

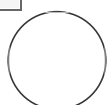


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Soil DNA Extraction Modified Protocol for Dryland Agroecosystems

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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.

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



Created: Sep 19, 2022

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

- 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 μL 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.