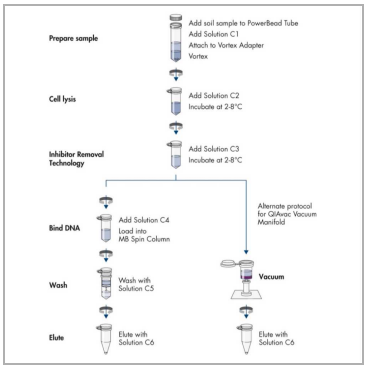

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Protocol status: Working
Protocol successful in several fish sedDNA studies

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QIAGEN DNeasy PowerSoil Kit

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ABSTRACT

For the isolation of microbial genomic DNA from all soil types

The DNeasy PowerSoil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples using patented Inhibitor Removal Technology® (IRT). This kit 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 types have also been used successfully with this kit. The isolated DNA has a high level of purity, which allows for more successful PCR amplification of organisms from the sample. PCR analysis has been performed to detect a variety of organisms including bacteria (e.g., Bacillus subtilis, Bacillus anthracis), fungi (e.g., yeasts, molds), algae and actinomycetes (e.g., Streptomyces).

Protocol used successfully to detect target fish species sedDNA by Olajos et al., 2018; Nelson-Chorney et al., 2019; Thomson-Laing et al., 2020; Shiragaki et al., 2021; and Naro-Maciel et al., 2022

MATERIALS

DNeasy PowerSoil Kit (50 preparations) already includes:
MB Spin Columns * 50
PowerBead Tubes * 50
Solution C1 6.6 mL
Solution C2 15 mL
Solution C3 15 mL
Solution C4 72 mL
Solution C5 30 mL
Solution C6 9 mL
Collection Tubes (2ml) 4 * 50
Quick Start Protocol

Additional equipments and reagents to be supplied by user:
Microcentrifuge (10.000 x g)
Pipettors (50 µl–500 µl)
Vortex-Genie 2 Vortex
Vortex Adapter for 24 (1.5-2.0 ml) tubes (cat. no. 13000-V1-24)
100% ethanol (for the QIAvac 24 Plus Manifold protocol only)
QIAvac 24 Plus Manifold

SAFETY WARNINGS

Solution C5 contains ethanol. It is flammable.
Do not use bleach to clean the inside of the QIAvac® 24 Plus manifold.

BEFORE START INSTRUCTIONS

Perform all centrifugation steps at room temperature (15–25°C).
If Solution C1 has precipitated, heat at 60°C until precipitate dissolves.

Sample preparation & cell lysis

1 **ADD** 0.25 g of soil sample to the PowerBead Tube

VORTEX gently to mix

2 **ADD** 60 µL of Solution C1

INVERT several times or vortex briefly

3 **SECURE** PowerBead Tubes horizontally using a Vortex Adapter for 24 (1.5–2.0 ml) tubes 10m

VORTEX at maximum speed for 00:10:00

4 **CENTRIFUGE** tubes at 10.000 x g for 00:00:30 30s

TRANSFER supernatant to a clean 2 mL Collection Tube

Inhibitor removal 25m 40s

5 **ADD** 250 µL of Solution C2 5m 5s

VORTEX for 00:00:05

INCUBATE at 2 °C to 8 °C for 00:05:00

6 **CENTRIFUGE** tubes at 10000 x g for 00:01:00 1m

AVOIDING the pellet, transfer up to 600 µL of supernatant to a clean 2 mL Collection Tube

7 **ADD** 200 µL of Solution C3 5m

VORTEX briefly to mix

INCUBATE at 2 °C to 8 °C for 00:05:00

8 **CENTRIFUGE** tubes at 10000 x g for 00:01:00 1m

AVOIDING the pellet, transfer up to 750 µL of supernatant to a clean 2 mL Collection Tube

Bind DNA 3m 5s

9 **SHAKE** to mix Solution C4 5s

ADD 1200 µL of Solution C4 to the supernatant

VORTEX for 00:00:05

10 **LOAD** 675 µL onto an MB Spin Column 1m

CENTRIFUGE at 10.000 x g for 00:01:00

DISCARD liquid flow-through

11 **REPEAT** step 10 twice, until all of the sample has been processed

Wash spin column 3m 5s

12 **ADD** 500 µL of Solution C5 30s

CENTRIFUGE at 10.000 x g for 00:00:30

DISCARD liquid flow-through

13 **CENTRIFUGE** again at 10.000 x g 00:01:00 1m

CAREFULLY place the MB Spin Column into a clean 2 mL Collection Tube. Avoid splashing any residual Solution C5 onto the column

Elute the DNA 3m 5s

14 **ADD** 100 µL of Solution C6 to the center of the white filter membrane 30s

CENTRIFUGE at 10000 x g for 00:00:30

DISCARD the MB Spin Column

DNA is now ready for downstream applications