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A Rapid and Simple Method for Preparation of RNA from Saccharomyces cerevisiae

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Most methods for isolation of RNA from yeast require tedious vortexing with glass beads, and give low yields when scaled down to 10 ml cultures (1). In addition, it is frequently desirable to prepare RNA from several different yeast strains grown under a variety of growth conditions, and preparations using glass beads are impractical when dealing with multiple samples.

Heating and freezing of yeast cells in the presence of phenol and SDS has been used for large scale isolation of RNA from yeast (2). We report here a mini-prep version of yeast RNA isolation using phenol and SDS that allows for the processing of a dozen samples in about 60 minutes, and provides enough RNA to run several northern blots. In our hands methods using glass beads are slower and give lower yields of RNA.

RNA was isolated as follows. Ten ml cultures were grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) to an OD_{600} of 2.5−5.0. We have also used YP 2% galactose, YP 4% ethanol/3% glycerol, YP 2% raffinose and a variety of minimal media. The cells were harvested by centrifugation and resuspended in 400 μl of 50 mM Na acetate pH 5.3, 10 mM EDTA ('AE buffer').

The resuspended cells were transferred to a 1.5 ml microcentrifuge tube and 40 μl of 10% SDS was added. The suspension was vortexed and an equal volume of fresh phenol, previously equilibrated with AE buffer, was added. The mixture was again vortexed and incubated at 65°C for 4 min. The mixture was then rapidly chilled in a dry ice/ethanol bath until phenol crystals appeared, and then centrifuged for 2 min at maximum speed in a microcentrifuge to separate the aqueous and phenol phases.

The upper, aqueous phase was transferred to a fresh microcentrifuge tube and extracted with phenol/chloroform at room temperature for 5 min. The extracted aqueous phase was then brought to 0.3 M Na acetate, pH 5.3, by adding 40 μl of 3 M Na acetate pH 5.3, after which 2.5 volumes of ethanol were added to precipitate the RNA. After washing with 80% ethanol, the pellet was dried and resuspended in 20 μl of sterile water and stored at −70°C until used. Throughout the preparation, normal precautions to avoid ribonuclease contamination were taken (3).

The isolation procedure was performed using seven different wild type yeast strains and over two dozen derivatives of these strains. Yields varied between 60 μg and 300 μg of RNA per 10 ml culture, with an average yield of 135 μg as quantitated by absorbance at 260 nm. Fig. 1a shows the quality of the RNA that is isolated using this method. No degradation of ribisomal RNA bands is seen, and recoveries of both high and low molecular weight RNA appear excellent.

Figure 1b shows the catabolite repression of mRNA transcription on a northern blot of total RNA prepared by the above procedure. The RNA is suitable for quantitative studies, and degradation of the mRNA was minimal when examined by northern hybridization with radioactive probes corresponding to 12 different yeast genes.

We have also successfully used RNA prepared by this procedure for quantitation in ribonuclease protection experiments (4) with the genes for apo-1-cytochrome c (CYC1, Ref. 5) and cytochrome c reductase subunit 9 (QCR9, Ref. 6). This procedure does not require a high-speed shaking apparatus to process several samples at once, nor does it require the use of expensive, ribonuclease free glass beads.

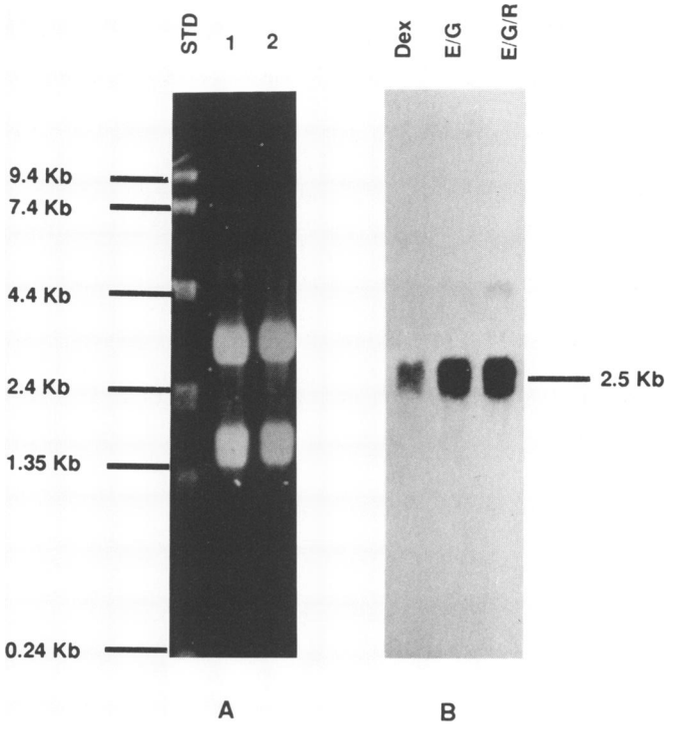

Figure 1. RNA derived from the heat/freeze RNA isolation. Panel A shows two preparations of RNA from different wild type strains of yeast, separated by agarose gel electrophoresis in the presence of formaldehyde (3) and stained with ethidium bromide. Standards are from Bethesda Research Labs. Panel B shows a northern blot (3) of 10 μg/lane of RNA from the yeast strain PSY142 (MATα, lys2−801, leu2−3, 121, ura3−52), grown on either 10% dextrose, 4% ethanol/3% glycerol or 4% ethanol/3% glycerol/2% raffinose as a carbon source. The separated RNA was probed with ATP1, the gene for the alpha subunit of the mitochondrial F_1 ATPase (7).

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