**Week 12: Confirm Genotype of Cloned DNA v2.0**

Learning Objectives for DNA Control Element Discovery

*Skills*

* Properly manipulate bacterial cultures to maintain clonality of cells.
* Quantify red fluorescent protein levels in populations of *E. coli* cells.
* Enter your results into DNA Registry.
* Perform a miniprep, Nanodrop the DNA, set up sequencing reactions.

*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.
* Use protocols for molecular biology to clone DNA.
* Interpret Synergy data for fluorescence and optical density.
* Design experiment to confirm cloned DNA was successful.

**Pre-Lab**

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 (Wall 325). You will start overnight cultures to be mini-preped in lab.

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

A) What is the objective of a plasmid mini-prep? What physical association does the *E. coli* genome have with its cell membrane?

B) What is required to perform Sanger DNA sequencing?

C) How is DNA quantified by spectrophotometry? What is the significance of a 260/280 ratio?

D) Describe the major types of biological molecules that must be separated from the plasmid

 DNA in order to be pure enough to be sequenced.

**Information: Confirm DNA Sequence Genotype of Cloned DNA v2.0**

In Lab

4) Miniprep your experimental plasmid DNA v2.0 using the cultures that grew overnight. One person from your group started these cultures yesterday. Follow the online protocol.

5) Nanodrop your plasmid DNA to quantify the DNA abundance and purity.

6) Prepare each miniprep for one sequencing reaction using the primer that is specific for the testing plasmid you used for this second iteration. Follow the online protocol.

7) Sign up to indicate which barcoded tubes were used for your sequencing reactions. Keep track of which sample was used for which barcoded tube.