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Fungal DNA Extraction from Fungal Plate

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

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to do fungal DNA extraction from fungal plate.

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.

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Please record all extractions correctly in the database. See database protocols for isolations for more information.

General Ex-N-Amp protocol:

1. Prepare primary vials number 1 to 8, and final extraction vials with database numbers.
2. Prepare primary strip tubes with 20uL of Ex-N-Amp (Sigma Aldrich extraction solution). This can be found in aliquots in the

shared box in the door of the small freezer.

3. Work with a cool rack, Ex-N-Amp and BSA are sensitive to temperature.
4. Scrape approximately 10uL of hyphae from fungal colony using a sterile pipette tip (20uL works best and is not used for much else) or scalpel.
5. Add fungal material to extraction solution and vortex. Do not scrape agar.
6. Centrifuge 3 seconds.
7. Run Ex-N-A protocol on a thermocycler (96C for 30 minutes). No more than 12 strip tubes at a time.
8. Add 20uL of BSA.
9. Vortex for 10-20 seconds.
10. Spin down with high rpm for 10 seconds.
11. Pipet off the top 20uL: that's your extract. It is often safe to dilute this a bit if you will need more than 20 uL, but this is not standard procedure.

Note: If BSA is needed, we have a stock of 2% molecular grade BSA in our freezer. This should be made into aliquots before using for samples. This is not standard, and only used in cases where it increases DNA amplification success.

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