APR 07, 2023

DOI:
dx.doi.org/10.17504/protocols.io.kxygx97mzg8j/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: QIAGEN 2023. QIAGEN DNeasy PowerSoil Pro Kit. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.kxygx97mzg8j/v1

Protocol status: Other
No published studies (as of April 2023) using this protocol to detect fish sedDNA
Unpublished studies on lake surface sediments report low detection of fish sedDNA
Protocol successful at detecting fish sedDNA collected in a stream during a fish migration

QIAGEN DNeasy PowerSoil Pro Kit

Forked from QIAGEN® DNeasy® PowerSoil® Pro

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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.

The DNeasy PowerSoil Pro Kit comprises a novel and proprietary method for isolating microbial genomic DNA from environmental samples. The kit uses QIAGEN's secondgeneration Inhibitor Removal Technology® (IRT) and is intended for use with environmental samples containing high humic acid content, including difficult soil types such as compost, sediment, and manure. Other more common soil and stool types have also been used successfully with this kit. Improved IRT combined with more efficient bead beating and lysis chemistry yields high-quality DNA that can be used immediately in downstream applications, including PCR, qPCR, and next-generation sequencing (16S and whole genome).

As of April, 2023 - no published studies successfully detecting fish sedDNA using only this kit (see Lakes ABPS protocol). Unpublished studies report poor DNA yields and low concentrations of fish sedDNA. Other studies targeting migratory fish sedDNA during a spawning run found sufficient fish sedDNA concentrations following this protocol.

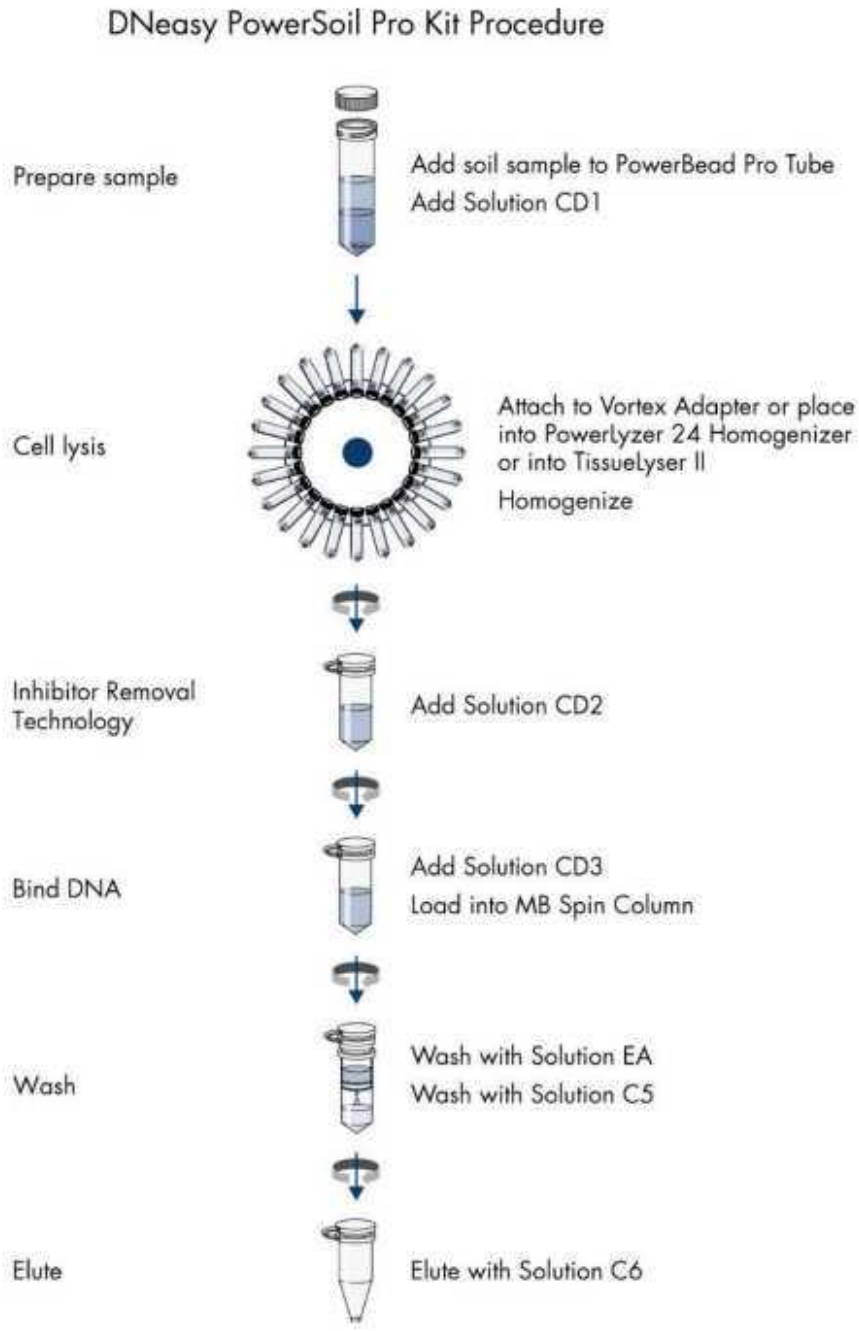
GUIDELINES

Created: Dec 21, 2022

Last Modified: Apr 07, 2023

PROTOCOL integer ID: 74332

Keywords: Qiagen, DNeasy, PowerSoil Pro, SedDNA, Sedimentary DNA, Fish



MATERIALS

DNeasy PowerSoil Pro Kit (50 preparations) already includes:

- PowerBead Pro Tubes * 50
- MB Spin Columns * 50
- Solution CD1 40 mL
- Solution CD2 15 mL
- Solution CD3 35 mL
- Solution EA 36 mL
- Solution C5 30 mL
- Solution C6 9 mL
- Microcentrifuge Tubes (2 mL) * 100
- Elution Tubes (1.5 mL) * 50
- Collection Tubes (2 mL) * 100

Equipment and Reagents to be Supplied by User

- Microcentrifuge (up to 16,000 x g)
- Pipettor (50-1000 ul)
- Vortex Genie
- Vortex Adapter for 24 (1.5-2 mL) tubes

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 INSTRUCTIONS

- 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).

Sample preparation & cell lysis

1 **SPIN** the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom

ADD up to 0.25 g of soil sample to the PowerBead Pro Tube

ADD 800 µL of Solution CD1

VORTEX briefly to mix

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 10m

VORTEX at maximum speed for 00:10:00

2.2 **USING** a PowerLyzer 24 Homogenizer, homogenize the soil at 2000 rpm for 00:00:00 1m

PAUSE for 00:00:30

HOMOGENIZE again at 2000 rpm for 00:00:30

2.3 **USING** a TissueLyser II, place the PowerBead Pro Tube into the TissueLyser Adapter 10m

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 00:05:00 at a speed of 25 Hz.

3 **CENTRIFUGE** the PowerBead Pro Tube at 15000 x g for 00:01:00 1m

TRANSFER supernatant to a clean 2 mL Microcentrifuge Tube (expect 500-600ul)

Inhibitor removal 1m 5s

4 **ADD** 200 µL of Solution CD2 5s

VORTEX for 00:00:05

5 **CENTRIFUGE** tubes at 15000 x g for 00:01:00 1m

AVOIDING the pellet, transfer up to 700 µL of supernatant to a clean 2 ml Microcentrifuge Tube

Bind DNA 1m 5s

6 **ADD** 600 µL of Solution CD3 5s

VORTEX for 00:00:05

7 **LOAD** 650 µL of the lysate onto an MB Spin Column 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the liquid flow-through

8 **REPEAT** step 7 to ensure that all of the lysate has passed through the MB Spin Column

CAREFULLY place the MB Spin Column into a clean 2 mL Collection Tube. Avoid splashing any flow-through onto the MB Spin Column

Wash spin column 4m

9 **ADD** 500 µL of Solution EA to the MB Spin Column 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the flow-through and place the MB Spin Column into the same 2 mL Collection Tube

10 **ADD** 500 µL of Solution C5 to the MB Spin Column 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the flow-through and place the MB Spin Column into a **NEW** 2 mL Collection Tube

11 **CENTRIFUGE** at 16000 x g for 00:02:00 2m

CAREFULLY place the MB Spin Column into a new 1.5 ml Elution Tube

Elute the DNA 1m

12 **ADD** between 50 µL and 100 µL of Solution C6 to the center of the white filter membrane 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the MB Spin Column

DNA is now ready for downstream applications