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Phenol-chloroform extraction for museum hides using Phase lock gel

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We use this protocol and it's working

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Abstract

This protocol outlines a method for extracting high-quality DNA from museum-preserved hides using a phenol/chloroform extraction process and phase lock gel tubes to optimize yield and purity. The procedure involves a multi-step workflow beginning with ethanol soaking to remove salts, followed by mechanical homogenization and enzymatic digestion of the sample to release nucleic acids. DNA is then purified through successive rounds of phenol/chloroform extraction using phase lock gel to efficiently separate phases and minimize contamination. Precipitated DNA is pelleted, rinsed with ethanol, and air-dried before being resuspended in low TE buffer for downstream applications. This method is tailored for challenging archival specimens, ensuring maximum DNA recovery while minimizing degradation and contamination.

Materials

General Laboratory Supplies

Labeled 2.0 mL tubes (screw-cap and snap-cap)
Kimwipes or similar lab wipes
Incubator (capable of maintaining 42°C and 55°C)
BeadBug™ Microtube Homogenizer (Benchmark Scientific, Inc.)
Next Advance 3.5 mm UFO Stainless Beads (Next Advance, Inc.)
Phase lock gel tubes (heavy gel, yellow) (2 per sample)
Centrifuge (capable of maximum speed for 2.0 mL tubes)

Reagents and Solutions95% ethanol

Fresh 95% and 70% ethanol
3M sodium acetate (NaOAc)
Isopropanol (cold if possible)
PCR-grade water
Lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, 25 mM sucrose, 100 mM NaCl, 1.0% SDS, 40 mM DTT)
Proteinase K (20 mg/mL)
Phenol:chloroform mixture (1:1)Alternatively: phenol:chloroform:isoamyl alcohol (25:24:1)
Low TE buffer (pH 8.0)

Optional Supplies

Ice (for isopropanol incubation at 0°C)
Timer (to ensure precise incubation and mixing times)
Rocking platform (for mixing samples during phenol:chloroform steps)

Day 1 - Ethanol Soak

1 Cut small pieces of the specimen (1 to 3 mm). Place the sample pieces in labeled 2.0 mL tubes.

2 Add 600 µL of 95% ethanol to each tube.
Note: Room-temperature ethanol removes more salts than ice-cold ethanol.

3 Ensure the sample is fully immersed in ethanol by gently flicking the tube.
- Allow the sample to soak for 2–3 hours. Pipette off the ethanol, then add another 600 µL of fresh ethanol.
- Repeat the process 2–3 times during the day.
- At the end of the day, add fresh ethanol and leave the samples soaking overnight at room temperature.

Day 2 - Digestion

4 Remove the sample from the ethanol soak and place it on a clean Kimwipe to pat off excess ethanol.
- Alternatively, for very small samples, place them in an incubator at 42°C for 30–60 minutes to dry.

5 Transfer the sample into a clean 2.0 mL screw-cap tube. Add one **Next Advance 3.5 mm UFO Stainless Bead** per tube.

6 Prepare the **BeadBug™ Microtube Homogenizer** (Benchmark Scientific, Inc.) as follows:
- Place it on a clean, flat, stable surface.
- Insert the 2.0 mL screw-cap tubes into the tube holder. Ensure tubes are fully inserted.
- Select the desired speed and time (maximum: 4000 rpm, 3 min).
- Close the lid and press "Start/Stop" to begin the cycle.

7 Add ~500 µL of lysis buffer (50 mM Tris pH 8.0, 50 mM EDTA, 25 mM sucrose, 100 mM NaCl, 1.0% SDS, 40 mM DTT).

8 Add 25 µL of 20 mg/mL Proteinase K to the tube.

9 Incubate the sample at 55°C for 24–48 hours with agitation or rotation, if possible.

Day 3 - Phenol/Chloroform Extraction

10 Spin the phase lock gel tubes (2 per sample) at maximum speed for 5 minutes.

11 Pipette ~500 µL of lysate into a 2.0 mL phase lock "heavy" gel tube containing 500 µL of a phenol:chloroform (1:1) mixture.
- Add ~200 µL of PCR-grade water (do not exceed the tube's capacity).
- Mix by rocking gently for 5 minutes until the mixture appears milky.

12 Centrifuge at maximum speed for 5 minutes to separate the phases.

13 Transfer the aqueous phase into a new phase lock "heavy" gel tube containing 500 µL of the phenol:chloroform (1:1) mixture.
- Add ~200 µL of PCR-grade water.
- Mix by rocking gently for 5 minutes until the mixture appears milky.

14 Centrifuge at maximum speed for 5 minutes to separate the phases.

15 Transfer the aqueous phase into a new 1.75 mL snap-cap tube (conical bottom).

16 Add 1/10 volume of 3M NaOAc to the aqueous phase and mix gently by rocking.
- Add 0.6 volume of cold isopropanol. Invert the tube several times to precipitate DNA.
- Add additional isopropanol if necessary.

17 Incubate the tube on ice overnight at 4°C (optional, but increases yield).

DAY 4 - Ethanol Rinse

18 Pellet the DNA by centrifugation at maximum speed for 30 minutes.

19 Carefully decant the liquid and rinse the pellet with 1 mL of 70% ethanol (cold if possible).
- Mix gently by rocking.

20 Pellet the DNA by centrifugation at maximum speed for 5 minutes.

21 Carefully decant the ethanol and rinse the pellet with 1 mL of 95% ethanol (cold if possible).
- Mix gently by rocking.

22 Pellet the DNA by centrifugation at maximum speed for 5 minutes.
- Carefully decant the ethanol and dry the DNA pellet in a vacuum chamber (no heat) overnight.

DAY 5 - DNA Resuspension

23 Preheat Low TE buffer (pH 8.0) to 55°C before resuspending the DNA.

24 Resuspend the DNA pellet as follows:
- Use 50 µL for small pellets.
- Use 100 µL for medium pellets.
- Use 200 µL for large pellets.

Notes:

25 **Phenol:Chloroform Mixture Preparation:**
Mix equal parts of saturated phenol and chloroform:isoamyl alcohol (24:1). Alternatively, use phenol:chloroform:isoamyl alcohol (25:24:1).