

## 7.03 Problem Set 4

Due before 5 PM on Friday, October 27

Hand in answers in recitation section or in the box outside of 68-120

**1.** In lecture we have seen in a qualitative way how different of Lac mutants behave. In this problem we will use some simple assumptions to develop a more quantitative description of Lac gene expression. Say that a wild type Lac<sup>+</sup> *E. coli* strain produces <1 unit of β-galactosidase when no inducer is present and 100 units of enzyme when an inducer such as IPTG is present. In addition assume that for merodiploids that carry two copies of the Lac operon that the total β-galactosidase is the sum of the enzyme expressed from each operon:

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	<1 unit	100 units
Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	<1 unit	200 units

Further assume that the amount of β-galactosidase expressed is inversely proportional to the activity of the Lac repressor protein. Thus a mutant in the promoter for the Lac I gene that expresses half of the amount of repressor protein (call this allele Lac I<sup>↓</sup>) will only give half the level of repression:

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
Lac I <sup>↓</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	50 unit	100 units
Lac I <sup>↓</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	<1 unit	200 units

**a)** Consider a Lac I<sup>d</sup> allele that interferes with the repressor headpiece binding to DNA but can still oligomerize. Assume that the subunits in a repressor tetramer mix at random and that a tetramer with one LacI<sup>d</sup> subunit has half the activity as a wild type tetramer and that tetramers with two or more LacI<sup>d</sup> subunits have no activity. Given these assumptions fill in the table below with the expected levels of β-galactosidase activity.

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
Lac I <sup>d</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		
Lac I <sup>d</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		

**b)** Now consider what would happen if you combined a Lac I<sup>s</sup> allele with a LacI<sup>d</sup> allele. Remember that a Lac I<sup>s</sup> mutation locks the repressor in a conformation where it binds tightly to the operator site regardless of whether inducer is present. Fill in the table below for this double mutant designated Lac I<sup>s-d</sup>.

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
Lac I <sup>s-d</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		
Lac I <sup>s-d</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		

**c)** Next consider what would happen if you combined a Lac I-↓ allele with a LacI<sup>d</sup> allele. This double mutant, designated LacI<sup>d</sup>-↓ should express LacI<sup>d</sup> protein at half the level as wild type. Fill in the table below.

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
LacI <sup>d</sup> -↓ O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		
LacI <sup>d</sup> -↓ O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		

**d)** Now you isolate a mutant in the promoter for the LacI gene that increases the amount of repressor protein ten-fold. For the purpose of this problem we will designate this allele is Lac I-↑, although in real life such alleles are called LacI<sup>q</sup>. Consider what would happen if you combined a Lac I-↑ allele with a LacI<sup>d</sup> allele. This double mutant, designated LacI<sup>d</sup>-↑ should express LacI<sup>d</sup> protein at ten times the level as wild type. By filling in the table below and comparing the results with **part c)** you should see a good example of how the degree to which an allele is dominant depends on the level of expression.

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
LacI <sup>d</sup> -↑ O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		
LacI <sup>d</sup> -↑ O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>		

**e)** Plasmid cloning vectors derived from R-factors usually are present at ten or more copies per cell. Imagine that you have cloned the Lac operon (without the LacI gene) into the vector pBR322. When this plasmid (pBR322 Lac O<sup>+</sup> Z<sup>+</sup> Y<sup>+</sup> A<sup>+</sup>) is in a wild type strain, you find to your surprise that although the operon contains an intact promoter and operator, the LacZ, LacY and LacA are not repressed properly. However when this plasmid is in a strain that carries a Lac I-↑ allele in the chromosome nearly normal regulation is restored.

	β-galactosidase activity	
	<u>- IPTG</u>	<u>+ IPTG</u>
Lac I <sup>+</sup> O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / pBR322 Lac O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	900 units	1000 units
LacI-↑ O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup> / pBR322 Lac O <sup>+</sup> Z <sup>+</sup> Y <sup>+</sup> A <sup>+</sup>	2 units	1000 units

Explain in simple qualitative terms why Lac I-↑ restores normal regulation to the Lac operon on a plasmid.

**2.** You are studying a new strain of *E. coli* that can utilize the disaccharide sucrose efficiently. You find that utilization depends on the enzyme sucrase, which is encoded by the gene *Suc1*. *Suc1* is not expressed unless sucrose is present in the growth medium.

**a)** You have isolated two mutations that prevent expression of sucrase, which you designate *SucA*<sup>-</sup> and *SucB*<sup>-</sup>. P1 phage mapping experiments using a Tn5 insertion linked to *SucA*<sup>-</sup> shows that the insertion is also linked to *SucB*<sup>-</sup>, but is not linked to *Suc1*. You construct an F' factor that carries the *SucA* *SucB* region of the chromosome and use this F' factor to perform a variety of tests shown below:

	sucrase activity	
	<u>- sucrose</u>	<u>+ sucrose</u>
wild type ( <i>Suc1</i> <sup>+</sup> )	-	+
<i>Suc1</i> <sup>-</sup>	-	-
<i>SucA</i> <sup>-</sup>	-	-
<i>SucA</i> <sup>-</sup> / F' <i>SucA</i> <sup>+</sup> <i>SucB</i> <sup>+</sup>	-	+
<i>SucB</i> <sup>-</sup>	-	-
<i>SucB</i> <sup>-</sup> / F' <i>SucA</i> <sup>+</sup> <i>SucB</i> <sup>+</sup>	-	+
<i>SucA</i> <sup>-</sup> <i>SucB</i> <sup>+</sup> / F' <i>SucA</i> <sup>+</sup> <i>SucB</i> <sup>-</sup>	-	+

Describe the basic genetic properties of the *SucA*<sup>-</sup> and *SucB*<sup>-</sup> mutations, explaining the rationale for your conclusions, and make a proposal for the type of regulatory functions affected by the *SucA*<sup>-</sup> and *SucB*<sup>-</sup> mutations.

**b)** Diagram two possible models for regulatory pathways for *Suc1* that can explain the behavior of the *SucA*<sup>-</sup> and *SucB*<sup>-</sup> mutations. For each model include a role for the inducer sucrose. Explain why or why not double mutant analysis could be used to distinguish between the two models.

**c)** Next, you isolate a third mutant, SucC<sup>-</sup>, which gives constitutive sucrose expression even in the absence of sucrose. The SucC<sup>-</sup> mutation is linked to the same Tn5 insertion described in **part a)** indicating that it is carried on the F' now designated F' SucA<sup>+</sup> SucB<sup>+</sup> SucC<sup>+</sup>. (although you should note that we do not know the order of the SucA<sup>+</sup> SucB<sup>+</sup> and SucC<sup>+</sup> alleles). Genetic tests of the SucC<sup>-</sup> mutation yield the following:

	sucrase activity	
	<u>- sucrose</u>	<u>+ sucrose</u>
SucC <sup>-</sup>	+	+
SucC <sup>-</sup> / F' SucA <sup>+</sup> SucB <sup>+</sup> SucC <sup>+</sup>	+	+
SucC <sup>-</sup> / F' SucA <sup>-</sup> SucB <sup>+</sup> SucC <sup>+</sup>	+	+
SucC <sup>-</sup> / F' SucA <sup>+</sup> SucB <sup>-</sup> SucC <sup>+</sup>	+	+

As above, classify the SucC<sup>-</sup> mutation in terms of its basic genetic properties and explain how you arrived at your conclusions.

**d)** Using the linked Tn5 you carry out two different P1 transduction experiments. You grow P1 on a Tn5 SucC<sup>-</sup> host and infect a SucA<sup>-</sup> recipient, selecting for Kan<sup>r</sup>. Among the Kan<sup>r</sup> transductants, about 10% show normally regulated sucrose expression, while the rest show either uninducible expression or half are constitutive for sucrose expression. When you use the same P1 lysate to infect a SucB<sup>-</sup> recipient, among 1000 Kan<sup>r</sup> transductants, about half are uninducible and half are constitutive for sucrose expression, but none show normally regulated sucrose expression. What do these linkage experiments tell you about the SucC<sup>-</sup> mutation. Be as specific as possible.

**e)** Finally, you construct a SucA<sup>-</sup> SucC<sup>-</sup> double mutant by P1 transduction (in real life this would not be trivial since there is no way to know a priori what the phenotype of this double mutant would be and you may want to think about how you might screen transductants for the double mutant). You find that the SucA<sup>-</sup> SucC<sup>-</sup> double mutant gives constitutive sucrose expression. Now using all of the information you have diagram the entire pathway for SucI regulation indicating the function of each of the elements affected by the SucA<sup>-</sup>, SucB<sup>-</sup>, and SucC<sup>-</sup> mutations and the inducer sucrose.