

SEED Academy, Spring 2008
Synthetic Biology Module

Homework #8
Due April 26, 2008

1) Project Question

Please answer the following specific questions related to your project. Keep a copy of your answers to put on your final project poster! It is best if you do this part on a computer. Remember you only have 3 weeks until you need to complete your poster.

- Son – What is the specific mechanism of how *Streptococcus mutans* causes cavities (chemical activity)? Do these bacteria play any positive role in the oral community? How does the activity of *Lactobacillus sporogenes* prevent the formation of cavities (i.e. how do the good bacteria attack the bad bacteria)? Are there specific nutritional requirements for *L. sporogenes* to live in the mouth? Are there any potential side effects of having *L. sporogenes* in the mouth?
- Kidus – What is the transparent chemical you will use to make the pattern? Please show the chemical structure. How will the bacteria recognize this chemical? Indicate the specific protein and how this protein responds in the presence and absence of the chemical. What colored chemical or protein will the bacteria make and how will the bacteria make this color?
- Joel – What is the chemical used to trace a path for the bacteria to follow? Please show the chemical structure. What protein will the bacteria sense this chemical and what are the outputs from this protein? HINT: Use your termite example and study this model system. How does the bacteria figure out direction (forward instead of backward)?
- Qin – What are the specific differences in the proteins between the hair color (amino acid residues, DNA sequence, chromophores, and/or protein structure)? What chemicals indicate good mood or bad mood, please give the structure? What constructs in the cell are responsible for sensing these compounds?
- Tu – What is the recommended daily dose of vitamin D (mg/kg body weight)? What is the solubility of vitamin D in water and what is the solubility in fat (in mg/mL)? What enzymes are required to produce vitamin D? How are you going to keep the level of vitamin D in the appropriate range (not too much or not too little)? What is the meat of the apple made out of (is it cells or extra-cellular components)? How will sunlight trigger vitamin D formation?

- Un Kei – What protein will you use to sense light and how does it behave in the presence and absence of light? What is the name of the protein you will use to generate light when it is dark? What is the source of this protein? What is the input energy source for producing light? How will you translate the input signal given off from the sensing protein to an output signal from the light-generating protein (please describe specific parts like DNA transcriptional devices, promoters, etc.)?
- Vannarith – How will you identify harmful microbes that should be killed? Please indicate the specific type of cell markers or chemical species you will use. We recommend targeting only one type of microbe. How will you kill this microbe? We talked about many general ideas in class ranging from antibiotics to viruses. Select which one you want to use and explain in detail how it will kill the cell. If it is an antibiotic, what is the mechanism of action and how will it be selective? If it is another means, what are the steps required to make it work?
- Shaena – How much gas do you need to blow up a balloon? If the balloon is going to rise off the ground, how much of the gas needs to be hydrogen (you will need to find the weight of a balloon for this, and how the volume changes as you add more gas)? How much pressure is required to put this much gas into a balloon? What is the protein that is capable of making and consuming hydrogen within a cell? What is the equilibrium value for the reaction catalyzed by this enzyme (i.e. how much hydrogen gas is around vs. hydrogen ions and electrons)?
- Ernest – How does the immune system remove a pathogen from the body? What cell types are responsible for this behavior? How do these specific cells recognize a cell as a foreign invader or a friendly cell? We recommend that you choose one specific problem to tackle (like the HIV or cancer idea). How would you identify the problem area you selected specifically?
- Nathashali – Select a project. Choose a project that is biologically feasible. We discussed several possible ideas in class. Email the instructors ASAP with your proposed project so that we can give you more specific direction.
- Rebekah – What system are you planning on designing for your final project? Email the instructors ASAP with your proposed project so that we can give you more specific direction.
- Peter – Are you planning on completing a project to finish this class? Please discuss with the professors immediately!

- 2) The sequence results have been posted to the class website at <http://openwetware.org/wiki/SEED/2008/Sequencing>. This includes both the raw chromatogram traces and the sequence read by the automated base-caller.

Analyze your four sequence results. If your sequencing results failed or you didn't sequence any samples, pick another group's 4 samples.

For each of your samples, answer the following questions:

- a) What was the expected promoter.RBS combination?
- b) Did the sample contain the expected promoter/RBS?
- c) Is the sequencing entirely correct (promoter.RBS.lacZ)? If so, roughly how many good bases were able to be read?
- d) Are there any mutations or unexpected results? If so, can you give a likely scenario for what happened?
- e) Examine the chromatogram data, especially for unexpected sequences, and "N" bases. Are there bases at which the base-caller made the wrong call? Does this change your analysis of what happened?

Here are some tools to help you with analysis. You will first need to figure out what the expected sequence should be. The sequences for the promoters and RBS and the lacZ gene are given at <http://openwetware.org/wiki/SEED/2008/Sequences>. Remember how you constructed these promoter.RBS.lacZ cassettes. We ran a PCR to attach the RBS which makes a perfect fusion between the RBS and lacZ, so you expect no extra bases in between the RBS and the lacZ. We then cut the RBS.lacZ piece with XbaI and the promoter piece with SpeI. Use your previous homework as a guide to determine the exact sequence that is expected in the junction between the promoter and RBS.

You can use BLAST2 <<http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi>> to compare the expected with the actual sequence. Use the following options: View option->mismatch highlighting; Masking color option->Red; Uncheck filter. For Sequence 1, enter the expected sequence that you figured out above. In Sequence 2, enter the sequence data as text.

Here are a couple free chromatogram viewers to look at the chromatograms.

Windows:

<http://www.techneesium.com.au/chromas233.exe>
<http://www.geospiza.com/finchtv/>

For Macs:

OSX: <http://mekentosj.com/4peaks/>, <http://www.geospiza.com/finchtv/>
http://www.appliedbiosystems.com/support/download/SeqAnal/installs/mac/EditView_1.0.1.sea.hqx

3) Beta-Galactosidase Activity

- a) Put the data you collected in lab into an Excel spreadsheet. If you collected the data, send a copy of the data to your lab partner and the instructors (seed-synbio@mit.edu).
- b) Plot the values of Abs_{420} against time for each of your samples (Put them in one chart in Excel). Do the graphs appear to be linear? Are the absorbance readings limited at any level? Attach the plot to this assignment
- c) Make a copy of the chart. On this chart, use only the data points which fall in the linear range. Add a linear regression to the data by right clicking on the data and selecting “Add Trendline.” Under the options, select “Show Equation” and “Show R^2 .” Attach the plot to this assignment.
- d) Record the slope of each linear regression of the data. The intercept should be near zero, if it isn't check your results. A non-zero slope may indicate a problem with your time measurements or incorrect blank readings for the spectrophotometer. The slope is the enzyme activity of the sample, not corrected for volume or cell density. Take the slope, and normalize it to find the Miller Units.

$$MillerUnits = 1000 \times \frac{(Abs_{420,t=2} - Abs_{420,t=1})}{(t_2 - t_1) \times v \times Abs_{600}} = 1000 \times \frac{slope}{v \times Abs_{600}}$$

The volume should be in milliliters. Remember that we used $20\mu\text{l} = 0.02\text{mL}$ of the sample. Report the Miller Units for each sample.

- e) Using each time point you collected, calculate the Miller units with the following equation:

$$MillerUnits = 1000 \times \frac{(Abs_{420})}{t \times v \times Abs_{600}}$$

Take the average of the Miller Units calculated at each time point to estimate the Miller Units for each sample. Report these numbers. Which method is more accurate and why?

- f) Plot the values you obtained for the Miller Units vs. each AHL induction concentration (0 and 10^{-11} M to 10^{-5} M) on a semi-logarithmic scale (x-axis only). You can treat no AHL as 10^{-12} M AHL. Describe the plot, what does the shape of the graph look like? Attach this plot to the assignment.

- g) Looking at your graph, what is the apparent *switch point*? The switch point is the level of AHL that gives half maximal output.
- h) The samples you measured contained the part F2620 (http://parts.mit.edu/registry/index.php/Part:BBa_F2620) controlling lacZ. F2620 has previously been characterized in front of GFP via fluorescence. Go to the part page for F2620 and look at the transfer function. Compare the transfer function of F2620.GFP with your F2620.lacZ data. Is it qualitatively and quantitatively similar? How do the switch points compare?
- 4) How long did you spend on homework this week?