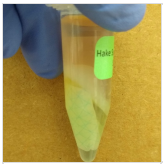

Sep 08, 2021

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

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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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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
- TlowE (Tris-HCl, EDTA, H2O)

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 safety equipment. 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

1 heat-shock Filters in 2 ml of Longmire buffer at 95C for 5' and then allow samples to cool to room temperature

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

3 incubation 56C at 120 rpm for 2h

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4 phase lock set up add ca. 800 µl of vacuum grease with a syringe onto the wall of the tube of the 5 ml tube

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

6 centrifugation shake well and spin 13.3 x g for 5' at 4C

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

8 

CI 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.**

9 centrifugation shake and spin 13.3 x g for 5' at 4C

10 CI add 2.2 ml of chloroform:isoamyl 24:1 and decant aqueous layer from step 9

11 centrifugation shake and spin 13.3 x g for 5' at 4C

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

13 mixing invert several times

14 precipitation overnight (or 2 h) at room temperature

15 centrifugation spin 13.3 x g for 30' at 4C

wash x2 pour liquid off slowly and add 800 µl of ice cold 70% EtOH

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16

17 centrifugation shake and spin 13.3 x g for 5' at 4C and repeat wash

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

19 resuspension once they are dry, resuspend in 100 µl TE buffer (warm - 37C)

20 storage store in the freezer (-20-80 C)

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