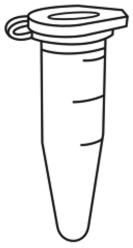
DNA Extraction Bench Protocol

This is an abbreviated protocol. Make sure to label all tubes and change pipette tips between samples.

Sample Preparation

- ❑ Remove the abdomen of the arthropod and cut off a small piece (roughly ~2 mm, or small enough to fit in the bottom of a microcentrifuge tube). If the specimen is smaller than a grain of rice, use the entire body.
- ❑ Place the specimen in a labeled 1.5 ml microcentrifuge tube.

Cell Lysis & DNA Precipitation

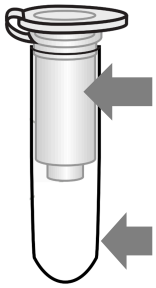


1.5 ml microcentrifuge tube

- ❑ Add 180 ul Buffer ATL and use a sterile pestle to grind the sample for 1 minute.
- ❑ Add 20 ul Proteinase K.
- ❑ Add 200 ul Buffer AL and immediately mix by vortexing for 10 seconds or pipetting up and down.
- ❑ Incubate for *at least* 15 minutes @ 56 °C. Longer incubation times (i.e., 2-3 hours) are most effective for cellular lysis.
- ❑ If arthropod debris is present, do a quick spin (~30 seconds) to pellet debris. Use a pipette to carefully transfer the supernatant to a new labeled 1.5 ml microcentrifuge tube. Discard the tube of cellular debris.
- ❑ Add 200 ul ethanol (96-100%) and mix by vortexing for 10 seconds or pipetting up and down.

Optional stopping point: store DNA in refrigerator (4 °C)

DNA Purification

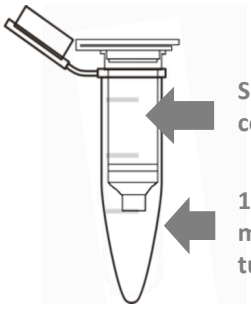


Spin column

2.0 ml collection tube

- ❑ Label a DNeasy spin column fitted with a 2.0 ml collection tube.
- ❑ Pipet the liquid containing ethanol-precipitated DNA into the DNeasy spin column.
- ❑ Centrifuge for 1 minute at ≥6,000 x g (8,000 rpm). Discard the flow through from the 2.0 ml collection tube.
- ❑ Add 500 ul of Buffer AW1 and centrifuge for 1 minute at ≥6,000 x g (8,000 rpm). Discard the flow through.
- ❑ Add 500 ul Buffer AW2 and centrifuge for 3 minutes at 20,000 x g (14,000 rpm).

DNA Elution



Spin column

1.5 ml microcentrifuge tube

- ❑ Transfer the spin columns to a labeled 1.5 ml microcentrifuge tube. Discard the 2.0 ml collection tube.
- ❑ Pipet 100 ul of Buffer AE directly onto the spin column membrane.
- ❑ Incubate at room temperature for 1 minute.
- ❑ Centrifuge at ≥6,000 g or 8,000 rpm for 1 minute.
- ❑ Discard the spin column and KEEP the labeled 1.5 ml tube.
- ❑ *Optional:* incubate the DNA for 1 hour @ 65 °C or overnight at room temperature.
- ❑ Store the eluted DNA frozen at -20 °C until PCR.