



Forensic Biology Section

Extraction of DNA with Phenol-Chloroform

1. Scope

- 1.1. Isolating DNA from nucleated cells using organic solvents, which consists of lysing cells with digest buffer coupled with protein digestion using proteinase-K enzyme. After digestion is complete, several phenol/chloroform/isoamyl alcohol washes are performed to remove any remaining proteins.
- 1.2. The extracted DNA (in aqueous solution) is then washed and concentrated in a DNA Ultra Filtration device.
- 1.3. Phenol-chloroform extractions are generally reserved for specimens with very high amounts of substrate (such as tissue samples or bone), but it can be utilized for any sample. The procedure is just more labor-intensive and requires labelling several sets of tubes.

2. Safety

- 2.1. CHEMICAL HAZARD. Wear chemical-resistant gloves and eye protection when handling phenol-chloroform solution. Work in well-ventilated area, preferably a total exhaust biosafety hood.
- 2.2. Treat all biological specimens as potentially infectious. Gloves and laboratory coat must be worn at all times. Follow Universal Precautions.

3. Specimens

- Tissue, muscle or organ (~1 - 3 cm³)
 - Bone (sample size varies)
- 3.1. NOTE: Sizes are only guidelines; evidentiary samples may be in limited supply. Add the maximum amount of evidentiary sample that can fit in a tube if very little biological material is present, trying to retain sufficient sample for replicate analysis when possible.

4. Reagents and Special Supplies

- DNA Ultra Filtration devices (Amicon Ultra 100k, Centricon-100 or Microcon-100)
- Digest Buffer (10 mM Tris-HCl, 10 mM EDTA, 50 mM NaCl, 2% SDS, pH 7.5)
- Molecular biology grade water
- Phenol/chloroform/isoamyl alcohol solution (25:24:1)
- Proteinase K (20 mg/ml)
- Tris-EDTA, “TE⁻⁴” (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)

5. Procedural Notes

- 5.1. This protocol is written for the use of 1.5 ml microcentrifuge tubes. If it is necessary to use larger tubes (such as 15 ml conical tubes), scale-up the proportions of reagents accordingly.
- 5.2. This protocol is written for the use of the Eppendorf model 5415C microcentrifuge (or equivalent). The “maximum speed” referenced in this protocol corresponds to approximately 15,000 rpm (approximately 14,000 to 15,000 g-force). When “2400 rpm” is referenced, the relative centrifugal force should be approximately 500 g.
- 5.3. If larger tubes are used (15 ml or 50 ml conical tubes), the rotor in the centrifuge will have to be changed to accommodate the larger tubes.



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6. Extracting Tissue, Muscle or Organ

- 6.1. Using a clean cutting surface for each different sample, dissect a sample approximately 1 mm square (or larger) and place in a labeled microcentrifuge tube
- 6.2. Add 500 μ l of Digest Buffer and 7.5 μ l of 20 mg/ml Proteinase K solution.
- 6.3. Incubate at 56° C for at least one hour. Digestion may continue overnight, up to 24 hours.
- 6.4. Add 500 μ l of phenol/chloroform/isoamyl alcohol. Vortex at least 15 seconds until an emulsion forms.
- 6.5. Spin in microcentrifuge for 3 minutes at maximum speed.
- 6.6. Transfer the upper (aqueous) phase to a new-labeled microcentrifuge tube. Do NOT transfer the white layer of protein that may be visible between the two layers.
- 6.7. Repeat steps 6.4 through 6.6 an additional two or three times until nothing is visible at the interface and the aqueous phase appears clear.
- 6.8. Transfer the entire upper (aqueous) phase containing extracted DNA to a DNA Ultra Filtration device (approximately 500 μ l). If the aqueous phase is more than 500 μ l, it can be concentrated using several DNA Ultra Filtration devices or up to 2 ml in a single Centricon-100. [Do not touch the membrane with the pipette tip or transfer any lower (organic) phase].
- 6.9. Spin the device at 2400 rpm for 15 minutes (the speed is the same whether using a microcentrifuge or a clinical centrifuge).
- 6.10. NOTE: almost all the liquid in the upper chamber should pass through the membrane. If a large amount of liquid remains in the upper chamber, the filter may be clogged; transfer the remaining liquid to a new DNA Ultra Filtration device and repeat step 6.9.
- 6.11. Add 500 μ l TE⁻⁴ to the retentate in the upper chamber and spin at 2400 rpm for 15 minutes (if using a Centricon-100, add 2 ml of TE⁻⁴).
- 6.12. Place sample reservoir upside down in a new vial. Spin at maximum speed for 3 minutes (or 5000 rpm if using clinical centrifuge).
- 6.13. Bring the recovered retentate volume up to 50 μ l with TE⁻⁴ if necessary.
- 6.14. Store in a refrigerator (1° C - 8° C) for short-term storage. Store in a freezer (-10° C or colder) for long-term storage.

7. Extracting Bone

- 7.1. Weigh and measure bone sample before extracting. Photo documentation is optional.
- 7.2. Using a clean surface for each different sample, scrape and sand the surface of the bone to remove debris and potential foreign contamination using Dremel-type tool with new, UV-irradiated sanding disks.
- 7.3. Cut a section of bone using Dremel-type tool with new, UV-irradiated cutting blades.
- 7.4. Attempt to quantify the bone section to be analyzed (weight, size, etc.).
- 7.5. Grind the bone sample in a blender cleaned with bleach and alcohol or scrape the bone surface if possible.



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- 7.6. Transfer the bone pieces to a 15 ml conical tube.
- 7.7. Add a measured amount of Digest Buffer sufficient to completely submerge the bone dust.
- 7.8. Add 15 μ l of 20 mg/ml Proteinase K solution for every ml of digest buffer added in step 7.7.
- 7.9. Incubate at 56° C for at least one hour. Digestion may continue overnight, up to 24 hours.
- 7.10. Add 500 μ l of phenol/chloroform/isoamyl alcohol. Vortex for at least 15 seconds until an emulsion forms.
- 7.11. Spin in a clinical centrifuge for 3 minutes at 5000 rpm.
- 7.12. Transfer the upper (aqueous) phase to a new-labeled 15 ml tube. Do NOT transfer the white layer of protein that may be visible between the two layers.
- 7.13. Repeat steps 7.10 through 7.12 an additional two or three times until nothing is visible at the interface and the aqueous phase appears clear.
- 7.14. Transfer the entire upper (aqueous) phase containing extracted DNA to a DNA Ultra Filtration device (approximately 500 μ l if extracted in microcentrifuge tubes). If the aqueous phase is more than 500 μ l, it can be concentrated using several DNA Ultra Filtration devices or up to 2 ml in a single Centricon-100. [Do not touch the membrane with the pipette tip or transfer any lower (organic) phase].
- 7.15. Spin the device at 2400 rpm for 15 minutes (the speed is the same whether using a microcentrifuge or a clinical centrifuge).
- 7.16. NOTE: almost all the liquid in the upper chamber should pass through the membrane. If a large amount of liquid remains in the upper chamber, the filter may be clogged; transfer the remaining liquid to a new DNA Ultra Filtration device and repeat step 7.15.
- 7.17. Add 500 μ l TE⁻⁴ to the retentate in the upper chamber and spin at 2400 rpm for 15 minutes (if using a Centricon-100, add 2 ml of TE⁻⁴).
- 7.18. Place sample reservoir upside down in a new vial. Spin at maximum speed for 3 minutes (or 5000 rpm if using clinical centrifuge).
- 7.19. Bring the recovered retentate volume up to 50 μ l with TE⁻⁴ if necessary.
- 7.20. Store in a refrigerator (1° C - 8° C) for short-term storage. Store in a freezer (-10° C or colder) for long-term storage.