Hair Organic Extraction for Mitochondrial or Nuclear DNA Testing

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 cryobox.

2.2 Fill out the Performed By tab for "Extraction Set Up in LIMS.

2.3 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.

2.4 Print an input LIMS label and attach it to the two extraction negative tubes.

2.5 Retrieve reagents for extraction and record all reagent lots in LIMS. Prepare the mastermix in the first extraction negative tube using the following table:

Reagents	Volume per sample (µL)	2 ENegs + 1 hair (µL)	2 ENegs + 2 hairs (µL)	2 ENegs + 3 hairs (µL)	2 ENegs + 4 hairs (µL)	2 ENegs + 5 hairs (µL)
Proteinase K (20 mg/mL)	15	45	60	75	90	105
DTT (1M)	37.5	112.5	150	187.5	225	262.5
20% SDS	3.75	11.25	15	18.75	22.5	26.25
Organic Extraction Buffer	94	282	376	470	564	658

2.6 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.

2.7 **Extraction WITNESS:** Have a witness verify the input sample labels and tube tops

2.8 Aliquot 150 µL of the master mix into the 1.5 mL microcentrifuge tube containing the second extraction negative tube and to each hair sample. Leave the remaining solution in the first extraction negative control tube.

2.9 Incubate the samples for 30 minutes in a 1400 rpm shaker at 56°C.

2.9.1 Record the instrument usage in LIMS and enter "56 degree incubation" for the Program. Record the temperature in the QC batch parameters in LIMS.

2.10 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. If samples require an overnight incubation, wrap each tube (extraction negatives and samples) in parafilm after the addition of 1M DTT. Hairs and control samples should be treated the same way. If performed, be sure to record the overnight incubation in the documentation, as follows:

2.10.1 "Hair(s) did not fully digest after the initial 30 minutes incubation period. Hair(s) did not fully digest after a total of 2 hours incubation period. 1 uL of 1M DTT was added to both negative controls [ENeg Name] and [Sample Name(s)] tubes and left to incubate overnight. [Initial and date]."

2.11 When the hair sample is completely digested, proceed with the purification in section 3.

2.12 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 the supernatant opaque and the hair sample translucent. If this happens, centrifuge the sample for 3-5 minutes at full speed. Collect and transfer the supernatant to a new tube, carefully without disturbing the pellet. Proceed to the purification step with the supernatant (see section 3). Record your actions in the extraction documentation as follows:

2.12.1 "Hair sample(s) 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

WARNING

Phenol Chloroform is toxic. Protective eyewear, mask, lab coat, and nitrile gloves should be worn when handling. All work must be conducted under a chemical fume hood.

3.1 Fill out the Performed By tab for Extraction Clean-Up in LIMS.

3.2 During the incubation, prepare and label for each sample: one Eppendorf Heavy Phase Lock Gel (PLG) tube, one Microcon® filter, four Microcon® collection tubes, and one 1.5 mL microcentrifuge tube for the final extract.

3.3 Remove the Phenol:Chloroform:Isoamyl Alcohol (25:24:1) (PCIA) from the refrigerator.

3.4 Obtain organic waste jug for disposal of any tubes or pipette tips that come in contact with PCIA.

3.5 Centrifuge PLG tubes at maximum speed for 30 seconds.

3.6 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 2.6.

3.6.1 NOTE: When pipetting PCIA, you must penetrate the top buffer layer and only aliquot the desired amount from the lower, clear organic layer. Place used pipette tips in the organic waste bottle.

3.7 Insert and label a Microcon® filter into a labeled Microcon® collection tube for each sample.

3.8 Prepare the Microcon® filter by adding 100 µL of TE^{-4} to the membrane. Set aside until step 3.12.

3.9 Print a small LIMS label for each output sample and affix to appropriate 1.5mL microcentrifuge tube.

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

3.11 **Purification WITNESS:** Have a witness verify your 1) input sample labels and tube tops, 2) PLG tube tops, 3) written tube top labels on Microcon® filters and tube tops on collection tubes, and 4) output sample labels and tube tops

3.12 Transfer each extracted sample (approximately 150µL) to the appropriate labeled PLG tube.

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

3.14 Centrifuge samples for 2 minutes at maximum speed to achieve phase separation.

3.15 Carefully transfer the aqueous phase (top layer) from the PLG tube to the prepared Microcon® filter. Do not disturb the PLG layer nor the Microcon® filter with the pipette tip.

3.16 Discard the used PLG tube containing the organic phase (bottom layer) into the organic waste bottle.

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

3.18 Transfer the Microcon® filter into a new labeled collection tube and add 400 µL of TE^{-4} to the filter of each Microcon®.

3.19 Spin again at 500 rcf for 20 minutes. After this spin, if liquid is still observed on the membrane of the filter, 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.

3.20 Add 400 µL of TE^{-4} to the filter of each Microcon® as a second wash step.

3.21 Spin again at 500 rcf for 20 minutes. After this spin, if liquid is still observed on the membrane of the filter, 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.

3.22 Add 20 µL of TE^{-4} to the filter of each Microcon®.

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

3.24 For mitochondrial DNA testing:

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

3.24.2 Transfer the eluted extract to a 1.5 ml microcentrifuge tube.

3.24.3 Adjust extract volume to 50 µL using TE^{-4}

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

3.24.5 Proceed with amplification of samples.

3.25 For nuclear DNA testing,

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

3.25.2 Transfer the eluted extract to a 1.5mL microcentrifuge tube.

3.25.3 The volume should be between 20 – 30 µL (preferably closer to 20µL) for the controls and hair samples. Extraction negatives elution volume should be equal to or less than the lowest hair sample elution volume (controls should be more concentrated than samples).

3.25.4 If the volume is >30 µL, prepare a new Microcon® filter and collection tube (see steps 3.7 – 3.8 above), transfer the eluted extract to this new Microcon filter 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.

3.25.4.1 Transfer samples to a 1.5 mL microcentrifuge tube.

3.25.5 Fill out the Performed by tab for “Extraction Run Completion” in LIMS.

3.25.6 Assign storage to extracts in LIMS and store extracts at 2-8°C or frozen.