| **Protocol Name/Title:**<br>**Ancient/Historic DNA Extraction from Bone/Teeth w/ Qiagen MinElute** | |
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| Based on:<br>**Dabney and Meyer 2019** | Last Edited Date:<br>08/05/2022 |

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## 1E. ANCIENT DNA EXTRACTION

### PRINCIPLE:

Ancient DNA extraction protocol based on Dabney and Meyer (2019) from *Ancient DNA: Methods and Protocols*. This protocol is designed to obtain fragments as short as 35 base pairs in length.

### CHEMICALS AND REAGENTS:

- 0.5 M EDTA pH 8.0
- 1 M Tris-HCl pH 8.0
- Proteinase K
- Guanidine hydrochloride
- 3 M sodium acetate pH 5.2
- PE buffer (Qiagen)
- Tween-20
- Isopropanol

### CONSUMABLES (see Note 1):

- 1.5 mL tubes
- 2.0 mL tubes
- 15 mL Falcon tubes
- 50 mL Falcon tubes
- MinElute PCR Purification Kit - spin columns
- Zymo-Spin V columns, Zymo Research

### Benchtop Bleach:

For cleaning work surfaces, make a fresh 1:5 dilution with distilled water.

### TRAINING REQUIRED:

- Clean-room protocol training
- Pipettes (Dependent on prior experience)
- Centrifuges (Dependent on prior experience)

Please see Lauren Clark prior to starting any work detailed in this protocol if you have not completed all the training listed above.

🖐 Indicates gloves should be changed and/or that clean gloves are required.
🧍 Indicates a potential 'break point' in the protocol.

**PROCEDURE:**

**1. Prepare Buffers** (*see* **Note 1):**

- Extraction buffer (.45 M EDTA, 0.25 mg/mL Proteinase K, 0.05% Tween-20 [final concentrations]).
    - Example for 10 mL (1 mL/sample):
        - 745 uL $H_2O$
        - 9 mL EDTA (0.5 M, pH 8)
        - 250 uL Proteinase K (10mg/mL)
        - 5 uL Tween-20

- Binding Buffer (5 M GuHCl, 40% Isopropanol [final concentrations]). (*See* **Note 2**)
    - Example for 100 mL (10 mL/sample)
        - 47.76 g GuHCl
        - 40 mL isopropanol
        - 50 uL Tween-20
        - Water to 60 mL

- Working TET Buffer
    - Example for 50 mL (made in advance for 1000 samples; stored at RT)
        - 100 µL EDTA (0.5 M, pH 8)
        - 500 µL Tris–HCl (1 M, pH 8)
        - 25 µL Tween-20
        - Water to 50 mL

**2. Prepare Samples**

1. Collect approx. 10-150 mg of powderized sample in a 2 mL tube 🖐. *See* **Note 3**.
    a. Note: Gloves ( 🖐 ) should be changed between the handling of each sample.

2. Add 1 mL of extraction buffer. Mix well by vortexing.

3. Incubate 16–24 hours (overnight), rotating at 37° C. *See* **Note 4** for setup instructions if using the alternative spin-column assembly. 🧍

**3. DNA Binding, Wash, and Elution**

1. For each sample and control, transfer ~10 mL of binding buffer to a labeled 15mL tube (*see* **Note 5**), and add 400µL 3M sodium acetate.

2. Centrifuge samples from step 3 (above) for 2 minutes at maximum speed in a benchtop centrifuge to pellet sample.

3. Transfer supernatant to the 15 mL tube containing the binding buffer. Mix gently by shaking. The pellet can be stored at -20° C until the conclusion of the project.

4. Pour the sample/binding buffer mixture into the reservoir of the spin-column assembly, and close the 50 mL tube with a screw cap. Centrifuge for 4 min at 400 x g. Rotate tubes 90° and centrifuge for another 2 min at 400 x g.

5. Remove the screw cap from the 50 mL tube and transfer the spin-column assembly to a clean 2 mL collection tube. Carefully remove and discard the extension reservoir. If desired, the 50 mL tube with flow-through can be stored at -20° C until the conclusion of the project.

6. Close and label the spin-column cap.

7. Perform a dry spin for 1 min at 3000 x g in a benchtop centrifuge. Discard any flow-through.

8. Add 750 µL PE buffer to each column. Centrifuge for 30s at 3000 x g. Discard flow-through.

9. Repeat step 8 for a total of two washes.

10. Perform a dry spin for 1 min at maximum speed (~16,000 x g), turning the columns in the centrifuge 180° relative to their previous orientation.

11. Transfer the column to a clean 1.5 mL tube.

12. Add 50 µL TET buffer directly onto the silica membrane. Let sit for 5 minutes.

13. Centrifuge 1 min at maximum speed.

14. Repeat steps 12 and 13 by transferring the eluate back onto the silica membrane, so that the final elution volume remains 50 µL.

15. Transfer the eluate (final DNA extract) to a clean 1.5 mL tube. Extracts should be at -20°C until the conclusion of the project.

## 4. Notes

1. Buffers can be UV irradiated before use.

2. Add salt to a 50 mL tube, and fill with water to 30 mL, using gradations on the tube. Mix to dissolve salt. Add remaining reagents.

3. More than 150 mg of bone powder is not recommended.

4. Treat extension reservoirs with bleach and rinse well with molecular biology grade water. Let dry and UV irradiate.

   Force extension reservoir into the opening of a MinElute spin column. Remove the extension reservoir-MinElute assembly from the MinElute collection tube, and place in a 50 mL conical tube. Save the collection tube for subsequent wash steps.

   The extension reservoir-MinElute assembly may become detached during centrifugation. It is advisable here to test the assembly with a dry run in the centrifuge.

5. Using the gradations on the tube is sufficient.