7.03 Problem Set 4

Due before 5 PM on Monday, October 30

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 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+ *E. coli* strain produces <1 unit of ß-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 ß-galactosidase is the sum of the enzyme expressed from each operon:

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac I^+ O^+ Z^+ Y^+ A^+	<1 unit	100 units
Lac I^+ O^+ Z^+ Y^+ A^+ / F' Lac I^+ O^+ Z^+ Y^+ A^+	<1 unit	200 units

Further assume that the amount of ß-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-$) will only give half the level of repression:

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac I-$ O^+ Z^+ Y^+ A^+	50 unit	100 units
Lac I-$ O^+ Z^+ Y^+ A^+ / F' Lac I^+ O^+ Z^+ Y^+ A^+	<1 unit	200 units

a) Consider a Lac I^{-d} 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^{-d} subunit has half the activity as a wild type tetramer and that tetramers with two or more LacI^{-d} subunits have no activity. Given these assumptions fill in the table below with the expected levels of ß-galactosidase activity.

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac I^{-d} O^+ Z^+ Y^+ A^+	**100 units**	**100 units**
Lac I^{-d} O^+ Z^+ Y^+ A^+ / F' Lac I^+ O^+ Z^+ Y^+ A^+	**~163 units**	**200 units**

Wildtype strains will always fully express ß-gal when an inducer (like IPTG) is present. When the inducer is absent, the lac repressor will (normally) bind to the operator of the lac operon and prevent expression. Dominant negative mutations in the lac repressor prevent the repressor from binding to the operator even in the absence of the inducer, due to a change in the operator binding site in the lac repressor gene product. Thus, the LacI^{-d} gene product cannot repress ß-gal expression and a constitutive phenotype is observed.

For a strain containing both Lac I^{-d} and Lac I^+ in the absence of the inducer:

In this particular case, the ratio of wild type Lac I gene product to mutant Lac I is 1:1, so each subunit in a given tetrameric repressor has a 50% chance of being normal and a 50% chance of being mutant.

The probability of a tetramer having four normal subunits is $(1/2)^4 = (1/16)$

The probability of a tetramer having three normal subunits and one mutant subunit is $4*(1/2)^1*(1/2)^3 = (4/16)$ (by binomial expansion)

The problem says that tetramers with 4 normal subunits have full repression activity, tetramers with 3 normal subunits have ½ of normal repression activity, and tetramers with 2 or more mutant subunits have no repression ability. The total probability of wildtype repression is, then, (1)*(1/16) + (1/2)*(4/16) = (3/16) (the sum of each probability x the amount of wildtype activity)

So, the probability of ß-galactosidase expression is 1 – 3/16 = 13/16, and the total amount of ß-galactosidase expression is (13/16)*200 units = 162.5 units.

b) Now consider what would happen if you combined a Lac I^s allele with a $LacI^{-d}$ allele. Remember that a Lac I^s 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^{s-d}.

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac I^{s-d} O^+ Z^+ Y^+ A^+	**100 units**	**100 units**
Lac I^{s-d} O^+ Z^+ Y^+ A^+ / F' Lac I^+ O^+ Z^+ Y^+ A^+	**~163 units**	**200 units**

If a Lac I^s allele is combined with a $LacI^{-d}$ allele, then the Lac repressor protein expressed from this gene will be unable to bind both the inducer and the operator. However, if the strain is unable to bind to the operator (the effect of the $LacI^{-d}$ allele), then the inducer has no real effect on the repressor. As such, a Lac I^s allele has no additional effect on a $LacI^{-d}$ allele, and expression patterns will be similar to those in part 1a).

c) Next consider what would happen if you combined a Lac I-$ allele with a $LacI^{-d}$ allele. This double mutant, designated $LacI^{-d}$ -$ should express $LacI^{-d}$ protein at half the level as wild type. Fill in the table below.

	ß-galactosidase activity	
	– IPTG	+ IPTG
$LacI^{-d}$ -$ O^+ Z^+ Y^+ A^+	**100 units**	**100 units**
$LacI^{-d}$ -$ O^+ Z^+ Y^+ A^+ / F' Lac I^+ O^+ Z^+ Y^+ A^+	**~121 units**	**200 units**

Again, the method and reasoning for this problem are equivalent to part 1a). The Lac I-$ allele just alters the mutant protein levels, which changes the probabilities.

For a strain containing both Lac I^{-d}-$ and Lac I^{+} in the absence of the inducer:

The mutant to wild-type subunit ratio is now 1:2

The probability of a tetramer having four normal subunits is now $(2/3)^4 = (16/81)$

The probability of a tetramer having three normal subunits and one mutant subunit is now
$4*(1/3)^1*(2/3)^3 = (32/81)$ (by binomial expansion)

The total probability of wildtype repression is, then, (1)*(16/81) + (1/2)*(32/81) = (32/81) (the sum of each probability x the amount of wildtype activity)

So, the probability of non-repression, or ß-galactosidase expression, is 1 – 32/81 = 49/81, and
the total amount of ß-galactosidase expression is (49/81)*200 units = 120.987654321 units.

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 $LacI^{q}$. Consider what would happen if you combined a Lac I-# allele with a $LacI^{-d}$ allele. This double mutant, designated $LacI^{-d}$ -# should express $LacI^{-d}$ 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.

	ß-galactosidase activity	
	– IPTG	+ IPTG
$LacI^{-d}$ -# O^{+} Z^{+} Y^{+} A^{+}	**100 units**	**100 units**
$LacI^{-d}$ -# O^{+} Z^{+} Y^{+} A^{+} / F' Lac I^{+} O^{+} Z^{+} Y^{+} A^{+}	**~200 units**	**200 units**

The method and reasoning for this problem are equivalent to part 1a). The Lac I-# allele also just alters the mutant protein levels, which changes the probabilities.

For a strain containing both Lac I^{-d}-# and Lac I^{+} in the absence of the inducer:

The mutant to wild-type subunit ratio is now 10:1

The probability of a tetramer having four normal subunits is $(1/11)^4 = \sim 0$

The probability of a tetramer having three normal subunits and one mutant subunit is $4*(10/11)^1*(1/11)^3$, which is also approx. 0.

Thus, there is almost no wildtype-like regulation, and even in the presence of a Lac I^{+} allele, a
$LacI^{-d}$ -# allele results in constitutive expression.

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^+ Z^+ Y^+ A^+) 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.

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac I^+ O^+ Z^+ Y^+ A^+ / pBR322 Lac O^+ Z^+ Y^+ A^+	900 units	1000 units
LacI-# O^+ Z^+ Y^+ A^+ / pBR322 Lac O^+ Z^+ Y^+ A^+	2 units	1000 units

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

Essentially, the normal levels of the LacI gene product (the Lac Repressor) are insufficient to repress every copy of the Lac operon present in the cell, because there are approximately ten copies of the plasmid in each cell. However, the Lac-# allele, which results in ten times as much of the repressor gene product, provides sufficient capability to repress the approximately ten copies of the Lac operon present in the cell.

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$^-$and SucB$^-$. P1 phage mapping experiments using a Tn5 insertion linked to SucA$^-$ shows that the insertion is also linked to SucB$^-$, 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		
	– sucrose	+ sucrose	**interpretation**
wild type (Suc1$^+$)	–	+	**sucrose is an inducer of the system**
Suc1$^-$	–	–	**Suc1- is uninducible**
SucA$^-$	–	–	**SucA- is uninducible**
SucA$^-$ / F' SucA$^+$ SucB$^+$	–	+	**SucA- is recessive**
SucB$^-$	–	–	**SucB- is uninducible**
SucB$^-$ / F' SucA$^+$ SucB$^+$	–	+	**SucB- is recessive**
SucA$^-$ SucB$^+$/ F' SucA$^+$ SucB$^-$	–	+	**SucA- and SucB- complement**

Describe the basic genetic properties of the SucA$^-$ and SucB$^-$ mutations, explaining the rationale for your conclusions, and make a proposal for the type of regulatory functions affected by the SucA$^-$ and SucB$^-$mutations.

SucA-: recessive, uninducible, trans-acting (positive regulator)
Because there is no sucrase activity in the presence or absence of sucrose in the SucA- mutant, we can conclude that the SucA- mutation produces an uninducible phenotype. That is, the system cannot be turned on in the presence or absence of inducer.

We know that SucA- is recessive based on the merodiploid, SucA$^-$ / F' SucA$^+$ SucB$^+$, which shows a phenotype of normal regulation. In this merodiploid, a wild-type copy of SucA is sufficient to restore normal regulation of Suc1 expression.

Finally, we can reason that SucA is trans-acting based on linkage analysis. Because the Tn5 insertion is linked to SucA but is unlinked to Suc1, we can conclude that SucA is unlinked to Suc1 as well. As a result, SucA must be a trans-acting factor.

Based on the conclusions above, SucA must encode for a net activator (positive regulator). The SucA- mutation must be a LOF mutation in the activator, resulting in an uninducible phenotype as is observed experimentally.

SucB-: recessive, uninducible, trans-acting (positive regulator)
Based on the same analysis as above, we can conclude that SucB- is recessive, uninducible, and trans-acting. As a result, SucB must also encode for a positive regulator.

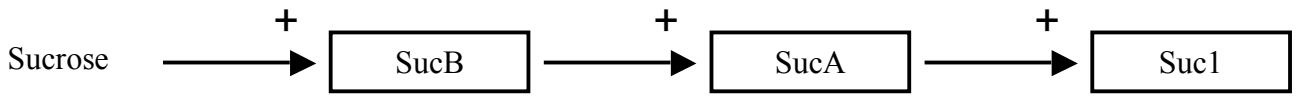
SucA- and SucB- are mutations in different genes:
Because SucA- and SucB- are recessive mutations, we can perform complementation analysis on the merodiploid, SucA$^-$ SucB$^+$/ F' SucA$^+$ SucB$^-$. Because this merodiploid strain displays a wild-type phenotype, SucA- and SucB- complement and are thus mutations in different genes.

b) Diagram two possible models for regulatory pathways for Suc1 that can explain the behavior of the SucA$^-$ and SucB$^-$ 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.

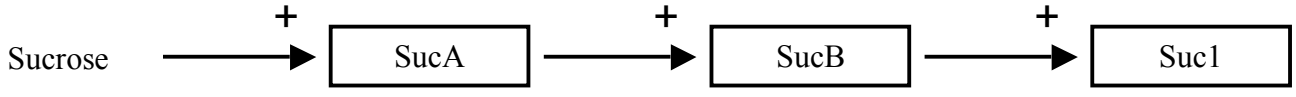
Based on the information provided in part (a), we know that SucA and SucB are both positive regulators. In addition, we know that sucrose is an inducer of the system.

It is important to note that we do not know the relative order of SucA and SucB. As a result, we can draw two possible models for the Suc1 regulatory pathway.

Model #1:



Model #2:



We cannot use double mutant analysis to distinguish between these two models because the SucA- and SucB- mutations have the same phenotype. In order to do epistasis analysis, the two mutations you are examining must have different phenotypes.

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

	sucrase activity		
	– sucrose	+ sucrose	**interpretation**
SucC$^-$	+	+	**SucC- is constitutive**
SucC$^-$ / F' SucA$^+$ SucB$^+$ SucC$^+$	+	+	**SucC- is dominant**
SucC$^-$ / F' SucA$^-$ SucB$^+$ SucC$^+$	+	+	
SucC$^-$ / F' SucA$^+$ SucB$^-$ SucC$^+$	+	+	

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

As indicated above, SucC- is a dominant mutation that produces a constitutive phenotype. We can conclude that SucC is a trans-acting factor because it is unlinked to Suc1. Based on this information, SucC- may be a mutation that results in a super-activator or a dominant-negative repressor. SucC may encode for a positive regulator or a negative regulator.

Complementation analysis cannot be employed here because SucC- is dominant.

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

SucC- encodes for a super-activator.

Cross #1:
In order to have a normally regulated Kan^r transductant, a crossover event must occur between SucA and SucC during homologous recombination between the host and recipient DNA. This occurs 10% of the time, indicating that SucA- and SucB- are not tightly linked. Based on the complementation analysis in part (a), we know that SucA and SucB are different genes.

Cross #2:
In order to have a normally-regulated Kan^r transductant, a crossover event must occur between SucB and SucC. No normally regulated transductants are observed. As a result, SucB- and SucC- must be tightly linked such that a crossover event between the two is extremely unlikely. The most likely scenario is that SucB- and SucC- are mutations in the same gene.

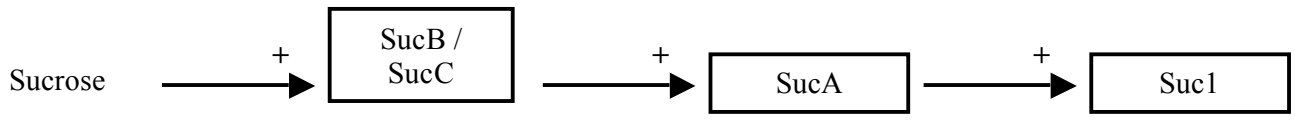
In part (a), we determined that SucB encodes for a net activator (positive regulator). Because the SucC- mutation is in the same gene as SucB-, we can conclude that SucC- encodes for a super-activator.

e) Finally, you construct a SucA$^-$ SucC$^-$ 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$^-$ SucC$^-$ double mutant gives constitutive sucrase expression. Now using all of the information you have diagram the entire pathway for Suc1 regulation indicating the function of each of the elements affected by the SucA$^-$, SucB$^-$, and SucC$^-$ mutations and the inducer sucrose.

There are two possible models to consider:

The double mutant, SucA- SucC-, gives constitutive sucrase expression. In epistasis analysis, the phenotype of the double mutant matches the phenotype of the downstream mutation. In this case, because SucC- gives constitutive sucrase expression, SucC must be downstream of SucA. Model #2 is correct.

Model #1:



Model #2: