DNA Transformation into *E. Coli*

1. Thaw aliquot of chemically competent *E. coli* (DH5α, Top10, DH10B, XL1-Blue, XL10-Gold, etc.) on ice for 10-15 minutes.

2. Chill your DNA or ligation mixes on ice for a few minutes.

3. Add ~2-3 µl of DNA or ligation mix (use a smaller volume if you are using supercoiled plasmid DNA) to a sterile chilled eppendorf tube.

4. Add 40-50 µl of competent E. coli to the DNA. Pipet up and down gently or briefly tap tube. DO NOT VORTEX! Let sit on ice for 15-30 minutes.

5. Heat shock at 42˚C for 45 seconds.

6. Return to ice for 1-2 minutes.

7. Add 0.25 ml of LB or SOC media (without antibiotics). Shake for 30 minutes-1 hour at 37˚C. I often do this in the eppendorf tubes in a thermomixer, but others prefer to transfer the DNA and media to a 14 ml Falcon tube and use a regular bacterial shaker. Some manufacturers of competent bacteria recommend the latter for the highest efficiency.

8. Plate bacteria on pre-warmed LB+antibiotic plates. Typically we use:

   LB+Ampicillin (50 µg/ml)- green stripe
   LB+Kanamycin (50 µg/ml)- blue stripe

   If you are going to also use blue/white selection, prepare plates by spreading 100 µl of 100mM IPTG and 20 µl of 50 mg/ml Xgal on the plates and letting these dry for AT LEAST 30 minutes before spreading on the bacteria.

   For routine cloning, spread the entire mix of bacteria, DNA, and media. For supercoiled plasmid DNA transformation, spread only a small amount (50 µl or less).

   Invert plates and incubate at 37˚C for ~16-18 hours. Do not let the plates incubate too long as satellite colonies may form. It's better to let the plates grow longer at cooler temperatures (i.e. room temp) than to let them overgrow.