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YEAST RNA PURIFICATION

1. Harvest ~10-20 ml cells at OD 0.5-1.0

   For SM (sulfometuron methyl) Inductions: grow ~5 ml O/N at 30 deg in GC (Glucose complete) –Val-Ile to saturation. Dilute back to OD ~0.3 in morning. When growing 20+ samples, I assume the O/N OD is around 6.0 based upon taking the ODs of random samples, so I dilute them all back 20-fold: 750 ul O/N in 15 ml fresh media (in the largest culture tubes). Grow for 5 hours, then induce for 90 min at 30 deg with 0.5 ug/ml final SM concentration. (4000X SM stock = 2 mg/ml in DMSO, stored at -20). I add DMSO only to non-induced controls.

   After inductions, harvest 1.5 ml for Western blot analysis (wash with water before quick freezing pellet or adding sample buffer).

   Harvest ~10 ml for RNA analysis. Spin down in 15 ml conical tubes, wash once with cold ~5 ml sterile water, quick freeze pellet. I remove any remaining water from the tube with a Pasteur pipette. Store pellet at -80 deg until ready to purify RNA.

2. Resuspend pellet in 600 ul TES (10 mM Tris pH 7.5, 10 mM EDTA, 0.5% SDS, filter sterilized, store at room temp) and transfer to eppy tube containing 400 ul acid phenol:chloroform (Ambion AM9722). Vortex 10 seconds then incubate for 60 min at 65 deg in shaking thermomixer (~ 1100 rpm).
3. Centrifuge at max speed for 10 min at 4 deg. Transfer aqueous layer to new tube containing another 400 ul acid phenol:chloroform. Vortex and spin again. Two phenol extractions should be sufficient to get rid of debris.
4. Transfer aqueous layer to new tube containing ~300 ul chloroform. Vortex 10 sec, spin for 10 min at 4 deg, and transfer ~200-250 ul to new tube.
5. Precipitate RNA (~300 ul) with 1/10 vol 3M NaOAc and 3 vol 100% EtOH on dry ice ~ 1 hr or at -20 deg O/N.
6. Pellet RNA at max speed for 15 min at 4 deg and wash pellet with 500 ul 80% EtOH.
7. Dry pellet in speed vac.
8. Resuspend pellet in ~100 ul RNase-free water (Ambion AM9938) and store at -80 deg. ***Note: RNAs may be hard to resuspend. Vortex well.***
9. Determine concentration and purity using Nanodrop spectrophotometer. Expect roughly 1.5 – 2.5 ug/ul.
10. (optional) Run ~ 1 ug RNA on a 1% agarose gel to ensure equivalent RNA quantities.

REMOVAL OF GENOMIC DNA

1. Assemble the following reactions in a 1.5 ml eppy tube:

10X Turbo DNase buffer	*5 ul*
Turbo DNase	*1 ul*
15 ug RNA	
RNase-free water	
Total volume	*50 ul*

2. Incubate at 37 deg for 30 min.
3. Quick spin, then add 5 ul DNase inactivation bead slurry to each tube. (ensure beads are resuspended before adding to tubes)
4. Vortex tubes and incubate for at least 2 minutes at room temp. Vortex at least 2 or 3 times during the incubation to ensure beads stay in suspension.
5. Pellet beads at 10K rpm for 1 min, then transfer 40 ul supernatant to new 1.5 ml tube and store DNased RNA at -80 deg. Be sure not to transfer any beads as they will inhibit cDNA synthesis.

cDNA SYNTHESIS

Use Roche's Transcriptor cDNA Synthesis kit.

1. Assemble the following reactions in a 0.2 ml PCR tube:

DNase-free RNA (1.1 ug)	*3.7 ul*
anchored oligo(dT)$_{18}$ (#5)	*1 ul*
RNase-free water	*8.3 ul*
Total volume	*13 ul*

2. Incubate at 65 deg for 10 min and hold at 4 deg. PCR program "C_DNA_1".
3. To each sample, add 7 ul of the following and mix by pipetting up and down 3 times:

5X reverse transcriptase buffer (#2)	*4 ul*
Protector RNase Inhibitor (#3)	*0.5 ul*
10 mM dNTPs (#4)	*2 ul*
Transcriptor Reverse Transcriptase (#1)	*0.5 ul*
Total volume	*7 ul*

4. Incubate tubes for 1 hr at 50 deg, 5 min at 85 deg, and hold at 4 deg. PCR program "C_DNA_2".
5. Store cDNA at -20 deg.
6. Dilute cDNA in RNase-free water for qPCR analysis. (1/25 dilution for SM induction strains, up to 1/100 dilution for other strains)