

Phenol-Chloroform Based RNA Extraction from Yeast

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Materials and Reagents

- 1. DEPC treated water
- 2. Phenol (TE)/Chloroform (1:1)
- 3. 3 M NaAc (pH 5.2)
- 4. NaOAc
- 5. 10 mM EDTA
- 6. 10% SDS
- 7. EtOH
- 8. Hydroxyquinoline
- 9. Complete buffer A (see Recipes)
- 10. Buffer A phenol or RNA phenol (see Recipes)
- 11. TE phenol (see Recipes)

Equipment

- 1. RNase free tubes
- 2. RNase-free plastic ware
- 3. Water bath

Procedure

A. Harvest Cells

- 1. Grow cells to OD_{600} 0.4-0.6 (mid-log phase).
- 2. Spin down 10 ml cell cultures for each sample.
- 3. Resuspend in 1 ml DEPC treated water, and transfer to RNase free tubes (*Note: screw caps are preferred, since snap-caps turns to pop-open in hot bath*).
- 4. Spin down cells, pour off supernatant, freeze at -80 °C or use right away.



B. RNA extraction

- 1. Prepare solutions:
 - a. Make 0.5 ml buffer A per sample + 1. Aliquot needed amount into RNase-free plastic ware. Add 1% DEPC just before use. Keep at room temp (RT).
 - b. Make 1.2 ml Phenol A per sample + 1. Aliquot phenol into RNase free plastic ware.
 Warm to 65 degrees.
 - c. Make 0.5 ml Phenol (TE)/Chloroform (1:1). Aliquot amount needed into RNase free plastic ware. Store at RT.
 - d. Prepare RNase free 3 M NaAc (pH 5.2), EtOH, 70% ETOH, and water.
- 2. Remove cells from -80 °C and immediately add 0.5 ml buffer A.
- 3. Add 600 µl of Phenol A (pre-warm and equilibrated to 65 °C). Add to all samples at once, putting tubes in 65 °C bath. Vortex two tubes at a time for 5 sec each. Keep rotating through tubes for 6 min.
- 4. Spin down tubes for 2 min. Place in water bath.
- Carefully remove the phenol layer using an RNase-free blue tip. You will be leaving the
 aqueous layer in the tube. Store in water bath while finishing extractions and add 600 μl of
 fresh phenol A.
- 6. Vortex tubes for another 6 min. Spin down 2 min. While spinning, label new set of RNase-free Epi tubes (snap-cap). Add 200 μl water and 500 μl Phenol (TE)/Chloroform to each tube.
- 7. Take off the aqueous layer and transfer to tubes containing water and phenol/Chloroform. Store in water bath.
- 8. Vortex each tube for about 5 sec then spin down for 2 min. While spinning label new set of Epi tubes.
- Put tubes back into water bath. Take off top aqueous layer making sure not to get any of the bottom layer (Note: this is important to avoid contaminating DNA, you have to sacrifice ~ 20 μl liquid near the interface). Store in water bath until all are complete.
- 10. Add 50 µl RNase-free 3 M NaAc and 1 ml EtOH. Mix. Store on ice 15 min.
- 11. Spin down at 4 °C for 15 min.
- 12. Wash with 1 ml 70% EtOH(RNase-free). Spin down for 10 min at 4 °C.
- 13. Pour off as much EtOH as you can, then add 400 μl water. Let sit in water bath for about 5 min. Add 40 μl NaAc and 800 μl EtOH, mix, and store on ice for 15 min.
- Wash with 70% EtOH as before, let dry inverted on paper towel under heat lamp.
- 15. Resuspend in 50 μl sterile water (25 μl if original culture volume was under 5 ml).
- 16. Let sit in water bath for 15 min. Vortex, spin down.
- 17. Dilute 5 μ l of RNA into 495 μ l water. Determine absorbance at A₂₆₀ and A₂₄₀.



Notes

- 1. Always wear gloves.
- 2. Always use RNase-free tubes, tips, plastic-ware, and solutions.
- 3. Always aliquot stock solutions from RNA shelf into an RNase-free container so you do not contaminate the stock.
- 4. When handling RNA, either keep tubes in ice bucket or water bath that is over 50 °C.

Recipes

- 1. Complete Buffer A
 - a. Buffer A stock

50 mM NaOAc

10 mM EDTA

Add 16.7 ml 3 M NaOAc (pH 5.2) to 20 ml 0.5 M EDTA to 963.3 ml water.

Add 0.1 % DEPC, stir O/N, autoclave.

b. Complete Buffer A (user solution)

Add 100 ml 10% SDS to 900 ml of Buffer A stock.

Add 1 % DEPC just before use.

2. Buffer A phenol or RNA phenol

Saturate phenol with Buffer A stock.

Add 0.1% hydroxyquinoline.

3. TE Phenol

Chloroform: Saturate phenol with 10 mM Tris-HCI (pH 8.0) and 1 mM EDTA.

Use 50% TE-Phenol and 50% chloroform.