Yeast RNA extraction with hot acidic phenol

SAFETY: When working with phenol always take extreme care. Make sure lids are fully closed on the ependorffs you are vortexing – phenol burns are not pretty ….

NOTE 1: From *Current Protocols in Molecular Biology* (1996) **Unit 13.12.** Protocol is well suited for obtaining well-suited for obtaining reproducible quantities of RNA from multiple sources. In addition these RNA preparations are largely devoid of contaminating DNA that partitions into the interface during the extraction step.

NOTE 2: All H_2O based buffers are made with DEPC-H_2O. ALWAYS wear clean gloves when working with RNA and segregate your reagents from general use.

1. Grow yeast cells in 10mls media of choice to mid-log phase ($OD_{600} \approx 1$). It is not advised to harvest RNA from later phase cells as yields vary.

2. Collect cells by centrifugation (packed cell volume (pcv) ≈ 250µl) and transfer to an ependorff tube with DEPC-ddH_2O (4°C). Collect by flash spin up to 10K and discard supernatant. Immediately freeze pellet on dry ice for long term storage at –80°C.

3. Resuspend cell pellet in 400µl TES solution. Add 400µl acid Phenol (H_2O buffered) (**BE CAREFUL**) cap the tube and vortex vigorously for 10 secs (check: if the tube is leaking the ink on the cap will smudge). Incubate 65°C 60', with vortexing every 15'.

TES solution 10mM Tris.CL pH 7.5
10mM EDTA
0.5%SDS
Stable at RT°, use DEPC-treated reagents (except Tris; it reacts with DEPC)

4. Place on ice 5'. Spin in microfuge 14000rpm 10', 4°C. Transfer aqueous top layer to a clean tube (avoiding the white protein phase). Add 400µl $CHCl_3$ and vortex vigorously 10secs. Spin in microfuge 14000rpm 10', 4°C. Transfer aqueous top layer to a clean tube (pipette carefully and don't get greedy: $CHCl_3$ has a nasty tendency to follow the tip)

5. Add 1/10 vol 3M NaAc pH5.2 and 2.5vol EtOH (-20°C). Precipitate –80oC >1hr. Spin in microfuge 14000rpm 10', 4°C. Carefully remove supernatant and wash pellet by vortexing in 70% EtOH (-20°C). Spin in microfuge 14000rpm 10', 4°C.

6. Resuspend pellet in 100% formamide (from 4°C). Try an equal volume of liquid to pellet first and move up from there. Most RNA should dissolve instantly. To aid solubilization allow to sit at RT° for 15m with pipetting every 5m. If sample is to be very concentrated, store at 4°C overnight.

7. Determine concentration by 1/100 in H_2O and $OD_{260/280}$ (OD_{260} 1 ≈ 40µg/ml for RNA). Remember to add formamide 1/100 to the blank.

NOTE 3: Resuspending RNA in formamide (Chomczynski, *Nucl Adids Res*) has several benefits over storage in ddH_2O or EtOH. First, formamide will protect the RNA from nucleases, allowing it to be stored at 4°C (or even RT°), although –20°C is generally used. Second. the samples can be concentrated up to 4ml/ml. Finally, the samples can be used immediately for northerns, RNase protection, or even RT-PCR.

NOTE 4: Formamide **NOT** formaldehyde (if you add the latter toss the sample – you just killed it)