High Efficiency Yeast Library Transformation Using RNA as a Carrier

1. Grow the recipient strain of yeast to mid-log ($1x10^7$). The better the media used the better the transformation efficiency, so use **YPD** when possible.

2. Pellet the cells and resuspend the pellets in **LiTE** (100mM LiAc in TE), the volume is not critical because this is a wash step.

3. Pellet the cells once more and this time resuspend the cells in 5ml of **LiSORB** for every 200ml of culture (LiSORB is LiTE containing 1M Sorbitol).

4. Incubate the cells for 15-30 minutes at 30°C with shaking. (Try not to go too long, cells begin to strarve and it decreases their survival)

5. Pellet the cells as above and resuspend in 500µl of **LiSORB** per 200ml of culture.
(I usually do large scale transformations with a liter of cells so the final volume is 2.5 mls)

6. After removing 100µl of cells for a negative control, add 2µg of **transforming DNA** and 200µg of **Yeast total RNA** for every 100µl of cells remaining. (50 ug DNA and 5 mg of total RNA carrier for 1 liters worth of cells)

7. Mix well then incubate for 10' at 30°C without shaking.

8. Add 900µl of **LiPEG** (LiTE containing 40% PEG 3350) for each 100µl of cells and mix well. This is 22.5 ml for the large scale. I usually do this in 125 ml or 250 ml flasks. This aids the heat shock step later.

9. Heat shock the tubes or flask the flask in a 42°C water bath for 12 minutes.

10. At this point cells can be plated out to check the transformation frequency. 5 ul on a plate should give 1000 or more transformants with a good transforming strain. However, in order to plate a large number of cells per plate, we generally take our transformation mixture and add it to 500 mls of dropout minimal media for an initial 1 liter culture and allow it to recover at 30°C for 4 hours. At this point cells have been established as transformants. They can be pelleted, concentrated, and frozen at -70°C in 9% DMSO and stored for future use. Storing small aliquots allows you to thaw them and plate them out later at an appropriate density for whatever purpose you might desire in the future. For activation domain transformations we recover in leu, trp, his triple dropout media. It is important not to have extra his around during plating since it interferes with the 3-AT selection. We have been successful plating directly from recovered or from frozen cells.

Our experience has taught us not try to pellet the cells through the PEG since this lowers the transformation frequency, and not to wait too long to plate or grow up the transformants since the PEG is toxic to the cells. However, everything will work to some degree so try any variation on this protocol that might save you time.