Organic Extraction for Mitochondrial or Nuclear DNA Testing

Refer to the current *Protocols for Forensic STR Analysis* manual for extraction, quantitation, amplification, and STR procedures currently on-line for other Nuclear DNA Operations.

PURPOSE: To isolate nuclear or mitochondrial DNA from the hair using an enzymatic digestion of the hair followed by an organic extraction.

A. Extraction for Mitochondrial and Nuclear DNA testing

1. Prepare hair for digestion by removing the appropriate microcentrifuge tube from the "To Be Extracted" cryobox. Record the Organic Extraction documentation.
2. Prepare the incubation solution in a 1.5ml tube using the following table. Label this tube with the extraction date and time as ENEGDDMMYY-HHMM.

Reagents	**1 hair + extraction negative**
Proteinase K (20mg/ml)	30 µL (15*2)
DTT (1M)	75 µL(37.5*2)
20% SDS	7.5 µL(3.75*2)
Organic Extraction Buffer	188 µL(94*2)

3. When extracting clumps of hair, or multiple hairs together, the total volume of the incubation solution can be increased 2- to 10-fold, to accommodate the size of the sample. Adjust the reagent volumes to accommodate these changes. Be sure to record such volume changes in the documentation.
4. Have the extraction tube set-up witnessed.
5. Aliquot 150 µLof the incubation solution into the 1.5ml tube containing the hair and leave the remaining solution in the original 1.5ml tube as the negative control.
6. Incubate samples for 30 min. in a 1400 rpm shaker at 56^{O}C. Record the number of the thermal mixer, the thermal mixer temperature setting and actual temperature in the documentation.
7. After 30 min., hairs should be dissolved. If not, incubate for a total of 1-2 hours. If hairs have not dissolved, add 1µLof 1M DTT and incubate overnight. Be sure to record this on the extraction documentation, if performed. Hairs and control samples should be both treated the same way. After overnight incubation, record the thermal mixer setting and temperature in the documentation.
8. When the hair sample is completely dissolved, proceed with the extract to the purification step (see part B)

9. The hair sample might not completely digest even after the overnight incubation. If the hair is chemically treated, straightened, or dyed, it might resist digestion. The incubation process might remove the pigment or coloring from a hair and leave it opaque. If this happens, record this observation in the documentation. Centrifuge the sample for 3-5 minutes at full speed. Collect the supernatant (extract) in a new tube, carefully without disturbing the pellet. Add the suffix "–R" to the sample name and label on the original tube containing the hair remain (pellet). Hair remains will be stored with the other sample. Proceed to purification step with the extract (see part B).

B. Purification of DNA for Mitochondrial and Nuclear DNA testing

1. During the incubation, prepare and label for each sample: one Eppendorf Heavy Phase Lock Gel (PLG) tube, one microcon filter, three microcon collection tubes, and one 1.5 ml tube for final extract. PLG tubes can be centrifuged for 30 seconds at maximum speed prior to sample addition.
2. After incubation, have the purification tube set-up witnessed.
3. Transfer each extracted sample to appropriate labeled PLG tube. PLG tubes make the phase separation between organic and aqueous layers of an organic extraction easier. To each PLG tube add an equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1 PCIA). The PCIA volume to be added should be 150µl unless the extraction volume has been increased in step A3. **PCIA is an irritant that is toxic. Its use should be confined to a certified fume hood. Gloves and a mask should be worn**.
4. Shake or briefly vortex the tube to achieve a milky emulsion.
5. Centrifuge the tube in a microcentrifuge for 2 minutes at maximum speed.
6. Insert Microcon DNA Fast Flow filter cup (blue) into labeled microcon tubes for each sample.
7. Prepare the Microcon concentrator by adding 100 µL of TE^{-4} to the filter side (top) of the concentrator.
8. Transfer the aqueous phase (top layer) from the PLG tube to the prepared Microcon concentrator. Do not disturb the PLG layer. Discard the PLG tube containing the organic layer into the organic waste bottle in the fume hood.
9. Spin the Microcon concentrator for 25 minutes at 500 rcf.
10. Transfer the Microcon filter cup into a new labeled Microcon tube and add 400µL of TE^{-4} to the filter side (top) of the concentrator.
11. Spin again at 500 rcf for 20 minutes. After this spin, if liquid is still observed on the membrane, spin again at 500 rcf for an additional 6 minutes. After this spin, if liquid is still observed on the membrane, continue spinning for a longer time.
12. Add 20 µL of TE^{-4} to the filter side (top) of the concentrator.

13. Invert the blue concentrator cup and place into appropriate microcon collection tubes. Spin at 1000 rcf for 3 minutes to collect samples.

- For mitochondrial DNA testing,
  - Using a pipettor, measure volume collected and record it.
  - Transfer samples to a 1.5 ml microcentrifuge tube for storage.
  - Adjust samples volume to 50 µL using TE^{-4} record these volumes.
  - Proceed with HVI-HVII amplification with 20µl of samples.
- For nuclear DNA testing,
  - Using a pipettor, measure the volume collected
  - The volume should be close to 20ul, in control and hair samples. If the volume is >30µl, prepare a new microcon filter and tube (see part 6 above) and spin at 500 rcf, control and hair samples, for an additional 6 minutes. After this spin, if liquid is still observed on the membrane, continue spinning for a longer time.
  - Measure the final volume collected and record it.
  - Transfer samples to a 1.5 ml microcentrifuge tube for storage
  - Send 2.5 µL of samples (neat) for nuclear quantification. If quantitation results show an insufficient amount of nuclear DNA for STR testing, the extract may then be used for mtDNA analysis.