TissueLyser II and EZ1 DNA Investigator Kit Bone/Teeth Extraction

1. Purpose

This document describes the procedures for performing a digest and extraction of bone and teeth samples on the TissueLyser II and the Qiagen EZ1 Advanced XL (EZ1) instrument.

2. Summary

Describes pre-processing reagent preparation. Materials and protocol for bone and teeth extractions is provided. This protocol is for extraction using the TissueLyser II and Qiagen EZ1 Advanced XL Extraction instrument with the Qiagen DNA Investigator Extraction Kit.

3. Procedure

Reagent Preparation

1. Bleach Solution
    a. Mix commercial bleach (6%) in equal parts with deionized water
    b. Store at room temperature.
2. Carrier RNA (cRNA)
    a. Add 310µL dH2O or TE to the vial of carrier RNA (310µg), provided in the EZ1 DNA Investigator kit.
    b. Transfer entire volume into 20µl aliquots.
    c. Store aliquots frozen.

Sample Preparation

1. If appropriate, remove the outside layer of the bone fragment with a new, clean piece of sandpaper (or other Dremel attachment).
2. Sample an approximately 2cm X 2cm fragment of bone using a Dremel equipped with a new, clean attachment.
    a. More than one fragment may be collected to increase the amount of bone powder available for downstream processing, based on the nature of the sample and previous analysis results, if available.
3. Submerge the fragment in the bleach solution inside a 50mL conical tube.
    a. Vortexing in the bleach solution may be performed for especially dirty samples.
4. Rinse the bone with sterile water for a total of three (3) washes by discarding the bleach solution from the conical tube and replacing with sterile water.
5. Allow to air dry overnight in a ventilated hood.

TissueLyser Operation

1. Place one bone fragment or up to two teeth in a Stainless-steel Grinding Jar with one stainless steel grinding ball.

2. Submerge the Stainless-steel Grinding Jar in a 95% alcohol/dry ice bath for 5-10 min prior to adding it to the TissueLyser II. To avoid damage to Grinding Jar and keep it moisture free, it should be placed in a sealed Ziploc bag (or similar) before submerging in alcohol/dry ice.
3. Place the grinding jar into the clamp of the TissueLyser II. Rotate the handwheel clockwise with two fingers until the grinding jar is seated in the clamp, is level, and does not move freely.
4. Continue to rotate the handwheel clockwise until 6-8 easily audible clicks are heard; the locking pin will rise and fall with each audible click.
    a. Ensure that the pin is in the fully lowered position before starting the instrument. If only processing one sample, place an empty grinding jar containing a stainless-steel grinding ball in the other clamp to ensure the instrument is balanced during operation.
5. Grind the fragment(s) for 2 minutes at 30 Hz.
6. Transfer the powder to a clean tube for storage.
7. Repeat steps 1-6 for all bone fragments/teeth sampled.
8. Clean the stainless-steel canisters with bleach followed by ethanol, once processing is completed.

Extraction Documentation

1. Open a DOC-307 Case Chronicle document and save a copy to the Batches in Progress folder on the Shared Drive as the Batch ID with the following naming format: YYMMDDAnalyst Initials (Example: 230215SW)
    a. If previous extractions were performed for this batch, then open the previously saved document.
2. Click on the Extraction Bones and Teeth tab of the saved Case Chronicle.
3. Enter the following information into the header of the document:
    a. Batch ID
    b. Performed by
    c. Date of extraction
    d. Reagent Lot Numbers
4. Enter the sample identification numbers for all samples that will be extracted in the batch
5. For forensic case samples, enter a reagent blank named with the following name convention: Numerical portion of the IMF Case Number without the place holder zeroes – Batch ID – RB (Example: 231-230215SW-RB) between each set of case samples included on the tray.

Item ID		Final Extraction Volume
IMF-23-00001-01-01-A		40
IMF-23-00001-02-01-A		40
231-230215SW-RB		40
IMF-23-00002-01-01-A		40

232-230215SW-RB		40

Digestion

1. Transfer up to 200 mg of powder to a 2mL conical bottom tube.
2. Create a master mix using 337.5µL Buffer ATL, 37.5µL Proteinase K, and 375µL 0.5M EDTA, pH 8.0 for each sample/reagent blank, plus overage, and mix thoroughly by vortexing.
3. Opening each tube individually, add 750µL to each sample.
4. Vortex vigorously to ensure all the bone powder is incorporated into solution.
5. After adding master mix to all samples associated with a forensic case, create one reagent blank by pipetting 500µL of the master mix into an empty tube between cases.
6. Place the tube in a thermomixer and incubate at 56°C overnight while shaking at maximum speed.
7. Centrifuge the tube for 5 minutes at maximum speed to pellet the bone powder.
8. Transfer the supernatant to a clean 2mL EZ1 sample tube (or other tube compatible with the EZ1 rack)
    a. The powder will absorb the digest buffer. At least 500µL is needed for the EZ1 Large-Volume Protocol.
9. Create a master mix using 400µL warm MTL buffer, 30µL 3M NaOAC, pH 5.2, and 1µL cRNA for each sample/reagent blank, plus overage, and mix thoroughly by vortexing.
10. Add 431µL to each sample and reagent blank.

    Note: Starting the EZ1 run with the samples still warm will help avoid possible precipitation.

EZ1 Instrument Run for Lysate Purification

1. Ensure the EZ1 Advanced XL DNA Investigator Flip-Cap Card is loaded in the instrument.
2. Turn the instrument on by clicking the toggle switch on the back panel of the instrument, next to the power cable.
3. Open the EZ1 door, press **START** on the control panel.
4. Set up the EZ1 instrument worktable:
    a. Invert reagent cartridges to mix the magnetic particles then tap the cartridges to deposit the reagents at the bottom of their wells and check that the magnetic particles are completely resuspended.
    b. Load the reagent cartridges into the cartridge rack by sliding the reagent cartridge into the rack and then pressing down until it clicks into place.
    c. Load opened elution tubes into the first row of the tip rack.

i. Prior to loading tubes, mark each with the associated item number that will be eluted into the tube.

d. Load tip holders and filter-tips into the second row of the tip rack.

e. Load open sample tubes into the third row of the tip rack.

i. Cutting off the lids can lower contamination risk if flip cap tubes are used.

3. Select the following parameters for the instrument run:

a. Press "3" for the "Large Volume Protocol"

b. Press "1" to elute in water

c. Press "1" to for a 40 µL elution volume

5. Press **Enter** to move through the instrument set-up verification prompts.

a. Review the set-up of the instrument to confirm it matches the prompts.

6. Close the EZ1 door.

7. Press **START** to start the protocol run.

8. After the run is finished, remove and cap the elution tubes, which contain the purified samples, and discard the sample-preparation waste and used cartridges.

a. Do not place the waste in a receptacle which also may contain bleach.

9. Perform Post Run UV Maintenance following the on-screen prompts.

4. References

EZ1® Advanced XL User Manual
EZ1® DNA Investigator® Handbook
TissueLyser II User Manual
User-Developed Protocol: Small Volume DNA Extraction from Bone or Teeth Using the EZ1® DNA Investigator® Kit and TissueLyser II (QIAGEN®, May 2020)

5. Definitions

N/A