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DNA extraction from mouthwash samples V.2

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protocol .

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DNA extraction

Ahmed A Shibl, Anique Ahmad, Tsedenia Denekew, Mamon Abd AlBaqi, Aashish Jha 2021. DNA extraction from mouthwash samples . **protocols.io** https://protocols.io/view/dna-extraction-from-mouthwash-samples-bzhip34e Ahmed Shibl

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QIAGEN - DNeasy PowerSoil Pro Kit
Ice
Vortex
BeadBeater
Centrifuge
Liquid N2

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

30m

1 Thaw mouthwash samples on ice for **00:30:00**

1.1 Transfer desired volume into 1.5 mL or 2 mL eppendorfs
1-2 mL

2 Centrifuge transferred samples at maximum speed at 4 ºC 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 high yield of DNA is required

4 Add **800 µL** CD1 and vortex to resuspend pellet

4.1 Spin down briefly

5 Transfer entire eppendorf content to PowerBead Pro tubes

6 Secure PowerBead Pro tubes onto the bead beater and run at maximum speed for **00:05:00** 5m

7 Centrifuge the PowerBead Pro tubes at maximum speed for **00:01:30** 1m 30s

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

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

10 Centrifuge the 2 mL microcentrifuge tube at maximum speed for **00:01:30** 1m 30s

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

12 Add **600 µL** 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 for **00:01:30** 1m 30s

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

14 Place spin column onto new collection tube, add **500 µL** EA, and centrifuge at maximum speed for **00:01:30** 1m 30s

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

16 Add **500 µL** C5 onto spin columns and centrifuge at maximum speed for **00:01:30** 1m 30s

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

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

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

20 Centrifuge at maximum speed for **00:01:00** , quantify using Qubit, flash freeze, and store at -20/80 ºC 1m