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Phenol-based RNA extraction from polycarbonate filters

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1 Works for me dx.doi.org/10.17504/protocols.io.bivuke6w

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ABSTRACT

This protocol is an acid-phenol-based method for extracting RNA from samples collected onto polycarbonate filters. Bead-beating is included to increase yields from difficult to lyse cells, for example, in some species of cyanobacteria. We have used it successfully in RNA-sequencing projects involving lab cultures of cyanobacteria and from freshwater and marine environmental samples.

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KEYWORDS

RNA extraction, polycarbonate filters, bead beating, cyanobacteria

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GUIDELINES

This protocol assumes that sample biomass has been appropriately collected onto polycarbonate filters and properly stored at -80 °C.

MATERIALS TEXT

frozen polycarbonate filters containing sample biomass
ice
Solution A (lysing buffer) (0.5% SDS, 20 mM sodium acetate, 10 mM EDTA)
Lysing Matrix E tubes (bead beating tubes)
basic phenol with 8-hydroxyquinoline (pH 7.8-8.2)
acid phenol:chloroform:isoamyl alcohol (125:24:1; pH 4.8)
chloroform
100% ethanol

70% ethanol
RNase-free water

SAFETY WARNINGS

Phenol is a very hazardous chemical. Use extreme caution and follow recommended manufacturer and institutional safety guidelines.

Phenol-based RNA Extraction

1 Add 700 µL of Solution A (lysing buffer) to an MP Biomedical Lysing Matrix E tube (or equivalent bead beating tube). Solution A consists of 0.5% SDS, 20 mM sodium acetate, and 10 mM EDTA in RNase-free molecular biology grade water.

2 Pre-chill tube containing Solution A on ice to prevent tube from overheating during bead beating.

3 Unfold polycarbonate filter (either frozen, or thawed but maintained on ice) and place into pre-chilled bead-beating tube.

4 Agitate tube on maximum speed for 40 s in Biospec Products Mini-beadbeater (or equivalent), then place on ice.

5 Add 500 µL basic phenol with 8-hydroxyquinoline (pH 7.8-8.2) plus 100 µL chloroform to tube.

6 Agitate tube on maximum speed for 40 s, then place briefly on ice to chill.

7 Centrifuge tube at maximum speed in benchtop centrifuge for 5 min.

8 Transfer as much of aqueous supernatant as possible from bead beating tube to new 2 mL centrifuge tube.

9 Re-extract aqueous supernatant twice with 500 µL of acid phenol:chloroform:isoamyl alcohol (125:24:1; pH 4.8).

10 Re-extract aqueous supernatant once with 500 µL basic phenol with 8-hydroxyquinoline plus 100 µL chloroform.

11 Re-extract twice with 500 µL pure chloroform to remove last traces of residual phenol.

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Ethanol Precipitation

12 Add sodium acetate to aqueous supernatant to a concentration of 0.3 M and mix.

13 Add 2-2.5x volume of ice-cold 100% ethanol and mix well.

14 Place in -80 °C freezer for at least 1 h, or preferably overnight.

15 Centrifuge at maximum speed at 0 °C for at least 30 min in refrigerated benchtop centrifuge. If you expect low yields, or wish to increase recovery, spin for 1 h at maximum.

16 Carefully decant ethanol supernatant without disturbing RNA pellet.

17 Wash pellet by adding 1 mL of ice-cold 70% ethanol and gently pipetting to dislodge RNA pellet.

18 Centrifuge at maximum speed at 0 °C for at least 15 min in a refrigerated benchtop centrifuge.

19 Gently and carefully aspirate as much of the 70% ethanol from the tube as possible taking caution not to accidently aspirate the RNA pellet.

20 Place the open tube in a 37 °C heating block for 3-5 min to evaporate all ethanol. Do not dry pellet to completion, as this can make RNA difficult to redissolve in water.

21 Dissolve the RNA pellet in an amount of RNase-free water appropriate for your expected yield and needed final concentration, typically about 50-100 µL.

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