

Transformation of Yeast Spheroplasts

1. Grow a 100 ml YPD overnight culture of cells till the $OD_{599}=1.5-2.0$
2. Spin down cells and wash once in sterile H_2O .
3. Form spheroplasts at $37^{\circ}C$ for 45 min in 20ml of spheroplast media.
4. Pellet the cells at 1100 rpm ($\sim 300 \times g$) in table top Beckman for 7 min.
5. **Gently** wash once in 1M sorbitol and pellet 5 min at 1100 rpm.
6. **Gently** resuspend in 20 ml of STC, and spin down cells for 5 min at 1100 rpm.
7. **Gently** resuspend in 2ml of STC. Aliquot 100 μ l of cells into 12 ml falcon tubes containing a mixture of plasmid DNA and carrier DNA which totals 5 μ g of DNA. I have used 0.2 μ g of plasmid DNA + 4.8 μ g of chick blood carrier DNA per tube and been very successful.
8. Incubate the tubes containing cells + DNA for 10 min at room temperature.
9. Add 1 ml of PEG-8000 buffer to each tube and gently mix to resuspend.
10. Incubate at room temperature for another 10 min and then harvest for 5 min at 1100 rpm.
11. Resuspend in 150 μ l of SOS media supplemented with 10 μ g/ml of selective amino acid (uracil for example if using *ura3-52* cells). Incubate at $30^{\circ}C$ for 20-40 min. I have used a 40 min incubation.
12. Add 7-8 mls of Top Agar, which is kept at $45-50^{\circ}C$, and invert tube to mix. The suspension was quickly poured onto SORB plates and incubated at appropriate temperature. I have directly placed the plates at restrictive temperature to select for complementing plasmids. Many colonies that develop will be embedded in the agar.
13. For controls, include a no DNA sample and a tube containing plasmid but no insert. This will control for revertants.

Stocks

STC media

1M sorbitol
10mM Tris-HCl, pH 7.5
10mM $CaCl_2$
mix and filter sterilize

SOS media

1M sorbitol
6.5mM $CaCl_2$

0.25% yeast extract
0.5% peptone
mix and filter sterilize

PEG-8000

20% PEG-8000
10mM Tris-HCl, pH 7.5
10mM CaCl₂
mix and filter sterilize

Top Agar (for 200ml)

1M sorbitol
2.5% agar
2.68g/200ml of yeast nitrogen base w/o amino acids
mix and sterilize, then add 10ml of sterile 40% glucose

SORB Plates

13.4 g of Difco Yeast nitrogen base without amino acids
164 g sorbitol
1 liter of water

place in 2 liter flask and sterilize

40 g Bacto-agar
164 g sorbitol
1 liter of water

place in 4 liter flask with stir bar and sterilize

60 g glucose
100 ml of water

sterilize

When above are cool enough to touch, mix all in the 4 liter flask and stir on warm setting till ~60°C. Pour some into a sterile liter flask and pour plates.