**Week 8: Compare Promoter v1 & v2; isolate plasmid DNA v1 & v2**

Learning Objectives for Promoter Discovery

*Skills*

* Analyze fluorometry and spectrophotometry data
* Generate graphs in Excel to display quantitative data
* Execute the miniprep procedure

*Cognitive*

* Integrate cell density and RFP fluorescence intensity to quantify promoter strength
* Summarize how plasmids are isolated from bacteria

**Pre-Lab**

Before you come to lab

1) Review 3 videos from the list for week 8 lab

2) One person/group comes to lab 4:30 Wednesday to boil oligos.

3) 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).

4) 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? What color would a colony be if it produced both RFP and GFP?

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

**Information: Quantify Phenotype and Start Genotyping**

In Lab

1) Isolate plasmid DNA using the online protocol.
[gcat.davidson.edu/GcatWiki/index.php/Miniprep\_Plasmid\_DNA\_for\_Bio113](http://gcat.davidson.edu/GcatWiki/index.php/Miniprep_Plasmid_DNA_for_Bio113)

2) Collect the phenotype data you need to compare the transcriptional potential of your v1 and v2 promoters. Construct the Excel graphs you will need for your final report.

3) Complete CATME.