Total RNA Isolation from *S. cerevisiae*

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Materials

50 ml size RNase-free Nalgene Oak Ridge Phenol-resistant Centrifuge Tubes
50 ml size Phase Lock Gel Tubes (Eppendorf 0032 005.330)
25% SDS (w / v)
AE Buffer (AE=50mM NaAcetate pH 5.2, 10mM EDTA)
Acid Phenol (pH 4.3, purchased as unbuffered saturated liquefied phenol (Fischer BP1751^{I}-400))
Chloroform (Fischer BP1145-1)
3 M NaAcetate (pH 5.2-5.6)

Protocol

1. Spin down the cells (approximately 250 ml at OD_{600}=0.5). Dump the supernatant.

2. Resuspend the pellet in 12 ml AE Buffer (AE=50mM NaAcetate pH 5.2, 10mM EDTA). Transfer to an RNase-free Nalgene Oak Ridge phenol-resistant centrifuge tube (50ml size).

3. Add 800 µl 25% SDS (w / v), 12 ml acid phenol (pH 4.3, purchased as unbuffered saturated liquefied phenol (Fischer BP1751^{I}-400)). Vortex to mix.

4. Incubate 10' in 65 °C water bath, vortexing every minute.

5. Incubate 5' on ice.

6. Spin down 15' at 10,000 rpm in an SS34 rotor. (Get Phase Lock tubes ready – Step 7)

7. Pour the supernatant into a **pre-spun** (5' at 1500 rpm) 50 ml Phase Lock Gel tube (Eppendorf 0032 005.330). Add 13 ml chloroform (Fischer BP1145-1) and shake to mix.

8. Spin down 10' at 3,000 rpm in a tabletop centrifuge (Beckman).

9. Pour the supernatant into a **new** RNase-free Nalgene Oak Ridge centrifuge tube.

10. Add 1 / 10 volume 3 M NaAcetate (pH 5.2-5.6) and equal volume of room temperature Isopropanol.

11. Spin down 35'-45' at 12,000 rpm in an SS34 rotor.

12. Wash the pellet with 5 ml 70% Ethanol and spin again 20' at 12,000 rpm.

13. Pour off the Ethanol. Dry the pellet in a speed vac (rotor off) on low heat for 15'.

14. Resuspend in 500 µl water and transfer to an RNase-free eppendorf tube. (Depending on the quantity of RNA, you may have to add more water and / or accelerate the solubilization by incubating a few minutes at 65 °C.)

15. Quantitate in a spectrophotometer at OD_{260nm} and run 1 ug on a 1% agarose gel. Expected yields should be around 2 mg of total RNA. (Pure RNA has an OD_{260} / OD_{280} ratio around 2.0) (Gel should show a light smear with 3 bands for the high molecular weight ribosomal subunits and bands for the low molecular weight tRNAs.)

    Note: A heavy smear down the lane or very bright bands at low molecular weight may be indicative of RNA degradation or RNase contamination and an additional chloroform extraction may be warranted.

16. Store the total RNA at –80 °C or continue with polyA RNA Isolation protocol.