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Introduction to LAMP

Contents

OptiGene
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Introduction to LAMP

Assay Design Overview

Loop-mediated isothermal amplification (LAMP) is a DNA amplification method that relies on a strand displacing DNA polymerase allowing amplification to occur under isothermal conditions. This is different to the more traditional method of polymerase chain reaction which requires repeated temperature cycling to allow amplification to take place. The LAMP method requires 4-6 primers that match 6-8 locations within the target DNA sequence (**Figure 1**) spanning a region of approximately 300-600bp.

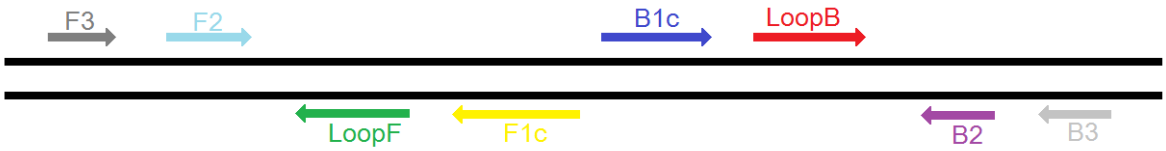

Figure 1: Diagram showing the location and direction of each primer site on a target for a LAMP reaction
FIP is made up of locations F1c and F2
BIP is made up of locations B1c and B1

For full details of the actual LAMP reaction using 4 primers can be found in Notomi, T. *et al.* (2000) Nucleic Acids Research 28(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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Typical Reaction

OptiGene LAMP mastermixes contain all necessary components for the reaction to take place (minus template and primers) and be detected by our Genie® instruments. This includes strand displacing DNA polymerase, dNTPs, an optimised reaction buffer and an intercalating dye allowing detection of double stranded DNA, using a Genie® instrument, as it is produced.

A typical reaction using our mastermixes (e.g. ISO-001 or ISO-004) would be set up as follows

	Individual reaction 1x 25µl	**Strip of 8 reactions 10x 25µl**
LAMP mastermix	15µl	150µl
10x Primer mix*	2.5µl	25µl
Template	0.1ng or 1x10^5 copies	Add individually
Water	To 25µl final reaction volume	To 250µl final reaction volume

*See **LAMP user guide – Assay Design & Primers** for details of primer concentrations and how to make a primer mix

Where the template is RNA we recommend adding 0.25U of AMV-RT to the reaction mix

A typical reaction involves an amplification step at 65°C for 20-30mins which allows DNA amplification to take place. This is then followed by an anneal step 98°C-80°C at 0.01°C/sec to give the anneal temperature of the amplicon produced

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Amplification Data

The Genie® instrument will display the reaction in real time. The level of double stranded DNA (dsDNA) at the start of the reaction is very low and the Genie® instrument uses this fluorescence level as a base line for the background fluorescence. As amplification takes place more dsDNA is produced and fluorescence levels increase as the dye intercalates with the dsDNA. This is monitored by the Genie® instrument which plots the increase in fluorescence on to a graph which gives an amplification curve. A typical dilution series can be seen in **Figure 2**. As levels of template decrease the amplification becomes slower and the detection of fluorescence over time becomes slower with a more gradual increase

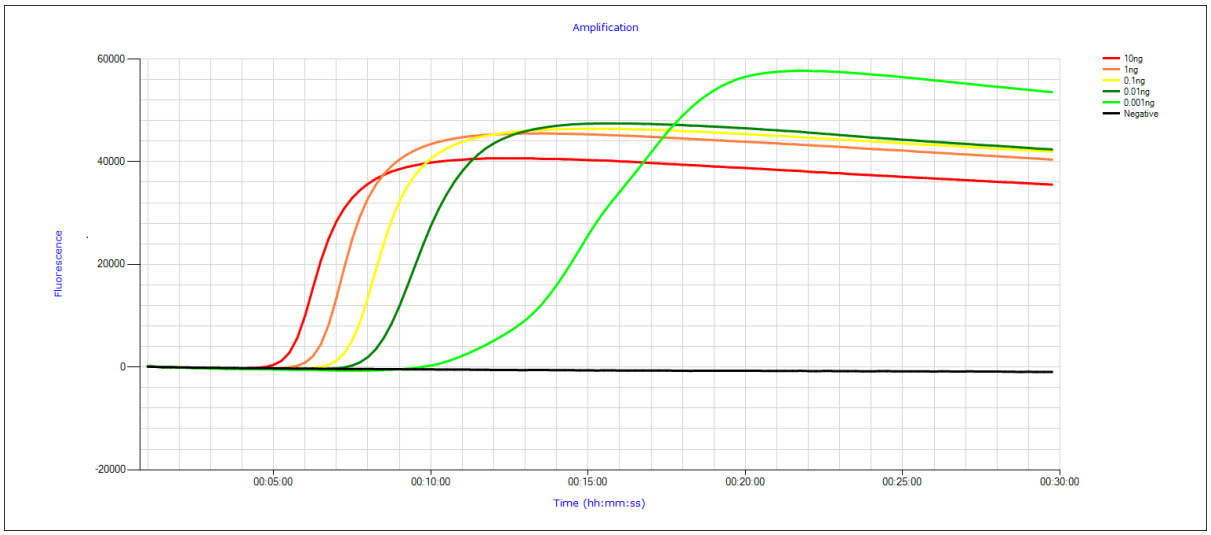

Figure 3. Amplification curve for a typical 1:10 dilution series of DNA template. As the concentration of template decreases with each dilution the detection of the assay gets slower until the last dilution no longer an evenly spaced amplification curve and has a much more gradual increase in fluorescence

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Amplification Rate (Genie Explorer software only)

Using our Genie Explorer software you can also analyse the amplification rate to give an idea of the efficiency of the reaction. **Figure 4** gives an example of the amplification rate plotted by the Genie Explorer software for a typical dilution series of template

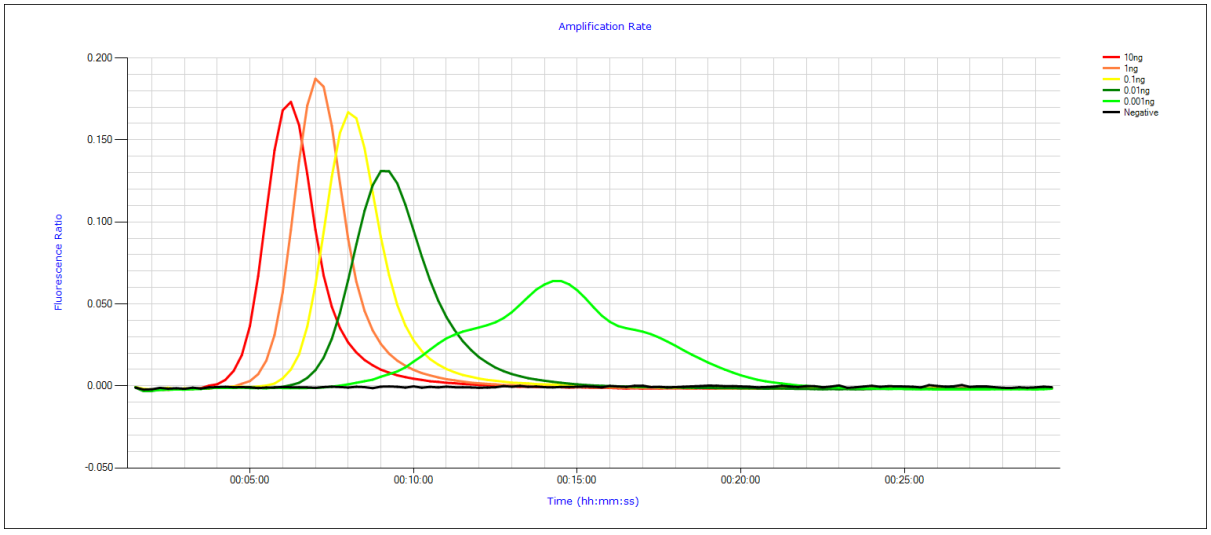

Figure 4 Amplification rate data for a typical 1:10 dilution series of template

- 10ng (red), 1ng (orange) and 0.1ng (yellow) have peaks that are tall, narrow and regular shaped indicative of an efficient amplification.
- 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 example, this concentration of template will not be reliably detected in replicates

Genie Explorer is available for free download off of our website

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

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Anneal Data

As confirmation of amplification of the correct target an anneal step is performed after the isothermal step. Each target will have its own signature anneal temperature which related to the GC content of the generated amplicon (**figure 5**). Positive amplification followed by an anneal temperature at the expected temperature is confirmation of a true positive in a sample.

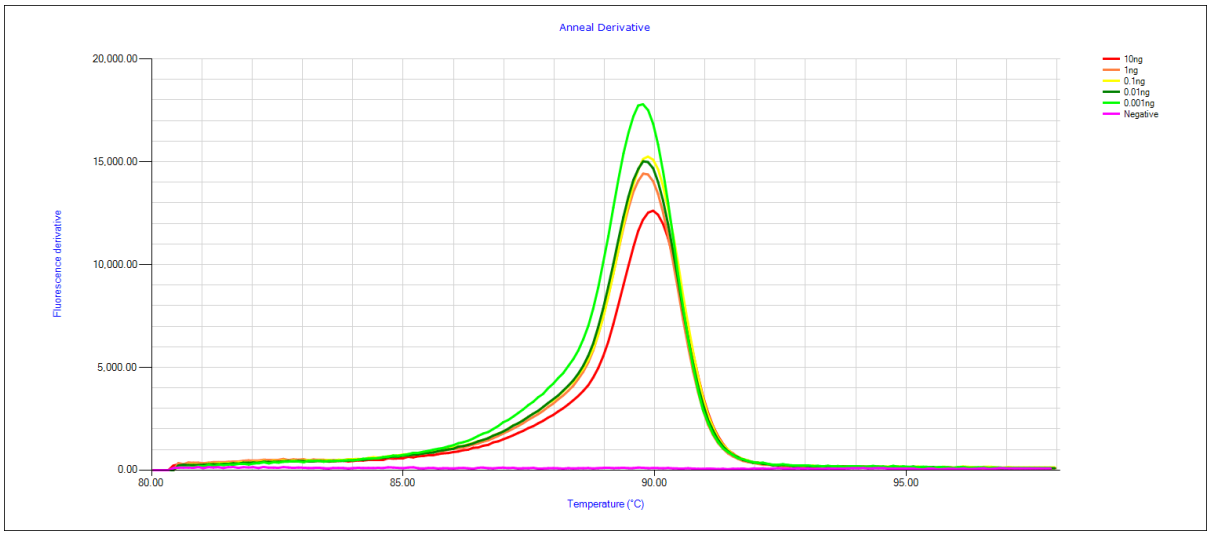

Figure 5 Anneal Derivative data for a typical dilution series
Even though amplification time is different for each template dilution their final anneal temperatures should always be the same providing the correct product has been amplified

Introduction to LAMP

Product Range

We sell a range of products to assist with running LAMP reactions:

- Genie® instruments as a platform to carry out LAMP reactions
    - These highly versatile instruments are designed to be used in the field or laboratory allowing you full flexibility in where you perform your assays
- A choice of different reagents allowing you to choose how you run LAMP which includes
    - A variety of diagnostic kits removing the need to design certain assays. Please see our website for the full range.
    - Mastermixes containing the necessary components required for a LAMP reaction allowing you to add add primer and template of your choice
    - Individual polymerases and buffers allowing you complete flexibility in running a LAMP reaction
        - **You will need to add an intercalating dye to your LAMP reaction mix in order for amplification to be detected by the Genie® instrument when using individual enzyme and buffers**
- Accessories to assist with running LAMP using OptiGene Reagents and a Genie® instrument.

Full details of the OptiGene product range can be found at http://www.optigene.co.uk/products/

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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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User Guides

For details of how to design an assay, set up a LAMP reaction using our mastermixes, template preparation and preparing primers please see our use guides

- **LAMP User Guide – Assay Design & Primers**
- **LAMP User Guide – Mastermixes and Assay Optimisation**