Competent yeast cells + LiAc transformation from liquid culture

Introduction

Protocol for LiAc yeast competent cell preparation and transformation, from Aalto University's Molecular Biotechnology group.

Materials

- 10x TE-buffer
- 1M LiAc
- 50% PEG-4000
- Solution A (1 ml 10xTE, 1ml LiAc, 8ml ddH2O = 10ml)
- Solution B (1 ml 10xTE, 1ml LiAc, 8ml 50% PEG-4000)

Procedure

Yeast cultivations

1. Make a 5ml liquid culture from yeast in YPD. Leave into 30°C O/N with shaking.
2. Measure absorbance
3. Dilute to get OD600=0,5. You will need 5 ml for each transformation reaction.
4. Grow on 30°C shaking until 1,5<OD<2.

Preparing competent cells

5. Harvest cells by centrifugation at 3900 rpm for 5 minutes RT (use Falcon tube)
6. Decant supernatant (collect to microbial waste)
7. Resuspend pellet with 30ml sterile H 2 O
8. Repeat centrifugation as described above
9. After decanting supernatant, resuspend pellet in 500 µl solution A (1x TE, 0.1% LiAc) and move to an Eppendorf tube.
10. Spin cells down (3 min at 3600 rpm in an Eppendorf centrifuge)
11. Per assay you'll need 100 µl suspended cells, so resuspend pellet in 100-300 µl solution A (1xTE, 0.1% LiAc)

Transformation of LiAc competent cells

12. Add 1-3 µl (600ng) of each plasmid DNA (the purer the DNA the less you need)

13. Mix plasmids with 3 µl salmon sperm [10 µg/ml], heat denatured (5 min at 100 °C) as carrier DNA
14. Add 100 µl cells (in solution A) to the tube
15. Add 1 ml solution B (1x TE, 0.1% LiAc, 40% PEG) and mix by inverting tube (no vortexing!)
16. Incubate for 1 h at 30 °C
17. Add 120 µl DMSO (invert tube to mix)
18. Heat shock cells for 10 min at 42 °C in a water bath
19. Cool down cells immediately on ice for 3 min
20. Spin down cells for 5 min at 3000 rpm in an Eppendorf centrifuge
21. Remove supernatant by pipetting
22. Resuspend pellet carefully in 100 µL ddH 2 O
23. Plate cells on selective medium and incubate at 30 °C (first transformants should be visible after 2 days).