**Week 7: Clone v2 Promoter**

Learning Objectives for Promoter Discovery

*Skills*

* Explain how Golden Gate Assembly works
* Describe how to clone a new promoter into plasmid J119137 (pClone Red)
[http://parts.igem.org/Part:BBa\_J119137](http://parts.igem.org/Part%3ABBa_J119137)

*Cognitive*

* Revise previous PPT flow chart showing all the major steps required to conduct a GGA experiment.

**Pre-Lab**

1) Watch 4 videos from list for week 7 lab (repeat of week 2 steps)
[www.bio.davidson.edu/people/macampbell/113/2iterationsGGAstudentF2022.html](https://www.bio.davidson.edu/people/macampbell/113/2iterationsGGAstudentF2022.html)

2) One person/group comes to lab 3:30 Wednesday to boil oligos.

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

A) How does the RNA polymerase know which way to transcribe?

B) How does your promoter v2 know which way to ligate?

C) What happens to your promoter v1 when doing GGA this time?

D) What 3 colony colors can you expect to see on the plates?

**Information: Quantify Phenotype and Start Genotyping**

In Lab

1) We will start GGA and let it run in the thermocycler.

2) You should revise your GGA PPT slides to add details that you missed the first time. You can use the notes space below the slides to add details in writing that you don’t want to clutter your visual slides. Also expand your PPT to include the new information for your promoter v2. Use the research proposal to add in your predictions for your promoter v2.

3) Plate GGA and positive control onto LB + amp media

4) Complete CATME.