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DNA extraction from pinned specimens

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1 Works for me dx.doi.org/10.17504/protocols.io.bnvame2e

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to extract DNA from pinned beetles.

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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Additional notes to DNeasy DNA extraction protocol.

Gentle surface washing is always a good idea, especially if the specimens are old.

If you want to keep the specimen in one piece I would poke a hole on each side of the mesothorax just above their first pair of procoxae. However if that is not needed, then separating the head/pronotum from the abdomen with forceps will work well.

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Long incubation times (12-20hrs) really seems to be the key. Do them overnight, that is set-up the incubation in the afternoon and then finish them the next morning.

As much light vortexing as you can reasonably get in helps too (Speed around 250 - just a bit to make the solution to move). We usually try to get a few vortexs in before leaving for the day and then a few the next day.

Per usual with non-destructive extractions, after incubation I rinse the beetle in the EtOH and then air dry it if you are going to re-pin them.

Lastly, always elute in a small volume, < 50ul, with old specimens will even perform a second elution with the first elute (2 elutions of 50 ul). Add same 50 ul again to the column 15 minutes to sit before centrifuge and then centrifuge again.

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