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DNA Extraction from Modern Dental Calculus

In 3 collections

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 We use this protocol and it's working

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ABSTRACT

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

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

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 MilliporeSigma (Sigma-Aldrich) 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 MilliporeSigma (Sigma-Aldrich) 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 Catalog #5404000537**
- ✂ Tube rotator **VWR International Catalog #444-0500P**
- ✂ Tube rotator **VWR International Catalog #444-0500P**
- ✂ Vortex-Genie® 2 EU-Stecker **VWR International 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.

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.

BEFORE START INSTRUCTIONS

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

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 HPLC 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

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

Note

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

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](#)

Note

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

Note

"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

Note

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.

- 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

Note

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)

Note

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 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

Note

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

Note

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](#)

32 Load 500 µL C5 Solution into spin filter unit

33 Centrifuge 00:01:00 at 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 μ L 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 μ L) with the Qubit ds BR Kit

39.1 Store the eluate at -20 °C