BASIC
PROTOCOL

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PREPARATION OF YEAST RNA BY EXTRACTION WITH HOT ACIDIC PHENOL

Yeast RNA can be isolated efficiently and directly from intact cells by extraction with acidic phenol (pH 5) and SDS at 65°C. Because this procedure does not require vortexing individual samples with glass beads (alternate protocol), which is tedious and a source of variability, it is well-suited for obtaining reproducible quantities of RNA from multiple samples. In addition, RNA preparations are largely devoid of contaminating DNA which partitions into the interface during the extraction step.

Materials

Yeast cells and desired medium (*UNITS 13.1 & 13.2*)
TES solution
Acid phenol
Chloroform
3 M sodium acetate, pH 5.3
100% and 70% ethanol, ice-cold
50-ml centrifuge tube (Falcon)
Centrifuge: tabletop *or* Sorvall equipped with an SS-34 rotor
Additional reagents and equipment for ethanol precipitation (*UNIT 2.1*) and spectrophotometric quantitation of cells and RNA (*APPENDIX 3*)

TES solution

10 mM Tris·Cl, pH 7.5
10 mM EDTA
0.5% SDS
Store indefinitely at room temperature

1. Grow yeast cells in 10 ml of desired medium to mid-exponential phase (OD_{600} = 0.5).
It is not advisable to prepare RNA from cells that have reached a higher density because as the stationary phase is approached, the results are less consistent and RNA yields will vary.
2. Transfer culture to 50-ml centrifuge tube and centrifuge cells 3 min at 1500 · *g* (7000 rpm in a tabletop centrifuge or SS-34 rotor), 4°C.
The time and speed of the centrifugation are not critical.
3. Discard supernatant, resuspend pellet in 1 ml ice-cold water. Transfer to a clean 1.5-ml microcentrifuge tube. Microcentrifuge 10 sec at 4°C, and remove supernatant. Proceed to step 4 or if desired immediately freeze pellet by placing tube in liquid nitrogen.
Liquid nitrogen may also be used to freeze the pellets. Although not essential, freezing is particularly useful when RNA is to be prepared from multiple cultures or multiple time points from a given culture; this permits simultaneous processing of the samples. The frozen cell pellets can be stored for months at –70°C. Thaw on ice just before continuing the procedure.
4. Resuspend cell pellet in 400 µl TES solution. Add 400 µl acid phenol preheated to 65°C and vortex vigorously 10 sec. Incubate 60 min at 65°C with brief vortexing for 10 sec every 15 min interval. *It is crucial to incubate for ≥30 min (with occasional vortexing) to obtain quantitative recovery of both large and small RNA species.*
5. Place on ice 5 min. Microcentrifuge 5 min at top speed, 4°C.
6. Transfer aqueous (top) phase to a clean 1.5-ml microcentrifuge tube, add 400 µl acid phenol, and vortex vigorously. Repeat step 5.

7. Transfer aqueous phase to a clean 1.5-ml microcentrifuge tube and add 400 µl chloroform. Vortex vigorously and microcentrifuge 5 min at top speed, 4°C.
8. **Transfer aqueous phase to a new tube, add 40 µl of 3 M sodium acetate, pH 5.3, and 1 ml of ice-cold 100% ethanol and precipitate** (usually done overnight). Microcentrifuge 5 min at top speed, 4°C.
9. **Wash RNA pellet by vortexing briefly in ice-cold 70% ethanol**. Microcentrifuge as before to pellet RNA. Dry RNA by keeping tube open for around 10 mins.
9. **Resuspend pellet in 50 µl H_2O**. Determine the concentration spectrophotometrically by measuring the A_{260} and A_{280}. Store at −70°C, or at −20°C if it is to be used within 1 year.
Make sure that the RNA is well dissolved; if necessary, heat the resuspended pellet at 65°C for 10 to 20 min and/or dilute further with more water. The yield from 10 ml of cells grown in YPD medium is ~300 µg. Cells grown in less optimal medium will yield less RNA per ml culture.

For RNA to be used in microarrays:
No need to quantitate RNA at this stage. Proceed to clean up this RNA with Qiagen RNasy mini kit.

Use the RNA resuspended in water (50 µl – add upto 200 µl water if necessary).
1. **Add ethanol to the RNA samples to make up the volume to 700 µl** and follow steps for 'RNA isolation from yeast' after this.

Note: Precipitates may be visible after addition of ethanol. This does not affect the procedure.

2. **Transfer the sample (usually 700 µl), including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 8000 x g(10,000 rpm). Discard the flow-through.**
Reuse the collection tube in step 3.
If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. Discard the flow-through after each centrifugation.
Optional: If performing optional on-column DNase digestion (see "Eliminating genomic DNA contamination", page 23), follow steps D1–D4 (page 69) after performing this step.

3. **Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x g(10,000 rpm) to wash the spin column membrane. Discard the flow-through.** Reuse the collection tube in step 4.
Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Be sure to empty the collection tube completely.
Skip this step if performing optional on-column DNase digestion.

4. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 8000 x g(10,000 rpm) to wash the spin column membrane. Discard the flow-through.**
Reuse the collection tube in step 5.
Note: Buffer RPE is supplied as a concentrate. Ensure that ethanol is added to Buffer RPE before use (see "Things to do before starting").

5. **Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 2 min at 8000 x g(10,000 rpm) to wash the spin column membrane.**
The long centrifugation dries the spin column membrane, ensuring that no ethanol is carried over during RNA elution. Residual ethanol may interfere with downstream reactions.
Note: After centrifugation, carefully remove the RNeasy spin column from the collection tube so that the column does not contact the flow-through. Otherwise, carryover of ethanol will occur.

6. Optional: **Place the RNeasy spin column in a new 2 ml collection tube (supplied), and discard the old collection tube with the flow-through. Close the lid gently, and centrifuge at full speed for 1 min.**
Perform this step to eliminate any possible carryover of Buffer RPE, or if residual flow-through remains on the outside of the RNeasy spin column after step 5.

7. **Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at 8000 x g(10,000 rpm) to elute the RNA.**

8. **If the expected RNA yield is >30 µg, repeat step 7 using another 30–50 µl RNase-free water, or using the eluate from step 7 (if high RNA concentration is required). Reuse the collection tube from step 7.**
If using the eluate from step 7, the RNA yield will be 15–30% less than that obtained using a second volume of RNase-free water, but the final RNA concentration will be higher.

I find that the second elution often has a better 260/280 and 260/230 ratio if the starting concentration of RNA was high. Quantitation is done using Nanodrop in Mello lab.
For additional confirmation of RNA quality, run 1 µl sample on a 1% TBE agarose gel.
Typically, for a microarray experiment, you need to submit around 500 µg /3 µl along with the gel picture.