

Yeast transformation on 96 well plates

Most of these steps can be performed using a Biomeck Robot (i.e. media distribution and pipetting)

Culture

- Distribute 100 μ l of YPD in a 96-multi well plate (U bottom)
- Add 10 μ l of a stationary phase culture in each well
- Incubate O/N at 30°C **without agitation** (to avoid cross-contamination) under a wet atmosphere (humidity supplied by a water bath for example)

Transformation

- centrifuge cells 5 minutes at 3000rpm
- remove the supernatant by quickly flipping the plate (over a sink)
- resuspend the pellet by smoothly vortexing the plate (there is enough liquid left to do so).
- add 100 μ l in each well of the following solution

Lithium Acetate	0,2 M	for 50 ml	10 ml of 1 M
PEG 3350	40%		20 g
DTT	100 mM		5 ml of 1 M

Containing

- 5 μ l carrier DNA (10mg/ml) (denatured 5 minutes at 95°C and then put on ice)
- 1 μ l replicative plasmid (1 μ g/ μ l).

You can test the optimal concentration for your plasmid. It is not necessarily required to increase it since the number of transformants/ μ g quickly reaches a plateau.

- **Carefully mix** by pipetting.
- incubate 30 minutes at 45°C.
- make 10 μ l drops on the appropriate media (we use large square plates)

You usually get between 1 and 20 transformants/drop, depending on the strain.

If you are using the FY1679 background, check that your transformants are not glycerol sensitive, as the heat shock can favour the appearance of gly⁻ colonies.