Yeast Transformation Protocol

This protocol works well for transforming PCR products for gene disruption.

Required Reagents:

- Stock Solutions
  - 1M Lithium Acetate (LiAc)
  - 10X TE
  - 50% PEG 3350
  - salmon sperm DNA (5mg/mL)
- 30°C incubator
- 42°C incubator
- fresh yeast
- transforming DNA

Solutions (Make fresh each time):

1. TE + 100 mM LiAc (Dilute stock LiAc and 10X TE in water 1:1:8).

2. PLATE: 1X TE, 100 mM LiAOC, 40% PEG (1:1:8 of stock solutions)

Protocol:

1. Grow yeast to be transformed overnight in YPD medium
2. Dilute overnight culture and grow to mid-log phase (a 1:100 dilution grown for 3 hours is usually about right)
3. Spin down 15 mL of cells
4. Wash cells in 0.5 ml of Solution 1 (TE + LiAOC)
5. Resuspend cells in 200 µl Solution 1 (TE + LiAOC)
6. Add transforming DNA (usually 40 - 50 µl of deletion cassette PCR product)
7. Add 20 µl freshly boiled ssDNA (boil on 95°C heat block for 5 min)
8. Add 1 mL PLATE solution and mix by inverting several times
9. Incubate at 30°C for 30 min, inverting a couple times during the incubation
10. Incubate at 42°C for 15 min, inverting a couple times during the incubation
11. Spin down cells and plate on selective media