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[Modified] DNeasy PowerSoil Pro Kit_Increased Sediment Volume & Inhibitor Removal Pre-Extraction

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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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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



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 Na₂PO₄*7H₂O, pH 8.0; final volume: 250mL

Note

1. ADD  33.508 g Na₂PO₄*7H₂O 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 $\text{Na}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$, 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 ($\text{Na}_2\text{PO}_4 \cdot 7\text{H}_2\text{O}$) 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 & cell lysis

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

Protocol references

Wash Buffer from Poulain et al., 2015; modified from Zhou, Bruns and Tiedje., 1996