**Week 10: Determine v2 Genotyping**

Learning Objectives for Promoter Discovery

*Skills*

* Convert DNA concentrations into volumes to pipet for desired amount of DNA

*Cognitive*

* Explain how dideoxy DNA sequencing is performed and analyzed

**Pre-Lab**

1) Watch 2 videos from list for week 10 lab

2) Download the “dilution calculation” Word file, the Excel template file and Nanodrop data Excel file.

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

A) How can you convert a DNA concentration into a volume needed to deliver 320 ng of DNA into a sequencing tube?

B) What is a dideoxy nucleotide? How does it differ from RNA and DNA nucleotides?

C) How many primers are used for DNA sequencing? Explain your answer.

D) What is a chromatogram that is produced during automated DNA sequencing?

Challenge to be discussed in lab groups: Using the information in the 4 questions above, discuss the major steps involved required to sequence your promoter. How can you be sure that your promoter will be sequenced and not some random portion of the plasmid?

**Information: Quantify Phenotype and Start Genotyping**

In Lab

1) Add one slide to your PPT file that illustrates how DNA sequencing works. You may combine your own drawings with screenshots from web sites. However, the more you draw, the better you will understand and remember.

2) Calculate how to set up the sequencing reactions using the Nanodrop Excel data and the Excel template file. The dilution calculation Word file helps you focus on the task at hand, and asks you one bonus question.

3) Discuss how you can determine if the promoter you actually cloned matches the desired sequence that you sent off to the company to synthesize. What online tools exist for aligning multiple DNA sequences?

4) Complete CATME.