

Bacterial Transformation

Last updated: December 10, 2010

Description: This procedure takes recently ligated plasmids containing our DNA constructs and injects (transforms) them in XL-1 bacteria by heat shock. You can also re-transform already prepped DNA.

Lab time: ~3 hours

1. Take XL-1 bacterial cells out of the -80C freezer (one for each ligation reaction)
 - a. Label each tube
 - b. Ice thaw for 10 minutes
2. Add 5ul of ligation reaction (only 0.5 ul if using already prepped DNA)
3. Ice for 30-45min
 - a. Pre-fill 42C dry bath holes with water (make sure water is fully heated before next step)
4. Heat shock at 42C for 45sec
5. Ice for 5 min.
 - a. Pre-fill 37C dry bath holes with water
6. Add 250-300ul of sterile LB media (no antibiotics)
*Note: Sterility is important. Flame top and cap of LB bottle/tube before and after. Use sterile pipette tips with pipette gun.
7. Incubate tubes at 37C for 1hr in dry bath
8. Plate onto a labeled dish
*Note: Just pour bacteria from tubes into dish. Then use sterile hockey puck (flamed with ethanol) to spread bacteria around dish. Be sure NOT to scorch bacteria by going immediately from the flame to the bacteria. Allow hockey puck to cool on the agar beside the bacteria for about 15 seconds before spreading.
9. Place dishes in the 37C incubator overnight.