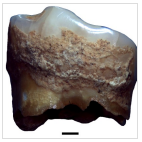

Dec 09, 2020

Ancient DNA Extraction from Dental Calculus with Consolidant Removal

Forked from Ancient DNA Extraction from Dental Calculus

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1 Works for me dx.doi.org/10.17504/protocols.io.bqbmmsk6

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ABSTRACT

Silica-based total DNA extraction protocol optimised for the recovery of ultra-short DNA molecules from archaeological dental calculus, modified from Dabney *et al.* (2013) *PNAS* (doi: 10.1073/pnas.1314445110) and adapted for dental calculus by Mann *et al.* (2018) *Scientific Reports* (doi: https://doi.org/10.1038/s41598-018-28091-9).

This protocol includes an optional acetone wash step to remove conservation consolidants (e.g. glue) from dental calculus that can sometimes be found on samples archived in museum collections.

DOI

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PROTOCOL CITATION

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FORK NOTE

FORK FROM

Forked from Ancient DNA Extraction from Dental Calculus, Zandra Fagernäs

KEYWORDS

Dabney, aDNA, extraction, DNA extraction, nucleic acids, ancient DNA, palaeogenetics, archaeogenetics, paleogenetics, archeogenetics, dental calculus

IMAGE ATTRIBUTION

Dental calculus deposit buildup on the molar of a Neanderthal; photo courtesy of Julie Arnaud, University of Ferrara

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Dec 09, 2020

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45133

GUIDELINES

Working in an Ancient DNA Laboratory

- All steps of the protocol should take place in a clean room facility specifically designed for ancient DNA.
- The researcher performing lab work should wear correspondingly suitable lab-wear, such as:
  - full-body suit with hood (e.g., Tyvek)
  - hairnet
  - face mask
  - two pairs of clean gloves (do not wear latex gloves if samples will be used for proteomic analyses)
  - clean shoes
  - protective glasses

- Sample processing should be carried out in separated work benches with integrated UV irradiation (e.g. Dead Air PCR work bench)
- Surfaces and equipment should be regularly decontaminated with e.g. bleach solution or Thermofisher's DNA AWAY (or similar) and irradiated with UV.
- All home-made buffers should be prepared in a separate, dedicated PCR-free ultra-clean room and UV-irradiated for 30 min.

Please see the following for more detailed guidance:

Llamas, B. et al., 2017. From the field to the laboratory: Controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. *STAR: Science & Technology of Archaeological Research*, 3(1), pp.1–14. Available at: https://doi.org/10.1080/20548923.2016.1258824.

Definitions

Stock-aliquot refers to a personal 'stock' (e.g. in a 50ml Falcon Tube) of reagents you can use across multiple sessions of this protocol. An 'aliquot' refers to a sub-aliquot of the stock, that is used for a single session of this specific protocol.

Protocol Specific Guidelines

This protocol requires the use of two rooms - a dedicated PCR-free ultra-clean buffer preparation room and a DNA extraction room. As calculus typically contains higher biomass than bones or teeth, it is preferable to do calculus extractions in a separate space when one is available. This reduces the possibility of contaminating extractions from lower biomass samples with oral bacterial DNA.

MATERIALS TEXT

MATERIALS

☒ Parafilm **Biozym Catalog #743311**

☒ pH indicator strips MQuant® Supelco® **Merck Millipore Catalog #1.09535.0001**

☒ Safe-Lock Tubes 1.5 ml PCR clean DNA LoBind **Eppendorf Catalog #0030108051**

☒ Safe-Lock Tubes 2 ml PCR clean DNA LoBind **Eppendorf Catalog #0030108078**

☒ 50 ml CELLSTAR® Polypropylene Tube 30/115 MM Conical Bottom Blue screw cap sterile skirt **greiner bio-one Catalog #210261**

☒ EDTA (0.5 M) pH 8.0-500 mL **Thermo Fisher Scientific Australia Catalog #AM9261**

☒ Ethanol Absolute **Merck Millipore Catalog #1009831011**

☒ Guanidine hydrochloride for molecular biology >=99% **Sigma Aldrich Catalog #G3272-500g**

☒ 2-Propanol for Analysis **Merck Millipore Catalog #1070222511**

☒ Proteinase K from Tritirachium album lyophilized powder >=30 units/mg protein **Sigma Aldrich Catalog #P6556-100MG**

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Sodium Acetate buffer solution 3 M pH 52 for molecular biology **Sigma Aldrich Catalog #S7899-500ML**

TE buffer (1X) pH 8.0 low EDTA for molecular biology 500ml **Panreac AppliChem Catalog #A8569,0500**

TWEEN 20 for molecular biology viscous liquid **Sigma Aldrich Catalog #P9416-50ML**

Water HPLC Plus **Sigma Aldrich Catalog #34877-2.5L-M**

High Pure Viral Nucleic Acid Large Volume Kit **Roche Catalog #05114403001**

Home Aceton ≥99.8% HiPerSolv CHROMANORM® für die HPLC Aceton ≥99.8% HiPerSolv CHROMANORM® **VWR Chemicals Catalog #20067.290P**

Aluminium foil 30cmx20m Schachtel 1 Ro/VE **Contributed by users Catalog #3760062**

Lab Equipment
Laboratory Fume Hood
PCR work bench (e.g. AirClean Dead Air PCR Werkbank, 48´´)
UV irradiation box or cross linker (e.g. Vilber Lourmat Bio-Link BLX-254)
Incubator with natural convection (e.g. Thermo Scientific Heratherm General Protocol Inkubator IGS100)
Overhead tube rotator (e.g. Stuart SB2/SB3 Rotator)
Centrifuge 50 ml (e.g. Thermo Scientific Heraeus Megafuge 8)
Centrifuge Rotor 50 ml (e.g. Thermo Scientific TX-400)
Centrifuge 1.5/2.0 ml (e.g. Eppendorf 5424)
Centrifuge Rotor 1.5/2.0ml (e.g. Eppendorf F-45-24-11)
Balance (e.g. Ohaus Adventurer balance AX1502)
Vortex mixer (e.g. Scientific Industries Vortex-Genie® 2)
Microwave (Optional)
Glass bottle (e.g., 500 ml)

Generic Reagents
Solution of household bleach (2-6% NaClO, then diluted to a working solution concentration of 0.2-0.5% NaClO)
Thermofisher DNA AWAY
Paper towels or tissues

SAFETY WARNINGS

Reagents
Household bleach solution (2-6%) diluted to a working concentration of 0.2-0.5 % NaClO in total
- H290 May be corrosive to metals.
- H314 Causes severe skin burns and eye damage.
- H411 Toxic to aquatic life with long lasting effects.
- EUH206 Warning! Do not use together with other products. May release dangerous gases (chlorine). Remove from surface after recommended incubation time with water-soaked tissue.

DNA AWAY
- H314 Causes severe skin burns and eye damage.

Note: Both bleach solutions and DNA AWAY are used for decontamintation. DNA AWAY is less corrosive than bleach and should be preferred for decontamination of sensitive equipments such as surfaces of electric devices.

GuHCl
- H302 Harmful if swallowed.
- H332 Harmful if inhaled.
- H315 Causes skin irritation.
- H319 Causes serious eye irritation.

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Ethanol
- H225 Highly flammable liquid and vapour.
- H319 Causes serious eye irritation.

Acetone
- H225 Highly flammable liquid and vapour.
- H319 Causes serious eye irritation.
- H336 May cause drowsiness or dizziness

Note: Should be used only in a laboratory fume hood.

Isopropanol
- H225 Highly flammable liquid and vapour.
- H319 Causes serious eye irritation.
- H336 May cause drowsiness or dizziness.

EDTA
- H373 May cause damage to organs through prolonged or repeated exposure.

Proteinase K
- H315 Causes skin irritation.
- H319 Causes serious eye irritation.
- H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
- H335 May cause respiratory irritation.

Sodium Acetate
- H139: Causes serious eye irritation

Kits
Check manufacturer's safety information for the High Pure Viral Nucleic Acid Large Volume Kit used in this protocol.

Equipment
UV radiation
- UV radiation can damage eyes and can be carcinogenic in contact with skin. Do not look directly at unshielded UV radiation. Do not expose unprotected skin to UV radiation.
- UV emitters generate ozone during operation. Use only in ventilated rooms.

ABSTRACT

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Silica-based total DNA extraction protocol optimised for the recovery of ultra-short DNA molecules from archaeological dental calculus, modified from Dabney *et al.* (2013) *PNAS* (doi: 10.1073/pnas.1314445110) and adapted for dental calculus by Mann *et al.* (2018) *Scientific Reports* (doi: https://doi.org/10.1038/s41598-018-28091-9).

This protocol includes an optional acetone wash step to remove conservation consolidants (e.g. glue) from dental calculus that can sometimes be found on samples archived in museum collections.

BEFORE STARTING

Planning
This protocol takes four days, out of which three include laboratory work and one is an incubation period.

Only the extraction buffer can be prepared within the DNA extraction room. All other home-made buffers must be prepared in a separate dedicated PCR-free ultra-clean room, and we typically UV-irradiate these for 30 min. Purchased kits should be DNA-free.

Check waste disposal guidance for all reagents in this protocol against your corresponding laboratory regulations.

Equipment
Make sure all necessary equipment is available (see Materials).

Abbreviations
EDTA = Ethylenediaminetetraacetic acid
GuHCl = Guanidinium chloride or Guanidine hydrochloride
HPLC = High Performance Liquid Chromatography (-Grade Water)
NaClO = Sodium hypochlorite
TE = Tris-EDTA
TET = Tris-EDTA-Tween (-buffer)
UV = Ultraviolet (radiation)

Samples
Ensure sample aliquots (2-5mg) of dental calculus (in 2ml tubes) are prepared in a dedicated sampling room, prior to the day you start this protocol.

Controls
Take along a positive control (sample of known performance) and a negative control (empty tube) in order to assess the performance of the protocol and the level of background contamination. Consider these two extra samples in your calculations for buffer preparations.

Day 1: Preparation of reagents (Buffer Prep Room)

1 Prepare cleaned workspace with all necessary reagents and equipment.

If lab-wide large-batch pre-prepared reagent stores are used, ensure to have made personal stock-aliquot of reagents such as UV-Water, EDTA, sodium acetate, and proteinase K in amounts sufficient for this extraction.

2 Prepare **binding buffer** calculating **10 mL / reaction** .

Reagent [Stock Concentration]	Final Concentration	Volume/reaction
GuHCl (1 mol=95.53 g)	5 M	4.77 g
UV HPLC-water		6 mL
Isopropanol (100%)	40%	4 mL
Total		**10 mL**

2.1 Weigh GuHCl and transfer into a glass bottle.

If you want to clean the area where GuHCl was used, first use water and then bleach solution. **Do not** use bleach directly as it reacts with GuHCl to produce toxic chlorine gas!

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2.2 Add UV-irradiated HPLC water (6 mL).

This reaction is endothermic and the tube will become very cold. Be aware of the unusual 'slushy' way of dissolving.

2.3 Gently shake horizontally in order to get the salt dissolved. If necessary, apply short (**00:00:10**) bursts in microwave (~400W) keeping the tube slightly unscrewed. Wait until bottle cools down between microwave bursts.

2.4 Pipette isopropanol (4 mL) to reach the final reaction volume (**10 mL**).

3 Prepare wash buffer by pipetting **40 mL** ethanol to the wash buffer from the High Pure Viral Nucleic Acid kit following manufacturer's instuctions and make an aliquot calculating **900 µl / reaction** .

4 Prepare **TET elution buffer** by making an aliquot of TE-buffer calculating **100 µl / reaction** and pipette Tween-20 accordingly to reach **0.05 % (v/v)** concentration to make 'TET'.

Because Tween-20 is highly viscous, we dilute it 1:10 in UV-HPLC water, and use this 10% dilution to add Tween-20 to the TE-buffer

5 Irradiate all buffers with UV for **00:30:00** without the lids.

UV irradiation can be done together with solutions from steps 1 (binding buffer), 4 (wash buffer), and 5 (TET buffer).

6 Store binding buffer in a fridge at **4 °C** overnight for day 4.

Label the bottle accordingly with the name, date and for the calculated amount of reactions. Buffer can be stored in a fridge for up to four weeks. Seal bottle with parafilm to avoid evaporation.

7 Dilute **proteinase K** powder (**100 mg**) in **10 mL** UV HPLC-water to a working concentration of **10 mg** / **1 mL** .

Store proteinase K solution at **-20 °C** for use on day 2.

Day 1: OPTIONAL Sample Consolidant Removal (Laboratory fume hood)

8 Add **500 µl** of acetone per sample

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Acetone wash is optional, and should only be used if the presence of consolidants (synthetic glues, conservation chemicals etc.) is observed on samples.

8.1 Soak for **00:15:00** with an open lid under the fume hood 15m

8.2 Centrifuge the samples for **15800 rcf, 22°C, 00:01:00**

8.3 Remove acetone with a pipette and discard. Leave the tube open so the remaining acetone is removed via evaporation until the calculus is dry.

Day 1: Sample DNA decontamination (DNA extraction room)

9 UV sterilize calculus. Irradiate samples for **00:01:00** , then shake the samples and irradiate for another **00:01:00** .

Aim for 2-5 mg starting material.

If samples were treated with acetone, just UV sterilize the calculus for 30sec.

If the sample is in powder-form, irradiate in the original tube with the lid open. If the sample is in large pieces, place calculus in an aluminium foil boat in the crosslinker to maximize exposure.

10 If the calculus was in a boat for irradiation place it back in a 1.5 mL or 2 mL tube. Add **1 mL** of 0.5 M EDTA to each sample, to remove surface contaminants.

This step should be skipped if the purpose of the study is to detect infectuous pathogens, as these are more likely to be located on the outer layer of the calculus deposit (however, this may lead to a higher proportion of environmental contaminants in the data).

10.1 Vortex and incubate on rotator at room temperature for **00:15:00** .

The incubation step can lead to some sample loss, as the calculus will start dissolving and releasing DNA. If the calculus is very powdery, the supernatant can be removed directly after vortexing, without any incubation, because the calculus solubilizes within the 15 min and you lose DNA while removing the EDTA.

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10.2 Spin tubes **00:02:00** at **18500 x g** .

10.3 Transfer supernatant to a separate tube, labelled "EDTA-wash". Store the "EDTA-wash" tube at **-20 °C** for potential future analyses.

Day 1: Decalcification (DNA Extraction Room)

11 Add **1 mL** of fresh 0.5 M EDTA to each sample pellet.

12 Seal tubes with Parafilm, rotate **Overnight (12-18h)** with low overhead rotation speed (e.g., 12-16 rpm) at room temperature. If the tubes will be exposed to UV irridation, such as whole-room irridation for sterilization, be sure to cover them with foil or otherwise shield them.

Post-incubation sample suspended in EDTA can safely be stored in a freezer (**-20 °C**) before isolation and clean-up.

Day 2-3: Protein digestion (DNA Extraction Room)

13 Remove Parafilm and make sure that the tubes have not leaked (if they have leaked, clean them).

The appearance of the calculus will vary depending on its initial state. Larger pieces will have barely changed at all, while powder will be almost gone.

14 Briefly centrifuge tubes to remove any liquid from the lids.

15 Add **50 µl** of 10 mg/ml proteinase K to each tube.

16 Vortex tubes, seal with Parafilm, and continue decalcification by rotating at low overhead rotation speed (e.g., 12-16 rpm) at room temperature for 24-48 hours.

After starting the incubation, we recommend beginning preparations for day 4, such as pre-labelling the falcon tubes that will be used in step 18.

Decalcification time can be adapted, so that the sample is completely decalcified before starting DNA isolation. After complete decalcification, the sample should appear buoyant and feathery, or disappear altogether.

Post-incubation suspension can safely be stored in a freezer (**-20 °C**) before isolation and clean-up.

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Day 4: DNA isolation and clean-up (DNA Extraction Room)

17 Prepare cleaned workspace with all necessary reagents and equipment.

18 For each reaction prepare one **50 mL** Falcon tube, one High Pure Extender Assembly (i.e. Falcon tube from kit containing funnel and purification column), two collection tubes from the kit, and one **1.5 mL** LoBind tube for final elution step.

19 In every **50 mL** Falcon tube pipette **10 mL** binding buffer and **400 µl** sodium acetate (UV-irradiated). Mix by inversion and measure pH (should be 5-6).

Add more sodium acetate if the pH is too high. If the pH is too low you can add sodium hydroxide.

20 

Remove parafilm from extraction tubes, then spin the tubes for **00:02:00** at **18500 x g** to pellet calculus.

If pellet is not solid, repeat centrifugation.

21 Pipette supernatant to respective **50 mL** Falcon tube, mix contents by inversion. If pellet is too fragile, repeat centrifugation before transferring supernatant. Store the calculus pellet at **-20 °C** .

22 Pipette binding buffer/extract mix to High Pure Extender Assembly.

23 

Spin at a maximum of **1500 rpm** for **00:08:00**

This RPM is specific to a 50 mL Thermo Scientific TX-400 Swinging Bucket Rotor. As this is a swing rotor, the rpm value maybe inconsistent for other models. Therefore this value must be adjusted on a per-machine basis. Convert the rpm to rcf (g) and determine the appropriate rpm for your instrument.

You can also turn the tube 180^{o} after **00:04:00** to ensure the liquid does not get stuck on the inner rim of the funnel

During this centrifugation step, we recommend preparing downstream steps, such as labelling of final elution

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tubes.

24 Pipette any liquids remaining in the funnel onto the column. Remove funnel from column and insert the column into a fresh 2 mL collection tube.

The flow-through stays in the collection tube of the 50mL High Pure Extender Assembly and can be discarded.

25 

Dry spin the column in the collection tube for **00:02:00** at **18500 x g** .

26 

Pipette **450 µl** wash buffer from the High Pure Viral Nucleic Acid kit to the column, and spin at **8000 x g** for **00:01:00** .

27 Remove column from the collection tube, discard the flow-through and the old collection tube, and put the column into a fresh 2 mL collection tube.

28 

Repeat washing step once ( ) reusing the collection tube, and discard flow-through.

Discard flow-through in one of two following ways:
- Remove all liquid in the collection tube with a pipette, or
- Pour off the liquid into a fresh waste tube, and pat the rim of the collection tube dry on a paper tissue or towl. Use just one spot on the paper tissue per sample. Be careful not to touch the rim of the tube on the waste container. Be sure to clean the surface with DNA Away or bleach after discarding the paper.

29 

Dry spin at **18500 x g** for **00:01:00**

To ensure the liquid does not get stuck on the inner rim of the funnel, you can optionally spin for **00:00:30** , turn the tube 180^{o}, and dry spin for another **00:00:30**

Day 4: Elution (DNA Extraction Room)

30 

Transfer column to a fresh **1.5 mL** LoBind Tube, pipette **50 µl** of Tris-EDTA-tween (TET) to the center of

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column, incubate for **00:03:00** on the benchtop, and spin **00:01:00** at **18500 x g** to elute the DNA.

Eluted DNA will be stored in this tube. Label tube on top and side accordingly.

31 Repeat elution step for a total elution volume of **100 µl** TET.

32 Store the DNA extracts at **-20 °C** .

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