Yeast LiOAc Transformation

1. From a fresh 5ml O/N YPD culture, dilute the yeast strain in YPD to an OD_{600}=0.2 in a 250ml flask. Grow cells with shaking at 30°C until they reach OD_{600}=1.0 (ODs from 0.7-3.0 have been used but cells must be in log phase growth). For 3 transformations grow 40-50ml culture. – Note that wild-type yeast doubling is about 90 min.
2. Pellet cells and resuspend to 1/4 of the original culture volume in freshly made 1X TE (pH 7.5), 1XliOAc solution. E.g. 10ml
3. Pellet cells and resuspend to a density of 2 X 10^9 cells/ml in TE-LiOAc. For 40ml culture this is 600µl.
4. Add 200µl of cells to 150µg salmon sperm DNA + DNA to transform and mix thoroughly. For integration use 200-500ng DNA. For simple plasmid transformation use 20-50ng.
5. Add 700µl of 1X TE, 1X LiOAc, 40%PEG solution and mix by stirring with the pipette tip – do not vortex.
6. Incubate 30 min 30°C in a water bath
7. Heat shock for 15 min in a 42°C water bath. Heat shock increases transformation efficiency by about 5X.
8. Spread cells directly from PEG misture after heat shock. **DO NOT PELLET CELLS** – this reduces transformation frequency by about 10-fold.

Stock solutions:

<u>10X TE</u>
100mM Tris pH 7.5
10mM EDTA

<u>10X LiOAc</u>
1M LiOAc

<u>50% PEG</u>
50% (w/v) PEG 3350

Filter sterilize all solutions, do not autoclave. With 50% PEG this is slow, but necessary. A 1.2µM prefilter often helps with PEG.

Alternate mini procedure:

At steps #4-5 scale down to the following:

4. Add 10-100ng plasmid DNA to 50µg salmon sperm DNA in ~ 10µl.
   Add 40µl cells and mix. Add 200µl 40%PEG, 1X TE, 0.1 M LiOAc solution and mix (pipette up and down slowly using a large bore (P1000) pipette tip).

The advantage of the mini procedure is that you can spread the entire reaction onto a single dropout plate.

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