Feather Extraction Protocol: Qiagen Blood & Tissue Extraction Kit

Follow Animal Tissue Spin-Column Protocol with the following modifications:

Day 1:

1. Wipe down lab bench with 10% Bleach and 70% Ethanol.
2. Turn on incubator/oven and make sure setting is at 56°C.
3. Check buffer ATL for white-yellowish precipitate. If precipitate exists, place buffer into incubator while incubator is warming up until precipitate has fully dissolved.
4. Remove a single feather from feather envelope. (For most cases a single feather tip is adequate)
5. On a weigh paper, petri dish, or weigh boat, hold down both distal and proximal end of feather and cut off tip of feather with razor blade. (Holding down both ends will prevent feather tip from flying off into the unknown).

cut

hold

1. Place feather back into envelope.
2. Using razor blade, split the feather tip right down the middle vertically.

cut

1. Place both halves into pre-labeled tubes.
2. Add 180μL Buffer ATL to tube with feather tip.
3. Add 20μL Proteinase K. Mix thoroughly by vortexing. Incubate at 56°C on a rocking platform overnight.
4. Check to make sure both halves of the feather tip are submerged in solution.
5. Write “1 tip extracted” or “1 tip left” and the date on the feather envelope.
   1. Let database manager know which samples you used so they can be updated in the database.

Day 2:

1. Check Buffer AL for precipitate. If precipitate exists, place inside incubator until dissolved.
2. Remove tubes from incubator and vortex.
3. Pre-mix 200μL buffer AL and 200μL ethanol (96-100%) per tube in a falcon tube.
4. Aliquot 400μL of the Buffer AL/Ethanol mix to each tube. Vortex thoroughly.
   1. A white precipitate may form after the addition of Buffer AL and ethanol; this does not interfere with DNA extraction. Just vortex the mix

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

1. Pipet the mix from step 2 (including any precipitate) into a DNeasy Mini spin column placed in a 2ml collection tube (provided).
2. Centrifuge for 1 min at ≥ 6000 x g (8000rpm)
3. Carefully remove column 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.
4. Place column into new collection tube and add 500μL Buffer AW1 to each tube.
5. Centrifuge for 1 minute at ≥ 6000 x g (8000rpm).
6. Discard flow-through and collection tube.
7. Place column into new 2mL collection tube and add 500μL Buffer AW2.
8. Centrifuge for 3 minutes at 20,000 x g (14,000rpm).
9. Discard flow-through and collection tube.
10. Place spin column in a clean, pre-labeled 1.7mL or 2mL micorcentrifuge tube.
11. Pipet 50μL Buffer AE directly onto membrane.
12. Incubate at room temperature for 5 minutes. This will allow elution buffer to thoroughly saturate membrane.
13. Centrifuge for 1 min at ≥ 6000 x g (8000rpm) to elute. This is the 1st elution.
14. Carefully remove tubes and column from centrifuge and check tube to make sure there is liquid.
    1. If there is no liquid in tube, re-centrifuge.
    2. If there is still no liquid, pipet another 50μL Buffer AE directly onto membrane, incubate at room temperature for 5 minutes, and centrifuge.
15. Place column in another pre-labeled micro-centrifuge tube. (This will be your second elution)
16. Pipet 100μL Buffer AE directly onto membrane.
17. Incubate at room temperature for 5 minutes.
18. Centrifuge for 1 min at ≥ 6000 x g (8000rpm) to elute. This is the 2nd elution.
19. Carefully remove tubes and column from centrifuge and check tube to make sure there is liquid.
20. Discard column.
21. Place extracts in freezer. Make sure to label box with proper information.
    1. Example: Sample type, your name, contact info, etc.