**113 Lab Learning Objectives**

**Week 4: synthetic lab #3**

Learning Objectives for Promoter Discovery

*Skills*

* Manipulate DNA to perform Golden Gate Assembly
* Transform bacteria and screen for phenotype.

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Describe the big idea of information based on lab experiences.
* Review the information contained within promoters.
* Explain how golden gate assembly method works.
* Use protocols for molecular biology to clone DNA.

**Bio113 Week 4**

Before you come to lab

1) At 4:30 pm on the Wednesday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Dana room 221). Make sure to bring your protocol from last week of how to prepare the oligos for boiling.

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

A) How will you ligate your new promoter into a plasmid for testing?

B) What will the plasmid need to contain if you want to determine if your promoter is working?

C) How is fluorescence of red fluorescent protein (RFP) measured?

D) How is a spectrophotometer used to measure cell density in a population of *E. coli*?

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**NOTE:** At 4:30 pm on the Wednesday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Dana room 221). Please be on time. We need to boil the oligos so we can ligate them in lab. See page 2 for details.

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**Week 4**

Before you come to lab

1) The afternoon before lab, one person has already boiled oligos and let cool slowly overnight.

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

In Lab: **(Start lab at this point)**

2) Do appropriate dilution (from 5 µM to 40 nM) of boiled and cooled oligos. You will ligate your promoter into receiving plasmid J119137.

3) You have been provided two tubes of a master mix for GGA. It already contains the receiving plasmid J119137, the BsaI and the ligase. The volume is 9 µL in each tube. You need one tube for your promoter (P) and one for a negative control (-). Add 1 µL of your freshly diluted promoter to the P tube and 1 µL water to the “-“ tube. Label your tubes. Put them in the thermocycler. GGA is program name.

4) Transform cells (zippy competent JM109) with 3 different DNAs:

a) experimental ligation DNA (with your promoter oligos added = P)

b) ligation negative control DNA (water added, not promoter = -)

c) transformation positive control DNA(+ tube; [pLac promoter+RBS+RFP](http://partsregistry.org/Part:BBa_J04450))

Transformation thaw competent cells for 6 minutes on ice

add all 10 µL of GGA to tube of thawed cells

return to ice ASAP and incubate 5 minutes

add 30 µL of SOC to cells, store at room temp

spread 90 µL of cells on LB amp plate (already labeled)

5) Plate each transformation on its own LB+amp plate.

6) Discuss as a group how to assay your promoter. How will test your promoter to know if it works the way you thought it would?

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