**Week 3: Design DNA Regulatory Sequence**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Find promoter or RBS sequence of interest based on the literature.
* Employ Oligator to produce oligos for construction of promoter

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Review the information contained within gene regulatory elements.
* Describe how golden gate assembly works.
* Demonstrate how to design a Golden Gate Assembly experiment.

**Pre-Lab**

Before you come to lab

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

A) How are type IIs restriction enzymes different from the more commonly used type II restriction enzymes?

B) Is Bsa I a type II or type IIs restriction enzyme? How did you find out?

C) What is DNA ligase?

D) What is bacterial transformation with regards to DNA engineering?

**Information: Design and Build a New Gene Regulatory Element**

In Lab:

1) Design your oligos to build your regulatory element using “Oligator” to [make oligos that will self-assemble](http://gcat.davidson.edu/iGem10/index.html). Make sure the longest oligo is no longer than 60 bases total. Be sure to include the sticky ends as part of your oligo length (to figure out sticky ends, see [http://parts.igem.org/Part:BBa\_J119137](http://parts.igem.org/Part%3ABBa_J119137)). Also, think about what we have learned in class about consensus sequences. How do your promoter’s -10 and -35 sites compare to the consensus for *E. coli*?

2) Take a screen shot of your Oligator output. Click on the “Ready-to-Order Format” button to copy your oligo sequences but you need to provide novel names such as Pmega\_top, Pmega\_bot. Send me (macampbell@davidson.edu) an email containing your sequences in text format and the name of the gene your promoter came from for the pClone groups. In addition, tell me in the email what type of information is encoded in your regulatory element (*i.e.* inducible, repressible, *constitutive*). I also need the name and concentration of any compound you need to regulate your regulatory element if it is not *constitutive* (🡨 look up this word if you don’t know it).

3) Make a [new **basic** part in the Campbell Lab](http://partsregistry.org/Add_a_Part_to_the_Registry) web page and document your design. Include the screen shot of the Oligator output and document how you learned about the regulatory element and its predicted behavior. Our part numbers range from BBa\_J100000 to BBa\_J100999 but Dr. C. will provide each group with the appropriate part number to use.

4) Read the [oligo assembly protocol](http://www.bio.davidson.edu/courses/Molbio/Protocols/anneal_oligos.html) (steps 1 – 6) and calculate how to prepare a 20 µL solution of your oligos so they will self-assemble into a functional promoter ready for ligation. Calculate how you will dilute the boiled oligos to a new concentration of 40 nM.

5) One person from each group will need to start the cells growing 4:30 pm Wednesday the day before lab. Come to Dr. C’s research lab (Wall 325) on time.