APPLICATION NOTE #201503

RNA Extraction from *Sacchromyces cervisiae* on the Bead Ruptor 24

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Via fermentation, *Saccharomyces cerevisiae* has been used for thousands of years for baking and in the production of alcoholic beverages. Particularly it is one of the first organisms to have its genome sequenced. As a result, yeasts like *S. cerevisiae* are one of the easiest single-celled organisms to study due to their short generation time and feasibility to culture. Also, many of their essential cellular processes are similar to those found in humans making it an excellent model organism for understanding biochemical, cellular and molecular interactions for eukaryotic organisms. Traditionally, yeast RNA and intracellular proteins are extracted from cells by enzymatic methods. These enzymatic methods can be time consuming and can lead to the denaturation of some intracellular proteins. Mechanical disruption is often needed to effectively lyse the cells and release biological molecules. Bead mill homogenizers, such as the Bead Ruptor 24, can quickly and effectively disrupt yeast cell walls for extraction of compounds such as RNA. Herein, we evaluate the potential for extraction of RNA from *S. cerevisiae* cells on the Bead Ruptor 24 bead mill homogenizer. The extraction efficiency and analyte integrity was evaluated.

Materials & Methods

Equipment

- **Qiagen RNeasy Mini Kit** (Cat #74104)
- **Bead Ruptor 24** (Cat #19-040)
- **Bead Ruptor 2ml Tube Carriage Kit** (Cat #19-010-310)
- **0.5mm Tough Micro-Organism Lysing Mix** (Cat #19-628)

RNA Extraction and Separation

A 40 ml nutrient broth culture of *S. cerevisiae* was grown for 3 days at 30°C. After incubation, the cell density at OD 600 nm was determined to be 0.013 resulting in 5.07 x 106 cells in the original culture. The cells were harvested by centrifugation at 12,000 rpm at 4°C for 5 minutes. The supernatant was removed and resuspended in lysis buffer with 2-mercaptoethanol (Bio-Rad #161-0710) provided from the RNeasy kit. The cell suspension was added to a 2 ml tube containing 0.5 mm glass beads and processed on the Bead Ruptor 24 for two 30 second cycles at 6 m/s with a 45 second dwell. The beads were allowed to settle and 300 µl of the lysate was removed and added to a clean 1.5 ml microcentrifuge tube. RNA extraction was carried out per the manufacturer's instructions using the Qiagen RNeasy Mini Kit. 1 µl of the 40 µl RNA eluant was quantified on a NanoDrop Spectrophotometer (Thermo Fisher) to determine RNA yields.

1 µl, 2 µl and 4 µl of the eluant was mixed with 5 µl of TBE/Urea sample buffer (Bio-Rad Cat# 161-0768) and heated at 70°C for 4 minutes. The samples were diluted to 10 µl with DD H20. The RNA was separated on 1.2% TBE agarose gel at 60 V and stained with ethidium bromide (Bio-Rad Cat#161-0433) for 30 minutes. The gel was washed with DD H20 for 10 minutes and visualized on a GelDoc EZSystem (Bio-Rad).

Results

Yeast cells have an extremely robust cell wall comprised of cross-linking polysaccharides and proteins. Lysing the cell wall is the primary obstacle when attempting to recover nucleic acids. In this application, we evaluated the capability of the Bead Ruptor 24 to disrupt yeast cells for the purpose of extracting high quality RNA from *S. cerevisiae*. The extraction of RNA from *S. cerevisiae* is critical as it is a common model organism used as a scientific tool in understanding the cellular and biochemical interactions of many organisms.

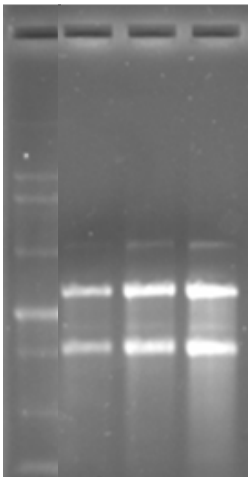

Figure 1 RNA Agarose Gel Electrophoresis of *S. cerevisiae* lysate: Lane 1. *New England Biolab ssRNA Ladder.* Lane 2: *1 µl extracted RNA*. Lane 3: *2 µl extracted RNA.* Lane 4: *4 µl extracted RNA*

After extraction, RNA yields were quantified by spectrophotometry. The RNA yield average was 224.4 ng/µl. The RNA was then separated and visualized by gel electrophoresis (Figure 1).

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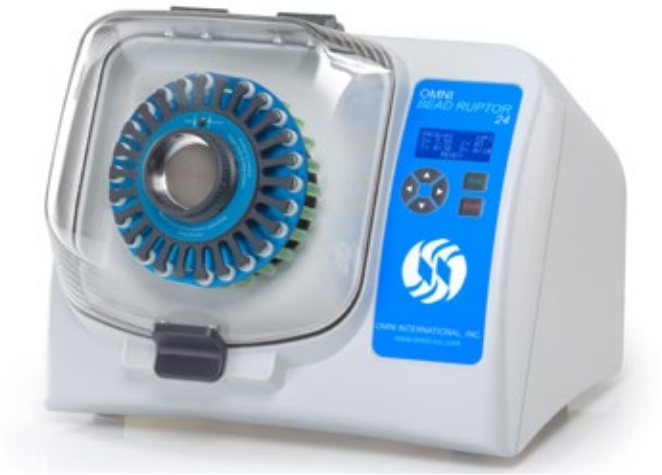
Results (cont.)

Based on the gel analysis, the extracted RNA was of good quality with a sharp and clear 26S and 18S rRNA bands. The 28S band is twice as intense as the 18S band indicating that the RNA is completely intact. The bands also depict the expected size of 2.0kb and 3.8kb for the 18S and 26S subunits respectively.

Conclusion

The Bead Ruptor 24 is capable of lysing *S. cerevisiae* is less than 5 minutes of processing time for the extraction of RNA in excess of 200 ng/µl concentrations. Gel electrophoresis indicated that the RNA was intact and at the expected molecular weight of the 28S and 18S ribosomal subunits.



Bead Ruptor 24: (Cat #19-040)

Bead Ruptor 2ml Tube Carriage Kit (Cat #19-010-310)

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