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

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Mikkel Pedersen[1]

[1]Centre for GeoGenetics, Natural History Museum, University of Copenhagen

Mark Louie Lopez
Umiversity of Victoria

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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 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 theeyes that encounter it.

Before start

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:

- [M] 68 millimolar (mM) *N*-lauroylsarcosine sodium salt
- [M] 50 millimolar (mM) Tris-HCl (pH 8.0)
- [M] 150 millimolar (mM) NaCl
- [M] 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 1M 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

Protocol references

Pedersen, M., Ruter, A., Schweger, C. et al. (2016). Postglacial viability and colonization in North America's ice-free corridor. Nature 537, 45–49. https://doi.org/10.1038/nature19085

Wales, N., Andersen, K., Cappellini, E., Avila-Arcos, M. C. & Gilbert, M. T. Optimization of DNA recovery and amplification from non-carbonized archaeobotanical remains.*PLoS One***9**, e86827 (2014)