2 hybrid yeast transformation (Gietz lab protocol)

1. Inoculate the yeast strain containing the first plasmid into the appropriate volume of the appropriate SC-omission medium in a flask and incubate at 30 ^{o}C overnight.

TRAFOR SCALE	10 X	30 X	60 X
Culture Size	25 mls	50mls	100 mls

2. Determine the cell titer and calculate the volume of cells that yields 2.5 x 10^8 cells for each 50 mls of YPAD culture needed

TRAFOR SCALE	10 X	30 X	60 X
YPAD culture Size	50 mls	150 mls	300 mls
# of Cells needed	2.5 x 10^8	7.5 x 10^8	1.5 x 10^9

3. Pour this culture volume into an appropriate sterile centrifuge tube and pellet the cells at 3000 x g for 5 min. Resuspend the cell pellet in the appropriate volume of pre-warmed (30^{o} C) YPAD and transfer to another sterile culture flask.

TRAFOR SCALE	10 X	30 X	60 X
YPAD culture Size	50 mls	150 mls	300 mls

4. Incubate at 30^{o} C while shaking at 200 rpm for 3 to 4 hrs until the cell titer reaches 2 x 10^7 cells/ml.
5. Harvest the cells by centrifugation at 3000 x g for 5 min.
6. Wash the cell pellet via resuspension with 1/2 volume of sdd water and collection by centrifugation as above.
7. Resuspend the pellet in the appropriate volume of 100 mM sterile LiAc and transfer to an appropriate centrifuge tube. Incubate for 15 min at 30^{o}C. Pellet the cells again by centrifugation and remove the supernatant.

TRAFOR SCALE	10 X	30 X	60 X
100 mM LiAc	3 mls	3 mls	6 mls

8. Add, in the order from top to bottom, the components of the transformation mix listed in the table below to a seperate tube and mix thoroughly by vortexing. Add the transformation mix to the cell pellet and vortex vigorously to resuspend the cell pellet. **Alternatively** you may also mix all components but the plasmid DNA

together. Add the entire volume of the transformation mix minus the plasmid DNA to the cell pellet and then add the plasmid DNA and mix. This will keep you from loosing any plasmid DNA when transfering the viscous liquid of the transformation mix on top of the cells.

TRAFOR SCALE	10 X	30 X	60 X
50% PEG	2.4 ml	7.20 ml	14.40 ml
1.0 M LiAc	360 µl	1.08 ml	2.16 ml
SS-DNA (2 mg/ml)	500 µl	1.50 ml	3.00 ml
Library plasmid DNA	A µl	B µl	C µl
sdd Water	340 - A µl	1.02 - B ml	2.04 - C ml

Please note:

The values for each scale up should be multiplied from the single reaction volumes. Previously the 60X scale up values for the LiAc, SS-DNA, and Plasmid DNA were NOT correct! (They were 90X scale, sorry) The numbers shown here are NOW correct! Thanks to the person that caught my error and sorry to all of you battling to get good 2HS screens done. In addition, Please note that we are now adding 2X the amount of carrier than in previous versions of this this page.

TIP:

i. The standard transformation reaction can be scaled up to 120 X a standard transformation reaction, however we rarely need to go to this scale.

ii. Use a a plastic pipet rather than a glass pipet to transfer the PEG solution as it adheres to the surface of glass pipets and hampers the delivery of an exact volume.

iii. The volume of sdd water and plasmid DNA may be adjusted, however, the total volume of these components must remain constant.

9. Vigourously vortex the cell pellet until it is totally resuspended, which should take about 1 min. If you have problems getting the pellet resusupended let it sit for 5 min and then vortex!
10. Incubate the transformation mix at 30^{o} C for 30 min.
11. Heat shock at 42^{o} C for time indicated by table below with mixing by inversion for 15 sec after every 5 min.

TRAFOR SCALE	10 X	30 X	60 X
Heat shock Time	30 min	40 min	45-60 min

TIP:

Heat shock of large scale transformations require the culture tube to be inverted several times every 5 minutes to equilibrate the temperature quickly in the larger volume.

12. Collect the cells by centrifugation as above. Gently resuspend the cell pellet in an appropriate volume of sdd water and plate onto SC ommission medium. For our **30 X** and **60 X** 2 hybrid screens we plate onto **100 large plates**. **(Yes! that is correct! A whole case of large plates)** This gives better transformation and library coverage! For the 10X screens we used less.

TRAFOR SCALE	10 X	30 X	60 X
Resuspension Volume	10 mls	40 mls	40 mls

TIP:

Transformations for the two-hybrid system which use the activation of the *HIS3* gene for genetic selection can be plated directly onto SC omission medium lacking Tryptophan, Leucine, and Histidine (Trp, Leu, His). The total number of transformants screened should be calculated by plating of a small aliquot (1- 2 µl) onto a pair of SC omission medium lacking Trp-Leu plates.

13. Incubate the plates for 3 - 5 days at 30^{o} C or until colonies appear. For some two hybrid Screen we wait as long as 14 days!