**Week 9: Submit Graded Research Proposal**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Develop new research proposal based on previous results.

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Revise experimental design for second iteration to refine DNA part characterization

**Pre-Lab**

Before you come to lab

1) Review your research proposal and come to lab with constructive criticism.

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

A) What benefits do you see by getting a second shot at discovering new DNA control elements?

B) How is DNA sequence generated using Sanger sequencing?

C) What is a chromatogram and how do you read it?

D) What is the function of a BLAST search?

 **Information: Finalize Research Plan Second Iteration**

In Lab

3) Finalize your research proposal and submit to Dr. C. before you leave lab. The final proposal that will be graded needs to contain these parts:

* previous results (yours or another group’s; include figure + legend)
* hypothesis of what the results mean
* new DNA sequence you want to test (two named oligos with sticky ends)
* figure of designed element in the testing plasmid
* new experiment to test your v2.0 DNA control element
* appropriate control *E. coli* strains
* predicted results from DNA control element v2.0 and controls (sketch + figure legend)

4) Design your oligos to build your promoter 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 (see [http://parts.igem.org/Part:BBa\_J119137](http://parts.igem.org/Part%3ABBa_J119137)). 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*?

5) 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. In addition, tell me in the email what type of information is encoded in your promoter (*i.e.* inducible, repressible, constitutive). I also need the name and concentration of any compound you need to regulate your promoter if it is not constitutive (🡨 look up this word if you don’t know it).

6) 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 promoter 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.

7) Show your DNA sequences to Dr. C. Once he has signed off on them, email the two sequences and their names to Dr. C.

8) 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.

9) 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.