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Version 1

QIAGEN® DNeasy® PowerSoil® Pro V.1

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Protocol status: In development

We are still developing and optimizing this protocol

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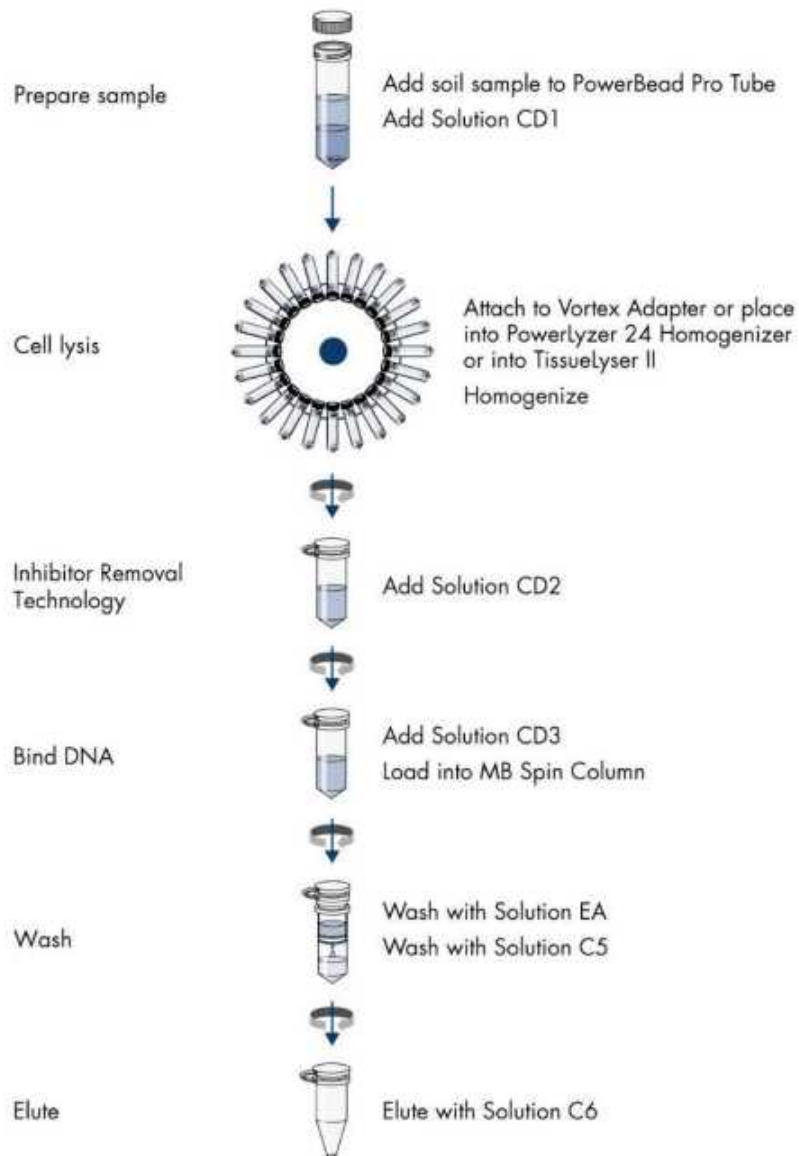
Keywords: microbial genomic dna from all soil type, microbial genomic dna, difficult samples such as compost, soil, dna, soil type

Abstract

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

Guidelines

DNeasy PowerSoil Pro Kit Procedure



Materials

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 start

- 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 μ L of Solution CD1. Vortex briefly to mix.

Note

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

Note

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

Note

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

**Note**

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

Note

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

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

Note

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

Note

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

Note

Expect 500–600 μL .

Note

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

Note

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.

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

1m

Note

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

1m

 15000 x g, 00:01:00 .

Note

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

1m

 15000 x g, 00:01:00 .

Note

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 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

**Note**

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.

Note

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

Note

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