

## **Transforming chemically competent cells**

1. Thaw cells on ice [DH5 $\alpha$  and Top10 cell lines are used for DNA amplification purposes. BL21(DE3) cell line is used for protein expression.]
2. Add DNA, gently mix (use 1  $\mu$ l – 2  $\mu$ l of prepped plasmid).
3. Let sit for 30 minutes on ice.
  - Note: If you are in a rush, you can shorten this incubation time to 5-10 min.
4. Incubate cells for 30 seconds at 42 °C.
5. Incubate cells on ice for 2 min.
6. Add 1 mL SOC (2XYT and LB are also suitable) at room temp.
7. Incubate for at least 1 hour in 37°C shaker. This can be extended up to 2 hours.
8. Spread 100-300  $\mu$ l onto a plate made with appropriate antibiotic. You can also make a “low” plate (using 50  $\mu$ l of culture) if you are worried about getting too many colonies.
9. Incubate plates overnight at 37 °C.
10. Save the rest of the transformed cells in liquid culture at 4 °C or on your bench. If nothing appears on your plate, you can try plating a larger volume of cells.

### **SOC Medium**

Add 20 ml of sterile 1 M glucose per liter of SOB medium immediately before use.  
Or make frozen aliquots and store at -20 °C.

### **SOB Medium**

1. Measure ~900ml of distilled H<sub>2</sub>O
2. Add 20g Bacto Tryptone
3. Add 5g Bacto Yeast Extract
4. Add 2ml of 5M NaCl
5. Add 2.5ml of 1M KCl
6. Add 10ml of 1M MgCl<sub>2</sub>
7. Add 10ml of 1M MgSO<sub>4</sub>
8. Adjust pH to 7.0 with 10N NaOH and adjust volume to 1 L with distilled H<sub>2</sub>O.
9. Autoclave to sterilize on LIQUID cycle 15 minutes.

### **2xYT Medium**

1. Measure ~900ml of distilled H<sub>2</sub>O.
2. Add 16g Bacto Tryptone.
3. Add 10g Bacto Yeast Extract.
4. Add 5g NaCl.
5. Adjust pH to 7.0 with 5N NaOH.
6. Adjust to 1L with distilled H<sub>2</sub>O.
7. Sterilize by autoclaving on LIQUID cycle.

## **LB (Luria-Bertani) Broth Recipe**

10 grams tryptone

5 grams of yeast extract

10 grams of NaCl

(NOTE: A premixed powder is also available from Fisher cat# BP1426)

1. Dissolve in 1 L of water.
2. Portion into flasks and cover with aluminum foil. Fill the flask to half its volume.
3. Autoclave 20-30 minutes on LIQUID cycle.