Stonybrook Arrays Protocol
Title: RNA Extraction from Fission Yeast
Version 1.0
Last updated 11/10/03 by Haiying Chen / Anna Oliva

1. Purpose

To extract and purify RNA from *S. pombe* for labeling reactions.

2. Reagent Preparation

Note: Always use gloves, separate solutions and special tubes/tips when working with RNA. Make all your reagents with DMPC(or DEPC) water (See 5.1).

2.1 DMPC water, 1 L (work in a hood)
Dissolve 1 ml DMPC in 99ml 50% ice old EtOH
Add 900ml dH_2O
Room temperature > 30 minutes
Autoclave

2.2 TES, 50 ml (10mM Tris pH 7.5; 10 mM EDTA pH 8, 0.5 % SDS)
(Tris, EDTA and SDS all made with DMPC water)

1 M Tris PH 7.5	0.5 ml
0.5M EDTA PH 8.0	1 ml
10% SDS	2.5 ml
DMPC water	46 ml

2.3 Sodium Acetate (NaOAc), 3M, PH 5.2

3. Protocol

Note: When working with phenol or chloroform, use a fume hood.

3.1 Grow *S. pombe* 25 ml/sample for early-log culture. (See 5.2)

3.2 Harvest cells: Add culture to cold centrifuge tubes with ice. Spin 4 min, 4300 rpm (~3k x g), 4ºC. Resuspend cell pellet in ~ 1 ml cold dH_2O, transfer to 2.0 ml tubes, spin ~ 13,000 x g (See 5.3), 30 sec, discard SN.

3.3 Cell pellets can be snap frozen (liquid N_2) and stored at -70 ºC.

3.4 Add 750 µl of TES to pellet in 2.0 ml tube, resuspend with pipetman, add 750 µl acidic phenol-chloroform, vortex 5 sec, place in 65 ºC heat block. Then do the next sample in the same way.

3.5 From the time the last sample is prepared, incubate samples at 65ºC for 1 hr (or longer), vortex 10 sec every 10 min.

3.6 Put on ice 4 min, vortex 20 sec, and spin for 18 min, 13,000 x g.

3.7 Prepare **2** sets of yellow phase-lock tubes, spin 1 min, 13,000 x g. Add 650 µl acidic phenol-chloroform/tube to the first set.

3.8 Add **650 µL** of the upper phase per sample from step 3.6 to the first set of phase-lock tubes, mix by inverting, spin for 7 min, 13,000 x g.

3.9 Add 600 µl of chloroform:isoamyl alcohol (24:1)/tube to the second set of phase-lock tubes.

3.10 Take **600** µl of the upper phase per sample from step 3.8 and add to the second set of phase-lock tubes, mix by inverting, spin 7 min @ ~13,000 x g.

3.11. Prepare normal 2 mL Eppendorf tubes with 1.5 mL of 100% EtOH (-20ºC) and 50 µl of 3 M NaOAc, pH 5.2.

3.12. Transfer **500** µl of the water phase to the tubes with EtOH, vortex 10 sec. leave @ -20ºC overnight or @ -70 ºC for 45 min.

3.13. Spin 10 min, ~13,000 x g. Discard SN, add 500 µl 70% EtOH (4ºC, made with DMPC water), don't vortex, spin 1 min. Aspirate most of SN, give a quick spin and remove rest of liquid with pipetteman. Air dry ~ 5 min.

3.14. Add 100 µl of DMPC water, and incubate 15 min @ 37ºC. Dissolve pellet by pipetting up and down (~30x) until no particles are left, then gently vortex 10 sec.

3.15. Measure $OD_{260/280}$: Rinse 50ul micro cuvette with 0.1 M NaOH, then 0.1 M HCl, and thoroughly with dH_2O. Dilute 1:100 (1 ul sample in 99 DMPC water), set reference with DMPC water. (For an accurate reading, OD_{260} should be between 0.1 and 1).

3.16. RNA conc. (ug /ul) = OD_{260} x 40 / 1000, expect a yield of 100-120 µg (See 5.2).

3.17. Purify 100 µg of each sample using RNeasy mini spin columns (Qiagen) as described in the RNeasy Mini Handbook. Elute twice with 40 µl RNase-free water.

3.18. Measure $OD_{260/280}$ of purified RNA: Same as 3.15 except making 1:50 dilution, expect a yield of ~80%. (See 5.2)

3.19. (Optional) Run 1 µg of purified RNA (~ 1 µl) on a 1% agarose gel (first scrub out the gel box with detergent and rinse thoroughly), use freshly prepared 1X TAE buffer, RNAse-free loading buffer (made with DMPC

water). You should see the ribosomal bands clean, distinct and without smears (running about 1.8 and 3.5 kb).

3.20. Store purified RNA in -70ºC. Use 25 μg for each labeling reaction, you can also do multiple reactions in the same tube (50 ug for 2x reaction, or 100 ug for 4x reaction).

4. Materials, suppliers, and ordering information

4.1. DMPC (Sigma D-5520)
4.2. 2 ml eppendorf tube (BioExpress C-3219-1)
4.3. Acidic phenol-chloroform (Sigma P-1944)
4.4. Phase lock tube, 2ml, heavy (Eppendorf 0032 005.132)
4.5. RNeasy mini kit (Qiagen 74106)

5. Detailed Protocol notes/discussion

5.1. DMPC is a substitute for DEPC. We choose DMPC since it is less toxic than DEPC. You can also use DEPC for this protocol.

5.2. We usually start with 25 ml of *S. pombe* in YES culture, OD_{600} 0.24 (6 OD in total, ~ $1.2x10^8$ cells). After hot phenol extraction, we get a RNA yield of 100-120 ug ($OD_{260/280}$ 1.7-1.8). After Qiagen purification, we get 70-90 ug ($OD_{260/280}$ 1.8-2.0). Yield may vary for cells grow in other types of media.

5.3. All centrifugation (except when harvesting cells) steps are done in a micro centrifuge at room temperature. For that centrifuge, 13,000 x g is roughly 13,000 rpm.

6. Protocol adapted from sources

7. Related web-links and references