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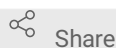
DNA extraction from mouthwash samples using Qiagen DNeasy PowersoilPro Kit

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1 Works for me



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ABSTRACT

This protocol describes how to extract DNA from mouthwash samples.

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MATERIALS TEXT

QIAGEN -DNeasy PowerSoil Pro Kit

Ice

Vortex

Omni Bead Ruptor Elite bead beater

Centrifuge

Liquid N₂


BEFORE STARTING

Set centrifuge to 4°C

Keep CD2 solution on ice or at 4°C

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** ^{5m}) for  **00:05:00**
- 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 ^{1m 30s} maximum speed ( **17000 x g**) for  **00:01:30**
- 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**) ^{1m 30s} for  **00:01:30**
- 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 ^{2m} 1.5mL eppendorf (elution) tube
- 19 Add  **50-100 µL** of nuclease-free water to the center of the spin column and leave at room ^{5m} temperature for  **00:05:00**
- 20 Centrifuge at maximum speed ( **17000 x g**) for  **00:01:00** , quantify using Qubit, flash ^{1m} freeze, and store at -20/80 °C