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We use this protocol and it's working

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

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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.

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