

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 μ 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 μ 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 μ 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.