High-throughput isolation of *Saccharomyces cerevisiae* RNA

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The yeast *Saccharomyces cerevisiae* has long been an important model organism for biological investigation (1). More recently, high-throughput approaches using deletion libraries and fusion protein libraries have provided powerful techniques with which to study the entire yeast genome and proteome (2–6). However, the methods typically used for preparing high-quality RNA samples from yeast are impractical to perform in a high-throughput format. These techniques involve either hot phenol extraction, mechanical disruption with glass beads, or lyticase treatments to break the cell wall and/or require additional organic extractions and alcohol precipitations to isolate the RNA (7–9). Handling large amounts of phenol poses safety risks, the mechanical lysis of yeast cells becomes tedious when working with large numbers of samples, and the multiwell plates typically used in high-throughput screens cannot be centrifuged at the high speeds required for RNA precipitation.

We have developed a method for isolating *S. cerevisiae* RNA in a 96-well format conducive to high-throughput studies. In this method, RNA is released from the cell upon digestion with proteinase K, and proteins and cell debris are precipitated with potassium acetate under low-speed centrifugation. RNA is then purified from the cleared extract using a commercially available nucleic acid binding plate in conjunction with a vacuum manifold.

To perform this method, *S. cerevisiae* cultures were grown 1 to 2 days in 0.5 mL selective medium in a 2-mL deep-well 96-well plate sealed with air-permeable tape. Cells were collected by centrifugation at 1200× *g* for 5 min (approximately 2500 rpm in a Beckman GS-6R centrifuge equipped with Micro Plus Carriers; Beckman Coulter, Fullerton, CA, USA), and the supernatants were discarded by aspiration, leaving approximately 25 µL liquid/well. The cell pellets were resuspended in the residual liquid by vortex mixing, and 300 µL proteinase K buffer [10 mM Tris, pH 8.0, 5 mM EDTA, pH 8.0, 150 mM NaCl, 1% sodium dodecyl sulfate (SDS), 0.4 mg/mL proteinase K (Sigma, St. Louis, MO, USA)] prewarmed to 65°C were added to each well and mixed thoroughly by gentle vortex mixing. Vigorous vortex mixing was avoided, as this can cause splashing, particularly with larger volumes. Protease digests were incubated for 15 min in a 65°C water bath with periodic mixing and then placed on ice for 20 min. Next, 175 µL ice-cold KOAc precipitation solution [3 M potassium acetate, 11.5% (v/v) glacial acetic acid] were added to each well, and the plate was vortex mixed for 10 s. Following centrifugation at 1650× *g* for 20 min, the supernatants (cleared extracts) were transferred to a clean deep-well plate, and 600 µL 100% ethanol were added to each well. The lysate/ethanol mixture was then transferred to a nucleic acid binding plate [96-well 800-µL UNIFILTER® Microplates (Whatman, Clifton, NJ, USA); similar plates from other manufacturers worked with comparable efficiency] that was assembled onto a vacuum manifold according to the manufacturer's instructions. Vacuum was applied until the cleared extract passed through the binding plate and into the waste receptacle of the manifold. If the binding plate did not hold the entire volume of lysate, the remaining volume was added, and the vacuum was reapplied as before. The binding plate was then washed by the addition of 500 µL/well wash buffer [10 mM Tris, pH 7.5, 60 mM KOAc, 60% (v/v) ethanol], and then the vacuum was applied until the liquid passed through the plate. An optional DNase treatment derived from a protocol designed to isolate RNA from mammalian tissue culture lysates (www.promega.com/tbs/tb294/tb294.pdf) was then performed by the addition of 25 µL/well DNase buffer [20 mM Tris, pH 7.5, 1 M NaCl, 10 mM $MnCl_2$, 0.1 U/µL RNase-free DNase (Sigma)] and a 10-min incubation at room temperature. To stop the reaction, 200 µL DNase stop solution [2 M guanidine isothiocyanate, 4 mM Tris, pH 7.5, 57% (v/v) ethanol] were added to each well, and the vacuum was applied until all the liquid passed through. This was followed by an additional wash step

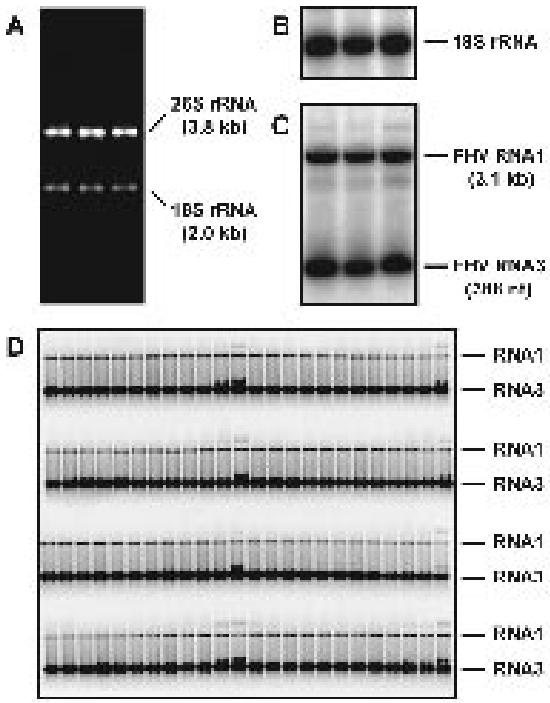

Figure 1. Northern blot analysis of RNA isolated from yeast. *Saccharomyces cerevisiae* strain BY4743 was transformed with a plasmid that induces the replication of flock house virus (FHV), and 96 independent cultures were each grown in 0.5 mL selective medium in a 96-well plate. RNA was isolated, and 2 µg each RNA sample were loaded on a denaturing 1.0% agarose gel and analyzed by Northern blot analysis using a probe that recognizes 18S rRNA or a probe that recognizes positive strands of both FHV RNA1 and FHV RNA3. Magnified views are shown for three representative samples visualized by ethidium bromide staining (A) and Northern blot analysis for 18S rRNA (B) or FHV RNAs (C). All 96 lanes from a Northern blot analysis detecting FHV RNA is shown in panel D. Unlabeled bands in panels C and D are double-stranded FHV RNAs. rRNA, ribosomal RNA.

using 500 µL wash buffer per well as described earlier. These optional DNase steps may be omitted if small amounts of DNA do not interfere with subsequent analyses. Residual wash buffer was removed by placing the nucleic acid binding plate on a microplate and centrifuging at 1200× *g* for 1 min. The binding plate was then placed on a clean microplate, 100 µL nuclease-free water were added to each well, and the plate was incubated for 1 min at room temperature. RNA was eluted by centrifugation at 1200× *g* for 1 min and quantitated by spectrophotometry and/or electrophoresis on an ethidium bromide-stained gel.

Using the above method, we produced RNA samples from 96 yeast cultures in <2 h with the aid of 12-channel pipets. On average, 24 µg RNA with an A_{260}/A_{280} ratio of 2.07 were isolated per 0.5 mL yeast culture grown to an OD_{600} of approximately 3.0. Upon agarose gel electrophoresis and Northern blot analysis, performed as previously described (10), bands in the RNA preps did not appear smeared, indicating that full-length intact RNAs were recovered (Figure 1). As an alternative to 96-well plates, single-tube nucleic acid binding matrices from various manufacturers have also been used successfully with this protocol. Thus, this method is suitable for isolating RNA from any number of samples, from individual cultures to high-throughput applications

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COMPETING INTERESTS STATEMENT

The authors declare no competing interests.

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