

SEED Academy Spring 2008
Synthetic Biology Module

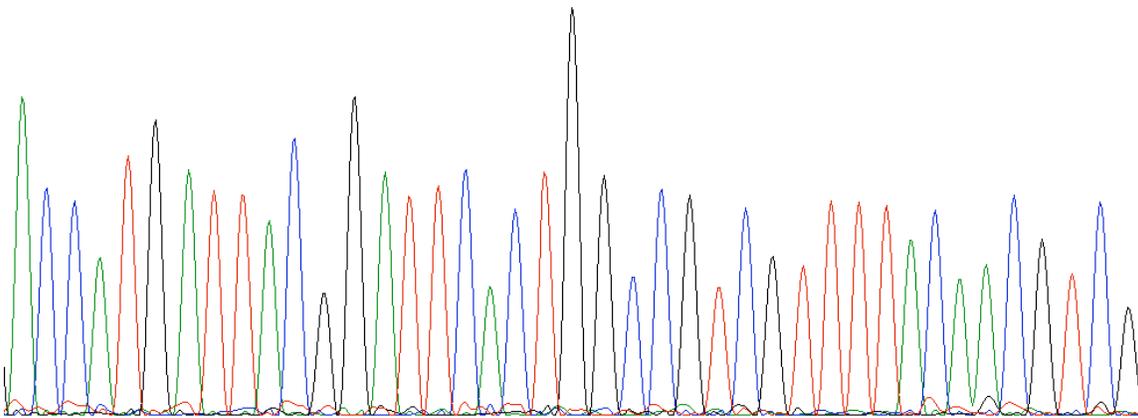
Homework #6
Due April 12, 2008

Note: This homework may seem a bit long. Please try to attempt all questions. Write down whatever work you are able to do. MAKE SURE you do at least several parts of question #2 as you may have to PRESENT INFRONT OF CLASS. As always, email seed-synbio@mit.edu if you have questions. Good luck!

- 1) DNA sequencing is used to read out the bases from DNA. Many methods have been developed but the currently most common method for sequencing is known as the dideoxynucleotide method or Sanger sequencing. Look up some information about both the classical method involving radioactively labeled nucleotides and the current method using fluorescently labeled nucleotides.
 - a) What does ddNTP stand for? How is it different from dNTP?
 - b) What does it mean when we refer to ddNTPs as chain terminators?
 - c) In the classical Sanger sequencing method, 4 separate reactions are run. Each reaction contains all of the dNTPs, DNA polymerase, the DNA to be sequenced, and a sequencing primer. What else is added to the 4 reactions?
 - d) Suppose we ran a sequencing reaction with only the nucleotides ddATP, dTTP, dCTP, and dGTP. Explain what would happen during the sequencing reaction and why this is not useful for sequencing.
 - e) Consider the following sequence. Suppose we use the primer 5'-TCAGT-3' and run a classical Sanger sequencing reaction. We set up 4 reactions and then run a gel to separate the bands. Draw a gel and the locations of the bands from the sequencing run. Be sure to label the four lanes.

5'-TCAGTAGCGTAGCACCTAG-3'
3'-AGTCATCGCATCGTGGATC-5'

- f) Current methods with different color fluorescently labeled nucleotides require only one reaction and use automated DNA sequencers. This is the method we will use in this class. Suppose we get the chromatogram as shown below. Write out the sequence this corresponds to. Green=A, Blue=C, Black=G, Red=T



- g) Using the previous DNA sequence, figure out what gene this sequence is likely from. To do this, you will use BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). You can think of BLAST as a DNA sequence search engine. Click on nucleotide blast. Enter your sequence in the top box. Under database, choose “Others” and the “nucleotide collection (nr/nt)” and hit BLAST. Can you figure out what gene this sequence corresponds to? Hint: this is a very commonly used gene and so appears in many plasmids. Try to find the BLAST matches that match a genome.

- 2) You have now each selected a topic for your final project and talked about it with the instructors. The format of your final project will be a poster presentation describing your system design. To get you ready for putting together your poster produce the following components (remember to keep a copy for your records, you probably want to do this on a computer if possible) **READ PART F) FIRST!!!**:
- a) Write one paragraph summarizing your project, what it will accomplish and why you want to do it.
 - b) Draw a schematic diagram of your system. Show all of the relevant inputs and outputs as well as the critical components you have identified so far, including fundamental parts to make your system, and any devices which you know you will need. Include both the specific parts which you know exactly how they will function and the abstract devices which you don't have fully defined yet.
 - c) Summarize the possible states of your system. What inputs (cues) will lead to what outputs (signals). Put this data in a table.
 - d) Draw a circuit diagram to explain the logic behind your schematic diagram. This diagram should not only contain all of the components of the system, but also how they interact with each other at all of the various possible levels and states.
 - e) Provide the DNA sequence for the two most critical parts in your system. If these parts are in the Registry for Standard Biological Parts, list their part numbers. If not list the source for the sequence (HINT: After you identify the part of interest, figure out the species it comes from and the name of the gene. Then you can search in KEGG, www.kegg.com or the NCBI Database, <http://www.ncbi.nlm.nih.gov/BLAST/>. If you get lost, ask for help)
 - f) Prepare a 5 minutes presentation about your project. **BE PREPARED TO PRESENT IN CLASS ON SATURDAY.** We will select people randomly based on the time available to discuss projects! We expect you to show us 2-3 of the above parts of this problem during your 5 minute presentation!