**Week 4: Ligating Gene Regulatory Sequence**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Manipulate DNA to perform Golden Gate Assembly
* Transform and plate bacteria, 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 and RBSs.
* Explain how Golden Gate Assembly method works.
* Use protocols for molecular biology to clone DNA.

**Pre-Lab**

Before you come to lab

1) At 4:30 pm on the Monday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Wall 325). Make sure to bring your protocol from last week of how to prepare the oligos for boiling.

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

A) How will you ligate your new DNA control element into a plasmid for testing?

B) What functional elements of the plasmid will you use when you clone your DNA into its plasmid?

C) How is the fluorescence of red fluorescent protein (RFP or mScarlet) 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 Monday before your lab, one person from each lab group MUST COME TO Dr. C’s research lab (Wall 325). 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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**Information: Cloning your Gene Regulatory Element into your Plasmid**

In Lab:

1) Monday, one person from your group boiled your oligos and let them cool slowly overnight.

**(Start lab at this point)**

2) Dilute (from 5 µM to 40 nM) your boiled and cooled oligos from yesterday. You will ligate your DNA control element into the receiving part J119137, J119384, J100397 or J100419.

3) You have been provided one tube of a master mix for GGA. It already contains the receiving plasmid, buffer, BsaI and DNA ligase. The volume is 36 µL. You need to pipet 9 µL into the tube for your experimental DNA (X) and 9 µL into the tube for your negative control (N). Add 1 µL of your freshly diluted (40 nM) boiled and cooled oligos to the X tube. Add 1 µL water to the “N” tube. Add a group label to both tubes so you can identify your tubes. Put both tubes in the thermocycler. GGA is the program name we will use – it takes about one hour.

4) Transform cells (zippy competent *E. coli*, strain JM109) with 3 different DNAs:

a) X: eXperimental ligation DNA (with your promoter oligos added)

b) N: Negative control ligation (water added instead of oligos)

c) P: Positive control DNA that will produce RFP or mScarlet protein

Transformation thaw competent cells for 6 minutes on ice

Gently but quickly add 50 µL of thawed cells to the tubes of DNA

return tubes of DNA + cells 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 (pre-labeled by you)

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

6) Discuss as a group how to assay your DNA regulatory element. How will you know if it works the way you thought it would? What sort of controls would you want to run? Next week you will use the protocol you design today.

7) One person from each group will need to start the cells growing 4:30 pm next Monday the day before lab. Come to Dr. C’s research lab (Wall 325) on time. Bring the protocol with you.