DNA Restriction:

1. Pre-heat PCR machine to 37 C
2. Fill wells of PCR machine with H2O
3. Thaw cutsmart buffer on ice
4. Map out desired cuts on a plasmid map with measurements
5. On ice, pipet 16 uL plasmid DNA, 2 uL cutsmart buffer, 1 uL restriction enzyme A, 1 uL restriction enzyme B (or 17 uL plasmid if only one cut-site) into a .5 mL PCR tube
6. Repeat for the secondary gene of interest (one backbone + one gene of interest)
7. Vortex both tubes
8. Incubate all reactions at 37 C for 2 hours in the PCR machine
9. With ~30 minutes left, prepare a gel electrophoresis gel
10. Run samples on gel electrophoresis with ladder (keep at least one well between different DNA)
11. Isolate and purify gel bands
12. Freeze or use for ligation

DNA Ligation:

1. Thaw T4-Ligase buffer on ice
2. While on ice, dispense cut 17.5 uL backbone DNA, 17.5 uL cut gene of interest, 4 uL T4-Ligase buffer, and 1 uL DNA ligase into a .5 mL PCR tube
3. Vortex contents and label tubes
4. Incubate at 16 C overnight (minimum of 30 minutes should suffice with minimal efficiency)
5. Proceed to transformation into bacterial chassis