RNA Isolation from Yeast

Tara Stuecker

Abstract

Protocol for obtaining high quality RNA from yeast using acid-phenol lysis at 65°C. Modifed from DOI: 10.1016/S0076-6879(02)50976-9 to use 10-15 ml of yeast culture. Expected yield of 100-200 µg of RNA. For qPCR or RNA-seq, we recommend DNase-treatment and further purification (e.g. Zymo RNA Purification Kit (R1057)).

Citation: Tara Stuecker RNA Isolation from Yeast. **protocols.io**
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Guidelines

This protocol is written for use with an incubating vortex, such as the MidSci Multi-Therm (MTH-1500). If this is not available, you can incubate samples in a 65°C water bath or heat block and vortex samples every 10-15 min during lysis.

Before start

Before starting experiment:

(1) Make or have ready RNase-free stock solutions.

(2) Set up incubating vortex in chemical hood and pre-warm to 65°C.

(3) Pre-label 4 sets of RNase-free 2 ml microcentrifuge tubes for each sample.

Materials

Phenol, Saturated (pH 4.3) BP1751I-400 by Fischer Scientific

Chloroform BP1145-1 by Fisher Scientific

Sodium Acetate AA11554-30 by Vwr

Tris-HCl, pH 8.0 (UltraPure) 15568025 by Thermo Fisher Scientific

EDTA (0.5 M, pH 8.0, nuclease-free) AM9260G by Thermo Fisher Scientific

Ethanol (100%, Molecular Biology Grade) BP2818500 by Fisher Scientific

Protocol

Collect Cells

Step 1.

1. Centrifuge 10 – 15 ml of yeast cells in a 15-ml conical tube at 1,500 rcf (room temperature) for 3 min.
2. Decant supernatant and freeze cells in liquid nitrogen.

NOTES

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We generally use mid-exponentially growing cells (OD_{600} ~0.3 - 0.6 on a Unico 1100RS Spectrophotometer).

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Cell pellets can be stored at -80°C for several months.

Lyse cells

Step 2.

1. To frozen cell pellet, add 800 µl of acid saturated phenol and 800 µl of lysis buffer.
2. Vortex until pellets are in solution.
3. Place tubes in MultiTherm (should be pre-heated to 65°C).
4. Vortex at maximum intensity for 45 min at 65°C.

NOTES

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To avoid RNA degradation, do not let cells thaw before adding phenol. Add the phenol first to frozen cell pellets, then the lysis buffer.

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Do not shake or otherwise disrupt the phases of the phenol bottle. There will be two layers: an upper aqueous layer containing buffer and a lower organic layer containing phenol. Make sure to pipette from the bottom layer. We recommend transferring the volume of phenol you will need for the day into a conical tube wrapped in foil (to protect phenol from light) using a serological pipette. This makes micropipetting easier and limits the possibility of a large phenol spill.

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Lysis Buffer

10 mM Tris-HCl pH 7.4

10 mM EDTA

0.5% SDS

Can be made in advance, autoclaved, and stored at room temperature.

Extract RNA

Step 3.

1. Transfer lysate to RNase-free 2-ml tube.
2. Centrifuge 10 min at max speed in microcentrifuge at 4°C.
3. Transfer top aqueous phase into fresh RNase-free 2-ml tube containing 800 µl phenol.
4. Vortex briefly and centrifuge 5 min at 5,000 rpm in a microcentrifuge at room temperature.
5. Transfer top aqueous phase into fresh RNase-free 2-ml tube containing 800 µl chloroform.
6. Vortex briefly and centrifuge 5 min at 5,000 rpm in a microcentrifuge at room temperature.
7. Transfer aqueous phase into fresh RNase-free 2-ml tube.

NOTES

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This protocol recommends two phenol extractions and one chloroform extraction. For each extraction, you will transfer the aqueous phase into a fresh set of tubes containing either phenol or chloroform. To reduce the chance of sample mixups, we recommend pre-labeling all your tubes before you start the experiment and pipetting phenol or chloroform into the next set of tubes during the preceeding centrifugation step.

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Be careful not to transfer any of the lower organic layer when removing the top aqueous layer. It is better to leave a little sample behind than to end up with protein contaminates.

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If a fluffy precipitate remains at the interphase for the second phenol extraction, repeat a third phenol extraction. Otherwise, proceed to the chloroform extraction.

Precipitate RNA

Step 4.

1. Add 75 µl 3 M NaOAc pH 5.2 (0.1 volume) and 1.8 ml 100% ethanol (2.5 volumes).
2. Place tubes at -20°C to precipitate RNA for >2 hours (**overnight works best**).

Next Day

1. Pellet RNA at max speed for 30 min in microcentrifuge at 4°C.
2. Carefully decant supernatant and add 1 ml 70% EtOH to wash pellet.
3. Centrifuge at max speed for 15 min at 4°C.
4. Remove ethanol using a 1-ml pipette (use RNase-free pipette tips).
5. Centrifuge briefly (1-2 min) at max speed to collect any residual ethanol to the bottom of the tube and remove that with a 10-ul pipette (use RNase-free pipette tips)..

NOTES
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The volume for Step 1 may exceed 2 ml depending upon how much aqueous layer you are able to pipette. To maximize the amount of sample you get without running out of room in the tube, we recommend pipetting NaOAc and 1 ml ethanol in tubes first. Then, transfer aqueus layer and toping off tubes with remaining ethanol until tubes are full.

Resuspend and Store RNA

Step 5.

Resuspend pellet in 50 µl RNase-free TE by pipetting up and down.

NOTES
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RNA concentration can be measured by spectrophotometer (Nanodrop) or Qubit. The A260/A280 ratio of purified RNA should be >2.0. The A260/A230 ratio should be ~2.2 or slightly above. You should see a clear peak around A260.

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Store RNA at -80°C.

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TE Buffer

10 mM Tris-HCl pH 8.0

1 mM EDTA

Warnings

Phenol is **very toxic**. It should be handled only in a chemical safety cabinet. Wear personal protective equipment, including eye protection, lab coat, long pants, closed toed shoes and NITRILE gloves. We

recommend you change your gloves often during the procedure to prevent phenol from penetrating gloves. We always place a box of gloves next to the chemical hood for easy access. Be sure to follow your Institution's guidelines for proper disposal of used phenol and phenol-contaminated plasticware.