BME 128L Spring Quarter 2015
Professor DuBois

Lab Week #3: Primer concentrations and PCR setup
Pre-lab Assignment for Week#4: Prep for Week 4 experiments

Due date: April 22, 2015

Goal: To determine DNA primer concentrations and set up PCR reaction

Methods:

1. Centrifuge your DNA primer tubes 1min at 10,000xg to ensure that the dry DNA is at the bottom of the tube.

2. Add the appropriate amount of milliQ water to the tube to make a 100µM solution.

3. Using the 100µM DNA primer sample and milliQ water, make a 100µl sample of a 10µM DNA primer sample.

4. Use the NanoDrop UV/VIS spectrophotometer to measure the concentration of your DNA primers.

5. Set up your PCR reaction using the guidelines discussed in class.

Pre-lab Assignment for Week#4:

1. What do you expect we will see on an agarose gel when we run the PCR product from today? Draw a picture of a gel with a ladder (New England Biolabs “Quick-Load Purple 2-Log DNA Ladder) and a lane with your PCR product. Label band sizes.

2. What will happen when we add DpnI enzyme to our PCR reactions?

3. We will purify the DNA from the PCR reaction using DNA clean-up columns (NucleoSpin). Why does DNA bind to the purification column? Why does it elute from the column? Explain the molecular mechanisms.

4. What chemical moietyes are required at the 5’ and 3’ ends of DNA for DNA ligase to work and ligate your PCR product into a closed plasmid?

5. You will transform your ligated DNA plasmid into chemically-competent E.coli. Why do we grow the E.coli on LB/Ampicillin plates? What would happen if we grew them on LB plates?

6. Safety question: we will use SybrSafe DNA stain in the agarose gel and chaotropic salts in the DNA clean-up experiment. Why are these chemicals unsafe?