BISHOP LAB (Modified from Red Book Protocol)
YEAST TRANSFORMATION
This protocol is optimized for 8 transformations. Adjust accordingly.

PREPARING COMPETENT YEAST

1. On day before transformations, set up overnight culture by inoculating a single colony from a fresh plate into 5mL YPDA
2. A few hours before doing transformations, inoculate 195mL YPDA with entire 5mL overnight culture (i.e. 1/40 dilution)
3. Grow cells at 30ºC until OD_{600} 0.1-0.2 – this will take 2-4 hours depending on how your specific strain is growing
   Note: Overgrowth will significantly decrease transformation efficiency!
4. Spin down cells at 3000rpm for 3 min at room temperature
5. Resuspend pellet in 50mL sterile H_2O and transfer cells to sterile 50mL conical tube
6. Spin as in step 4
7. Resuspend pellet in 5mL 0.1M LiAc
8. Spin as in step 4
9. Resuspend pellet in 400μL 0.1M LiAc (enough for 8 transformations)

TRANSFORMATIONS

1. Aliquot 50μL yeast per transformation tube
2. Add 50μL 2mg/mL carrier DNA (salmon sperm or similar)
3. Add transformation DNA and mix well by pipeting up and down
   Note: Set up a negative control tube using only water.
4. Add 600μL PEG/LiAc and mix well by pipeting up and down
   Note: PEG mix is viscous so be sure to pipet slowly in order to get all of PEG into the tube and well mixed with the yeast.
5. Incubate at 30ºC for 30 minutes (water bath is preferable)
6. Add 70μL DMSO
7. Heat shock immediately by incubating at 42ºC for 15 minutes (water bath is preferable)
   Note: Set a timer! If incubated for much longer than 15 minutes at 42ºC, yeast will die from heat shock.
8. Spin tubes at maximum speed in a microcentrifuge for 1 minute at room temperature
9. Resuspend in 100-400μL sterile H_20
10. Plate 100-200μL cells per selective plate
11. Incubate at 30ºC for two days

YPDA
10g yeast extract
20g bacto peptone
10mL 0.1% adenine

Dissolve in 1L H_20
Adjust pH to 7 with NaOH
Autoclave
Add 50mL sterile 40% glucose per liter

PEG/LiAc Mix
77.4mL 50% PEG (3350)
11mL 1M LiAc
11.6mL ddH_20