LiAc/SS-DNA/PEG TRANSFORMATION

Introduction

This protocol was created by Justin D Smith and edited by Hannah Shen. This is a standard lithium acetate protocol for transforming yeast with miniprep DNA. We use this protocol to get *up to 22 million transformants/ µg of plasmid DNA*.

Materials

- YPD
- 1M LiAc
- 100mM LiAc
- 50% w/v PEG
- 10mg/mL SS-DNA
- SCM-minus plate

LiAc/SS-DNA/PEG TRANSFORMATION (Quick and dirty)

Check two days before transformation that there is enough of all plasmids.

1. Inoculate 5mL of liquid YPD or 15mL SCM and incubate with shaking overnight at 30°C. Use 50mL Falcon tube

2. Inoculate into 50mL YPD (5mL cells/transformation) to make OD_{600}=0.1. Use 250mL sterile flask

3. Incubate the culture at 30°C on a shaker at 200 rpm until OD_{600}~0.6-1.0. This will take a **minimum** of 4 hours.

4. Harvest the culture in a sterile 50mL centrifuge tube at 3000 x g (5000 rpm) for 5 min. Keep cells cold from this step.

5. Pour off the medium, resuspend the cells in 25mL of sterile water and centrifuge again.

6. Pour off the water, resuspend the cells to a final volume of 500µL (2 x 10^9 cells/ml) -- about 400µL of 100mM LiAc.

   Use 400µL 100mM LiAc if OD_{600} =0.6, and 800µL if OD_{600} =1.2, OD from step 3.

10. Vortex the cell suspension and pipette 50µL samples into labelled microcentrifuge tubes. Pellet the cells at top speed 15 sec and remove the LiAc with a micropipette.

11. The basic "transformation mix" consists of:

    240 µl PEG (50% w/v)

    36µL 1.0M LiAc

    50µL SS-DNA (10mg/mL)

    X µl Plasmid DNA (0.1 - 10µg)

    34-X µl Sterile ddH_2O 360µL TOTAL

    Note: Make PEG, LiAc and SS-DNA into a mastermix if different plasmids are used, mastermix = number of sampels + 1

12. Vortex each tube vigorously until the cell pellet has been completely suspended. Usually takes about 1 min.

13. Incubate at 30°C for 30 min.

14. Heat shock in a water bath at 42°C for 30 min.

15. Microfuge at 6-8000 rpm for 15 sec and remove the transformation mix with a micropipette. Discard as biowaste and Li+ chemical waste.

16. Add 1ml of YPD media to all tubes and incubate for 2 hours at 30°C in shaker.

17. Centrifuge at 14’000 rpm for 15 secounds.

18. Pipette 200µL of sterile water into each tube and resuspend the pellet by pipetting it up and down gently.
19. Plate transformation mix onto selective plates.
20. Incubate the plates for 2 - 4 days to recover transformants.