

Transformation Protocol:

1. Use pre-prepped competent *E. coli* cells (usually DHF α λ pir). Stocks are in the minus 80° freezer. Thaw cells on ice for ~ 30 mins.
2. Add 5-10 μ l of ligation mix to thawed cells, mix gently, and incubate on ice for at least 30 minutes. While you're waiting, pre-heat a hot block (with added water) to 42°C.
3. Remove the tubes from the ice and immediately put them in the 42° hot block for 90 seconds. Return the tubes to ice and incubate for ~ 5 mins.
4. Add 0.5 ml of LB medium to the cells, transfer to a sterile falcon tube, and incubate at 37° (with shaking) for one hour.
5. Plate the entire culture (should be ~700 μ l) on LB (or BHI) plates containing the appropriate antibiotic (50-100 μ l per plate).
6. Incubate overnight at 37°C. The next morning, pick resultant colonies, make freezer stocks, and make a plasmid prep (for PCR and restriction enzyme confirmation that the strain has picked up the correct plasmid).