Sep 10, 2022

QIAGEN® DNeasy® PowerSoil® Pro

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dx.doi.org/10.17504/protocols.io.bp2l69411lqe/v1

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ABSTRACT

For the isolation of microbial genomic DNA from all soil types, including difficult samples such as compost, sediment, and manure.

DOI

dx.doi.org/10.17504/protocols.io.bp2l69411lqe/v1

EXTERNAL LINK

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

PROTOCOL CITATION

Hsin-Mao Wu 2022. QIAGEN® DNeasy® PowerSoil® Pro. **protocols.io** https://protocols.io/view/qiagen-dneasy-powersoil-pro-cgecttaw

CREATED

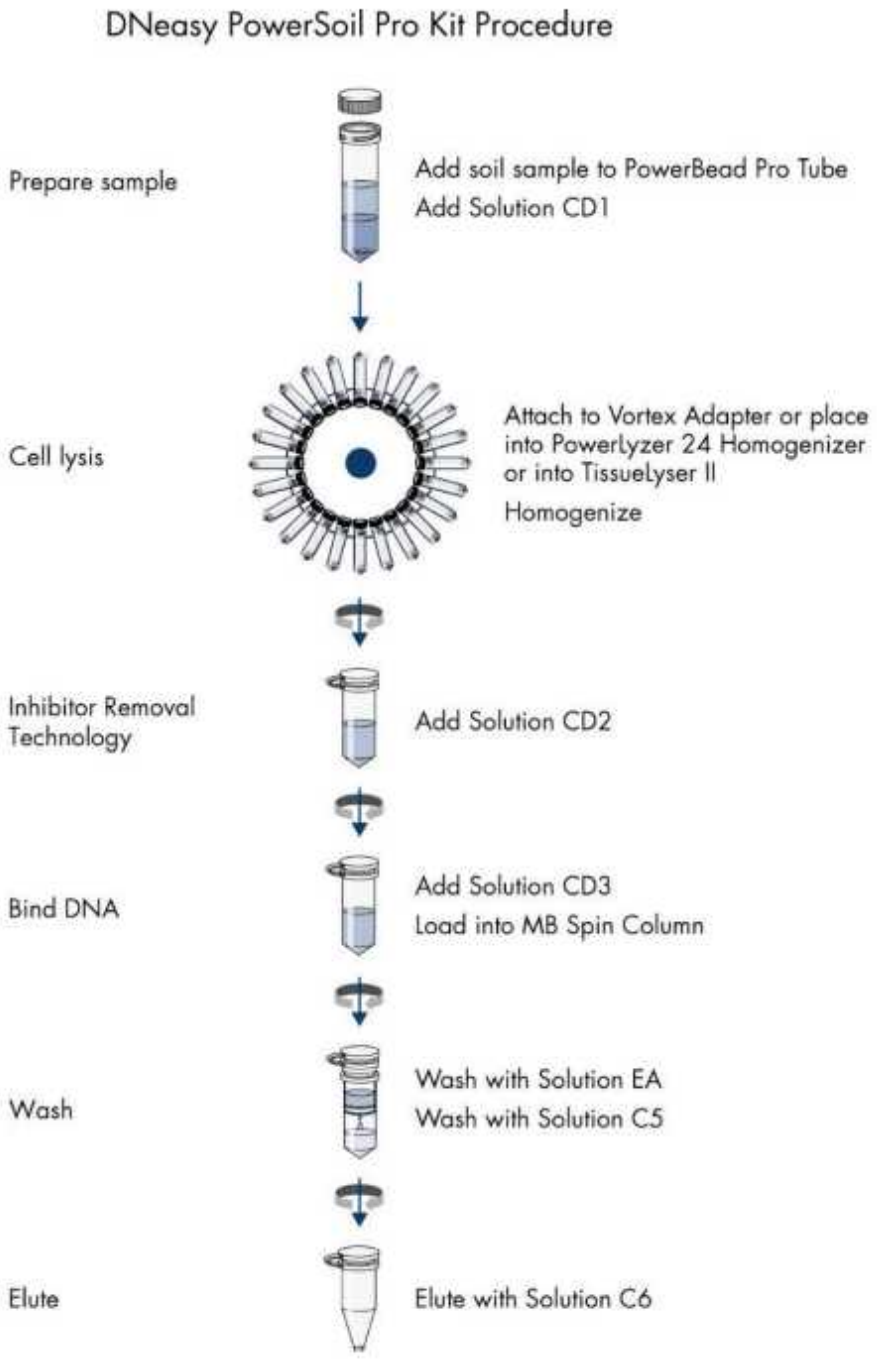
Sep 10, 2022

LAST MODIFIED

Sep 10, 2022

PROTOCOL INTEGER ID

69796



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MATERIALS TEXT

1. PowerBead Pro Tubes
2. MB Spin Columns
3. Solution CD1
4. Solution CD2
5. Solution CD3
6. Solution EA
7. Solution C5
8. Solution C6
9. Microcentrifuge Tubes (2 ml)
10. Elution Tubes (1.5 ml)
11. Collection Tubes (2 ml)
12. Microcentrifuge
13. Pipettor

SAFETY WARNINGS

1. Solution EA and Solution C5 are flammable
2. DO NOT add bleach or acidic solutions directly to the sample preparation waste.

BEFORE STARTING

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Prepare sample & Cell lysis

1 Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to **250 mg** of soil and **800 µL** of Solution CD1. Vortex briefly to mix.

After the sample has been loaded into the PowerBead Pro Tube, the next step is a homogenization and lysis procedure. The PowerBead Pro Tube contains a buffer that will (a) help disperse the soil particles, (b) begin to dissolve humic acids, and (c) protect nucleic acids from degradation. Gentle vortexing mixes the components in the PowerBead Pro Tube and begins to disperse the sample in the buffer.

2 Homogenize samples thoroughly using one of the following methods:

2.1 Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for **00:10:00** . 10m

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If using Vortex Adapter for more than 12 preps simultaneously, increase the vortexing time by 5–10 min.

Using the Vortex Adapter will maximize homogenization, which can lead to higher DNA yields. Avoid using tape, which can become loose and result in reduced homogenization efficiency, inconsistent results, and reduced yields.

2.2 Use a PowerLyzer 24 Homogenizer. PowerBead Pro Tubes must be properly balanced in the tube holder of the PowerLyzer 24 Homogenizer. We recommend homogenizing the soil at **2000 rpm, 00:00:30** , pausing for **00:00:30** , then homogenizing again at **2000 rpm, 00:00:30** . 1m 30s

Homogenizing samples at higher speeds (up to 4000 rpm) may increase yields but result in more fragmented DNA.

2.3 Use a TissueLyser II. Place the PowerBead Pro Tube into the TissueLyser Adapter Set 2 x 24 (cat. no. 69982) or 2 ml Tube Holder (cat. no. 11993) and Plate Adapter Set (cat. no. 11990). Fasten the adapter into the instrument and shake for **00:05:00** at speed 25 Hz. Reorient the adapter so that the side that was closest to the machine body is now furthest from it. Shake again for 5 min at a speed of 25 Hz. 5m

Vortexing/shaking is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents from step 1 and mechanical shaking introduced at this step. Randomly shaking the beads in the presence of disruption agents will cause the beads to collide with microbial cells and lead to the cells breaking open.

3 Centrifuge the PowerBead Pro Tube at **15000 rpm, 00:01:00** . 1m

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4 Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).

Expect 500–600 µl. The supernatant may still contain some soil particles.

Inhibitor removal 1m 5s

5 Add **200 µL** of Solution CD2 and vortex for **00:00:05** . 5s

Solution CD2 contains IRT, which is a reagent that can precipitate non-DNA organic and inorganic material including humic substances, cell debris, and proteins. It is important to remove contaminating organic and inorganic matter that may reduce DNA purity and inhibit downstream DNA applications.

6 Centrifuge at **15000 x g, 00:01:00** . Avoiding the pellet, transfer up to **700 µL** of supernatant to a clean 2 ml Microcentrifuge Tube (provided). 1m

Expect 500–600 µl.

The pellet at this point contains non-DNA organic and inorganic material including humic acids, cell debris, and proteins. For best DNA yields and quality, avoid transferring any of the pellet.

Bind DNA 1m 5s

7 Add **600 µL** of Solution CD3 and vortex for **00:00:05** . 5s

Solution CD3 is a high-concentration salt solution. Because DNA binds tightly to silica at high salt concentrations, Solution CD3 will adjust the DNA solution salt concentration to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the MB Spin Column filter membrane.

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8 Load **650 µL** of the lysate onto an MB Spin Column and centrifuge at **15000 x g, 00:01:00** . 1m

DNA is selectively bound to the silica membrane in the MB Spin Column in the presence of high salt solution. Contaminants pass through the filter membrane, leaving only DNA bound to the membrane.

9 Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.

10 Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.

Wash 4m

11 .Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at **15000 x g, 00:01:00** . 1m

Solution EA is a wash buffer that removes protein and other non-aqueous contaminants from the MB Spin Column filter membrane.

12 Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

13 Add **500 µL** of Solution C5 to the MB Spin Column. Centrifuge at **15000 x g, 00:01:00** . 1m

Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the MB Spin Column. This wash solution removes residual salt, humic acid, and other contaminants while allowing the DNA to stay bound

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to the silica membrane.

14 Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).

15 Centrifuge at up to **16000 x g, 00:02:00** . Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided). 2m

This spin removes residual Solution C5. It is critical to remove all traces of Solution C5 because the ethanol in it can interfere with downstream DNA applications, such as PCR, restriction digests, and gel electrophoresis.

Elute 1m

16 Add **50 µL** ~ **100 µL** of Solution C6 to the center of the white filter membrane.

Placing Solution C6 in the center of the small white membrane will make sure the entire membrane is wet. This will result in a more efficient and complete release of the DNA from the MB Spin Column filter membrane. As Solution C6 passes through the silica membrane, DNA that was bound in the presence of high salt is selectively released by Solution C6 (10 mM Tris), which lacks salt.

17 Centrifuge at **15000 x g, 00:01:00** . Discard the MB Spin Column. The DNA is now ready for downstream applications. 1m

We recommend storing the DNA frozen (−30 to −15°C or −90 to −65°C) as Solution C6 does not contain EDTA. To concentrate DNA, refer to the Troubleshooting Guide

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