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Protocol status: Other
Protocol successful at detecting fish sedDNA from stream sediment collected 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 & Inhibitor Removal Pre-Extraction

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ABSTRACT

Protocol (wash buffer plus modified extraction) unsuccessful at detecting fish sedDNA from lakes in Maine, USA
Protocol successful at detecting fish sedDNA collected from streams during an anadromous fish sea-run migration

Keywords: Sedimentary
DNA, SedDNA, Fish

Wash buffer reagents

1 **CREATE** 0.5M EDTA, pH 8.0; final volume: 250mL

Note

1. ADD 52.025 g Ethylenediamine Tetraacetic Acid, Tetrasodium Salt Dihydrate (EDTA) to a volumetric flask
2. ADD 200 mL DI water to dissolve
3. TITRATE to pH 8.0 with Hydrochloric Acid (HCL, approx. 9.5 mL)
4. ADD DI water to bring final volume to 250 mL
5. AUTOCLAVE solution

2 **CREATE** 1M Tris-HCl, pH 8.0; final volume: 500mL

Note

1. ADD 78.8 g Tris-HCl to a volumetric flask
2. ADD 400 mL DI water to dissolve
3. RAISE pH to 8.0 with 10 N NaOH
4. ADD DI water to bring final volume to 500 mL
5. AUTOCLAVE solution

3 **CREATE** a batch of 0.5M $Na_2PO_4*7H_2O$, pH 8.0; final volume: 250mL

Note

1. ADD 33.508 g $Na_2PO_4*7H_2O$ to a volumetric flask
2. ADD 200 mL DI water and heat to dissolve
3. RAISE pH to 8.0 with 10 N NaOH (approx. 1.5 mL)
4. ADD DI water to bring the final volume to 250 mL
5. AUTOCLAVE solution

4 **CREATE** 10N NaOH; final volume: 40mL

Note

1. ADD 16 g NaOH to a volumetric flask
2. ADD 40 mL of DI water to dissolve

5 **MIX** Inhibitor Removal Wash Buffer; final volume: 500mL

Note

1. 10 mL 0.5M EDTA, pH 8.0
2. 25 mL 1M Tris-HCl, pH 8.0
3. 50 mL Na_2PO_4*$7H_2P$, pH 8.0
4. 415 mL DI water

AUTOCLAVE solution

Final buffer contains 10mM EDTA pH 8.0 + 50mM Tris-HCl pH 8.0 + 50mM sodium phosphate dibasic heptahydrate (Na_2PO_4*$7H_2O$) pH 8.0

PCR inhibitor removal via wash buffer

10m 30s

6 **WEIGH** sediment samples into a centrifuge-safe tube

ADD approximately 3x the volume of Wash Buffer to the sediment samples

Note

Account for loss of sediment during wash process (e.g., wash 5g if you plan to extract 3g of sediment)
If you measure 5g sediment, add ~15 mL of Wash Buffer

7 **VORTEX** sample at maximum speed for 00:00:30 10m 30s

CENTRIFUGE at 1760 x g for 00:10:00

DISCARD supernatant

8 **REPEAT** steps 6 and 7 three to four times

PROCEED with DNA extractions

Modified PowerSoil pro extraction - sample preparation & c..

23m

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

ADD 0.5 g of washed sediment to the PowerBead Pro Tube

ADD 800 µL Solution CD1

VORTEX briefly to mix

10 **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

11 **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

23m

12 **ADD** 200 µL of Solution CD2

VORTEX briefly to mix

13 **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

23m

14 **ADD** 600 µL of Solution CD3

VORTEX briefly to mix

15 **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

16 **REPEAT** step 15 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 23m

17 **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

18 **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

19 **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 23m

20 **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

21 **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