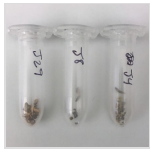

Dec 09, 2020

DNA Extraction from Modern Dental Calculus

Franziska Aron[1]

[1]Max Planck Institute for the Science of Human History

1 Works for me dx.doi.org/10.17504/protocols.io.7p8hmrw

WarinnerGroup

Franziska Aron

ABSTRACT

Protocol for DNA extraction from modern dental calculus samples for Illumina sequencing. This protocol uses the Qiagen PowerSoil DNA extraction kit.

DOI

dx.doi.org/10.17504/protocols.io.7p8hmrw

PROTOCOL CITATION

Franziska Aron 2020. DNA Extraction from Modern Dental Calculus. **protocols.io** https://dx.doi.org/10.17504/protocols.io.7p8hmrw

KEYWORDS

DNA, extraction, dental calculus

CREATED

Sep 26, 2019

LAST MODIFIED

Dec 09, 2020

PROTOCOL INTEGER ID

28128

GUIDELINES

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 a Biological Safety Laboratory level S2 due to handling of human substrates.

MATERIALS TEXT

Consumables

1.5ml Eppendorf DNA LoBind tubes **Contributed by users**

Qubit™ dsDNA BR Assay Kit **Thermo Fisher Catalog #Q32853**

EDTA (0.5 M) pH 8.0 **Life Technologies Catalog #AM9261**

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

2 ml LoBind Tubes **Eppendorf Catalog #0030108078**

DNeasy PowerSoil Kit (100) **Qiagen Catalog #12888-100**

Rotilabo®-Mikropistille **Carl Roth Catalog #YE15.1**

Proteinase K from Tritirachium album **Merck Millipore Sigma Catalog #P2308-10MG**

Roti®-Tape-Markierband **Carl Roth Catalog #AK65.1**

Falcon 50 ml **greiner bio-one Catalog # 210261**

Equipment

Centrifuge 5424 R refrigerated with Rotor FA-45-24-11 rotary knobs 120 V/50 – 60 Hz (US) **Eppendorf Centrifuge Catalog #5404000537**

Tube rotator **VWR international Ltd Catalog #444-0500P**

Tube rotator **VWR international Ltd Catalog #444-0500P**

Vortex-Genie® 2 EU-Stecker **Vwr Catalog #444-5900P**

Generic Reagents

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

SAFETY WARNINGS

Location

Work must be performed in BSL-S2 safety lab (Germany, or equivalent for your country).

Wear nitrile gloves, a lab coat and lab safety glasses.

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.

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

Note: Bleach can corrode sensitive equipments such as surfaces of electric devices.

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.

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

ABSTRACT

Protocol for DNA extraction from modern dental calculus samples for Illumina sequencing. This protocol uses the Qiagen PowerSoil DNA extraction kit.

BEFORE STARTING

Planning
This protocol requires the use of a Biological Safety Laboratory level S2 due to handling of human substrates.

This protocol takes ~3 days (including multi-day incubation).

Prepare a cool tube-rack for 1.5ml and 2ml tubes by placing it at +4°C for the DNA-clean up. This will speed up reaction time with a 'cold start'.

Modern dental calculus samples should be stored at least at +4°C or preferably at -20°C to preserved DNA after sampling.

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

Equipment

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

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

Abbreviations
EDTA = Ethylenediaminetetraacetic acid
HPLC = High Performance Liquid Chromatography (-Grade Water)

Controls
Consider taking along a positive control (sample of known performance) and a negative control (tube with HLPC water instead of DNA) in order to assess the performance of the protocol and the level of background contamination. Take into account these two extra samples in your calculations for buffer preparations.

Preparation Day 1

1 Place a 1.5ml/2ml tube rack at **4 °C** for the incubation steps on the final days

2 Prepare a 1:10 dilution of household bleach solution (~6% hypochlorite) in a 50ml-Falcon tube

3 Label for each sample one 2ml-Eppendorf Safe-Lock DNA LoBind-Tube
and one 1.5ml-Eppendorf Safe-Lock DNA LoBind-Tube

Sample Preparation

4 Weigh out **3 mg** to **5 mg** of the calculus and transfer it into a 2ml-Eppendorf Safe-Lock DNA LoBind-Tube (use special accuracy scales)

(OPTIONAL) EDTA Wash

5

Note this step is optional, however it can be useful to remove exogenous fluids such as blood.

Add **1 mL** of **0.5 Molarity (M)** EDTA and let it rotate on a tube rotator for **00:15:00** at **Room temperature**

6 Centrifuge for **00:01:00** **9400 x g** at **Room temperature**

7 Remove supernatant

Optional: Transfer the supernatant to a clean 1.5ml loBind Tube and stored it for future analysis at **-20 °C**

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

Lysis

8 Crush the calculus with a sterile metal pestle within the tube.

8.1 Clean the metal pestle after every sample by incubating for **00:02:00** in a household bleach dilution , then wiping clean with HPLC-water and drying with a clean paper towel **go to step #8**

Sterile plastic pestles can be used instead of metal pestles, but they must be changed between each sample and not re-used.

9 

Add **250 µl** **0.5 Molarity (M)** EDTA and **12.5 µl** Proteinase K (**10 mg /ml**)

10 

Let it rotate in the dark at **Room temperature** for **24:00:00 up to 48h** (optional: over the weekend) until the calculus fully decalcifies and either disappears or becomes buoyant (floating and feathery in appearance)

Preparation for Final Day

11 Retrieve the spin filter columns (provided in the kit) from fridge to warm them up to **Room temperature** for use at step 26

"provided in the kit" refers to: DNeasy PowerSoil Kit (100) Cat No./ID: 12888-100
A spin filter column is a spin filter unit sitting in a collection column. The spin filter unit contains the membrane.

12 Label for each sample one Power Bead tube, one spin filter column and three 2ml collection tubes (all provided in the kit)

DNA_Clean_up

13 Remove the tubes from the rotator and briefly centrifuge the extraction tubes

This centrifugation step is just made to remove the liquid from the lid.

14 Transfer everything with a **1000 µl** pipette tip (supernatant and pellet) to the Power Bead tubes

15 Invert to mix.

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

16 Add **60 µl** C1 solution, invert several times to mix.

17 Secure tubes horizontally on a flat-bed vortex pad with tape, vortex at maximum speed for **00:10:00**

18 

Centrifuge at **9400 x g** for **00:00:30** at **Room temperature**

Make sure the Power Bead tubes can rotate without rubbing, 10.000 x g is the maximum, otherwise the tubes may break

19 Transfer supernatant to the 2ml collection tube (provided in the kit)

Expect **400 µl** to **500 µl**

19.1 Save power bead tubes at **-20 °C** as backup. These can be thrown out once you know the extraction worked

20 Add **250 µl** C2 Solution to the supernatant and vortex **00:00:05** at maximum speed

21 Incubate at **4 °C** for **00:05:00** on the pre-cooled rack (**4 °C**)

22 

Centrifuge **9400 x g** for **00:01:00** at **Room temperature**

23 Transfer **600 µl** of the supernatant to a clean 2ml collection tube (provided in the kit), avoid transferring the pellet.

24 Add **200 µl** C3 solution and vortex briefly

25 

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

Incubate the tubes at **4 °C** for **00:05:00** on the pre-cooled rack (**4 °C**)

26 

Centrifuge the tubes at **9400 x g** for **00:01:00** at **Room temperature**

27 Transfer **750 µl** of the supernatant to a clean 2ml collection tube (provided in the kit)

27.1 Store remaining supernatant at **-20 °C**

We save this as backup until confirmation that the extraction worked. If extraction was successful, this can be discarded.

28 Gently shake C4 Solution to mix

29 Add **1.2 mL** C4 Solution to supernatant and vortex for **00:00:05**

The solution should not exceed the rim of the tube.

30 Bind the DNA on the membrane of spin filter unit:

30.1 Load approximately **675 µl** into spin filter unit

30.2 

Centrifuge **00:01:00** at **9400 x g** at **Room temperature**

30.3 Discard flow through

31 Repeat until the entire solution has been passed through the spin filter unit (2-3 times) **go to step #30.1**

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw

32 Load **500 µl** C5 Solution into spin filter unit

33 

Centrifuge **00:01:00** **9400 x g** at **Room temperature**

34 Discard flow through

35 

Dry spin **00:01:00** **9400 x g** at **Room temperature**

Elution

36 Place the spin filter unit into a clean 2ml collection tube (provided in the kit)

37 Pipette **100 µl** C6 Solution into center of the membrane in the spin filter unit and incubate for **00:01:00**

38 

Centrifuge **9400 x g** for **00:00:30** at **Room temperature**

39 Remove spin filter unit and quantify the DNA (**3 µl**) with the Qubit ds BR Kit

39.1 

Store the eluate at **-20 °C**

Citation: Franziska Aron (12/09/2020). DNA Extraction from Modern Dental Calculus. https://dx.doi.org/10.17504/protocols.io.7p8hmrw