

## **Bacterial Transformation**

1. Turn on the 42 degree water bath
2. aliquot 10-50 ng of DNA into chilled(on ice) eppendorf tubes
3. get competent cells from -70 freezer and thaw on ice (approx 10 min)
4. aliquot 300  $\mu$ L of competent cells into each tube
5. mix by tapping gently
6. incubate on ice for 30 min.
7. heat shock at 42 degrees for 45 sec.
8. place tubes on ice for 2 minutes
9. add 700  $\mu$ L of LB media (non-selective / no amp) and allow cells to recover for 1 hour at 37 degrees.
10. spin down briefly (top speed 10 seconds)
11. decant 500  $\mu$ L LB and resuspend the pellet in the remaining solution
12. plate on selective media with positive and negative controls.  
for example: amp plasmid plated on LB amp, no amp, and no plasmid on LB amp.
13. incubate at 37 degrees overnight.

### Procedure for Transformation from TA cloning KIT

1. Centrifuge the vials LB containing the ligation reactions briefly and place them on ice.
2. Pipette 2  $\mu$ l of each ligation reaction directly into the competent cells **DH5** and mix by stirring gently with the pipette tip. Incubate the vials on ice for 30 minutes. Store the remaining ligation mixtures at -20°C.
3. Heat shock for exactly 30 seconds in the 42°C water bath. Do not mix or shake. Remove the vials from the 42°C water bath and place on ice for 2 minutes.
4. Add 800  $\mu$ l of LB medium (at room temperature) to each tube.
5. Shake the vials horizontally at 37°C for 1 hour at 225 rpm in a rotary shaking incubator. Place the vials with the transformed cells on ice.
6. Spread 1/4 cells and 3/4 cells from each transformation vial on separate, labeled LB agar plates containing 50  $\mu$ g/ml of ampicillin and 50  $\mu$ l 20mg/ml x-gal and 20  $\mu$ l 100m MIPTG.
7. Make sure the liquid is absorbed, then invert the plates and place them in a 37°C incubator for at least 18 hours. Plates should then be shifted to +4°C for 2-3 hours to allow for proper color development.

### IMPORTANT

Transformed INV F' cells may appear very small after overnight growth when compared to other *E. coli* strains. The transformants may need to grow an additional 2-3 more hours before selecting colonies for analysis.

### Results of Transformation

For an insert size of 400-700 bp, you should obtain 50-200 colonies per plate depending on the volume plated and of these, approximately 80% should be white on X-Gal plates or X-Gal/IPTG plates. Note that ligation efficiency depends on insert size. As insert size increases, the efficiency will decrease.