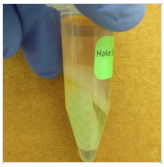

Sep 09, 2021

eDNA extraction: phenol-chloroform-isoamyl alcohol DNA purification from filters stored in Longmire buffer

Forked from eDNA extraction: phenol-chloroform-isoamyl alcohol DNA purification from filters stored in Longmire buffer

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Ana Ramón-Laca

DISCLAIMER

ABSTRACT

This is an organic DNA extraction method for filters preserved in 2 ml of Longmire buffer that uses a phase lock to allow easy decanting of the aqueous layer instead of pipetting.

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PROTOCOL CITATION

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FORK NOTE

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53112

GUIDELINES

To make TlowE:
To make 50 ml
1. 500 µl of 1 M Tris-HCL, pH 8.0
2. 10 µl of 0.5 M EDTA, pH8.0
3. up to 50 ml of nuclease free H2O

MATERIALS TEXT

- proteinase k 20 mg ml^{-1}
- Dow Corning high vacuum grease 5.3 oz
- Phenol-chloroform-isoamyl (25:24:1)
- Chloroform:isoamyl 24:1
- Isopropyl
- Ethanol (95%)
- 5 ml LoBind tubes Eppendorf
- Pipettes and tips (5 ml, 1 ml, 200 µl)
- 1.5 ml LoBind tubes Eppendorf
- Zymo One-Step inhibitor removal plates
- Incubator
- Centrifuge with a rotor with capacity for 5 ml tubes
- Tube racks (5 ml, 2 ml)
- Tube squeezing tool and sterile 15 ml syringes with wings or rings for grease dispensing
- Laboratory chemical fume hood or biosafety cabinet
- 5 M NaCl
- 1 M Tris-HCL, pH 8.0
- 0.5 M EDTA, pH8.0
- H_2O

SAFETY WARNINGS

Phenol and Chloroform are very corrosive and irritant chemicals. Always perform these extractions in fume hoods, wear a lab coat, safety glasses, nitrile gloves and any other PPE. Also, always use resistant plasticware. Please refer to Safety Datasheets for more information. Phenol is volatile and can burn your skin and damage your eyes. Chloroform can make you faint and can even be lethal

DISCLAIMER:

DNA extraction 2h 50m

1 **heat-shock** Filters in **2 mL** of at **95 °C** for **00:05:00 min** and then allow samples to cool to room temperature 5m

Longmire lysis buffer
by Ana Ramón-Laca

PREVIEW RUN

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1.1 975 ml double-distilled water

1.2 100 ml of 1 M Tris-HCL, pH 8.0

1.3 200 ml of 0.5 M EDTA, pH8.0

1.4 2 ml of 5 M NaCl

1.5 25 ml of 20% SDS (w/v) Filter the buffer with an autofill PES bottle top filtration device (sterile 500ml, 0.22 µm)

2 **digestion** add **100 µl** proteinase K (final concentration **1 mg/ml**)

3 **incubation** **56 °C** at **120 rpm** for **02:00:00** 2h

4 **phase lock set up** add ca. **800 µl** of vacuum grease with a syringe onto the wall of the 5ml tube without touching the lysate to avoid cross-contamination

Eppendorf 5415R Refrigerated Centrifuge
Refrigerated Centrifuge
Eppendorf **EP-5415R**

5 ⚠☠

add 2 ml of phenol-chloroform-isoamyl (25:24:1) pH **8**

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pH is critical for good results

6 shake well and centrifuge **13300 x g, 4°C, 00:05:00** 5m

7 place ca. **800 µl** of vacuum grease in 2 sets of empty tubes for CI. (These can be prefilled for convenience)

8 ⚠

add **2.2 mL** of chloroform:isoamyl 24:1 and decant aqueous layer from step 6.

Important!! only add the chloroform just before use (while the tubes with PCI are in the centrifuge) so it does not affect the phase lock if grease is at the bottom.

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9 shake well and centrifuge **13300 x g, 4°C, 00:05:00** 5m

10 **go to step #8**

11 add **2 mL** of isopropanol (can be prefilled for convenience), add **80 µl** of **5 Molarity (M)** NaCl and decant aqueous layer from step 11

12 invert several times

13 precipitation overnight (or 2 h) at room temperature

14 centrifuge **13300 x g, 4°C, 00:30:00** 30m

15 pour liquid off slowly and add **800 µl** of ice cold **70 % (v/v)** EtOH

16 shake well and centrifuge **13300 x g, 4°C, 00:05:00** and **go to step #15** 5m

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17 pour liquid off slowly and allow tubes to dry for 1 h or until dry

18 once they are dry, resuspend in **100 µl** TE buffer (warm **37 °C** to favor elution)

19 store in the freezer (**-20-80 °C**)

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