**Week 3: Testing Phenotype of Cloned DNA**

Learning Objectives for Promoter Discovery

*Skills*

* Apply lab protocols to methods used to quantify strength of a promoter.

*Cognitive*

* Integrate fluorescence and absorbance data to determine promoter strength.
* Review the information contained within promoters.

**Pre-Lab**

Before you come to lab

1) Watch 2 videos from list for week 3 lab

2) Predict what you will see from your colonies on the positive control plasmid (J04450: [http://parts.igem.org/Part:BBa\_J04450](http://parts.igem.org/Part%3ABBa_J04450)), the negative control plasmid (J119137 + water), and your experimental plate (J119137 and your promoter).

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

A) How can you be sure that *E. coli* cells in a colony contain a plasmid?

B) What phenotypes would indicate the plasmid contains your promoter?

C) How does RNA polymerase know which way to transcribe when it binds to a promoter?

D) How can we use RFP or GFP to measure the strength of transcription from a promoter?

Challenge to be discussed in lab groups: Using the information in the 4 questions above, explain to a non-biology friend why we are picking some colonies and not others. Furthermore, why are we growing them in liquid media when they have already formed colonies on solid media?

**Information: Start Overnight Cultures of Transformed *E. coli***

In Lab:

1) Today’s lab is much simpler than last week’s. Today all we are doing is starting cell cultures growing overnight in liquid LB amp (100 µg/mL). You should read about your promoter and predict how it will work. Discuss if we need to add anything to the media before starting the cell cultures.

2) Each lab group will assemble as set of PPT slides (not Google slides) that describes the major steps we are taking today. Add these slides to the previous slides you made last week. Each slide will represent one step. Your task is to construct your own understand of picking the right colonies, what needs to be added to the LB media, why we are growing them in liquid media, and how we will measure promoter strength. The goal is NOT to generate one file collectively as fast as possible. You should show your share your PPT file with the instructor before leaving lab. This will be the bulk of lab time. You may use images from the GGA presentation.

3) Make sure your slides from last week included how dsDNA is formed from two oligos and how we can directionally-clone your promoters into J119137 (pClone Red).