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Soil DNA Extraction Modified Protocol for Dryland Agroecosystems

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McKenzie L. Stock[1], Paul Gabriel[1], Jennifer J. Randall[1], Nicole Pietrasiak[2]

[1]New Mexico State University; [2]University of Nevada Las Vegas

McKenzie L. Stock
New Mexico State University

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Protocol status: Working
We use this protocol and it's working

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Abstract

The Qiagen DNEasy PowerLyzer PowerSoil Kit is widely used in DNA based sample processing and extraction procedures. However, dryland soils are typically high in salts and secondary metabolites, which can cause interferences with A 260/230 quality of extracted DNA. We modified the Qiagen manufacturer protocol to account for high soil salinity by including additional washing steps and extra centrifugation time. Quality DNA was extracted from agricultural soils using this protocol which passed the quality checks for next generation amplicon sequencing.

Troubleshooting

Sample Prep

1 Soil samples should be stored cold, at least -20 C (ideally at -80 C).

Homogenization

3m

2 Fill a **12 ml homogenization vial** approximately 2/3 full with soil **(Spex SamplePrep 6133PC-T)**. Include three 6.35 mm diameter chrome steel beads **(BioSpec Products Cat. No. 11079635c)**.

3 Homogenize at 4000 rpm for 00:00:10 using the **SPEX SamplePrep 1200 Genolyte** homogenizer with the **single-vial attachment for 12mL vials**. Re-homogenize as needed at 4000 rpm for 00:00:10 to avoid the soil from thawing and sticking together.

20s

DNA Extraction

17m 10s

4 This protocol is modified from the Qiagen manufacturer protocol for the **DNeasy PowerLyzer PowerSoil Kit**.

Equipment

DNeasy PowerLyzer PowerSoil Kit	NAME
DNA Extraction Kit	TYPE
Qiagen	BRAND
12855-50	SKU
https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/microbial-dna/dneasy-powerlyzer-powersoil-kit/	LINK

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5 Add approximately 80 µL of soil sample by volume to the PowerBead Tube provided.

6 Add 750 µL of PowerBead Solution to the PowerBead Tube.

7 Add 60 µL of Solution C1 and invert several times or vortex briefly.

8 Bead beating: Spex 1200 Genolyte homogenizer: Place the PowerLyzer Glass Bead Tubes into the **tube holder attachment for the homogenizer (holds 6)**. The PowerBead Tubes must be balanced on the tube holder. Run the samples at a time and RPM suitable for your soil type. For our samples, 3000 rpm for 00:00:35 . 35s

9 Centrifuge Bead Tubes at 10,000 x rcf for 00:03:00 . Note: We increased the centrifugation time to ensure that no sediment/soil is left in suspension, improving DNA quality downstream. 3m

10 Transfer the supernatant to a clean 2 ml collection tube. Note: Expect 400-500 µL . Supernatant may still contain some soil particles.

11 Add 250 µL of Solution C2 and vortex for 5 sec. Incubate at 2-8°C for 5 min.

12 Centrifuge the tubes at 10,000 x rcf for 1 min. Avoiding the pellet, transfer up to 600 µL of supernatant to a clean 2 ml collection tube.

13 Add 200 µL of Solution C3 and vortex briefly. Incubate at 2-8°C for 00:05:00 . 5m

14 Centrifuge the tubes at 10,000 x rcf for 00:03:00 . Avoiding the pellet, transfer up to 750 µL of supernatant to a clean 2 ml collection tube. Note: We increased the centrifugation time to ensure that no sediment/soil is left in suspension, improving DNA quality downstream. 3m

15 Add 1200 µL of Solution C4 to the supernatant and vortex for 00:00:05 . 5s

16 Load 675 µL of the supernatant onto a MB Spin Column and centrifuge at 10,000 x rcf for 00:01:00 . Discard the flow through and add an additional 675 mL of supernatant. 1m

17 Centrifuge at 10,000 x rcf for 00:01:00 . Load the remaining supernatant onto the MB Spin Column and centrifuge at 10,000 rcf for 00:01:00 . Note: A total of three loads for each sample processed are required. 2m

18 Add 500 µl of Solution C5 and centrifuge at 10,000 x rcf for 00:00:30 . Discard the flow through. Repeat this step once more: add 500 µL of Solution C5 and centrifuge at 10,000 x rcf for 00:00:30 . Discard the flow through. Note: We repeated this washing step to minimize salt contamination, which helps improve DNA A260/230 values. 1m

19 Centrifuge again at 10,000 x rcf for 00:01:00 . 1m

20 Carefully place the spin filter in a clean 2 ml collection tube. Avoid splashing any Solution C5 onto the MB Spin Column.

21 Add 50 µL of Solution C6 to the center of the white filter membrane. Note: We used the minimum recommended amount of Solution C6 to yield greatest DNA concentrations.

22 Centrifuge at 10,000 x rcf for 00:00:30 . Discard the MB Spin Column. 30s

23 The DNA is now ready for downstream applications.