YEAST TRANSFORMATION - LIAC METHOD

1. Inoculate 50 ml of **YPD** with a single colony of yeast cells. Shake at 30°C for ~2 days.

2. Sub 5 ml of the culture into 100 ml of **YPD** in 250 ml flask and shake at ~250rpm for 4 – 5 hr. Can scale down to 50 ml if necessary.

3. Harvest cells by spinning culture at ~3500 rpm at 4°C for 1'.

4. Wash pellet in 50 ml of sterile water.

5. Suspend cells in 1 ml of sterile water and transfer to microfuge tube.
   Pellet cells with a 13K pulse.

6. Wash cells in 1 ml of **LiAcTE**.

7. Suspend cells in 300 – 700 μl of **LiAcTe**. Volume depends on number of
   transformations needed. Obviously larger volume = lower competency
   of cells, but this is not usually a problem.

8. Add 10 μl of 10 $mgml^{-1}$ herring sperm DNA per 100 μl of yeast cells. Mix well.

9. Take 50 μl of yeast cells. Add 100 ng – 1 μg of plasmid in volume of 1-2 μl. Add 300 μl **LiAcPEG**. Mix by flicking.

10. Gently shake cell/plasmid mix at 30°C for 10'.

11. Place at 42°C for 10'.

12. Plate ~100µl of reaction onto a yeast selection plate. This is **SD media** with carbon source and all amino acids apart from that which acts as a selection agent for your plasmid.

13. Leave at 30°C for 2 days. Colonies should be nice and fat by then.

NOTE: It is very easy to co-transform 2 plasmids together. Just plate on media lacking both selection agents.

YPD (1L) pH to 5.8
20 g Difco peptone
10 g yeast extract
15 g agar (ONLY FOR SOLID)

Autoclave. Then add sterile carbon source (usually glucose) to final of concentration of 2% before using.

LiAcTE (Made from stocks of 10 X LiAc and 10 X TE)
10 X LiAc = 1 M LiAc adjusted to pH 7.5 with dilute acetic acid. Autoclaved.
10 X TE = 0.1 M Tris-HCl, 10 mM EDTA pH 7.5. Autoclaved.

LiAcPEG.
Made from stocks of LiAc (as above) and 50% PEG 4000.
Make up 50% PEG 4000 then filter sterilise.

SD media (1L)
6.7 g Yeast nitrogen base without amino acids
15 g agar (IF FOR PLATES).

Selection plates
Melt SD media.
Add autoclaved carbon source to final of 2%.
Add 100 X 'dropout' amino acid solution.
Add rest of amino acids, omitting your selection agent.

Pour plates.

100 X amino acids

Threonine	2g
Tyrosine	300mg
L-Isoleucine	300mg
Valine	1.5g
Adenine hemisulphate Salt	200mg
Arginine HCl	200mg
Lysine HCl	300mg
Methionine	200mg
Phenylalanine	500mg

To 100 ml with water

Add 1st 2 amino acids slowly to small amount of water. Then add rest of amino acids. Autoclave.

Selection amino acids

100 X histidine HCl	200mg
100 X leucine	200mg
100 X tryptophan	200mg
100 X uracil	200mg

All in 100 ml of water

Autoclave. Store all amino acids in dark at 4°C. Stable for ~ 1 year. The uracil tends to drop out of solution, so good idea to aliquot and prewarm before adding to media.