Protocol: One-Step Plasmid Transformation of Yeast

This low-efficiency transformation protocol is routinely used for the transformation of yeast cells with plasmid DNA. Before starting, ensure that you have a healthy culture of yeast, a miniprep of sufficient concentration (>100 ng/μl) of the plasmid, One-Step Buffer, and solid agar media for the appropriate selection.

Protocol:

1. Grow liquid culture of strains for transformation overnight.
2. Centrifuge 1 ml of overnight culture at 6,000 x g for 30 seconds.
3. Aspirate or pipette off the media supernatant.
4. To the pellet of yeast cells, add:
    a. 100 μl of One-Step Buffer
    b. 10 μl of freshly denatured salmon sperm DNA*
    c. 2 μl of miniprepped plasmid DNA (from Qiagen Miniprep)
5. Mix by stirring with the tip of a pipette. The One-Step Buffer is goopy, pipetting and vortexing are not recommended, but do resuspend the cells well by stirring.
6. Incubate at 43°C for 30 minutes.
7. Following the incubation, centrifuge cells at 6,000 x g for 30 seconds.
8. Pipette off the transformation solution. Resuspend the cell pellet in 200 μl of sterile water and plate immediately onto selective solid agar media.

* To denature salmon sperm DNA, aliquot a small volume (in slight excess of what is required for your transformations) into a PCR tube and incubate for 98°C on the PCR machine for 5 minutes. Following the incubation, return the salmon sperm DNA immediately to ice to chill.

Recipe for One-Step Buffer:

Stock Solution	Volume	Final Concentration
50% Polyethylene glycol 3350 (PEG_{3350})	8 ml	40%
2M Lithium Acetate (LiOAc)	1 ml	200 mM
1M Dithiothreitol (DTT)	1 ml	100 mM

One-Step Buffer is stored at -20°C in 1 ml aliquots. ***Do not re-use or re-freeze One-Step Buffer***; simply dispose of the aliquot when done.

Note on yeast plasmids:

The common yeast plasmids share a nomenclature that describes the features of the plasmid. For example, the plasmid pRS416 is a new series plasmid that contains a centromere for stable transformation and the URA3 gene for selection.

First Number (MCS)	Second Number	Third Number (Auxotrophy)
There are two versions of the multiple cloning site: The 300 series is older. The 400 series is newer.	This describes the expected plasmid copy number: 0: For genomic integration 1: Contains centromere for low copy stable transformation 2: Contains a 2μ ORI for multi-copy over-expression	This describes the selection to be used: 3: HIS (histidine) 4: TRP (tryptophan) 5: LEU (leucine) 6: URA (uracil)