**Yeast Two-Hybrid Screening**

**Yeast transformation**

1. Resuspend 1 colony AH109 in 50μL sterile dH2O
2. Add 273μL PEG/LiAc mix, 5μL prey miniprep, 5μL 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 200μL 0.9% NaCl (filter sterilised)
10. Plate 100μL 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

800μL 40% PEG 100μL LiAc 100μL TE

**Handy Hints**

Rinse DO supplement off weigh boat into media

Dissolve w/stirring for 15min, then autoclave 121°C, 15min