**Week 10: Ligating Gene Regulatory Sequence v2.0**

Learning Objectives for DNA Control Element 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 and RBSs.
* Explain how Golden Gate Assembly method works.
* Use protocols for molecular biology to clone DNA.
* Generate overall conclusions of the multi-week laboratory module to identify new promoters that could be useful in synthetic biology research.

**Pre-Lab**

Before you come to lab:

1) Review how Golden Gate Assembly (GGA) works. What is the outcome from this procedure?

2) Review the transformation protocol and what it accomplishes.

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

A) In what order does RNA polymerase bind to the -35 and -10 sequences?

B) What information is encoded in the promoter other than -35 and -10 sequences?

C) What are the sequence requirements for an RBS? What is the optimal spacing between an RBS and a start codon?

D) How does RNA polymerase “know” which direction to transcribe when it binds to a promoter?

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**NOTE:** At **4:30 pm on the Monday before your lab next week**, 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 v2.0 into your Plasmid**

In Lab:

1) On 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 use GGA to ligate your DNA control element into the receiving plasmid (J119137, J119384, J100397 or J100419).

3) You have been provided one tube of a master mix (40 µL) for GGA. It already contains the receiving plasmid, BsaI and ligase with buffer. Pipet 9 µL of the master mix into the tube labeled (X) eXperimental and 9 µL of the master mix into the tube labeled (N) for negative control. 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. Put them in the thermocycler. GGA is the program name we will use – it takes about one hour.

4) Transform cells (zippy competent 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 (J04450) or mScarlet (J100434)

Transformation thaw competent cells for 6 minutes on ice

Gently & quickly add all 50 µL of thawed cells to tubes with DNA

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 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 before lab. Come to Dr. C’s research lab (Wall 325) on time. Bring your experimental design with you.