Yeast Transformation (high efficiency)

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Hahn lab standard method for yeast transformation.
DMSO addition increases the efficiency of transformation.

This method was optimized for use with S. cerevisiae strains that are derivatives of S288C. You may need to optimize the method to your particular yeast strain and DNA used in the transformation by changing the number of cells and/or amount of DNA used and number of cells plated.

1. Grow yeast overnight in YPD (or other suitable selective media) at 30 deg. For most applications, growth in selective media (rather than YPD) is not required if the cells that are used to inoculate the overnight are taken from selective media.

2. Inoculate 1.5 ml overnight culture in 50 ml YPD media. Grow 4 hrs at 30 deg. This works well for strains with near wild type growth rates. If strains grow substantially slower, inoculate with more cells or let cells grow longer in this step. *Aiming for A600 ~ 0.5-1.0 but this is rarely measured.*

3. Spin down 40 ml cells. Resuspend in 1 ml sterile water. Transfer to sterile eppendorf tube. Wash cells 3x with 1 ml TE/LiOAc solution. This solution is stable for years so no need to make fresh.

Depending on the purpose of the transformation, cells can be resuspended in different volumes of TE/LiOAc. The transformation works best if more cells are used, but the tradeoff is that some selective media have a high background and if too many cells are plated, it's difficult to pick the transformants from the background.

For integration of linear DNA into the chromosome (lower efficiency with high background) we suggest resuspending the washed cells in 1.5 ml TE/LiOAc.

One can use more cells if transforming with self-replicating plasmids where the transformation has higher efficiency and low background (e.g., resuspend washed cells in 300-500 microliters TE/LiOAc).

4. Set up the transformation in sterile eppendorf tubes containing:

- DNA (0.05 - 0.1 microgram purified plasmid DNA) or (~ 1/5 of DNA from a PCR reaction where linear DNA has been amplified for chromosomal DNA integration). *Note, DNA from PCR reactions should be purified by EtOH precipitation or some other means before yeast transformation.*
- 10 microliters single strand salmon sperm DNA. Heat this DNA to 90 deg for a few min before adding. Let the tubes cool for a min or so before adding the cells to the transformation tubes.
- 50 microliters washed competent cells from above
- 300 microliters PEG/LioAC/TE

Mix well and incubate 30 deg for 45 min.

Add 40 microliters DMSO. Mix well and incubate at 42 deg for 20 min with shaking in a heat block.

Spin cells for a ~5 sec. Wash 1x with 1 ml sterile H2O. Use a pipetman to mix cells as the pellets are difficult to resuspend by vortexing alone.

Resuspend cells in 300 microliters H2O. For chromosomal integration, plate 100 microliters on each of three plates. For plasmid transformation, plate 100 microliters to one plate.

Incubate plates for 2-3 days at the permissive temperature.

100 ml TE/LioAc
1 ml 100x TE, pH 7.5
10 ml 1M LiOAc
Autoclave or filter sterilize

100 ml PEG/TE/LiOAc
40 g PEG 4000
1 ml 100x TE, pH 7.5
10 ml 1M LiOAc
H2O to 100 ml final
Filter sterilize.

Adapted from:
Gietz et al, (1992) Nuc Acids Res. 20, 1425.
Schiestl, R.H. and Gietz, D. (1989) Curr. Genet. 16, 339-346
Hill et al, (1991) Nuc. Acids Res. 19, 5791