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 A negative control needs to be processed with every extraction batch. Obtain two empty 1.5 mL screw cap tubes for the extraction negatives and manually label them as Extraction Negative 1 and Extraction Negative 2

2.2 Fill out the "Extraction Set Up" performed by tab in LIMS. This will add the date and time to the extraction negatives for the batch.

2.3 Prepare hair for digestion by removing the 1.5 mL screw cap tube from the cryobox.

2.4 Centrifuge hair sample contained in the 1.5 mL screw cap tube at maximum speed for 30 seconds to pull the hair sample to the bottom of the tube.

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

2.7 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.

2.8 Aliquot 150 µL of the master mix into the 1.5 mL screw cap 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 longer 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 clean up 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 with the clean up of 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 Phenol Chloroform and Microcon Clean up

****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 During the incubation, remove the Phenol:Chloroform:Isoamyl Alcohol (25:24:1) (PCIA) from the refrigerator.

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

3.3 Once incubation is complete, fill out the Performed By tab for Extraction Clean-Up in LIMS.

3.4 Vortex and centrifuge all tubes for a quick spin.

3.5 Obtain and label 1.5 mL microcentrifuge tubes for the phenol-chloroform extraction clean-up step.

3.5.1 Extraction negatives and samples in 1.5 mL screw cap tubes only require one 1.5 mL microcentrifuge tube each.

3.6 Add an appropriate volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1 PCIA) to the microcentrifuge tube which is equal to the volume of lysate (e.g. 150 µL) but this may be adjusted as needed

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 Obtain and label a Microcon® DNA Fast Flow filter and collection tube for each sample.

3.8 Prepare the Microcon® filters by adding 100 µL of TE^{-4} to the membrane located on the filter side (top) of each concentrator. Set aside until step 3.14.

3.9 Obtain and label a 1.5 mL screw cap tube for the final DNA extract of each sample.

3.10 **Purification WITNESS:** Have a witness verify your 1) input sample labels and tube tops, 2) tube top labels on the 1.5 mL microcentrifuge tubes containing PCIA 3) tube top labels on Microcon® filters and collection tubes, and 4) output sample labels and tube tops

3.11 Pipette and transfer each lysate (approximately 150µL) to the .respective labeled 1.5 mL microcentrifuge tube containing PCIA.

3.12 Shake the 1.5 mL microcentrifuge tubes vigorously by hand or by inversion to form a milky colored emulsion. **Note: Do NOT vortex the microcentrifuge tubes.**

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

3.14 Carefully transfer the aqueous phase (top layer) to the prepared Microcon® filter. To do so, slowly lower the pipette tip just beneath the surface of the aqueous phase and aspirate the top layer.

When transferring the aqueous layer, do not let the pipette tip touch the interphase (middle layer) or organic phase (lower layer). Leaving behind ~10-20 µL of the aqueous phase is acceptable to avoid accidentally aspirating the interphase.

3.14.1 Note: When transferring the aqueous phase (top layer), position and hold the tube at eye level, being careful not to disturb the contents – even gentle agitation can disrupt the interphase.

3.14.1.1 Troubleshoot: If the interphase is accidentally disrupted and/or aspirated, deposit the aliquot back into the 1.5 mL microcentrifuge tube and repeat steps 3.13 and 3.14.

3.15 Discard the used microcentrifuge tubes with the remaining PCIA into the organic waste bottle.

3.16 Spin the Microcon® concentrators for 25 minutes at 500 rcf.

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

3.18 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, spin for additional time in 6-minute increments.

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

3.20 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, spin for additional time in 6-minute increments.

3.21 When the sample is ready to elute, add 20 µL of TE^{-4} to the filter of each Microcon®.

3.22 Separate the filter from the used collection tube, invert the Microcon® filter and place into a new labeled collection tube.

3.23 Spin the inverted filters at 1000 rcf for 3 minutes.

3.24 Remove and discard the sample filter.

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

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

3.25.2 If the volume is > 30µL, prepare a new Microcon® filter and collection tube (steps 3.7-3.8), transfer the eluted extract to this new filter and spin at 500 rcf for 6 minutes. If liquid is still observed on the membrane, continue to spin in 6-minute increments. Follow steps 3.21 – 3.25. Transfer final extract to screw-cap tube.

3.26 If the hairs were extracted for mitochondrial DNA testing, adjust the final elution volumes of the extraction negatives and samples to 30 µL using TE^{-4}.

3.27 If the hairs were extracted for nuclear DNA testing, the elution volumes can remain between 20 – 30 µL.

3.28 Fill out the Performed by tab for "Extraction Run Completion" in LIMS.

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