Apr 18, 2023

Lakes ABPS Protocol - Optimized protocol for the extraction of fish DNA from freshwater sediments (Thomson-Laing et al., 2022)

DOI
dx.doi.org/10.17504/protocols.io.yxmvm24d6g3p/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.yxmvm24d6g3p/v1

Protocol Citation: Georgia Thomson-Laing 2023. Lakes ABPS Protocol - Optimized protocol for the extraction of fish DNA from freshwater sediments (Thomson-Laing et al., 2022). **protocols.io**
https://dx.doi.org/10.17504/protocols.io.yxmvm24d6g3p/v1

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

Created: April 17, 2023

Last Modified: April 19, 2023

Protocol Integer ID: 80636

Keywords: lakes abps protocol, lake sediment samples by an alkaline lysis method, fish dna from freshwater sediment, fish seddna from surface sediment, comparison of multiple seddna extraction method, lake sediment, range of lake sediment, multiple seddna extraction method, freshwater sediment, detecting fish seddna, protocol for the extraction, fish dna, sediment sample, surface sediment, abps protocol, optimized abps protocol, alkaline buffer, highest concentrations of target genet, extraction, alkaline lysis method, lake, sediment, multiple powersoil extraction, dna, abp

Abstract

DNA was extracted from lake sediment samples by an alkaline lysis method with ethanol precipitation adapted from method described by Kuwae et al. (2020); Sakata et al. (2020b); Sakata et al. (2020a).

In a comparison of multiple sedDNA extraction methods, the ABPS (Alkaline buffer - power soil) protocol yielded the highest concentrations of target genets across a range of lake sediments. This protocol was further optimized (65C incubation temperature, pooling of multiple PowerSoil extractions) to overcome technical challenges related to co-precipitation of organic content in lake-surface sediments.

The optimized ABPS protocol is called the "Lakes ABPS protocol"

This protocol has proven to be successful at detecting fish sedDNA from surface sediments in multiple systems for multiple species.

Troubleshooting

Alkaline extraction

1h 51m

1 **INTO** a 50 mL tube add:
10 g of sediment sample
6 mL sodium hydroxide (0.33M)
3 mL Tris-EDTA (pH 8)

2 **VORTEX** for 00:01:00 — 51m

INCUBATE at 65 °C for 00:50:00

3 **ALLOW** samples to cool to Room temperature — 1h

CENTRIFUGE at 15000 x g for 01:00:00

Ethanol precipitation

2h

4 **TRANSFER** 7.5 mL of supernatant to a new 50 mL tube

ADD 7.5 mL of Tris HCl (1M, pH 6.7) to neutralize

5 **ADD** 1.5 mL sodium acetate (3M, pH 5.2) — 1h

ADD 30 mL of molecular grade 100% ethanol

INCUBATE samples at -20 °C for < 01:00:00

6 **CENTRIFUGE** samples at 10000 x g for 01:00:00 — 1h

DISCARD supernatant

RETAIN precipitated pellet

DNeasy PowerSoil extraction

7 **EXTRACT** the total pellet using multiple DNeasy PowerSoil DNA Isolation Kit extractions **following the manufacturer's instructions**

Note

0.25-0.5 g of pellet per extraction

POOL resultant DNA elutes

DNA is now ready for downstream applications

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