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DNA Extraction from Modern Dental Plaque on Gauze

DNA Extraction from Modern Dental Calculus

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Protocol for DNA extraction from modern dental plaque dried on gauze samples for Illumina sequencing. This protocol uses the Qiagen PowerSoil DNA extraction kit, but applies modifications to improve DNA recovery. Specifically, solution C2 is not used to avoid uneccessary DNA loss during inhibitor removal.

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DNA Extraction from Modern Dental Calculus, Franziska Aron

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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.

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**

Bead Ruptor Elite
Omni International 19-040E

Generic Reagents

Paper towels or tissues

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
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.

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

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

This protocol takes ~1 day.

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 plaque 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
HPLC = High Performance Liquid Chromatography (i.e., HPLC-grade water)

Controls
Consider taking along a positive control (sample of known performance) and a negative control (tube with HLPC-grade 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 Label for each sample one 2ml-Eppendorf Safe-Lock DNA LoBind-Tube
and one 1.5ml-Eppendorf Safe-Lock DNA LoBind-Tube

3 Place solution C6 in an incubator to pre-heat it to **37 °C** .

Preparation for Final Day

4 Retrieve the spin filter columns (provided in the kit) from fridge to warm them up to **Room temperature** for use at step 25

"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

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membrane.

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

Be sure to label both the top and sides of the PowerSoil bead tube. The labeling on the top may be rubbed off during bead-beating with the Omni BeadRuptor.

Sample Preparation

6 Sterilize scissors by wiping with 75% ethanol, and then wiping them with HPLC water, then wipe dry. Use the scissors to clip a small piece of gauze with plaque.

Do not take more than half of the plaque, in case more is needed for another extraction later.

7 Place the piece of gauze with plaque in a PowerSoil bead tube using forceps sterilized by wiping with 75% ethanol, and then wiping them with sterile water.

Change gloves and sterilize scissors and forceps between each sample.

8 Make two extraction blank PowerSoil bead tubes per extraction batch, one with nothing added, and a second with a small section of sterile gauze added.

DNA_Clean_up

9 Invert the PowerSoil bead tubes to mix and fully submerge gauze with plaque.

10 Add **60 µL** C1 solution, invert several times to mix.

11 Add **25 µL** proteinase K (**10 mg/mL**), and rotate tubes several times to mix.

12 Place tubes in an OmniBeadRuptor, secure them, and close the lid. Run the BeadRuptor at 2.9 m/s for **00:10:00**

Make sure the Power Bead tubes are secure, but do not close the securing lid too tightly, 2.9 m/s is the maximum, otherwise the tubes may break.

13

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

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14 Transfer **600 µL** supernatant to the 2ml collection tube (provided in the kit), but leave the gauze in the bead tube.
Be careful not to transfer any beads or dust from the beads.

14.1 Save power bead tubes at **-20 °C** as backup.

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

15 Add **200 µL** C3 solution and vortex briefly

16 

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

17 

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

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

18.1 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.

19 Gently shake C4 Solution to mix

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

The solution should not exceed the rim of the tube.

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

21.1 Load approximately **675 µL** into spin filter unit

21.2 

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

21.3 Discard flow through

22 Repeat until the entire solution has been passed through the spin filter unit (2-3 times)

23 Load **500 µL** C5 Solution into spin filter unit

24 

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

25 Discard flow through

26 

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

Elution

27 Place the spin filter unit into a clean 2mL collection tube (provided in the kit), or if preferred, a 1.5 mL LoBind tube

28 Pipette **100 µL** C6 Solution (pre-warmed to **37 °C**) into center of the membrane in the spin filter unit and incubate for **00:01:00**

29 

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

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30 Remove spin filter unit and quantify the DNA (**3 µL**) with the Qubit ds BR Kit following the manufacturer's protocol

30.1

Store the eluate at **-20 °C**

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