Transformation of Yeast
(This protocol is taken from Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual, 2005 Edition)

1. Inoculate 5 ml of liquid YPD or 10 ml of SC and incubate with shaking overnight at 30C.
2. Count overnight culture and inoculate 50ml of YPD to a cell density of 5 X 10^6 cells/ml of culture.
3. Incubate the culture at 30C on a shaker at 200 rpm until it is at 2 x 10^7 cell/ml. This typically takes 3-5 hours. This culture will give sufficient cells for ten transformations.

   Notes:

   i. It is important to allow the cells to complete at least two divisions.
   ii. Transformation efficiency remains constant for three to four divisions.
4. Harvest the culture in a sterile 50-ml centrifuge tube at 3000g (2500 rpm) for 5 minutes.
5. Pour off the medium, resuspend the cells in 25 ml of sterile H2O, and centrifuge again.
6. Pour off the H2O, resuspend cells in 1.0 ml of 100mM lithium acetate (LiAc), and transfer the suspension to a sterile 1.5-ml microfuge tube.
7. Pellet the cells at top speed for 5 seconds and remove the LiAc with a micropipette.
8. Resuspend the cells to a final volume of 500 μl (2 x 10^9 cells/ml), which is about 400 ml of 100 mM LiAc.

   Note: If the cell titer of the culture is grater than 2 x 10^7 cell/ml, the volume of the LiAc should be increased to maintain the titer of this suspension at 2 x 10^9 cells/ml. If the titer of the culture is less than 2 x 10^7 cells/ml, decrease the amount of LiAc.
9. Boil a 1.0-ml sample of single-stranded carrier DNA for 5 minutes and quickly chill in ice water.

   Note: It is not necessary or desirable to boil the carrier DNA every time. Keep a small aliquot in your freezer box and boil after three or four freeze/thaws.
10. Vortex the cell suspension and pipette 50-μl samples into labeled microfuge tubes. Pellet the cells and remove the LiAc with a micropipette.
11. The basic “transformation mix” consists of the following ingredients; carefully add them ***in the order listed***:

    240 μl of PEG (50% w/v)
    36 μl of 1.0M LiAc
    25 μl of single-stranded carrier DNA (2.0 mg/ml)
    50 μL of H2O and plasmid DNA (0/1-10μg)

    Note. The order is important here. The PEG, which shields the cells from the detrimental effects of the high concentration of LiAc, should go in first.
12. Vortex each tube vigorously until the cell pellet has been completely mixed. This usually takes 1 minute.
13. Incubate for 30 minutes at 30C.
14. Heat shock for 20-25 minutes at 42C.

Note: The optimum time can vary for different yeast strains. Test this if you need high efficiency from your transformations.

15. Microfuge at 6000-8000 rpm for 15 seconds and remove the transformation mix with micropipette.
16. Pipette 0.2-1.0 ml of sterile H2O into each tube and resuspend the pellet by pipetting it up and down gently.
17. Plate from 200-μl aliquots of the transformation mix onto selective plates.