**Week 2: Clone A Promoter to Test Its Strength**

Learning Objectives for DNA 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*

* Generate a flow chart showing all the major steps required to conduct a GGA experiment.

**Pre-Lab**

Before you come to lab

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

2) Find your set of oligos using this key:

* blue lab group = entB promoter
* green lab group = glp promoter
* red lab group = hutP promoter
* yellow lab group = pbp1b 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) CATME has placed you in a lab group. The algorithm maximizes several categories of diversity and minimizes scheduling conflicts. You will work in this group each week for the entire semester. Each week, you will use CATME to evaluate your own contribution to the group effort as well as the other 3 members of your group. Over the weekend, each person will see their anonymized ratings to offer you feedback on how to be an effective team player.

2) 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 = glp promoter
* red lab group = hutP promoter
* yellow lab group = pbp1b promoter

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

4) I will give a presentation on GGA and answer 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