**Week 8: Clone v2 Promoter**

Learning Objectives for Promoter Discovery

*Skills*

* Build a new promoter from modified oligo sequences
* Ligate your new promoter into pClone Red
* Transform and plate your cells

*Cognitive*

* 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)

**Pre-Lab**

1) Watch 4 recap videos from list for week 8 lab (repeat of week 2 steps)
<<https://www.bio.davidson.edu/people/macampbell/113/2iterationsGGAstudent_S2024.html>>

2) One person/group comes to lab 4:20 Monday 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) Plate GGA and positive control onto LB + amp media