**Week 9: 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
<<https://www.bio.davidson.edu/people/macampbell/113/2iterationsGGAstudent_S2024.html>>

2) One person/group comes to lab 4:20 Monday to start cells growing overnight.

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.