HUMAN BONE SAMPLE EXTRACTION

Background

A human bone sample can be tested as a means of determining parentage or identity. The sample may be a product of medical examiner collection of remains, crime scene evidence collection, product of conception, or other human bone.

Summary of Procedure

The cells in human bone are a source of nuclear DNA. Slicing the bone into small pieces and grinding will increase the material surface area and increase the efficiency of DNA extraction. The addition of stain extraction buffer and Pro K will release the nuclear DNA material from the cells. The nuclear DNA material can be isolated by using the organic method of extraction to remove released protein material.

Sample Handling

Forensic samples may be in limited supply. Retain sufficient sample for replicate analysis.
Label all samples with complete identifying information.

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- ***Safety Manual***, *Universal Precautions*
- ***Quality Assurance Manual***, *General Sample Control and Forensic Sample Preservation Policy*
- ***Analytical Procedures Manual***, **Forensic Evidence Handling**.

Reagents and Materials

See **Appendix B** for reagent preparation

Molecular Biology Grade Water
Ethanol
Stain Extraction Buffer
20mg/mL Proteinase K
2M DTT

Sterile transparent 2.0mL micro-centrifuge tubes and rack
Razor blade
Forceps
Dremel Tool with sanding barrel attachment and rotary cutting bit
6750 Freezer/Mill
Grinding Vial Set
Liquid Nitrogen
Commercial Blender (optional)
Disposable bench paper
Lab Coat
Gloves
Kimwipes
Permanent marker
Vortex
Nucleic Acid Workstation
Biological safety cabinet
Micro-centrifuge

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the **Bone Sample Extraction Worksheet** is required for documentation. All information must be completed.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions. This negative control consists of all reagents used in the procedure but contains no DNA sample.

Procedure

1. Describe the sample received in the case notes. Note the description of the sample container, specific markings, color of the bone, specific descriptions related to preservatives added if applicable, liquid volume and any other unique identification.

2. Open the container in the biological safety cabinet with proper safety equipment.

3. Recommendations for bone and tooth follow, however, depending on the condition of the sample some steps may not be necessary and some steps, such as bleaching and UVing can be changed at the processor's discretion.

Cleaning of bone is dependent on the type and quality.

- Remove any tissue/ debris from the bone with a sterile razor (tissue should be preserved for possible DNA analysis)
- Rinse with MBG water (several seconds)
- Rinse with ethanol (several seconds)
- Dry with Kimwipe
- UV 30 min
- Sand top layer of bone (1-2mm) to remove contaminates
- Repeat steps a-c
- Cut off approximately 5mm slices from bone using (enough to create 2.5g of bone powder) or sample using a grid pattern

Cleaning of teeth is dependent on the type and quality.

- Soak the tooth in 20mL of regular Clorox bleach for 20 minutes
- Soak the tooth in 20mL of MBG water for 20 minutes
- Rinse with ethanol
- Dry the tooth under UV, 10 minutes on each side

4. Pulverize the bone slices and/or teeth, using a mortar and pestle, or sample bone in a manner in which pieces will easily fit into the 6750 Freezer/Mill center cylinder.
   Note: A commercial blender may be used for pulverization. If a blender is used, proceed to step 10 after pulverizing.

5. Add the sample to the grinding vial set. Be sure to leave enough room in the cylinder for the impactor to move back and forth.

6. Slowly pour liquid nitrogen into the Freezer/Mill chamber up to the fill line. Place the grinding vial into the hole of the assembly. Additional samples can be added to the upper rack of the lid assembly so that up to three vials are cooling at the same time. Slowly close the lid of the mill and fasten.

7. Press the run button on the Freezer/Mill key pad. A pre-cooling period of 10 minutes is pre-programmed, followed by a pulverizing cycle of 3 minutes and a rest cycle of 1 minute. The pulverizing and rest cycle are repeated 3 times.

8. Once the program is completed, remove the grinding assembly using the extractor and allow the assembly to reach room temperature. If multiple grinding assemblies were placed in the mill, place a new sample in assembly hole. The pre-cooling period does not need to be repeated since the sample has already been cooled. To modify the pre-cooling period, press the T3 key and enter a value of one minute. Press enter. Press run.

9. Once the grinding assembly has reached room temperature, remove the pulverized sample.

10. Weigh out multiple 0.50g samples of the ground material. Place each of the five samples into an orange top 15mL conical tube.

11. Add 10mL of 0.5 M EDTA pH 8 to each tube. Place on a rocker. Continue until the samples look suspended in the EDTA.

12. After the EDTA wash is complete and the last EDTA supernatant has been removed, add approximately 500uL of Stain Extraction Buffer, 20uL of Proteinase K, and 20uL of 2M DTT. These volumes may be increased if the bone material soaks up the reagents. Note the volumes of the above reagents added to the samples on the **Bone Sample Extraction Worksheet**

13. Vortex and incubate the samples at 56°C for approximately six (6) hours, with intermittent vortexing every hour.

14. At the end of the six (6) hour incubation add approximately 20uL of Proteinase K and 20uL of 2M DTT to each sample and incubate overnight.

15. Proceed with **Organic Extraction** starting with the addition of PCI to separate and remove unwanted proteins from the nuclear DNA material. It is highly recommended that a Centricon be used for filtering steps and that samples be quantitated separately first before any pooling of extract.