**113 Lab Learning Objectives**

**Week 2: synthetic lab #1**

Learning Objectives for Promoter Discovery

*Skills*

* Sign up for one user account (DNA Parts Registry, M Campbell Lab Page)
* Search PubMed to find promoter of interest

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Evaluate publications to find a suitable promoter.
* Explain how DNA fragments are cloned.
* Review the information contained within promoters.

**Bio113 Week 2**

Before you come to lab:

1) Create an account on the [Campbell lab wiki page (http://igem.org/Lab.cgi?id=66)](http://igem.org/Lab.cgi?id=66)

2) Listen to this 6 minute information story about synthetic biology: <http://www.npr.org/templates/story/story.php?storyId=90014997>

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

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

B) What is a plasmid? What parts are essential to its function?

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

D) What is a restriction enzyme?

**Information: Design and Build a New Promoter (an 8 week project)**

In Lab:

1) Watch [movie of restriction enzyme](http://www.dnalc.org/view/15476-Genetic-engineering-inserting-new-DNA-into-a-plasmid-vector-3D-animation-with-with-basic-narration.html) cutting DNA (<http://www.dnalc.org/view/15476-Genetic-engineering-inserting-new-DNA-into-a-plasmid-vector-3D-animation-with-with-basic-narration.html>)

See the [structure of a restriction enzyme bound to DNA](http://bioinformatics.org/firstglance/fgij/fg.htm?mol=1RVC). (<http://bioinformatics.org/firstglance/fgij/fg.htm?mol=1RVC>)

Discuss sticky ends. How do we connect two segments of DNA with compatible sticky ends?

2) Search PubMed for papers using terms such as “inducible promoter escherichia coli” or “repressible promoter escherichia coli” or “constitutive promoter escherichia coli”. Download and save the PDF files for later use. You are looking for a promoter that you could manipulate experimentally in Bio113 lab. Use quotation marks (see above) to find exact matches to phrases rather than independent words as happens when you don’t use quotation marks.

3) Identify how the promoter could be experimentally regulated by you. For pragmatic reasons, avoid regulatory mechanisms such as “expose cells to X-rays” or “put cells in space were there is no gravity”, *etc*.

4) Working in your lab group, find your gene’s promoter DNA sequence by looking upstream from the start transcription site. You might find the promoter described in a published paper which would be better than just guessing its location. You can also try some online genome web sites to find the DNA sequence. You may not finish this part today. You will have more time to work on this next week. Get as far as you can though.

Resources you could use to find promoter sequences:

* [*E. coli* genome browser](http://microbes.ucsc.edu/cgi-bin/hgGateway?org=Escherichia+coli+K12&db=eschColi_K12&hgsid=581372)
* [Promoters in Registry of Standard Parts](http://partsregistry.org/cgi/partsdb/pgroup.cgi?pgroup=Other_Regulator&show=1)
* [Partial list of *E. coli* gene promoters](http://margalit.huji.ac.il/promec/prom.seq.final.html)

**Reference**

<http://www.oxfordgenetics.com/SiteContent/Store/bacterial-promoter-information>