Organic Extraction		
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Organic Extraction

***NOTE:** This protocol allows for use of either nDNA QC'd reagents and Mito QC'd reagents.

1 Sample Incubation

1.1 An extraction negative needs to be processed with every batch of extractions. Obtain three empty 1.5 mL Eppendorf tubes for the extraction negatives and manually label them as Extraction Negative 1, Extraction Negative 2, and Extraction Negative 3.

1.1.1 For bone samples, obtain three 50 mL conical tubes for extraction negatives.

1.2 Following the tables below, prepare the master mix in a microcentrifuge tube or conical tube and mix thoroughly by swirling or vortexing *very briefly*.

1.3 **Incubation WITNESS:** Have a witness verify the input tube top and sample labels.

***NOTE: The addition of DTT is optional in the incubation of pseudo-exemplars, depending on the nature of the particular sample.**

For bone samples:

	Per bone (~2g dust)	**1 sample (N+ 2)**	**3 samples (N+ 2)**	**5 samples (N+ 2)**
Organic Extraction Buffer	2370 µL	7.11 mL	11.85 mL	16.59 mL
20% SDS	300 µL	900 µL	1.5 mL	2.1 mL
1.0 M DTT	120 µL	360 µL	600 µL	840 µL
Proteinase K (20 mg/mL)	210 µL	630 µL	1.05 mL	1.47 mL
Total Incubation Volume *per Extraction Negative*				*1000 µL*
Total Incubation Volume *per bone sample*:				*3000 µL*

For liquid blood, dry blood and bone marrow samples:

	1 Sample	**5 Samples**	**10 Samples**	**15 Samples**
Organic extraction buffer	400 µL	2.0 mL	4.0 mL	6.0 mL
20% SDS	10µL	50 µL	100µL	150 µL
Proteinase K (20 mg/mL)	13.6 µL	68 µL	136 µL	204 µL
Total Incubation Volume *per sample*:				*400 µL*

For tissues (POC/muscle), paraffin embedded tissue (e.g. microdissection), and pseudo-exemplars:

	Per tissue	**1 sample (N+ 2)**	**3 samples (N+ 2)**
Organic extraction buffer	395 µL	1185 µL	1975 µL
20% SDS	50 µL	150 µL	250 µL
1.0 M DTT	20 µL	60 µL	100 µL
Proteinase K (20 mg/mL)	35 µL	105 µL	175 µL
Total Incubation Volume *per sample*:			*500 µL*

For teeth samples:

	Per tooth	**1 sample (N+ 2)**	**3 samples (N+ 2)**	**5 samples (N+ 2)**
Organic Extraction Buffer	790 µL	2.37 mL	3.95 mL	5.53 mL
20% SDS	100 µL	300 µL	500 µL	700 µL
1.0 M DTT	40 µL	120 µL	200 µL	280 µL
Proteinase K (20 mg/mL)	70 µL	210 µL	350 µL	490 µL
Total Incubation Volume *per sample*:				*1000 µL*

1.4 Add the appropriate incubation volume of master mix to each sample tube and Extraction Negative tube. Vortex tubes briefly. Make certain the substrate, tissue, or swab is totally submerged. **Note: Reagent volumes may be adjusted in order to accommodate the size or nature of a particular sample.**

1.5 Wrap each tube (Extraction Negatives and samples) in parafilm after the addition of mastermix.

1.6 Place tubes in a shaking 56°C heat block and incubate overnight.

1.7 Proceed to Section 2: Phenol Chloroform and Microcon Clean up® cleanup.

2 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.

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

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

2.3 Vortex and centrifuge the incubated microcentrifuge tube samples at high speed for 1 minute. Vortex and centrifuge bone dust, incubated in 50 mL conical tubes, for 5-10 minutes at 1000 RPM in Eppendorf Centrifuge Model 5810.

2.4 Obtain and label one prepared Eppendorf Phase Lock Gel (PLG) tube per sample, including the extraction negative.

2.4.1 NOTE: For bone samples, label as many tubes to accommodate the total volume of incubation buffer per sample. For example, if you incubated 2g of bone dust with 3 mL of incubation buffer, you will need 6 PLG tubes.

2.5 Centrifuge PLG tubes at maximum speed for 30 seconds.

2.6 Label Microcon® filters for each sample. Prepare the Microcon® concentrators by adding 100 µL of TE^{-4} to the filter side (top) of each concentrator. Set aside until step 2.13.

2.7 Add a volume of Phenol:Chloroform:Isoamyl Alcohol 25:24:1 (PCIA) to the PLG tube which is equal to the volume of incubation buffer (typically 400 µL) to be added from the sample.

2.7.1 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.

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

2.9 Pipette the sample supernatant (typically 400 µL) to the PLG tube already containing PCIA. For bone dust samples, pipette several aliquots of the supernatant into multiple PLG tubes. **Note: Do not disturb bone pellet.**

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

2.11 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).

2.12 If the sample is discolored, contains particles in the aqueous phase, or contains a lot of fatty tissue, transfer the top layer (aqueous phase) to a new PLG tube and repeat Steps 2.7-2.9.

2.12.1 NOTE: The aqueous layer from bone and teeth will usually be discolored. Only repeat the phenol-chloroform clean-up steps if any dust or particles are present in the aqueous layer. If it is not necessary to repeat the clean-up step, go to Step 2.13.

2.13 Carefully transfer the aqueous phase (top layer) to the prepared Microcon® concentrator. Be careful not to let the pipette tip touch the gel.

2.13.1 Discard used PLG tubes into the organic waste bottle.

2.14 Spin the Microcon® concentrators for 12-24 minutes at 500 x g, which is approximately 2500 RPM. (On Eppendorf Centrifuge Model 5415D, spin at 0.6 RCF or 2600 RPM).

2.14.1 Ensure that most fluid has passed through filter. If it has not, spin for additional time, in 8-minute increments. If fluid more than ~2µL remains, transfer sample to a new filter and microcon again.

2.15 Discard the wash tubes and place the concentrators into a new collection tube.

2.16 Add 400 µL of TE^{-4} to the filter side of each Microcon® concentrator.

2.17 Spin again for 12 minutes at 500 x g. (On Eppendorf Centrifuge Model 5415D, spin at 0.6 RCF or 2600 RPM).

2.17.1 Ensure that most fluid has passed through filter. If it has not, spin for additional time, in 8-minute increments. If fluid more than ~2µL remains, transfer sample to a new filter and microcon again.

2.18 Add 40 µL of TE^{-4} to the filter side of each Microcon® concentrator. For bone samples, add only 10-20 µL of TE^{-4} to each filter side to ensure smallest elution volume.

2.19 Invert sample reservoir and place into a new labeled collection tube. Spin at 1000 x g, which is approximately 3500 RPM, for 3 minutes. (On Eppendorf Centrifuge Model 5415D, spin at 1.2 RCF or 3600 RPM).

2.20 Measure the approximate volume recovered and record the value. **Combine bone elutants before measuring volume.**

2.21 Discard sample reservoir and adjust sample volume depending on the starting amount and expected DNA content as follows using TE^{-4}.

2.21.1 Samples may be microcon'ed again to further concentrate low DNA content samples.

Sample type	**Final Volume**
High DNA content (Small amounts of blood, fresh tissue, bone marrow, oral swabs, and dried bloodstains); differential lysis samples	200 µL
Medium DNA content (Formalin fixed tissue, dried bone, teeth, samples from decomposed or degraded remains, some reference samples)	100 µL
Low DNA content (Teeth, samples from decomposed or degraded remains, pseudo-exemplars)	50 µL

2.22 Transfer samples to newly labeled 1.5mL microcentrifuge tubes for storage. Record the approximate final volume.

2.23 As needed, pipette aliquots of neat and/or diluted extract (using TE^{-4}) into microcentrifuge tubes for real-time PCR analysis to determine human DNA concentration.

2.24 Store the extracts at 2 to 8°C or frozen.

2.25 In the LIMS system, navigate to the Data Entry page, assign the samples to a storage unit (cryobox), and indicate which samples are complete.

NOTE: See Microcon® troubleshooting (in the appropriate section of the STR manual) as needed.