Lab 7: Sequence Reaction Setup

Objectives:

- Amplify the DNA fragment using BigDye Terminator nucleotides to prepare the sample for sequencing.

Before Lab:

- Read the section of your text on PCR reactions so that you understand what you'll be doing in the lab. Take a look at this link for more information on the general PCR uses in forensics - http://learn.genetics.utah.edu/content/labs/pcr/
- ***Important Note*** - you will not be running normal PCR. The purpose of this lab is to run a sequencing reaction. This will amplify your DNA fragment into many different sized strands to be read by the sequencer. Review the following links to make sure you understand what you will be doing. There are lots of visual and interactive aids in these links. Check them out!
    - http://www.wiley.com//college/pratt/0471393878/student/animations/dna_sequencing/index.html
    - http://www.dnalc.org/resources/animations/cycseq.html
    - http://scidiv.bellevuecollege.edu/gb/Bio_275/biology275_tools.html#genseq.
- This is specific information on the plasmid that our *P. fluorescens* DNA library was cloned into.
    - http://lucigen.com/store/pSMART-HC-Kan/, click on the "More Info" tab and scroll to the bottom of the page to the plasmid illustrations (we are using the pSMART HCKan).
- Make sure that you have the primer sequences for your lab book. See this reference:
    - http://lucigen.com/Vector-Sequences.html , scroll to the bottom to view the SL1 and SR2 sequences.
- Make sure that you understand using the BigDye Terminator reagents in PCR to prepare the sequencing reaction. Use the procedures and reagents listed in chapter 3 of the manufacturer's handbook on BigDye Terminator Reactions. Note that we are conducting the reaction in microcentrifuge tubes and we do not have large DNA templates. http://www3.appliedbiosystems.com/cms/groups/mcb_marketing/documents/generaldocuments/cms_081527.pdf
- You may need to copy and past this link into your browser for it to work.
- Begin to write a protocol for the sequencing reaction using the information above. Include what each step and reagent is and what it does in the tube. The Big Dye terminator contains all of the materials needed to run the sequencing reaction except for primers.

In Lab:

- Follow the procedures in your protocol with any modifications discussed at the start of class.