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[Modified] DNeasy PowerSoil Pro Kit_Increased Sediment Volume & Optional Concentration

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Protocol status: Other
Protocol successful at detecting fish sedDNA collected in a stream during a fish migration Protocol unsuccessful at detecting fish sedDNA from Maine lakes

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Abstract

Protocol (both increased sediment amount up to 2.0g as well as concentrating DNA post-extraction) unsuccessful at detecting fish sedDNA from lakes in Maine, USA.

Both protocols successful at detecting fish sedDNA collected in streams during anadromous fish sea-run migrations

Modified PowerSoil Pro extraction - sample preparation & cell lysis

31m

1 **CENTRIFUGE** sediment samples briefly to separate pore water

DISCARD pore water to retain only sediment samples

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

ADD up to 2.0 g of wet sediment to the PowerBead Pro Tube

Note

Qiagen recommends .25 g
In this test, 0.25 g , 0.5 g , 1.0 g , and 2.0 g of sediment were tested

ADD 800 µL Solution CD1

VORTEX briefly to mix

3 **SECURE** PowerBead Pro Tubes horizontally to a 1.5mL-2.0mL Vortex Adapter

20m

VORTEX for 00:10:00

ROTATE tubes so caps are oriented in opposite direction

VORTEX for another 00:10:00

4 **CENTRIFUGE** PowerBead Pro Tube at 15000 x g for 00:01:00

1m

TRANSFER all supernatant to a clean 2 mL Microcentrifuge Tube

Modified PowerSoil Pro extraction - inhibitor removal

31m

5 **ADD** 200 µL of Solution CD2

VORTEX briefly to mix

6 **CENTRIFUGE** at 15000 x g for 00:01:00

1m

AVOIDING the pellet, transfer **all** supernatant to a clean 2 mL Microcentrifuge Tube

Modified PowerSoil Pro extraction - bind DNA

31m

7 **ADD** 600 µL of Solution CD3

VORTEX briefly to mix

8 **LOAD** 650 µL of the lysate onto a MB Spin Column 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the liquid flow-through

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

CAREFULLY place the MB Spin Column into a clean 2 mL Collection Tube

Modified PowerSoil Pro extraction - wash spin column

31m

10 **ADD** 500 µL of Solution EA to the MB Spin column 1m

CENTRIFUGE at 15000 x g for 00:01:00

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

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

CENTRIFUGE at 15000 x g for 00:01:00

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

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

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

Modified PowerSoil Pro extraction - elute the DNA

31m

13 **ADD** 100 µL of Solution C6 to the center of the white membrane in the MB Spin Column 2m

INCUBATE at Room temperature for 00:01:00

CENTRIFUGE at 15000 x g for 00:01:00

14 **PIPETTE** the liquid flow-through and re-add it to the center of the white membrane in the MB Spin Column 2m

INCUBATE at Room temperature for 00:01:00

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the MB Spin Column

DNA is now ready for downstream applications

(Optional) DNA Concentration with PALL Nanosep 30K Centrifugal Devices 2m

15 **ENSURE** that the sample reservoir is firmly placed into the filtrate receiver

PIPETTE 50-100 µL of DNA extract into the sample reservoir

CAP the Nanosep device

16 **CENTRIFUGE** at 5000 x g for 00:02:00 2m

RECOVER concentrated sample from the sample reservoir with a micropipette

TRANSFER concentrated sample to a new 1.5 mL Microcentrifuge Tube

Concentrated DNA is now ready for downstream applications

Note

If the sample appears to have "spun dry", recover the sample by pipetting ~ 20uL of elution buffer onto the membrane and recovering with a micropipette