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Protocol status: Working Protocol used successfully by Sales et al., 2021 to detect fish sedDNA

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QIAGEN DNeasy PowerMax Soil Kit

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ABSTRACT

For the isolation of microbial DNA from large quantities of soil - great for samples with low microbial load

The DNeasy PowerMax Soil Kit comprises a novel and proprietary method for isolating genomic DNA from environmental samples using Inhibitor Removal Technology® (IRT). With this kit, it is possible to process samples that have proven difficult in the past due to high levels of humic-like substances. The isolated DNA has a high level of purity, which allows for successful PCR amplification from samples. Total DNA isolated from various soil types has been successfully amplified using PCR with primers specific for bacteria (Bacillus subtilis, Bacillus anthracis), fungi (yeast, mold), and actinomycetes (Streptomyces).

Using the DNeasy PowerMax Soil Kit, environmental samples are added to a bead-beating tube with a kit-supplied proprietary buffer for rapid and thorough homogenization. Cell lysis and DNA exposure occur by mechanical and chemical methods. Extracted genomic DNA is captured on a silica membrane in a spin column format. The DNA is washed and eluted from the membrane and is ready for PCR and other downstream applications.

Protocol successfully used by Sales et al., 2021 to characterize fish species diversity and richness in water and sediment samples

MATERIALS

DNeasy PowerMax Soil Kit (10 preparations) already includes:
MB Maxi Spin Columns * 10
PowerMax Bead Pro Tubes * 10
PowerBead Solution 20 mL
Solution C1 6.6 mL * 2
Solution C2 28 mL * 2
Solution C3. 44 mL
Solution C4 330 mL
Solution C5 30 mL * 4
Solution C6 66 mL
Collection Tubes (50ml) 5 * 8
Quick Start Protocol * 1

Additional equipments and reagents to be supplied by user:
Centrifuge capable of spinning 50 ml tubes at 2500 x g using swing-out rotor
Pipettes (1ml and 10 ml)
Vortex-Genie 2 Vortex
Vortex Adapter for 2 (50 ml) tubes (cat. no. 13000-V1-50)

SAFETY WARNINGS

Solution C5 contains ethanol and is flammable.
DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Sample preparation & cell lysis

1h 27m 30s

1 **ADD** 15 mL of PowerBead Solution to a PowerMax Bead Pro Tube 1m

ADD up to 10 g of soil sample to the PowerMax Bead Pro Tube containing PowerBead Solution

VORTEX vigorously for 00:01:00

Note

Refer to manufacturer's Troubleshooting Guide before deciding on the amount of soil to process. However, higher volumes of sediment (10 g) have shown better detection rates for fish sedDNA.

2 **ADD** 1.2 mL of Solution C1 to the PowerMax Bead Pro Tube 30s

VORTEX vigorously for 00:00:30

3 **PLACE** PowerMax Bead Pro Tube on a vortex adapter 40m

VORTEX for 00:10:00 at the highest speed

ALTERNATIVELY, place the tube in a shaking water bath set at 65 °C and shake at maximum speed for 00:30:00

4 **CENTRIFUGE** at 2500 x g for 00:03:00 at Room temperature 3m

TRANSFER supernatant to a clean collection tube

Inhibitor removal

1h 27m 30s

5 **ADD** 5 mL of Solution C2 10m

INVERT twice to mix

INCUBATE at 2 °C to 8 °C for 00:10:00

6 **CENTRIFUGE** at 2500 x g for 00:04:00 at Room temperature 4m

AVOIDING the pellet, transfer the supernatant to a clean collection tube

7 **ADD** 4 mL of Solution C3 10m

INVERT twice to mix

INCUBATE at 2 °C to 8 °C for 00:10:00

8 **CENTRIFUGE** at 2500 x g for 00:04:00 at Room temperature 4m

AVOIDING the pellet, transfer the supernatant to a clean collection tube

Bind DNA

1h 27m 30s

9 **SHAKE** to mix Solution C4

ADD 30 mL of Solution C4 to supernatant

INVERT twice to mix

10 **FILL** an MB Maxi Spin Column with the solution from step 9 2m

CENTRIFUGE at 2500 x g for 00:02:00 at Room temperature

DISCARD the liquid flow-through

11 **ADD** a second volume of supernatant to the same MB Maxi Spin Column 2m

CENTRIFUGE at 2500 x g for 00:02:00 at Room temperature

DISCARD the liquid flow-through

REPEAT until the entire volume has been processed. This will take up to **4 total spins**

Wash spin column

1h 27m 30s

12 **ADD** 10 mL of Solution C5 3m

CENTRIFUGE at 2500 x g for 00:03:00 at Room temperature

DISCARD the liquid flow-through

13 **CENTRIFUGE** again at 2500 x g for 00:05:00 at Room temperature to remove residual Solution C5 5m

CAREFULLY place the MB Maxi Spin Column in a new collection tube. Avoid splashing any residual SolutioN C5 onto the column

Elute the DNA

1h 27m 30s

14 **ADD** 5 mL of sterile Solution C6 to the center of MB Maxi Spin Column membrane 3m

CENTRIFUGE at 2500 x g for 00:03:00 at Room temperature

DISCARD the MB Maxi Spin Column

DNA is now ready for downstream applications