OptiGene
Unit 5 Blatchford Road ♦ Horsham ♦ West Sussex ♦ RH13 5QR ♦ UNITED KINGDOM
T: +44 (0)1403-274980 ♦ F: +44 (0)1403-271017 ♦ E: info@optigene.co.uk ♦W: www.optigene.co.uk

LAMP User Guide – Mastermixes & Assay Optimisation

Contents

Unit 5 Blatchford Road ♦ Horsham ♦ West Sussex ♦ RH13 5QR ♦ UNITED KINGDOM
T: +44 (0)1403-274980 ♦ F: +44 (0)1403-271017 ♦ E: info@optigene.co.uk ♦W: www.optigene.co.uk

LAMP User Guide – Mastermixes & Assay Optimisation

Lamp Reagents

OptiGene offer a variety of different reagents allowing flexibility and choice in how you carry out your LAMP reactions.

Isothermal Mastermixes

OptiGene sell a variety of mastermixes to assist with Isothermal amplification. All that you will need to add is primer and templates to complete your own assay. For information of which mastermix is most suited for your application please visit our website at

http://www.optigene.co.uk/isothermal-mastermix-selection-guide/

Mastermixes in our range include

- Our standard Mastermixes which can be used following the template preparation methods detailed in this guide. Alternatively you can lyse and/or purify the template with a method of your choice. Our range of standard mastermixes include the following:
  - **ISO-001**
  - **ISO-004**
  - **ISO-001Tin**

- Our Lyse 'n' LAMP mastermixes require lysis using KOH (***CORROSIVE***). This method is versatile enough to work on many sample types without the need for a lengthy purification protocol at the lab bench. Our Lyse 'n' LAMP range include
  - **ISO-001LNL**
  - **ISO-004LNL**

This guide is focused on how to use and optimise your LAMP assay using these mastermixes

LAMP User Guide – Mastermixes & Assay Optimisation

Individual Reagents

If even more flexibility is required then OptiGene also offer a wide range of enzymes and buffers allowing you to make your own bespoke assay. For full details please visit our website at

http://www.optigene.co.uk/polymerase-selection-guide/

Diagnostic Kits

OptiGene have a range of readymade diagnostic kits preventing the need to design and optimise your own reaction. The ranges we offer include

- Clinical kits from the Amplex BioSystems GmbH range of eazyplex® tests
  - http://www.optigene.co.uk/amplex-easyplex/
- Plant Health and pest diagnostic kits
  - http://www.optigene.co.uk/plant-health-pests-diagnostics-3/

LAMP User Guide – Mastermixes & Assay Optimisation

Template Preparation for Standard Mastermixes

Our standard mastermixes are robust and can use a variety of crude samples providing adequate lysis of the cells has occurred releasing the DNA template. If the chosen lysis method is not sufficient to release the template DNA then we recommend using our Lyse 'n' LAMP preparation and mastermix. The Lyse 'n' LAMP mastermix rely on a quick and simple lysis method which is more likely to release template DNA from crude samples. As an alternative to following these methods you could use your own preferred lysis and/or template purification protocols.

In situations where it is essential that very low copy numbers are detected we suggest that a concentration step of the sample or an enrichment step is used to reduce the likelihood of false negatives.

NB: Some media contain components that are fluorescent and will interfere with the detection of DNA amplification

Purified Samples & Controls

Our mastermixes can be used with most laboratory prepared purified samples. We recommend adding at least 0.01ng or $1x10^3$ copies although this is assay specific. Details on how to optimise your template concentration can be found in this guide on page 15 under **Assay Optimisation – Template Concentration**

Bacterial colonies

Use a sterile inoculation loop to remove a single bacterial colony and resuspend in nuclease free water, vortex and heat for 95°C for 5-10mins. Allow contents to cool before opening the tube. An aliquot of the prepared sample can be used directly in a 25µl LAMP reaction.

If this method does not appear to sufficiently lyse the template then we recommend using our Lyse 'n' LAMP mastermix and lysis methods

LAMP User Guide – Mastermixes & Assay Optimisation

Swabs

Thoroughly swab the site with a clean sterile swab and twizzle in a sufficient volume of nuclease free water. Heat at 95°C for 5-10min. Allow contents to cool before opening the tube. An aliquot of the prepared sample can be used directly in a 25µl LAMP reaction.

If this method does not appear to sufficiently lyse the template then we recommend using our Lyse 'n' LAMP mastermix and lysis methods

Liquid Culture

Heat an aliquot of liquid to 95°C for 5-10mins. Allow contents to cool before opening the tube. An aliquot of the prepared sample can be used directly in a 25µl LAMP reaction.

NB: Some media contain components that are fluorescent and will interfere with the detection of DNA amplification

If this method does not appear to sufficiently lyse the template then we recommend using our Lyse 'n' LAMP mastermix and lysis method

Plant Material

We recommend lysis using our Plant Material Lysis Kit catalogue number **EXT-001**

Alternatively, for some plant assays, we have available some readymade diagnostic kits. Full details can be found at
http://www.optigene.co.uk/plant-health-pests-diagnostics-3/

Food Samples

We recommend using our Lyse 'n' LAMP mastermix and lysis method for use with food samples

LAMP User Guide – Mastermixes & Assay Optimisation

Template Preparation for Lyse 'n' LAMP Mastermixes

The Lyse 'n' LAMP mastermixes, **ISO-001LNL** and **ISO-004LNL**, allow crude samples to be used directly in a LAMP reaction after a simple lysis step. The lysis method uses KOH (***CORROSIVE***) and the mastermix has been designed to require the addition of an extra 60mM KOH (***CORROSIVE***), in the form of the prepared template, to the final reaction mix in order for the enzyme to work.

The Lyse 'n' LAMP mastermixes will not work in the absence of 60mM KOH in the final reaction mix

In situations where it is essential that very low copy numbers are detected we suggest that a concentration step of the sample or an enrichment step is necessary to prevent the occurrence of false negatives.

NB: Some media contain components that are fluorescent and will interfere with the detection of DNA amplification

Purified Samples & Controls

This mastermix requires an additional 60mM KOH (***CORROSIVE***) be added to the 25µl LAMP reaction. If the purified sample is in a standard buffer or water then then extra KOH will need to be added to the reaction. We recommend 2.5µl of 0.6M KOH (***CORROSIVE***)

	1x 25µl
Lyse 'n' LAMP Mastermix	15µl
10x primer mix	2.5µl
Template in water/buffer	0.01-10ng
0.6M KOH (*CORROSIVE***)**	2.5µl
Water	To 25µl

LAMP User Guide – Mastermixes & Assay Optimisation

Bacterial Colonies

Use a sterile inoculation loop to remove a single bacterial colony and resuspend in 0.3M KOH (***CORROSIVE***), seal tube and vortex briefly. Heat sample for 10mins at 95°C (optional). Allow contents to cool before opening the tube. 5µl of prepared sample can be used directly in a 25µl Lyse 'n' LAMP reaction

Swabs

Thoroughly swab the site with a clean sterile swab and twizzle in a sufficient volume of 0.3M KOH (***CORROSIVE***). Vortex and seal tube. Heat at 95°C for 10mins (optional). Allow contents to cool before opening the tube. 5µl of prepared sample can be used directly in a 25µl Lyse 'n' LAMP reaction

Liquid Culture

Add liquid culture to 0.6M KOH (***CORROSIVE***) at a ratio of 1:1. For example: 500µl of liquid culture to 500µl 0.6M KOH (***CORROSIVE***), seal tube and vortex briefly. Heat sample for 5mins at 95°C (optional). Allow contents to cool before opening the tube. 5µl of prepared sample can be used directly in a 25µl Lyse 'n' LAMP reaction

NB: Some media contain components that are fluorescent and will interfere with the detection of DNA amplification

Plant Material

We recommend lysis using our Plant Material Lysis Kit catalogue number **EXT-001** with our standard mastermixes. We do not recommend the use of Lyse 'n' LAMP method for plant materials

Alternatively, for some plant assays, we have available some readymade diagnostic kits. Full details can be found at
http://www.optigene.co.uk/plant-health-pests-diagnostics-3/

LAMP User Guide – Mastermixes & Assay Optimisation

Food Samples

Place a clean disposable petri dish on top of the cutting template (**Figure 1)**. Using the guide cut a small sample (~0.5mm^3) and discard remaining sample. Cut up the sample further to increase surface area and add to 1ml 0.3mM KOH (***CORROSIVE***), seal tube, vortex and heat for 10mins at 95ºC. Allow sample to cool and contents to settle before opening. 5µl of prepared sample can be used directly in a 25µl Lyse 'n' LAMP reaction

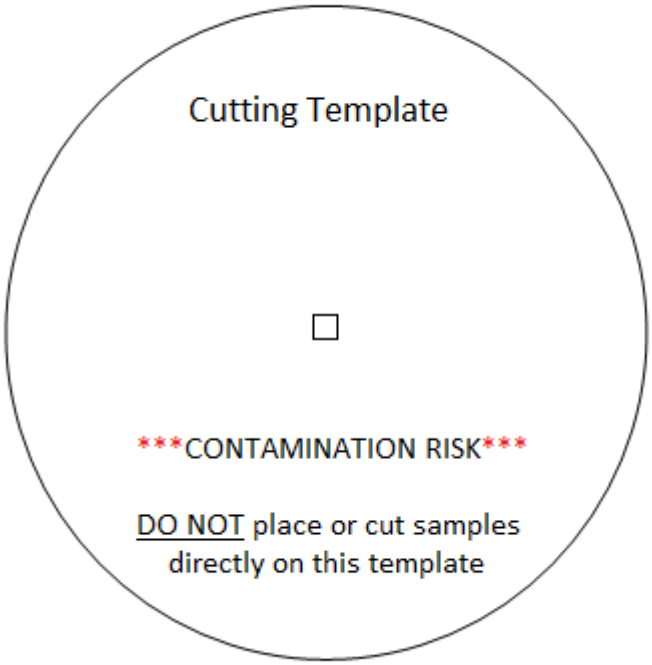


Figure 1. Cutting Template
Make a photocopy of this template, cut out and place underneath a clear sterile petri dish to serve as a guide to the size of food sample required. To avoid cross contamination of samples avoid cutting directly on the template

LAMP User Guide – Mastermixes & Assay Optimisation

Preparation for a LAMP reaction

- Pre-chill Genie® strip holders (cat. No: **GBLOCK-03**)

- Thaw all reagents on ice

- Re-suspend lyophilised primer pellet to 100µM with 10mM Tris pH 8.0 and make a 10x primer mix. See our **OptiGene LAMP User Guide – Assay Design & Primers** for details of how to make a primer mix

- Prepare template by the desired method as detailed above.

- Set up a Reaction mastermix as detailed below

LAMP User Guide – Mastermixes & Assay Optimisation

Preparing a Reaction Mastermix

We recommend you make a mastermix containing all components of a reaction that are to be kept constant, dispensing this into individual Genie® well tubes and then adding the variable (e.g. template). In doing so this will

- Reduce well to well variation
- Increase reproducibility from one run to the next
- Reduce instances of pipetting error
- Decrease the number of pipetting steps
- Speed up setting up a LAMP reaction

A typical mastermix could be as follows

	Individual rxn	Strip of 8 rxns	2x Strips of 8 rxns
	1x 25µl	10x 25µl	18x 25µl
LAMP Mastermix	15µl	150µl	270µl
10x primer mix	2.5µl	25µl	45µl
Template (0.1ng/µl)	5µl	Added individually	Added individually
Water	2.5µl	25µl	45µl

Dispense 20µl of the prepared mastermix into each well followed by 5µl of lysed template. Close each cap, making sure the lid is locked securely into place and load into the Genie® instrument.

*****Once a Genie® tube has been securely sealed it should never be reopened. There is a huge contamination risk with the number of copies generated in a LAMP reaction if the tube is opened*****

For a standard LAMP reaction run an isothermal amplification step of 65°C for 20-30mins followed by an anneal step of 98-80°C

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation

Our mastermixes already offer you the most optimum reaction conditions in terms of buffer and DNA polymerase. However, with some assays speed is very important or you may find that other assays are slow due their design. Factors that can influence amplification include

- Poor assay design
- Poor DNA target sequence
  - GC Rich
  - AT rich
  - Repeats
  - Secondary structure
- Poor sample quality
  - Inhibitors
  - Poorly lysed
  - Sample too dilute
  - Copy numbers too low
  - Components interfering with amplification detection

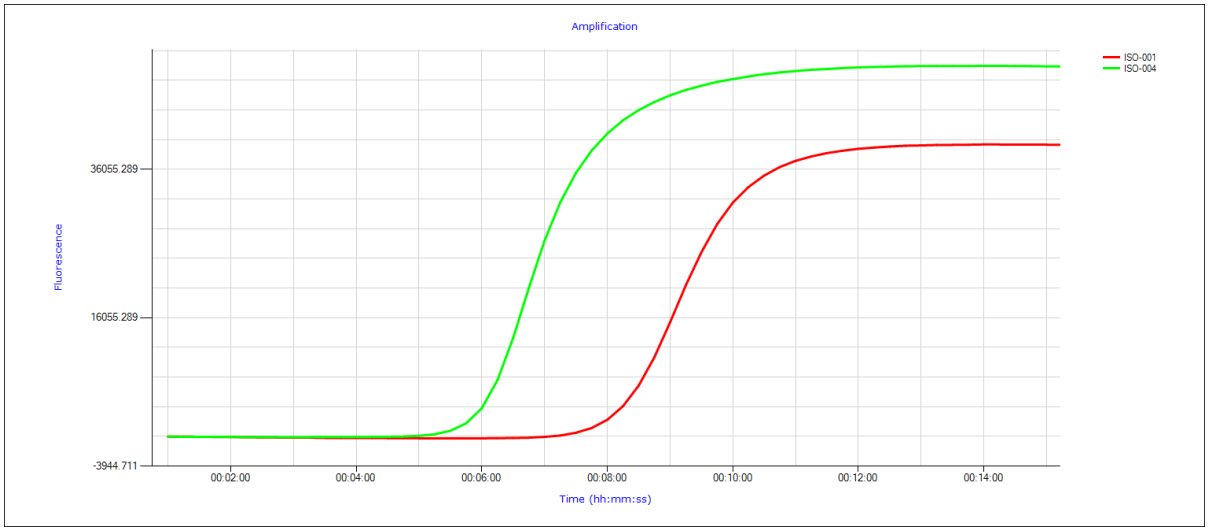
It is possible for an assay to be improved by taking into account some or all of the following

- Isothermal Mastermix choice
- Primer concentrations
- Isothermal reaction temperature
- Template concentrations
- Sample Quality
  - Presence of inhibitors in a sample
  - Incomplete lysis
  - Copy number of DNA target
  - Components interfering with amplification detection

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Mastermix

We offer two main mastermixes for LAMP; **ISO-001 and ISO-004**. These are also available in the Lyse 'n@ LAMP range. ISO-001 contains OptiGene's proprietary novel GspSSD LF DNA polymerase and ISO-004 contains an engineered GspSSD LF DNA polymerase allowing for faster amplification time. If a faster assay is required then this can be achieved by switching from ISO-001 to ISO-004 isothermal mastermix with no other change to the assay conditions. Figure 2 shows the difference between running ISO-001 and ISO-004 isothermal mastermix using the same primers and template



Mastermix	Peak amplification
ISO-001	09:00
ISO-004	06:30

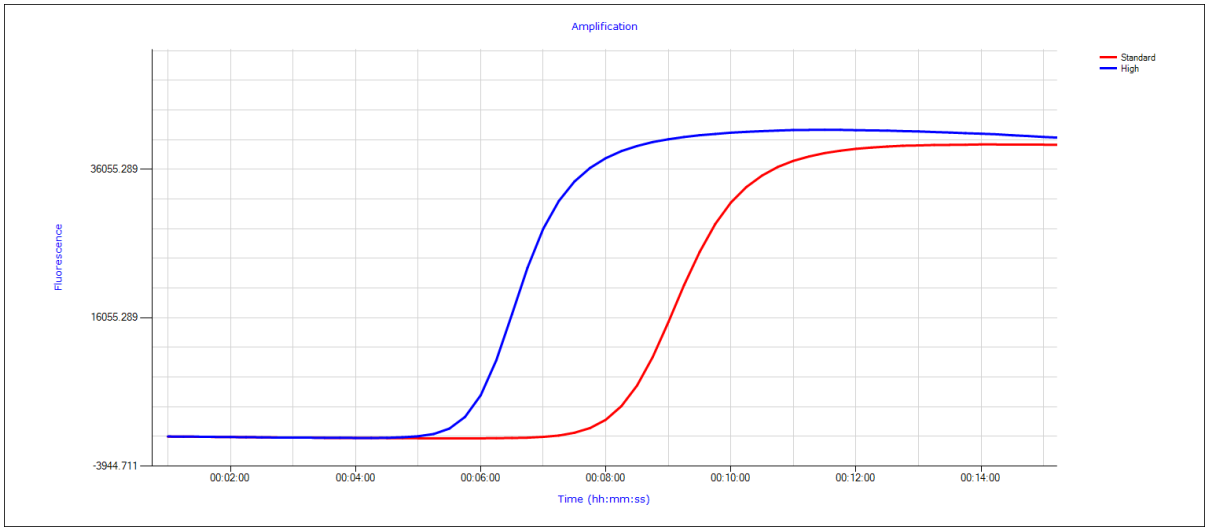
Figure 1. amplification curve of an *E.coli* LAMP assay running our standard primer concentration and 0.1ng of purified *E.coli* template.
Red – ISO-001
Green – ISO-004

In this example by changing the isothermal mastermix from ISO-001 to ISO-004 the time to peak amplification was decreased by 2mins 30sec. The time difference seen between the mastermixes is assay dependent. Changing mastermix is recommended for situations where an assay is particular slow, due to assay design, or if a faster assay is required.

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Primer Concentrations

Our standard primer concentrations, as detailed in **OptiGene LAMP User Guide – Assay Design & Primers**, are suitable for most assays to give a reasonable amplification time. However, in some situations a faster assay is required. Increasing the primer concentrations can help speed up an assay as showing in **figure 3**



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

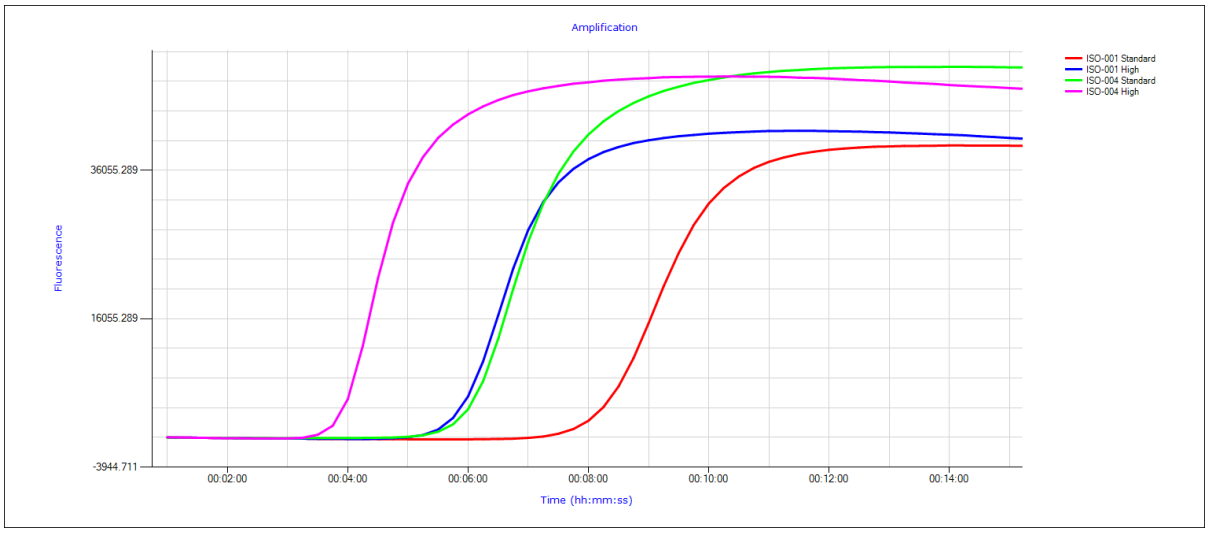
Figure 3 an *E.coli* LAMP assay using ISO-001 isothermal mastermix with 0.1ng of purified *E. coli* template.

Red - our standard primers concentrations (0.2µM F2 /B2, 0.8µM FIP/BIP and 0.4µM LoopF/LoopB
Blue – our high primer concentrations (0.2µM F2 /B2, 2.0µM FIP/BIP and 1.0µM LoopF/LoopB

In this assay increasing the primer concentrations has decreased the time to peak amplification by 2mins 30sec

LAMP User Guide – Mastermixes & Assay Optimisation

Increasing the primer concentration can be applied using any of our isothermal mastermixes. So to increase the speed of a reaction further you could combine ISO-004 isothermal mastermix along with our high primer concentrations as seen in **figure 4**



Primer conc.	ISO-001	ISO-004
Standard	09:00	06:30
High	06:30	04:15

Figure 4. Effect of combining different mastermixes and different primer concentrations

Red – ISO-001 and standard concentration primers
Blue – ISO-001 and high concentration primers
Green – ISO-004 and standard concentration primers
Pink – ISO-004 and high concentration primers

The fastest reaction is achieved by combining ISO-004 with the higher concentrating of primers

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Template Concentrations

Ensuring you have sufficient template with no inhibitors can improve the efficiency of an assay. A dilution series of the template can be carried out and analysis of the data can help you select the most suitable concentration of template for your requirements. **Figure 5** shows the amplification data from a typical 1:10 dilution series of template

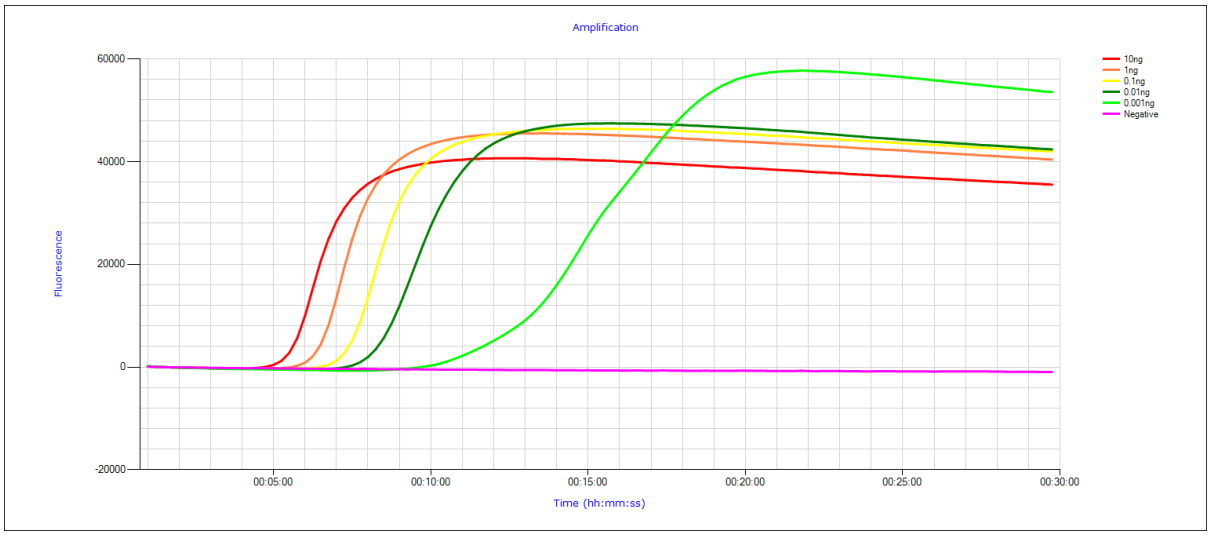


Figure 5 Amplification curve for a typical 1:10 dilution series of template using ISO-001

The final dilution in this dilution series is amplifying late with an amplification curve that has a shallower gradient suggesting this concentration is not ideal for the assay.

Choosing templates between 10-0.01ng would be most suitable for this particular assay.

LAMP User Guide – Mastermixes & Assay Optimisation

Further confirmation of the most suitable level of template can be given by analysing the amplification rate (**figure 6**) using our Genie Explorer software

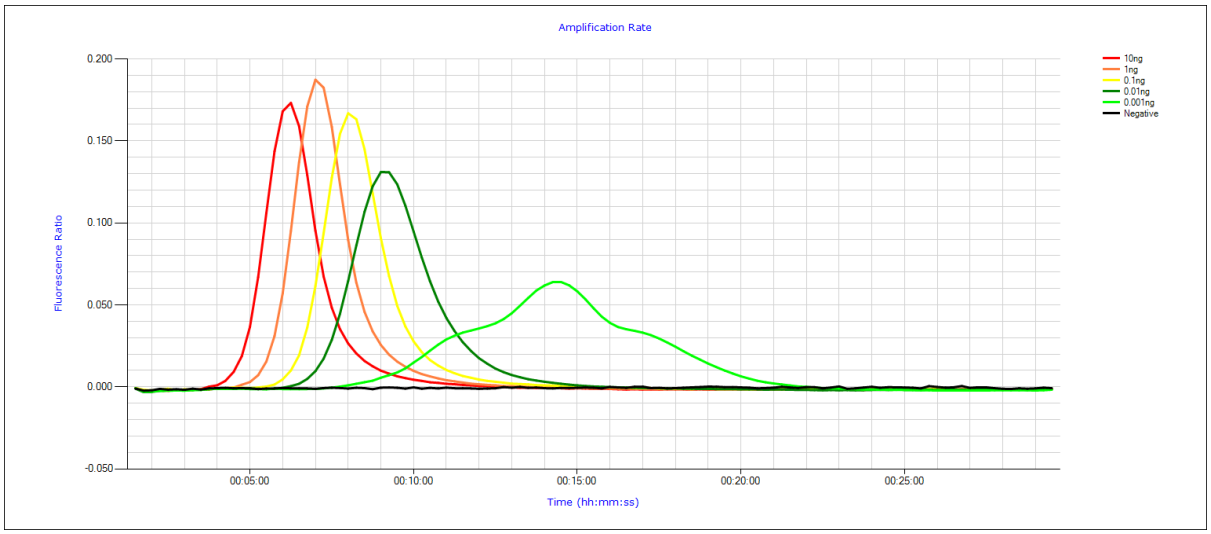


Figure 6 Amplification rate data for a typical 1:10 dilution series of template

- 10ng (red), 1ng (orange) and 0.1ng (yellow) have peaks that are tall, narrow and regular indicative efficient amplification. The
- 0.01ng (dark green) has a peak that is lower, broader but still a regular shape compared to the higher dilutions. This concentration of template should still be reliably detected in replicates
- 0.001ng (light green) shows a poor amplification rate with a very shallow and irregular peak. It is possible that, for this assay, this concentrating of template will not reliably be detected in replicates

Please note that the amplification rate data is not available if analysing data from the Genie® instrument itself and can only be carried out using our Genie Explorer software. Genie Explorer is available for free download off of our website

http://www.optigene.co.uk/support

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Isothermal Reaction Temperature

We recommend running the isothermal amplification step of LAMP at 65°C as this temperature is the most optimum for our DNA polymerase. However, in some assays, the primers require temperatures quite different to those of the DNA polymerase. Running a thermal gradient for your assay can indicate what temperature is best for the isothermal amplification step for your individual assay.

Our Genie® user manual details how to set up a temperature gradient on the Genie®. Set up one single mastermix containing all reaction components an example of which can be seen in **table 1**. Pipette 25µl into each well, cap tubes and run a thermal gradient of your choice

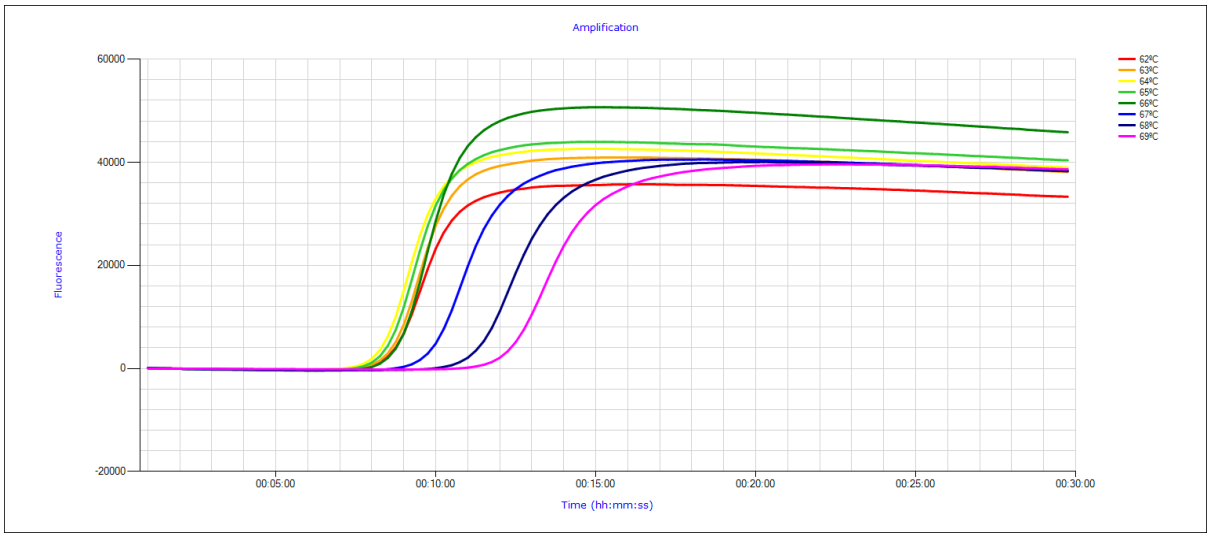
	10x 25µl (for strip of 8 rxns)
Isothermal mastermix **10x primer mix** **Template (0.1ng/µl)**	15µl 2.5µl 10µl
Water	65µl

Table 1. Example of a mastermix for running a thermal gradient on the Genie® instrument.

It is essential that all components are kept constant and that the only variable is the well temperatures as controlled by the Genie®. 0.1ng of template per reaction has been used in this example but this will be assay dependent. The level of template used should allow for a slow enough assay to see well to well changes with the different temperatures

LAMP User Guide – Mastermixes & Assay Optimisation

Analysis of the isothermal data and the peak amplification time (**Figure 7**) will show which temperature is optimum for your reaction



Name	Time
62°C	09:15
63°C	09:15
64°C	09:00
65°C	09:00
66°C	09:30
67°C	10:30
68°C	12:00
69°C	13:00

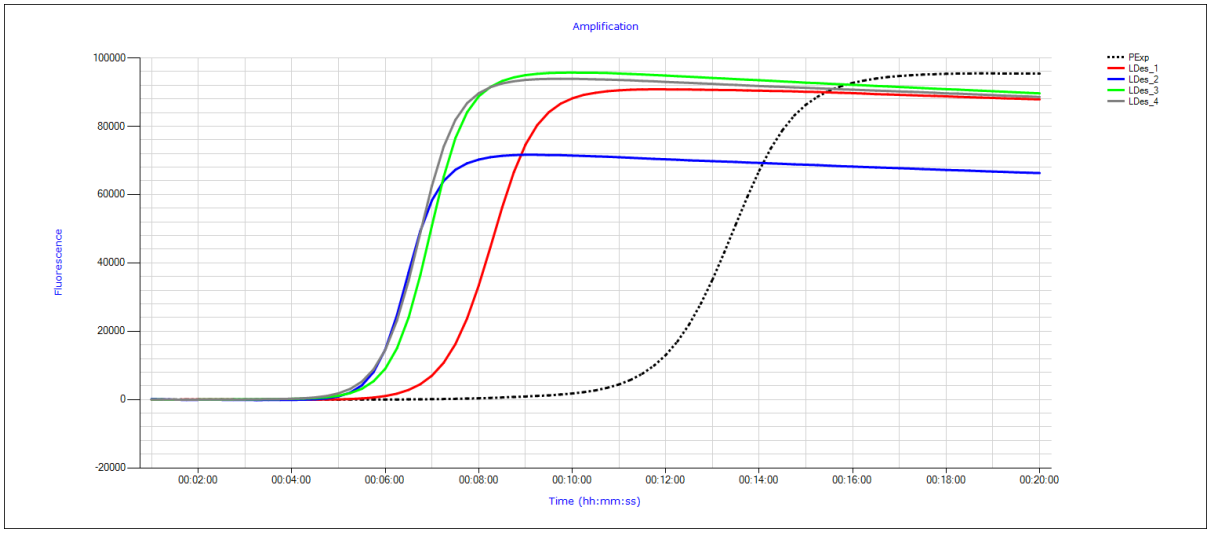
Figure 7. Amplification data for an *E. coli* LAMP assay using ISO-001 and our standard concentration of primers.

65°C is the optimum reaction temperature for the assay.

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Primer Design

An alternative improvement solution, that involves changing the assay, is to re-do the primer design. Using Premier Biosoft LAMP Designer software will ensure that assays are designed with Loop primers which speed up the reaction. Where possible use the default settings on the LAMP Designer software which will offer several different assays for the same region. It is worth selecting 4 different assay designs, with primers in different locations, for your desired sequence and testing all 4 to see which is best for speed (**figure 8**), sensitivity and specificity. Please see **OptiGene LAMP user guide – Assay Design & Primers**



Name	Time	Temp
PExp	13:00	88
LDes_1	08:00	88.5
LDes_2	06:30	88.7
LDes_3	06:45	89.4
LDes_4	06:30	89.2

Figure 8. Comparing 5 different LAMP assay designs for the same lambda DNA target
PExp is the original Primer Explorer designed assay
LDes are Lamp Designer designed assays
Even though they are designed for the same target and all contain 6 primers the speed varies.

LAMP User Guide – Mastermixes & Assay Optimisation

Choosing the best assay design cannot be done by just analysis of the primers and locations and has to be completed by running some experiments. Determining the best assay should take into account a compromise between

- Speed
  - The time at which the assay reaches peak amplification. Altering the primer location within a given target can have improve the speed of the assay
- Sensitivity
  - What is the lowest concentration that the assay can reliably detect. This means that replicates of this lowest level should always produce a positive result. See Page 15 **assay optimisation – Template concentration** within this guide
- Specificity
  - Ensure your assay will detect all strain variations that you require while not detecting any similarly related organisms that could potentially be present in a sample

LAMP User Guide – Mastermixes & Assay Optimisation

Assay Optimisation – Quality of Sample

The quality of the sample plays an important role in the efficiency of the LAMP assay. It is important to take this into consideration as running a poor quality sample will likely lead to false negatives

Inhibitors

Where there are inhibitors present in the sample amplification could be delayed or absent despite plenty of DNA target being present. Inhibitors therefore increase the likelihood of false negatives occurring. In order to determine if your sample has inhibitors present you will need a known working primer set and template, designed for an unrelated assay, to serve as a control. Two assays need to be run together at the same time.

Assay 1 - Run the control template and primers in a standard LAMP reaction

Assay 2 – Run the control template and primers, as before, but also add your sample

	Assay 1	Assay 2
Isothermal Mastermix	15µl	15µl
Control DNA	0.1ng	0.1ng
10x Control Primer mix	2.5µl	2.5µl
Sample	None	Your usual sample volume
Water	To 25µl	To 25µl

It is very important that your control primers will NOT amplify any of the DNA in your sample in this experiment as you are looking to see what effect the sample has on normal amplification of another target

Interpreting the results

Amplification Time	Result
Assay 1 = Assay 2	No Inhibitor
Assay 1 < Assay 2	Inhibitor present
Assay 2 shows no amplification	Sample completely inhibitory

If a sample is found to be very inhibitory to LAMP then a purification step will be necessary to avoid false negatives

Incomplete Lysis

LAMP User Guide – Mastermixes & Assay Optimisation

If the sample has not been lysed correctly then the LAMP reaction could be slow or false negatives could occur

Ensure sample is fully lysed by running our Lyse 'n' LAMP mastermix and lysis methods or using our standard isothermal mastermixes with a lysis and purification method of your choice

Low Copy Number

If the sample has a low copy number it may be necessary to perform a concentration step or an enrichment step in order for the LAMP assay to detect the DNA

Poor Amplification Detection

In some instances the crude sample may contain components that fluoresce or interfere with the intercalating dye and therefore can prevent normal detection of amplification by the Genie® instrument. This can be determined by using the Genie Explorer software and looking at the amplification data with and without normalisation. If the assay fluorescence is very high, with no normalisation, it is likely that the crude sample does contain components that interfere with detection. This can also be seen in situations where it appears that no amplification has occurred when analysing the amplification fluorescence data however, after running an anneal step, a genuine anneal peak can be seen.