Guide

LavaLAMP: technical guide for new users

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BIOSEARCH™ TECHNOLOGIES

GENOMIC ANALYSIS BY LGC

Guide

LavaLAMP: technical guide for new users

Contents

Guide

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1. Purpose of this document

The purpose of this document is to introduce loop-mediated isothermal amplification (LAMP), to outline the benefits and limitations of the technology, and to provide the technical information required for you to get LavaLAMP™ up and running in your own laboratory.

2. Abbreviations

Listed below are the abbreviations used within this document and their definitions.

BIP	backward inner primer
BL	backward loop primer
B3	backward outer primer
DNA	deoxyribonucleic acid
FIP	forward inner primer
FL	forward loop primer
F3	forward outer primer
LAMP	loop-mediated isothermal amplification
LB	loop-B primer
LF	loop-F primer
NTC	no template control
PCR	polymerase chain reaction
RNA	ribonucleic acid
TTR	time to results
T_m	melting temperature

3. Introduction to LAMP

LAMP is a technique that amplifies DNA by performing synthesis of looped DNA. It typically requires two pairs of primers[1], with optional loop primers.[2] The cycles of strand displacement and annealing lead to exponential accumulation of double-stranded DNA.

Figure 1 outlines the components and steps in a typical LavaLAMP reaction.

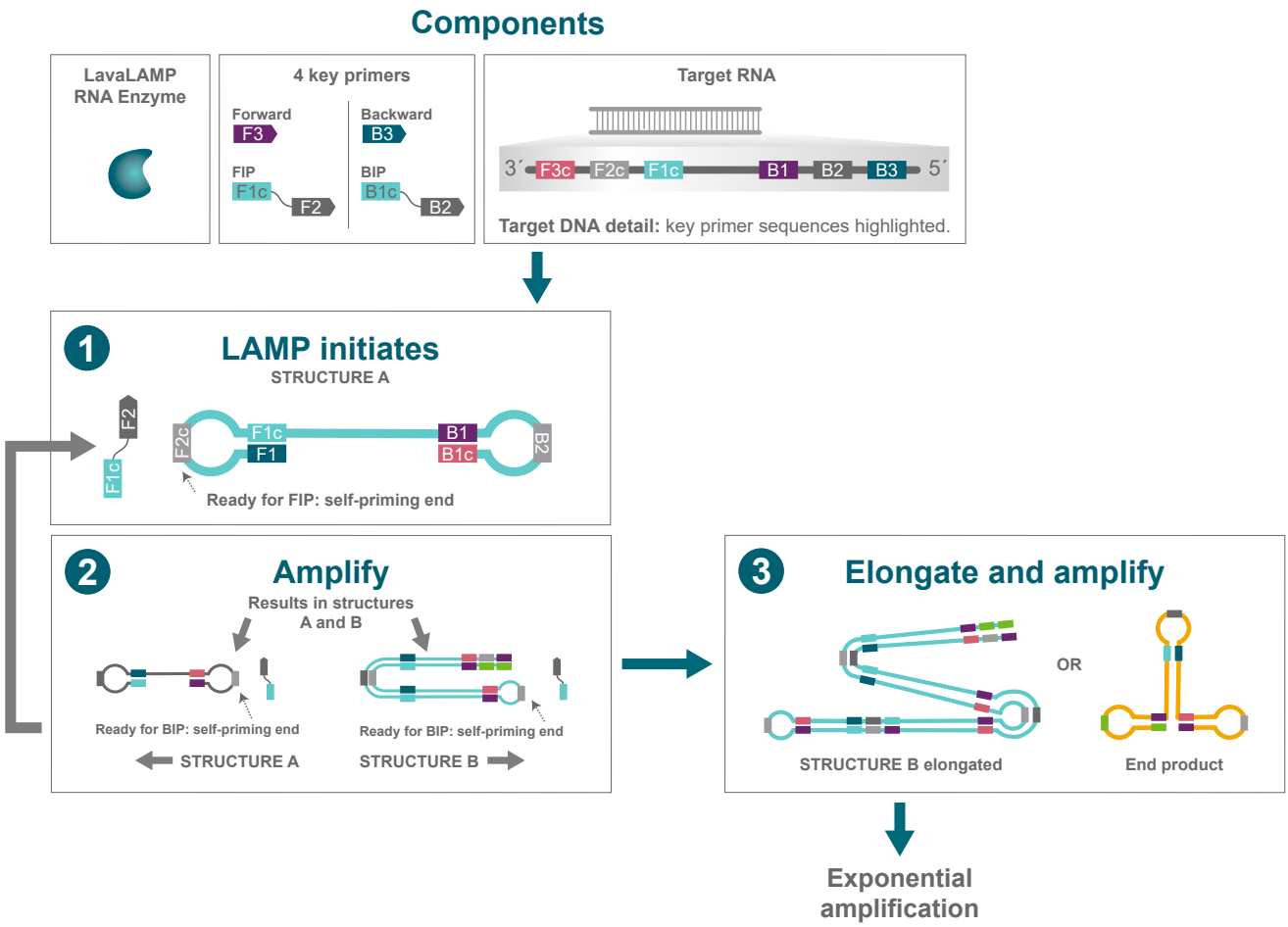

Figure 1. Components and reaction steps for LavaLAMP. This schematic is for LavaLAMP RNA, but the process is the same for LavaLAMP DNA, just double-stranded DNA is used as starting template. For simplicity, four of the six key RNA LAMP primers are illustrated. Amplification can be enhanced by the addition of two primers; loop-F primer (LF) and loop-B primer (LB).

The process of a typical LAMP assay development is illustrated in Figure 2. Detailed guidance for each of these steps is provided in this document. If you are a completely new user of LAMP, it is recommended to use the positive control (provided in LavaLAMP kits) to perform a verification of your laboratory set up and process prior to working with your own samples and assays. More details on this step can be found in section 4.3.

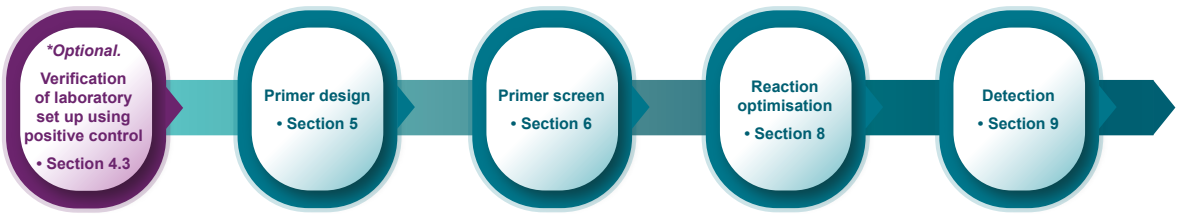

Figure 2. LAMP assay development typical workflow. Verification of laboratory set-up using the supplied positive control is an optional and recommended first step for users who are new to LAMP.

3.1 LAMP vs PCR

LAMP distinguishes itself from PCR in a few ways:

1. LAMP reactions are performed at a single temperature, meaning that a thermal cycler instrument is often unnecessary. This makes the technique appealing for point of care applications and in difficult test environments.
2. The LAMP reactions are more tolerant of inhibitors, such as heme, ferritin and polyphenol, than PCR reactions.[3,4]
3. Detection of amplification is often determined by turbidity, colour change, fluorescence of intercalating dyes or pH change rather than with agarose gel electrophoresis.[5,6,7]

3.2 Challenges of LAMP

Despite the benefits of LAMP, there are a few challenges:

1. It is inherently more difficult to get a set of six primers to 'play well together' and to result in an effective LAMP reaction.
2. Significant up-front work is required with primer design when compared to other DNA amplification techniques (e.g. PCR, which only requires designing two primers). Most researchers design multiple sets of six primers and test to determine which work well, with the expectation that some sets will fail. More details on primer design and primer screening can be found in section 5 and section 6, respectively.

3.3 LavaLAMP product portfolio

LGC Biosearch Technologies offers both the LavaLAMP DNA Master Mix and the LavaLAMP RNA Master Mix. These master mixes greatly simplify reaction optimisation by limiting optimisation to target-specific components and conditions such as LAMP primer design, target concentration and reaction temperature. Our LavaLAMP enzyme and buffer combination function optimally at a higher temperature (68-74 °C) compared with other LAMP kits, so difficult templates can be detected with greater confidence.

For the more experienced user, Biosearch Technologies also offers both the LavaLAMP DNA Component Kit and the LavaLAMP RNA Component Kit. The individual reagent format of these kits enable complete control and offer the opportunity for further optimisation. As this document is intended primarily for new users of LAMP, we will focus on the LavaLAMP Master Mixes.

The LavaLAMP Master Mixes from Biosearch Technologies contain our unique Green Fluorescent Dye for template detection. This dye utilises fluorometric detection and can give a quantitative result by utilising a real-time thermal cycler. The instrument's setting for Fluorescein (FAM) or SYBR Green can be used, or the instrument should be capable of emitting 490 nm light and detecting 520 nm light. The two component kits can also be supplied with Green Fluorescent Dye, if required. Table 1 details the kits and master mixes available from Biosearch Technologies.

Ordering information

Cat no.	Size	Description
30066-1	**200 rxn**	LavaLAMP DNA Master Mix
30067-1	**200 rxn**	LavaLAMP DNA Master Mix with Dye
30086-1	**200 rxn**	LavaLAMP RNA Master Mix
30087-1	**200 rxn**	LavaLAMP RNA Master Mix with Dye
30076-1	**500 rxn**	LavaLAMP DNA Component Kit
30077-1	**500 rxn**	LavaLAMP DNA Component Kit with Dye
30096-1	**500 rxn**	LavaLAMP RNA Component Kit
30097-1	**500 rxn**	LavaLAMP RNA Component Kit with Dye
30078-2	**200 rxn**	Green Fluorescent Dye
30078-1	**500 rxn**	Green Fluorescent Dye

Table 1. LavaLAMP Master Mixes and Kits available from Biosearch Technologies. Master Mix kits contain: LavaLAMP DNA or RNA Master Mix, DNA or RNA Positive Control LAMP Primer Mix, and DNA or RNA Positive Control as appropriate for each DNA or RNA kit. The LavaLAMP DNA or RNA Master Mix with Dye also contains Green Fluorescent Dye for fluorescent detection of amplified DNA. Component kits contain: 10X LavaLAMP DNA or RNA Buffer, LavaLAMP DNA or RNA Enzyme, Magnesium Sulfate, 100 mM, DNA or RNA Positive Control LAMP Primer Mix and DNA or RNA Positive Control as appropriate for each DNA or RNA kit. The LavaLAMP DNA or RNA Component Kit with Dye also contains Green Fluorescent Dye for fluorescent detection of amplified DNA. Note: Standard LavaLAMP reactions are 25 µL.

4. LavaLAMP reagents

4.1 Reagents required to run LavaLAMP reactions

Tables 2 and 3 detail the reagents that are required to run LavaLAMP reactions for DNA and RNA respectively, using the LavaLAMP Master Mix products.

Components
Nuclease-free water
LavaLAMP DNA Master Mix (2X)
Target-Specific Primer Mix, 10X • 2 µM each F3 and B3 primers • 8 µM each LF and LB primers • 16 µM each FIP and BIP primers
DNA Positive Control LAMP Primer Mix **(optional)**
Green Fluorescent Dye **(optional)**

Table 2. Reagents required for DNA LavaLAMP reactions. Use of the DNA Positive Control LAMP Primer Mix and the Green Fluorescent Dye are optional

Components
Nuclease-free water
LavaLAMP RNA Master Mix (2X)
Target-Specific Primer Mix, 10X • 2 µM each F3 and B3 primers • 8 µM each LF and LB primers • 16 µM each FIP and BIP primers
RNA Positive Control LAMP Primer Mix **(optional)**
Green Fluorescent Dye **(optional)**

Table 3. Reagents required for RNA LavaLAMP reactions. Use of the RNA Positive Control LAMP Primer Mix and the Green Fluorescent Dye are optional

Details on the positive control primer mixes, and when it is appropriate to use these, can be found in section 4.3.

4.2 Customer requirements

To run LavaLAMP in your own laboratory, the following components are required in addition to those outlined in section 4.1:

- Target DNA/RNA
- Thermal cycler or heat block
    - We recommend using instruments with heated lids.
    - We recommend using calibrated instruments.

Optional: While not required, we recommend using Green Fluorescent Dye to detect amplified DNA. See section 9 for more details. Green Fluorescent Dye is packaged separately from the master mix. Do not combine the dye with the master mix until you are setting up the reaction (section 7).

4.3 Positive controls

The positive controls provided with all our LavaLAMP kits are designed to be used for two main purposes. These are i) initial verification of laboratory set up and processes for new users of LAMP, and ii) troubleshooting if you are experiencing any issues with your reactions.

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If you are completely new to LAMP, we recommend using the positive control reactions that are provided to set up a trial reaction before you begin working with your own DNA and assays. Each kit includes the necessary DNA/RNA template and LavaLAMP assay primers to run a trial reaction.

A LavaLAMP reaction involving the provided positive control should be set up as detailed in Table 4.

Component	Volume (µL)
Nuclease-free water	8
LavaLAMP DNA/RNA Master Mix	12.5
DNA/RNA positive control LAMP primer mix	2.5
Green Fluorescent Dye (optional) (If not using, replace volume with nuclease-free water)	1
Positive control DNA/RNA	1
Total reaction volume	25

Table 4. Reaction set up for a LavaLAMP positive control reaction

Detailed information on how to prepare and combine these reagents can be found in the appropriate product user manual (see Section 12 for links).

The DNA positive control requires amplification to be performed at 74 °C for 30 to 60 minutes while the RNA positive control requires amplification to be performed at 68 °C for 30 to 60 minutes. See section 6 for more details on performing the LavaLAMP reaction.

The positive controls can also be used for troubleshooting issues with your own LavaLAMP assays. Running a positive control reaction will enable you to verify that all components other than the designed primers are functioning correctly.

Please note that it may not be possible to run a positive control reaction alongside your own LavaLAMP assays as designed because the controls must be amplifed at specific temperatures. We do not advise amplifying the positive control reactions at any other temperature.

If you need additional positive controls and primers, information on composition and how to order these items are in the following sections.

4.3.1 LavaLAMP DNA positive control

The LavaLAMP DNA Positive Control template contains the following:

- 25,000 copies per µL in Low TE
- 0.1 pg/µL M13mp18 ssDNA (the target)
- 1.0 ng/µL Salmon Sperm ssDNA (the carrier)

The primer sequences for the Control primer set are shown in Table 5:

M13-FIP	TAATCAGTGAGGCCACCGAGTA-CAAAGAAGTATTGCTACAACGG
M13-BIP	CTGGCGTACCGTTCCTGTC-CCTCGTTAGAATCAGAGCG
M13-FL	GAGTCTGTCCATCACGCAA
M13-BL	TAATCGGCCTCCTGTTTAGC
M13-F3	GGCGGTAATATTGTTCTGGATA
M13-B3	TTGACGAGCACGTATAACG

Table 5. Primer sequences for the LavaLAMP DNA positive control set

4.3.2 LavaLAMP RNA positive control

The LavaLAMP RNA Positive Control template contains the following:

- 30,000 copies per µL in Low TE {10 mM Tris (pH 7.5), 0.1 mM EDTA} *E. coli* Bacteriophage MS2 RNA (target)
- 1.0 ng/µL t-RNA from baker's yeast (carrier)

The primer sequences for the Control primer set are shown in Table 6.

MS2 FIP	GCCCAAACAACGACGATCGGTAA-AACCAGCATC CGTAGCCT
MS2 BIP	GCACGTTCTCCAACGGTGCT-GGTTGCTTGTTCA GCGAACT
MS2 FL	CCAGAGAGGAGGTTGCCAA
MS2 BL	TGCAGGATGCAGCGCCTT A
MS2 F3	TGTCATGGGATCCGGATGTT
MS2 B3	CAATAGAGCCGCTCTCAGAG

Table 6. Primer sequences for the LavaLAMP RNA positive control set

5. Primer design

5.1 Conversion from alternative LAMP assays to LavaLAMP

If converting an established alternative LAMP assay to LavaLAMP, consider that the LavaLAMP enzyme requires 68-74 °C for optimal activity. The T_m of the primers should be checked, and the design(s) tested empirically. If the T_m of the primers are too low, or existing designs do not work, a redesign will be required.

If you are new to LAMP and starting from scratch, we encourage you to spend some time understanding how to design primers optimally.

5.2 Initial considerations

A LavaLAMP assay requires a set of 6 primers:

- F3 – Forward Outer Primer
- B3 – Backward Outer Primer
- FL – Forward Loop Primer
- BL – Backward Loop Primer
- FIP – Forward Inner Primer
- BIP – Backward Inner Primer

The inclusion of loop primers improves LAMP results by increasing the speed and sensitivity of the assay.[2, 8, 9] Figure 3 shows a visualisation of primer design for a LavaLAMP assay.

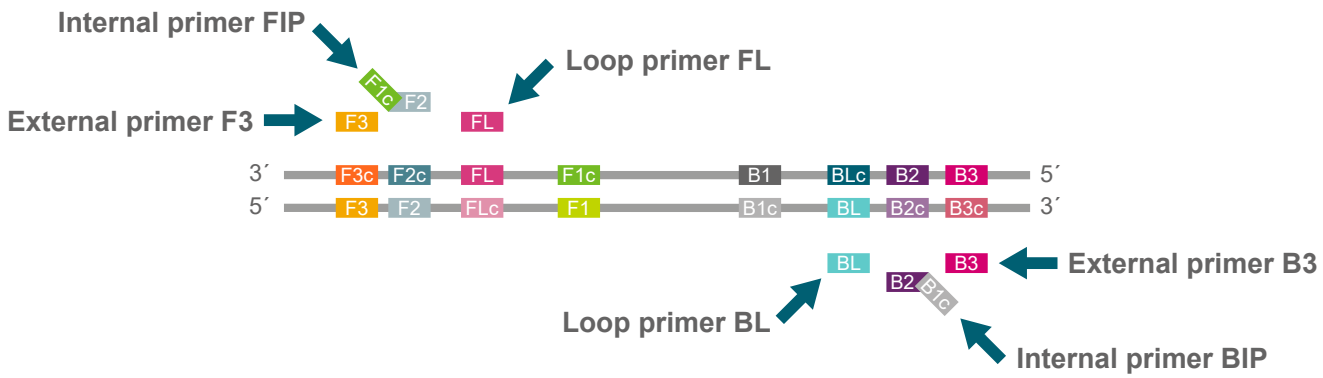

Figure 3. Primer visualisation for LavaLAMP assay design

We recommend designing multiple sets of six primers and testing these in parallel. Even with the best *in silico*-designed primer sets, one should expect at least some of the sets of six primers to fail to produce acceptable LAMP results. The characteristics of the target sequence will influence the design success rate. Simple sequences with 50% GC and no mutations should be easier to design to than complex sequences.

5.3 Primer characteristics

Key factors of successful LavaLAMP primer design include primer T_m and primer length. Table 7 details optimal characteristics for each of the six primers in a LavaLAMP assay. Please note that these optimal characteristics are for guidance only. Depending on the target sequence, designs may have to vary from these temperatures and lengths.

Primer(s)	Length (mer)	T_m (°C)
F3/B3	15-25	66 °C
FIP/BIP	15-25	71 °C
FL/BL	15-22	68 °C

Table 7. Optimal primer design characteristics.

5.4 Additional primer design considerations

The list below details other factors that should be considered when designing primers for LAMP:

- Primers are specified 5′ to 3′.
- Aim for 40-60% GC content
- Target amplicon should be ≤400 base pairs
- Avoid runs of 3 or more of one base, or dinucleotide repeats (e.g. ACCC or ATATATAT), as both can cause mis-priming. Runs of 3 or more Gs may cause issues with primer synthesis and HPLC purification.
- Primer pairs should have similar T_ms, with a maximum difference of 5 °C, and should not be complementary to each other.
- Avoid regions of secondary structure; namely intra-primer homology (more than 3 bases that complement within the primer).
- In general, select the largest ΔG value for dimer analysis minimum of -3.5 for optimal design. Select the smaller ΔG value for the ends of primers maximum of -4 for optimal design. (ΔG limits are suggestions for PrimerExplorer)
- For hairpins, the melting temperature (T_m) should be lower than the annealing temperature of the reaction; on average it should range between 68 °C and 74 °C. The T_m for the strongest hairpin should be at least 68 °C and below the reaction temperature of the LavaLAMP reaction.

Additional tips for successful primer design include:

- Poly T linkers e.g. GGCACATGGTCCCGTTCCTGATTTTTTAGCGCCAGACGGGATTCG
    - Some reports suggest the addition of a Poly T linker in the FIP and BIP between F2-F1C and B2-B1C can improve loop formation and reaction speed.
- Mutations
    - It is preferred not to have a mutation within a primer
    - Primer locations least impacted by having a mutation:
        - 5′ ends of F3, B3, F2 and B2
        - 3′ ends of F1C and B1C
        - Internal regions
- Degenerate nucleotides
    - A degenerate primer is a mix of oligonucleotide sequences in which some positions contain a number of possible bases.
    - Degenerate nucleotides can help if there is a mutation within a primer region.
    - Note that the T_m of primers with degenerate bases can vary across the different designs.
- Inosine bases
    - As inosine can pair with any natural base, it can be useful in primer design where there is a polymorphism in the target sequence. Rather than multiple designs in one reaction (as for degenerate primers), use of inosine bases means one primer is used to target all sequence variations and this primer has one T_m.

5.5 Primer design tools

Below are a number of tools that you may find helpful for primer design.

- LAMP Designer by Premier Biosoft, is available for purchase at: http://www.premierbiosoft.com/isothermal/lamp.html
- PrimerExplorer by Eiken is a free online application that can be accessed at: https://primerexplorer.jp/e/

6. Primer design screen

Once you have designed several sets of primers for your chosen target (see section 5 for guidance on primer design), the next step is to test these primers. Each individual set of primers will have its own temperature optimum, and some sets will work more efficiently than others. It is unlikely that all designs will work, and this step is to determine which set(s) are the most suitable to proceed with.

Please note: primer concentration is critical. Always independently confirm the concentration of received and reconstituted primers before preparing your own working solutions.

We recommend performing a primer design screen, where all sets of primers are tested alongside each other against a temperature titration. In this way, you can screen for functionality across temperature, and as a result can select the best starting design based on speed (TTR) and delta TTR (i.e. background amplification minus positive amplification).

To perform a primer design screen against a temperature titration, you will need to set up multiple reactions for each set of primers and run each one at a different temperature. The recommended amplification temperature for LavaLAMP reactions is between 68 °C and 74 °C. Depending on the primer set characteristics, you may choose to run seven reactions per primer set at 1 °C increments (i.e. 68 °C, 69 °C, 70 °C, 71 °C, 72 °C, 73 °C and 74 °C). If your thermal cycler has a gradient option, this can be utilised for this process. With an automated gradient, the temperatures programmed are unlikely to be exactly 1 °C increments (e.g. may be 68 °C, 69.2 °C, 70.4 °C, etc) but this is acceptable for the purposes of screening. The temperature deemed optimal for amplification can subsequently be checked in an individual run. Details on how to set up reaction mixes and run the reactions can be found in section 7.

Once this is completed for all your designed primer sets, you can select the best starting design and use the amplification temperature at which it performed best. Further optimisation of the primer set can then be considered (see section 8).

7. The LavaLAMP reaction

The protocols for performing LavaLAMP reactions are similar when using DNA or RNA template. Section 6 outlines the key steps required to perform a LavaLAMP reaction. Please view the full DNA or RNA Master Mix manual as appropriate for full product-specific information. A no template control (NTC) should be run alongside experimental samples.

7.1 Protocol overview

1. Thaw components and keep on ice.
2. Mix each component by vortexing.
3. Prepare reaction mixes. Table 8 details reaction mix requirements for a DNA LavaLAMP reaction.

	NTC	Experimental
Component	**Volume (µL)**	**Volume (µL)**
Nuclease-free water	8	8
LavaLAMP DNA Master Mix (2X) OR LavaLAMP RNA Master Mix (2X)	12.5	12.5
Target-Specific Primer Mix, 10X	2.5	2.5
Green Fluorescent Dye **(optional)**	1	1
Total volume	24	24

Table 8. Reaction mix requirements for LavaLAMP DNA reaction

4. Mix the prepared reaction mix and add to the required number of PCR tubes.
5. Add target DNA or positive control template DNA to each tube along with water and mix.
6. Using a heat block or thermal cycler, perform the amplification reaction. Incubate the reactions as follows:

Step	Temperature	Time
1. Amplification	**Experimental and NTC**: 68-74 °C **DNA positive control template**: 74 °C **RNA positive control template**: 68 °C	30-60 minutes
2. Hold **(optional)**	4 °C	∞

Table 9. Temperature requirements for the LavaLAMP DNA reaction

7. Stop the amplification reaction.
8. Detect the amplified product.

7.2 Advanced protocols

Generally, LavaLAMP users begin successfully with the Master Mix. Once a user has gained confidence using the Master Mix there is often the desire to further optimise LAMP. For that we offer Component Kits for both DNA and RNA.

7.3 Laboratory guidance when performing LavaLAMP

- Always wear gloves while handling components. Set up reactions using good laboratory techniques to minimise cross contamination.
- Calculate the total volume of each reagent required for the planned experiment and verify that sufficient volume is available before proceeding to reaction setup.
- Set a thermal cycler or heat block to the desired temperature. If using a heat block, we recommended using 0.2 mL PCR tubes and monitoring the temperature closely.
- Biosearch Technologies encourages all users to perform an NTC reaction with each primer set. A Positive Control reaction is recommended to confirm proper setup before proceeding to run your own samples, and can also be used to aid troubleshooting as required.

8. LAMP assay optimisation

After identifying a set of six primers that function with LavaLAMP reasonably well, optimisation is often possible. The four goals for optimisation are:

1. Increased reaction speed (decrease overall TTR).
2. Decreased non-specific amplification (slower TTR from negative samples).
3. Improved sensitivity (detect low copy inputs).
4. Increased separation between positive and NTC TTR

Below are a range of factors that can be adjusted when optimising an assay. Those marked with an asterisk (*) will have the most impact:

- *Primers:
    - Design
    - Concentration
    - Ratio (1:4:8) - decreasing a single primer can decrease background. If one of the loop primers is causing the background, removal can solve the problem.
- *Varying magnesium concentration
- Titrating the concentration of LavaLAMP enzyme added;
- Additives (e.g. Betaine or Triton)
    - Be careful; if you wish to lyophilise, some additives are not compatible.

9. LAMP assay detection

For LAMP assay detection, we do not recommend colorimetric detection. The three methods we recommend are as follows:

- Fluorescent assays in real-time detection instruments: monitor reaction fluorescence using the FAM channel to detect amplified product.
- End-point fluorescent assays: measure fluorescence in a fluorometer using the FAM channel to detect amplified product.
- Non-fluorescent end-point assays: agarose gel (visual), spectrophotometer (turbidity, OD600). Note: turbidity is a low-cost option and is field deployable, but is single-use only and not as sensitive as other methods. Agarose gel is more applicable for diagnostics, and for troubleshooting e.g. determining whether NTC amplification is due to contamination or primer dimerisation.

Detection of Green Fluorescent Dye requires a real-time amplification instrument or a fluorometer for end-point analysis. This should be capable of excitation at 490 nm and measuring fluorescence at 520 nm.

- The following instruments have been successfully used with Green Fluorescent Dye: AmpliFire (Agdia), CFX96 and iQ5 Thermo Cyclers (Bio-Rad), ESEQuant TS2 (Qiagen), Genie II (OptiGene), and the ABI 7500 Real-Time PCR System (Thermo Fisher Scientific).
- Fluorescent dyes such as V13-01184, EvaGreen and SYTO-13 have also been used successfully with the LavaLAMP DNA Component Kit, but SYBR Green Dye will not work acceptably. Optimisation of dye concentration is necessary to produce the fastest TTR.

You can view detailed examples of LavaLAMP DNA results in our product manual here.

10. Frequently asked questions

Is LavaLAMP lyophilisable?

LavaLAMP is lyophilisation-compatible due to being glycerol-free and containing stabilising reagents. These reagents also allow for production of the desirable 'fluffy cake' when lyophilised.

We do not provide lyophilisation advice beyond the conditions described in Table 10. Here is a link to some useful information that may help.

Step	Conditions
Thermal treatment	Temp -50 °C
	240 min
Freeze, condenser vacuum	Temp -50 °C
	Additional Time 10 min
	Condenser -50 °C
	Vac 2000 mTorr
Drying cycle	Temp -40 °C for 360 min at 50 mTorr HOLD
	Temp -20 °C for 120 min at 50 mTorr HOLD
	Temp +0 °C for 120 min at 50 mTorr HOLD
	Temp +20 °C for 120 min at 50 mTorr HOLD
Secondary drying	Setpoint at +50 °C
	Post Heat +20 °C
	Time 60 min
	Vac 0050

Table 10. Conditions used for lyophilisation of LavaLAMP using a SP Scientific Wizard 2.0. These conditions have been used successfully by Biosearch Technologies at a small scale.

Tell me more about the detection with Green Fluorescent Dye. Can I substitute the Green Fluorescent Dye for other dyes?

Our Green Fluorescent Dye will absorb at 490 nm and emit at 520 nm, so your instrument's setting for SYBR Green or FAM may be used. Syto-13 (Molecular Probes) and EvaGreen (Biotium) have also been tested with the LavaLAMP DNA Master Mix and perform well.

In-house results demonstrate that the V13-01184 dye from Dyomics is stable in the LavaLAMP Master Mix. If you require master mix stability with the dye present, or wish to lyophilise master mix and dye together, we recommend that this dye is used.

Our Green Fluorescent Dye is not SYBR Green and SYBR Green will not work well with our LavaLAMP kits.

Can I use a colorimetric detection method with LavaLAMP?

We strongly recommend that colorimetric detection is not used; LavaLAMP kits work best using fluorescent detection.

The LavaLAMP enzyme/buffer combination are not well suited for use with colorimetric detection systems. Most colorimetric detection systems rely on a pH change as the LAMP reaction proceeds. As dNTPs are used to synthesise the looped DNA, inorganic pyrophosphate is released and there is a pH change in the reaction mix. An indicator dye changes colour as the pH changes. Optimal (shortest) TTR with these systems requires a reaction mix with very low buffering capacity, however, the LavaLAMP kits employ a reaction mix with a significantly higher buffering capacity. Thus, most colorimetric detection systems will not work optimally with the LavaLAMP products.

How do you run an analytical gel for LavaLAMP?

You may use a 4" long gel (typical of a minigel apparatus)

- 1% standard agarose
- 1X TBE
- 100 Volts

Perform electrophoresis until the Bromophenol Blue is ~1" from the bottom of the gel.

If you choose to add Ethidium Bromide to the gel when casting, also include it in the gel running buffer.

Alternatively, you may stain the gel after electrophoresis. Use SYBR Gold for optimal signal-to-noise ratio (for strongest bands against darkest background), however SYBR Green and other stains will also work admirably.

11. Summary

For the successful implementation of LavaLAMP:

1. Design multiple sets of primers for each target as LAMP primer design can have varying success.
2. Read the protocols and order the required consumables and reagents. Before running your test samples, use the provided positive control to verify your laboratory set up.
3. Perform a primer design screen to select the best starting design, and perform a temperature titration to establish the most efficient amplification temperature.
4. Primer concentration is critical – always independently confirm the concentration of received and reconstituted primers before preparing your own working solutions.
5. Run an NTC each time.
6. Follow guidance on LAMP assay optimisation.
7. View the two webinars listed in section 12 for more help and information.

12. Useful links

To order LavaLAMP products, please visit our website. Below are links to the product-specific pages for each of our LavaLAMP products.

- LavaLAMP DNA Component Kit
- LavaLAMP DNA Master Mix for Amplification
- LavaLAMP RNA Component Kit
- LavaLAMP RNA Master Mix

We have some additional resources that you may find helpful:

- Webinar 1: LAMP: Primer Design and Assay Optimisation Webinar
- Webinar 2: LAMP: Assay Development Challenges and Solutions Webinar
- Data sheet: LavaLAMP DNA and RNA Isothermal Amplification Kits

If you require additional support, please contact our technical support team at techsupport@lgcgroup.com.

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