DNA Extraction Protocol

Class Materials

- ❑ Water bath, 95 °C
- ❑ Vortexer *(optional)*
- ❑ Mini-centrifuge (10,000 x *g*)
- ❑ Float rack
- ❑ Metal tongs
- ❑ Ice bucket or cooler

Materials per Group

- ❑ 2 Arthropod specimens
- ❑ +/- *Drosophila* controls
- ❑ Distilled water
- ❑ Gloves
- ❑ Kimwipes (or paper towels)
- ❑ Petri dish
- ❑ Dissecting tools (tweezers/scalpel)
- ❑ Cell lysis buffer
- ❑ DNA elution buffer
- ❑ Isopropanol, cold
- ❑ 70% Ethanol
- ❑ 1.5 ml microcentrifuge tubes
- ❑ Tube rack
- ❑ Sterile pestles
- ❑ Pipette (20-200)
- ❑ Pipette tips
- ❑ Waste cup for tips
- ❑ Waste cup for liquids
- ❑ Sharpie
- ❑ Paper towels
- ❑ Parafilm

Sample Preparation

1. Use tweezers to carefully transfer the arthropod to a Petri dish.
2. Rinse with water using either a squirt bottle or a 20-200 ul pipette.
3. Blot dry excess liquid. *(See Note 2.1)*
4. Remove the abdomen of the insect 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.
5. Place the specimen in a labeled 1.5 ml microcentrifuge tube.

Samples should be small enough to fit in the bottom of a tube.

Note 2.1: *DNA precipitates in the presence of alcohol. Therefore, remove as much preservative as possible. If the arthropod is large or has a thick/tough exoskeleton, dissect out the reproductive tissues.*

Cell Lysis

6. Add 100 ul Cell Lysis Buffer to the first tube.
7. Use a sterile pestle to grind the sample for 1 minute. *(See Note 2.2)*
8. Add an additional 100ul of Cell Lysis Buffer. Grind a few more times, then dip the pestle in the arthropod lysate to rinse and remove remaining debris. Change pestle and pipet tip; move on to the next sample and repeat Steps 6-8.
9. Heat samples @ 95° C for 5 minutes. *(See Notes 2.3 & 2.4)*
    - Wrap the caps with Parafilm to prevent lids from popping off while heating.
    - Place each of the tubes into a foam rack and place the rack directly in the beaker of hot water.
    - Use tongs or a long metal rod to remove the rack from the hot water.

Use a clean pestle for each arthropod.

Note 2.2: *This is the most critical step of the entire protocol. Thorough grinding is necessary in order to obtain high DNA yield. Use muscle power and grind each sample thoroughly.*

Note 2.3: *Do not over-boil. Extended incubation at 95° C can lead to denaturation and degradation of DNA.*

Keep water boiling during incubation.

Note 2.4: *While samples are incubating, label new tubes containing 150 ul cold isopropanol in preparation for Step 13.*

Cellular Debris Removal

10. Place tubes in ice bucket or refrigerator to cool to room temperature.
11. Vortex tubes for 20 seconds. If you do not have a vortexer, mix tubes by tilting back and forth ~ 50 times. This can be done with the microcentrifuge tubes still in the foam carrier (or in a tube rack) by placing one hand on the top and one on the bottom and gently inverting.
12. Centrifuge for 2 minutes to pellet debris. *(See Note 2.5)*

Centrifuge at 10,000 x g or use the default high speed.

Note 2.5: *Always keep the centrifuge balanced! Space samples evenly across the rotor. If unable to properly balance the rotor, fill a labeled tube with water and use it as a balancer.*

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DNA Precipitation & Purification

13. Use a pipet to carefully transfer 150 ul of supernatant to a new tube containing an equal amount (150 ul) of cold isopropanol. *(See Note 2.6)*

Note 2.6: *If you disturb the pellet, repeat step 12. If you are unable to collect 150 ul, add 50-100ul additional lysis buffer to the tube and repeat steps 11-12.*

This is an optional STOPPING POINT. Store DNA in freezer (-20 °C) for an extended period.

14. Gently mix samples by inverting approximately 50 times.
15. Centrifuge for 5 minutes to pellet genomic DNA. *(See Note 2.7)*
16. Carefully pour the supernatants into a waste cup and invert tubes on a paper towel to air dry for 1 minute. Be careful not to reinvert the tubes prior to placing them on the paper towel as this could cause ethanol to flow back against, and possibly dislodge, the pellets.
17. Add 100 ul of 70% ethanol to each pellet.
18. Invert tubes 10 times to wash the DNA.
19. Centrifuge for 1 minute.
20. Carefully pour off the supernatants and invert tubes on a clean paper towel to air dry for about 10 minutes. *(See Note 2.8)*

Note 2.7: *To easily locate the pellet, orient the hinge of the tube to point away from the middle of the centrifuge. The DNA pellet may be seen near the bottom of the tube under the hinge.*

Note 2.8: *Pellet may be loose so watch carefully and pour slowly. If the pellet begins to dislodge, add more ethanol and re-spin.*

Allow residual ethanol to drain and/or evaporate from each tube.

DNA Elution

21. Add 50ul of TE Buffer to each tube.

Recommended: Re-hydrate DNA by incubating samples at 65 °C for up to 1 hour or overnight at room temperature.

Storage

22. Store at 4 °C for a few weeks or -20 °C indefinitely.

Helpful Tips

- The DNA pellet may not be visible by step 16—that is OK. Continue with the procedure and the pellet will become more visible by step 20. It should appear as a small white dot under the hinge of the microcentrifuge tube. You may have to hold the tube up to the light in order to see the pellet.
- To ensure optimal results:
    1. Grind, grind, grind!
    2. Avoid contamination by changing tips between each reagent, sample.
    3. Keep the rotor balanced.