**Week 3: Clone A Promoter to Test Its Strength**

Learning Objectives for DNA Promoter Discovery

*Skills*

* Generate dsDNA from paired oligos
* Transform competent cells with GGA ligation mixture
* Spread transformed cells onto selection media (LB + ampicillin)

*Cognitive*

* Generate a flow chart showing all the major steps required to conduct a GGA experiment.
* Explain how Golden Gate Assembly works
* Describe how to clone a new promoter into plasmid J119137 (called pClone Red)
[http://parts.igem.org/Part:BBa\_J119137](http://parts.igem.org/Part%3ABBa_J119137)

**Pre-Lab**

Before you come to lab

1) Watch 7 videos for week 3 lab
<<https://www.bio.davidson.edu/people/macampbell/113/2iterationsGGAstudent_S2024.html>>

2) Find your set of oligos from the online files using this key:

* blue lab group = *entB* promoter
* green lab group = *pbp1b* promoter
* red lab group = *glp* promoter
* yellow lab group = *hutP* promoter

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

A) What are the -10 and -35 regions of a promoter?

B) How are type IIs restriction enzymes different from the more commonly used type II restriction enzymes? Determine if Bsa I is a type IIs or a type II.

C) What is T4 DNA ligase? How is it used to clone DNA?

D) What are oligonucleotides (often referred to as oligos)?

**3) One person from each group will come to lab at 4:30 pm the day before your normal to assemble a promoter from 2 oligos.**[www.bio.davidson.edu/courses/Molbio/Protocols/anneal\_oligos.html](https://www.bio.davidson.edu/courses/Molbio/Protocols/anneal_oligos.html)

**Information: Testing A Promoter**

In Lab:

1) Each group has been assigned a promoter. You should already have found the two oligo sequences used to generate your promoter.

* blue lab group = *entB* promoter
* green lab group = *pbp1b* promoter
* red lab group = *glp* promoter
* yellow lab group = *hutP* promoter

2) Someone from your group mixed your paired oligos and started them boiling. They were left to cool overnight.

3) Right away, we will begin GGA and let it run in the thermocycler.

4) While it is running, I will give a presentation on GGA and answer your questions. Then you will use a paper exercise to help you visualize how GGA works at the molecular level.

5) Each lab group will assemble as set of PPT slides (not Google slides) that describes the major steps of GGA. Each slide will represent one step. Your task is to use the slide preparation to construct your own understand of GGA. The goal is NOT to generate one file collectively as fast as possible. You should show your share your PPT file with the instructor before leaving lab.

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