reagents from other vendors. For example, the kit may instruct users to use a Qiagen purification kit to isolate mRNA from a samples collected using a provided sample collection device. The kit may comprise spin column technology (e.g. RNeasy Plus Micro Kit) or magnetic bead-based technology (e.g. ARCTURUS® PicoPure® RNA Isolation Kit, Dynabeads® mRNA DIRECT™ Micro Kit) that may isolate mRNA, total RNA, or total nucleic acids. The disclosed kit may contain a squeegee or cell scraper to enhance sample removal from the provided sample collection device when using a kit or reagents from another vendor. The kit may comprise a storage device for a collected specimen. The storage device may be a sample collection tube, an Eppendorf, a container, or any device that is suitable for storing substances. The kit may contain instructions to use a cDNA synthesis kit from another vendor. As an example, the cDNA synthesis kit may contain the SuperScript® III reverse transcriptase, AffinityScript RT, M-MuLV RNase H+ reverse transcriptase, RE3 Reverse Transcriptase, or Quantiscript Reverse Transcriptase with dNTPs in a compatible buffer. The disclosed kit may contain primers to perform cDNA synthesis.

Uses

The primers disclosed herein may contain a reporter label comprising a tag, fluorescence label, a magnetic bead, or a barcode. The reporter label may be targeted to specific nucleic acids. The reporter label may be used to identify nucleic acids. The primers may be used for sequencing of targeted nucleic acids with or without amplification of the nucleic acids. The sequencing may be any sequencing technologies known in the art. The disclosed kit may provide instructions for performing sequencing using reagents enclosed therein. The disclosed kit may contain instructions to perform cDNA synthesis using random oligonucleotide primers, poly-A primers, or analyte-specific primers. The disclosed kit may contain instructions for the user to amplify RNA or cDNA using enclosed reagents, or reagents provided by another vendor. For example, the instructions may direct users to

use enclosed primers to perform analyte-specific amplification using reagents provided by another vendor. The amplification could be performed using PCR, quantitative PCR (QPCR), real-time PCR, digital PCR (dPCR), digital droplet PCR (ddPCR), or isothermal amplification. The real-time PCR reagents from another vendor could consist of Thermo Scientific TaqPath™ qPCR Master Mixes, which can be provided as general purpose reagents. Synthesis of mRNA to cDNA and subsequent amplification can be performed using a kit, for example the TaqPath™ 1-Step RT-qPCR Master Mix. RNA may also be directly used as targets. Analyte-specific probes and fluorescent reporters can also be used. Alternatively, primers without analyte-specific probes can be used, which would be compatible for an intercalating fluorescent reporter, for example a SYBR dye.

The products of the amplification methods described herein can be analyzed using a next-generation sequencing platform. The next-generation sequencing platform can be a commercially available platform. Commercially available platforms include, e.g., platforms for sequencing-by synthesis, ion semiconductor sequencing, pyrosequencing, reversible dye terminator sequencing, sequencing by ligation, single-molecule sequencing, sequencing by hybridization, and nanopore sequencing. Platforms for sequencing by synthesis are available from, e.g., Illumina, 454 Life Sciences, Helicos Biosciences, and Qiagen. Illumina platforms can include, e.g., Illumina's Solexa platform, Illumina's Genome analyzer, which are described in Gudmundsson et al., Genome-wide association and replication studies identity four variants associated with prostate cancer susceptibility. Nat. Genet. 2009 41:1122-1126, Out et al. Deep sequencing to reveal new variants in pooled DNA samples. Hum. Mutat. 2009 30:1703-12, Turner, Massively parallel exon capture and library-free resequencing across 16 genomes. Nat. Methods 2009 6:315-6, U.S. Patent Application Publication nos. 20080160580 and 20080286795, and U.S. Pat. Nos. 6,306,597, 7,115,400, and 7,232,656, which are hereby incorporated in their entireties. 454 Life Science platforms include, e.g., the GS Flex and GS Junior, and are described in U.S. Pat. No. 7,323,305, which patent is hereby incorporated in its entirety. Platforms from Helicos Biosciences include the True Single Molecule Sequencing platform. Platforms for ion semiconductor sequencing include, e.g., the Ion Torrent Personal Genome Machine (PGM) and are described in U.S. Pat. No. 7,948,015, which patent is hereby incorporated in its entirety. Platforms for pyrosequencing include the GS Flex 454 system and are described in U.S. Pat. Nos. 7,211,390; 7,244,559; 7,264,929, which patents are hereby incorporated in their entireties. Platforms and methods for sequencing by ligation include, e.g., the SOLiD sequencing platform and are described in U.S. Pat. No. 5,750,341. Platforms for single-

molecule sequencing include the SMRT system from Pacific Bioscience and the Helicos True Single Molecule Sequencing platform.

The products produced using the methods described herein can be used as biomatter for various uses known to those of skill in the art. Examples include, but are not limited to, assembly of macro and micromaterials, DNA synthesis, transcript generation, protein generation, information storage, information capture, and information transduction.

The methods described herein can be used with barcodes. Barcodes can have a variety of different formats. For example, barcodes can include non-random, semi-random, and/or random nucleic acid and/or amino acid sequences, and synthetic nucleic acid and/or amino acid sequences. One or more barcodes can be used as part of the target sequence, or can be introduced to the LAMP product via primers.

A barcode can be attached to an analyte or to another moiety or structure in a reversible or irreversible manner. A barcode can be added to, for example, a fragment of a deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sample before or during sequencing of the sample. Barcodes can allow for identification and/or quantification of individual sequencing-reads (e.g., a barcode can be or can include a unique molecular identifier or "UMI").

Barcodes can spatially-resolve molecular components found in biological samples, for example, at single-cell resolution (e.g., a barcode can be or can include a "spatial barcode"). In some embodiments, a barcode includes both a UMI and a spatial barcode. In some embodiments, a barcode includes two or more sub-barcodes that together function as a single barcode (e.g., a polynucleotide barcode). For example, a polynucleotide barcode can include two or more polynucleotide sequences (e.g., sub-barcodes) that may be separated by one or more non-barcode sequences.

Detection systems are known in the art, and include optical assays (including fluorescence and chemiluminescent assays), enzymatic assays, radiolabeling, surface plasmon resonance, magnetoresistance, cantilever deflection, surface plasmon resonance, etc. In some embodiments, the products made by the methods described herein can be used in additional assay technologies.

In one embodiment, the surface can be a chip containing synthetic oligonucleotides. The concatemerized nucleic acid (the product of the methods disclosed herein) can be cleaved by digestion. Such means are known to those of skill in the art. This released nucleic acid can then be used as probes for hybridization, or for gene assembly. The concatemers can be barcoded, such that each concatemer comprises a unique sequence.

This barcoded concatemerized nucleic acid can be cleaved by digestion and used for gene assembly, as described herein.

In some embodiments, the products of the method described herein can be attached to solid supports for detection. Similarly, bead arrays as described below may be used.

In one embodiment, the present invention provides arrays, each array location comprising at a minimum a covalently attached strand displacement reporter, also referred to herein as a "capture probe". By "array" herein is meant a plurality of nucleic acid probes in an array format; the size of the array will depend on the composition and end use of the array. Generally, the array will comprise from two to as many as 100,000 or more reporters, depending on the size of the electrodes, as well as the end use of the array. Preferred ranges are from about 2 to about 10,000, with from about 5 to about 1000 being preferred, and from about 10 to about 100 being particularly preferred. In some embodiments, the compositions of the invention may not be in array format; that is, for some embodiments, compositions comprising a single capture probe may be made as well. In addition, in some arrays, multiple substrates may be used, either of different or identical compositions. Thus, for example, large arrays may comprise a plurality of smaller substrates. Nucleic acids arrays are known in the art, and can be classified in a number of ways; both ordered arrays (e.g. the ability to resolve chemistries at discrete sites), and random arrays (e.g. bead arrays) are included. Ordered arrays include, but are not limited to, those made using photolithography techniques (Affymetrix GeneChip™), spotting techniques (Synteni and others), printing techniques (Hewlett Packard and Rosetta), origami pads, paperfluidics, electrode arrays, three dimensional "gel pad" arrays, etc. Liquid arrays may also be used.

By "substrate" or "solid support" or other grammatical equivalents herein is meant any material that can be modified to contain discrete individual sites appropriate for the attachment or association of nucleic acids. The substrate can comprise a wide variety of materials, as will be appreciated by those in the art. including, but not limited to glass, plastics, polymers, metals, metalloids, ceramics, organics, etc. When the solid support is a bead, a wide variety of substrates are possible, including magnetic materials, glass, silicon, dextrans, plastics, etc.

Chemically derivatized particles, plates, cartridges, tubes, magnetic particles, or other solid phase matrix with specificity to the assay components can also be used. The binding surfaces of microplates, tubes or any solid phase matrices include non-polar surfaces, highly polar surfaces, modified dextran coating to promote covalent binding, antibody coating, affinity media to bind fusion proteins or peptides, surface-fixed proteins

such as recombinant protein A or G, nucleotide resins or coatings, and other affinity matrix are useful in this invention.

Platforms for multi-well plates, multi-tubes, holders, cartridges, minitubes, deep-well plates, microfuge tubes, cryovials, square well plates, fitters, chips, optic fibers, beads, and other solid-phase matrices or platform with various volumes can be accommodated on an upgradable modular platform for additional capacity. This modular platform includes a variable speed orbital shaker, and multi-position work decks for source samples, sample and reagent dilution, assay plates, sample and reagent reservoirs, pipette tips, and an active wash station.

The instrumentation can include a detector, which can be a wide variety of different detectors, depending on the labels and assay. In a preferred embodiment, useful detectors include a microscope(s) with multiple channels of fluorescence; plate readers to provide fluorescent, electrochemical and/or electrical impedance analyzers, ultraviolet and visible spectrophotometry detection with single and dual wavelength endpoint and kinetics capability, fluorescence resonance energy transfer (FRET), luminescence, quenching, two-photon excitation, and intensity redistribution; CCD cameras to capture and transform data and images into quantifiable formats; and a computer workstation.

These instruments can fit in a sterile laminar flow or fume hood, or are enclosed, self-contained systems, for cell culture growth and transformation in multi-well plates or tubes and for hazardous operations. The living cells may be grown under controlled growth conditions, with controls for temperature, humidity, and gas for time series of the live cell assays. Automated transformation of cells and automated colony pickers may facilitate rapid screening of desired cells. Flow cytometry or capillary electrophoresis formats can be used for individual capture of magnetic and other beads, particles, cells, and organisms.

The flexible hardware and software allow instrument adaptability for multiple applications. The software program modules allow creation, modification, and running of methods. The system diagnostic modules allow instrument alignment, correct connections, and motor operations. The customized tools, labware, and liquid, particle, cell and organism transfer patterns allow different applications to be performed. The database allows method and parameter storage. Robotic and computer interfaces allow communication between instruments.

The present invention can be used to diagnose and detect a wide variety of pathogens and disorders that have nucleic acid-based genetic material and/or genetic components. The system and method of the present invention can be used to detect and

diagnose molecular diagnostic targets arising in the fields of oncology, cardiovascular, identity testing and prenatal screening,

Preferably, biological sample is derived from a biological fluid, such as but not limited to blood, saliva, semen, urine, amniotic fluid, cerebrospinal fluid, synovial fluid, vitreous fluid, gastric fluid, nasopharyngeal aspirate and/or lymph.

A biological sample can be a tissue sample, a water sample, an air sample, a food sample or a crop sample. Preferably, the biological sample analysis detects any one or more of water-born pathogen, air-born pathogen, food-born pathogen or crop-born pathogen.

The pathogen detectable by the system and method of the present invention can come from a variety of hosts. The host, whether biological or non-biological, should be capable of supporting replication of an infectious agent by allowing the infectious agent to replicate in or on the host. Examples of such hosts include liquid or solid in vitro culture media, cells or tissues of animals, plants or unicellular organisms, whole organisms including mammals such as humans.

The kits and methods of the present disclosure can be employed in one of more of the following areas. In one embodiment, the kits and method of the present invention can be employed in the area of defense against biological weapons. For example, The kits and methods of the present disclosure can be used for point-of-incidence and real-time pathogen-detection. In another embodiment, The kits and methods of the present disclosure can be employed in the area of life sciences. For example, the disclosed kits and methods can be used as and with a portable analytical instrument. In another embodiment, the kits and method of the present disclosure can be employed in the area of clinical diagnostics. For example, the present disclosure can be used to diagnose and/or identify pathogens by doctors, nurses or untrained users in hospitals, homes or in the field. The present disclosure can also be used for genotyping an organism, thereby determining predisposition to genetic diseases, if any, or antibiotic resistance, if any. The present disclosure can also be used to determine pathogens present in a patient and the sensitivity and resistance profiles of those pathogens to various antibiotics. The present disclosure can also be used as a drug monitoring device, a prognostic indicator of disease, and a theragnostic device. In another embodiment, the system and method of the present disclosure can be employed in the area of industrial and agricultural monitoring. For example, the present disclosure can be used to monitor and/or detect pathogens born by food, crops, livestock, and the like. In another embodiment, the system and method of the present disclosure can be employed in the area

of forensics. For example, the present disclosure can be used to genetically identify an individual.

In one embodiment, genetic disorders and disorders having a genetic component can be diagnosed by employing the system and method of the present disclosure. For example, numerous oncogenes have been identified, including p53, implicated in the development of breast, colorectal and other cancers; c-erbB2, associated with breast cancer development and metastasis; and BRCA1 , involved in 50% of all inherited breast cancers, and also associated with increased risk for prostate and other cancers. Screening for the these genetic markers can be accomplished using the system and methods described herein.

Devices

The disclosures described herein can be configured or utilized in products or devices that include but are not limited to handheld devices, computer tablets, notebooks, smart phones, implantable devices (implantables), ingestible devices (ingestibles), wearable devices (wearables) and injectable devices (injectables).

The device or system can include or be operably coupled to system instructions, e.g., embodied in a computer or computer readable medium. The instructions can control any aspect of the device or system, e.g., to correlate one or more measurements of signal. A system can include a computer operably coupled to the other device components, e.g., through appropriate wiring, or through wireless connections. The computer can include, e.g., instructions that control amplification, e.g., using feedback control as noted above, and/or that specify when images are taken or viewed by the optical train. The computer can receive or convert image information into digital information and/or signal intensity curves as a function of time, determine concentration of a target nucleic acid analyzed by the device, and/or the like. The computer can include instructions for normalizing signal intensity to account for background, e.g., for detecting local background for one or more regions of the array, and for normalizing array signal intensity measurements by correcting for said background. Similarly, the computer can include instructions for normalizing signal intensity by correcting for variability in array capture nucleic acid spotting, uneven field of view of different regions of the array, or the like. The computer can also comprise a display unit for displaying information received from the signal output unit.

EXAMPLES

To further illustrate the principles of the present disclosure, the following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and

description of how the compositions, articles, and methods claimed herein are made and evaluated. They are intended to be purely exemplary of the invention and are not intended to limit the scope of what the inventors regard as their disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperatures, etc.); however, some errors and deviations should be accounted for. Unless indicated otherwise, temperature is °C or is at ambient temperature, and pressure is at or near atmospheric. There are numerous variations and combinations of process conditions that can be used to optimize product quality and performance. Only reasonable and routine experimentation will be required to optimize such process conditions.

Example 1: Universal Long LAMP

Universal LAMP can be used to capture almost any target nucleic acid sequence (few tens to hundreds of base pairs) within LAMP concatemers without having to design new LAMP primers for each target. Referring to Figure 1, target capture is achieved via two chimeric single stranded oligonucleotide primers whose 3'-end portions (shown in pink) are complementary to either the antisense strand (forward target primer) or the sense strand (reverse target primer) of the desired non-cognate target nucleic acid. Binding sites for these two primers on the target nucleic acid may be separated by a few tens to hundreds of base pairs. The longest target amplified efficiently thus far via long LAMP is 231 bp.

The 5'-ends of these forward and reverse target primers are extended by addition of the left and right halves, respectively, of a universal LAMP template (structurally organized as 5'-F3-F2-F1-B1-B2-B3-3' where each F or B domain is a primer interacting sequence). The left half includes the 5'-F3-F2-F1-3' sequence of the universal template sense strand while the right half is comprised of the 5'-B3c-B2c-B1c-3' sequence of the antisense strand. It is also feasible to bisect the left and right halves of the universal LAMP template sequence into two overlapping portions and append only the 3'-end portions to the respective target-specific primer. The 5'-end portions can then be supplied as separate 'outer' primers that bind to complementary regions in the chimeric target-specific primers and recreate the intact left and right halves of the universal LAMP template upon their extension.

Long LAMP can be operated in a two-pot or a one-pot reaction. In the two-pot process the first step is comprised of PCR wherein the two long chimeric primers are used to PCR amplify the target sequence into amplicons of discrete unit size comprising the target sequence flanked by the left and right halves of the LAMP template. In the second step, these PCR amplicons are used as templates for universal LAMP primers to produce

concatemeric LAMP amplicons. In the one-pot method, the target sequence is isothermally incubated in a single reaction comprising both the chimeric target specific primers and the universal LAMP primers yielding concatemeric end products containing inverted repeats of the target sequence flanked by regions of the universal LAMP amplicon.

A study was devised to observe whether universal LAMP amplicons could tolerate long insertions between their F1 and B1 regions. In the experiment, templates flanked by the left and right halves of a universal LAMP template were generated by PCR. These templates were then added to LAMP reactions containing universal LAMP primers. Following 2h amplification at 65 °C, the amplicons were purified, prepared for sequencing using the Rapid Barcoding kit and sequenced using the Mk1c platform. The experimental results (shown in Figures 2-18) demonstrated the synthesis of several potent universal LAMP assays which can be implemented in a variety of applications.

Referring to Figures 2-5, LAMP template sequence from previously designed LAMP assays for the dsrA gene of sulfate reducing bacteria was used as the universal template for this experiment. The left and right halves of the SRB template were used to create chimeric primers specific for *Chlamydia trachomatis* ORF2 gene (top panel). Long LAMP was performed in a two-step process where PCR was used in the first step to attached the universal LAMP template left and right halves to the ORF2 amplicon. In the second step this PCR product was amplified by LAMP using SRB LAMP primers (FIP+BIP+F3+B3) to create concatemeric LAMP amplicons. These amplicons were purified using Ampure XP magnetic beads and then prepared for nanopore sequencing using the Oxford nanopore rapid barcoding kit. This kit uses a transposase to simultaneously cleave DNA and attach barcoded sequencing tags to the cleaved ends. The prepared libraries were sequencing using R9 flow cells and the Mk1c platform. The sequence reads were mapped to the expected target sequence and formation of LAMP concatemers containing the target sequence was verified (middle panel). A couple of example alignments are depicted in the bottom panel.

Some of the reads from Figures 2-5 were also used as BLAST query against the nucleotide database to confirm that the sequence reads could be used to identify the valid target (results in Figure 6).

In Figures 7-10, a similar experiment as shown in Figures 2-6 was performed using a different target sequence: Fusobacterium FN1868 gene. Some of the reads seen in Figures 7-10 were used as BLAST query against the nucleotide database to confirm that the sequence reads could be used to identify the valid target (Figure 11).

Figures 12-14 show similar two-step long LAMP experimental pipeline as shown in Figures 7-10 with the following differences: The target nucleic acid being tested is a synthetic sequence derived from MERS CoV. The universal LAMP template left and right halves being used here were derived from Ebolavirus LAMP assay instead of the SRB assay used in the previous experiment.

Figure 15 shows two additional in-house designed LAMP amplicons, NRP2 and WSP, were tested successfully as universal templates using the two-step experimental process and targets described in the previous slides. Taken together, these data suggest that several different LAMP amplicons have the ability to serve as universal templates. This ability is not restricted to one or two specific amplicons.

Figure 16 describes the development of one-step isothermal execution of Long LAMP in one-pot reactions. The NRP2 amplicon was used as the universal template by appending its left and right halves to chimeric target-specific forward and reverse primers (named *.PU.fwd and *.PU.rev). One-pot reactions that included both the target specific chimeric primers and the NRP2 LAMP primer mix comprised of FIP, BIP, F3, and B3 were seeded with either no templates or with 3 ng of a plasmid template. The impact of 40 mM guanidinium hydrochloride, gp32 single stranded DNA binding protein, and additional target-specific primers on the invasion of the chimeric primers was evaluated.

Long LAMP amplicons produced in the presence or absence of specific target templates in one-pot isothermal reaction conditions were purified and subjected to nanopore sequencing. Figure 17 summarizes results from one-pot reactions that did not receive additional primers, gp32, or GnHCl. In the presence of the target templates, concatemeric amplicons that had incorporated the target sequence were observed. Amplicons produced in the absence of the target template did not demonstrate the target sequence. Inclusion of additional primers, gp32, or GnHCl did not alter the reaction outcomes.

Example 2: One Pot Isothermal Universal LAMP

One pot isothermal universal LAMP can capture and amplify target sequences. Applications include multiplex universal LAMP - MLST (*E. coli* SNP: Figures 2-5) and 18S v9.

Corollary non-universal applications include, but are not limited to: single or multiplex LAMP using target-specific LAMP primers designed with long (>100 bp) gaps between the F1 and B1 regions; target-specific OSDs provide an immediate yes/no diagnostic readout; and samples showing a positive result are directly processed for nanopore sequencing.

Figure 18 summarizes data from experiments designed to evaluate detection limit of long LAMP. NRP2-based universal long LAMP one-pot isothermal reactions were seeded with either no templates or received indicated amounts of the target plasmid template. Following 2h isothermal amplification at 65 °C, the amplicons were purified and sequenced. Universal amplicons that had incorporated the target sequence were observed only in the presence of 3 ng or 30 pg of the target template. Lower template amounts did not result in capture of the target sequence in universal amplicons. Control reactions containing 3 ng of target templates and either only the NRP2 LAMP primers or only the chimeric target primers did not produce amplicons bearing concatemerized target sequences.

In one embodiment, the one pot long LAMP reaction can first be subjected to four cycles of PCR using Taq or Vent to allow formation of the initial universal LAMP dumbbell amplicon with the captured target. Subsequent addition of Bst DNA pol and isothermal incubation can yield concatemeric amplicons. Inclusion of denaturants like formamide may promote primer invasion.

The methods and compositions of the appended claims are not limited in scope by the specific methods and compositions described herein, which are intended as illustrations of a few aspects of the claims and any methods and compositions that are functionally equivalent are within the scope of this disclosure. Various modifications of the methods and compositions in addition to those shown and described herein are intended to fall within the scope of the appended claims. Further, while only certain representative methods, compositions, and aspects of these methods and compositions are specifically described, other methods and compositions and combinations of various features of the methods and compositions are intended to fall within the scope of the appended claims, even if not specifically recited. Thus, a combination of steps, elements, components, or constituents can be explicitly mentioned herein; however, all other combinations of steps, elements, components, and constituents are included, even though not explicitly stated.

References

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[4] WO 2013/171140A1

[5] US 11,118,206 B2

CLAIMS

What is claimed is:

1. A method of amplifying a target nucleic acid sequence using a universal loop-mediated isothermal amplification (LAMP) target sequence, the method comprising:

a) providing a target nucleic acid sequence, wherein said target nucleic acid sequence comprises a target-specific hybridization region;

b) carrying out a nucleic acid amplification step using a forward primer and a reverse primer, wherein both primers comprise a universal LAMP handle, wherein said universal LAMP handles comprise a universal amplification support region comprising one or more regions for hybridization of universal priming sequences, thereby producing a universal LAMP target sequence, wherein said universal LAMP target sequence comprises a target sequence flanked by universal handles on both a 3' and 5' terminus of the target sequence; and

c) carrying out amplification using LAMP with universal priming sequences which recognize the universal support region, thereby producing concatameric amplicons comprising amplified copies of the target nucleic acid sequence.

2. The method of claim 1, wherein the target sequence is 10-1000 nucleotides in length.

3. The method of claim 2, wherein the target sequence is 20-350 nucleotides in length.

4. The method of any one of claims 1-3, wherein in step b), amplification is carried out using PCR or an isothermal reaction.

5. The method of any one of claims 1-4, wherein steps b) and c) are carried out using one-pot synthesis.

6. The method of any one of claims 1-5, wherein steps b) and c) are both carried out at about 65°C.

7. The method of any one of claims 1-6, wherein step b), carrying out nucleic acid amplification using universal handles, further comprises:

i) hybridizing a forward universal handle (F-strand) to the target sequence, wherein the F-strand comprises in 3' to 5' order, a hybridization region, an F1 region, a loop region, an F2 region, and an F3 region, and further wherein the hybridization region hybridizes with the target sequence, and the F1, F2, and F3 regions are primer-interacting sequences; and

ii) hybridizing a reverse universal handle (B-strand) to the target sequence, wherein the B-strand comprises in 3' to 5' order, a hybridization region, a B1 region, a loop region, a B2 region, and a B3 region, and further wherein the hybridization region hybridizes with the target sequence and the B1, B2, and B3 regions are primer-interacting sequences.

8. The method of any one of claims 1-7, wherein the F-strand and the B-strand bind the target sequence at the 3' and 5' ends of the target, respectively.

9. The method of any one of claims 1-8, wherein the hybridization region of the F-strand is at a 3' terminus of the F-strand.

10. The method of any one of claims 1-9, wherein the hybridization region of the B-strand is at a 3' terminus of the B-strand.

11. The method of any one of claims 1-10, wherein both the loop region of both the F-strand and the B-strand comprises a self- complementary hairpin structure which is generated during LAMP.

12. The method of any one of claims 1-11, wherein step b), LAMP reaction, comprises steps of:

i) providing a forward inner primer (FIP) which complements the F2 region of the universal target sequence at a 3' terminus of the FIP, and further wherein said FIP comprises a sequence which complements the F1 region of the universal LAMP target sequence at a 5' end of the FIP;

ii) amplifying a universal LAMP target sequence using FIP as a primer;

iii) providing an F3 primer, wherein said F3 primer complements the F3 region of the universal LAMP target sequence, wherein said F3 complementary region is at a 5' and 3' end of the F3 primer;

iv) initiating amplification using the F3 primer and producing a product therefrom, which displaces the product of step ii);

v) providing a backward inner primer (BIP) which complements the B2 region of the target sequence at a 3' terminus of the BIP, and further wherein said BIP comprises a sequence which complements the B1 region of the universal LAMP target sequence; amplifying a universal LAMP target sequence using BIP as a primer;

vi) providing a B3 primer, wherein said B3 primer complements the B3 region of the universal LAMP target sequence, wherein said B3 complementary region is at a 5' and 3' end of the B3 primer;

vii) initiating amplification of the B3 primer and producing a product therefrom, which displaces the product of step v);

vii) allowing hybridization of the F1 region and its complement and the B1 region and its complement; thereby forming a double-loop barbell product;

viii) allowing binding of the FIP primer at the F2 complementary region and carrying out amplification thereof; and

ix) allowing binding of the BIP primer at the B2 complementary region and carrying out amplification thereof.

13. The method of any one of claims 1-12, wherein loop primers can be used during LAMP amplification.

14. The method of any one of claims 1-13, wherein the target sequence is not circularized prior to amplification.

15. The method of any one of claims 1-14, wherein two or more target sequences or sequence variants are detected concurrently, and wherein two or more sets of universal handle sequences are used.

16. The method of claim 15, wherein the two or more target sequences are present in the same contiguous nucleotide sequence or on two different nucleotide sequences.

17. The method of claim 16, wherein the two or more target sequences or sequence variants are derived from one organism or from two or more different organisms or are synthetic in origin.

18. The method of any one of claims 1-17, wherein after amplification, at least one extension product is detected.

19. The method of claim 18, wherein one or more extension products are detected in real time.

20. The method of claim 18 or 19, wherein the product is detected using a hybridization probe.

21. The method of any one of claims 18-20, wherein the product is detected using fluorescence.

22. The method of any one of claims 18-21, wherein a signal primer is used to detect amplification.

23. The method of any one of claims 18-22, wherein the product is detected by sequencing.

24. The method of any one of claims 18-23, wherein product is used as biomatter for assembly of macro, micro, and nanomaterials, DNA synthesis, transcript generation, protein generation, information storage, information capture, and information transduction.

25. The method of any one of claims 1-24, wherein concatemeric amplicons are generated on a surface.

26. The method of claim 25, wherein the surface is a chip containing synthetic oligonucleotides.

27. The method of claim 25 or 26, wherein concatameric amplicons are released from the surface by digestion.

28. The method of claim 27, wherein concatameric amplicons released from the chip surface are used as probes for hybridization.

29. The method of claim 27, wherein concatameric amplicons released from the chip surface are used for gene assembly.

30. The method of any one of claims 1-29, wherein the target nucleic acid sequence comprises a barcode.

31. The method of any one of claims 1-30, wherein the universal LAMP handle comprises a barcode.

32. The method of any one of claims 1-31, wherein concatemeric amplicons are barcoded, such that each concatemeric amplicon contains a unique sequence.

33. The method of claim 32, wherein barcoded, concatameric amplicons are cleaved by digestion.

34. The method of Claim 33, wherein cleaved concatameric amplicons are used for gene assembly.

35. A kit comprising:

a) a forward universal LAMP handle comprising a forward nucleic acid extension sequence (F-strand), wherein the F-strand comprises in 3' to 5' order, a hybridization region to a target nucleic acid sequence, an F1 region, a loop region, an F2 region, and an F3 region, and further wherein the hybridization region hybridizes with the target sequence, and the F1, F2, and F3 regions are primer-interacting sequences; and

b) a reverse universal LAMP handle comprising a reverse nucleic acid extension sequence (B-strand), wherein the B-strand comprises in 3' to 5' order, a hybridization region, a B1 region, a loop region, a B2 region, and a B3 region, and further wherein the hybridization region hybridizes with the target sequence and the B1, B2, and B3 regions are primer-interacting sequences.

36. The kit of claim 35, wherein the kit further comprises primers needed for LAMP reaction.

37. The kit of claims 35 or 36, wherein the kit further comprises loop primers.

38. The kit of any one of claims 35-37, wherein the kit further comprises reagents needed for LAMP reaction.

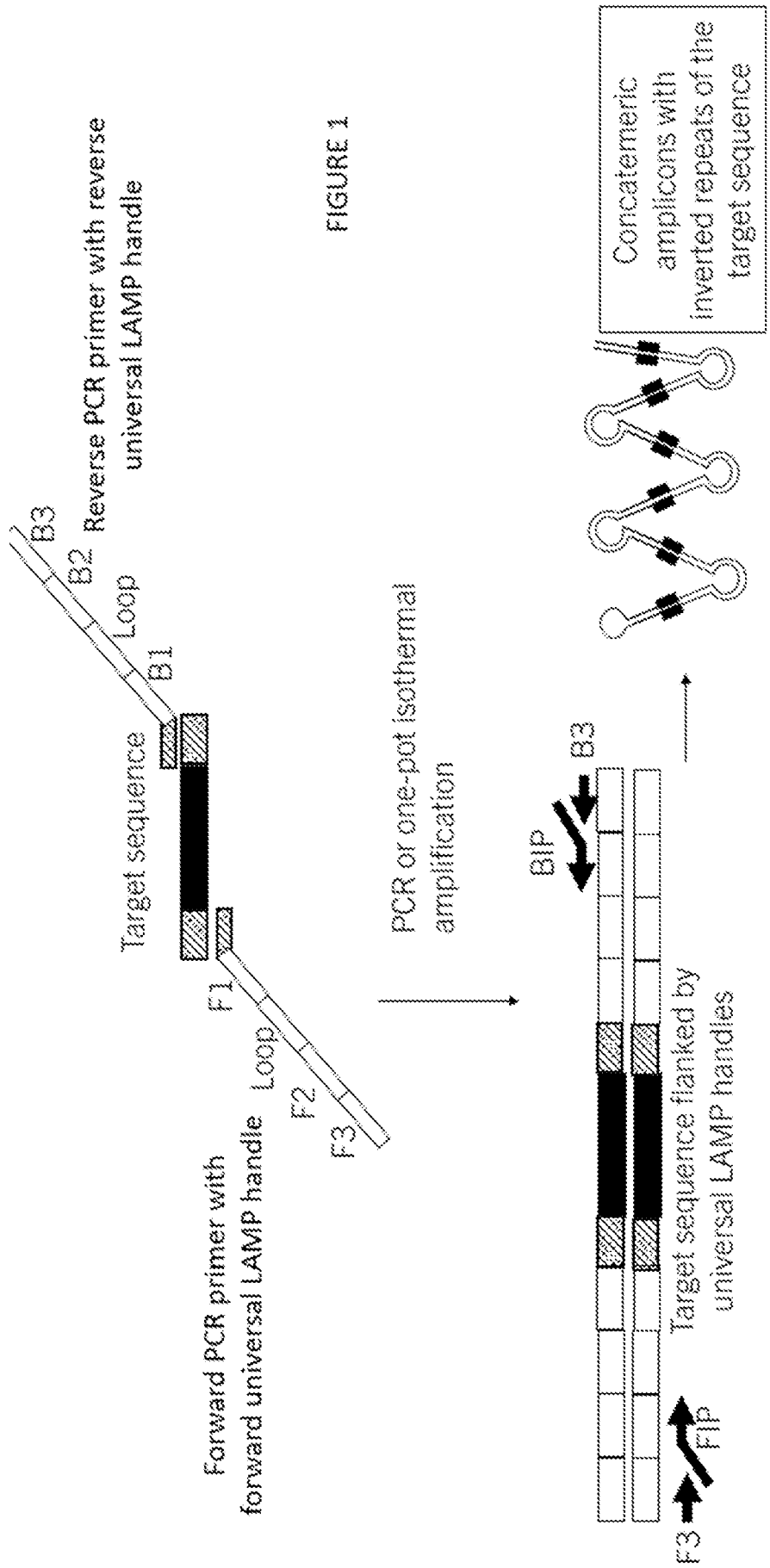

Reverse PCR primer with reverse universal LAMP handle
B3
B2
Loop
B1
Target sequence
F1
Loop
F2
F3
Forward PCR primer with forward universal LAMP handle
PCR or one-pot isothermal amplification
BIP
B3
FIP
F3
Target sequence flanked by universal LAMP handles
Concatemeric amplicons with inverted repeats of the target sequence

FIGURE 1

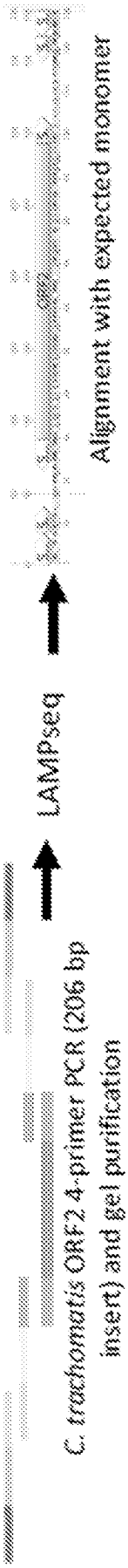


FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5

BLAST query of concatemer hits *C. trachomatis*

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chlamydia trachomatis strain CH2_mutantL2(434/Bu#0) plasmid,	381	8034	53%	2e-99	100.00%	7676	CP064431.1
Chlamydia trachomatis strain CH1_mutantL2(434/Bu#0) plasmid,	381	8034	53%	4e-99	100.00%	7676	CP064430.1
Chlamydia trachomatis strain CH3_mutantL2(434/Bu#0) plasmid,	381	8034	53%	2e-99	100.00%	7676	CP064429.1
Chlamydia trachomatis strain CH5_mutantL2(434/Bu#0) plasmid,	381	8034	52%	2e-99	100.00%	7676	CP064427.1

FIGURE 6

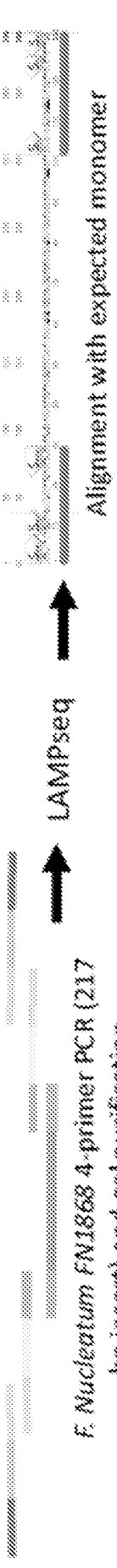


FIGURE 7

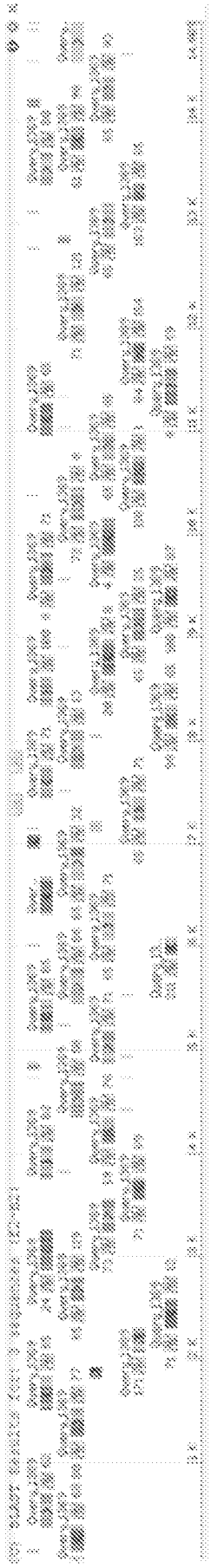


FIGURE 8

No F3 or B3 sequences in concatemers

FIGURE 9

FIGURE 10

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
	Fusobacterium nucleatum subsp. nucleatum ATCC 23726 chromosome, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2299539	CP028109.1
	Fusobacterium nucleatum subsp. nucleatum strain 25586 chromosome, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2180101	CP028101.1
	Fusobacterium nucleatum subsp. nucleatum strain ChDC F317, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2233010	CP022122.1
	Fusobacterium nucleatum subsp. nucleatum strain KCOM 1250 chromosome, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2290405	CP012717.1
	Fusobacterium nucleatum subsp. nucleatum ChDC F316 strain KCOM 1322 chromosome, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2227288	CP012716.1
	Fusobacterium nucleatum subsp. nucleatum ATCC 25586, complete genome	Fusobacterium ...	401	8834	42%	1e-105	100.00%	2174500	AE009951.2

FIGURE 11

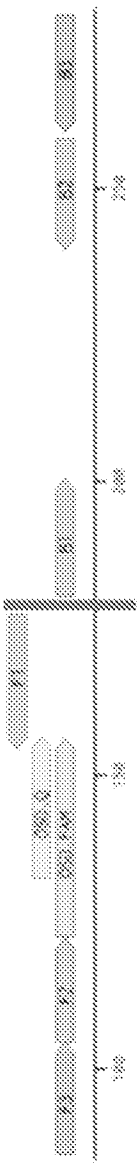


FIGURE 12

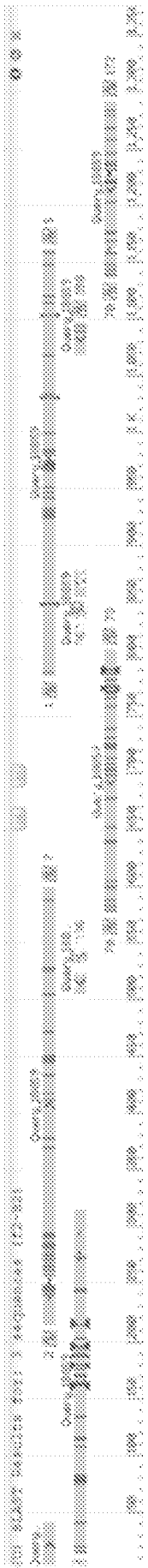


FIGURE 13

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Middle East respiratory syndrome-related coronavirus isolate Riyadh_1764_2015, partial genome	Middle East resp...	285	839	46%	1e-71	93.78%	29816	MN481986.1
Middle East respiratory syndrome-related coronavirus isolate Riyadh_1760_2015, partial genome	Middle East resp...	285	839	46%	1e-71	93.76%	29818	MN481985.1
Middle East respiratory syndrome-related coronavirus isolate Riyadh_1758_2015, partial genome	Middle East resp...	285	839	46%	1e-71	93.76%	29818	MN481983.1
Middle East respiratory syndrome-related coronavirus isolate Riyadh_1760_2015, partial genome	Middle East resp...	285	839	46%	1e-71	93.78%	29816	MN481982.1
Middle East respiratory syndrome-related coronavirus isolate Riyadh_1737_2015, partial genome	Middle East resp...	285	839	46%	1e-71	93.78%	29816	MN481981.1

FIGURE 14

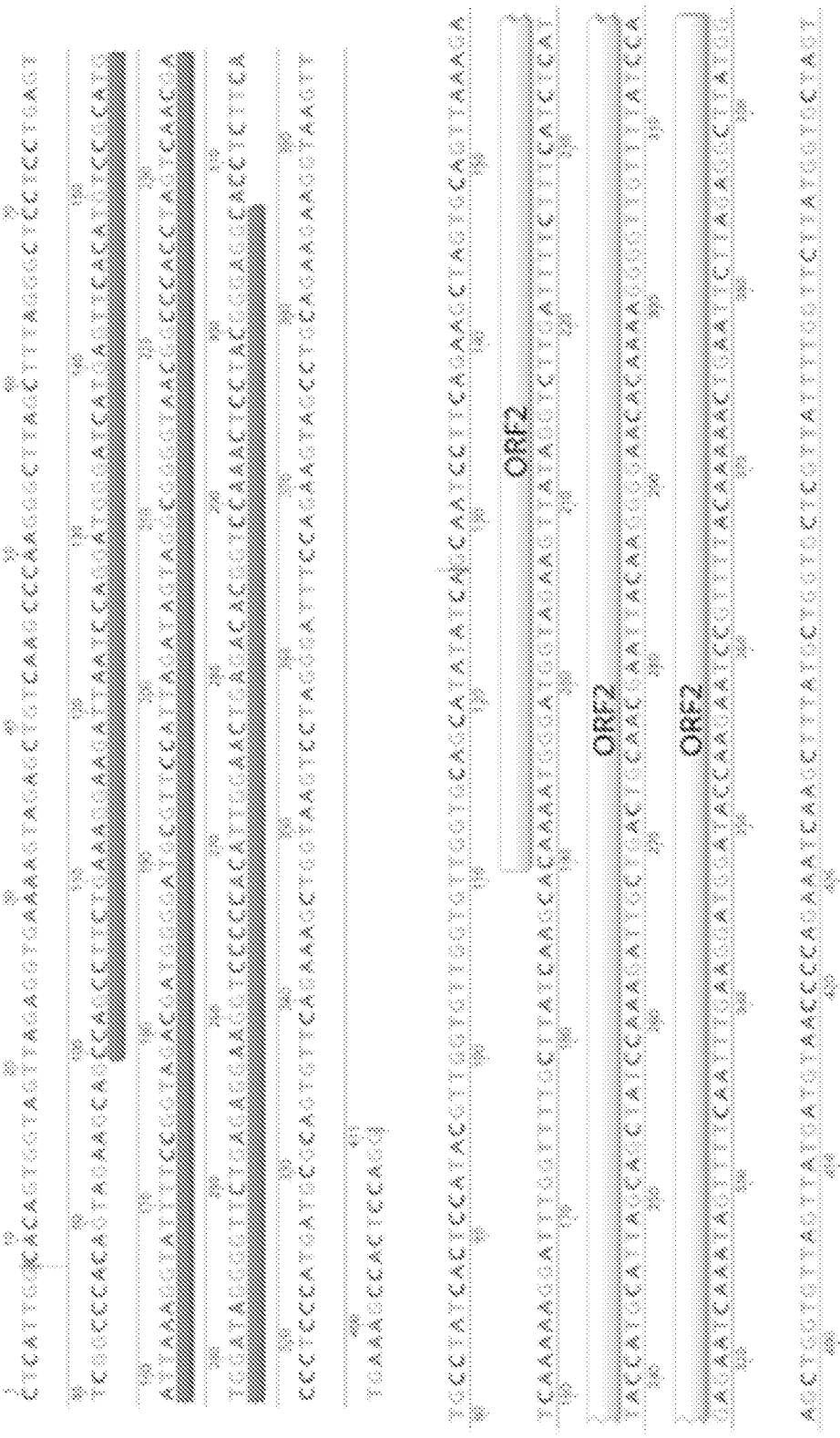


FIGURE 15

NRP2
FIGURE 16
Plasmid (1 ng/uL) / water 3
10X isothermal buffer 2.5
10 mm dNTP 2.5
4-LAMP NRP2 primer mix (normal) 2
10 uM mix Fla.PU.fwd 1
10 uM mix Fla.PU.rev 1
5 uM each mix of invasion primers / water 0.5
gp32 (10 ug/ul) / water 1
200 mM GnHCl / water 5
5M betaine 4
100 mM mgSO4 0.5
bst 2.0 (8 u/ul) 2.00
65 C, 120 min → 95C, 2 min → barcode and sequence
Invasion F
Invasion R1
Fla.PU.REV
Invasion R2
Condition 1 2 3 4 5 6
invasion primers N Y Y Y N N
gp32 N N Y N Y N
GnHCl N N N Y N Y

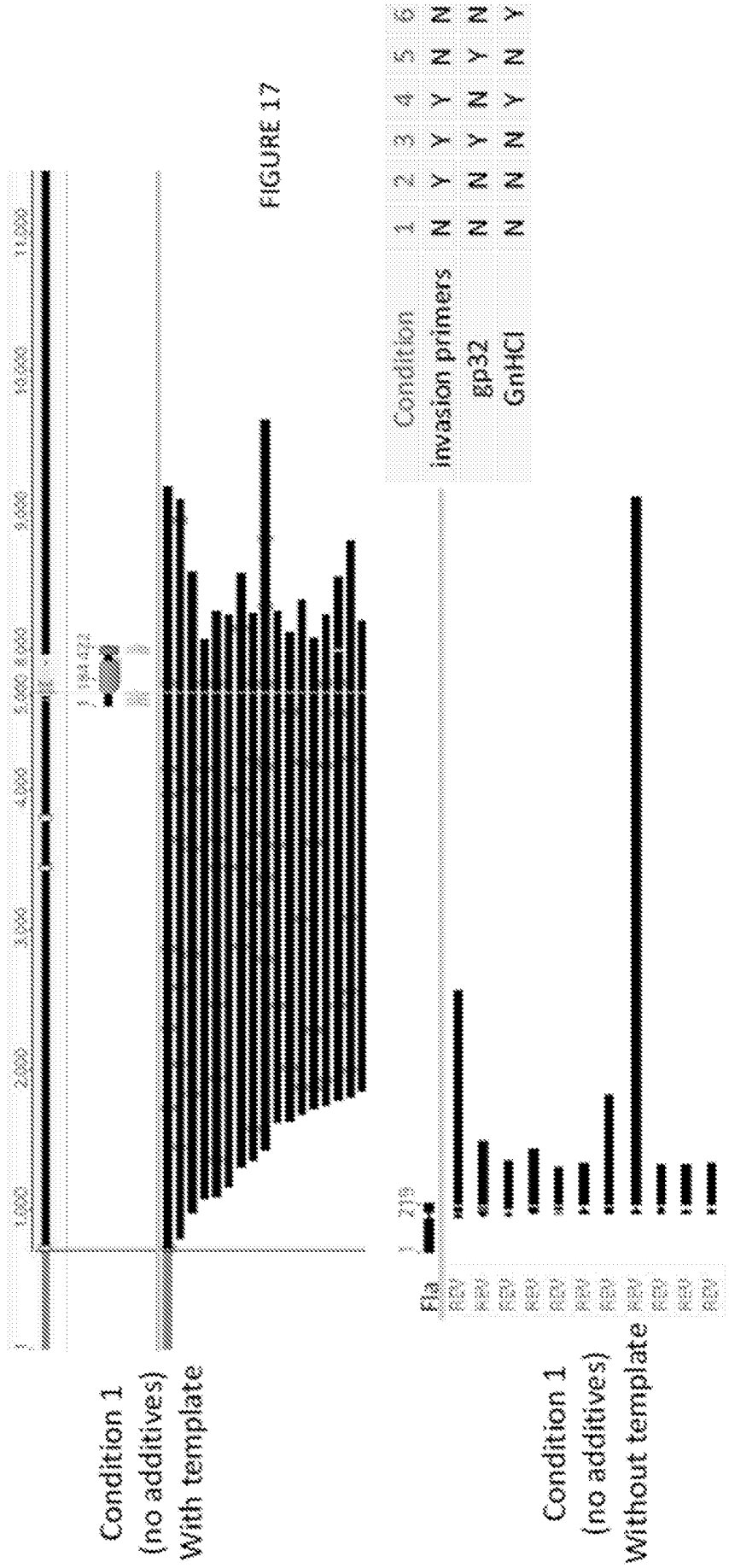

Condition 1
(no additives)
With template
Condition 1
(no additives)
Without template
Condition
invasion primers
gp32
GnHCl
1
2
3
4
5
6
N
Y
N
N
N
N
Y
N
Y
N
N
N
N
Y
Y
N
Y
N
N
N
Y
N
N
Y

FIGURE 17

FIGURE 18A

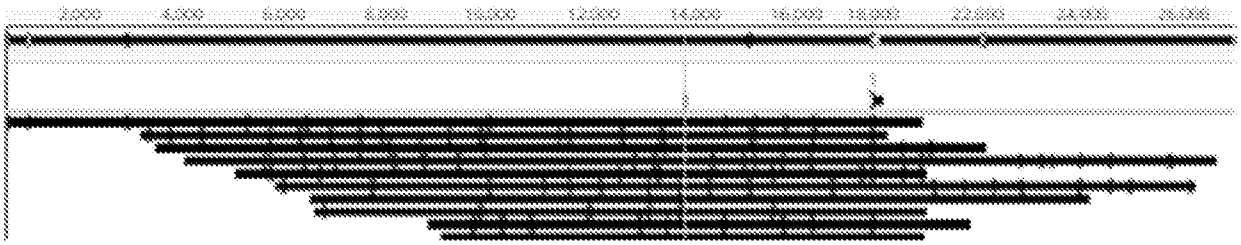


FIGURE 18B

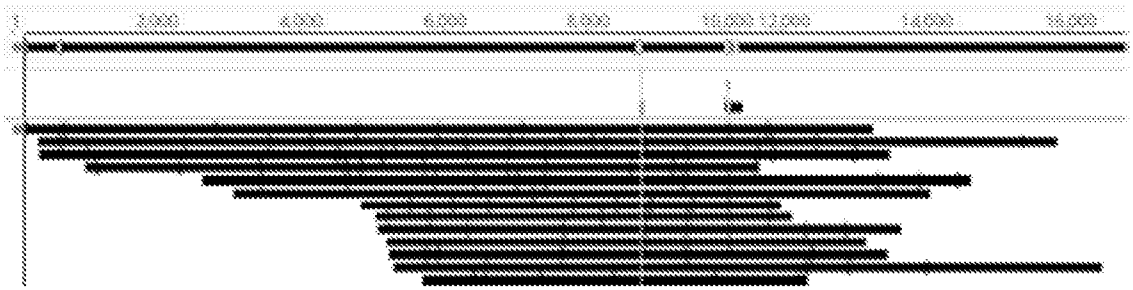


FIGURE 18C

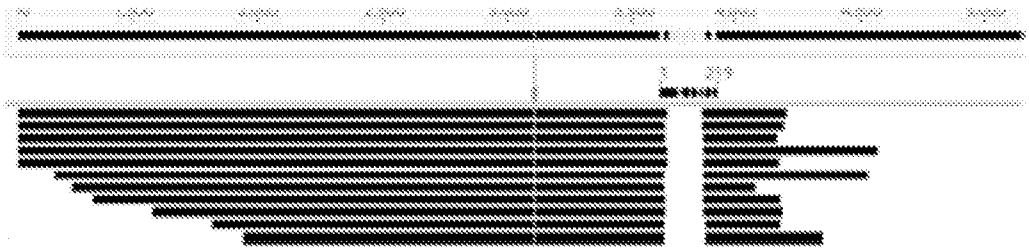


FIGURE 18D

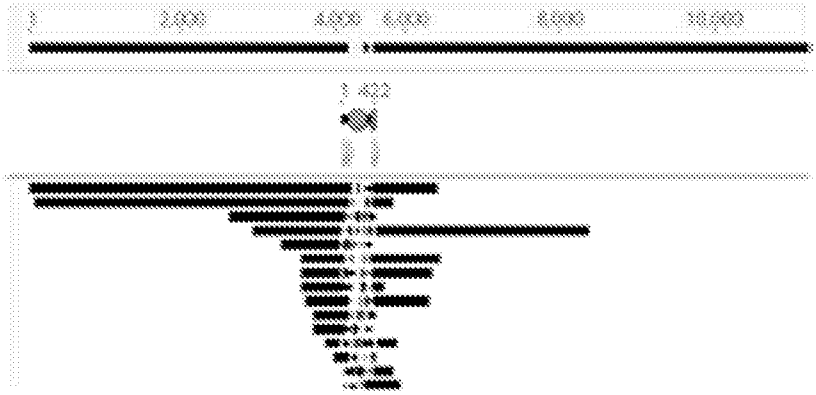


FIGURE 18E

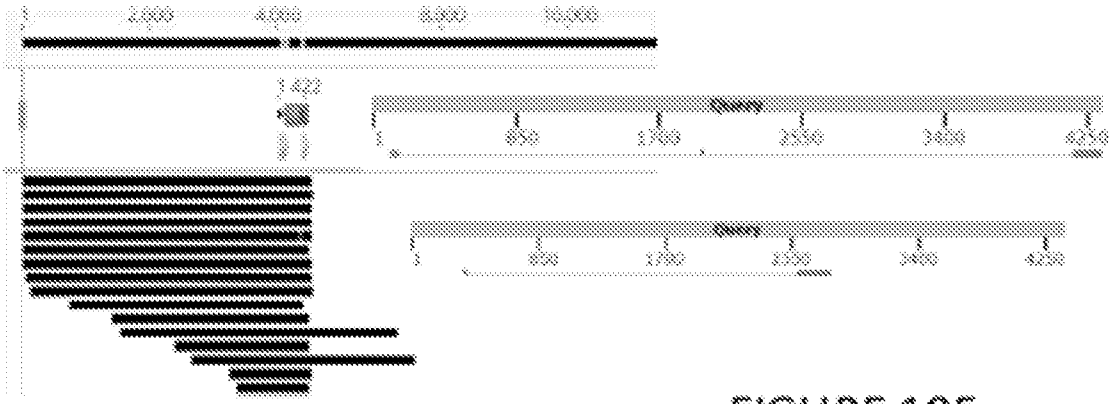


FIGURE 18F

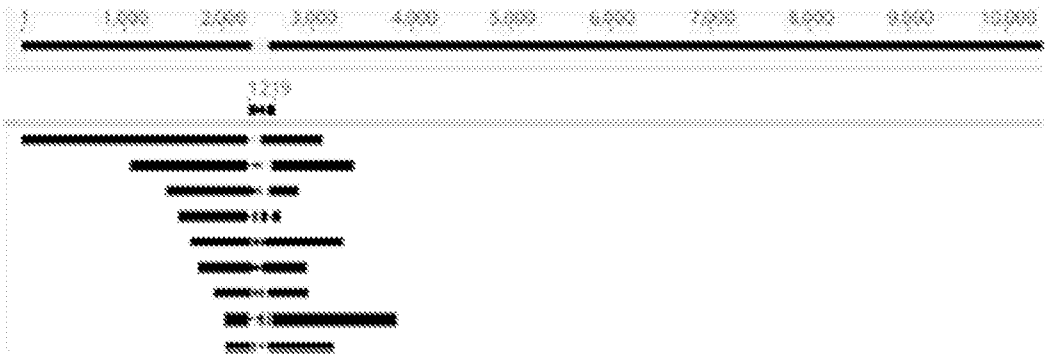


FIGURE 18G

FIGURE 19

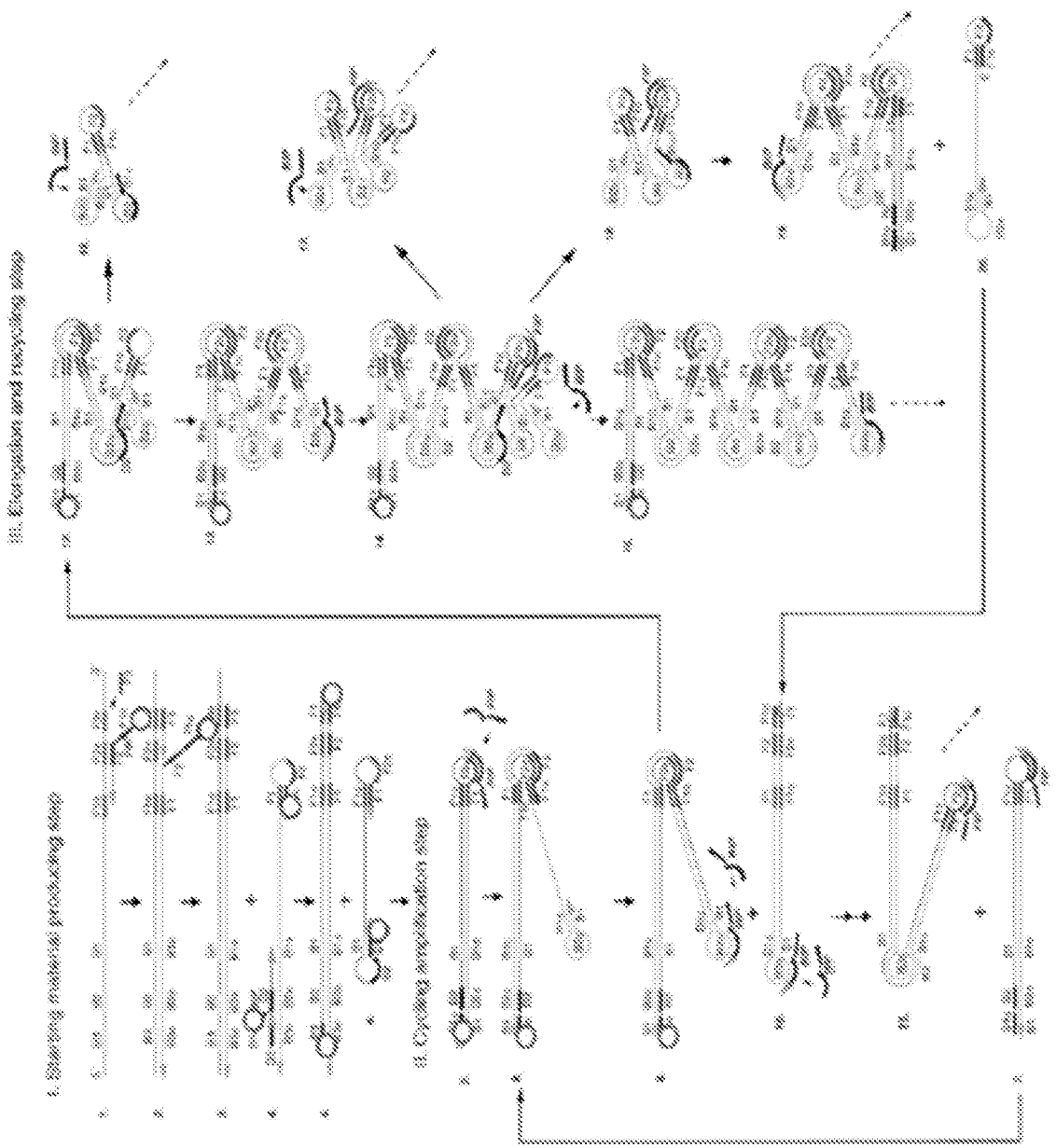


FIGURE 20

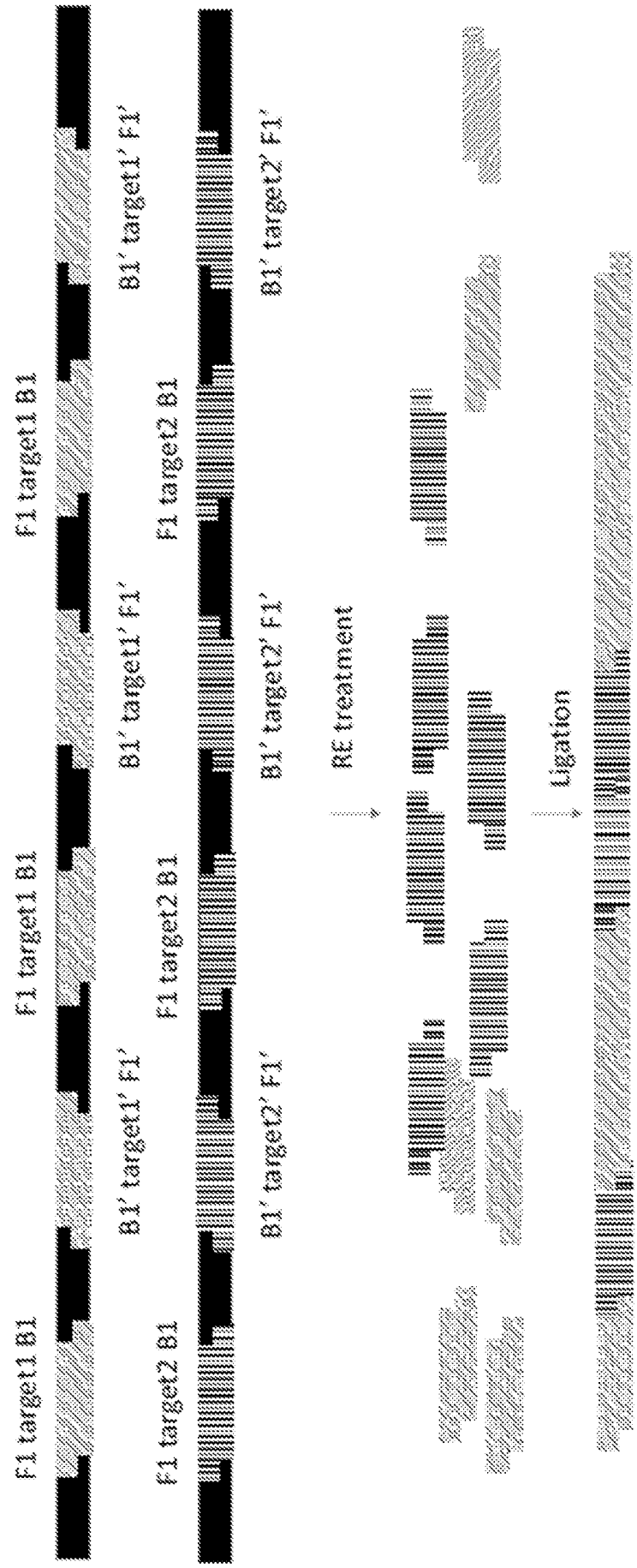

F1 target1 B1
F1 target2 B1
B1' target1' F1'
B1' target2' F1'
RE treatment
Ligation

FIGURE 21

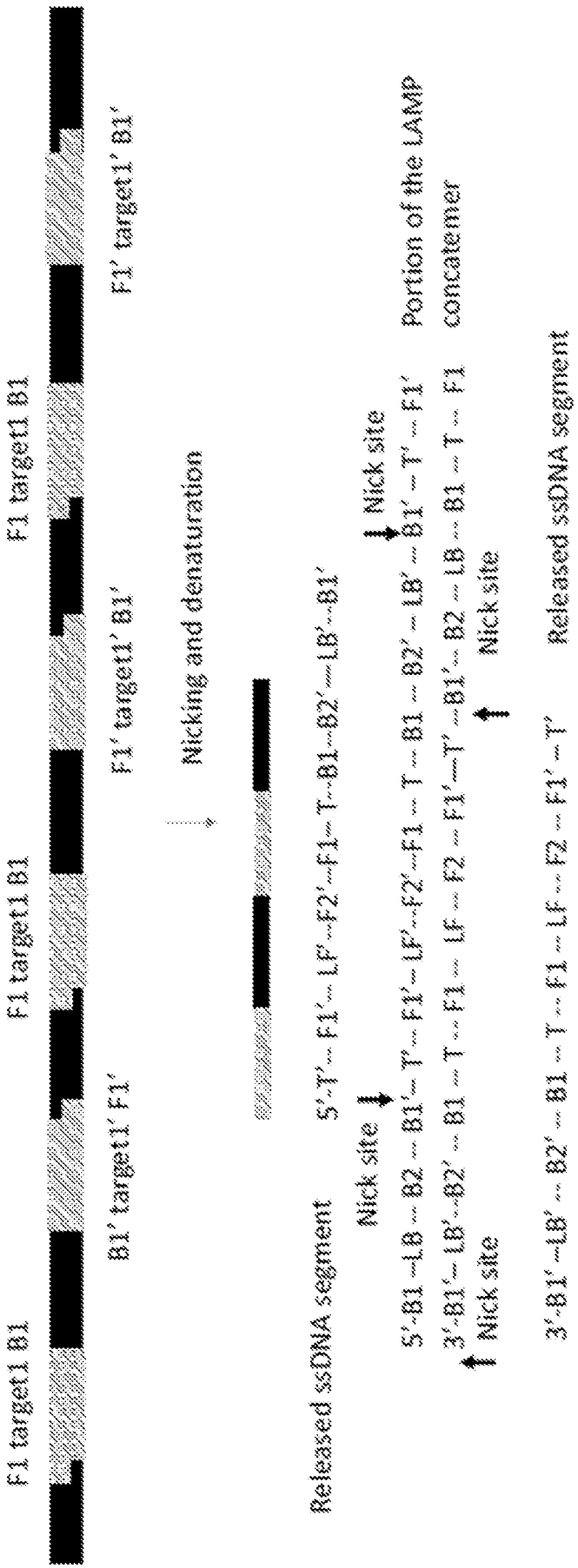

F1 target1 B1
F1 target1 B1
F1 target1 B1
B1' target1' F1'
F1' target1' B1'
F1' target1' B1'
Nicking and denaturation
Released ssDNA segment
5'-T'-- F1'-- LF'--F2'--F1-- T--B1--B2'—LB'--B1'
Nick site
Nick site
5'-B1 –LB -- B2 -- B1'-- T'-- F1'-- LF'--F2'--F1 -- T -- B1 -- B2' -- LB' -- B1' -- T' -- F1'
Portion of the LAMP concatemer
3'-B1'-- LB'--B2' -- B1 -- T -- F1 -- LF -- F2 -- F1'—T' --B1'-- B2 -- LB -- B1 -- T -- F1
Nick site
Nick site
3'-B1'—LB' -- B2' -- B1 -- T -- F1 -- LF -- F2 -- F1' -- T'
Released ssDNA segment

FIGURE 22

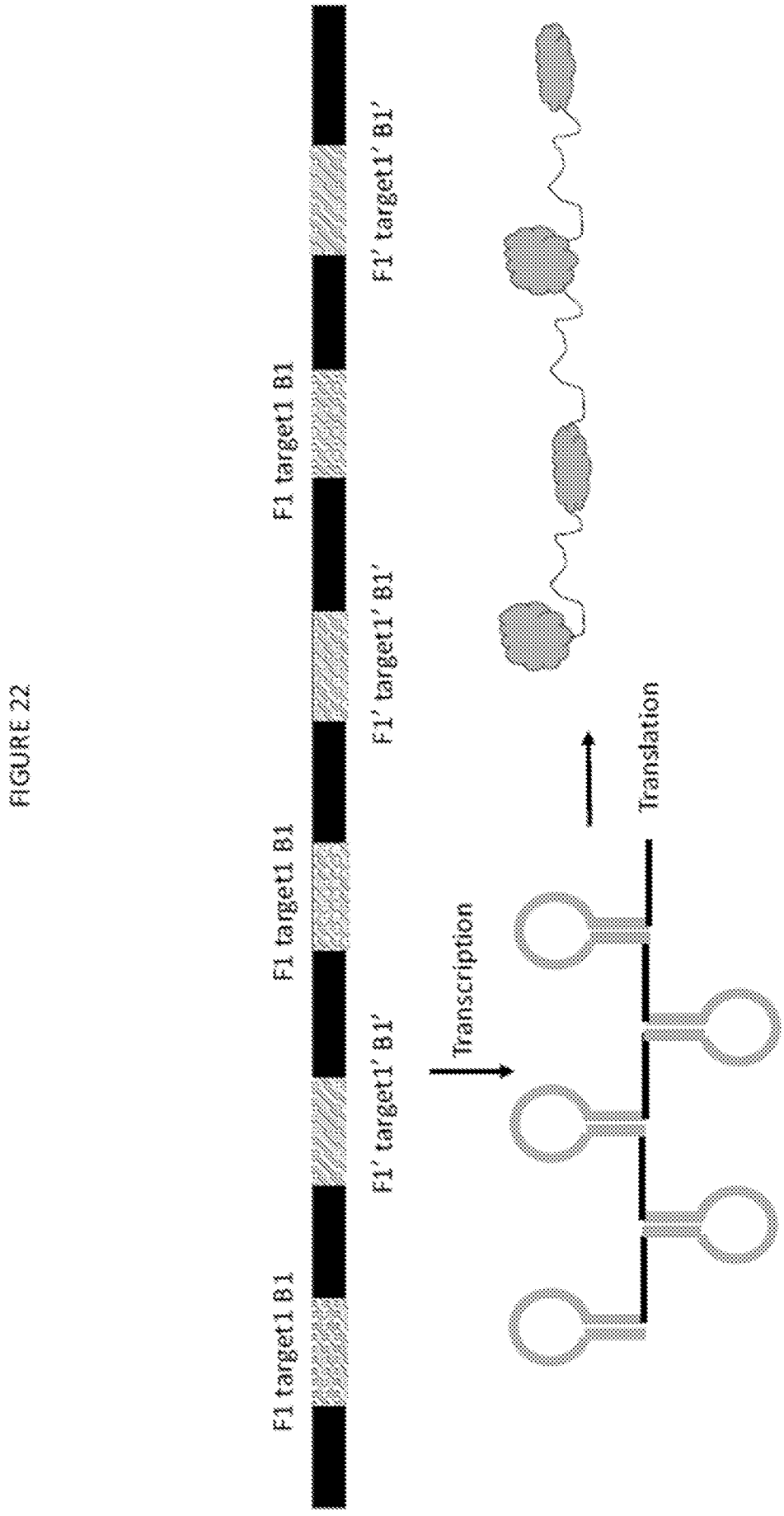
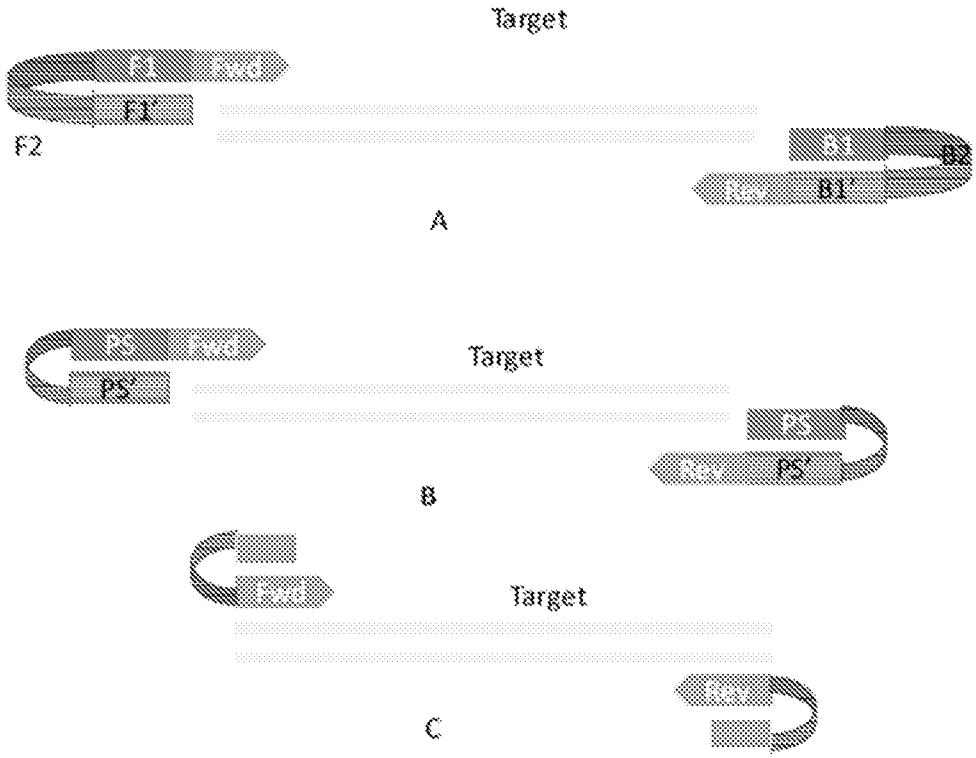

F1 target1 B1
F1' target1' B1'
F1 target1 B1
F1' target1' B1'
F1 target1 B1
F1' target1' B1'
Transcription
Translation

FIGURE 23A-C



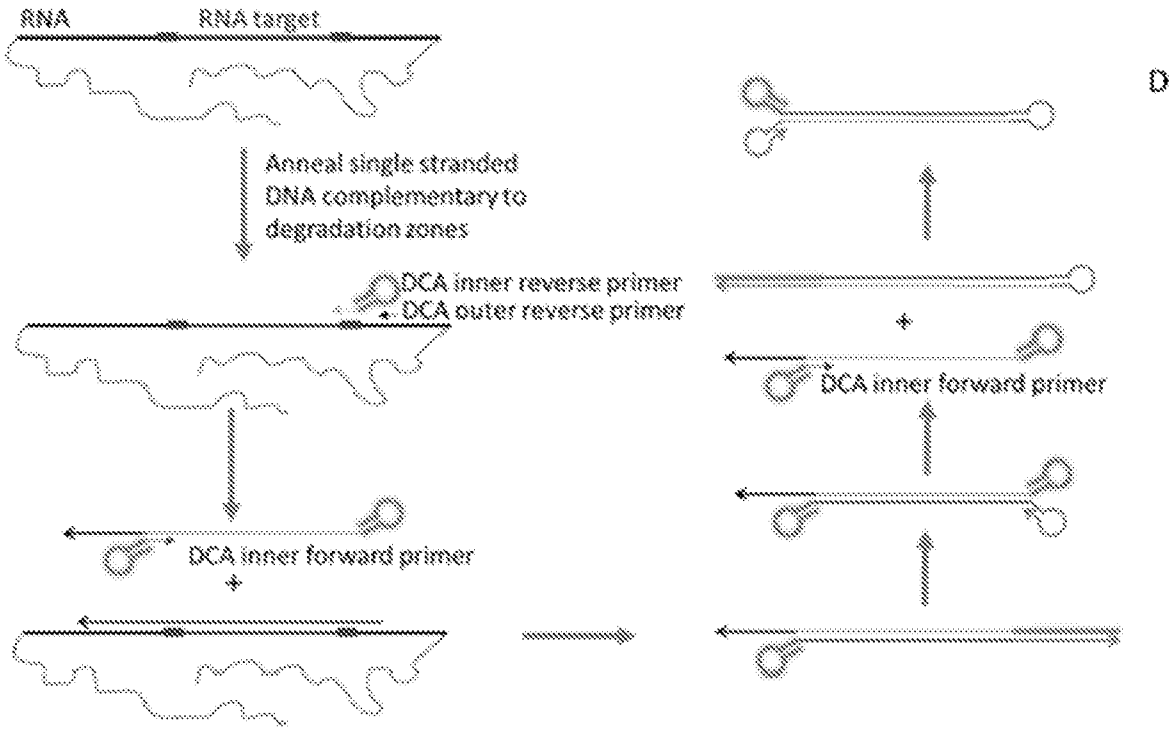


FIGURE 23D