Small-scale protein prep in *S. pombe*

1. Grow yeast in liquid culture to desired density (we grow to 0.5 at OD_{595})

2. Pellet 20 ml by centrifuging at 3000 rpm for 10 minutes at 4°C (increasing the time up to 20 minutes or centrifuging at room temperature won't do any harm).

All subsequent steps should be carried out with cold solutions on ice.

3. Wash once in water and resuspend pellet in 200 ul protein lysis buffer (or other appropriate buffer) in a 1.5 ml screwcap tube.

4. Add 0.5 mm glass beads (we use acid washed glass beads from Sigma, 425-600 um) to lysis buffer meniscus.

5. Vortex aggressively at 4°C for 15 min using a multi-tube holder. The buffer should be yellow and most yeasts appear broken by microscopy (If you vortex for 15 minutes or more you may need not to confirm by microscopy).

6. Puncture screwcap tube bottom with a needle. The needle can by heated with a flame to facilitate puncturing.

7. Place the punctured tube in a fresh 1.5 ml tube.

8. Spin for 10 seconds at maximum speed in a microcentrofuge to drain lysate into the fresh tube. Then spin the collected lysate for 5 min at maximum speed in a microcentrofuge to pellet insoluble material.

9. Collect the supernatant in a new 1.5 ml tube. The Supernatant can be used as a clear cell lysate for subsequent biochemistry.

10. Quantify the protein concentration using your method of choice. We use the Bio- Rad DC protein assay

If you started with 20 ml of 0.5 OD_{595} cultured cells you may get 150 – 300 ug of protein or more.

Protein Lysis Buffer

50 mM Tris (pH 7.5)

150 mM NaCl

5 mM EDTA

10% Glycerol

1 mM phenylmethylsulphonylfluoride (PMSF) – added fresh

References

Forsburg and Rhind (2006). Yeast 23: 173-183