A Simple RNA Preparation Procedure from Yeast for Northern Blot Using Hot Phenol

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[Abstract] Compared to several expensive RNA extraction kits, the following protocol provides an economic and simple method for researchers to extract yeast RNA. This method can achieve RNA quality that is sufficient for most northern blot studies in yeast.

Materials and Reagents

1. W303a cell line
2. Sodium acetate trihydrate ($CH_3CO_2Na \cdot 3H_2O$) (Sigma-Aldrich, catalog number: 236500)
3. Phenol (C_6H_5OH) (Sigma-Aldrich, catalog number: P1037)
4. EDTA ($Na_2EDTA \cdot 2H_2O$) (Sigma-Aldrich, catalog number: ED2SS)
5. Acetic acid (CH_3COOH) (Sigma-Aldrich, catalog number: 320099)
6. Chloroform ($CHCl_3$) (Sigma-Aldrich, catalog number: 472476)
7. 8-hydroxyquinoline (C_9H_7NO) (Sigma-Aldrich, catalog number: 252565)
8. NaCl (Thermo Fisher Scientific, catalog number: S641-500)
9. Synthetic complete (SC) medium
10. SDS (Sigma-Aldrich, catalog number: L3771)
11. Isoamyl alcohol (Sigma-Aldrich, catalog number: W205710)
12. Ethanol (Thermo Fisher Scientific, catalog number: 64-17-5)
13. DEPC water
14. Phenol-AE buffer (see Recipes)
15. Phenol: $CHCl_3$ /AE-Na (see Recipes)
16. $CHCl_3$: Isoamyl alcohol (24:1) (see Recipes)

Equipment

1. Standard bench-top centrifuge
2. Microfuge
3. Shaker
4. 1.5 eppendorf tube

5. Liquid nitrogen

Procedure

1. Prepare reagents according to recipes. Note that everything is in DEPC water.
2. Inoculate W303a cells expressing different TOR1-RR variants in 2 ml SC medium overnight.
3. Subculture the cells starting from OD_{600}=0.1 in 10 ml SC media, shake vigorously at 30 °C, 300 rpm for around 4-6 h until OD_{600}=0.4-0.5.
4. Collect the cells by spinning down without freezing on ice. Discard supernatant.
5. Re-suspend cells with 1 ml water and transfer to a 1.5 eppendorf tube, quickly spin down at 3,000 *x g* for 15 sec.
6. Re-suspend cell pellet in 400 µl of AE buffer at room temperature.
7. Add 40 µL 10% SDS (final around 1%) and vortex briefly at room temperature (RT).
8. Immediately add 500 µl hot phenol/AE (put in 65 °C for 10 min before use), vortex vigorously for 1 min.
9. Incubate at 65 °C for 5 min. Briefly vortex every 30 sec.
10. Immediately freeze by dumping into liquid nitrogen (labels won't get off).
11. Wait to thaw at RT (put in 30 °C to thaw may crack the tube).
12. Centrifuge for 10 min on a standard laboratory microfuge at 20,000 *x g* at RT.
13. Transfer around 400 µl supernatant to a new eppendorf tube. Recycle the lower phenol fraction carefully following the chemical safety protocol in your laboratory.
14. Add equal volume (400 µl) phenol: $CHCl_3$/AE-Na. Vortex vigorously for 1 min at RT.
15. Spin down at 20,000 *x g* for 5 min in a standard laboratory microfuge.
16. Transfer supernatant (around 350 µl) to a fresh 1.5 ml eppendorf tube.
17. Add $CHCl_3$: isoamyl alcohol (24:1). Vortex vigorously for 1 min at RT.
18. Transfer aqueous supernatant to fresh 1.5 ml microfuge tube. If white cloudy precipitate is observed between aqueous phase and organic phase, repeat steps 17-18.
19. Add 1/10 volume of 3 M NaOAc (pH 5) and vortex vigorously. Add 2.5 volumes of ethanol. Vortex again.
20. Place at -20 °C for at least 30 min.
21. Spin down in the microfuge at 20,000 *x g*, 15 min at 4 °C. RNA pellet is usually visible.
22. Add ice-cold 75% EtOH, place at 4 °C for around 10 min. Vortex and spin down on microfuge 20,000 *x g*, 15 min at 4 °C.
23. Discard supernatant. Suck out the liquid droplets in the tube.
24. The white RNA pellet will turns clear when it dries out. Add 30-50 µl ddH_2O (DEPC) immediately after it becomes clear.

25. Do not let the RNA over-dry, which will make it difficult to dissolve. If RNA pellet is over-dry, dissolve RNA at 37 °C for 30 min.
26. Store RNAs at -80 °C for more than 2 months.

Recipes

1. Phenol-AE buffer
   Make Acetate-EDTA(AE) buffer
   50 mM NaOAc
   10 mM EDTA (pH 5.0)
   Liquefied phenol equilibrated with equal volume of AE buffer. Store at 4 °C.
2. Phenol: $CHCl_3$ /AE-Na
   Make AE-Na buffer
   10 mM NaOAc
   2 mM EDTA
   100 mM NaCl (pH 6.0)
   50% phenol/AE
   50% $CHCl_3$
   0.25% 8-hydroxyquinoline
   Store at 4 °C.
3. $CHCl_3$: Isoamyl alcohol (24:1)

Acknowledgments

This protocol was adapted from and used in Wei and Zheng (2009) and Wei *et al.* (2009).

References

1. Wei, Y. and Zheng, X. F. (2009). *Sch9* partially mediates TORC1 signaling to control ribosomal RNA synthesis. *Cell Cycle* 8(24): 4085-4090.
2. Wei, Y., Tsang, C. K. and Zheng, X. F. (2009). Mechanisms of regulation of RNA polymerase III-dependent transcription by TORC1. *EMBO J* 28(15): 2220-2230.