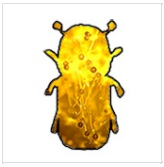

Nov 06, 2020

LAMP Master Mix + Reaction Protocol

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1 Works for me dx.doi.org/10.17504/protocols.io.bm27k8hn

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes the LAMP (Loop-mediated isothermal amplification) master mix and reaction.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

THIS DOCUMENT ACCOMPANIES THE FOLLOWING PUBLICATION

Villari, C., Mahaffee, W. F., Mitchell, T. K., Pedley, K. F., Pieck, M. L., & Hand, F. P. (2017). Early detection of airborne inoculum of Magnaporthe oryzae in turfgrass fields using a quantitative LAMP assay. Plant disease, 101(1), 170-177.

DOI

dx.doi.org/10.17504/protocols.io.bm27k8hn

DOCUMENT CITATION

Caterina Villari 2020. LAMP Master Mix + Reaction Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bm27k8hn

MANUSCRIPT CITATION please remember to cite the following publication along with this document

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CREATED

Oct 06, 2020

LAST MODIFIED

Nov 06, 2020

DOCUMENT INTEGER ID

42815

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LAMP Master Mix + Reaction Protocol

Materials:
Variable volume pipettors with filter tips
Small Rubbermaid container to hold ice and the PCR rack (or NUNC ice block)
Optical real time PCR plates, or flat topped 0.1 ml PCR tubes (plastic PCR tube opener)
2.0 ml tubes, clear and dark
0.5 ml dark tubes
Centrifuge
Vortex
DEPC Ultrapure Water
OptiGene Isothermal Master Mix (ISO-001nd)

Primers and probes:
See LAMP reaction template and primer info excel files for specific details on the primers.
The fluorescent probe is FAM with the black hole quencher strand.

Primer and Probe Dilution (work in the "clean - no DNA" hood):

- When new primers and probes are received, dissolve them to a concentration of 100µM in Ultrapure DNA- and RNA-free water. To do this, spin down the tubes for a few seconds at max speed to collect the contents and add the proper volume of ultrapure water. To determine the volume of water to add, multiply the number of nmoles of primer by 10 and add this volume (in µl) of water. This will yield a 0.1nmole/µl concentration, which equals 100pmoles/µl and 100µM=100pmoles/µl; hence, you now have a 100uM primer concentration.

NOTE: Allow the hydrating primer tubes to sit at room temperature for at least 20 minutes following the addition of the water to allow for complete hydration of the DNA.
FIP, BIP, Forward Loop, and Reverse Loop are all used at a concentration of 100µM.
F3 and B3 are used at a concentration of 10µM; make a 1:10 dilution using ultrapure water of F3 and B3 to yield 10µM working stocks of these primers.

- Mix and aliquot your fresh primers out into volumes that you will be using to make master mix batches. *LAMP primers are sensitive to freeze thaw.* This will minimize the freeze-thaw cycles that the primers are subjected to (maximum of 2 to 3 freeze-thaw cycles per primer tube). Follow table below for primer mix

- FL-F and Q-strands are stored frozen in dark 0.5 mL tubes as 100 µM aliquots that need to be diluted to 10 µM working concentrations (see table below). Do not re-freeze: store working concentration probes in 4ºC in a dark box and use within 1 month. Work with the hood light off when aliquoting the probes.

Master mix preparation (work in the "clean- no DNA" hood, light off):

1. LAMP is very sensitive and can be easily contaminated. It is best to prepare your master mix in a hood that has not been exposed to target DNA ("clean" hood). Avoiding making master mix after being exposed to target DNA. Wash hands well. Anything (including gloved hands) used in the "clean" hood should be cleaned with RNAse-Away (or 10% Bleach) upon entering. In the "clean" hood, use only dedicated equipment (i.e., pipettes, tips, rack, vortex, lab coat, etc.). Keep the dedicated equipment in a close box, stored in a place far from potential contamination.
2. Determine amount of master mix and probe mix needed, including treatments and controls. Always include a positive control to demonstrate that all the components were added and were functioning. Always include a negative control (no template or TE Buffer) to test that the components are not contaminated. Add several extra volumes (3-5) of MM to account for loss from tip transfer. Add additional extra volumes to the probe mix in order to have enough volume for the MM (Note: the amount per sample computed in the probe mix in less than the one actually used in the MM).
3. Put on gloves. Wipe down inside of hood and all equipment with RNase-away. Prepare the rubbermaid container and work on ice.
4. Take the "black" box containing fluorescent probes out of 4ºC. Probes are UV sensitive. make sure you work with the hood light off.
5. Mix the required amount of Fluorescent Probe and Quencher Strand in a new dark PCR tube (follow table below). Place mix in black box and return to 4ºC until needed for addition to master mix.

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6. Completely thaw following components: primer mix, water, and Optigene master mix
7. Label a dark 2 mL tube for the Master Mix. Add components in the order listed on the Lamp Template.
8. Remove fluorescent probe "black" box from the 4C, add the required amount (mixture) to the Master Mix, and discard the tube. Avoid exposing fluorescent probes to sun or artificial light as much as possible.
9. Vortex a minimum speed the tube in horizontal setting for approx. 3 minutes and spin down in centrifuge (13,000 RPM) for 30 seconds (until bubbles are gone).
10. Working on ice (or NUNC ice block), aliquot the master mix into 0.1 ml PCR tubes or optical plates. If possible, use the Eppendorf repeater stream for consistency. Cover strips with a lid if they have no caps.

Using a polystyrene box (to protect from light), carry the Master Mix strips in the "PCR assembly" hood.

The following is a sample template. See each reaction file for specifics.

PROBE MIX:	1X (µl)
FL - F Strand [10uM]	0.2
Q Strand [10uM]	0.4

LAMP MIX:	1X (µl)
Water	1.5
MM	15
Primer mix (0.7 x primer)	2.8
Probe mix	0.7
Template	5
Total	25

Primer mix stock x 57 reactions (1 plate)	
FIP [100uM]	40
BIP [100uM]	40
F3 [10uM]	40
B3 [10uM]	40

Probe stock x 200 reactions	
Q strand	
Q Strand [100uM]	8
Water (add only before use)	72
FAM strand	
FL - F Strand [100uM]	4
Water (add only before use)	36

Keep stocks frozen. Upon adding water, keep in dark at 4°C and use within one month.

LAMP assembly and reaction (work in the "PCR assembly" hood, light off):

12. Anything (including gloved hands) entering in the hood should be cleaned with RNAse-Away (or 10% Bleach). In the "PCR assembly" hood, use only dedicated equipment (i.e., pipettes, tips, rack, vortex, etc.). Keep the dedicated equipment in a close box, stored in a place far from potential contamination.
13. Keep strips not currently in use covered under the lid.
14. Transfer 5 µL of the extracted DNA into each tube. For field samples, two or three technical replicates are typically used
15. Always add the positive control DNA AFTER adding the field samples DNA and NEVER open the negative control PCR tubes.
16. Spin all the sample-containing PCR tubes down using a mini-centrifuge
17. Run the LAMP reaction in the Real-time machine

Real time settings	
Protocol	Start by loading previous LAMP run, name each specific reaction uniquely
Experiment type	Presence/absence
Fluorophore	FAM, Be sure to remove the threshold probe or any other from the file
Threshold	default
Baseline start	default
Baseline end	default
Temperature	See each specific reaction for testing temperatures. 65 works well for now
Reaction volume	25 uL
Data collection	Check that data is collected at least every “cycle”. Can do every 30 sec
Number of cycles	At least an hour to check for late amplification

NOTE: Do not open any LAMP reaction tube after the reaction is completed since this may contaminate the work area. If a gel run is needed, open the tube in a different room and consider the potential contamination of dresses, hair and other personal items. Aerosols of LAMP products are very stable and will remain as contaminants in the area for weeks.

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