RNA extraction from *Saccharomyces cerevisiae* on the Omni Bead Ruptor Elite bead mill homogenizer.

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 Omni Bead Ruptor Elite™ bead mill homogenizer, 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 Omni Bead Ruptor Elite bead mill homogenizer. The extraction efficiency and analyte integrity was evaluated.

Materials & methods

Equipment

- **Omni Bead Ruptor Elite bead mill homogenizer** (Cat # 19-042E)
- **Omni Bead Ruptor Elite 2 mL Tube Carriage** (Cat # 19-373)
- **Tough Micro-organism Lysing Mix 0.5 mm Glass** (2 mL Tubes) (Cat # 19-622)

For research use only. Not for use in diagnostic procedures.

Omni Bead Ruptor Elite bead mill homogenizer

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 10^6 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 commercially available lysis buffer supplemented with 2-mercaptoethanol per commercially available kit instructions. The cell suspension was added to a 2 mL tube containing 0.5 mm glass (Cat # 19-622) beads and processed on the Omni Bead Ruptor Elite bead mill homogenizer for two 30 second cycles at 6 m/s with a 45 second dwell. RNA extraction was carried out per manufacturer's instructions using a commericially available Yeast RNA extraction kit. 1 µL of the 40 µL RNA eluant was quantified on a NanoDrop Spectrophotometer (Thermo Fisher) to determine RNA concentration and purity.

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 ddH_2O. 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 ddH_2O 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 Omni Bead Ruptor Elite bead mill homogenizer to disrupt yeast cells for the purpose of extracting 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.

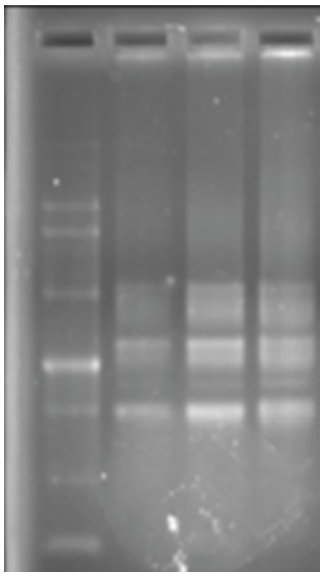

Figure 1: RNA Agarose Gel Electrophoresis of *S. cerevisiae* lysate: Lane 1. *New England Biolab ssRNA Ladder*. Lane 2: *1 µL extracted RNA*. Lane 3: *2 µL extracted RNA*. Lane 4: *4 µL extracted RNA*

After extraction, RNA concentrations were quantified by spectrophotometry. The average RNA concentration was 224.4 ng/µL. The RNA was then separated and visualized by gel electrophoresis (Figure 1). Based on the gel analysis, the extracted RNA presented with sharp and clear 28S 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 2.0 kb and 3.8 kb for the 18S and 28S subunits, respectively.

Conclusion

The Omni Bead Ruptor Elite bead mill homogenizer 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.

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