APPLICATION NOTE

Budding yeast homogenization: Reproducible protein extracts with protein functioned retained.

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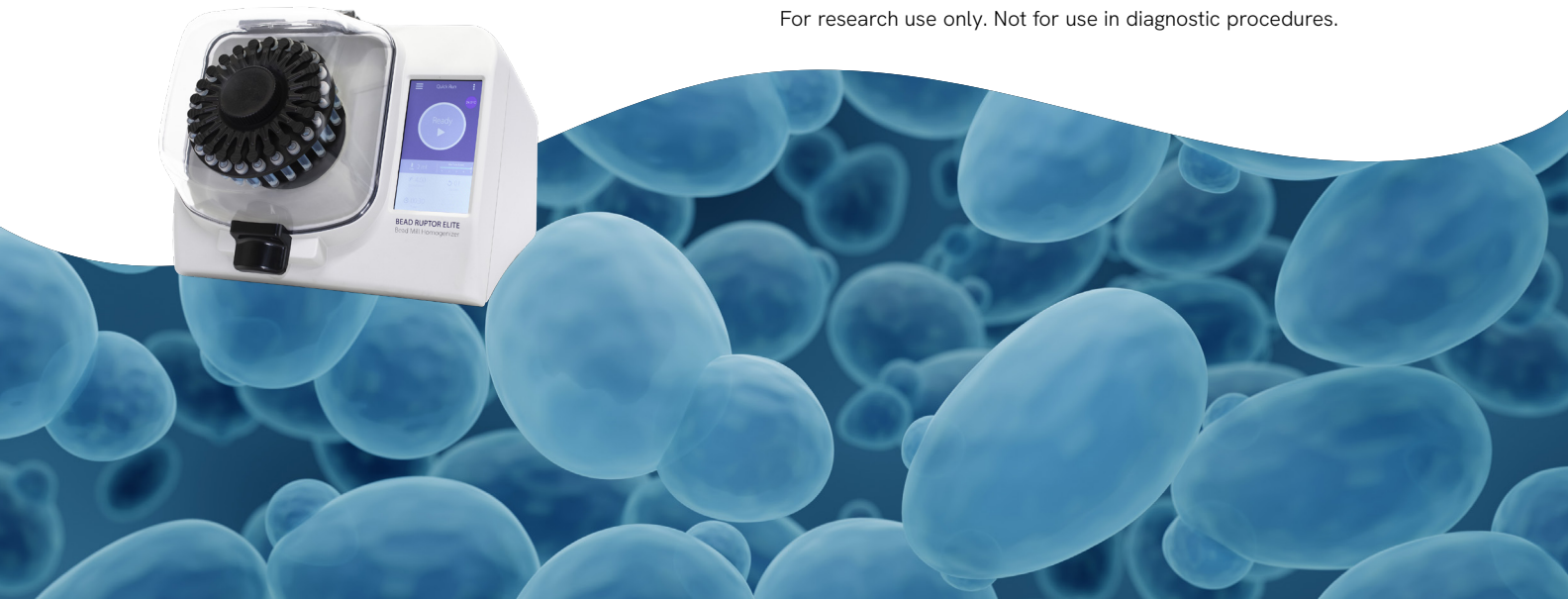
Saccharomyces cerevisiae has historically been heralded as an excellent model organism in molecular research due to its quick reproduction cycle, large population study abilities, ease of growth, and well documented genome[1, 2]. Therefore this common baker's yeast is a familiar organism in many research labs.

Despite the great research benefits of the organism, many complications arise when working with the yeast due to a robust cell wall[3]. The yeast cell wall is a highly evolved organelle that is adaptive and responsive to environmental stress. The effectiveness of the strong, but elastic cell wall is highlighted by its preservation across many other yeast species. Although beneficial for the organism, the cell wall proves to be an obstacle for researchers interested in analyzing its molecular components due to its difficult disruption.

Bead mill homogenization, as performed by the Omni Bead Ruptor Elite bead mill homogenizer, offers a robust and quick method for disrupting yeast cells. Bead mills are particularly attractive because of the automated nature of their operation, eliminating variation caused by human error. Their ability to process a large number of samples at once eliminates sample processing bottlenecks, creating the potential for high throughput studies. The Omni Bead Ruptor Elite bead mill homogenizer provides researchers with a rapid, efficient, and reliable method for disrupting yeast cells in a sterile and controlled manner.

Omni Bead Ruptor Elite bead mill homogenizer



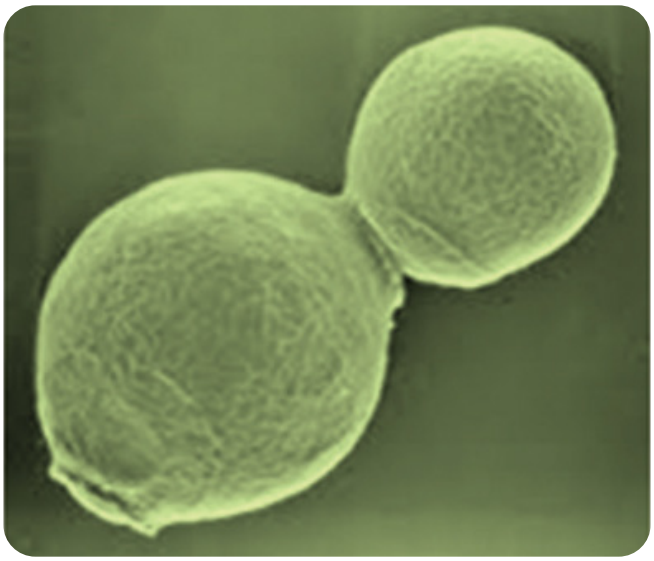


Figure 1. Budding yeast.

Despite the vigorous nature of bead mill homogenization, downstream molecular analyses methods are unaffected and samples are open to a variety of methods including PAGE, Western blotting, protein functional studies, and nucleic acid purification. Herein we describe the methods used by Szymanski and Kerscher to disrupt yeast cells for western blotting and protein functional studies.

Materials and 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) (Cat # 19-622)

Procedure

Yeast cultures were transformed with plasmids carrying a 6xHIS V5-tagged SUMO ligase Siz1, GST tagged Slx5 and a HA tagged Siz1Δ440. Cells were isolated by centrifugation and resuspended in PBS with a protease inhibitor cocktail. Following resuspension, cells were prepared for storage or downstream analysis by high-speed centrifugation and snap-freezing in liquid nitrogen.

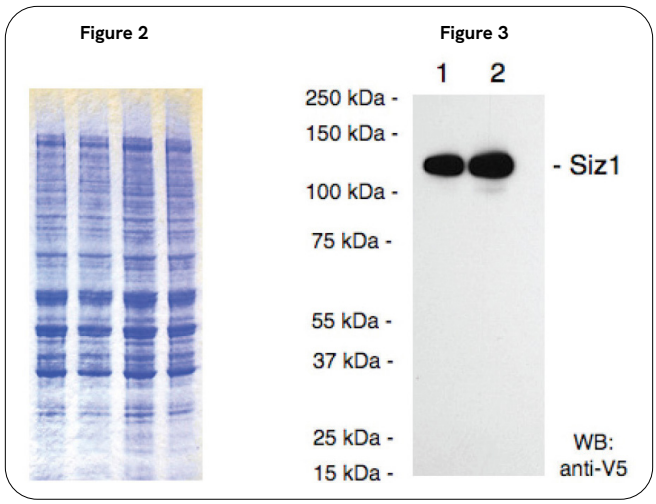


Figure 2. SDS-PAGE stained with Coomassie blue of whole cell extracts demonstrating reproducible protein recoveries.

Figure 3. Western blot of Siz1 shows no protein degradation or shearing.

Frozen pellets were added to 2 mL tubes containing 0.5 mm glass beads (Cat # 19-622) and 500 µL of an ice-cold lysis buffer. Samples were homogenized through bead beating on the Omni Bead Ruptor Elite bead mill homogenizer at 5.5 m/s for 20 seconds, six times, with 1 minute incubation on slushy ice between each run. Homogenates were clarified by centrifugation then protein precipitation was performed by addition of TCA. Protein pellets were resuspended and stored at -80 °C for future use. Protein samples were separated by SDS-PAGE to demonstrate the reproducibility of the protein extractions (Figure 2). Western blotting was then performed using an anti-V5 antibody to demonstrate the presence of the V5-tagged SUMO ligase Siz1 in the whole cell lysate (Figure 3).

Syzmanski and Kerscher further evaluated if bead milling would impact a proteins tertiary structure and thus its function.

Slx5 and Siz1Δ440 both possess a RING domain that has been reported to naturally coordinate with Zn^{2+} ions. Both proteins were suspended in either a non-denaturing or denaturing buffer then subjected to affinity capture through immobilized metal affinity chromatography. The captured proteins were then eluted and separated via 1D-PAGE and detected through Western blotting using either an anti-GST or anti- HA antibody.The integrity of the proteins tertiary structure was demonstrated by the fact that the protein was detected under native conditions but absent under denaturing conditions (Figure 4).

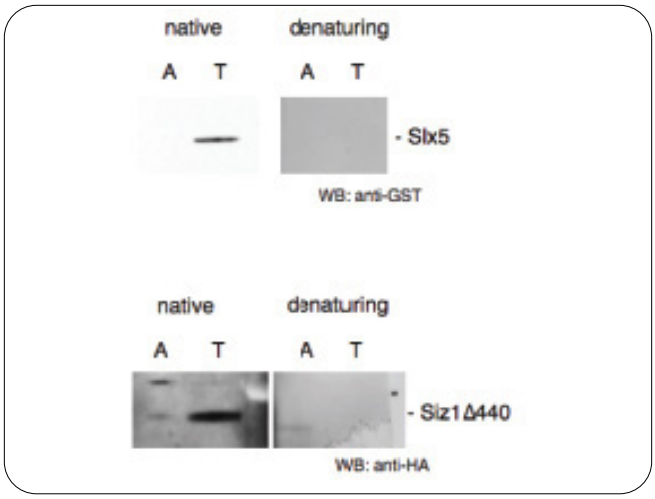


Figure 4. Western Blot for Slx5 and Siz1Δ440 shows recovery following resin-based purification under native conditions, but not denaturing conditions.

Conclusions

Syzmanski and Kerscher's study established a foundation of procedures from which future yeast research can be built upon. In their study, they show the benefit of using bead mill homogenization as a method to efficiently disrupt yeast in a reproducible and conservative manner. This technology provides researchers with a fast, user-friendly mechanism to perform high-throughput studies, eliminating sample prep bottlenecks.

References

1. Botstein D, Chervitz SA, & Cherry JM. (1997). Yeast as a Model Organism. Science. 277(5330): 1259–1260.

2. Ashburner et al. (2000). Gene Ontology: tool for the unification of biology. Nature Genetics. 25: 25–29.

3. Klis FM, Boorsma A, & De Groot PWJ. (2006). Cell wall construction in Saccharomyces cerevisiae. Yeast. 23: 185–202.

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