**Week 5: Testing Phenotype of Cloned DNA**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Properly manipulate bacterial cultures to maintain clonality of cells.
* Quantify red fluorescent protein levels in populations of *E. coli* cells.
* Enter your results into DNA Registry.

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Describe the big idea of information based on lab experiences.
* Review the information contained within promoters and RBSs.
* Use protocols for molecular biology to clone DNA.
* Interpret Synergy data for fluorescence and optical density.
* Design experiment to confirm cloned DNA was successful.

**Pre-Lab**

Before you come to lab

1) At 4:30 pm on the Wednesday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Wall 325). Make sure to bring your protocol from last week of how you plan on testing the function of your DNA control element.

2) Predict what you will see from your cells with the experimental treatment.

3) Answer each of these four questions in two sentences or less.

A) Are promoters more like on/off switches, or rheostats?

B) Design an experiment to confirm you successfully cloned your DNA control element into the plasmid. (not a question, but a challenge)

C) How will you calculate the level of fluorescence of your cells to take into account that different treatments may produce different densities of cells?

D) What would happen if a weak RBS were transcribed into mRNA?

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**NOTE:** At 4:30 pm on the Wednesday before lab this week, one person from each lab group MUST COME TO Dr. C’s research lab (Wall 325). Please be on time. We need to start your transformed cells growing +/- any treatment conditions.

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**Information: Determine Phenotype from your Transformed *E. coli***

In Lab:

1) Yesterday, one person from your group started multiple overnight cultures of transformed *E. coli* so that your lab group can determine the phenotype of each strain of cells (different genotypes). The P sample contained part J04450 from the Registry.

**(Start lab at this point)**

2) Take photos of your plates with colonies from last week, and your tubes of cells that have grown overnight. These photos can be useful in your presentations a few weeks from now.

3) Confirm successful cloning of DNA control element. Start PCR by putting 23 µL of the green master mix with 2 µL of the overnight cultures for the DNA template. Each group will be supplied with tubes containing everything except the template. Templates should include negative control (N), and three experimental strains (X1, X2, X3) as well as the known strong and known weak standards\* provided to each group (but not pClone). You will run a gel next week to determine the size of your PCR products.

4) Try loading a practice gel so you will be more experienced when loading the real gel next week with your PCR products from today.

5) Make a glycerol stock of your three eXperimental clones by mixing in sterile 1.5 mL tubes, 75 µL of sterile glycerol with 425 µL of your overnight cultures. This will give you a 15% glycerol stock that we can store at -80° C; glycerol prevents lethal ice crystals from forming inside the cells. To pipet glycerol, microwave it for 30 seconds to reduce viscosity; pipet from the top.

6) Use the Synergy machine and a 96-well plate to measure how much RFP each strain of cells is producing, and cell density. Be sure to include wells of the appropriate control strains. Each well should contain 200 µL of liquid.

7) Use Excel to quantify the amount of RFP fluorescence/number of cells in each population. Calculate 3 separate ratios, then average the ratios and produce standard deviation (STDEV) and then standard error (STDEV/SQRT(n)); n = sample size. You will generate a graph with these data and upload a screenshot of the graph for use on the Registry under the “experience” tab.

7) Enter your results into the [Registry of DNA parts](http://parts.igem.org/cgi/partsdb/pgroup.cgi?pgroup=lab&group=Campbell%20M%20Lab), including a graph for your new promoter. Search for your part number and log in to make changes. The wiki code to center an image and limit its size to 500 pixels is:

<center>

[[File:name\_no\_spaces.png|500px]]

</center>

**\*Strong and Weak Standards**

rClone

J100321 rClone Red with strongest basic RBS (RR4) = 67.9

J100323 rClone Red with very weak simple RBS (BI) = 8.5

actClone

Part J100309 contains wildtype *ompC* promoter

Part J100314 contains scrambled version of wt promoter

repClone

Part J100306 contains wildtype *Ptet* promoter

Part J100312 contains scrambled version of wt promoter

P positive control

J04450