Yeast total RNA isolation protocol

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November 2, 2012

Use RNase-free dH_2O throughout.

1. Grow up 5ml liquid yeast culture to OD 2.0. Spin at 3000g for 4min at room temperature. Remove supernatant and resuspend pellet in 500μl RNA lysis buffer (10mM Tris-HCl pH8.5, 5mM EDTA, 2% SDS, 2% stock 2-mercaptoethanol).

2. Transfer liquid to 1.5ml tube and put on heat block at 83°C with 450rpm mixing for 20mins (to disrupt cells and denature proteins).

3. Spin down for 5mins at 12,000g.

4. Take supernatant into new tube and add 550μl of Phenol pH8. Vortex for 15 mins[1].

5. Spin down for 2mins at 12,000g. There should be a lower phenol phase, a cloudy interphase, and an upper aqueous phase: RNA partitions mostly into the upper phase. Transfer upper aqueous phase (roughly 200μl) to new 1.5ml tube (labeled tube N). Add 250μl RNA lysis buffer to previous tube (labeled tube P) and vortex tube P for 5mins.

6. Add 250μl chloroform to tube P to suck off phenol from water phase (This completes a phenol:chloroform extraction of RNA from the original sample). Vortex for 3 mins, then spin 2mins at 12,000g and transfer aqueous phase from tube P to tube N. Discard tube P.

7. A second phenol extraction: add 550μl phenol to tube N. Vortex 5mins, spin 2mins at 12,000g.

8. Transfer aqueous phase to another 1.5ml tube, discard tube N. Add 550μl phenol:chloroform pH4.5. Vortex 3mins, then spin 2mins at 12,000g.

9. Transfer 450μl aqueous phase to yet another 1.5ml tube, add 200μl of 0.6M Sodium acetate pH4.5, mix by flicking, and spin briefly. Add 600 μl of phenol:chloroform pH4.5. Vortex 5mins, then spin 2 mins at 12,000g.

10. Transfer 350μl aqueous phase to a fresh 1.5ml tube, add 30 μl 5M Ammonium acetate and 1.1ml ethanol. Mix well, and precipitate at -80°C for 20mins. The sample may be left for longer, for example overnight, at this point if a pause is desired.

11. Remove the sample from freezer. Cold spin (4°C) for 15 mins at 12,000g.

12. Thoroughly remove ethanol from pellet, and add 700μl 80% ethanol. Cold spin for 2 minutes at 12,000g. Repeat the ethanol wash and cold spin. This removes all traces of salt, SDS, etc.

13. Dry pellets thoroughly, i.e., pipette off ethanol, removing all liquid. If necessary, dry with the tubes open on a 37°C heat block (if the RNA sample is pure, this should not degrade the RNA). Resuspend pellet in 50 μl H_2O.

14. To check the quality of the RNA, pour a 1% agarose gel on RNA-free equipment, and run using NEB RNA loading dye. Heat loading dye and H_2O to 95°C for 5 minutes, and then cool, to reduce the possibility of contamination. Mix 1μl sample, 5μl H_2O, and 6μl 2X loading dye for each well. Perform a 2X serial dilution of the sample for more precise quantification.

[1] To find out how much sample is lost in the first centrifugation, resuspend pellet in 500μl of RNA lysis buffer, and repeat 3 previous steps. Label tube distinctly and proceed in parallel with the main sample.