Nov 20, 2020

PCR for amplification of bark beetles or fungal DNA

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1 Works for me dx.doi.org/10.17504/protocols.io.bnvbme2n

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to conduct PCR of bark beetles or fungal DNA.

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.

DOI

dx.doi.org/10.17504/protocols.io.bnvbme2n

DOCUMENT CITATION

Demian F Gomez, You Li, Caroline Storer 2020. PCR for amplification of bark beetles or fungal DNA. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnvbme2n

CREATED

Oct 23, 2020

LAST MODIFIED

Nov 20, 2020

DOCUMENT INTEGER ID

43651

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Materials:

DNA Extractions
Forward and reverse primers
Premix taq from freezer
Empty Eppendorf tube
Eppendorf tube of PCR water

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Positive control (Specimen already known to be high quality)
Negative control - PCR water
Two 96-well plates
Eppendorf vial rack

Preliminary Procedure:
• Primers need to be diluted from 10 micromolar to 1 micromolar to be stocked in the freezer for 1-2 months.
• Put your sample information in the database to make new sample numbers for the PCR.
• Label the strip tubes with the new sample numbers using an alcohol resistant marker and put them into a PCR plate. Add extra labels at the end for positive and negative controls.
• Look in the database to find primers in the freezer.
• Use the PCR excel chart to determine the amount of reagents you need to use in the master mix (the final volume of the master mix should equal 25 uL).
• UV the PCR hood for 15 minutes before starting to do the PCR.

Master Mix Procedure:
o Add x uL of Taq to the empty Eppendorf tube
o Add x uL of each primer to the tube, pipetting up and down to mix
o Add x uL of PCR water to the tube
o Vortex the master mix for 5 seconds

PCR Procedure:
• Vortex the extractions for 1 second, and then centrifuge to eliminate any bubbles.
• Make sure that the DNA extractions are in the same order that the empty PCR tubes are in.
• Pipette 23 or 24 uL of master mix into each PCR tube using the same tip.
• Pipette 1 or 2 uL of each DNA extraction into the PCR tubes. Use a different pipette tip each time, and mix the liquid up and down with the pipette. Try not to introduce bubbles. Close each cap when done pipetting so that none are skipped or none have double the correct amount added.
• Add 1 or 2 uL of PCR water only to the negative control tube.
• Close the tops of all the vials and make sure that all of them are closed. Sometimes they do not close all the way even if they look closed.
• Tap the plate to the table to bring drops in the tubes down.
• Centrifuge strip tubes for 3 seconds to make sure there are no bubbles.
• Put the tubes into the smaller holes of the thermocycler. Make sure the caps are completely closed. Add other tubes of the same kind to balance it.
• Run the program called for in the PCR protocol, and set a timer to be able to take the PCR products out and put them in the freezer.
• Put the extractions and any unused reagents back in the freezer in your box.
• The PCR products should be used as soon as possible.
• Clean everything with soapy water or mild bleach and UV the hood.

Ensure you enter all PCR runs into the PCR form on the isolations database. You can use the form linked here to properly plan your PCR master mix and database entries. Use the database entry rows at the top to fill in your samples for one run, including a positive control (known working template for these conditions), an extraction negative control (negative control from extraction as template), and a PCR negative control (no template). Then put in the number of reactions in the master mix form, this will give you the necessary amount of reagents to make the master mix for your reactions plus two additional.

It is recommended you input your PCR database entries before beginning mixing and your PCR run, this will reserve the spots so that the PCR IDs do not get taken while you are working.

Extract-N-Amp PCR ready premix

Typical master mix:
- PremixTAQ 12.5uL, found in freezer door. Fisher Catalog: RR003A
- Primer F (Conc. 10uM) 1uL
- Primer R (Conc. 10uM) 1uL
- PCR H2O, aliquots made by lab manager and stored in shared reagent box in door of small freezer. 9.5uL
- DMSO, aliquots made by lab manager and stored in shared reagent box in door of small freezer. 1uL

Please keep aliquots you open and use in your box after initial use.
Primer stock (100uM) locations can be found the Primer_locations table in the isolations database, these are in the ThermoFisher -

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80C freezer and will need to be diluted into a 10uM aliquot.
Multiply volumes by number of samples + 2 to make a small amount extra (form above does this). Use 25uL master mix and 1uL template per sample. PCR cycle will vary with primers used.
Common primers and cycle combinations used in our lab. Number in the cycle title is usually annealing temperature.

- ITS1F/ITS4 – PCR_53
- LR0R/LR5 – PCR_55_safe (Dreaden_LSU)
- Bt2a/Bt2b – PCR_53

Another master mix:

- XNA template 4uL (if less, add XNA solutions up to 4 uL!)
- primer F 0.5 uL (ideally 0.4uM)
- primer R 0.5 uL (ideally 0.4uM)
- XNA PCR ready premix 10 uL
- water 5 uL (or add up to 20 uL total)

Typical cycling conditions:

- 1) 94C, 3 min
- 2) 94C, 45s
- 3) xC, 45s
- 4) 72C, 1:30min
- 5) go to 2 34x

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