SEED Academy, Spring 2008
Synthetic Biology Module

Homework #7
Due April 19, 2008

1) Project Question

a. Draw 3 graphical figures that can go on your final poster. Include appropriate captions.

b. Layout a rough sketch of a poster with the content that it will have. For example, a title, your name, project summary, the figures from above, etc. You can leave some of the content empty if you don't know the information yet, but specify what you want to go there (e.g. "a description of a gene that will respond to chemical X").

2) Transformation Efficiency. The Viable cell titer contained 7 cells in the 10 μL of the 5^{th} serial 10X dilution. You transformed 10 μL of 100 pg/μL ligation product with an expected length of 5000 bp and a molecular weight of 650 Dalton per base.

If you obtained 100 colonies on the plate…

a. What is the transformation efficiency (the number of ligation products recovered in cells)?

b. What fraction of viable cells obtained a plasmid with antibiotic resistance?

If you obtained 10,000 colonies on the plate…

c. What is the transformation efficiency?

d. What fraction of viable cells obtained a plasmid with antibiotic resistance?

3) Enzyme Activity

a. Expression Levels

i. mRNA

You are using a plasmid which is maintained in the cell at 5 copies per cell and a promoter which recruits RNA polymerase at a rate of 10 per second per plasmid. Assume that every second 1/4th of the mRNA in existence is degraded.

What is the steady concentration of mRNA [#/cell] (HINT: A steady concentration will be reached when the RNA production by the polymerase equals the rate of degradation)?

ii. Protein

Use the steady mRNA concentration from above (if you did not get an answer above, use a value of 1 mRNA per cell). The ribosome binding site initiates translation of the mRNA at a rate of 2 per second per mRNA. Assume that every second, 1/10th of the protein in existence is degraded.

What is the steady concentration of protein [#/cell]?

b. Time duration enzyme rate (Beta-Gal Miller Equation)

Read the background and protocol for Saturday's experiments:
http://openwetware.org/wiki/Beta-Galactosidase_Assay_(A_better_Miller)

1 Miller Unit = $$1000 * \frac{(Abs_{420} - (1.75 * Abs_{550}))}{(t * v * Abs_{600})}$$

i. Explain the purpose of each term in the equation above (not just what the variable stands for but why it is in the equation). Include: Miller Unit, Abs_{420}, Abs_{550}, Abs_{600}, t, and v.

ii. What chemical is responsible for the color in the Miller assay? How does this work?

iii. Why is it important to keep careful track of the time?

iv. Complete the following table & indicate the rank of the various cultures in the per cell Beta-galactosidase activity (1 having the most activity per cell).

Sample #	Abs420	Abs550	Abs600	t (min)	v (uL)	Miller Units	Activity Rank
1	0.5	0.1	0.2	50	0.02		
2	0.75	0.1	0.2	50	0.02		
3	0.75	0.1	1	50	0.02		
4	1	0.2	0.1	10	0.02		
5	1	0.2	0.1	50	0.02		
6	1.5	0.5	1	100	0.02		
7	1.5	0.2	1	100	0.04		
8	1.75	1	1	20	0.04		

c. Enzyme Kinetics

i. Look up the Michaelis-Menten equation, write it below and explain what all of the terms mean. Briefly explain in your own words how this equation is derived.

ii. How does the Miller Equation (Part b) relate to this equation (Hint: The Miller Equation takes values we can read in lab and calculates something pertaining to the Michaelis-Menten equation. What is the result of the Miller Equation and how does this factor into Michaelis-Menten)?

4) How long did you spend on homework this week?