# *S.cerevisiae* transformation

**NOTE 1:** This is one of those protocols where the numbers are a little flexible – and it should really be idiot proof : if it didn't work you did something really stupid.

**NOTE 2:** Are you sure that the strain to be transformed doesn't already contain the resistance marker or auxophic marker you're about to cover ?

**1.** Grow 10ml culture to $OD_{600} \approx 1.0$ (assuming WT-like growth, 10AM: inoculate a loopful of a relatively fresh strain into YPD, should be right by 4PM). Collect by centrifugation: 5min, 2000rpm in a tabletop centrifuge (should get ≈ 200µl pcv) (see **NOTE 3**: this step may not be required).

**NOTE 3:** For everyday transformations (plasmid into strain), you can use a small loopful (20µl **pcv**; packed cell volume) of *S.cerevisiae* (direct from a plate less than a month old and stored appropriately at 4°C) and 2µl of Qiagen grade DNA (miniprep from a pRS series plasmid: 1.5mls overnight, 50µl elution ≈ 100ng/ml). This should give you ≥50 colonies. If you want targeted homologous recombination you need to step it up a bit (as in **Step #1** above).

**2.** Discard supernatant. Collect pellet by resuspending in 750µl $ddH_2O$. Transfer to a sterile ependorff on ice. Collect by centrifugation in a table-top microfuge (a flash-spin up to 10K should be enough). Discard the supernatant and resuspend pellet in 1ml LiTE. Collect by flash-spin centrifugation. Resuspend in < 500µl LiTE (enough for about 10 transformations).

**3.** Prepare ependorff tubes containing the DNA of interest (5µl PCR product, excised fragment, whatever). Add 5µl sheared, salmon sperm DNA (10mg/ml; this is usually stored at –20°C in 500µl aliquots. Boil an aliquot (5m, 100°C) and store at 4°C for repeated use). Add 50µl of the cell suspension from **Step #2** and mix well. Add 250µl PLATE. Mix well.

**4.** Incubate 30°C for 15m with occasional agitation (flick the tube if you remember). Heat shock 42°C for 60-90m with occasional agitation (try and flick the tube once or twice).

**5.** Flash spin to collect cell pellet. Note this will take a little longer because of the PEG. Also the quality of the pellet will be different, with a significant amount along the side of the tube. Remove the supernatant and resuspend in 250$\mu$l $ddH_2O$. Plate all as appropriate.

**NOTE 4:** If transforming a resistance marker do not plate directly. Rather resuspend pellet from **Step #5** in 500µl YPD. Incubate (30°C, 3-4 hours) to allow gene expression and plate all as appropriate. If using KanMX (G418 selection; 200µg/ml) it is recommended to replica the plate after 24 hours (it reduces the background significantly). With Nat (Noursethricin; 100µg/ml) this is not necessary.

## Buffers:

**1xLiTE** 0.1 M Lithium Acetate (1M stock)
1x TE (10 mM Tris-HCl pH7.9, 1 mM EDTA)

**PLATE** 40% Polyethylene Glycol 3500 or less (50% Stock)
0.1 M Lithium Acetate (1M Stock)
1x TE (10 mM Tris-HCl pH7.9, 1 mM EDTA)