7.03 Problem Set 4

Due before 5 PM on Thursday, October 23

Hand in answers in recitation section or in the box outside the class

1. **(a)** You have isolated two mutations in the Lac operon that cause constitutive expression of Lac genes. You designate these mutants Lac1$^-$ and Lac2$^-$. Making use of an F' that carries the Lac operon with the LacY gene mutated, you construct strains that you test for both ß-galactosidase activity and Lac permease activity with results shown below.

	ß-galactosidase activity		Lac permease activity	
	– IPTG	+ IPTG	– IPTG	+ IPTG
Lac 1$^-$ Lac Z$^-$ / F' LacY $^-$	–	+	+	+
Lac 2$^-$ Lac Z$^-$ / F' LacY $^-$	+	+	+	+

Classify each mutation as dominant or recessive and as cis- or trans-acting, giving the experimental result that allows you to arrive at each conclusion. Finally, deduce what type of Lac mutation best fits the properties of Lac 1$^-$ and of Lac 2$^-$.

(b) The first Lac promoter mutations that were isolated were "leaky" mutations that decreased, but did not entirely eliminate, the promoter function. The table below shows the behavior of such mutants giving the quantity of ß-galactosidase activity and Lac permease activity produced.

	ß-galactosidase activity		Lac permease activity	
	– IPTG	+ IPTG	– IPTG	+ IPTG
Lac$^+$ (wild type)	2	100	2	100
LacP$^-$	1	8	1	10

The LacP$^-$ mutations map very close to LacO^c nevertheless researchers concluded that LacP and LacO represent functionally distinct sites on the DNA. Explain how the results shown above would lead to such a conclusion.

(c) You have isolated two mutations that show decreased expression of the Lac operon. However, unlike like the promoter mutations described in part (b) these mutations don't respond to the inducer IPTG. These mutations, designated Lac3$^-$ and Lac4$^-$, are evaluated for the quantity of ß-galactosidase and permease activity expressed with or without IPTG:

	ß-galactosidase activity		Lac permease activity	
	– IPTG	+ IPTG	– IPTG	+ IPTG
Lac 3$^-$	12	12	12	12
Lac 3$^-$ / F' Lac I$^+$LacZ $^-$	1	12	na	na
Lac 4$^-$	8	8	9	9
Lac 4$^-$ / F' Lac I$^+$LacZ $^-$	8	8	na	na
			(na = not assayed)	

Mapping experiments reveal that Lac3$^-$ and Lac4$^-$ are different short deletions located in the region before the start of the LacZ gene. Given the data shown above suggest which genetic element(s) in addition to part of the promoter has been deleted in each mutant. Explain your reasoning.

2. You are studying a new strain of *E. coli* that can utilize the disaccharide melibiose very efficiently. You find that utilization depends on the enzyme melibiase, which is encoded by the gene Mel1. Mel1 is not expressed unless melibiose is present in the growth medium.

(a) You have isolated a mutation that causes constitutive melibiase activity, which you designate MelA$^-$. P1 phage mapping experiments using a Tn5 insertion linked to Mel1 show that MelA$^-$ is not linked to Mel1. Moreover you find that when an amber suppressor is introduced into a MelA$^-$ mutant, normal melibiase regulation is restored. Classify the MelA$^-$ mutation in terms of its basic genetic properties explaining the rationale behind your conclusions. Based on these properties make a proposal for the type of regulatory functions affected by the MelA$^-$ mutation.

(b) Next you isolate a mutation, designated MelB$^-$, which gives uninducible melibiase activity. Mapping experiments show that MelB$^-$ is linked to Mel1. Using an F' factor that carries the chromosomal region surrounding Mel1, you perform the following genetic tests:

	melibiase activity	
	– melibiose	+ melibiose
wild type (Mel1$^+$)	–	+
Mel1$^-$	–	–
MelB$^-$	–	–
MelB$^-$ / F' Mel$^+$	–	+
Mel1$^-$ / F' Mel$^+$	–	+
MelB$^-$ / F' Mel1$^-$	–	+

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

(c) Diagram two possible models for regulatory pathways for Mel1 that can explain the behavior of the MelA$^-$ and MelB$^-$ mutations. For each model include a role for the inducer melibiose.

(d) You next construct a MelA$^-$ MelB$^-$ double mutant, which gives the following behavior:

	melibiase activity	
	– melibiose	+ melibiose
MelA$^-$ MelB$^-$	–	–

Which of your two models is consistent with this new data?

(e) Next, you isolate a third mutant, MelC$^-$, which gives constitutive melibiase expression. The MelC$^-$ mutation is closely linked to Mel1 and MelB$^-$. Genetic tests of the MelC$^-$ mutation yield the following:

	melibiase activity	
	– melibiose	+ melibiose
MelC$^-$	+	+
MelC$^-$ / F' Mel$^+$	+	+
MelC$^-$ Mel1$^-$/ F' Mel$^+$	+	+
MelC$^-$ / F' Mel1$^-$	+	+

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

(f) A MelB$^-$ MelC$^-$ double mutant shows uninducible melibiase activity. Assuming that MelC$^-$ mutations affect the same gene as MelB$^-$ mutations, propose two different possible mechanisms to explain the behavior of MelC$^-$. Your answer should include a diagram showing the entire pathway for Mel1 regulation indicating the function of each of the elements affected by the MelA$^-$, MelB$^-$, and MelC$^-$ mutations and the inducer melibiose. Finally, propose some type of experiment(s) that would allow you to distinguish the two possible mechanisms.