

## 2005 7.03 Problem Set 4

Due before 5 PM on FRIDAY, October 28, 2005.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

**1.** For each of the following merodiploid strains, predict the number of units of enzyme activity that will be displayed by a strain of the given genotype, grown under the given conditions. Assume that, when no repressor is bound to DNA, 100 units of  $\beta$ -galactosidase activity are produced from each functional copy of the **LacZ** gene. Assume that, when repressor is fully bound to DNA, only 1 unit of enzyme is produced for each functional copy of **LacZ**. The presence of **Lac I<sup>d</sup>** protein will fully prevent any other forms of the repressor in the same cell from binding to DNA. The strain genotypes are written in the following format:

on the chromosome/ on the F' factor

**\*\*Note** that whenever strains are denoted this way anything not listed on the chromosome is wild-type, and anything not listed on the F' factor is absent from that plasmid.\*\*

	β-galactosidase activity	
	-IPTG	+IPTG
Lac O <sup>-</sup> P <sup>-</sup> Z <sup>+</sup> / F' Lac I <sup>-d</sup> O <sup>+</sup> P <sup>+</sup> Z <sup>+</sup>	_____	_____
Lac Y <sup>-</sup> / F' Lac I <sup>-</sup> O <sup>+</sup> P <sup>+</sup> Z <sup>+</sup>	_____	_____
Lac I <sup>-d</sup> / F' Lac I <sup>+</sup> O <sup>+</sup> P <sup>+</sup> Z <sup>+</sup>	_____	_____
Lac I <sup>-</sup> / F' Lac I <sup>S</sup>	_____	_____
Lac O <sup>-</sup> Z <sup>+</sup> / F' Lac I <sup>-</sup> O <sup>+</sup> P <sup>+</sup> Z <sup>+</sup>	_____	_____
Lac P <sup>-</sup> Z <sup>-</sup> / F' O <sup>+</sup> P <sup>+</sup> Z <sup>+</sup>	_____	_____

**2.** You are studying the regulation of a bacterial gene that encodes a toxin that is necessary for the successful infection of mice by the bacteria. This gene is turned on by a signal that is produced by the bacteria only when they are at a high enough cell density to successfully infect and colonize a mouse. You name the toxin-encoding gene *virR*.

**(a)** Why does it make sense for the bacterium to keep expression of the VirR protein off when the bacteria are growing at low cell density? (Think in terms of why it might be that genes are regulated at all, as opposed to having all genes be constitutively expressed.)

You isolate four strains of this bacterium, each of which harbors a single mutation: *virW*<sup>-</sup>, *virX*<sup>-</sup>, *virY*<sup>-</sup>, or *virZ*<sup>-</sup>. The *virX*<sup>-</sup> and *virY*<sup>-</sup> mutations cause *virR* to be constitutively expressed (regardless of whether the bacteria are at high cell density or low cell density). The *virW*<sup>-</sup> and *virZ*<sup>-</sup> mutations prevent all *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You make the following strains and note their phenotype:

Strain #	Genotype	Expression of VirR:	
		At low cell density	At high cell density
1.	Z <sup>-</sup> / F' Z <sup>+</sup>	off	on
2.	Y <sup>-</sup> R <sup>-</sup> / F' R <sup>+</sup>	on	on
3.	Y <sup>-</sup> Z <sup>-</sup>	off	off
4.	X <sup>-</sup> / F' X <sup>+</sup> R <sup>-</sup>	off	on
5.	W <sup>-</sup> Z <sup>+</sup> / F' W <sup>+</sup> Z <sup>-</sup>	off	on
6.	W <sup>-</sup> / F' W <sup>+</sup>	off	on
7.	X <sup>-</sup> W <sup>-</sup>	on	on
8.	Z <sup>-</sup> / F' Z <sup>+</sup> R <sup>-</sup>	off	on
9.	X <sup>-</sup> / F' X <sup>+</sup>	off	on
10.	X <sup>-</sup> Z <sup>-</sup>	on	on

You find that the *virW* and *virY* loci are very tightly linked to each other by cotransduction mapping, and then you do DNA sequencing and find that these two mutations are two alleles of the same gene.

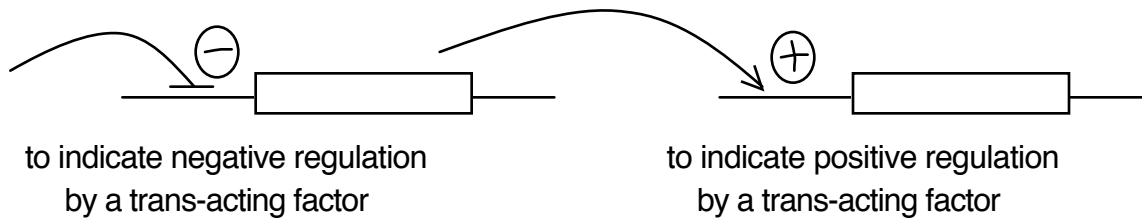
**(b)** Classify the *virX*<sup>-</sup> mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

**(c)** Classify the *virY*<sup>-</sup> mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

**(d)** Classify the *virZ*<sup>-</sup> mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

**(e)** Which strains will you use to order *virX*, *virY*, and *virZ* in a pathway for *virR* regulation? List all of the strains you will use by strain number.

**(f)** Given all of the information in this question, draw a linear genetic pathway that shows the pathway by which the *virR* gene is regulated. Be sure to include the wild-type functions of *virR*, *virW*, *virX*, *virY*, and *virZ*. Also include the signal of high cell density. For each model, be sure to use the proper notation of:



**(g)** Which of the proteins (VirX, VirY, and/or VirZ) might potentially bind to DNA sequences in the *virR* gene that lie upstream of the *virR* coding region?

**(h)** In a sentence, describe the specific function that might be performed by the protein(s) listed in part **(g)** when they are bound to DNA sequences in the *virR* gene.

**3.** In the previous problem, you found two different mutant alleles (*virW*<sup>-</sup> and *virY*<sup>-</sup>) of one gene that regulates expression of the *virR* gene. The *virY*<sup>-</sup> mutation causes *virR* to be constitutively expressed, and the *virW*<sup>-</sup> mutation prevents *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You want to isolate a Tn5 Kan<sup>r</sup> transposon insertion that is linked to the *virW/Y* gene.

**(a)** Why might you want to isolate such a transposon insertion? What would an insertion like that help you do that you can't do currently?

You have a collection of 2,000 bacterial strains, each of which harbors a single Tn5 insertion somewhere in the genome. This collection is called a transposon library. If you grow P1 phage on this collection of bacteria, you can collect a group of phage, each of which contains a different piece of DNA from the transposon library. You then infect *virW*<sup>-</sup> bacteria with this group of phage, and then select for infected bacteria that are now Kan<sup>r</sup>.

**(b)** You screen through your new collection of bacteria, and find that one colony of bacteria now properly regulates the expression of *virR*. This colony harbors a Tn5 insertion near to the *virW/Y* gene. How would you measure the cotransduction distance between the Tn5 insertion and the *virY* locus? Fill in the blanks in the following paragraph to show what experiment you would do:

You grow P1 phage on bacteria of the genotype \_\_\_\_\_.

You use the resulting phage lysate to infect bacteria of the genotype \_\_\_\_\_.

You select for transductants that can grow on plates containing \_\_\_\_\_.

You then screen the transductants that grow on those plates for their ability to properly regulate *virR* expression. You screen 100 colonies, and find that 30 have the phenotype of \_\_\_\_\_, so you conclude that the cotransduction distance between Tn5 and *virY* is 30%.

You now do two different crosses to determine the order of the transposon insertion you have isolated, the *virW* locus, and the *virY* locus.

The first cross: You grow P1 phage on *virY*<sup>-</sup> bacteria containing the Tn5 insertion. You use the resulting phage lysate to infect *virW*<sup>-</sup> bacteria. You select for KanR transductants and find that 200 transductants grow. 62 of those express *virR* constitutively and 138 do not ever express *virR*.

The second cross: You grow P1 phage on *virW*<sup>-</sup> bacteria containing the Tn5 insertion you isolated. You use the resulting phage lysate to infect *virY*<sup>-</sup> bacteria. You select for KanR transductants and find that 200 transductants grow. 131 of those express *virR* constitutively, 65 do not ever express *virR*, and 4 properly regulate *virR* expression.

**(c)** Draw a map showing the relative order of the *virY* and *virW* loci and the site of the Tn5 insertion.

**(d)** Draw the recombination events that occurred between the transduced DNA and the bacterial chromosome to create a regulated transductant from the second cross. Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.

**(e)** What is the genotype of a transductant created by a quadruple crossover event between the transduced DNA and the bacterial chromosome in the second cross? Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.