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

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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 &

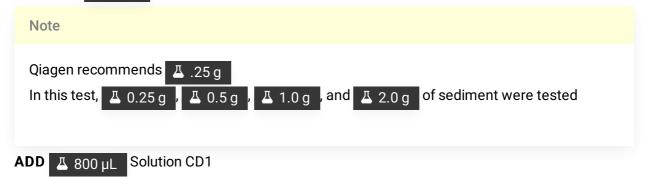


1 **CENTRIFUGE** sediment samples briefly to separate pore water

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 Z 2.0 g of wet sediment to the PowerBead Pro Tube



VORTEX briefly to mix

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

20m

ROTATE tubes so caps are oriented in opposite direction

4 CENTRIFUGE PowerBead Pro Tube at 4 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 Δ 200 μL of Solution CD2

VORTEX briefly to mix

1m

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

Modified PowerSoil Pro extraction - bind DNA

31m

7 ADD Δ 600 μL of Solution CD3

VORTEX briefly to mix

8 LOAD Δ 650 μL of the lysate onto a MB Spin Column

1m

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 Δ 500 μL of Solution EA to the MB Spin column

1m

CENTRIFUGE at <u>■ 15000 x g</u> for 00:01:00

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

11 ADD \angle 500 μ L of Solution C5 to the MB Spin Column

1m

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

 2m

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

Modified PowerSoil Pro extraction - elute the DNA

31m

ADD Δ 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

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

DISCARD the MB Spin Column

DNA is now ready for downstream applications

(Optional) DNA Concentration with PALL Nanosep 30K Cent..

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

PIPETTE 4 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 \sim 20uL of elution buffer onto the membrane and recovering with a micropipette