# 6/10/2015

### Aims for today:

- 1. Miniprep pDawn
- 2. Design SapI primers
- 3. Design Compatibility Systems
- 4. Pick Colonies for Biobrick, inoculate, glycerol stock
- 5. Colony PCR, pDawn + LacZ, Glycerol stock, miniprep
- 6. Redigest pDawn (with extreme controls)
- 7. Reattempt Ligation of pDawn+LacZ
- 8. Colony PCR of BioBrick Transformation
- 9. Run gel of colony PCR products
- 10. Run gel of newly digested pDawn, Overnight ligation.

## Accomplishments:

- 1. Miniprep pDawn
- 2. Re-digest of pDawn (with extreme controls)
- 3. Re-attempt Ligation of pDawn+LacZ
- 4. Colony PCR of pDawn + LacZ
- 5. Gel extract of newly digested pDawn
- 6. Transformed lux box BioBrick, pDawn + lacZ, just pDawn backbone (for miniprep and
- 7. making our own glycerol stock) into NEB turbo again [had just cut pDawn backbone and just digested lacZ insert transformed as well as controls]
- 8. Plated transformed colonies and placed into incubator (will look at results tomorrow)

## Aims for tomorrow:

- 1. Run gels of colony PCR from 6/10/15 and see if the lacZ was ligated into pDawn backbone
- 2. Finish designing cloning of luxbox biobrick into pACBB-eGFP (plasmid that is compatible with pDawn)
- 3. Pick colonies for most recent BioBrick Transformation, Inoculate, glycerol stock, miniprep
- 4. Colony PCR of new pDawn + LacZ transformation
- 5. Induce luxbox transformed E. coli with arabinose (growing up transformed E. coli to saturation, back diluting 1:100, growing up to OD600 of 0.3, then inducing with low to high arabinose concentrations)
- 6. If transformation results not looking good, we will need to re-digest lacZ insert and pDawn, and re-ligate pDawn into lacZ

## **Questions:**

- 1. How exactly does the two-plasmid transformation work? Do we transform the NEB turbo with one and then make it competent again and transform it with the second?
- 2. Is there any way we can check for incomplete digestion (because the gel resolution doesn't allow us to see that, since the part we cut out is only a few bases big)?