RNA extraction and precipitation for 47 mm PES and polycarbonate filters

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Working

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ABSTRACT

This protocol is a modified version of Bryn Durham et al., 2015 for extracting RNA from filters.

This protocol has been tested with 0.22 μm pore size Durapore filters and 2.0 μm polycarbonate filters. Custom synthesized RNA transcript standards are added at the time of extraction and are recovered post-sequencing for quantitative metatranscriptome analysis (Satinsky et al., 2013). To prevent RNA degradation, work in a clean environment with asceptic practices and avoid possible introduction of RNases to samples and reagents.

DOCX

Phenol Chloroform Protocol for RNA Extraction final used for matrix summer 2017217.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

RNA is easy to work with; you just need to be very clean and fast with the samples. Do not let the samples thaw, do the samples one at a time.

Use RNase-free solutions and tools.

Most of the steps require things to be on ice, keep that in mind.

This protocol uses a lot of microtubes, make sure you have the right ones at all time and before starting. The low binding DNA microtubes do not make a pellet.

*Make sure you turn the cooling microcentrifuge to 4C before starting.

MATERIALS

NAME	CATALOG #	VENDOR
0.1 mm Zirconia/Silica Beads	11079101z	Bio Spec Products Inc.
0.5 mm Zirconia/Silica Beads	11079105z	Bio Spec Products Inc.
0.5mm diameter glass beads	SI-BG05	Scientific Industries, Inc.
Proteinase K	EO0491	Thermo Fisher Scientific

SAFETY WARNINGS

Materials used in this protocol are hazardous and should be handled and disposed of appropriately.

BEFORE STARTING

Combust all beads (4 hours @ 500 Celsius).
Clean the work station and all materials to eliminate RNAses.

1 Prepare a 2.0 ml tube (low-binding) for each sample, with each tube containing 400 µl beads (mix of 200 µl 0.1 mm zirconium beads, 100 µl 0.5 mm glass beads, 100 µl 0.5 mm zirconium beads.

Glass beads are only needed when processing phytoplankton. If extracting from bacteria only then replace glass beads with more 0.5 mm zirconium.

2 Cut membrane filters using a RNAse free scissor.

3 Add T1E1 buffer with SDS 0.6%, Proteinase K 120 ng/µl. Vortex for 10 minutes, incubate at RT for 50 minutes. Add 964 µl of T1E1 buffer, 30 µl of 20% SDS (20% SDS stock), and 6 µl of proteinase K, total 1000 µl.

T1E1 buffer: 10 mM Tris + 10 mM EDTA

4 Add the RNA standard - 0.5% of expected yield

5 Transfer supernatant to a new 2.0 ml microtube (low-binding). Be careful not to transfer the beads.

6 Centrifuge for 1 min at 8,000 rpm. If you have beads in your sample, repeat 5.

7 Add Acidic Phenol:Chloroform:Isoamyl alcohol (25:24:1) at a 1:1 volume. Shake until emulsified, don't vortex.

8 Incubate the mixture on ice for 15 min.

9 Centrifuge at 13,400 rpm for 15 min, 4 Celsius. Collect supernatant in a new 2.0 ml tube.

10 Add 1 volume of Chloroform:Isoamyl alcohol (24:1), shake until emulsified.

Chloroform by itself will also work but it will create foam.

11 Incubate the mixture 15 min, on ice.

12 Centrifuge at 13,400 rpm for 15 min, 4 Celsius. Collect supernatant in a new tube.

13 Repeat Chloroform cleanup (steps 10, 11, and 12).

14 Add 1 volume of isopropanol. Mix with a 21g syringe and shear DNA (8-10 times). Incubate at -20 Celsius overnight.

15 Centrifuge 30 min, 13,400 rpm, 4 Celsius. Remove supernatant, being careful not to lose the pellet.

You can transfer the supernatant to a new microtube, in case you lose the pellet you can recover it. This pellet almost always sticks to the microtube, the wash step is the one that mostly gets lose.

16 Add 1.0 ml 75% ethanol.

17 Centrifuge 10 min, 13,400 rpm, 4 Celsius. Discard supernatant, being careful not to lose the pellet.

18 Repeat ethanol wash (steps 16 and 17).

19 Short spin (~15s), remove remaining supernatant.

20 Repeat the short spin and make sure there is no ethanol in the sample.

If the pellet is wet it will look white, if it's dry it will look clear.

21 Resuspend in ~30 ul RNAse free water.

This is depending on your experiment and needs, wait from 30 min to 1 hour on ice to take a reading, RNA takes time to dissolve in the sample.

22 Proceed to other applications or store at -80 Celsius.

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