HAIR EXTRACTION

Background

This protocol outlines a method to prepare loose human hairs and mounted human hairs for organic DNA extraction.

Summary of Procedure

The cells in human hair are a source of nuclear DNA. Washing the hair will remove any styling products or other debris to increase the efficiency of DNA extraction. The addition of stain extraction buffer, DTT 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. If multiple hairs are collected from an item, only a representative sample should be tested.
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
100% Ethanol
Stain Extraction Buffer
1M DTT
20mg/mL Proteinase K

Microscope
Forceps
50mL conical tubes
Nutator/Rocker
1.7 Microcentrifuge tubes
Razor blades
Heat Block
Vortex
Lab Coat
Gloves
Kimwipes
Sterile Disposable Pipette Tips
Pipettes
Disposable Bench Paper
Permanent marker
Microcentrifuge

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 **Hair Extraction Worksheet** and **Hair Root Identification Evidence Processing Worksheet** are 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. Examine the hair under a microscope noting the possible presence of foreign material on the hair. Take a photograph(s) of the hair including the root, tip and any area of interest. Clean the hair, if necessary.

2. **Cleaning loose hairs:** Fill a clean 50mL conical tube with MBG water. Place the hair into the conical tube, invert the tube and rock on a nutator for at least 2 hours. Wash each hair to be analyzed separately in fresh MBG water. If the material associated with the exterior of the hair may be probative then the hair may be washed in a 1.7mL microcentrifuge tube with MBG water. After washing, remove the hair from the tube and retain for further testing. Centrifuge the wash for 5 minutes then discard all but 50uL of the supernatant.

   Recovering and cleaning mounted hairs: Remove the cover slip, pick up the hair with clean forceps and wash in a 1.7mL microcentrifuge tube containing 100% ethanol; then wash in a 1.7mL microcentrifuge tube containing MBG water.

3. Once the hair is ready for analysis, cut off about 5 to 10mm of the proximal (root) end for digestion and place in a 1.7mL microcentrifuge tube. Because the hair may contain cellular material on the surface that may or may not have originated from the hair donor, it is advisable to cut off about 5 to 10mm of the shaft next to the root for separate analysis as a control and place in a 1.7mL microcentrifuge tube. The remaining shaft will be retained for possible additional testing.

4. Add 500uL of Stain Extraction Buffer, 20uL 1M DTT and 20uL 20mg/mL Pro K to the microcentrifuge tube containing the hair.

5. Incubate at 56°C for 6 to 8 hours. Hair will soften but not completely dissolve after this initial incubation. Vortex for approximately 30 seconds and centrifuge for 5 to 10 seconds.

6. Add an additional 20uL 1M DTT and 20uL 20mg/mL Pro K. Incubate at 56°C for 6 to 8 hours or overnight until hair is completely dissolved. Vortex approximately 30 seconds.

7. Spin the sample in a microcentrifuge for 1 minute at 13,200 rpm at room temperature to remove pigment and particles. Remove the supernatant and place in a new sterile 1.7mL microcentrifuge tube. Discard the pellet.

8. Proceed with organic extraction/isolation to separate and remove unwanted proteins from the nuclear DNA material. Only one PCI step is required.