Lithium Acetate Transformation

Protocol provided by Computational Systems Biology Lab, BSSE

Materials

- Growth medium (eg. YPD)
- Yeast strain to be transformed
- Sterile ddH2O
- 4M Lithium acetate, filter sterilized
- 0.2M Lithium acetate, filter sterilized
- 0.2M Lithium acetate in 20% glycerol, sterile
- 0.4M Tris buffer, pH 7.5, sterile
- 0.08M EDTA, sterile
- High molecular weight, single-stranded carrier DNA (2ml/ml), sterile
- Transforming DNA (digested plasmid, circular plasmid or PCR product)
- 58% PEG 3350, sterile
- DMSO
- Selection plates

Protocol

Time required: 5 minutes to prepare overnight culture the day before. On the day of the protocol 2 hours of bench time followed by 2 days of incubation before colonies appear.

1. Day 0: Start a 5mL o/n culture of the strain to be transformed in the appropriate medium.
2. Day 1: Dilute the culture in growth medium such that it reaches 20 million cells per mL in 5 hours (5mL per transformation).
3. Harvest 5mL of cells per transformation by centrifugation at 3000g for 3 minutes.
4. Resuspend each tube in 1.4mL 0.2M LiAc, then centrifuge at 3000g for 3 minutes.
5. Meanwhile prepare master mix fresh (makes 1mL):
    - 200μL 4M LiAc
    - 100μL 0.4M Tris
    - 100μL 0.08M EDTA
    - 100μL ssDNA
    - 500μL ddH2O
6. Resuspend cells in 100μL 0.2M LiAc supplemented with 20% glycerol.
7. For each transformation, take 100μL of the cells and add 100μL of the master mix. Pipette gently to mix.
8. Add up to 500ng of DNA in a volume of 16μL.
9. Add 240μL of 58% PEG-3350. Pipette gently to mix.
10. Incubate on the wheel at room temperature for 30 minutes.
11. Add 50μL of DMSO.

12. Heatshock on the heat block at 42°C for 30 minutes.
13. Cool down on ice for 5 minutes.
14. **If transforming uncut plasmid**: transfer 60μL of transformation mix into a new tube and add 250μL of medium.
15. **If transforming digested plasmid or PCR product**: Spin down cells for 1 minute at 2000g. Resuspend in 300μL of medium.
16. Incubate at room temperature for 10 minutes for auxotrophic markers, 4 hours for antibiotic resistance markers.
17. Plate and incubate at 30°C for 2 days. Small colonies should appear on the second day.