**Week 2: Clone New 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 6 videos from list for week 2 lab

2) Find your set of oligos using this key:

* blue lab group = lac promoter
* green lab group = ompC promoter
* red lab group = ospA promoter
* yellow lab group = tet pomoter

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

A) What is a restriction enzyme? How is it used to clone DNA?

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) Summarize the major steps involved in Golden Gate Assembly.

Challenge to be discussed in lab groups: Using the information in the 4 questions above, integrate how GGA allows you to remove the existing promoter in plasmid J119137 and clone a new on in its place. ([http://parts.igem.org/Part:BBa\_J119137](http://parts.igem.org/Part%3ABBa_J119137))

**Information: Testing A Promoter**

In Lab:

1) CATME has placed you in a lab group. The algorithm maximizes diversity and minimizes scheduling conflicts. You will work in this group each week for the entire semester. Each week, you will evaluate your own contribution to the group effort as well as the other 3 members of your group. 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 = lac promoter
* green lab group = ompC promoter
* red lab group = ospA promoter
* yellow lab group = tet pomoter

3) I will give a presentation on GGA and answer questions. (GGA\_method\_pCloneRed F2020.key)

4) 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. This will be the bulk of lab time. You may use images from the GGA presentation.