Phenol-chloroform extraction of DNA from avian museum specimens

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Materials

- 1X STE Buffer (Sodium Chloride-Tris-EDTA)
- Buffer ATL (Qiagen)
- Proteinase K
- 1M DTT (store in aliquots at -20˚C, avoid freeze/thaw cycles)
- Ultrapure Phenol:Chloroform:Isoamyl Alcohol 24:25:1 (Thermo Fisher Scientific)
- Phase Lock Gel Light (Quanta Bio)
- AMPure XP beads or substitute (Serapure)
- Rare-earth magnet stand (Promega Magnesphere or Thermo Fisher Scientific DynaMag)
- Fume hood
- 100% and 70% EtOH
- 3M NaOAc (Sodium Acetate)
- 10mM Tris-HCl, pH 7.5
- 10mM Tris-HCl pH 8.0

Recommended Materials

- Thermomixer C (Eppendorf)
- HulaMixer Sample Mixer (Thermo Fisher Scientific)
- Qubit Fluorometer (Thermo Fisher Scientific)
- CryoGrinder System (OPS Diagnostics)

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Tissue collection

Toe pad removal

1. Place bird specimen on a piece of foil with the ends curled up.
2. Choose the most easily accessible toe with the largest toe pad.
3. Stabilize the legs of the specimen and use a new sterile scalpel blade and sterilized forceps, cut toe pad as close to the bone as possible without breaking it.
4. Place toe pad in clean 1.7 ml tube.
5. Change foil and scalpel blade and flame sterilize forceps between each sample.
6. Safe stopping point: you may stop at this step and store toe pads dry in the freezer.

Skin punch removal

1. Gently sweep aside the breast feathers to expose apterium.
2. Use a new sterile scalpel blade and sterilized forceps to cut a piece of featherless skin approximately 5 x 5 mm.
3. Place skin punch in clean 1.7 ml tube.
4. Gently move feathers back into place to cover the location of skin punch removal.
5. Change scalpel blade and flame sterilize forceps between each sample.
6. Safe stopping point: you may stop at this step and store skin punches dry in the freezer.

Bone removal and grinding

1. Stabilize the legs of the specimen and use a new sterile scalpel blade to cut the skin closest to the feather line, around the distal end of the tibiotarsus.
2. Gently remove the leg from the body of the specimen.
3. Use sterilized scissors to cut a small piece off the bone.
4. Place bone in a clean 1.7 ml tube.
5. Change scalpel blade and flame sterilize scissors between each sample.
6. Once bone sampling is no longer necessary, take steps to restore specimen to original condition by either gluing or sewing the leg back to the skin.
1. Safe stopping point: you may stop at this step and store bone dry in the freezer.
2. Fill CryoCooler with liquid nitrogen.
3. Place sterile mortars into the CryoCooler to chill for at least 15 minutes.
4. Place bone into a chilled mortar and allow to chill for a few minutes in the CryoCooler.
5. Use a sterilized pestle on the CryoGrinder to grind bone into a fine powder.
6. Scrape bone powder into clean 1.7 ml tube.
7. Use a different sterilized mortar and pestle for each sample.
8. Safe stopping point: you may stop at this step and store bone powder dry in the freezer.

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Sample wash and digestion

***NOTE:** Perform all steps with a negative control.

Sample wash

1. Preheat Buffer ATL on heat block at 56˚C to dissolve precipitate.
2. Add 500 µl 100% ethanol to each tube.
3. Incubate samples on a thermomixer at room temperature at 1000 rpm for 5 minutes.
4. Remove ethanol and discard.
5. Add 500 µl of 1X STE Buffer to each tube.
6. Incubate on a thermomixer at room temperature at 1000 rpm for 5 minutes.

Sample digestion, day 1

1. To new 1.7 ml tubes add:
    a. 180 µl Buffer ATL
    b. 20 µl Proteinase K
2. Transfer tissue sample to new tube containing Buffer ATL and ProK with flame sterilized forceps.
3. Vortex well and place in thermomixer at 56˚C at 1000 rpm.
4. Incubate samples on a thermomixer at 56˚C at 1000 rpm for ~2 hours.
5. Remove from thermomixer and using a separate mini pestle for each sample, mash tissue in tubes (REPEAT this step every few hours if necessary).
6. Vortex and return to thermomixer and incubate at 56˚C at 1000 rpm overnight or until fully digested.

Sample digestion, day 2

1. Remove from thermomixer and vortex
    a. If sample is completely digested move to phenol-chloroform addition.
    b. If not, move to step 2.
2. Add 20 µl ProK.
3. Vortex and return to thermomixer and incubate at 56˚C at 1000 rpm for at least 1 hour.
4. If the sample has not completely digested, add 25 µl 1M DTT (in a fume hood).
5. Vortex and return to thermomixer and incubate at 56˚C at 1000 rpm for about 1 hour.
6. Continue incubation mashing with mini pestle every few hours if necessary until sample is completely digested.

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Phenol-chloroform addition and precipitation

Perform all phenol-chloroform addition and precipitation steps in a fume hood.

***NOTE:** *If you added DTT or more ProK, you will need to change some of the reagent volumes in the following steps.*

1. Spin down Phase Lock Gel Light tubes at 12,000rpm for 30 seconds.
2. Vortex samples after removing from incubation.
3. Transfer each sample to a pre-spun Phase Lock Gel tube.
4. Add 200 µl Phenol:Chloroform:Isoamyl Alcohol (24:25:1) ****or volume equal to sample volume after all additions.*
5. Mix sample thoroughly by gently rotating tube for at least 10 minutes inside the fume hood (can be placed on a HulaMixer).
6. After the 10 minutes of mixing, open the lid to each tube to vent gas that has built up in each tube.
7. Centrifuge at 14,000rpm for 15 minutes.
8. Label tubes for final storage (include initial tube number).
9. Sweep aside the top layer of gel with a sterile pipette tip and KEEP supernatant (top solution) by pouring into final storage tubes.
10. Add 20 µl 3M NaOAc to each tube ****or 0.1x the supernatant volume.*
11. Add 500 µl cold 100% ethanol ****or 2x the volume.*
12. Mix well and store in -20˚C overnight or at -80˚C for 2 hours.

Precipitation

1. Centrifuge at 14,000rpm for 10 minutes.
2. Remove supernatant and keep the DNA pellet.
3. Add 500 µl cold 70% ethanol to the samples without disturbing the DNA pellet.
4. Centrifuge at 14,000rpm for 10 minutes.
5. Remove 70% ethanol without disturbing the pellet.
6. Dry the DNA pellet in the fume hood (2-3 hours).
7. Re-suspend pellet in 100 µl 10mM Tris-HCl pH7.5 and store in the freezer.

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Post extraction high ratio bead clean

Prior to any other processes, clean your samples with Serapure beads to get rid of any residual reagents/PCR inhibitors.

1. Add 400 µl (4x) Serapure solution to each sample.
2. Pipette up and down or vortex gently to mix.
3. Incubate for 5 minutes at room temperature in the dark.
4. Move tubes to magnet stand and let sit for 3 minutes or until solution is clear.
5. Using a separate filter tip for each sample, aspirate liquid from each tube and discard. Be careful not to discard any beads.
6. Add 800 µl fresh 80% EtOH in the stand without disturbing the beads.
7. Incubate 30 seconds.
8. Using a separate filter tip for each sample, aspirate EtOH from tubes and discard.
9. Add 800 µl fresh 80% EtOH in the stand without disturbing the beads.
10. Incubate 30 seconds.
11. Using a separate filter tip for each sample, aspirate EtOH from tubes and discard.
12. Allow tubes containing Serapure beads to dry for approximately 5 minutes or until there is no longer a smell of EtOH. Once dry, the beads should appear more matte than shiny, but avoid letting them over dry and appear cracked.
13. Add 52 µl 10mM Tris-HCl pH 8.0 to each tube.
14. Pipette up and down or vortex gently to mix.
15. Incubate for 5 minutes at room temperature in the dark.
16. Move tubes to magnet stand and let sit for 3 minutes or until solution is clear.
17. Using a separate filter tip for each sample, aspirate liquid from each tube and transfer to **a new clean tube**.
18. Quantify 2 µl using a Qubit Fluorometer.