RNA Extraction from Fission Yeast

Pombe Post-Genomics

Caution: always use gloves, separate solutions and special tubes/tips when working with RNA

1. Harvest cells (usually 25 ml of OD_{600} ~0.2, adjust volume according to OD). Centrifuge 2 min at 2000 rpm and discard SN. Snap freeze pellet (liquid nitrogen or dry ice/ethanol). Alternatively, filter cells and snap freeze filter disc. Store cells at -70°C.
2. Thaw cells on ice (~5 min). Add 1 ml of pre-chilled DEPC water, resuspend cells, and transfer to 2 ml Eppendorf tubes. Spin 10 sec at 5000 rpm and remove SN.
3. To pellet add 750 µl of TES (adjust if total cells are >5 ODs), resuspend cells with pipette, immediately add 750 µl acidic phenol-chloroform (refrigerated, Sigma P-1944), vortex, and incubate in 65°C heat block (use fume hood!). Then do the next sample in the same way.
4. Incubate all samples in 65°C heat block for 1 hr, vortex 10 sec every 10 min.
5. Place samples on ice for 1 min, vortex 20 sec, and centrifuge for 15 min at 14,000 rpm at 4°C.
6. Pre-spin 2 ml yellow phase-lock (heavy) tubes (Eppendorf) for 10 sec. Add 700 µl of acidic phenol-chloroform.
7. Take 700 µl of the water phase from step 5 and add to the phase-lock tubes from step 6, thoroughly mix by inverting (no vortexing), and centrifuge 5 min at 14,000 rpm at 4°C.
8. Pre-spin 2 ml phase-lock tubes as in step 6. Add 700 µl of chloroform:isoamyl alcohol (24:1)(under fume hood, Sigma C-0549).
9. Take 700 µl of the water phase from step 7 and add to the phase-lock tubes from step 8, thoroughly mix by inverting (no vortexing), and centrifuge 5 min at 14,000 rpm at 4°C.
10. Prepare normal 2 ml Eppendorf tubes with 1.5 ml of 100% EtOH (-20°C) and 50 µl of 3 M NaAc pH 5.2.
11. Transfer 500 µl of water phase from step 9 to the tubes from step 10, vortex 10 sec. Samples can be precipitated at -20°C overnight (or at -70°C for 30 min).
12. Centrifuge for 10 min at 14,000 rpm at RT. Discard SN, add 500 µl 70% EtOH (4°C, made with DEPC water), don't vortex, just add, and spin for 1 min (same tube orientation!). Aspirate most SN, spin 5 sec, and remove rest of liquid with pipette. Air dry 5 min at RT.
13. Add 100 µl of DEPC water, and incubate 1 min at 65°C (or 10 min at RT). Dissolve pellet first by pipetting up and down (~30x) until no particles are left, then gently vortex 10 sec.

14. Measure $OD_{260/280}$: add 5 µl to 995 µl DEPC water (1:200), set reference with water in 500 µl glass cell, then measure RNA (OD should be >0.1). Rinse cell with 0.1M NaOH, 0.1M HCl, and thoroughly with ddH_20.
15. Expect ~400 µg of RNA in total, but it may be less for RNA isolated under some conditions. Use 100 µg of your RNA for Qiagen purification (see step 16). Measure the volume of the remaining RNA, add 3 volumes of 100% EtOH and store at -70°C as a backup.
16. Purify 100 µg of each of your RNAs using RNeasy mini spin columns (Qiagen) as described in the RNeasy Mini Handbook (p. 48-49). Elute twice with 30 µl RNase-free water. Keep on ice!
17. Run 2 µl of purified RNA on a 1% agarose gel (wipe gel apparatus/tray with RNAse-Zap, rinse with water and use new TBE buffer, use RNAse-free loading buffer made with DEPC water). You should see the two ribosomal bands clean, distinct and without smears.
18. Measure $OD_{260/280}$ of purified RNA: add 2 µl to 100 µl DEPC water (1:50), set reference with water in 50 µl glass cell, then measure RNA (OD should be >0.1; ratios 260/280 >1.8). Rinse cell with 0.1M NaOH, 0.1M HCl, and thoroughly with ddH_20.
19. Add DEPC water to every sample such that the end concentration is 20 µg RNA/13.9 µl.
20. From each sample, use ~50% of your RNA to make up a reference pool by combining equal amounts (e.g. 40 µl) from every timepoint. Mix and make up 12.9 µl aliquots stored at -70°C (ready to use for labeling).
    Make up 13.9 µl aliquots if not using bacterial control RNA for labeling.
21. With the rest of your RNA, make up 12.9 µl aliquots of each sample and immediately store at -70°C (ready to use for labeling).
    Make up 13.9 µl aliquots if not using bacterial control RNA for labeling.

TES: 10mM Tris pH 7.5; 10mM EDTA pH 8; 0.5% SDS
(do not treat Tris stock with DEPC, just use DEPC treated water to make solution; store at RT)