

Protocol for *Candida albicans* Homogenization in the Bullet Blender®

The protocol described in this document is for the use of the Bullet Blender® for the homogenization of cultures of the diploid fungus *Candida albicans*. This protocol was used for protein extraction and does specify wash solutions and buffers, however you may choose buffers and otherwise alter the protocol as appropriate for your downstream application (RNA / DNA extraction, etc.).

Materials Required: *C. albicans*, aspirator, Bullet Blender®, homogenization buffer, pipettor, microcentrifuge tubes, 0.5mm zirconium beads (part GB05)

Instructions

1. Take 50ml of *C. albicans* culture at an OD_{600nm} of 1.0.
2. Harvest by vacuum filtration, resuspend in ice-cold 20% TCA, and incubate on ice for 30min.
3. Centrifuge culture to yield a cell pellet.
4. Wash the pellet 2x in alkaline-buffered acetone (three parts 3M Tris, pH 8.8, to seven parts acetone) or other wash of your choice.
5. Air-dry the pellet. Resuspend in 8M urea (or lysis buffer of your choice).
6. Add a volume of beads equal to the volume of the pellet.
7. Set controls for **SPEED 8** and **TIME** to **5** minutes. Press **Start**.
8. After the run, remove tubes from the instrument.
9. Visually inspect samples. If homogenization is unsatisfactory, run for another two minutes at the **SPEED 10**.
10. 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.

References:

Rauceo JM, Blankenship JR, Fanning S, Hamaker JJ, Deneault JS, Smith FJ, Nantel A, Mitchell AP. [Regulation of the *Candida albicans* cell wall damage response by transcription factor *Sko1* and PAS kinase *Psk1*](#). Mol Biol Cell. 2008 Jul;19(7):2741-51.

This protocol is an adaptation of the protocol in the referenced article.