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DNA extraction from mouthwash samples using Qiagen DNeasy PowersoilPro Kit

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We use this protocol and it's working

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Abstract

This protocol describes how to extract DNA from mouthwash samples.

Materials

QIAGEN -DNeasy PowerSoil Pro Kit
Ice
Vortex
Omni Bead Ruptor Elite bead beater
Centrifuge
Liquid N_2

Before start

Set centrifuge to 4ºC
Keep CD2 solution on ice or at 4ºC
Prepare and label collection tubes, microcentrifuge tubes, MB spin columns, and powerbead tubes

1 Thaw the sample on ice for 00:30:00 30m

1.1 Transfer the desired sample volume 1-2 mL into a 1.5mL or 2mL eppendorf tube

2 Centrifuge the transferred sample at maximum speed (17000 x g) at 4°C for 00:10:00 10m

3 Discard the supernatant carefully without disturbing the pellet

3.1 Repeat Step 1.1 if more volume is needed to see a pellet or a higher yield of DNA is required

4 Add 800 µL of CD1 and vortex to resuspend pellet

4.1 Spin down briefly

5 Transfer entire eppendorf content in a PowerBead Pro Tube

6 Secure PowerBead Pro tube onto the bead beater and run at maximum speed (17000 x g) for 00:05:00 5m

7 Centrifuge the PowerBead Pro tube at maximum speed (17000 x g) for 00:01:30 1m 30s

8 Transfer the supernatant carefully, without disturbing the pellet, into a clean 2mL microcentrifuge tube

9 Add 200 µL of CD2 into the 2mL microcentrifuge tube and vortex for 00:00:10 10s

10 Centrifuge the 2mL microcentrifuge tube at maximum speed (17000 x g) for 00:01:30 — 1m 30s

11 Transfer the supernatant carefully without disturbing the pellet, into a clean 2mL microcentrifuge tube

12 Add 600 µL of CD3 into the 2 mL microcentrifuge tube and vortex for 00:00:10 — 10s

13 Load 650 µL of the lysate onto MB spin columns and centrifuge at maximum speed (17000 x g) for 00:01:30 — 1m 30s

13.1 Discard flow-through and repeat step 13 to consume all the lysate

14 Place the spin column onto a new collection tube, add 500 µL of EA, and centrifuge at maximum speed (17000 x g) for 00:01:30 — 1m 30s

15 Discard flow-through and place the spin column back into the collection tube

16 Add 500 µL of C5 onto the spin column and centrifuge at maximum speed (17000 x g) for 00:01:30 — 1m 30s

17 Discard flow-through and place the spin column back into the collection tube

18 Centrifuge at maximum speed (17000 x g) for 00:02:00 and place spin column into 1.5mL eppendorf (elution) tube — 2m

19 Add 50-100 µL of nuclease-free water to the center of the spin column and leave at room temperature for 00:05:00 — 5m

20 Centrifuge at maximum speed (17000 x g) for 00:01:00 , quantify using Qubit, flash freeze, and store at -20/80 °C 1m