4.1 PURPOSE:

4.1.1 To isolate and purify DNA from bone and teeth samples.

4.2 RESPONSIBILITY:

4.2.1 Forensic Science Examiners from the CT DESPP Division of Scientific Services who have been trained in the discipline of Mitochondrial DNA Analysis according to the Mitochondrial DNA Section Training Manual.

4.3 SAFETY:

4.3.1 Use appropriate measures for the proper handling of physical evidence following the Mitochondrial DNA General Evidence Procedures (mtDNA SOP-01) and according to the Laboratory Safety Manual.

4.4 Extraction from Teeth

4.4.1 Initial Assessment of Teeth

4.4.2 Teeth will be photo-documented prior to any processing. If they need to be cut, the resulting pieces will also be photographed before extraction. Samples may be cleaned using water, 5% Terg-a-zyme™, or 1% SDS prior to extraction based on the nature of the evidence. If soft tissue is present, DNA may be extracted from the soft tissue using the tissue extraction protocol (mtDNA SOP-03). Upon removal of the soft tissue, the resulting piece(s) will be photographed before extraction.

4.4.3 If several teeth are available for analysis, the order of preference for tooth selection for DNA extraction is as follows:

4.4.3.1 Non-restored molar

4.4.3.2 Non-restored premolar

4.4.3.3 Non-restored canine

4.4.3.4 Non-restored anterior tooth

4.4.3.5 Restored molar

4.4.3.6 Restored premolar

4.4.3.7 Restored canine

4.4.3.8 Restored anterior tooth

4.5 Extraction from Bone

4.5.1 Bones will be photo-documented prior to any processing. If they need to be cut, the resulting piece(s) will also be photographed before extraction. Samples may be cleaned using water, 5% Terg-a-zyme™, or 1% SDS prior to extraction based on the nature of the evidence.
If soft tissue is present, DNA may be extracted from the soft tissue using the tissue extraction protocol (mtDNA SOP-03). Upon removal of the soft tissue, the resulting piece(s) will be photographed before extraction. DNA may be extracted from bone marrow using the tissue extraction protocol (see section 4.8.3). Upon removal of the bone marrow, the resulting piece(s) will be photographed before extraction.

4.5.2 If several bones are available for analysis, the order of preference for bone selection for DNA extraction is as follows:

4.5.2.1 Long bone (take a wedge-shaped section to avoid cutting bone in two)

4.5.2.2 Rib (mid-section)

4.5.2.3 Other

4.6 Preparation of Reagent Blank and Cylinder

4.6.1 Thoroughly clean the cylinder, metal end plugs, and impactor. (options: 20% in-house bleach or 10% stabilized bleach, Terg-a-zyme™, DNA Off™). Rinse with dH_2O. Expose the cylinder, end plugs,
and impactor to UV light for a minimum of 15 minutes.

4.6.2 Prepare the reagent blank by swabbing the inside of the cylinder, end plugs, and impactor with an appropriate number of sterile, dry cotton swabs.

4.6.3 Place a portion of one swab in a 2 mL extraction tube. Add ~1.6 mL of 0.5 M EDTA. Agitate tube at room temperature for up to 24 hours.

4.7 Cutting a Tooth

Note: Wear protective eyewear for these steps.

4.7.1 Using a rotary tool with an emery disk, sand the outer surface of the tooth so that the outer surface appears free of debris. Discard the emery disk.

4.7.2 Using the rotary tool with a separating disk, cut the tooth horizontally at the cemento-enamel junction being careful to avoid any restorations that may be present. Make an additional horizontal cut in the tooth slightly apical to the original cut so that a small cross section of root can be separated from the remainder of the tooth. Alternatively, the entire root may be used. Discard the separating disk.

4.7.3 Examine tooth for the presence of soft tissue. If any is present, remove it from the pulp chamber and place it in a sterile 2 mL extraction tube. If required, the material can be extracted following the protocol for DNA Extraction from Body Fluids and Tissues (mtDNA SOP-03).

4.7.4 Proceed to Section 4.9 for pulverization of tooth.

4.8 Cutting a Bone

Note: Wear protective eyewear for these steps.

4.8.1 Using a rotary tool with an emery disk, sand the outer surface of the bone so that the outer surface appears free of debris. If bone has been cut prior to submission or is hollow, also sand interior surface. Discard the emery disk.

4.8.2 Cut the bone using a rotary tool with a separating disk to a sample measuring approximately 1-2 cm X 1-2 cm. Discard the separating disk.

4.8.3 If there is material within the marrow space, remove it with forceps and place it in a sterile 2 mL extraction tube. If required, the material can be extracted following the protocol for DNA Extraction from Body Fluids and Tissues (mtDNA SOP-03).

4.9 Pulverization of Teeth and Bones

4.9.1 Teeth and bones can be pulverized using the SPEX Freezer/Mill. Assemble the cleaned sample vial by inserting one end-plug into the cylinder. End-plugs should be inserted concave side in. Place the impactor in the sample cylinder along with the cut portion of the bone or

tooth. Insert the second end-plug.

4.10 SPEX Freezer/Mill:

4.10.1 Chemical Hazard: Liquid nitrogen can be hazardous. Use cryogenic gloves, appropriate clothing and protective eyewear when handling liquid nitrogen. Be careful not to expose face or hands to liquid nitrogen.

4.10.2 Place the cylinder containing the sample in a 6770 freezer/mill.

4.10.3 Keeping the top of the freezer/mill open, fill it with liquid nitrogen (~ 5L). Close the mill and allow it to sit for approximately ten minutes. After the allotted time, reopen the mill and top it off with liquid nitrogen to the fill line. Close the mill and latch it.

4.10.4 Set the freezer/mill program as follows:

Pre cooling period: 1 minute
Running period: 5 minutes
Cooling period: 2 minutes
Rate: 15 cps
Cycles: 1

4.10.5 Do not open the lid of the freezer/mill during pulverization.

4.10.6 When the pulverizing cycle is finished, open the lid, remove the cylinder with the Extractor and Cylinder Opener, and inspect the sample. If sample is not sufficiently pulverized, reinsert for additional pulverizing. Additional liquid nitrogen may be necessary.

4.10.7 Once the cylinder containing the sample has been removed from the freezer/mill, the cylinder may be left to warm to room temperature before removing the sample, or the cylinder may be opened while still cold. Align the pins on the cylinder end-plug with the slots on the Extractor. Turn the Extractor handle clockwise to release the end-plug.

4.10.8 Remove the impactor and empty the pulverized sample into a sterile 2 ml extraction tube.

4.10.9 Once the freezer/mill has completely cooled and the liquid nitrogen has evaporated, wipe down the interior of the freezer/mill.

4.11 Extraction of DNA

4.11.1 Carefully pour a portion of the pulverized sample (up to ~1/8 – 1/4 of the tube) into a sterile 2 mL extraction tube(s).

4.11.2 Add ~1.6 mL of 0.5M EDTA to each tube. Close tube(s) carefully and mix gently to suspend powder.

4.11.3 Agitate tubes at room temperature for up to 24 hours (8-16 hours is optimal).

4.11.4 Centrifuge tube(s) for one minute at ~8000 X g to pellet powder.

4.11.5 Remove supernatant and discard.

4.11.6 Wash RB swab and powder by adding ~1 mL dH_20 to each tube. Close lid and mix briefly. Centrifuge tubes for one minute at 8000 X g to pellet powder. Remove supernatant and discard.

4.11.7 Repeat water wash step two more times for a total of three washes (or more as needed).

4.11.8 Add ~300 µL SEB/DTT and 2 µL of proteinase-K [600 U/mL] to RB and powder. Mix briefly.

4.11.9 Incubate powder and RB in SEB/DTT with agitation up to overnight (6-8 hours is optimal) in a 56 ºC oven.

4.11.10 Pulse-spin tube(s) in a centrifuge. Place the RB swab in a filter basket, insert basket(s) into extraction tube(s), and pulse-spin. Discard RB swab.

4.11.11 Add ~300 µL AL buffer to each sample and RB tubes. The solution may appear very viscous. Mix briefly. Incubate tubes for 10 minutes at 70ºC. Centrifuge briefly.

4.11.12 Add ~400 µL ethanol to tube. Mix and centrifuge briefly.

4.11.13 Carefully transfer ~500 µL mixture (avoiding any precipitate) directly to QIAamp™ spin column. Avoid touching pipette tip to lip of column and do not touch pipette tip to filter in column. Centrifuge column for one minute at 8000 X g. Discard the collection tube and transfer the spin column to a new collection tube.

4.11.14 Transfer remaining ~500 µL mixture (avoiding any precipitate) directly to QIAamp™ spin column. Avoid touching pipette tip to lip of column and do not touch pipette tip to filter in column. Centrifuge column for one minute at 8000 X g. Discard the collection tube and transfer the spin column to a new collection tube.

4.11.15 Add ~500 µL buffer AW1 directly to QIAamp™ spin column. Centrifuge column for one minute at 8000 X g. Discard the collection tube and transfer the spin column to a new collection tube.

4.11.16 Add ~500 µL buffer AW2 directly to QIAamp™ spin column. Centrifuge column for three minutes at full speed.

4.11.17 Discard the collection tube and transfer the spin column to a new collection tube. Centrifuge column for one minute at full speed.

4.11.18 Transfer spin column to sterile 1.5 mL tube for elution of DNA. Add sufficient AE buffer for recovery of ~ 60 µL. Incubate at room temperature for five minutes. Centrifuge column for one minute at 8000 X g to collect DNA extract.

4.11.19 Using a sterile pipette tip, determine the volumes of the RB and the sample extracts. The elution volumes shall be documented manually on QRM-5. The volume of the RB must not exceed the volume of the sample. If necessary, add AE buffer to bring the sample up to the volume of the RB.4.11.20 Store extracted DNA at 4 ^{o}C.

4.11.21 See mtDNA WI-05 for further instructions on bone and tooth extraction.