Generating highly concentrated yeast whole cell extract using low-cost equipment.

Evelyn Sattlegger (e.sattlegger@massey.ac.nz)
Sattlegger Lab, Institute of Natural Sciences, Massey University, Auckland, New Zealand
Jyothsna Visweswaraiah
Sattlegger Lab, Institute of Natural Sciences, Massey University, Auckland, New Zealand
Martina Dautel
Sattlegger Lab, Institute of Natural Sciences, Massey University, Auckland, New Zealand

Method Article

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Abstract

This protocol describes a cost effective method for generating highly concentrated yeast whole cell extracts \(WCE) at concentrations of 50 µg protein/µl or 0.3 A_{260nm}/µl. Highly concentrated extracts are used for many applications such as protein purification, purification of protein complexes \(in particular when interactions are weak), protein-protein interaction studies, and velocity sedimentation studies. Normally highly concentrated extracts are generated with homogenizers or the French press. However, such equipment 1) is expensive, 2) is not available to every researcher, and/or 3) often cannot be used for cells from small culture volumes. The procedure described here can be used for breaking cells originating from cultures as small as 100 ml, although a culture volume of at least 200 ml gives the best result. Furthermore, this method is also effective for breaking formaldehyde crosslinked cells.

Reagents

Reagents • Breaking buffer. The composition depends on the experiment planned after cell breakage. For protein-protein interaction assays we used 30 mM HEPES, pH 7.4, 50 mM KCl, 10 % Glycerol \[1]. For velocity sedimentation assays we used 20 mM TrisHCl, pH 7.5, 50 mM KCl, 10 mM $MgCl_2$ \[2]. • Breaking buffer with protease inhibitors. Add inhibitors as required for your particular application. For example, we used 1 complete tablet without EDTA \(Roche) per 50 ml breaking buffer, 1 mM phenylmethylsylfonyl fluoride, 0.1 mg/ml pepstatin, 1 mM dithiothreitol or 2-mercaptoethanol. • Yeast cell pellets, obtained from a culture grown to A_{600nm}=1 or less, washed with ice cold water or breaking buffer. Pellet may be fresh or stored at -20°C or -80°C. • If required, reagents to determine protein concentration \(here we used the Bradford method). **Material** • Round base tubes, 13 ml, 16x100mm, polypropylene, \(Sarstedt, order No 62.515.006), or round base tubes with similar diameter. • 1.5 ml microfuge tubes. • Acid washed and autoclaved glass beads, 0.5 mm diameter. • Cuvettes for spectrophotometer.

Equipment

• Vortexer, optimally 3000 rpm. If more than 2 samples are being processed preferably use 2 vortexers. It is important that the vortexers have cup heads. Do not use platform heads. • Microfuge centrifuge. • Centrifuge that holds the above 13 ml round base tubes \(e.g. Heraeus multifuge 1S-R). If not available transfer samples to microfuge tubes and use microfuge centrifuge instead. • Spectrophotometer to determine protein concentration or to determine A_{260nm}.

Procedure

1. Cell pellets should be in round base tubes, no more than 0.8 g of wet weight cell pellet per tube \(equivalent to a 300 ml yeast cell culture grown to A_{600nm}=1). **2.** If cell pellet is frozen, thaw in a mixture of ice and water. **3.** Dissolve pellet in 200 µl of ice cold breaking buffer with inhibitors. **4.** Add 700 µl volume of acid washed glass beads. **5.** Vortex sample at maximum speed for 30 sec. Important: See Figure 2 for the exact procedure. Efficient breakage is visible by the sample forming a

whitish film and tiny bubbles on the inside wall of the tube \(see Figure 1). **6.** Place sample in a mixture of ice and water for 30 sec. **7.** Repeat steps 5 and 6 seven more times. Tip: If more than one sample needs to be processed the time spent for cell breakage can be shortened by vortexing 2 samples at a time on 2 vortexers for 30 sec. While these samples rest for 30 sec the next 2 samples can be vortexed. **8.** Spin samples at 4°C for 3 min at low g \(e.g. 2,500g). If a centrifuge for round base tubes is not available, transfer samples to microfuge tubes and spin in microfuge centrifuge at low speed. **9.** Transfer supernatant to fresh microfuge tubes. **10.** Spin in microfuge centrifuge at 4°C for 10 min at 10,000 rpm. **11.** Transfer supernatants to fresh tubes. Note: A highly concentrated WCE has an opaque appearance. A yellowish colour is due to pigments and does not necessarily indicate good breakage. **12.** Depending on your subsequent application, determine the protein concentration e.g. via the Bradford method, or determine the A_{260nm}, using breaking buffer alone as reference.

Timing

Approximately 1 hour: **Thawing and preparation of cells** – 15 min; **Cell breakage** - 8 min for 4 samples if using 2 vortexers; **Centrifugations** – 20 min.

Troubleshooting

Inefficient breakage: **a)** Make sure cells were harvested in exponential phase. **b)** Do not use more than 0.8 g of wet weight cell pellet per round base tube. **c)** Use round base tubes of the indicated size. **d)** Do not use more than 700 µl volume of glass beads of the correct size \(0.5 mm diameter). **e)** Make sure the tubes are being positioned on the vortexer as described in Figure 2, and the vortexers have cup heads.

Anticipated Results

The anticipated result is highly concentrated yeast whole cell extract. Expected are protein concentrations of 50 µg/µl or ribonucleotide concentrations of 0.3 A_{260nm}/µl.

References

\[1] E. Sattlegger, Barbosa, JARG, Moraes, MCS, Martins, RM, Hinnebusch, AG, and Castilho BA, Gcn1 and actin binding to Yih1: Implications for activation of the eIF2 kinase Gcn2. Journal of Biological Chemistry \(in press). \[2] E. Sattlegger, and A.G. Hinnebusch, Polyribosome binding by GCN1 is required for full activation of eukaryotic translation initiation factor 2 alpha kinase GCN2 during amino acid starvation. J Biol Chem 280 \(2005) 16514-21.

Figures

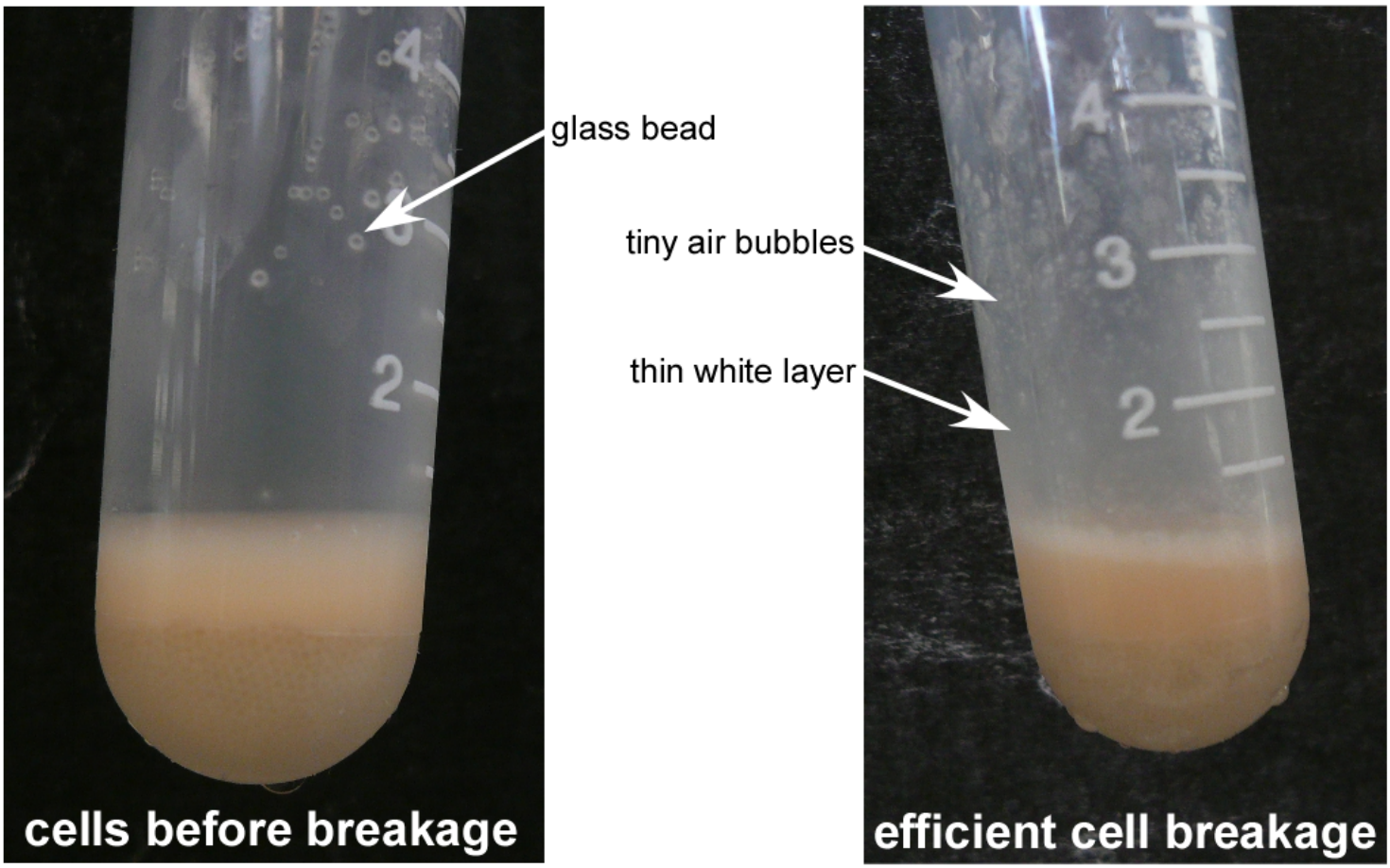

Figure 1

Photographs of samples before and after cell breakage. After efficient cell breakage tiny air bubbles (smaller than the 0.5 mm glass beads) and a thin white layer of cell debris coat the inside wall of the round base tube.

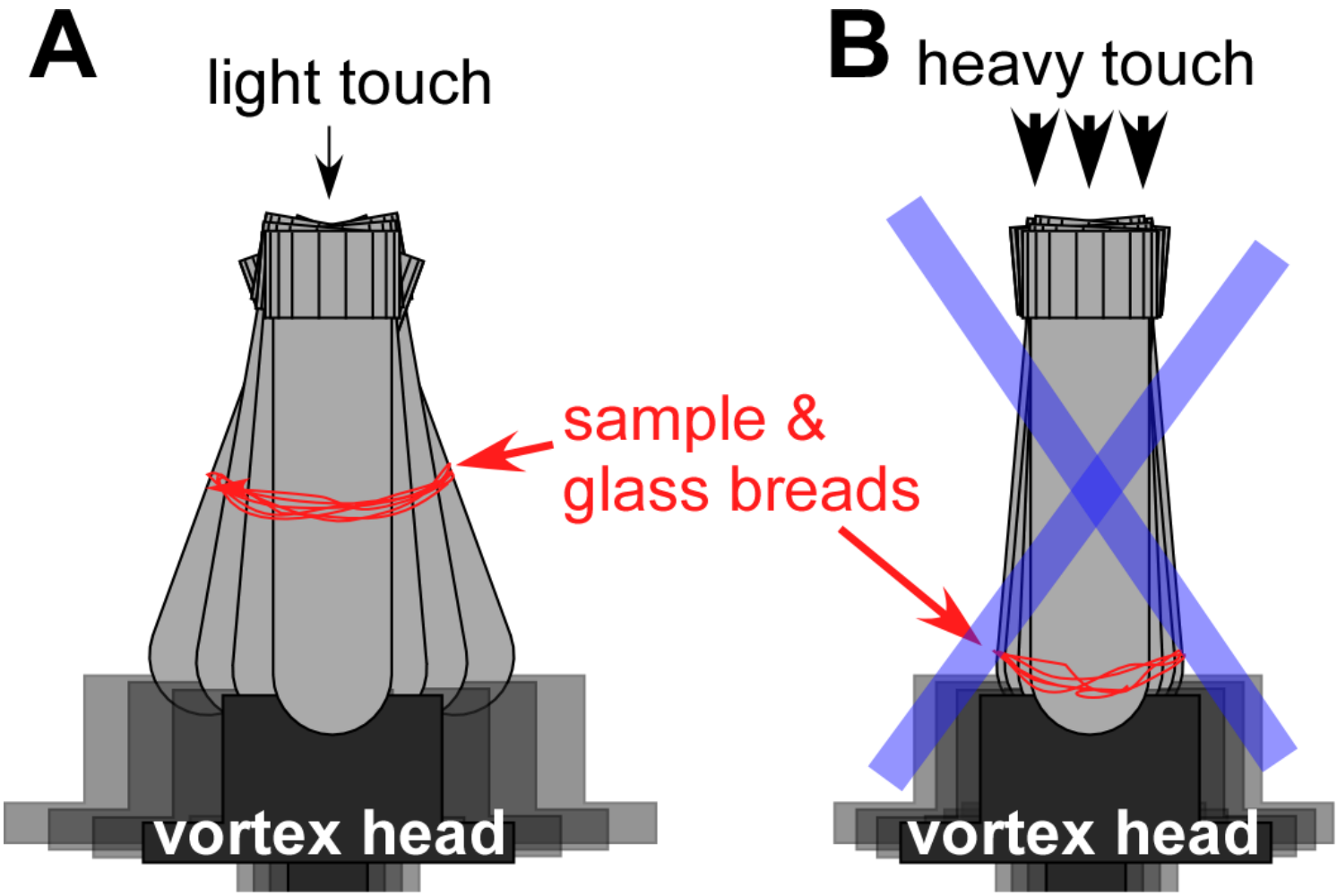

Figure 2

Illustration of round base tubes rotating on vortex cup heads. *A* Holding the tube on the cup head very gently allows maximum movement of the tube. This results into maximum movement of the sample and glass beads in the tube (indicated in red). *B* Pressing the tube onto the cup head restricts movement of the tube. This results in the sample and the glass beads racing around only at the bottom of the tube instead of further up in the tube (indicated in red).