DNA extraction from mouthwash samples V.1

Ahmed A Shibl[1], Anique Ahmad[1], Tsedenia Denekew[1], Aashish Jha[1]

[1]New York University, Abu Dhabi

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protocol .

Ahmed Shibl
New York University, Abu Dhabi

DNA extraction

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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

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

Transfer desired volume into 1.5 mL or 2 mL eppendorfs

1.1 **1-2 mL**

2 Centrifuge transferred samples at maximum speed at 4 ºC 10m

2.1 Repeat step 1.1 if more volume is needed to see a pellet or a high yield of DNA is required

3 Discard the supernatant carefully without disturbing the pellet

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

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 **00:01:00** 1m

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 **00:01:00** 1m

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 **00:01:00** 1m

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 **00:01:00** 1m

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

16 Add **500 µL** CD5 onto spin columns and centrifuge at maximum speed for **00:01:00** 1m

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 tube 2m

19 Add **50-100 µL** nuclease-free water to the center of the spin column and leave at room temperature for around **00:07:00** 7m

20 Centrifuge at maximum speed for **00:01:00** , measure with Qubit and flash freeze DNA 1m

with liquid N2