MYXOCOCCUS TOTAL RNA PREPARATION PROTOCOL

Adapted from existing protocols by Vinh Pham.
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MATERIALS:

10% SDS
1M NaOAc pH 5.2
Phenol, Unbuffered/Water-saturated
24:1 Chloroform:Isoamyl Alcohol
3M NaOAc/1mM EDTA pH 8.0 (seal top with Parafilm to minimize evaporation)
100% Ethanol (store at -20ºC)
70% EtOH/10mM Tris pH 7.4/10mM NaCl (seal top with Parafilm)
DEPC-treated water
RQ1 DNAse (1U/µl) Promega cat# M6101

PROCEDURE:

1. Grow cells to Klett 80-100. Pellet the desired amount of cells and resuspend in 500µl TPM. For developmental samples, spot 50-60 spots of 20µl each onto 150mm TPM agar plates and transfer fruiting bodies/spores/cells at the desired time point to a 1.5ml µfuge tube containing 500µl TPM.

   Note: The more cells, the greater the RNA yield. For volumes >2.0ml, pellet the cells in multiple 1.5ml µfuge tubes (1' at max speed) and resuspend in a single volume of 500µl TPM.

2. Freeze at –80ºC.

   Note: Ideally, cells should be quick frozen by immersing the µfuge tube in liquid nitrogen for ~1'.

3. Pre-heat 10% SDS, 1M NaOAc pH 5.2, and phenol at 64ºC.

4. Add 100µl SDS, mix, and shake every 10" for 1-2min. at 64ºC.

   Note: Adding pre-heated SDS and pipetting to resuspend will thaw the sample.

6. Add 110µl NaOAc.

7. Add 1ml phenol. Shake vigorously every 30"-1' for 4-6' at 64ºC.

8. Spin on countertop microfuge for 10' at 14Krpm. Remove aqueous layer to new tube.

9. Repeat 1ml phenol extraction.

   Note: Perform additional extractions with phenol, as needed. Use phenol with a pH below 7.8 to minimize chromosomal DNA contamination. DNA enters the organic phase below this pH.

10. Add 1ml RT chloroform/IAA. Spin for 10' at 14Krpm. Remove aqueous layer to new tube.

11. Add 130µl NaOAc-EDTA.

12. Add 1ml ice-cold 100% ethanol.

    Note: The sample can be stored at –20ºC if desired.

13. Spin for 25' at 10Krpm.

14. Wash pellet 3X with 500µl EtOH/Tris/NaCl.

    Note: Do not disrupt pellet between washes. Simply add the wash solution to the pellet, let sit for 1-5', then spin for 1' at max speed on a countertop µfuge.

Note: To prepare, autoclave	*1M Tris, pH 7.6*	*1.76ml*
	NaCl	*0.116g*
	dH_2O	*51.3ml*
	Adjust to pH 7.4	
Allow to cool.		
Add	*95% EtOH*	*147ml*

15. Resuspend in 100µl DEPC-treated water.

    Note: Add diethyl pyrocarbonate (DEPC) to a final concentration of 0.1% to distilled water, incubate at 37ºC for 1hr, and inactivate by autoclaving.

16. Add 5µl RQ1 DNase (RNase-free) & incubate at 37ºC for 1hr to remove DNA contamination.

    Note: This step is essential if the RNA sample is to be used in quantitative experiments, e.g., RNA "slot blots" or real-time RT-PCR experiments.

17. Incubate at 64ºC for 5' to inactivate.