

E. coli Transformation

1. Get an ice bucket
2. Thaw as many aliquots of *E. coli* as necessary on ice. Nova blue cells are for DNA work, and can be found at the bottom left of the first room's -80. RIPL cells are for protein expression work.
3. Put a transformation tube on ice
4. Put 4 uL of plasmid or ligation reaction at the bottom of the transformation tube, and let them chill on ice for a bit.
5. Once thawed, add 50 uL of *E. coli* to the transformation tube.
6. Leave on ice for 20 minutes
7. heat shock at 42 C for 45 seconds (30 sec on 42 C)- try if 45 doesn't work.
8. Return to ice for 5 minutes (ice for 2 min)
9. Add 200 uL of Lb to the transformation tube and shake for 1 hour. ~~Make an ethanol cloud before opening the LB container~~
10. Halfway through the incubation, get out a plate with the appropriate antibiotic and place it in the 37 C incubator to warm up
11. Pippette 150 uL of cells onto the plate after the 1 hour incubation, and spread with an L spreader or spreader beads
12. Incubate overnight at 37 C

3000 rpm for 5 min- spin down the culture,
leave 100 ul at the bottom and resuspend
purity and concentration