A Dohlman Lab Protocol

PLATE Transformation

"PLATE" is an acronym of the names of ingredients in the transformation solution: PEG, Lithium Acetate, Tris, and EDTA.

-Use sterile technique and sterile solutions throughout this method.-

1. In a 15 ml culture tube, inoculate 2-3 ml of the appropriate media with a single colony of the yeast strain to be transformed. This is the starter culture.
***Various wild-type and mutant yeast strains can be obtained from Research Genetics (Huntsville, AL) or ATCC (Manassas, VA).

2. Grow the starter culture at 30 with shaking (250 rpm) until it reaches saturation.
***This takes anywhere from 1 to 6 days, depending on the strain and the media.

3. Place 0.5 ml of the saturated culture in a sterile microfuge tube.

4. Collect the cells by centrifuging at 16,000 x g for 30 sec.

5. Aspirate the supernatant.

6. Add 10 ul of sonicated salmon sperm DNA (10 mg/ml stock) (Stratagene, #201190).
***The DNA must be single-stranded, which can be achieved by boiling for 5 min (a 100°C heat block works well) and immediately chilling on ice.
***The DNA only needs to be boiled every 3 to 4 times it is used (as long as it remains on ice when thawed).

7. Add 1-2 ug of the plasmid DNA to be transformed and vortex.

8. Add 500 ul of PLATE solution. Mix by inverting or by pipetting gently. Do not vortex.

PLATE solution
40% PEG3350 (w/v)
100 mM lithium acetate (LiAc)
10 mM Tris, pH 7.5
0.4 mM EDTA

9. Leave at room temperature or 30oC for 15 min, then heat shock 42oC for 15 min, then ice for 2 min. Alternatively leave at room temperature or at 30oC for

24-48 hours.

10. Collect the cells by centrifuging at 16,000 x g for 30 sec.

11. Aspirate the supernatant.

12. Resuspend the cells in 200 ul of sterile water by pipetting gently and thoroughly.

13. Spread on solid media that will select for the plasmid.

14. Incubate at 30° until colonies appear.
***This takes around 2 to 6 days, depending on the yeast strain and the plasmid's nutritional marker. In our experience, it usually takes ~ 2 days for *LEU2* plasmids and ~ 6 days for *URA3* plasmids.

15. Restreak 2-3 transformed colonies onto a new plate.

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