

Yeast Two-Hybrid Screening

Yeast transformation

1. Resuspend 1 colony AH109 in 50 μ L sterile dH₂O
2. Add 273 μ L PEG/LiAc mix, 5 μ L prey miniprep, 5 μ L bait
3. Heatshock 15min, 45°C
4. Spin at 8000rpm, 1min
5. Take off supernatant
6. Add 1 ml YPDA + leave O/N
7. Spin 8000rpm, 1min
8. Take off supernatant
9. Resuspend in 200 μ L 0.9% NaCl (filter sterilised)
10. Plate 100 μ L onto selective and non-selective plates

Media

YPD (starter) plates (1 litre)

20g Peptone
10g Yeast extract
20g Agar
950ml dH₂O
pH 6.5

Autoclave, cool to 55°C, add 50ml 40% dextrose (filter-sterilised)

YPDA (1 litre)

20g Peptone
10g Yeast extract
Autoclave, then cool to 55°C and add;
15ml 0.2% Adehemisulphate (Sigma A9126)
50ml 40% Dextrose (filter sterilised)

Non-Selective Plates (1 litre)

1.7g Nitrogen base
5g Ammonium Sulphate
8g Agar
0.64g -L/-T DO Supplement
Autoclave, then add;
50ml 40% Dextrose

Selective Plates (1 litre)

1.7g Nitrogen base
5g Ammonium Sulphate
8g Agar
0.60g -H/-L/-T DO Supplement

Autoclave and cool to 55°C
50ml 40% Dextrose
1ml α -gal

0.2% Adehemisulphate
20mg in 10ml

PEG/LiAc Mix
800 μ L 40% PEG 100 μ L LiAc 100 μ L TE

Handy Hints

Rinse DO supplement off weigh boat into media
Dissolve w/stirring for 15min, then autoclave 121°C, 15min