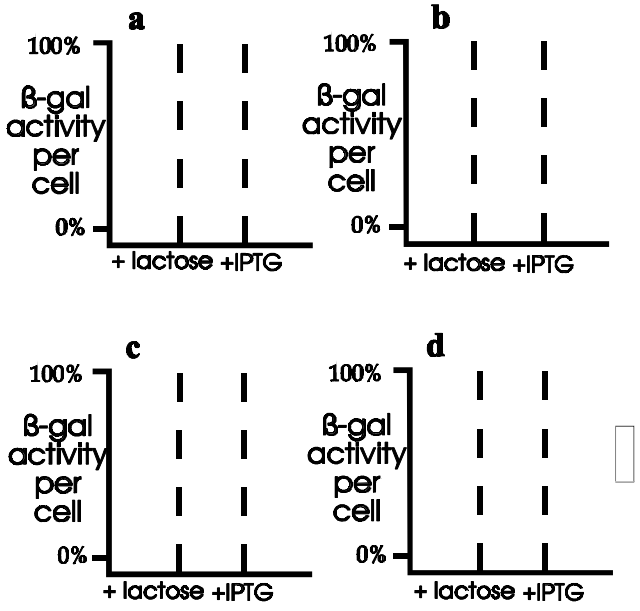
Bio 213 GENETICS (Fall 2000)
Problem Set 8: Transcription and Regulation (Prokaryotic)

8.1. Strains of *E. coli* were constructed by mating different F'*lac* plasmids into bacteria with various genetic compositions. The combinations are given below. For each, predict (by drawing on the graphs supplied) the ß-galactosidase activities that would result from the sequential addition of lactose and then IPTG. If a genotype is not given for a gene or element, then it is presumed to be wild-type(+).

a. $i^+ z^- y^+$ **F(**$i^- z^+ y^-$**)**
b. $i^- z^- y^-$ **F(**$i^+ o^c z^+ y^-$**)**
c. $i^- z^- y^+$ **F(**$i^- p^- z^+ y^+$**)**
d. $i^+ z^- y^-$ **F(**$i^- z^+ y^-$**)**



***8.2.** F'*lac* plasmids of different genotypes are mated into *E. coli* of different genotypes, as shown below. For each resulting strain, predict the β-galactosidase phenotype: answer **I** if β- galactosidase can be induced by IPTG, answer **C** if activity is constitutive, and answer **N** if there is no β-galactosidase activity under any circumstances. Predict also the growth phenotype: answer **Lac$^+$** if the strain can grow on lactose as the sole carbon source and **Lac$^-$** if it cannot. If a genotype is not given for a gene or element, then it is presumed to be wild-type (**+**).

a. $i^+z^-y^+ i^-z^+y^+$
b. $i^+z^+y^+ i^+o^cz^-y^+$
c. $i^+z^-y^+ i^-o^cz^+y^+$
d. $i^+z^-y^- i^-z^-y^+$
e. $i^-z^+y^- i^-z^+y^+$
f. $i^sz^+y^+ i^+o^cz^-y^+$
g. $i^+p^-z^+ i^-z^-$
h. $i^+o^cz^-y^+ i^+z^+y^-$
i. $i^+p^-o^cz^-y^+ i^+z^+y^-$
j. $i^-p^-o^cz^+y^+ i^-z^+y^-$

8.3. Static regulation (part I): Although we have focused on only the most common control point (transcriptional initiation) over gene expression, many other strategies are employed by cells. Just as RNA polymerase, Lac repressor, and CAP all have their specific binding sites on DNA, so do bacterial ribosomes bind to specific base sequences on mRNA. The ribosome looks for a specific sequence around six bases before the start codon to which it can attach. It attaches by base pairing (remember that ribosomes contain RNA). It pairs using a region near the 3' end of a piece of ribosomal RNA: ...AUUCCUCCA.... Examine the five genes within pUR3 (Appendix I of Lab 2) and find the one with the worst predicted ribosome site (with the fewest contiguous bases complementary to the ribosomal RNA region).

8.4. Static regulation (part II): Compare the sequences (Appendix I of Lab 2) of the promoters preceding *bla, lacI,* and *lacZ* with the consensus promoter sequence (Brooker, p.342). Which gene has the worst predicted promoter? (By the way, can you find a promoter preceeding *lacY*, and *lacA*? Why (not)?

8.5. Static regulation (part III): Expression of ß-galactosidase needs to vary according to needs, but some genes are needed at a continuously low or high level. Rationalize your answers to Problems 9.4 and 9.5 in terms of the cell's need for the protein encoded by the genes you considered.

8.6. Notice that in pUR3 (Appendix I of Lab 2) all five genes on the plasmid use the upper strand as the nontemplate strand (in bold). Coincidence? What would be the result if *lacY* used the lower strand? What if *lacI* used the lower strand?

8.7. Match the following mutant bacterial strains with mutations that may have caused the observed characteristics.

a. All twenty genes normally turned on by osmotic shock are not induced.

b. The *gal* operon, normally expressed only in the presence of galactose, is expressed whether or not galactose is present.

c. Several genes normally repressed by the presence of glucose are not expressed whether or not glucose is present.

d. ß-galactosidase is not made even though *lacZ* is transcribed normally.

e.The *glnA* gene, normally induced by nitrogen-starvation, cannot be induced under any condition (expression is constant and low). The strain is otherwise normal.

A. Mutation in a gene encoding a core subunit of RNA polymerase

B. Mutation in an operator

C. Mutation in a gene encoding a positive-acting regulator

D. Mutation in a binding site for a positive-acting regulator

E. Mutation in a gene encoding a repressor

F. Mutation in a ribosome-binding site

8.8. The gene *lacI*, encoding the Lac repressor, is right next to the *lac* operon. Do you think regulation would be affected if *lacI* were distant from the *lac* operon? Do you think regulation would be affected if the *lac* operator were distant from the *lac* operon?

8.9. *E.coli* is grown on lactose alone and then switched to a medium containing glucose but no lactose. ß-galactosidase activity is, of course, high prior to the switch, but it remains high for several hours afterwards. Why?

8.10. *E. coli* living its life out in an intestine is first bathed in a meal of pecan pie (high glucose), which after some time is washed down with lots of milk (low glucose, high lactose). Take the point of view of a molecule of lactose and describe its life

course from swimming freely in the intestine to its final metabolism. Considering the state of the *lac* operon in *E. coli* prior to the nutritional shift, what immediate problem must lactose face in order to accomplish its tasks? Speculate on how this problem is solved.

8.11. A single operon in the photosynthetic bacteria *Rhodobacter capsulata* contains genes encoding the photosynthetic reaction center and those encoding the much more plentiful antenna proteins (which funnel light energy to the reaction centers). Speculate on ways this difference in protein levels might be achieved.

***8.12.** Consider the following information about wild-type *E. coli* and a particular mutant strain:

A. The presence of the sugar galactose induces in the wild type strain the expression of three genes: *galE, galT,* and *galK*.

B. The mutant has very low levels of expression of all three genes, whether or not galactose is present.

C. When an F-plasmid carrying wild-type copies of all three genes (plus adjacent DNA) is introduced into the mutant, the resulting strain still has very low levels of expression (as in **B**).

D. The mutant (without F-plasmid) is exposed to a mutagen, producing a second mutation in the strain. The resulting strain now expresses high levels of the three genes, even in the absence of galactose.

Indicate which of the above statements support (are consistent with), oppose (contradict) , or are uninformative about each of the hypotheses below:

Hypothesis	Supports	Opposes	Neutral
Example: *galE, T,* and *K* are part of an operon.	ABD		C
a. The original mutant has a defective promoter.			
b. The original mutant has a defective operator.			
c. The original mutant has a defective repressor.			
d. (Insert your own plausible hypothesis here)			

e. Using *lac* as a model, make up names for the two mutations described in **B** and **D**.

***8.13.** The region upstream from the *lac* operon of *E. coli* was mutated such that the CAP binding site could no longer bind CAP. The mutant was called *L8.*

a. What expression of ß-galactosidase would you expect from mutant *L8*?

A. No expression
B. Low expression without lactose. No different with lactose.
C. Low expression without lactose. Higher expression with lactose, but still low.
D. Low expression without lactose. High expression with lactose.
E. High expression without lactose. No different with lactose.
F. High expression without lactose. Even higher with lactose.

Mutant *L8* was mutagenized a second time to increase the maximal level of expression. This mutant was called *L8UV5*. In 1997, Kimata et al reported the results of their study of this strain and the basis of diauxic growth (see Figure below).

b. The level of ß-galactosidase activity shown represents:

A. The amount of lactose that enters the cell and is broken down
B. The amount of exogenously supplied substrate (e.g. ONPG) that is broken down by enzyme liberated from broken cells
C. The amount of enzyme that is not bound to repressor
D. The amount of ß-galactosidase protein present in the cell

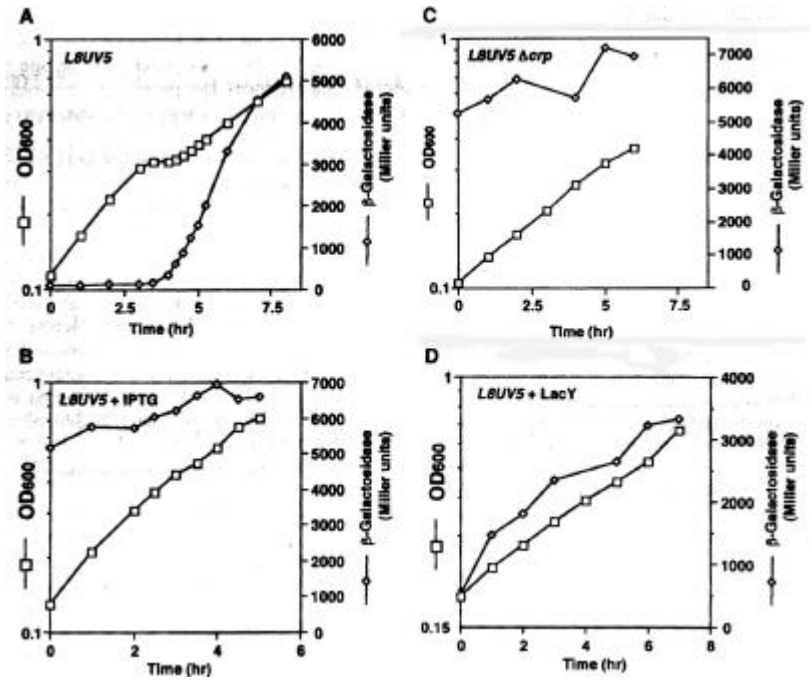


Figure for Problem 9.13. An *E. coli* strain carrying the *L8* and *UV5* mutations was grown with a mixture of glucose and lactose. Square symbols represent measurements of the optical density of the suspension, indicative of the amount of cell growth. Diamond symbols represent measurements of ß-galactosidase activity. (**A**) No addition. (**B**) IPTG added at 0 hrs. (**C**) Strain also has *crp* mutation and is unable to make CRP protein. (**D**) Strain carries a plasmid with a *lacY* gene. This strain makes significantly more Lac permease than normal

For questions **c**-**f**, indicate which of the graphs support (are consistent with), oppose (contradict), or are uninformative about each of the hypotheses below. Consider each graph to stand alone.

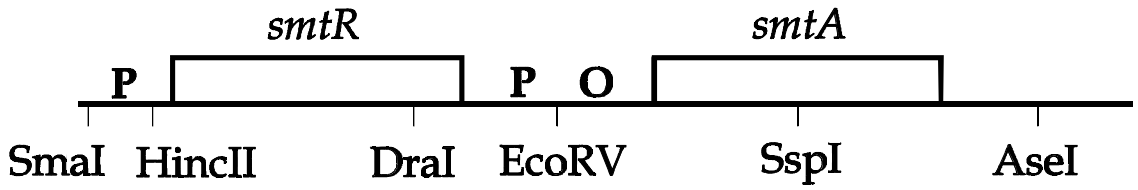
c. The diauxic effect, the sequential utilization of sugars in a mixture, is mediated by the binding of CAP to a site near the *lac* promoter.

d. The presence of CAP protein is essential to the diauxic effect.

e. The binding of repressor to the *lac* operator is required for the diauxic effect.

f. Diauxie occurs because glucose decreases the transport of lactose into the cell, enabling the Lac repressor to maintain residence on the operator.

8.14. You want to market a home testing unit that will enable households living nearby mining operations to monitor minute levels of toxic heavy metals. Needless to say, your customers will want to know about heavy metals before they reach levels that are lethal. Your plan is to make use of a gene from the bacterium *Synechococcus* PCC 7942, which is highly resistant to heavy metals. *Synechococcus* achieves its resistance by producing in large quantitities a protein, metallotheionein, that binds to heavy metals and prevents them from acting on the cell. The protein is encoded by *smtA*. *smtA* is preceded by *smtR*, which encodes a repressor of the gene. You have cloned the *smt* region and from the sequence and other experiments deduced the following map:



Your goal is to clone a portion of this region into pUR3 in such a way that the resulting plasmid could be inserted into *Synechococcus* and used to detect heavy metals. The test you envision is that the customer adds suspect water to a test tube containing *Synechococcus* carrying the plasmid you've made. Then, the customer adds ONPG. The test is positive or negative depending on the resulting color of the liquid in the tube.

Describe in detail how you would make the desired plasmid and what problems you might encounter in using it successfully. For example, what might cause the plasmid in *Synechococcus* to give false positives or negatives?