MYXOCOCCUS TOTAL RNA PREPARATION PROTOCOL
Adapted from existing protocols by Vinh Pham.
Last modified: February 12, 2002

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 -20°C)
- 70% EtOH/10mM Tris pH 7.4/10mM NaCl (seal top with Parafilm)
- DEPC-treated water
- RQ1 DNAs (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 –80°C.

   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 64°C.

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

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

5. Add 110µl NaOAc.

6. Add 1ml phenol. Shake vigorously every 30°-1' for 4-6' at 64°C.

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

8. 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.

9. 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 –20°C if desired.*


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*

<table>
<thead>
<tr>
<th>Solution</th>
<th>Volume</th>
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<tbody>
<tr>
<td>1M Tris, pH 7.6</td>
<td>1.76ml</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.116g</td>
</tr>
<tr>
<td>dH₂O</td>
<td>51.3ml</td>
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<tr>
<td>Adjust to pH 7.4</td>
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</tbody>
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   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 37°C for 1hr, and inactivate by autoclaving.*

16. Add 5µl RQ1 DNase (RNase-free) & incubate at 37°C 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 64°C for 5’ to inactivate.