DNeasy PowerSoil Pro Kit modification for soil fungal community barcoding

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Protocol status: Working
We use this protocol and it's working well for extracting fungal DNA from field collected soils.

DISCLAIMER

This protocol has been used extensively for high yield fungal DNA from field collected soils. It deviates from the manufacturers recommended conditions for maximum rpm for mechanical cell lysis. Although we acknowledge this, to date we have not found any issues or degradation to the DNA isolated through this method.

ABSTRACT

Here we provide a modified protocol for use with the Qiagen PowerSoil Pro Kit that has been used with great success for obtaining fungal DNA from field collected soil samples for downstream barcoding.

MATERIALS

Qiagen DNeasy Powersoil Pro Kit
Qiagen Buffer ATL

Microcentrifuge
Biospec mini-beadbeater (or equivalent)
p200 pipette
p1000 pipette

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PROTOCOL integer ID: 86465

1 Centrifuge the PowerBead Pro Tubes briefly to ensure contents have settled to the bottom. 5s

2 Weigh out 250 mg of soil and add to the Powerbead Pro Tube. Add 750 µL of Solution CD1 and 50 µL of Solution ATL (ordered seperately from the kit contents). Vortex to homogenize. 5s

3 Place samples in a Biospec Mini-beadbeater (or equivalent) at 3800 rpm for 1 min. 1m

4 Remove samples and place on ice for 5 min. 5m

5 Repeat Step 3. 1m

6 Centrifuge tubes at 15,000 x g for 1 min. 1m

7 Transfer supernatant (~500-600 µL) to a 2 mL microcentrifuge tube, avoiding debris as much as possible. Keep samples on ice or in a cold microcentrifuge tube block.

8 Add 200 µL of Solution CD2 and vortex for 5 sec. 5s

9 Centrifuge tubes at 15,000 x g for 1 min. 1m

10 Transfer up to 700 µL to a new 2 mL microcentrifuge tube, avoiding the pellet at the bottom.

11 Add 600 µL of Solution CD3 and vortex for 5 sec. 5s

12 Pipette 650 µL of lysate onto the MB Spin Column membrane and centrifuge at 15,000 x g for 1 min. 1m

13 Discard the flow-through and repeat Step 12 with the remaining sample. 1m

14 Place the MB Spin Column in a new 2 mL collection tube and add 500 µL of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min. 1m

15 Discard flow-through and using the same collection tube, add 500 µL of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min. 1m

16 Place the MB Spin Column in a new 2 mL collection tube and centrifuge at 16,000 x g for 2 min. 2m

17 Place the MB Spin Column in a 1.5 mL Elution Tube and pipette 60 µL of Solution C6 (or water), ensuring it is placed directly onto the filter membrane. Allow tubes to sit for 5 min at room temp. 5m

18 Centrifuge tubes at 15,000 x g for 1 min, then discard MB Spin Column. DNA is now ready and should be stored at -20^{o}C. 1m