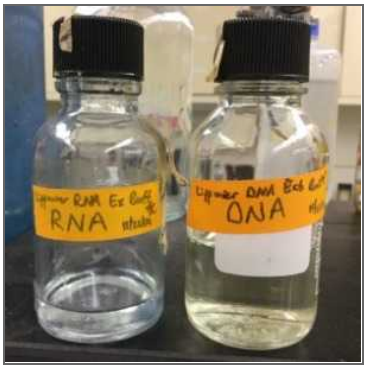

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OPEN ACCESS

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MANUSCRIPT CITATION: Lippmeier, J.C., Crawford, K.S., Owen, C.B., Rivas, A.A., Metz, J.G. and Apt, K.E. (2009), Characterization of Both Polyunsaturated Fatty Acid Biosynthetic Pathways in *Schizochytrium* sp.. Lipids, 44: 621-630. https://doi.org/10.1007/s11745-009-3311-9

Labyrinthulomycete total RNA extraction protocol - hot phenol

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Protist Research to Optimize Tools in Genetics (PROT-G) | Collier Lab

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ABSTRACT

Modified from Lippmeier et al. 2009; developed as part of the labyrinthulomycete JGI Community Sequencing Project and Gordon and Betty Moore Foundation Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP)

SAFETY WARNINGS

Be super careful with hot phenol! It's near the boiling point of phenol, so just a little too hot can be explosive.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 86355

Preparing biomass and reagents

1 Grow up cells, collect, and freeze rapidly - preferably in liquid nitrogen. Store biomass at -80C if not extracting immediately.
This protocol has worked so far for two different thraustochytrids (*Aurantiochytrium limacinum* ATCC MYA-1381, *Schizochytrium aggregatum* ATCC 28920) and two *Aplanochytrium* strains (PBS06 and PBS07).

2 Prepare extraction buffer
100 mM Tris-HCl pH 7.5
1.5 M NaCl
50 mM Na_2-EDTA pH 8.0
20 g per liter CTAB (cetyltrimethylammonium bromide)
8 mg per ml DTT (dithiothreitol) - ADD FRESH JUST BEFORE USE
(note, if you keep these stocks in glass bottles, first soak the bottles with 0.1 M NaOH to inactivate RNAses)

Prepare Tris-equilibrated phenol, pH 4.5-4.8, by warming to 65 C
Prepare acid phenol:chloroform:isoamyl alcohol (pH 4.5-4.8, 125:24:1) by warming to 65 C
Set up to incubate the extraction step at 65 C

Prepare 8M LiCl

Chill 100% ethanol and 70% ethanol

Prepare TE or nuclease-free water

Get a bucket of ice

Extraction steps - REPEAT 3 TIMES (do 3 extractions)

3 Suspend ~1000 micrograms wet weight biomass per ~10 ml extraction buffer by vortexing (these amounts would yield tens of micrograms of total RNA)

3.1 Incubate at 65 C for 5 minutes, mix by vortex or inverting 2 or 3 times

4 Add equal volume hot (65 C) acid phenol/chloroform/isoamyl alcohol, votes to mix well

4.1 Incubate at 65 C for 5 minutes, mix by vortex or inverting 2 or 3 times

5 Cool the mixture in ice

6 Centrifuge to separate the phases 4 C, 6,000g, 15 minutes

7 Move the top phase to a new tube, avoiding the interface

Precipitation steps

8 Add an equal volume of isopropanol and incubate overnight at 4 C

8.1 If remaining DNA forms a large fluffy precipitate, remove by spooling onto glass rod

9 Collect precipitate by centrifugation 4 C, 16,000g, 30 minutes
(may require distributing the sample among several 1.5 ml microfuge tubes and/or multiple spins for each tube to process the volume)

10 Wash each pellet with 0.5 ml ice-cold 75% ethanol twice, centrifuging 4 C 16,000g, 5 minutes

10.1 Allow the pellets to air dry

11 Dissolve pellets in an appropriate volume, analyze by appropriate method (spectrophotometry, Bioanalyzer); store frozen at -80 C

Optional cleanup steps

12 If the RNA contains particulates that do not dissolve, they can be removed by centrifugation 4 C, 6,000g, 5 minutes and transferring the supernatant to a fresh tube.

13 Even if the RNA does not have obvious DNA, a DNAse treatment step is nonetheless recommended

14 If the RNA contains large amounts of DNA or other contaminants, it can be removed by selectively precipitating the RNA with 17 microliters of 8M LiCl per 50 microliters RNA

14.1 Incubate -20 C 60 minutes

14.2 Collect RNA by centrifugation 4 C, 16,000g, 20 minutes

15 Wash each pellet with 0.5 ml ice-cold 75% ethanol twice, centrifuging 4 C 16,000g, 5 minutes

15.1 Allow the pellets to air dry

16 Dissolve pellets in an appropriate volume, analyze by appropriate method (spectrophotometry, Bioanalyzer); store frozen at -80 C