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CTAB–Chloroform DNA Extraction from Ethanol-Preserved Freshwater Phytobentos

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We use this protocol and it's working

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Abstract

This protocol describes a CTAB-based extraction of eDNA from particulate material pelleted from an ethanol-preserved river/stream water sample. It uses a single chloroform extraction, isopropanol precipitation, two 70% ethanol washes, and elution in Qiagen EB buffer. The protocol is adapted from Strand et al. (2019) with modifications for a pellet-based workflow.

Guidelines

- Contamination prevention (recommended): Use filter tips, wear gloves, change gloves frequently, and decontaminate work surfaces with a bleach-based solution before and during the work.
- Pellet handling: DNA pellets may be small and can be nearly invisible to the naked eye. Use extra care when removing the isopropanol supernatant after precipitation and during the 70% ethanol wash steps to avoid pellet loss.
- Optional clarification step: If the recovered aqueous phase remains visibly turbid after chloroform separation, an additional chloroform extraction can be performed to improve phase clarity before precipitation.

Materials

Reagents

- CTAB buffer (pH 8.0): 100 mM
- Tris-HCl, 20 mM EDTA, 1.4 M NaCl, 2% (w/v) CTAB (no additives)
- Proteinase K (20 mg/mL)
- Chloroform, pure (p.a.)
- Isopropanol (p.a.)
- 70% ethanol (prepared from p.a. ethanol + autoclaved deionized water)
- Qiagen EB buffer (10 mM Tris-Cl, pH ~8.5)

Consumables

- 2.0 mL microcentrifuge tubes (standard snap-cap “Eppendorf” type)
- Filter tips
- Gloves

Equipment

- Microcentrifuge for 2.0 mL tubes (able to run at ~13,500 rpm / max speed)
- Temperature-controlled device (heat block, water bath, or incubator) capable of 65 °C
- Vortex mixer
- Vacuum concentrator (optional; alternative is heat block drying)

Safety warnings

- Chloroform is toxic and volatile: handle in a fume hood; dispose of organic waste properly.
- Ethanol and isopropanol are flammable: keep away from ignition sources.
- Bleach-based disinfectants are irritants/corrosive: wear gloves and avoid mixing with acids.

Before start

- Pre-cool isopropanol and 70% ethanol at −20 °C.
- Preheat a heat block, water bath, or a temperature-controlled incubator (suitable for microcentrifuge tubes) to 65 °C.
- Decontaminate the work surface and equipment and organise a clean workflow to minimise contamination.

Pellet preparation from ethanol-preserved sample (2 mL aliquot)

33m 30s

1 Resuspend particulates in the ethanol-preserved sample container by manual shaking until homogenized. 30s

2 Transfer 2 mL of homogenized ethanol mixture into a 2.0 mL microcentrifuge tube. 30s

3 Centrifuge at 13500 rpm, Room temperature, 00:20:00 (max speed). 21m

4 Carefully decant the supernatant without disturbing the pellet. 30s

5 Dry the pellet for 00:10:00 using either a vacuum concentrator or a heat block (open cap), ensuring ethanol is removed. 11m

CTAB lysis and Proteinase K digestion

2h 35m

6 Add 500 µL CTAB buffer (room temperature) to the dried pellet. 30s

7 Vortex continuously for 00:15:00 . 16m

8 Freeze at -20 °C until fully frozen (typically 01:00:00); samples may be stored at -20 °C Overnight and processed the next day. 1h 1m

9 Thaw at 65 °C for 00:15:00 . 16m

10 Add 4 µL 20 µL Proteinase K ([M] 20 mg/mL) and vortex briefly. 30s

11 Incubate at 65 °C for 01:00:00 . 1h 1m

Chloroform extraction

19m

12 Add 400 µL chloroform and mix gently with the pipette tip. 1m

13 Centrifuge at 13500 rpm, Room temperature, 00:15:00 . 16m

14 Carefully transfer ~ 300 µL of the upper aqueous phase to a new 2.0 or 1,5 mL tube using a fresh filter tip, avoiding the interphase. 2m

Isopropanol DNA Precipitation

33m

15 Add 400 µL ice-cold isopropanol (stored at -20 °C) and mix by inverting the tube several times.

16 Incubate 00:15:00 at 4 °C . 16m

17 Centrifuge for 13500 rpm, Room temperature, 00:15:00 . 16m

18 Remove supernatant carefully without losing the pellet. 1m

Ethanol washes

15m

19 Add 500 µL ice-cold 70% ethanol (stored at -20 °C) and briefly vortex. 1m

20 Centrifuge at 13500 rpm, Room temperature, 00:05:00 . 6m

21 Carefully remove supernatant. 30s

22 Repeat etanol wash once more go to step #19 . 7m 30s

Drying and elution 12m

23 Dry the pellet (open cap) for ~ 00:10:00 in a vacuum centrifuge or on a 65 °C heat block (avoid airborne contamination). 11m

24 Dissolve DNA pellet in 30 µL EB buffer (or sterile milliQ water), vortex and spin down. Let the DNA dissolve for at least 1 hour before further analysis (or store in fridge/freeze). 1m

Protocol references

Strand, D. A., Johnsen, S. I., Rusch, J. C., Agersnap, S., Larsen, W. B., Knudsen, S. W., Møller, P. R., & Vrålstad, T. (2019). *Monitoring a Norwegian freshwater crayfish tragedy: eDNA snapshots of invasion, infection and extinction*. Journal of Applied Ecology, 56(7), 1661–1673. https://doi.org/10.1111/1365-2664.13404