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Low Biomass, high contamination Illumina DNA prep using DNeasy PowerSoil (Pro) Kit

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Sonia L. Ghose: Originally adapted from.;

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ABSTRACT

This is an addendum to the already optimized DNeasy PowerSoil Kit. This can also be applied to the PowerSoil Pro Kit.
Use this protocol for Low Biomass or high contamination samples.

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GUIDELINES

Using some small tweaks to an already optimized protocol you can get your DNA from contaminated samples!

MATERIALS TEXT

DNeasy Powersoil Kit (Adjusted name to PowerLyzer Kit) - https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powerlyzer-powersoil-kit/

DNeasy Powersoil Pro Kit https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powersoil-pro-kit/

100% ethanol

BEFORE STARTING

Make sure you have all the components of the kit ready to go.

1 Add sample into the PowerBead tube.

2 Add 60µL of Solution C1.

2.1 Make sure the sample has not precipitated. If so, heat sample to 60ºC until precipitate is dissolved into solution.

3 Vortex to mix and incubate at 65ºC for 10 minutes. 10m

4 Bead beat at "homogenize" setting: 90 seconds bead beating, 60 seconds rest, then another 90 seconds (3 minutes total of actual beating). 4m

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5 Centrifuge at 10,000 x g for 30 s. 30s

6 Remove the supernatant or upper aqueous layer if you have one, to the new tube.

7 Add 100 uL Solution C2 and 100µL of Solution C3. Vortex to mix. Incubate at 4ºC or on ice for 5 minutes. 5m

7.1 *This is for low humic soils (100µL of each). If high humic soil, add 150µL C2 followed by 150µL C3.

8 Centrifuge to pellet (1 min at 10,000 x g.) and transfer the supernatant to a new tube. Avoid pellet. 1m

9 Ideally you will have 650µL of lysate, and can add 650µL of Solution C4 and 650µL of 100% ethanol.

9.1 Make sure you mix C4 solution well. If new kit add ethanol.

9.2 If you have 700µL, add 700µL of Solution C4 and 600µL of 100% ethanol. (Keep it as close to 1:1 as possible)

10 Load the lysate to the spin filter 650µL at a time and bind in three steps, alternating with centrifugation (1 min at 10,000 x g). Discard flow-through each time. 3m

11 If the membrane is not stained brown, wash with 650µL of 100% ethanol (centrifuge 1 min at 10,000 x g and discard flow-through). 1m

11.1 If the membrane is stained brown, per sample, prepare a mix of 300µL of

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Solution C4 and 370µL of 100%
ethanol. Wash the column with this mixture first. Follow this wash with the 100% ethanol wash.

12 Wash with 500µL of solution C5 (centrifuge 1 min at 10,000 x g and discard flow-through) 1m

13 Dry the spin column for 2 minutes 10,000 x g. Transfer to a clean tube. 2m

14 Elute in 60µL of buffer C6 (Heat this buffer to 60ºC before eluting). Let the buffer sit on the membrane 5
minutes before elution. 5m

15 Then centrifuge for 1 min 10,000 x g into your storage tube. 1m

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