7.03 Problem Set 4

Due before 5 PM on Friday, April 1

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

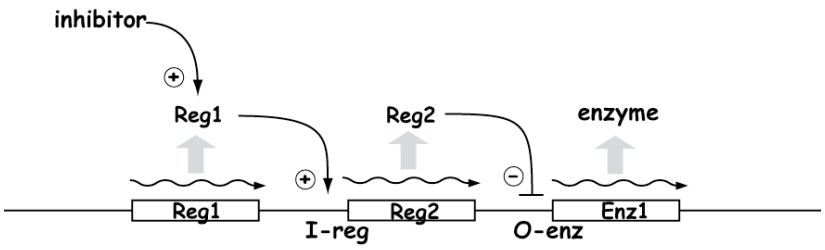
1. You have isolated a Tn5 insertion, which carries resistance to kanamycin (Kan^r), that is linked to the Lac operon and you wish to know on which side of the Lac operon the insertion lies (that is where the insertion lies relative to the known orientation of the Lac operon. Since you have available a $LacZ^-$ mutation (which gives uninducible ß-galactosidase expression) and $LacO^c$ mutation (which gives constitutive ß-galactosidase expression), you decide to perform a 3-factor cross to determine the relative order of $LacZ^-$ and $LacO^c$ with respect to the Tn5 insertion, realizing that this will give you the position of the insertion relative to the orientation of the Lac operon.

For the 3-factor analysis you set up two reciprocal crosses. First you grow P1 on a strain with the $LacO^c$ mutation and the Tn5 mutation and then use this lysate to infect a $LacZ^-$ mutant selecting for Kan^r. From 100 Kan^r transductants examined, 60 show uninducible ß-galactosidase expression, 36 show constitutive ß-galactosidase expression, and 4 show normally regulated ß-galactosidase expression. For the reciprocal cross, you grow P1 on a strain with the Lac Z^- mutation and the Tn5 insertion and then use this lysate to infect a $LacO^c$ mutant selecting for Kan^r. From 100 Kan^r transductants examined, 65 show uninducible ß-galactosidase expression and 35 show constitutive ß-galactosidase expression.

a) Use the principle of a 3-factor cross that the rare recombinant class (requiring four crossovers) will allow you to deduce the order of the markers. You will find this much easier if you diagram each cross for both possible orders of $LacO^c$ and $LacZ^-$ and then determine the expected phenotypes for each of the four possible recombinant classes (three from double crossovers and one from a quadruple crossover).

b) Next, use the same crosses to determine the distance between the Tn5 insertion and the $LacO^c$ mutation and between the Tn5 insertion and the $LacZ^-$ mutation. Express the measured distances as cotransduction frequencies.

2. The diagram below outlines the regulatory circuit in *E. coli* that controls transcription of the gene **Enz1**, which encodes an easily assayed enzyme. **Enz1** expression is shut off when an **inhibitor** molecule is present and is genetically controlled by two genes **Reg1** and **Reg2**. **Reg1** encodes a transcriptional activator molecule that when bound by **inhibitor** will bind to an initiator site on the **Reg2** gene (I-reg) and activates transcription of **Reg2**. **Reg2** encodes a repressor protein that binds to an operator for the **Enz1** gene (O-enz) and will repress transcription of **Enz1**.



Note that these genes lie very close together and all mutations in this region are very tightly linked by P1 transduction. You have at your disposal an F' factor carrying this region of the *E. coli* chromosome. You also have a recessive mutation in **Enz1** that does not express the enzyme and you have the capability of constructing a variety of strains with desired combinations of alleles on either the chromosome or F' factor.

For each of the mutants described below: i) give the expected phenotype with respect to **Enz1** expression (regulated, constitutive, or uninducible), ii) determine whether you would expect the mutant to be dominant or recessive, and iii) whether the mutation would be cis-acting or trans-acting with respect to **Enz1** expression. To answer ii) and iii) describe the strain(s) that you would construct to do the test and the expected outcome(s).

a) A "super-activator" allele of **Reg1** that causes the repressor to bind to I-reg even in the absence of inducer.

b) A loss of function allele of **Reg1**.

c) A dominant negative allele of **Reg1**.

d) A loss of function allele of **Reg2**.

e) A dominant negative allele of **Reg2**.

f) A mutation in **I-reg** that prevents **Reg1** binding.

g) A mutation in **O-enz** that prevents repressor binding.

3. You are studying a new *E. coli* strain that can grow on sucrose. In these cells sucrose utilization depends on the enzyme sucrase, encoded by the gene **Suc1**. You find that sucrase expression is negatively controlled by the presence of glucose in the growth medium and glucose is absent from the medium as shown below. You have a mutation in the **Suc1** gene itself (**Suc1**$^-$) that gives uninducible sucrase activity under all conditions.

	Sucrase activity	
	–glucose	+glucose
Suc1$^+$ (wild type)	+	–
Suc1$^-$	–	–

Next, you isolate a set of regulatory mutants by transposon mutagenesis.

a) Mutant **Suc2⁻::Tn5** was isolated by Tn5 insertion and gives constitutive expression of sucrase as shown below (this mutant is designated **Suc2⁻::Tn5** to show that it is caused by a Tn5 insertion).

	Sucrase activity	
	–glucose	+glucose
Suc2⁻::Tn5	+	+

You grow P1 phage on an **Suc2⁻::Tn5** mutant. After infection of an **Suc1⁻** mutant with the resulting phage lysate, you select for Kan^r. All of the Kan^r transductants exhibit uninducible sucrase activity under all conditions. Based on this information propose the type of regulatory functions affected by the **Suc2⁻::Tn5** mutation.

b) Mutant **Suc3⁻::Tn10** was isolated as a Tn10 insertion and gives uninducible expression of sucrase as shown below. You grow P1 phage on an **Suc3⁻::Tn10** mutant. After infection of an **Suc2⁻::Tn5** mutant with the resulting phage lysate, you select for Tet^r. All of the Tet^r transductants are also Kan^r, and exhibit constitutive expression of sucrase as shown below.

	Sucrase activity	
	–glucose	+glucose
Suc3⁻::Tn10	–	–
Tet^r Kan^r transductants	+	+

From all of the information at hand, draw a diagram for a regulatory pathway showing the normal function of both the **Suc2** and **Suc3** gene products, and the inhibitor glucose.

4. In the StarGenetics problem PS4-4 you will be analyzing a regulatory pathway that controls the expression of a **yeast** enzyme required for maltose utilization that is induced when maltose is present in the medium. To facilitate the study of this enzyme, the LacZ gene has been fused to the gene for the enzyme so that the enzyme ß-galactosidase is expressed under exactly the same conditions for which the enzyme would be expressed. Expression of ß-galactosidase can be detected by blue color that forms when yeast are plated on medium that has X-Gal (you can choose regular X-Gal plates or X-Gal + Mal, which has the inducer sucrose added). You are provided with MAT**a** and MATalpha strains carrying this LacZ reporter construct (labeled "Rep a" and "Rep alpha"). You have isolated three mutants in the reporter strain (MAT**a** genetic background) that fail to express ß-galactosidase (labeled Mut1, Mut2, and Mut3). Finally, you have isolated a revertant of Mut1 strain that gives constitutive expression of ß-galactosidase (labeled Rev1). All of these mutants carry a leu2 mutation. You are also provided with wild type yeast strains for mating type testing (no reporter) that carry a lys9 mutation (these strains are labeled MATa and MATalpha).

To analyze the regulatory circuit that controls enzyme regulation you will need to perform appropriate crosses to determine whether the regulatory mutations are dominant or recessive and whether they are linked to the reporter gene or not (i.e. whether they are cis- or trans- acting with

respect to reporter expression). In addition you will need to perform the appropriate double mutant analysis to determine the order of action of the relevant genes. A crucial part of this analysis will be to separate the revertant mutation (which is constitutive) from the original uninducible Mut1 mutation. You will then need to ascertain the linkage relationship and epistasis relationship between the revertant ans each of the original uninducible mutants (Mut1, Mut2, and Mut3). In the end you should be able to construct a model for enzyme regulation that explains the behavior of all of the relevant gene products assuming that they work together in a single linear pathway. Your model should include an explanation for the behavior of the revertant mutation and for the inducer maltose.