

2005 7.03 Problem Set 4 KEY

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 β -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^{-d}** 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⁻ P⁻ Z⁺ / F' Lac I^{-d} O⁺ P⁺ Z⁺ 100 100

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will never be expressed because it has no functional promoter. There is also another LacZ reporter that is fully wild-type (O⁺ P⁺ Z⁺).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and dominant negative repressor floating around. The dominant negative repressor will “soak up” and inactivate any wild-type repressor, so there is actually no functional repressor in the cell.

Together, this means that there is one functional reporter gene, and no functional repressor protein. Thus the one functional reporter will always express b-gal.

Lac Y⁻ / F' Lac I⁻ O⁺ P⁺ Z⁺ 2 200

First consider what forms of the LacZ reporter are in the cell. In this cell, there are two LacZ reporters that are both fully wild-type (O⁺ P⁺ Z⁺).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and non-functional repressor floating around. The wild-type repressor can float around and bind to any LacZ reporter that has a functional operator sequence.

Together, this means that there are two functional reporter genes, and functional repressor protein. Thus the two functional reporters will both show normal inducible expression of b-gal.

Lac I^{-d} / F' Lac I⁺ O⁺ P⁺ Z⁺ 200 200

First consider what forms of the LacZ reporter are in the cell. In this cell, there are two LacZ reporters that are both fully wild-type (O⁺ P⁺ Z⁺).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and dominant negative repressor floating around. The dominant negative repressor will “soak up” and inactivate any wild-type repressor, so there is actually no functional repressor in the cell.

Together, this means that there are two functional reporter genes, and no functional repressor protein. Thus the two functional reporters will both always express b-gal.

Lac I⁻ / F' Lac I^S 1 1

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that is fully wild-type (O⁺ P⁺ Z⁺) on the chromosome.

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is non-functional repressor and super-repressor floating around. The super-repressor will always bind to all reporters in the cell, and constantly repress them.

Together, this means that there is one functional reporter gene, and super-repressor protein. Thus the one functional reporter will always be repressed.

Lac O⁻ Z⁺ / F' Lac I⁻ O⁺ P⁺ Z⁺ 101 200

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will always be expressed because it has no functional operator. There is also another LacZ reporter that is fully wild-type (O⁺ P⁺ Z⁺).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and non-functional repressor floating around. The wild-type repressor can float around and bind to any LacZ reporter that has a functional operator sequence.

Together, this means that there is one functional reporter gene and one constitutive reporter gene, and functional repressor protein. Thus the one functional reporter will show normal inducible regulation, and the other reporter will be constitutive.

Lac P⁻ Z⁻ / F' O⁺ P⁺ Z⁺

1

100

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will never be expressed because it has no functional promoter. There is also another LacZ reporter that is fully wild-type (O⁺ P⁺ Z⁺).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor being expressed from the chromosome.

Together, this means that there is one functional reporter gene, and functional repressor protein. Thus the one functional reporter will show normal inducible expression of b-gal.

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.)

It takes energy and resources to produce an mRNA and a protein from a gene, so an organism may want to express a gene only when it can be used, and may want to repress expression of that gene when it is useless.

In this case, when the bacteria are at a low cell density, the production of the toxin (VirR) is unnecessary because the bacteria aren't be able to successfully colonize the mouse at low cell density. This means the VirR product would be wasted if it was produced.

You isolate four strains of this bacterium, each of which harbors a single mutation: *virW*⁻, *virX*⁻, *virY*⁻, or *virZ*⁻. The *virX*⁻ and *virY*⁻ mutations cause *virR* to be constitutively expressed (regardless of whether the bacteria are at high cell density or low cell density). The *virW*⁻ and *virZ*⁻ 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 ⁻ / F' Z ⁺	off	on
2.	Y ⁻ R ⁻ / F' R ⁺	on	on
3.	Y ⁻ Z ⁻	off	off
4.	X ⁻ / F' X ⁺ R ⁻	off	on
5.	W ⁻ Z ⁺ / F' W ⁺ Z ⁻	off	on
6.	W ⁻ / F' W ⁺	off	on
7.	X ⁻ W ⁻	on	on
8.	Z ⁻ / F' Z ⁺ R ⁻	off	on
9.	X ⁻ / F' X ⁺	off	on
10.	X ⁻ Z ⁻	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⁻* 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.

Strain 9 shows that *virX⁻* is recessive & Strain 4 shows that *virX⁻* is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 9 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virX⁻* is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 4 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virX* passes the trans test.

(c) Classify the *virY⁻* 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.

Strain 6 shows that *virW⁻* is recessive & Strain 5 shows that *virW⁻* is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 6 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virW⁻* is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 5 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virW* passes the trans test.

virY and *virW* are alleles of the same gene. This means that, if *virW* is trans, then *Y* is also trans. Once you deduce that *virY⁻* is dominant, Strain 2 confirms that *virY⁻* is trans, but this is not necessary.

The *virW⁻* mutation gives a recessive phenotype of uninducible. This means that the wild-type function of *virW* is that it is an activator protein. The fact that *virW⁻* is recessive tells us that *virW⁻* is a loss-of-function mutation, and we can deduce the wild-type function of a gene from loss-of-function mutations. Since *virY⁻* is an allele of a gene that encodes an activator protein, and *virY⁻* gives a constitutive phenotype, *virY⁻* must be a superactivator allele. Superactivator alleles are gain-of-function alleles that show dominant phenotypes.

(d) Classify the *virZ⁻* 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.

Strain 1 shows that *virZ⁻* is recessive & Strain 8 shows that *virZ⁻* is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 1 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virZ⁻* is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 8 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virZ* passes the trans test.

(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.

Strains 3, 7, and 10. We use double mutant strains to order genes into pathways – strains that lack function in two different genes are used for epistasis tests, and epistasis tests are how we order genes into pathways.

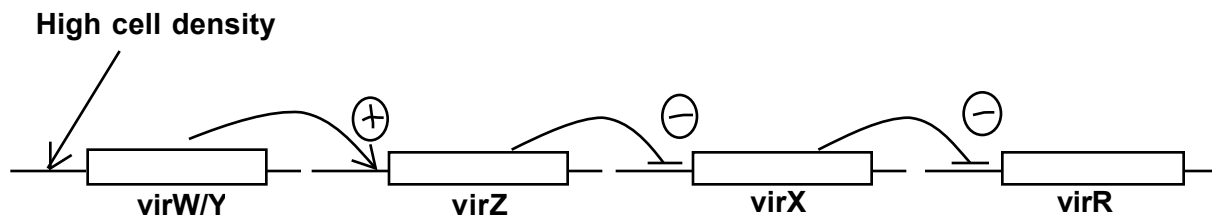
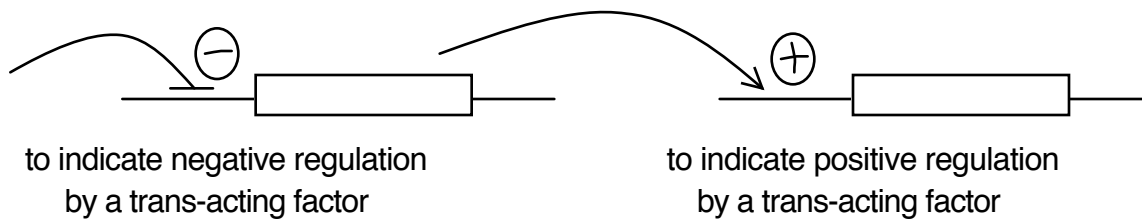
To get the order of the genes in the pathway:

Strain 7 and strain 10 both show the $virX^-$ phenotype so we know that $virX$ is downstream of both $virW/Y$ and $virZ$.

Strain 3 has the $virZ^-$ phenotype, telling you that $virZ$ is downstream of $virY/W$.

$VirW/Y$ must be the most upstream gene to act in the pathway.

(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:



To assign positive or negative signs to the arrows, we need to consider the net function (positive or negative) of each wild-type gene on $virR$. Do this by working from right to left, so that we start with the gene closest to $virR$.

$VirX$ is a net repressor because X is trans and, when you lose function in X , you get a constitutive phenotype.

$VirZ$ is a net activator because Z is trans and, when you lose function in Z , you get an uninducible phenotype. Since we know that $virX$ acts after $virZ$, then to get positive regulation of $virR$, $virZ$ must act negatively on $virX$.

VirW is a net activator because W is trans and, when you lose function in W, you get an uninducible phenotype. This means that virWY must act positively on virZ to get a final net output of positive regulation of virR.

Finally, high cell density acts positively on virR expression, so high cell density must act positively on virYW.

(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?

VirX

Since VirX acts last in the pathway, it is the only one with the potential to bind the DNA sequences that are upstream of the virR region. All other genes in the pathway operate indirectly on virR.

(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.

VirX acts negatively on VirR, so it is a repressor of VirR (as long as there are no further unknown regulatory components in the virR regulation pathway). Repressor proteins prevent RNA polymerase from binding to the promoter and thereby allowing transcription of RNA from a gene.

3. In the previous problem, you found two different mutant alleles (*virW*⁻ and *virY*⁻) of one gene that regulates expression of the *virR* gene. The *virY*⁻ mutation causes *virR* to be constitutively expressed, and the *virW*⁻ 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^r 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?

It would us to move the virW and virY loci from one strain to another. We can't select for virW and virY, so it is extremely hard to move the virWY gene around between cells. We need to move this gene around in order to map the virY and virW loci, and make strains with different genotypes at virW and virY. If we isolate a transposon that is linked to virWY, then we can move the transposon around by selecting for it, and since the transposon is linked to virWY, then virWY will come along with the transposon sometimes.

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*⁻ bacteria with this group of phage, and then select for infected bacteria that are now Kan^r.

(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:

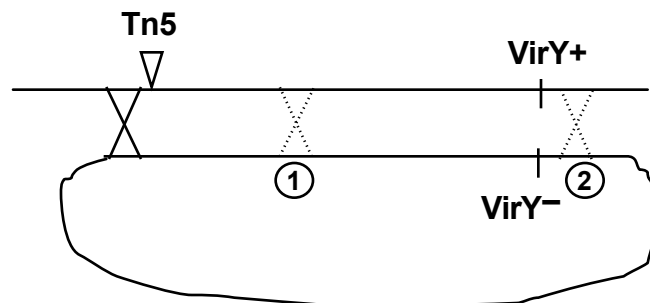
You grow P1 phage on bacteria of the genotype _____ **Tn5 (Kan^r) VirW/Y+** _____.

You use the resulting phage lysate to infect bacteria of the genotype _____ **VirY-** _____.

You select for transductants that can grow on plates containing _____ **kanamycin** _____.

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 _____ **regulated virR expression** _____, so you conclude that the

cotransduction distance between Tn5 and *virY* is 30%.



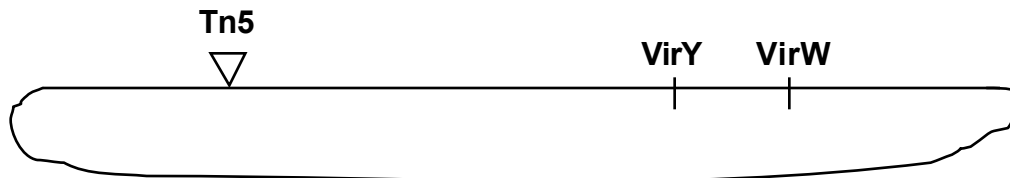
If the second Xover is at position 1, then VirY+ will not be cotransduced with the Tn, and you will get constitutive expression of virR. If the second Xover is at position 2, then VirY+ will be cotransduced with the Tn, and you will get regulated expression of virR.

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

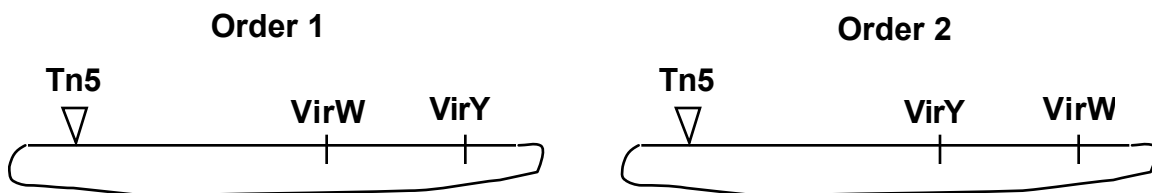
The first cross: You grow P1 phage on *virY*⁻ bacteria containing the Tn5 insertion. You use the resulting phage lysate to infect *virW*⁻ bacteria. You select for KanR transductants and find that 200 transductants grow. 62 of those express *virR* constitutively and 138 do not ever express *virR*.

The second cross: You grow P1 phage on *virW*⁻ bacteria containing the Tn5 insertion you isolated. You use the resulting phage lysate to infect *virY*⁻ 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.

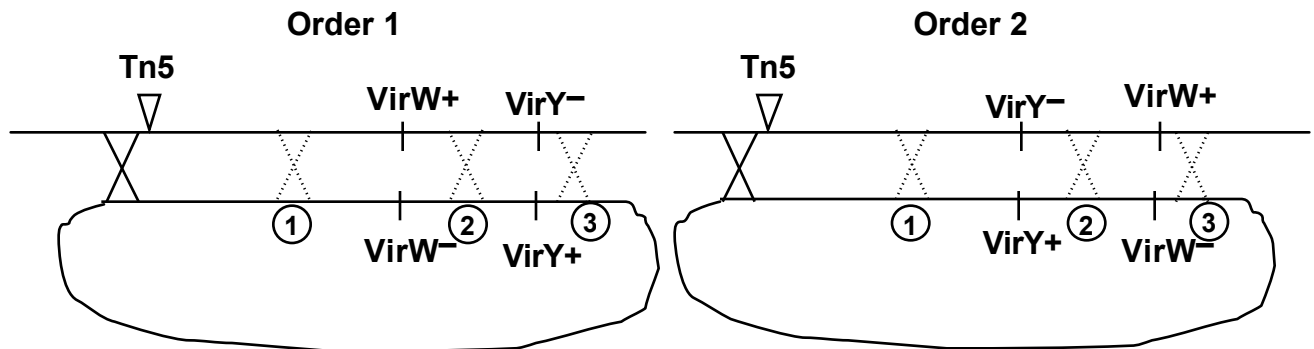
(c) Draw a map showing the relative order of the *virY* and *virW* loci and the site of the Tn5 insertion.



Since *VirW* and *VirY* are in the same gene, the transposon can't be between them. If the transposon were between them, then it would disrupt the function of the *virWY* gene. This is not possible because we isolated a colony in part (b) that contained the Tn but showed regulated expression of *VirR*. This means there are two possible remaining map orders:



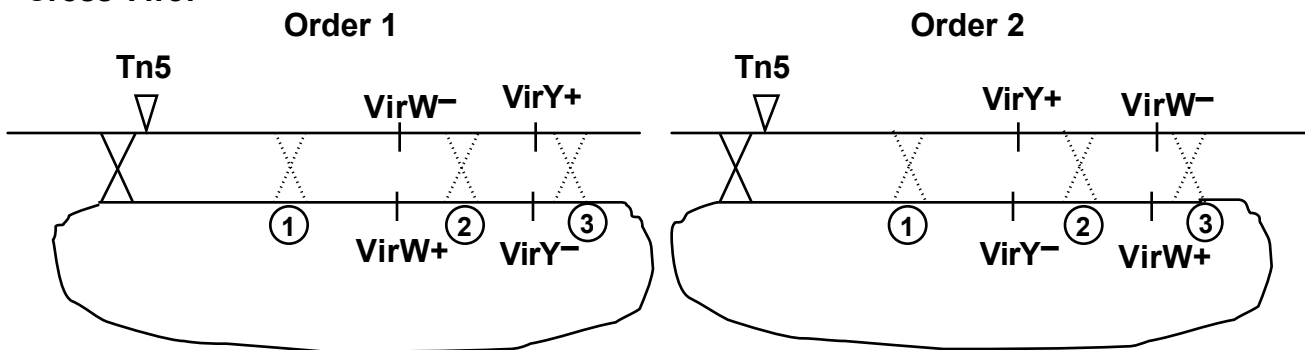
In each of the crosses, one of the crossovers must occur to the left of Tn5 because it is what we selected for. There are three possible locations for the other cross over events. Cross One:



Possible Genotypes from Cross 1:

The 2 nd crossover is at	For Order 1	For Order 2
1	Tn5, virW ⁻ , virY ⁺	Tn5, virW ⁻ , virY ⁺
2	Tn5, virW⁺, virY⁺	Tn5, virW ⁻ , virY ⁻
3	Tn5, virW ⁺ , virY ⁻	Tn5, virW ⁺ , virY ⁻
Quadruple Xover	Tn5, virW ⁻ , virY ⁻	Tn5, virW⁺, virY⁺

Cross Two:

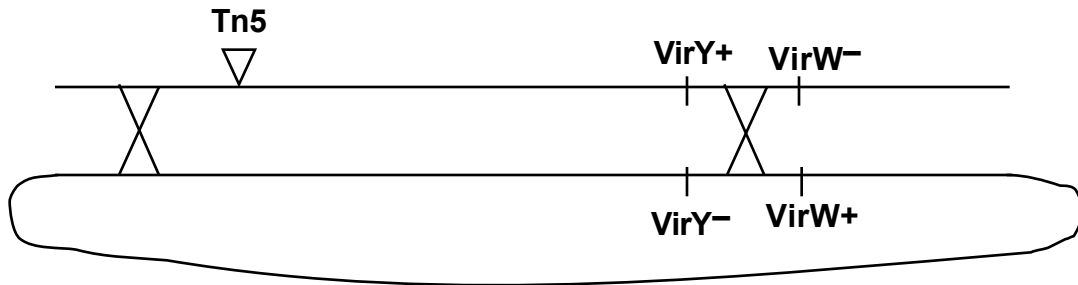


Possible Genotypes from Cross 2:

The 2 nd crossover is at	For Order 1	For Order 2
1	Tn5, virW ⁺ , virY ⁻	Tn5, virW ⁺ , virY ⁻
2	Tn5, virW ⁻ , virY ⁻	Tn5, virW⁺, virY⁺
3	Tn5, virW ⁻ , virY ⁺	Tn5, virW ⁻ , virY ⁺
Quadruple Xover	Tn5, virW⁺, virY⁺	Tn5, virW ⁻ , virY ⁻

If the Order One is correct, then there should be more wild-type transductants in cross 1 than in cross 2. If Order Two is correct, then there should be more wild-type transductants in cross 2 than in cross 1. This is because a quadruple crossover event is much less likely to occur than a double crossover event. Since the actual data tell us that cross 2 has more wild type transductants than cross 1, Order Two must be correct.

(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.

Tn5, VirY⁻, VirW⁻

