

Protocol for *Saccharomyces* Homogenization in the Bullet Blender™ 50

The protocol described in this document is for the use of the Bullet Blender™ for the homogenization of *Saccharomyces* cultures (*cerevisiae*, *pombe*, etc.). You may utilize this protocol as a starting point for other fungi, aslo. This protocol does not specify a particular buffer - you may choose which is most appropriate for your downstream application (nucleic acid isolation, protein extraction, etc.).

Materials Required: yeast, cell culture hood, aspirator, Bullet Blender™ 50, [beads \(zirconium oxide – 0.15mm or stainless steel, 0.2mm\)](#), homogenization buffer, 50mL centrifuge tubes, and pipetor.

Instructions

1. Pour yeast culture into a 50mL centrifuge tube.
2. Centrifuge culture to yield a cell pellet (2000g for one minute).
3. Completely aspirate the supernatant liquid. Place tube on ice.
4. You may use a larger cell pellet than yielded from 50mL culture by loading more culture, then repeating steps 1 through 3. **NOTE:** Increasing pellet may require a longer homogenization time to yield the same homogenization efficiency.
5. Inspect the volume of the pellet. It should be 4mL or less in order to get efficient homogenization.
6. Add a volume of 0.15mm zirconium oxide beads equal to the volume of the pellet. See **NOTES** below.
7. Add buffer (2 volumes of buffer for every volume of cells).
8. Close centrifuge tubes.
9. Place tubes into the Bullet Blender™.
10. Set controls for **SPEED 8** and **TIME** to **9** minutes. Press **Start**.
11. After the run, remove tubes from the instrument.
12. Inspect samples. If homogenization is unsatisfactory, run for another six minutes at **SPEED 10**.
13. Proceed with your downstream application.

SAFETY NOTE!!!

When using a centrifuge to separate your homogenate from the debris and beads, make sure your tubes are balanced.

NOTES

Different species of yeast and different applications will be amenable to different bead types. Cell density, cell size, and buffer composition will affect homogenization and variation of the bead selection is an easy way to empirically determine what works best.