

96-Well Plate Yeast Transformation (1/09 GP version)

(Adapted from Gietz and Woods (2005) *Methods Mol. Biol.* **313**, 107-120)

(**Note:** put a shaking platform in an incubator and adjust the temperature to 42°C before starting the transformation)

1. Grow an overnight yeast culture to $\sim 2 \times 10^7$ cells per ml in YPD liquid medium. We use $\sim 4 \times 10^7$ cells/well of transformation, and therefore need a 200 ml culture grown to $\sim 2 \times 10^7$ cells per ml for each 96-well plate.
2. Harvest the cells in 50 ml conical tubes at 2500 rpm for 5 minutes in a tabletop centrifuge or 4K rpm for 5 min in Sorvall.
3. Dump the YPD medium. To remove the remaining YPD, wash the cells by resuspending the pellets in sterile water, combining the cells into one tube, and pelleting again as above.
4. Dump the water, and wash the cells once with sterile 0.1M LiOAc.
5. Pellet the cells, dump out the 0.1M LiOAc, and resuspend the cells in fresh sterile 0.1M LiOAc to final volume of 1 ml per 96-well plate.
6. Add the 1 ml cell suspension to TRAF0 mix without the PEG (see table below) and gently mix to disperse. Aliquot 50 microliters of the transformation mix to each well of a Falcon #3077 plate using a 12-channel pipetter and a sterile tray. Each well should have $\sim 4 \times 10^7$ cells.
7. Add plasmid DNA to each well using a 12-channel pipetter. We are currently using ~ 60 ng of plasmid DNA per well to be safe, but adjust this amount to the transformation efficiency of your strain. With healthy strains we can get enough transformants using only 10 ng of DNA. Some of the DNA plates should have empty wells; in those plate positions you could add control vector or any positive controls that generate the desired phenotype in your starting strain.
8. Shake the plate at ~ 200 rpm for 2 minutes to allow the DNA to mix with the cells.
9. Add 100 microliters of 50% PEG 3350 to each well with a multi-channel pipetter.
10. Incubate plates at 42°C for 1 hour on a shaking platform at ~ 220 rpm (optimal incubation time might be strain-dependent, test each strain before doing the large-scale transformation). The Gietz transformation protocol recommends 3 hr at 42°C, but we found no difference between 1 hour and 3 hour incubations; the shorter incubation might help to get transformants when using slowly growing or temperature-sensitive strains.
11. Pellet cells in the 96-well Falcon plate at 2500 rpm for 5 minutes at room temp; remove the TRAF0 mix with a multipipetter, ensuring to not to disturb the pellet.
12. Add 14 microliters of sterile water to each well, and resuspend the pellet by shaking on multi-vortexer for ~ 2 minutes at a setting that will resuspend the cells without splashing into adjacent wells.

13. Spot 7 microliters of the resuspended cells onto SC-Leucine plates to select for transformants. We use a 12-channel pipetter to spot the cells, typically spotting duplicate rows onto the SC-Leu plate, offset at a 45 degree angle. To help generate evenly spaced rows, the plate is first lightly touched with a sterile 96-prong replicator (V&P scientific #VP407) leaving a light imprint of the replicator positions in the agar, using the V&P #381 tray copier to position the spots. The cells are then spotted onto that imprint using the multi-channel pipetter. We use Nunc Omni-plates (Nunc # 242811) filled with ~35 ml of SC-Leu agar.

14. Incubate plates at 30°C until transformants appear. With the relatively large number of cells plated per spot, background growth can be relatively high. Note that the transformation method used in the Jones *et al* paper resulted in low background growth but required greater amounts of plasmid DNA. We also find that transformants near the center of the plate take longer to appear than the transformants near the edge of the plate, but other labs report similar edge effects. We have not figured out how to get uniform growth. If you have ideas on how to get more uniform growth of transformants across the entire plate, please pass them along.

15. After the transformants appear, we first transfer them into 100 microliters of sterile water in Falcon #3072 plates using the VP407 96-prong replicator. The cells are dispersed in the water by gently shaking on a platform shaker for ~2 minutes.

16. The dispersed cells are pinned to selective plates in duplicate rows using the VP407 96-prong replicator. We pin them twice onto each spot to ensure even distribution of the cells.

17. Hope, pray, chant, perform the secret genticist's dance, or wish upon a star that a few plasmids generate the desired phenotype. Note that some plasmids might not generate transformants in your mutant background. These could be due to simple pipetting errors during the large-scale transformation, but they could also be examples of synthetic dosage lethality (SDL)(see Meth. Enzymol 350: 316-326) where overexpression causes lethality in a specific mutant background, but not in wild-type cells.

18. Re-transform any suppressor or SDL candidates individually by your standard transformation protocol to confirm the overexpression phenotype.

| <u>#of plates</u> | <u>1</u> | <u>2</u> | <u>4</u> | <u>9</u> | <u>18</u> | <u>per well</u> |
|-------------------|-------------|-------------|-------------|-------------|--------------|-----------------|
| 50% PEG3350 | 10 ml | 20 ml | 40 ml | 90 ml | 180 ml | 100 µl |
| 1M LiOAc | 1.5 ml | 3 ml | 6 ml | 13.5 ml | 27 ml | 15 µl |
| H ₂ O | 0.5 ml | 1 ml | 2 ml | 4.5 ml | 9 ml | 5 µl |
| 2mg/ml ssDNA | 2 ml | 4 ml | 8 ml | 18 ml | 36 ml | 20 µl |
| cells | <u>1 ml</u> | <u>2 ml</u> | <u>4 ml</u> | <u>9 ml</u> | <u>18 ml</u> | <u>10 µl</u> |
| | 15 ml | 30 ml | 60 ml | 135 ml | 270 ml | 150 µl |

Notes: ssDNA is a 2 mg/ml stock of salmon sperm carrier DNA (Sigma D1626) boiled for 10 minutes, then put on ice for 5 minutes. It doesn't have to be boiled fresh every time, but don't keep freezing and thawing it either.

Have an incubator ready at 42°C before starting the procedure.

1M LiOAc

51g LiOAc·2H₂O

463 mls H₂O

500 mls final (should be between pH 8.4 - 8.9)
(filter sterilize or autoclave)

50% PEG 3350

125 g PEG 3350

~145 ml H₂O (to 250 ml final vol.)

Heat at low setting to get into solution
Autoclave