Biology 211
Lab 5
Plasmid Purification

Objectives:

- To spin down *E. coli* cells prior to alkaline lysis.
- To execute alkaline lysis DNA isolation of a plasmid containing a piece of *Pseudomonas fluorescens* DNA

Before Lab:

- Write out a protocol for spinning down your culture. This is a simple procedure where you will use a centrifuge to spin your bacterial cells to the bottom so that you can discard the broth. Note that you will need to transfer the cells from 3ml of broth into one 1.5ml tube. How will you do this? Leave room to add details during lab.
- We are using a Qiagen Miniprep kit to isolate our plasmid DNA. For information on the procedure we will follow, look at the Qiaprep Miniprep Handbook. We will be using the QiaPrep spin procedure in a microfuge (found on page 20, with a visual partial explanation on page 19). Use this information to introduce this lab in your lab notebook. The handout also gives some background information starting on page 11. Note that you will need this procedure for the lab.
- For more information about plasmid minipreps and what you will find in each solution, look here:
    - http://bitesizebio.com/13516/how-dna-extraction-rna-miniprep-kits-work/
    - http://www.csub.edu/~kszick_miranda/miniprep.doc

- Prepare the protocol for your lab notebook. Make sure that you indicate what the chemicals are for each step of the procedure and indicate what they are doing to the cell to get the DNA out. This information should be included either in your background information or in your methods section.

In Lab:

- Spin down your culture using your protocol.
    - Additional notes:
        - Check the turbidity of your broth culture. If it is not turbid, discard the culture and start a new inoculation.
        - Transfer all of your culture to a 1.5ml tube.
        - When discarding supernatant, be careful to not disturb the pellet. It is ok to leave a small amount of supernatant.
- Prepare your DNA following the instructions in the Qiagen handout. Again, write out a detailed protocol as you conduct the lab that goes beyond the information on the handout.
- Make sure that your discussion includes a 'big picture' view of why this lab is important in sequencing the genome of *Pseudomonas fluorescens* and an error analysis of mistakes you made, changes to your protocol, and how it affected your results