**Feather Extraction Protocol (Single Tube)**

*2021 Version 2: Modified 10/26/2021*

**Consumables**

**Product Supplier Cat. #**

DNeasy Blood & Tissue Kit Qiagen 69506

Low retention 1.5mL tubes Any

DTT (dithiothreitol) Fisher FERR0861

Ultrapure DNAse/RNAse-free Water Thermo Fisher 10977015

**Labware**

Centrifuge capable of 8000rpm

Incubator and shake plate (or similar) capable of 56°C

Pipettes and filter tips

**Important Notes**

1. DTT degrades quickly in aqueous solution. Remove only what is needed from freezer.
2. This protocol follows Qiagen’s DNEasy Blood and Tissue Kit extraction protocol for Animal Tissue in a Spin-Column with a few modifications.

**Feather Cutting**

1. Wipe down lab bench with 10% Bleach and 70% Ethanol.
2. Label 1.5mL tubes with number of samples to extract and make note of which is which in lab notebook. For each razor blade, we will use the blade three times, using different parts each time:

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1. Pull out feathers from envelope. Use the opening of the envelope to hold down the feathery tip and your finger to hold down the calamus end. Cut the calamus tip off with the razor blade. For down feathers, cut 5 tips. Only 1 tip is sufficient for a tail feather. (Holding down both ends will prevent feather tip from flying off into the unknown).

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1. Place the rest of the feather back into envelope.
2. Place the cut calamus tip into pre-labeled tubes.
3. Write “# tips used/ total # tips in envelope used” on the feather envelope (i.e. 1 of 2 tips used).

**DNA Extraction**

**Day 1:**

1. Turn on incubator and make sure setting is at 56°C.
2. Remove 1M DTT from freezer and allow to thaw.
3. Check buffer ATL for white-yellowish precipitate. If precipitate exists, place buffer in incubator until precipitate has fully dissolved.
4. Add 180μL Buffer ATL to tubes.
5. Add 20μL Proteinase K.
6. Add 10 μL of 1M DTT.
7. Mix thoroughly by vortexing. Spin down tubes and check to make sure the tip is fully submerged in solution. Incubate at 56°C on a rocking platform overnight (at least 12 hours).

**Day 2:**

1. Remove tubes from incubator and vortex. Spin down and make sure that tips are fully dissolved. If not fully dissolved, add an additional 10μL of DTT and incubate for another hour until fully dissolved.
2. Check Buffer AL for precipitate. If precipitate exists, place inside incubator until dissolved.
3. Pre-mix 200μL buffer AL and 200μL Ethanol (96-100%) per sample in a falcon tube. Add 10% for pipette error.
4. Aliquot 400μL of the Buffer AL/Ethanol mix to each tube. Vortex thoroughly. A white precipitate may form after the addition of Buffer AL and ethanol. This does not interfere with DNA extraction. Vortex the mix.

*\*\*\*Essential to vortex mix immediately after the addition of Buffer AL and ethanol\*\*\**

1. Pipette the mix from step 4 (including any precipitate) into a DNeasy Mini spin column placed in a 2mL collection tube (provided in Qiagen kit).
2. Centrifuge for 1 min at ≥ 6000 x g (8000rpm).
3. Pipette the flow-through back onto the filter and centrifuge again for 1 min at ≥ 6000 x g (8000rpm).
4. Carefully transfer column to new collection tube and discard flow-through and collection tube.
   1. If at any point during this extraction, the membrane of the column gets wet from flow-through, re-centrifuge for 1 minute.
   2. This centrifugation step ensures that no ethanol will be carried over during the following elution. Ethanol may interfere with subsequent reactions.
5. Add 500μL Buffer AW1 to each tube.
6. Centrifuge for 1 minute at ≥ 6000 x g (8000rpm).
7. Place column into new collection tube and discard flow-through and collection tube.
8. Add 500μL Buffer AW2.
9. Centrifuge for 3 minutes at 20,000 x g (14,000rpm).
10. Discard flow-through, replace in same collection tube, and repeat centrifugation.
11. Discard flow-through and collection tube.
12. Place spin column in a pre-labeled 1.5mL or 2.0mL microcentrifuge tube for storage. Tube label should include BGP ID#, species code, date of extraction and location.

*NOTE: Depending on the total elution volume you use, you may use a new collection tube and transfer the final volume to a 1.5mL tube once the extraction is complete.*

1. Pipette 200μL of Buffer AE directly onto membrane.
2. Incubate at room temperature for 5 minutes. This will allow elution buffer to thoroughly saturate membrane.
3. Centrifuge for 2 min at ≥ 6000 x g (≥8000rpm) to elute.
4. Pipette another 200μL Buffer AE directly onto membrane.
5. Incubate at room temperature for 5 minutes.
6. Centrifuge for 2 min at ≥ 6000 x g (≥8000rpm).
7. Carefully remove tubes and column from centrifuge and check tube to make sure there is about 400μL of elution in tube and no remaining liquid above filter. If there is still liquid above the filter, centrifuge again for 2 min at ≥ 6000 x g (≥8000rpm).

*NOTE: To ensure maximum DNA yield, can repeat elution with an additional 200μL Buffer AE for a total volume of 600μL.*

1. If a collection tube was used for elution rather than the final extraction tube, transfer total volume from collection tube into pre-labeled 1.5mL microcentrifuge tube for storage. Tube label should include BGP ID#, species code, date of extraction and location.
2. Discard column and collection tube.
3. Place extracts in appropriate extraction box/well in freezer.