**Week 10: Testing Phenotype of Promoter v2**

Learning Objectives for Promoter 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 Monday 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 promoter.

2) Predict what you will see from your cells with the experimental promoter v2.

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

A) What will the Synergy machine measure for this week’s experiment?

B) How can you account for different cell densities with regards to fluorescence?

C) What would you like to know about your V1 and V2 promoters that PCR cannot detect?

D) Is it possible your DNA worked as expected but you do not see with your eyes any RFP? Explain your answer.

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**NOTE:** At 4:30 pm on the Monday 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:

3) Monday, 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).

**(Start lab at this point)**

4) Take photos of your 3 plates with colonies from last week, and your cells that have grown overnight. These photos can be useful in your final written lab report.

3) Spot 2 µL of all 3 eXperimental culture tubes onto a single LB amp plate to save the cells for next week. Be sure to match the plate labels (on bottom) with the tube labels.

5) Use the Synergy machine and a 96-well plate to measure how much fluorescence each strain of cells is producing, and cell density. Be sure to include wells of the appropriate control strains.

6) Use Excel to quantify the amount of fluorescence/number of cells in each population. 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 DNA control element. 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>

8) One person from each group will need to start your experimental cells strains growing at 4:30 pm this FRIDAY April 3. Come to Dr. C’s research lab (Wall 325) on time.