DNA Extraction from Fresh/Frozen Butterfly Tissue using the Qiagen DNeasy Blood and Tissue Kit

This protocol is based on the manufacturer's handbook for the DNeasy Blood and Tissue Kit (July 2006) with minor modifications and written in reference to our butterfly study system. Methods are suitable for freshly collected, frozen, or dried specimens. Protocol maintained by Zachary Nolen.

Procedure

Day 1 - Tissue Lysis

1. Using a **decontaminated scalpel or microscissors** and set of tweezers, remove wings, abdomen, legs, and antennae. Store these in a labelled tube or envelope. Transfer **thorax** and **head** to **Eppendorf LoBind tube**. This step can be done in advance if the tissue is then stored at -80°C.
2. Add **180 µl Buffer ATL** to tube with tissue. **Crush** tissue using a **decontaminated pestle** by grinding against the sides of the tube. Break tissue down as much as possible.
3. Add **20 µl Proteinase K**, **mix** by vortexing and incubate at **56°C** in agitated water bath **overnight**.

Day 2 - DNA Extraction

1. Add **4 µl RNase A** to sample, **mix** by vortexing, and incubate for **2 min** at **room temperature**.
2. (***Optional***) If sample has particulate matter remaining that may clog the spin column, centrifuge at **5000 x g** for **5 min** and **transfer supernatant** to a **new LoBind tube** and discard the pellet.
3. Mix **200 µl Buffer AL** with **200 µl 96-100% EtOH** in a **separate tube**. Prepare enough to be used with all samples being processed.
4. Add **400 µl** of the **AL/EtOH mixture** to each sample tube and **mix** thoroughly by vortexing.
5. Pipette the mixture from Step 4 into a **DNeasy Mini spin column** in a 2 ml collection tube. Centrifuge at **6000 x g** for **1 min**. Discard flow through and collection tube.
6. Place the spin column in a new 2 ml collection tube, add **500 µl Buffer AW1**, centrifuge at **6000 x g** for **1 min**. Discard flow through and collection tube.
7. Place the spin column in a new 2 ml collection tube, add **500 µl Buffer AW2**, centrifuge at **20 000 x g** for **3 min** to dry the DNeasy membrane. Discard flow-through and collection tube.
    - **Note**: Ensure spin column does not touch flow through on removal from the collection tube. If it does, place in a new spin column and repeat centrifugation to dry.
8. Place the spin column in a clean and **labeled LoBind tube**. Add **100 µl Buffer AE** directly to the column membrane. Incubate at **room temperature** for **1 min**, then centrifuge at **6000 x g** for **1 min** to elute.
9. **Transfer elution** from LoBind tube **back into the spin column**, and **place the column back into the LoBind tube**. Incubate at **room temperature** for **1 min**, then centrifuge at **6000 x g** for **1 min** to elute again. Label each tube with the elution number, these are your extractions.

Additional notes: According to Qiagen, higher elution volumes (200 µl in handbook) will increase DNA yield, but reduce overall concentration. We use a lower volume and reuse the elution to increase concentration.

Required Materials and Reagents

In Kit:

- DNeasy Mini Spin Columns (1 per sample)
- Buffer ATL
- Proteinase K Solution (20 mg/ml)
- Buffer AL
- Buffer AW1
- Buffer AW2
- Buffer AE

User Supplied

- Microtube pestle (1 per sample)
- Eppendorf LoBind Tubes (1.5 or 2.0 ml)
- RNase A (100 mg/ml)
- Ethanol (96 - 100%)

Last revised 11 December 2020