Biology 211
Lab 4 - Begin Culture

Before Lab:

- We will culture *E. coli* in LB broth. Find information on what LB stands for, what LB broth is, what is in it and why it is good for bacterial growth (a good place to start is here http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2168924/).
- This is specific information on the plasmid that our DNA library was cloned into http://lucigen.com/store/pSMART-HC-Kan/, click on the description tab and scroll to the bottom of the page to the plasmid illustrations (we are using the pSMART HCKan).
- Our LB broth will contain Kanamycin. Find information on what Kanamycin is and why it is important for our experiment.
- Find information on how to inoculate a broth with *E. coli*. Many videos are available such as:
  - http://www.youtube.com/watch?v=R48YZlkLHsg
  - http://www.youtube.com/watch?v=7PLGDpfdeNc
- Write a protocol for starting a bacterial culture using the information and links above.
- The colony that you will culture is from a genomic library that was prepared for you. Summarize the important steps in the making of this genomic library that you are getting your clone from. Use this resource starting with Gene Cloning Part 1 and going through Genomic Libraries.

http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=959197140&topicorder=8&maxto=16&minto=0

In lab:

- Follow the protocol you brought to class, with any modifications based on the discussion in class.
- For each step involving a container, pre-loosen all lids before transferring solutions.
- Calculate the amount of kanamycin that was added to the LB broth (this has already been done). The kanamycin started at a concentration of 30mg/ml and is currently at a concentration of 30ug/ml. Include this calculation in your materials or methods section.
- Include a brief description of every material used, how it works, and its purpose

In your background:

- Include the plasmid map for the plasmid we are using and discuss the importance of the various parts of the plasmid.
- Why are we concerned with making billions of *E. coli* cells when we're really trying to sequence *Pseudomonas fluorescens L5.1-96?*
- Include background information on making genomic libraries.
- A protocol to follow in lab.

In your results:

- Your clone number
- A description of the visual appearance of your falcon tube

In your discussion:

- A 'big picture' view of why this lab is the first step in sequencing your plasmid
- An error analysis of mistakes you made, changes to your protocol, and how it affected your results