**Items needed**

1 - DNA Plasmid

1- Plate of *E. coli* K12 derived DH5a or DH10B or BL21 bacteria

2 - LB Agar plates

5mL Transformation buffer (10% PEG 8000, 5% DMSO, 25mM CaCl2)

18g LB Amp Agar or LB Kan Agar

20 plates

250mL glass jar

10 inoculation loops

50 - 1.5mL microfuge tubes

10 Dropper pipettes

Restreak the DH5a, DH10B or BL21 cells every 1-2 months. Fresh cells will provide better transformation efficiency.

18g of LB Agar should pour ~20 plates from 500mL

If you have a centrifuge start at (1) otherwise start at (8)

1. Grow up 5mL overnight culture in LB and the appropriate antibiotic
2. Spin down at ~3000rpm
3. Decant.dump out LB supernatant
4. Resuspend cells in 500uL to 1mL of Bacterial Transformation Buffer
5. Spin down cells at ~3000RPM
6. Decant.dump out transformation buffer supernatant
7. Add 100uL transformation buffer to the cell pellet and gently resuspend them
8. If you do not have access to a centrifuge using an inoculation loop scrape enough bacteria to almost fill the loop and mix it into 100uL transformation buffer.
9. Add 500ng+ of plasmid to the microfuge tube containing the transformation buffer and place at 4C or in the fridge for 30 minutes.
10. “Heat Shock” the bacteria by placing them in water that is ~42C for 30 seconds and then let them sit at 37C(room temperature is fine if you don’t have a 37C incubator) for 1-3 hours (in this step the bacteria that have accepted the DNA will produce antibiotic resistant proteins for selection and replicate hopefully creating more bacteria with the plasmid)
11. Take 100uL of your transformation and put it on a LB agar plate with the correct antibiotic to select for your plasmid
12. Incubate overnight at 37C or ~24 hour at room temperature(RT)(if using the pVIB.pJE202 bioluminescent plasmid incubate only at RT)