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🌐 General DNA Extraction with Sigma Extract-N-Amp

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Abstract

This protocol describes general DNA Extraction with Sigma Extract-N-Amp.

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.

Troubleshooting

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

Spin-down protocol (for beetles)

Keep BSA and the Ex-n-A solution cold, on ice or in a pre-frozen rack.

With BSA as a binding agent

- beetle body parts (legs work well from specimens killed with 95% EtOH)
- 40uL of Extraction Solution per sample (in yellow tray in the freezer)
- run protocol Ex-n-Amp on thermocycler (96C for <u>30 minutes</u>)
- add equal volume(to extract solution) of 3% BSA (will bind extra stuff)— (40uL of Extraction Solution:40uL of BSA)
- vortex thoroughly
- spin down, store the upper half (30uL) as your final sample
- use the 0.5-1.0uL of this supernatant for PCR

BSA from Fermentas, #00066587, 20mg/mL =~2%

General Ex-N-Amp protocol for fungi:

1. Prepare strip tubes with 40uL of Sigma Aldrich extraction solution. This can be found in aliquots in the shared box in the door of the small freezer.
2. 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.
3. Add fungal material to extraction solution and vortex.
4. Run Ex-N-A protocol on a thermocycler (96C for 30 minutes).
5. Spin down with high rpm.
6. Pipet off the top 25uL: that's your extract. It is often safe to dilute this a bit if you will need more than 25 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.

BSA from Fermentas, #00066587, 20mg/mL =~2%. We don't use the Dilution Solution from the manufacturer, as it seems to inhibit PCR.