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. Aliqout 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.
5. 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.
9. 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.
14. 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 µl of RNA into 495 µl water. Determine absorbance at A_{260} and A_{240}.

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.