

# Extraction Protocol for Body Feathers and Patagial Biopsies<sup>1</sup>

- 1) Prepare 100 mg/ml DTT solution.

Measure 0.100 g of DTT and add to 1 ml water in 1.5 ml microtube. Shake or vortex to dissolve.

Discard DTT solution after use as it does not do well in storage.

- 2) Place up to 25 mg tissue in 1.5 ml microtube, and add 180 ul **Buffer ATL**.
- 3) Add 20 ul **Proteinase K** and 25 ul **DTT** solution. Mix by vortexing for ~15 sec. Incubate @ 55 °C in shaking water bath (or equivalent) until tissue is completely lysed.

Lysis is usually complete in 2-3 h, but may be carried out overnight.

- 4) Remove samples from water bath. Vortex for 15 sec. Add 200 ul **Buffer AL** to sample and vortex for 30 sec. Incubate at 70 °C for 10 min.
- 5) Add 200 ul **ETOH** (96-100%) to sample, and mix by vortexing. White precipitate may form with addition of ETOH. Be sure to add all precipitate to spin-column.
- 6) Place **Buffer AE** into 55 degree water bath (heating the elution buffer will increase DNA yields from the membrane).
- 7) Pipet mixture from step 5 into DNeasy spin column sitting in 2 ml collection tube (provided). Centrifuge at 8000 rpm for 1 min. Discard flow-through and collection tube.
- 8) Place DNeasy mini-column in a new 2 ml collection tube (provided), add 500 ul Buffer AW1, and centrifuge at 8000 rpm for 1 min. Discard flow through and collection tube.
- 9) Place DNeasy mini-column in a 2 ml collection tube (provided), add 500 ul **Buffer AW2**, and centrifuge at 10,000 rpm for 3 min

Be careful that spin-column does not contact the flow-through in this step (leading to carryover of ETOH). If this happens, discard flow through and centrifuge again at 8000 rpm for 1 min.

- 10) While these sample are spinning, remove the tops from a number of microtubes equivalent to the number of samples you are processing.

---

<sup>1</sup> Modified from Qiagen Inc. DNeasy Kit instructions

11) Go get **Buffer AE** from the water bath.

12) Place DNeasy column in clean 1.5 ml microtube with top removed. Pipet 60-80 ul **Buffer AE** directly onto membrane (for adult feathers or patagial tissue). Incubate at room temp. for 1 min. Centrifuge at 10,000 rpm for 1 min to elute.

Close tube, label, and store. If needed, carry out second elution using clean 1.5 ml microtube and 60 ul Buffer AE as above. Close tube, label and store.