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[Modified] Lake ABPS Protocol - University of Maine

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Grayson Huston[1]

[1]Maine Center for Genetics in the Environment, University of Maine

G Grayson Huston
University of Maine

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Protocol status: Working
We use this protocol and it's working

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Abstract

A modified version of the Lake ABPS protocol as described in Thomson-Laing et al. 2022

Protocol successful at detecting fish sedDNA collected from lake **surface** sediments, as well as river sediments during an anadromous fish sea-run migration

Troubleshooting

Alkaline Lysis & Ethanol Precipitation

4h 1m

1 **CENTRIFUGE** sediment samples at 5250 x g for 00:05:00 — 5m

DISCARD pore water using a sterile pipette, so only sediment remains

2 **ADD** 10 g of wet sediment to a sterile 50 mL tube

ADD 6 mL sodium hydroxide (NaOH, 0.33M) to the sample

ADD 3 mL Tris-EDTA (pH 8.0) to the sample

3 **VORTEX** sample at max speed for 00:01:00 — 56m

INCUBATE sample at 65 °C for 00:55:00

ALLOW samples to cool to Room temperature

4 **CENTRIFUGE** samples at 5250 x g for 01:00:00 — 1h

TRANSFER 7.5 mL of supernatant to a new, sterile 50 mL tube

5 **ADD** 7.5 mL Tris-HCl (pH 6.7)

ADD 1.5 mL sodium acetate (3M, pH 5.2)

ADD 30 mL molecular grade ethanol

6 **INCUBATE** samples at -20 °C for 01:00:00 — 1h

7 **CENTRIFUGE** sample at 5250 x g for 01:00:00 — 1h

DISCARD supernatant

ALLOW remaining ethanol to evaporate off of concentrated pellet before proceeding to next step

PowerSoil Pro Extraction on Concentrated Pellet - sample preparation & cell lysis

29m

8 **WEIGH** the concentrated pellet and split it into multiple 0.5 g replicates

TRANSFER each 0.5 g replicate into a PowerBead Pro Tube

9 **ADD** 800 µL of Solution CD1 to each PowerBead Pro Tube 20m

SECURE PowerBead Pro Tubes horizontally to a Vortex Adapter

VORTEX for 00:10:00

ROTATE tubes so caps are oriented in the opposite direction

VORTEX for another 00:10:00

10 **CENTRIFUGE** sample at 15000 x g for 00:02:00 2m

TRANSFER all supernatant to a clean 2 mL Microcentrifuge Tube

PowerSoil Pro Extraction on Concentrated Pellet - inhibitor removal

29m

11 **ADD** 200 µL of Solution CD2

VORTEX briefly to mix

12 **CENTRIFUGE** at 15000 x g for 00:01:00 1m

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

PowerSoil Pro Extraction on Concentrated Pellet - bind DNA

29m

13 **ADD** 600 µL of Solution CD3

VORTEX briefly to mix

14 **LOAD** 650 µL of lysate onto an MB spin column 1m

CENTRIFUGE at 15000 x g for 00:01:00

DISCARD the liquid flow-through

15 **REPEAT** step 14 to ensure all the lysate has passed through the MB Spin Column

CAREFULLY place the MB spin column into a clean 2mL collection tube

PowerSoil Pro Extraction on Concentrated Pellet - wash spin column 29m

16 **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 the same 2 mL Collection Tube

17 **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

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

CAREFULLY place the MB spin column into a **new** 2mL Collection Tube

19 **ADD** 50-100 µL of Solution C6 to the center of the white membrane in the MB Spin Column

Note

Adjust the amount of Solution C6 added to each replicate so that the final volume, once all replicates are pooled together (step 21), totals 200ul

For example, if at Step 8 sample A weighed 1.0g and was split into two 0.5g replicates: A1 and A2. At this step (Step 19), A1 and A2 would each receive 100ul Solution C6, so that when they are pooled together, their total volume is 200ul

If sample A was split into three 0.5g replicates (A1, A2, and A3), each would receive approximately 66ul of Solution C6

20 **CENTRIFUGE** at 15000 x g for 00:01:00 1m

DISCARD the MB Spin Column

POOL all replicates into a sterile 1.5 mL Microcentrifuge Tube

DNA is now ready for downstream applications

Note

For best results in qPCR, use ~ 6 µL of extracted DNA template per PCR reaction

Protocol references

Thomson-Laing, G., Howarth, J.D., Vandergoes, M.J., & Wood, S.A. (2022). Optimised protocol for the extraction of fish DNA from freshwater sediments. Freshwater Biology, 67, 1584-1603. https://doi.org/10.1111/fwb.13962