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 Pedersen, M., Ruter, A., Schweger, C. et al. Postglacial viability and colonization in North America's ice-free corridor. *Nature* 537, 45–49 (2016).
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Protocol status: Working
 We use this protocol and it's working

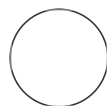
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Modified Organic Extraction Protocol (Pedersen et al., 2016)

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ABSTRACT

A modified version of the organic extraction protocol used in Wales et al., 2014.

Protocol successful at reconstructing the biodiversity of 15 kya sediment core, including a few fish species

GUIDELINES

Phenol should be handled with care as it is corrosive and toxic.
 Chloroform is toxic if swallowed or inhaled. It can cause severe and irreversible health effects, including death

MATERIALS

Lysis buffer
 Proteinase K
 MO BIO's PowerClean DNA Clean Up Kit
 Phenol: Chloroform: Isoamyl alcohol
 Ethanol
 TE Buffer
 30 kDa Amicon Ultra-4 centrifugal filters

SAFETY WARNINGS




Skin exposure to large amounts of phenol or chloroform can cause sores. Skin exposure to lesser amounts of phenol or chloroform can cause irritation. Phenol and chloroform can irritate the eyes that encounter it.

BEFORE START INSTRUCTIONS


Review the manufacturer's Safety Data Sheet and additional chemical information.
 Ensure that a written experimental protocol including safety information is available.


Sample preparation & cell lysis


53m 40s

1 **ADD**  2 g of sediment sample to a sterile spin tube

40s





ADD  170 µg of proteinase K to the sample


ADD  3 mL of lysis buffer to the sample



VORTEX sample at max speed for  00:00:40


Note

Lysis buffer:

-  68 millimolar (mM) *N*-lauroylsarcosine sodium salt
-  50 millimolar (mM) Tris-HCl (pH 8.0)
-  150 millimolar (mM) NaCl
-  20 millimolar (mM) EDTA (pH 8.0) and,

Immediately before extraction, for each  30 mL of lysis buffer add:

-  1.5 mL 2-mercaptoethanol
-  1 mL 1 M DTT

2 **ADD** an additional  170 µg of proteinase K to each sample

INCUBATE  Overnight at  37 °C

Inhibitor removal

53m 40s

3 **REMOVE** PCR inhibitor using MOBIO (MO BIO Laboratories, Carlsbad, CA) C2 and C3 buffers following the manufacturer's protocol

3m

Note

Link to MO BIO's PowerClean DNA Clean Up Kit Handbook:

<https://www.qiagen.com/us/resources/resourcedetail?id=a757e687-7b7a-4801-96bb-6267620414de&lang=en>

CENTRIFUGE samples at  10000 x g for  00:03:00

TRANSFER supernatant to a sterile microcentrifuge tube


Phenol:Chloroform extraction

53m 40s

4 **ADD** equal volume of Phenol: Chloroform: Isoamyl alcohol (25: 24: 1) solution to samples

15m

ROTATE samples gently at  Room temperature for  00:10:00

CENTRIFUGE at  3200 x g for  00:05:00

DISCARD supernatant

5 **REPEAT** step 4

TRANSFER supernatant to a Millipore 15 mL Amicon Ultra filter for concentration and purification

DNA concentration and purification

40m



6 **CENTRIFUGE** filter at  4000 x g for  00:10:00

10m

DISCARD liquid flow-through


7 **ADD**  500 µL EB Buffer (Qiagen) to the filter

10m


CENTRIFUGE at  4000 x g for  00:10:00

DISCARD liquid flow-through

8 **REPEAT** step 7

9 **ADD**  50 µL molecular grade water to the filter

20m

INCUBATE at  Room temperature for  00:10:00

CENTRIFUGE at  4000 x g for  00:10:00

DISCARD filter

DNA is now ready for downstream applications