# *S. cerevisiae* acetone powder

**NOTE:** Used for the pre-adsorption of polyclonal antisera, this can reduce backgrounds significantly. If you raise an Ab to protein Yfg, a powder will be made to the *YFG* deletion strain and used to adsorb a batch of the appropriate Yfg anti-sera: if you were to use a WT strain, you'd obviously just adsorb the specific signal.

**1.** Grow 50ml culture of the appropriate Δ strain to confluence (late log, $OD_{600} \approx 2$). Collect cells by centrifugation.

**2.** Resuspend cells in 1x PBS at a density of 10 – 30 $OD_{600}$ / ml. Add zymolase (≈ 1mg/1000 ODs) and incubate with gentle shaking 10 – 20 min 30°C

**3.** Place on ice 10m. Don't worry if spheroplasting is incomplete: it's really only necessary to weaken the cell wall.

**4.** Flick pellet to resuspend and add 4 vol acetone (-20°C). Vortex to a single cell suspension and put on ice 30 min.

**5.** Collect by centrifugation and resuspend pellet in acetone (-20°C). This may take a bit of work and involve some crushing with a large wooden applicator and extreme vortexing. Put on ice 30 min.

**6.** Collect by centrifugation and transfer pellet to mortar. Leave until dry (should be pretty quick – acetone is very volatile but can speed up using fume hood).

**7.** Grind to a fine powder using pestle. Store powder at RT° in a foil-wrapped falcon tube.

**8.** Use powder in undiluted sera at 10mg/ml and adsorb overnight at 4°C with rotation

**9.** Centriguge 12000rpm, 10min, 4°C. Remove adsorbed sera to a new ependorff and compare with pre-adsorbed sample.