Protocol Name/Title: **Ancient/Historic DNA Extraction from Bone/Teeth w/ Qiagen QiaQuick**	
Based on: **Yang et al. 2008**	Last Edited Date: 9/14/2022

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2E. ANCIENT DNA EXTRACTION

PRINCIPLE:

Ancient DNA extraction protocol based on Yang et al. (2008) - "Wild or domesticated: DNA analysis of ancient water buffalo remains from North China". This protocol is designed to obtain fragments as short as 50 base pairs in length.

CHEMICALS AND REAGENTS:

- 0.5 M EDTA pH 8.0
- Proteinase K
- QiaQuick Purification kit and buffers (PE, PB, and EB buffers)
    - Prepared according to the manufacturer's directions
- 10% SDS (*see* **Note 1**)

CONSUMABLES:

- 1.5 mL tubes
- 2.0 mL tubes
- 15 mL Falcon tubes
- 50 mL Falcon tubes (optional – *see* **Note 2**)
- Amicon 10kDA centrifugal filter units
- QiaQuick Purification Kit - spin columns

Benchtop Bleach:

For cleaning work surfaces. Approximately 1:5 dilution with distilled water.

TRAINING REQUIRED:

- AbLab-specific clean-room protocol training (required for all new researchers)
- 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. Locate reagents for buffers:

- Extraction/lysis buffer (.5 M EDTA, 0.5 mg/mL Proteinase K, 0.25% SDS [final concentrations]).
    - Example for 10 mL (3 mL/sample):
        - 8.5 mL EDTA (0.5 M, pH 8)
        - 250 uL Proteinase K (10 mg/mL)
        - 250 uL SDS (10%)

2. Prepare Samples

1. Collect approx. 100-200 mg of powderized or cut sample in a 15 mL tube. *See* **Note 3.**
    a. Note: Gloves () should be changed between the handling of each sample.

2. Add 3 mL of extraction buffer to each sample and blank tube. Mix well by vortexing.

3. Incubate 16–24 hours (overnight), rotating at 50° C.

3. DNA Binding, Wash, and Elution

****Prior**

1. Centrifuge samples at maximum for 6 min in a large benchtop centrifuge to pellet sample. If desired, the pellet can be stored at -20° C until the conclusion of the project.

2. Transfer 2.5 mL of supernatant to a 10kDA Amicon tube and centrifuge at maximum speed until less than 100uL remains above the filter. **Notes: this might take upwards of 2-3 hours.

3. Prep for incubation step by setting the instrument to 70°C.

4. Transfer 500uL of PB buffer into the Amicon tube and gently pipette the sample/buffer mixture up and down to mix.

5. Transfer 600uL of this mixture to a Qiagen QiaQuick spin column.

6. Spin down mixture in 2mL spin column at 13,000krpm for 3 minutes in a micro centrifuge. Discard flow-through.

7. Add 400 µL PE buffer to each column. Spin down for 1 min at 6krpm. Discard flow-through.

8. Repeat step 7 for a total of two washes.

9. Perform a dry spin for 3 min at maximum speed, turning the columns in the centrifuge 180° relative to their previous orientation.

10. Transfer the column to a clean conical collection tube.

11. Add 100 uL EB buffer. *See* **Note 4**.

12. Incubate sample at 70°C for 4 minutes.

13. Centrifuge 1 min at maximum speed.

14. 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.

15. Repeat steps 11-13 by for two eluates.

4. Notes

1. Sodium dodecyl sulfate (SDS) is a detergent and is optional in ancient DNA extraction. It is recommended that SDS if the bone or tooth sample has not been cleaned prior to extraction or appears to be particularly 'dirty' during the sample preparation. Residual dirt on the sample can increase the likelihood of co-extraction of humic acids, which can inhibit a PCR reaction.

2. 50mL Falcon tubes are only necessary if it is preferable to complete a pre-digestion of the bone or teeth samples.

3. More than 200 mg of bone powder is not recommended.

4. The volume of the eluate can be reduced (down to 50uL) to concentrate the amount of DNA in the extract.