| Organic Extraction for Mitochondrial or Nuclear DNA Testing | | |
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# Organic Extraction for Mitochondrial or Nuclear DNA Testing

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

## 1 Purpose

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

## 2 Extraction for Mitochondrial and Nuclear DNA testing

2.1 Prepare hair for digestion by removing the appropriate microcentrifuge tube from the "To Be Extracted" cryobox.

2.2 Centrifuge hair sample contained in the microcentrifuge tube at maximum speed for 30 seconds to pull the hair sample to the bottom of the tube.

2.3 Print a input LIMS label and attach it to the extraction negative tube. Prepare the mastermix in the extraction negative tube using the following table:

| Reagents | Volume per sample (µL) | Eneg + 1 hair (µL) | Eneg + 2 hairs (µL) | Eneg + 3 hairs (µL) | Eneg + 4 hairs (µL) | Eneg + 5 hairs (µL) |
|---|---|---|---|---|---|---|
| Proteinase K (20 mg/mL) | 15 | 30 | 45 | 60 | 75 | 90 |
| DTT (1M) | 37.5 | 75 | 112.5 | 150 | 187.5 | 225 |
| 20% SDS | 3.75 | 7.5 | 11.25 | 15 | 18.75 | 22.5 |
| Organic Extraction Buffer | 94 | 188 | 282 | 376 | 470 | 564 |

2.4 When extracting clumps of hair, or multiple hairs together, the total volume of the master mix 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.

2.5 **Extraction WITNESS:** Have a witness verify the input tube top names and sample labels.

2.6 Aliquot 150 µL of the master mix into the 1.5 mL microcentrifuge tube containing the hair sample and leave the remaining solution in the extraction negative control tube.

2.7 Incubate the samples for 30 minutes in a 1400 rpm shaker at 56°C. Record the name of the thermal mixer and actual temperature in LIMS.

2.8 After 30 minutes, the hairs should have dissolved. If not, incubate for a total of 1-2 hours. If hairs still have not dissolved, add 1 µL of 1M DTT and incubate overnight. Hairs and control samples should be treated the same way. Be sure to record the overnight incubation in the documentation, if performed:

2.8.1 "Hair did not fully digest after 30 minutes. Hair incubated a total of 2 hours and still was not fully digested. 1M DTT (1 uL) was added to both [Eneg Name] and [Sample Name] tubes and left to incubate overnight. [Initial and date]."

2.9 When the hair sample is completely dissolved, proceed with the extract to the purification section 3.

2.10 The hair sample might not have completely digested 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, centrifuge the sample for 3-5 minutes at full speed. Collect the supernatant in a new tube, carefully without disturbing the pellet. Proceed to purification step with the supernatant (see section 3). Record your actions in the extraction documentation:

2.10.1 "Sample did not fully digest after overnight incubation. Proceeded with extraction using supernatant. [Initial and date]."

# 3 Purification of DNA for Mitochondrial and Nuclear DNA testing

3.1 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 microcentrifuge tube for the final extract.

3.2 Centrifuge PLG tubes at maximum speed for 30 seconds. (On Eppendorf Centrifuge Model 5415D, spin at 16.1 rcf or 13.2 rpm).

3.3 Insert and label Microcon® DNA Fast Flow filter cup (blue) into labeled Microcon® collection tubes for each sample.

3.4 Prepare the Microcon® concentrator by adding 100 µL of $TE^{-4}$ to the filter side (top) of each concentrator. Set aside until step 11.

3.5 Print a small LIMS label for each output sample and attach it to appropriate tube.

3.6 After incubation, perform a quick spin on the incubated samples if condensation is observed.

3.7 **Purification WITNESS:** Have a witness verify your 1) input tube top names, 2) PLG tubes, 3) Microcon® filters, and 4) output tube top names and sample labels.

3.8 Transfer each extracted sample (typically 150µL) to the 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 150 µL unless the extraction volume has been increased in step A4.

3.8.1 ****WARNING**:** PCIA is an irritant that is toxic. Its use should be confined to a certified fume hood. Gloves and a mask should be worn.

3.9 Shake the PLG tube vigorously by hand or by inversion to form a milky colored emulsion. **Note: Do NOT vortex the PLG tube.**

3.10 Centrifuge samples for 2 minutes at maximum speed to achieve phase separation. (On Eppendorf Centrifuge Model 5415D, spin at 16.1 rcf or 13.2 rpm).

3.11 Carefully transfer the aqueous phase (top layer) from the PLG tube to the prepared Microcon® concentrator. Do not disturb the PLG layer.

3.12 Discard the used PLG tube containing the organic layer into the organic waste bottle.

3.13 Spin the Microcon® concentrator for 25 minutes at 500 rcf.

3.14 Transfer the Microcon® filter cup into a new labeled Microcon® collection tube and add 400 µL of $TE^{-4}$ to the filter side (top) of each concentrator.

3.15 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.

3.16 Add 20 µL of $TE^{-4}$ to the filter side (top) of each Microcon® concentrator.

3.17 Invert the Microcon® concentrator and place into a new labeled Microcon® collection tube. Spin at 1000 rcf for 3 minutes.

3.18 **For mitochondrial DNA testing**:

3.18.1 Using a pipette, measure the approximate volume recovered and record the value.

3.18.2 Transfer samples to a 1.5 ml microcentrifuge tube.

3.18.3 Adjust samples volume to 50 µL using $TE^{-4}$

3.18.4 The sample volume can be readjusted to ≥25µL if necessary for samples containing very low amounts of mtDNA.

3.18.5 Proceed with HVI-HVII amplification with 20 µL of samples.

3.19 **For nuclear DNA testing**,

3.19.1 Using a pipette, measure the approximate volume recovered and record the value.

3.19.2 The volume should be close to 20 µL, in control and hair samples.

3.19.3 If the volume is >30 µL, prepare a new Microcon® filter and collection tube (see steps 3-4 above) and spin samples 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. Measure the final volume recovered and record the value.

3.19.4 Transfer samples to a 1.5 mL microcentrifuge tube.