**Week 8: Start Testing Promoter v2 Function**

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) Review 1 video from the list for week 8 lab

2) Predict what you will see from your colonies on the positive control plasmid (J04450: <http://parts.igem.org/Part:BBa_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) Were the -10 or -35 sites of your promoter affected by your v2 changes?

B) To which site does RNA polymerase bind first, -10 or -35? What are the functions of these two binding sites?

C) Why might some of your colonies be neither red nor green?

D) Does the production of RFP or GFP affect *E. coli* growth rate?

Challenge to be discussed in lab groups: Using the information in the 4 questions above, hypothesize what colors you will see on the plates and how each color could be produced. Would it be possible for colonies to appear yellow due to the production of GFP and RFP? Speculate how this could happen. (<http://parts.igem.org/Part:BBa_J119137>)

**Information: Quantify Phenotype and Start Genotyping**

In Lab

1) Revise your GGA PPT slides to add in the data from the plates today. You can use the notes space below the slides to add details in writing that you don’t want to clutter your visual slides.

2) Discuss how you will quantify the strength of your v2 promoter and compare it to your v1 promoter. What additional information would you want to know before you conclude anything about your v2 and v1 promoter strengths?

3) Complete CATME.