

FBS08- Organic DNA Extraction

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1. Scope

- 1.1. This procedure describes the isolation of deoxyribonucleic acid (DNA) from biological specimens presumed not to have sperm, recovered from evidentiary items for nuclear DNA typing.

2. Background

- 2.1. Biological samples contain a number of substances in addition to DNA. DNA molecules must be separated from other cellular material before they can be examined. Cellular proteins that package and protect DNA in the environment of the cell can inhibit the ability to analyze the DNA. DNA extraction methods separate proteins and other cellular materials from the DNA molecules.

3. Safety

- 3.1. Wear personal protective equipment (e.g., lab coat, gloves, mask, eye protection), when carrying out standard operating procedures.
- 3.2. Read Material Safety Data Sheets to determine the safety hazards for chemicals and reagents used in the standard operating procedures.

4. Materials Required

- 4.1. Digest Buffer (FBR35)

- 4.2. Proteinase K (10 mg/ml) (FBR36)
- 4.3. Phenol/Chloroform/Isoamyl Alcohol (PCI)
Note: This reagent and its waste must be handled in a fume hood.
- 4.4. TE Buffer
- 4.5. Microcentrifuge
- 4.6. 1.5mL and 2.0 mL Microcentrifuge tubes
- 4.7. Microcon devices

5. Standards and Controls

- 5.1. At least one reagent blank (i.e., extraction control) must be prepared and processed in parallel with each set of evidentiary specimens for DNA typing purposes. The reagent blank(s) (RB) is comprised of all the reagents used in the analytical process and carried through the same extraction, quantitation, amplification and electrophoretic typing procedures as the evidence samples. If more than one extraction method is used then at least one reagent blank must be processed for each type of procedure. The reagent blank(s) implemented as part of the organic DNA extraction procedure performed on the evidence is named the RB. The RB will always be the last sample processed in a set.
- 5.2. In order to maintain a separation in time and space between questioned and known samples:
 - 5.2.1. At no time will questioned and known samples be simultaneously incubating in the same heat block.
 - 5.2.2. At no time will questioned and known samples be simultaneously extracted in the organic fume hood.

6. Calibration

- 6.1. Not applicable

7. Procedures

- 7.1. Place each sample into a labeled, sterile 2.0 ml microcentrifuge tube.

- 7.2. To each sample tube, pipette 400 μL Digest Buffer and 12 μL of Proteinase K (10 mg/mL) solution. Vortex and quick-spin in a microcentrifuge. Incubate on the heat block at 56°C for a minimum of 2 hours to overnight.
- 7.3. **OPTIONAL:** Additional Digest Buffer and Proteinase K, in the proper concentrations, may be added to the sample, if needed.
- 7.4. **Note:** The Digest Buffer and Proteinase K may be aliquoted as a master mix. If the master mix method is used, this aliquot expires at the end of the prepared date.
- 7.5. After incubation, vortex and spin-down the sample tubes. Transfer the substrate to a filterless basket in a 2.0mL microcentrifuge tube and spin for 3 – 5 minutes at maximum speed. Save the substrate in a new sterile, labeled microcentrifuge tube.
- 7.6. In a fume hood add 500 μl of PCI solution. Thoroughly mix (vortex or invert by hand) to form a transiently homogenous suspension.
 - 7.6.1. All nuclear DNA isolation steps in which Phenol/Chloroform/Isoamyl Alcohol is used must be performed in a fume hood.
 - 7.6.2. To ensure a homogeneous solution, the Phenol/Chloroform/Isoamyl Alcohol reagent will be removed from the refrigerator and allowed to equilibrate to room temperature before beginning extractions.
- 7.7. Microcentrifuge the samples at room temperature for 5 minutes at maximum speed to separate the two phases. Proceed to Step 7.9 if the upper aqueous phase is clear.
- 7.8. **Note:** If the aqueous phase is not clear (e.g., cloudy, dark in color or colored from dyes), due to incomplete phase separation, then transfer the upper aqueous phase to a new sterile 1.5 ml microcentrifuge tube. Repeat steps 7.6 – 7.7 an additional 2 to 3 times, until the interface is clean and the aqueous phase is clear. For these additional extractions, the lower Phenol/Chloroform/Isoamyl Alcohol layer may be removed and discarded, eliminating the need for a new microcentrifuge tube.
- 7.9. Assemble the Microcon and label the specimen reservoir.
- 7.10. Add 100 μL TE Buffer to upper reservoir to pre-wet the membrane.
- 7.11. **Note:** When adding sample and/or buffer to specimen reservoir be cautious to avoid touching the filter with the pipette tip.
- 7.12. Transfer the entire aqueous phase to the upper reservoir containing TE Buffer. Microcentrifuge at 500 x g for at least 10 minutes.

- 7.12.1. Caution will be taken to prevent microcon filters from exceeding their specified limitations. Excessive g-force may result in leakage or damage to the centrifugal device.
- 7.13. Remove the upper specimen reservoir from the tube, discard the effluent in the lower reservoir, and re-insert the upper reservoir into the Microcon tube. Alternatively, the upper reservoir may be placed into a new appropriately labeled Microcon tube and the original tube containing the effluent discarded.
- 7.14. Add 200 μ L of TE Buffer to the upper reservoir.
- 7.15. Microcentrifuge at 500 x g for at least 10 minutes. If liquid remains, additional spin(s) may be performed.
- 7.16. Label a new set of Microcon recovery tubes.
- 7.17. When the fluid has been drawn completely through the filter, add approximately 10-25 μ L of TE buffer to reservoir, and invert the upper reservoirs into the recovery tubes. Microcentrifuge for 3 minutes at 500 x g. Be certain that the caps on the tubes are all facing inward in the microcentrifuge to avoid possible snapping of the caps.
- 7.18. Remove and discard filter and close final Microcon tube. The contents of the final Microcon tube will be transferred to an appropriately labeled sterile microcentrifuged tube. Add additional TE buffer, if necessary, to bring the final sample volume to at least 25 μ L. Note: A higher recovery volume of ~ 50 μ L is recommended for samples of expected high DNA concentration, e.g. reference samples.
- 7.19. Reagent Blanks must be less than or equal to the lowest volume (highest concentration) of an associated sample within an extraction set.
- 7.20. Store the samples frozen.
- 7.21. If needed, a sample may be re-concentrated by following steps below:
 - 7.21.1. Assemble the Microcon and label the specimen reservoir.
 - 7.21.2. Add the DNA sample to the upper reservoir, being cautious to avoid touching the filter with the pipette tip.
 - 7.21.3. Repeat steps 7.15 – 7.19.
 - 7.21.4. **Note:** If a sample is to be re-concentrated, the associated reagent blank must also be re-concentrated as well.

8. Sampling

8.1. Not applicable

9. Calculations

9.1. Not applicable

10. Uncertainty of Measurement

10.1. Not applicable

11. Limitations

11.1. The quantity and quality of the DNA present within any biological material ultimately determines if a nuclear DNA isolation is successful.

12. Documentation

12.1. Organic Extraction Sample Sheet (Document Control Number: 1579)

13. References

- 13.1. Comey, C.T., Koons, B.W., Presley, K.W., Smerick, J.B., Sobieralski, C.A., Stanley, D.M., and Baechtel, F.S. DNA extraction strategies for amplified fragment length polymorphism analysis. *Journal of Forensic Sciences* (1994) 39: 1254-1269.
- 13.2. Millipore Corporation. Microcon[®] Centrifugal Filter Devices User Guide. Millipore Corporation, Billerica, MA, 2000.
- 13.3. Forensic Science Laboratory Quality Assurance Manual (Current Version)
- 13.4. Forensic Biology Unit Quality Assurance Manual (Current Version)
- 13.5. DFS Departmental Operations Manuals (Current Versions)
- 13.6. FSL Laboratory Operations Manuals (Current Versions)
- 13.7. FBR35 - Digest Buffer (Current Version)