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LAMP User Guide – Assay Design & Primers

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LAMP User Guide – Assay Design & Primers

Assay Design - Overview

A LAMP assay relies on 4-6 primers that target 6-8 locations within a given DNA sequence of approximately 300-600bp (**Figure 1**). Designs based on 4 primers contain 2 outer primers (F3 and B3) and two inner primers (FIP and BIP). An assay that has 6 primers has the addition of two loops (LoopF and LoopB) in the design, these serve to speed up the isothermal reaction time.

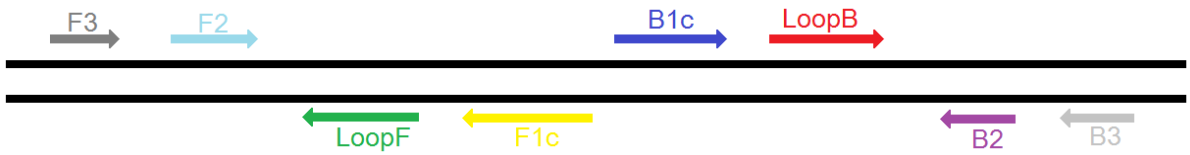

Figure 1: Diagram showing the location and direction of each primer site on a target for a LAMP reaction
FIP is made up of locations F1c and F2
BIP is made up of locations B1c and B1

For full details of what happens in a LAMP assay can be found in Notomi. T *et al.* (2000) Nucleic Acids Research28 (12) e63

Details of the LAMP reaction using 6 primers can be found in *Nagamine, K. et al.* (2002) Mol Cell Probes. 16:223-9

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Assay Design – Custom Design Service

OptiGene offers a chargeable custom assay design service. We can look into designing assays for a range of different requirements including

- Detecting individual species
- Generic assays to detect a group of closely related organisms
- DNA or RNA based targets
- SNP detection

Please note that the ability to design an assay is dependent on a number of factors including but not limited to:

- Sequencing data availability
- Strain variation
- Similarly related organism
- The DNA sequence composition of the target

Please contact us for more information at
http://www.optigene.co.uk/custom-design-service/

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Assay Design - Software

If you choose to design your own assays we recommend using Premier Biosoft LAMP Designer software. LAMP Designer creates assays based on 6 primers as it automatically incorporates loops into the design. This gives a faster assay than those designed without loops.

LAMP Designer has default settings for optimum primer design as well as giving the user the flexibility to be able to alter settings when required. Altering from default settings can make the reaction slower and less sensitive

LAMP Designer is available from our website
http://www.optigene.co.uk/lamp-designer/

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Assay Design – Target Sequence

Choosing your target DNA sequence is the most important part of LAMP assay design. Careful selection is required to ensure that the reaction will detect exactly what is required without amplifying other closely related, non-desired targets. The following aspects will need to be taken into consideration

- **Strain to strain variation in the sequence – avoiding false negatives**
  - Organisms such as viruses and bacteria often have sequence variation from strain to strain. The assay needs to be conserved enough to detect all strains in order to avoid false negatives. We recommend creating a consensus sequence of your desired gene from all desired strains and picking a conserved region. It is likely that you will need to increase the 'maximum number of ambiguous bases in an amplicon' in LAMP designer advanced search parameters settings. LAMP designer will never create a degenerate primer set, but increasing ambiguity allows for differences in the amplicon where the primers do not actually anneal
    - If designing for a genus or a group of species rather than an individual organism you will need to choose a conserved gene and create a consensus of all species to be detected

- **Closely related organisms – avoiding false positives**
  - If there are closely related organisms likely to be found in the sample then care needs to be taken to not choose a conserved gene that will be very similar in these closely related organisms. BLAST analysis will show if the sequence you have picked is highly conserved across other organisms. BLAST however is limited in that it will only analyse species where sequence data has been deposited so should not be relied upon for specificity. You cannot tell just by analysing the design whether or not the assay will be specific enough for your requirements. This can only be done by testing the assay against desired and undesired targets.

- **Nucleotide repeats and hairpins**
  - Avoid long chains of single nucleotides within primers and/or amplicons that are likely to cause strong hairpins, especially those that will not be resolved at the 65°C isothermal reaction temperature.

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- **Presence of variable number tandem repeats**
  - Repeats falling within a primer and/or amplicon can cause problems with primer dimers, mis-priming and incorrect LAMP amplicon formation. All of which have the potential of interfering with efficient strand displacing during the reaction

- **GC content**
  - Try to avoid regions that are very AT or GC rich. If there is no other target choice then LAMP Designer settings can be changed from default to try to design an assay.
    - AT rich templates will need longer primers and altered settings to decrease the allowed GC content of the final amplicon
    - GC rich templates will need shorter primers and altered settings to increase the allowed GC content of the final amplicon

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Primers - Primer Concentrations

Due to their different role within the LAMP reactions, each primer needs to be at a different concentration for optimal amplification. The primer concentrations we recommend, for a standard assay, can be seen in **Table 1**. However these concentrations can be altered, if necessary, to speed up a LAMP reaction if the assay is particularly slow or a faster assay is required. **Table 2.** Shows our recommended primer concentrations to speed up a reaction. The differences between these primer concentrations on amplification time can be seen in **Figure 2**.

Altering primer conditions is one of many ways that a reaction speed can be increased. For more information please see our **LAMP User Guide – Mastermixes and Optimisation**

Primer	Primer concentration[1]
F3	0.2µM
B3	0.2µM
FIP	0.8µM
BIP	0.8µM
LoopF	0.4µM
LoopB	0.4µM

Table 1. Recommended primer concentrations for a standard LAMP reaction.
[1] concentration of the primer in the final 25µl LAMP reaction

Primer	Primer concentration[2]
F3	0.2µM
B3	0.2µM
FIP	2.0µM
BIP	2.0µM
LoopF	1.0µM
LoopB	1.0µM

Table 2. Recommended primer concentrations for speeding up a LAMP reaction.
[2] concentration of the primer in the final 25µl LAMP reaction

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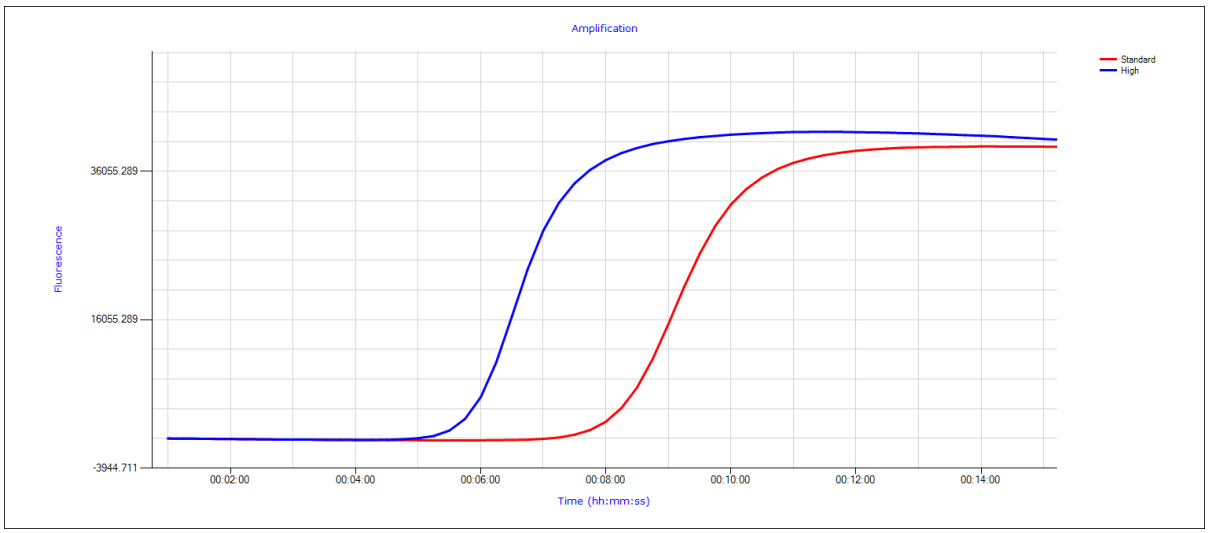

Primer conc.	Peak amplification
Standard	09:00
High	06:30

Figure 2: An *E. coli* LAMP assay using ISO-001 and 0.1ng template
Red – our recommended standard primer concentration recommendations
Blue – higher primer concentrations for speeding up assays
In this example increasing the primer concentration has decreased the time to peak amplification by 2mins 30sec

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Primers - Preparing Primer Stocks

Primers can be ordered on the smallest scale with standard de-salting purification. They usually arrive from the manufacturer in a lyophilised state. We recommend diluting primers to a 100micromolar (µM) working stock using 10mM Tris pH8.0 (@ 25°C)

Each primer should detail the amount of lyophilised product, in nmoles, on the side of the tube or on the certificate of analysis. This will be different for every individual primer as it is related to the synthesis yield/efficiency

In order to make a 100µM stock you will need to add 10x the amount of 10mM Tris pH8.0 (@ 25°C) as the nmoles yield detailed on the tube.

Working example

A lyophilised primer pellet of 32.4nmoles will need 324µl 10mM Tris pH8.0 (@ 25°C) to make a 100µM working stock

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Primers - Calculating volume of primer to add to an individual LAMP reaction

We do advise making a 10x primer mix containing all 6 primers that can then be added to the individual LAMP reactions rather than adding the primers separately. However, to calculate the amount of primer that would need to be added to each LAMP reaction the following formula can be used

$$\frac{\text{LAMP reaction volume X required primer concentration}}{\text{primer stock concentration}} = \text{volume of primer to add}$$

Working example

For a 25µl LAMP reaction requiring the FIP at a concentration of 0.8µM and using a stock primer at a concentration of 100µM

$$\frac{25\mu l \times 0.8\mu M}{100\mu M} = 0.2\mu l$$

So, 0.2µl of FIP would have to be added to the LAMP reaction and, once all other LAMP components and primers added, the final volume made to 25µl with nuclease free water

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Primers - Primer Mixes

We recommend making a primer mix containing all necessary LAMP primers prior to setting up a LAMP reaction. A single primer mix can then be used for several reactions allowing for consistency across reactions, reducing pipetting frequency, avoiding pipetting small volumes and ultimately reducing user error. This can therefore help to increase reliability and repeatability of your LAMP reactions.

Making a 10x primer mix

We recommend making a 10x primer mix, 2.5µl of which should be added to every 25µl LAMP reaction. Once the final concentration of primer in an individual LAMP reaction has been decided multiply this by 10 to calculate the concentration required in a 10x primer mix. **Table 3** shows our recommended concentration for each primer

Primer	Primer conc. in LAMP reaction[3] (1x)	Primer conc. in primer mix (10x)
F3	0.2µM	2µM
B3	0.2µM	2µM
FIP	0.8µM	8µM
BIP	0.8µM	8µM
LF	0.4µM	4µM
LB	0.4µM	4µM

Table 3. Our recommended concentrations for each primer
[3] this is the concentration of primer that is required in the final 25µl LAMP reaction mix

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You will also need to know the number of reactions you want to make in order to calculate the final volume of primer mix to be made **Table 4**

Number of reactions	10x primer mix[4] volume
1	2.5µl
10	25µl
25	62.5µl
50	125µl
100	250µl
250	625µl
500	1250µl

Table 4 – the volume of primer mix required for different numbers of reactions
[4] The primer mix will include all necessary primers at their relevant concentrations and then made up to the final volume with 10mM Tris pH8.0 (@ 25°C)

Each lyophilised primer should be made to a 100µM working stock and then the following formula can be used to calculate the amount of each primer needed for the 10x primer mix

$$\frac{\text{primer conc. in 10x mix} \quad X \quad \text{final primer mix volume}}{\text{Primer stock conc.}} = \text{volume of individual primer to add}$$

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Working example making a 10x primer mix using our recommended concentrations

Each primer has been diluted to a 100µM working stock

Need 100 reactions (250µL) of a 10x primer mix. The concentration of each primer within the 10x primer mix is as follows:

- F3 – 2µM
- B3 – 2µM
- FIP – 8µM
- BIP – 8µM
- LoopF – 4µM
- LoopB – 4µM
-

This formula was used to calculate the amount of each primer needing to be added to make the 10x primer mix.

$$\frac{\text{primer conc. in 10x mix} \times \text{final primer mix volume}}{\text{Primer stock conc.}} = \text{volume of individual primer to add}$$

- F3

$$\frac{2\mu m \times 250\mu l}{100\mu M} = 5\mu l \text{ of F3}$$

- B3

$$\frac{2\mu m \times 250\mu l}{100\mu M} = 5\mu l \text{ of B3}$$

- FIP

$$\frac{8\mu m \times 250\mu l}{100\mu M} = 20\mu l \text{ of FIP}$$

- BIP

$$\frac{8\mu m \times 250\mu l}{100\mu M} = 20\mu l \text{ of FIP}$$

- LoopF

$$\frac{4\mu m \times 250\mu l}{100\mu M} = 10\mu l \text{ of LoopF}$$

- LoopB

$$\frac{4\mu m \times 250\mu l}{100\mu M} = 10\mu l \text{ of LoopB}$$

After all the primers have been added 10mM Tris pH8.0 (@ 25°C) is added to bring the final volume up to 250µl. In this example 180µl 10mM Tris pH8.0 (@ 25°C) will need to be added

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Summary of how to make standard primer mixes

Primer	Primer working stock conc.	Primer conc. in 25µl LAMP rxn	Primer conc. in 10x primer mix	Vol. of primer (1 rxn)	Vol. of primer (10 rxns)	Vol. of primer (25 rxns)	Vol. of primer (50 rxns)	Vol. of primer (100 rxns)	Vol. of primer (250 rxns)	Vol. of primer (500 rxns)
F3	100µM	0.2µM	2µM	0.05µl	0.5µl	1.25	2.5	5	12.5	25
B3	100µM	0.2µM	2µM	0.05µl	0.5µl	1.25	2.5	5	12.5	25
FIP	100µM	0.8µM	8µM	0.20µl	2.0µl	5.00	10.0	20	50.0	100
BIP	100µM	0.8µM	8µM	0.20µl	2.0µl	5.00	10.0	20	50.0	100
LoopF	100µM	0.4µM	4µM	0.10µl	1.0µl	2.50	5.0	10	25.0	50
LoopB	100µM	0.4µM	4µM	0.10µl	1.0µl	2.50	5.0	10	25.0	50
10mM Tris pH8.0 (@ 25°C)				1.8µl	18µl	45µl	90µl	180µl	450µl	900µl
Final volume				2.5µl	25µl	62.5µl	125µl	250µl	625µl	1250µl

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Summary of how to make more concentrated primer mixes for speeding up amplification

Primer	Primer working stock conc.	Primer conc. in 25µl LAMP rxn	Primer conc. in 10x primer mix	Vol. of primer (1 rxn)	Vol. of primer (10 rxns)	Vol. of primer (25 rxns)	Vol. of primer (50 rxns)	Vol. of primer (100 rxns)	Vol. of primer (250 rxns)	Vol. of primer (500 rxns)
F3	100µM	0.2µM	2µM	0.05µl	0.5µl	1.25µl	2.5µl	5µl	12.5µl	25µl
B3	100µM	0.2µM	2µM	0.05µl	0.5µl	1.25µl	2.5µl	5µl	12.5µl	25µl
FIP	100µM	2.0µM	20µM	0.5µl	5.0µl	12.5µl	25µl	50µl	125µl	250µl
BIP	100µM	2.0µM	20µM	0.5µl	5.0µl	12.5µl	25µl	50µl	125µl	250µl
LoopF	100µM	1.0µM	10µM	0.25µl	2.5µl	6.25µl	12.5µl	25µl	62.5µl	125µl
LoopB	100µM	1.0µM	10µM	0.25µl	2.5µl	6.25µl	12.5µl	25µl	62.5µl	125µl
10mM Tris pH8.0 (@ 25°C)				0.9µl	9µl	22.5µl	45µl	90µl	225µl	450µl
Final volume				2.5µl	25µl	62.5µl	125µl	250µl	625µl	1250µl