DNA EXTRACTION ON THE EZ1® ADVANCED XL – Teeth and Bone

Background

A human bone sample can be tested as a means of determining parentage or identity. The sample may be a product of medical examiner collection of remains, crime scene evidence collection or other human bone.

The EZ1® Advanced XL and the EZ1® DNA Investigator Kit reproducibly automate purification of genomic DNA from 1-14 samples encountered in forensic applications. Purification is efficient, and purified DNA is suitable for use in downstream analyses, such as quantitative PCR and STR analysis, with high signal-to-noise ratios.

The magnetic-particle chemistry is similar to that currently used at the Marshall University Forensic Science Center (MUFSC) in Promega's DNA IQ method, and can provide a higher yield when Carrier RNA, included in the kit, is utilized. The EZ1 Advanced XL performs all steps of DNA purification, post-digestion, and can accommodate an input volume of 250µL for extraction.

Magnetic-particle technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. DNA is isolated from lysates in one step through its binding to the silica surface of the particles in the presence of a chaotropic salt. The particles are separated from the lysates using a magnet. The DNA is then efficiently washed and eluted in water or TE buffer.

Summary of Procedure

This protocol is for the extraction of teeth and bone samples. The cells in human bone are a source of nuclear DNA. Slicing the bone into small pieces and pulverization will increase the material surface area and increase the efficiency of DNA extraction. The addition of buffer ATL, EDTA, Pro K, and DTT, followed by a 24 hour incubation at 56 degrees Celsius, will release the nuclear DNA material from the cells. The nuclear DNA material can then be purified and concentrated on the EZ1® Advanced XL instrument. This protocol was based on the user developed protocol "Forensic "Quick Bone DNA extraction" protocol using the EZ1 DNA Investigator Kit", developed by the North Louisiana Criminalistics Laboratory and the EZ1® Investigator Handbook, Fourth Edition, April 2009 from Qiagen. Minimal changes were made to accommodate both the large volume protocol on the EZ1 and also in house quantitation results of approximately 60 samples (teeth and bone).

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, *Forensic Evidence Handling*

After extraction is complete, samples are securely stored at 2-8C.

Warnings and Precautions

Buffer G2 contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. This reagent is **not** compatible with disinfecting agents which contain bleach.

Reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful, and irritants.

Reagents and Materials

Sterile transparent 2.0mL micro-centrifuge tubes and rack
Razor blade
Forceps
Dremel Tool with sanding barrel attachment and rotary cutting bit
6770 Freezer/Mill
Grinding Vial Set
Liquid Nitrogen

EZ1® Advanced XL instrument
EZ1® DNA Investigator Kit (48 reactions)
- 48 Reagent Cartridges
- 50 Tip Holders
- 50 Filter-Tips
- 50 2mL Sample Tubes (Digestion)
- 50 1.5mL Elution Tubes
- 310µg Carrier RNA

Additional Buffer ATL (260mL bottle, available separately from the EZ1® DNA Investigator Kit)
Additional Buffer MTL (260mL bottle, available separately from the EZ1® DNA Investigator Kit)
Sodium Acetate pH 5.0
20mg/mL Proteinase K
1M Dithiothreitol (DTT)
0.5 M EDTA pH 8.0
Pipettes (1000µL, 200µL, 40µL, and 10µL)
Pipette tips
Heat block
1.7mL or 2mL micro-centrifuge tubes
Permanent marker
Kimwipes
Vortex
Centrifuge
Protective eyewear
Gloves
Lab coat

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the ***EZ1 Sample Extraction Worksheet - Teeth and Bone*** is required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (TF- Optional): Positive control(s) are processed and run with each set of extractions and used to ensure the extraction, amplification and typing procedures are working as expected.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions and consists of all reagents used in the procedure but contains no DNA sample.

Procedure

1. Describe the sample received in the case notes. Note the description of the sample container, specific markings, color of the bone, specific descriptions related to preservatives added if applicable, liquid volume and any other unique identification.

2. Open the container in the biological safety cabinet with proper safety equipment.

3. Recommendations for bone and tooth follow, however, depending on the condition of the sample some steps may not be necessary and some steps, such as bleaching and UVing can be changed at the processor's discretion.

   Cleaning of bone is dependent on the type and quality.
   a) Remove any tissue/ debris from the bone with a sterile razor (tissue should be preserved for possible DNA analysis)
   b) Rinse with MBG water (several seconds)
   c) Rinse with ethanol (several seconds)
   d) Dry with Kimwipe
   e) UV 30 min
   f) Sand top layer of bone (1-2mm) to remove contaminates
   g) Repeat steps above as needed
   h) Use a Dremel Tool to make a rectangular, grid-like pattern, cut in the middle of the bone, such that it can be split into 4-8 squares ~2cm^2.

   Cleaning of teeth is dependent on the type and quality.
   a) Soak the tooth in 20mL of regular Clorox bleach for 20 minutes
   b) Soak the tooth in 20mL of MBG water for 20 minutes
   c) Rinse with ethanol
   d) Dry the tooth under UV, 10 minutes on each side

4. These pieces of bone and/or teeth should be cut in a manner which allows the sample to easily fit inside the Freezer/Mill cylinder so that the impacting rod can move freely inside the assembly. **Note: A commercial blender may be used for pulverization. If a blender is used, proceed to step 10 after pulverizing. Refer to page 6 for grinding vial assembly.**

5. Add the sample to the grinding vial set. Be sure to leave enough room in the cylinder for the impactor to move back and forth.

6. Slowly pour liquid nitrogen into the Freezer/Mill chamber up to the fill line. Place the grinding vial into the hole of the assembly. Additional samples can be added to the upper rack of the lid

assembly so that up to three vials are cooling at the same time. Slowly close the lid of the mill and fasten.

Caution: Liquid nitrogen is susceptible to overflowing when closing the lid. Wear proper protective gear and take caution. Ensure the workspace is clear of any tangible items that may be hazardous or cause damage if in contact with liquid nitrogen.

7. Press the run button on the Freezer/Mill key pad. A pre-cooling period of 10 minutes is pre-programmed, followed by a pulverizing cycle of 3 minutes and a rest cycle of 1 minute. The pulverizing and rest cycle are repeated 3 times. Lower the rate cycle to 10 to reduce the production of heat during the pulverizing cycle.

8. Once the program is completed, remove the grinding assembly using the extractor and allow the assembly to reach room temperature. If multiple grinding assemblies were placed in the mill, place a new sample in assembly hole. The pre-cooling period does not need to be repeated since the sample has already been cooled. To modify the pre-cooling period, press the T3 key and enter a value of one minute. Press enter. Press run.

9. Once the grinding assembly has reached room temperature, remove the pulverized sample.

10. Weigh out multiple samples of the pulverized material (target sample weight ~0.20 grams). Place each sample into a labeled microcentrifuge tube.

11. Samples are ready for incubation.

Incubation:

- Pre-warm thermomixer to 56°C
- Reagent blank starts at this step in the procedure

1. Add 675uL of Buffer ATL, 75uL of Proteinase K, 750uL of 0.5 M EDTA pH 8, and 60uL of 1M DTT to each tube. Place on a rocker or invert several times to mix. Continue until the samples look suspended in the master mix. These volumes may be increased if the bone material soaks up the reagents. Note the volumes of the above reagents added to the samples on the **EZ1 Sample Extraction Worksheet-Teeth and Bone**.

2. Incubate the samples at 56°C on the thermomixer set at 900rpm for approximately twenty-four (24) to forty-eight (48) hours.

3. At the end of the twenty-four (24) to forty-eight (48) hour incubation centrifuge at 13,200rpm for 10 minutes and split the supernatant into three 500uL aliquots.

4. While the sample is still **warm**, add 400uL of **warm** Buffer MTL, 30uL of 3M Sodium Acetate pH 5.0, and 1uL of Carrier RNA and follow the remainder of the **EZ1 Sample Extraction Worksheet-Teeth and Bone** with the large volume protocol**.**

EZ1 Setup:

- When a new kit is opened:
  - Tip Holders, Filter-Tips, Sample Tubes and Elution Tubes must be cross-linked at 6J/cm^2.
  - 310µL of nuclease-free water must be added to the dehydrated carrier RNA. Dispense carrier RNA into 30µL aliquots, and store frozen.

Before starting:

- Allow Carrier RNA aliquot to equilibrate to room temperature.

1. Label all micro-centrifuge tubes, EZ1 sample tubes and EZ1 elution tubes with permanent marker or printed labels.

2. Switch on the EZ1 workstation.

3. Press “START” to start the protocol setup.

4. Choose the appropriate protocol.
    - Press “3” for Large-Volume Protocol (for volumes up to 500uL and substrate removed)

5. Choose water or TE as the elution buffer and a 40uL elution volume. Press any key to start the worktable setup.

6. Open the workstation door and invert 1-14 reagent cartridges at least twice to mix the magnetic particles. Tap cartridges to deposit reagents at the bottom of the well. Load reagent cartridges into the cartridge rack, (Make sure they click in place.)

7. Load 1-14 opened elution tubes (labeled) into the first row of the tip rack.

8. Load 1-14 tip holders containing filter-tips into the second row of the tip rack.

9. Load 1-14 opened sample tubes containing digested samples into the back row of the tip rack.

10. Close the workstation door and press “START” to begin the purification procedure.

11. “Protocol Finished” should display when it is finished.

12. Open the workstation door and remove the elution tubes containing purified DNA. Replace tube caps and store samples appropriately.

13. Remove spent cartridges, tip holders, filter-tips and samples tubes and discard appropriately.

14. Close the workstation door and follow the prompts to UV irradiate the workstation for 30 minutes.

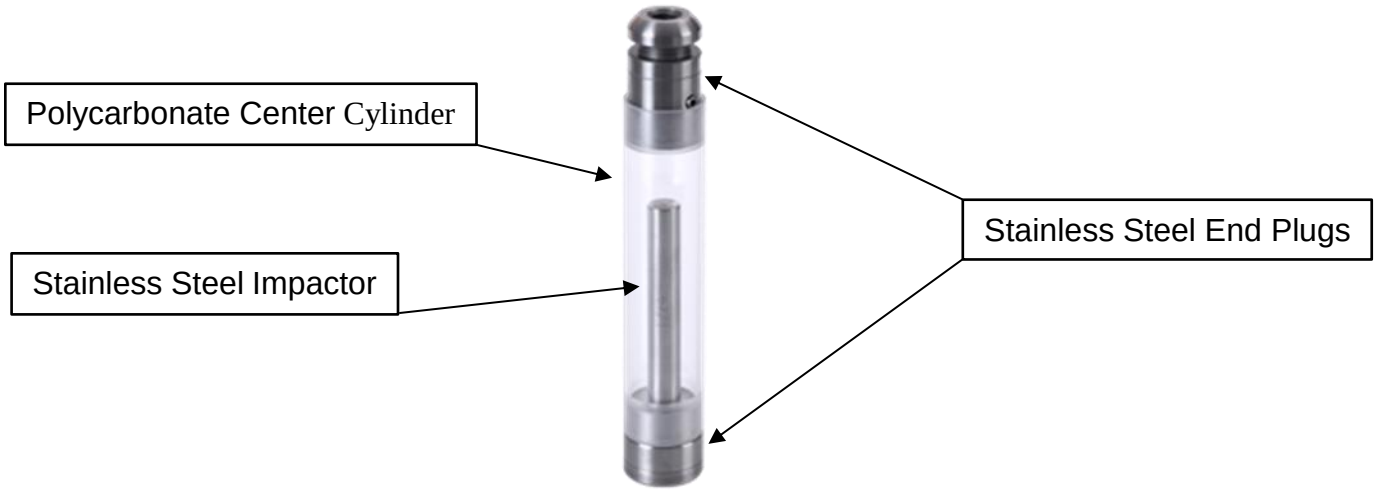
Comments on Storage

Non-amplified DNA must be stored separately from amplified product. Store samples at 2-8°C.

Grinding Vial Assembly

Grinding Vial Components:

- Stainless steel end plugs
- Impactor (stainless steel)
- Polycarbonate center cylinder (plastic)
- Channel lock pliers



1. Take the rounded edge stainless steel end plug and place the flat edge into the polycarbonate center cylinder until it touches (or nearly touches) the notches.
   Note: Make sure you do not observe any cracks forming in the polycarbonate cylinder. If cracks are observed do not continue.

2. Place the bone inside the cylinder. Then, place the impactor into the cylinder.

3. Take the flat edge stainless steel end plug and place the concave flat edge into the polycarbonate center cylinder.

4. Shake the assembled grinding vial to ensure the impactor can move freely.
   Note: If the impactor cannot move freely and a metal pounding sound cannot be heard than there is too much sample in the vial. A portion of the sample will need to be removed before proceeding.

Grinding Vial Disassembly

1. After removing the grinding vial from the 6770 Freezer Mill, thaw overnight to prevent damages.

2. Use the channel lock pliers to remove the stainless steel end plugs by gripping the plug and twisting until removed.

3. After disassembly, place all components into a can of distilled water.

Technical Assistance

For information and assistance regarding the performance or applications, contact the Qiagen Technical Service Department 1-800-362-7737.

Reference

1. EZ1® Investigator Handbook, Fourth Edition, April 2009. Qiagen

2. User Developed, Protocol. "Forensic "Quick Bone DNA extraction" protocol using the EZ1 DNA Investigator Kit". Developed by the North Louisiana Criminalistics Laboratory; 1115 Brooks St.; Shreveport Louisiana, 71101.
3. Small Grinding Vial Set, Part #: 6751, SPEX® SamplePrep, Metuchen, NJ.