Procedure for DNA Extraction of Bone and Teeth

1.0 Purpose – This procedure specifies the steps for pre-processing and DNA extraction of bone and teeth samples.

2.0 Scope – This procedure applies to Forensic Scientists in the Forensic Biology Section who perform DNA extractions of bone and teeth samples for forensic casework.

3.0 Definitions – See Section Definition List

4.0 Equipment, Materials, and Reagents

- Tris/EDTA Solution (TE)
- Promega Bone DNA Extraction Kit (Demineralization buffer, 1-Thioglycerol, Proteinase K Solution, Lysis Buffer)
- Drill bit
- Dremel grinding wheel
- Coffee grinder
- Dremel tool (or equivalent)
- Calibrated pipets (various sizes)
- ART Pipet Tips (or equivalent, various sizes)
- Thermomixer
- Autoclaved microcentrifuge tubes (various sizes)
- 2 mL tubes with a lyse & spin basket (or equivalent)
- Sterile tubes (various sizes)
- Microcon 100 Filters and corresponding centrifuge tubes (or equivalent)
- Vortex mixer
- Various lab equipment (various disposable conical tubes, lab tape, lab coat, lab gloves, microcentrifuge tubes and rack, wipes, etc.)
- 10% bleach solution

5.0 Procedure

5.1 Overview

5.1.1 All known samples shall be extracted separately from unknown samples. Thermomixers shall be designated exclusively for either “knowns” or “unknowns.”

5.1.2 The amount of sample take shall be determined based upon the scientist’s evaluation of sample (e.g., age, condition, and type of sample).

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5.1.3 No more than 30 total samples (knowns and unknowns) shall be extracted as a batch. Exemptions to this item limit shall be approved only with written documentation from the Technical Leader.

5.1.4 Negative extraction control

5.1.4.1 For each case, a reagent blank shall be prepared each time an extraction set is begun (i.e., knowns and unknowns). This blank will consist of the reagents used in the extraction process and shall be treated the same as other samples throughout the entire process. Also, the final volume of this control shall be the same as the forensic sample(s) brought up in the most minimal volume and amplified using the maximum volume.

5.1.4.2 Due to the amount of sample needed for an extraction (i.e., number of swabs, size of the cutting) it may be necessary to split the extraction of a sample across multiple extraction tubes. In such instances, multiple reagent blanks will need to be created. At the point when the separate extractions of the sample are recombined, prior to amplification, the same number of reagent blanks must also be combined. This ensures that the same quantity of reagents have been processed in the reagent blank as the given sample.

5.1.4.3 It is acceptable to run more than one reagent blank in anticipation of having to re-run or dilute samples for amplification.

5.1.4.4 If additional extractions are performed, the associated negative extraction controls shall have a unique identifier (different date will suffice as identifier).

5.1.4.5 The requirement for extracting a reagent blank concurrently with each case applies to samples extracted on or after July 1, 2009. If the sample is resubmitted for additional analysis and the extraction occurred prior to this date, there may not be reagent blanks present to reprocess. A note will be added to the case notes that details this exception. A blank consisting of the same amp grade water being used in the sample(s) will be prepared and processed for these samples. Cases processed after this date shall require reagent blanks for any reprocessing requests to proceed.

5.1.4.6 All tubes shall be labeled with a unique identifier.

5.2 Preprocessing of Bone/ Teeth – Sample preparation shall be performed in a Biological Safety hood.

5.2.1 If tissue and/or marrow is associated with the submitted bone, and the sample is not degraded, then take a sample and place in a separate labeled microcentrifuge tube(s) for DNA extraction.

5.2.2 Cleaning the bone.

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5.2.2.1 Small bones/tooth: If the bone/tooth is soiled, it may be necessary to remove any debris or associated dirt from the bone or part of the bone prior to cutting/grinding. The bone/tooth shall be placed in a weigh boat and a new toothbrush and sterile dH2O used to physically remove any excess dirt.

5.2.2.2 Large bones: The area of the bone to be cut and used in analysis shall be ground off mechanically using a Dremel tool (cleaned with 10 % bleach) and a new grinding bit to remove soil and dirt.

5.2.2.3 Using a cutting tool (e.g., sterile scalpel blade or Dremel tool with a cut-off wheel) remove any associated tissue on the bone to be processed. Note: It is helpful to remove the tough fibrous membrane, the periosteum, prior to processing because the removal aids in the extraction process.

5.2.3 Sample for DNA extraction using one of the following methods.

5.2.3.1 Cutting/Grinding

5.2.3.1.1 Remove a cross-sectional wedge or rectangle of bone using a cutting tool (e.g., Dremel tool with a cut-off wheel). Avoid cutting the bone in half unless necessary due to size; this preserves the bone for further anthropological study.

5.2.3.1.2 Obtain a new coffee grinder.

5.2.3.1.3 Wipe down inside and outside with a Kim-wipe wetted with fresh 10 % bleach. Wipe down a second time with 100 % alcohol. Allow to dry.

5.2.3.1.4 Place the tooth/cut sample into the cleaned grinder and grind the sample to a fine powder. Note: for small bones that are too small for the coffee grinder, a mortar and pestle may be used to grind the bone to a powder for DNA extraction.

5.2.3.2 Drilling

5.2.3.2.1 Using a decontaminated drill bit and a cleaned Dremel tool, drill 4 to 5 holes through the bone. To decontaminate the drill bit, wipe down with a Kim-wipe wetted with fresh 10 % bleach. Then, wipe down a second time with 100 % alcohol. Allow to dry.

5.2.3.2.2 Collect the powder produced from the drilling for analysis.

5.3 DNA Extraction

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5.3.1 Aseptically transfer 0.025 g to 0.050 g of drilled bone powder or 0.1 g to 0.2 g of ground bone powder to a 2 mL tube or Lyse & Spin basket for extraction. Place any excess bone or tooth powder into a separately labeled tube and save.

5.3.2 Prepare bone lysis cocktail A as follows (volume for n=1 sample):

400 µL Demineralization Buffer
40 µL Proteinase K Solution
10 µL 1-Thioglycerol

5.3.3 Add 400 µL of bone lysis cocktail A to each tube containing bone powder. Vortex for approximately 10 seconds to mix.

5.3.4 Incubate the tube containing the sample at 56 °C for approximately 2.5 hours in a thermomixer set to approximately 700 rpm. Based on sample type incubation may extend to overnight.

5.3.5 Vortex for approximately 10 seconds to mix. Spin in a microcentrifuge at high speed for 5 minutes. If using a Lyse & Spin basket, remove the basket and discard into a biohazard waste container. If bone powder was placed directly into a 2 mL tube, carefully transfer the supernatant to a newly labeled 2 mL tube, taking care not to disturb the bone pellet.

5.3.6 Prepare bone lysis cocktail B as follows (volume for n=1 sample):

990 µL Lysis Buffer
10 µL 1-Thioglycerol

5.3.7 Add 800 µL of bone lysis cocktail B to each tube containing lysate from step **5.2.3.5.** Vortex for approximately 10 seconds to mix.

5.3.8 Divide the liquid from each digested sample into two (2) 2 mL microcentrifuge tubes. Each tube should contain approximately 550 µL.

5.3.9 Proceed with DNA purification using the Large Volume protocol the EZ1 Advanced XL or EZ2 Connect Fx according to the Forensic Biology Procedure for DNA Extraction.

5.3.10 Once the DNA purification has been completed on the EZ1/EZ2 instrument and the sample tubes have been removed combine/concentrate the sample according to the Forensic Biology Procedure for DNA Extraction.

5.3.11 The DNA is ready to use or can be stored refrigerated at 2-8 °C until quantitation.

5.4 Storage of DNA Extracts - Store the samples at 4 °C (short term) or frozen (long term.) Prior to use of samples after storage, they shall be vortexed and then centrifuged for 5 seconds.

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6.0 Limitations – N/A

7.0 Safety

7.1 Wear eye protection when using power tools.

8.0 References

Forensic Biology Section Procedure for DNA Casework Training
Forensic Biology Section Procedure for DNA Extraction
Forensic Biology Section Evidence Handling Procedure

9.0 Records – N/A

10.0 Attachments – N/A

Revision History		
Effective Date	Version Number	Reason
02/71/2023	1.0	Initial document.

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