15-minute DNA extraction from beetle legs for PCR barcoding

Chase Mayers

1Cornell University

Protocols Bark Beetle Mycobiome

ABSTRACT

This method allows for quick extraction of DNA from beetle specimens for routine PCR amplification of barcoding genes such as COI (cytochrome oxidase subunit 1) for beetle ID. It is likely to also be effective for other high-copy genes such as other mitochondrial genes or ribosomal genes. It may not yield enough DNA to amplify large fragments or low-copy genes, and is certainly not appropriate for next-generation sequencing. However, for quick ID of beetle vouchers (for example, for beetles from which fungal cultures were isolated in a mycological study) it's hard to beat a ~15 minute DNA extraction.


GUIDELINES

The manufacturer's protocol recommends 200 µL of PrepMan per extraction, but we found good results with 50 µL volumes, which quadruples the number of extractions possible from a single bottle of PrepMan and results in relatively inexpensive DNA extractions.
**MATERIALS**

**PrepMan™ Ultra Sample Preparation Reagent**
Thermo Fisher Catalog #4318930

---

**PROTOCOL integer ID:**
42332

1. **Use a pipette to transfer** 50 µL of PrepMan Ultra Sample Preparation Reagent into a small microcentrifuge tube. Label the tube appropriately.

   *If possible, this should be done in a sterile environment and away from PCR equipment to avoid contaminating the PrepMan reagent with unwanted DNA.*

2. Using sterile forceps, remove one leg (or multiple) from the beetle and transfer to the PrepMan reagent.

   *Fresh, dried, and ethanol-pickled beetle specimens have all yielded usable results with varying success rates.*

3. Using a sterile transfer needle (or other tool), thoroughly crush the leg(s) against the inner wall of the microcentrifuge tube.

4. Incubate the microcentrifuge tube at 100 °C for 00:10:00.

5. **Optional:** Centrifuge the microcentrifuge tube briefly and pipette the supernatant to a new microcentrifuge tube. Label appropriately.
The liquid in the tube is now ready to be used as template for PCR. It can be stored at \(-20^\circ C\).

Typically we used