## **Sample Collection**

- Transform a culture using the isolated plasmid that has been sequenced.
- Transfer culture into a 10 ml tube and allow it to grow for 2 hours
- Fill 37 10 ml tubes with 4 ml of LB media and ampicillin
- Add inducers according to table
- Inoculate 36 of the tubes with the DH5 $\alpha$ Pro cells and the last with TOP10 cells. NOTE: This construct needs to be remade in between the 3 and 6 hour marker so that the culture can be transferred to fresh media after the 6 hour samples are taken. This prevents the cells from entering the stationary phase
- Samples are taken every 3 hours for a 12 hour time period.
- Samples are collected based on OD about 250 µl for a low OD or 100 µl for a high OD
- Spin cells at 5K rpm for 5 minutes
- Discard the supernatant, then re-suspend in approximately 1 ml of PBS.
- Spin cells at 5000 RPM for 5 minutes
- Discard supernatant and re-suspend in Paraformaldahyde
- Incubate culture at 25 °C for 30 minutes
- Spin at 5000 RPM for 5 minutes and discard supernatant
- Re-suspend in PBS and refrigerate at 4 °C
- Take measurements of relative green florescent protein using a FACSCalibur