Name Answer Key

Student ID#_____

Bacterial Genetics,
BIO 4443/6443
Fall Semester 2003
Exam IV

You have isolated a strain of the naturally competent bacteria, Seracia marcessans from the stables of a horse farm that is resistant to three different antibiotics, chloramphenicol, penicillin, and rifampicin. Some concern is expressed from the horse farmers because all three antibiotics are used at the farm up the road as a supplement in the pig feed. They ask you to see if you can identify where the multiply resistant bacteria came from. All three resistance genes map to the same small region of the chromosome. To begin your characterization, you decide to determine the order three resistant genes on the chromosome using a three facor cross.

You prepare chromosomal DNA from the resistant strain of bacteria, and transform the DNA into a wild type strain that is susceptible to all three antibiotics.

Following transformation, you plate the cells on plates that contain chloramphenicol and select 200 of the colonies that grow.

You then take each of these colonies and replica plate them onto plates that contain either penicillin or rifampicin, keeping track of the genotype of each cell. For the 200 colonies that were Cam^R, you find they have the following genotype for the remaining two anitbiotics.

91 colonies are $Pen^S\ Rif^S$
53 colonies are $Pen^R\ Rif^R$
51 colonies are $Pen^S\ Rif^R$
5 colonies are $Pen^R\ Rif^S$

1.) What is the cotransformation frequency of *Pen* with *Cam*? (2pts)

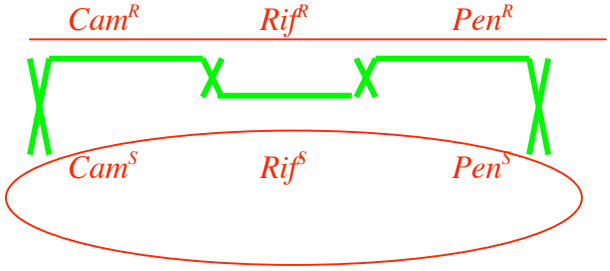
When Cam^R is selected for, how often does it also bring in Pen^R...
(53 + 5 Pen^R colonies) / (200 Cam^R colonies) =
58 Pen^R colonies / 200 Cam^R colonies =
0.29 Pen^R colonies/ Cam^R colony or *Pen* is 29% cotransducable with *Cam*

2.) What is the cotransformation frequency of *Rif* with *Cam*? (2pts)

When Cam^R is selected for, how often does it also bring in Rif^R...
(51 + 53 *Rif* colonies) / (200 Cam^R colonies) =
104 Rif^R colonies / 200 Cam^R colonies =
0.52 Pen^R colonies/ Cam^R colony or *Rif* is 52% cotransducable with *Cam*

3.) What is the most likely map order for these three genes? Show your reasoning. (6pts)

only 5 colonies had the rare phenotype of $Cam^R\ Pen^R\ Rif^S$, so this probably involved the most exchanges



This gene order requires that 4 exchanges must occur whereas the other two possible gene orders would only require 2 exchanges.

4.) For a typical lytic phage, describe two types of gene products that you would expect to be expressed early after infection? (4pts)

Gene products that are associated with directing transcription to the phage genes, phage DNA replication, proteins that shutdown the host's metabolism or degrade the host genome, proteins that prevent the degradation of the invading viral DNA.

5.) For a typical lytic phage, describe two types of gene products that you would expect to be expressed late after infection? (4pts)

Gene products that are associated with packaging the phage genomes into capsids, proteins that make up the head tail or fiber components of the phage capsids, proteins involved in cell lysis.

6.) Describe two properties of a phage's reproductive cycle that would make it desirable to use for generalized transduction? (4pts)

1. It should NOT degrade the host genome during its lytic cycle.
2. It should have a general or sloppy packaging system such as the "head full" system used by T4. Packaging systems that recognize specific sequences, such as the cos site of phage lambda, would generally reduce the frequency and randomness of packaging chromosomal fragments.

Most mutations that make *E. coli* resistant to the antibiotic rifampicin are found in the *rpoB* gene of the RNA polymerase. You have just isolated a new rif^R mutant and to your suprize, your conjugational mapping experiment suggests that the mutation is on the opposite side of the chromosome from the *rpoB* gene. Your mutation does map nearby both *pro* and *lac*, however. Since this represents a possibly novel mechanism of bacterial resistance, you decide to map your rif^R mutation in a little more detail and set up a transduction experiment.

To the best of your knowledge, your mutant is wild type for all other genes besides the rif^R mutation. You infect the mutant rif^R pro^+ lac^+ *E.coli* with P1 bacteriophage and make a P1 lysate.

You then use the lysate to infect a rif^S pro^- lac^- recipient and select for rif^R transductants. Of the rif^R transductants, you find that 80% are pro^+ and 20% are lac^+.

7.) What are the two possible orders that these three genes could be in? (4pts)

rif cotransduces with *pro* much more frequently than with *lac* so *pro* must be much closer to *rif* than *lac*. So it could be either...

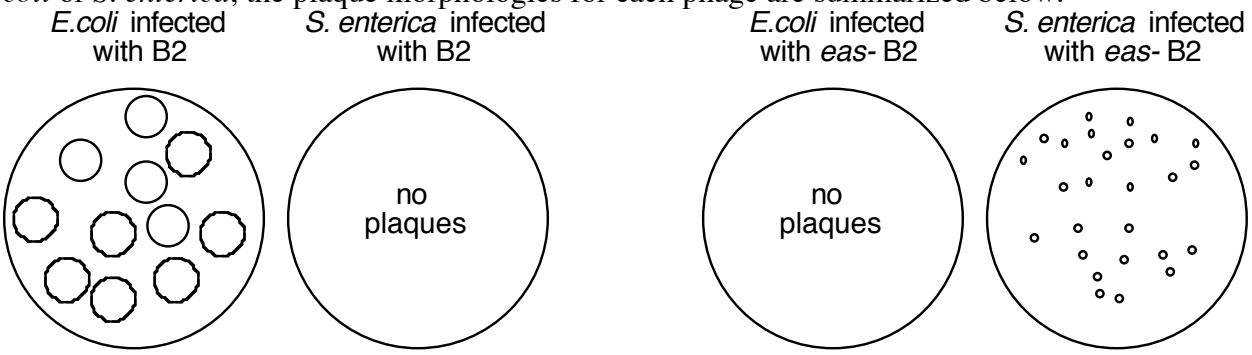
rif----pro----------lac or *pro----rif----------lac*

8.) You repeat the infection, this time selecting for lac^+ transductants. Of the lac^+ transductants, 20% are rif^R and 40% are pro^+.

Where does the rif^R gene map in relation to *pro* and *lac*? (4pts)

lac and *pro* cotransduce more frequently than do *lac* and *rif*. Looking at the above two choices, only the first one *rif----pro----------lac* places the *pro* closer to *lac* than *rif* is to *lac*.

Normally Bacteriophage B2 only infects and replicates in *Escherichia coli*. However, after using ethidium bromide to mutagenize the phage, you have isolated a mutant, *eas1,* that is no longer able to replicate in *Escherichia coli*, but it now can replicate and lyse *Salmonella enterica,* although the plaques it forms appear smaller. When you let the phage infect either *E. coli* or *S. enterica*, the plaque morphologies for each phage are summarized below.

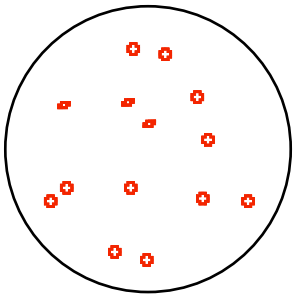


9.) You decide to determine how many genes might be involved in determining the host restriction in this phage. As a first step, you try to isolate more *eas-* mutants. You first mutagenize the phage by treating them with ethidium bromide and then screen for your mutants. Briefly, describe how would screen for *eas-* mutant B2 phage. In addition, indicate what species of bacteria you would infect and CLEARLY draw what the desired mutants would look like. (6pts)

Allow the mutagenized phage to infect *Salmonella enterica*. Those that can grow (and form small plaques) are your mutants

Species Infected > *Salmonella enterica*

Morphology of phage to be selected >



The mutants you obtain are through your screen are all *non-leaky*. This is in contrast with another colleague who did the exact same screen, and isolated several leaky mutants following mutagenesis with bromo-deoxyuridine.

10.) Name two general classes of mutations that would be expected to produce primarily nonleaky mutations. Indicate why these classes of mutations are often nonleaky. (4pts)

1.) Deletions tend to cause drastic changes in the protein because they can remove large portions (or all) of it.

2.) Framshifts tend to cause drastic changes in the protein because they change every amino acid in the protein that is located downstream of the mutation is changed.

11.) Name a general class of mutations that would be expected to generate some leaky mutations in your colleagues screen. Indicate why these classes of mutations can be leaky (2pts)

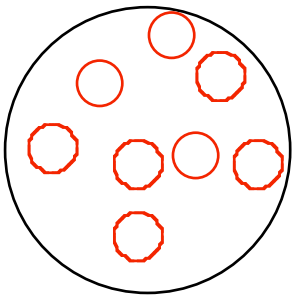
Base substitution often cause less dramatic changes in the protein because they only change a single amino acid, which may or may not be in a critical region.

12.) You would also like to see if you can isolate suppressors of your *eas1* mutation, so you again decide to mutagenize the *eas1* phage with ethidium bromide prior screening for your mutants. Briefly, describe how would screen for suppressor mutants of *eas*-phage. In addition, indicate what species of bacteria you would infect and CLEARLY draw what the desired phage would look like. (6pts)

Allow the mutagenized *eas-* phage to infect *Escherichia coli*. Those that can grow (and form large plaques) must have suppressor mutations.

Species Infected > *Escherichia coli*

Morphology of phage to be selected >



13.) Considering your answer to #10, what does the fact that you were able to isolate suppressor of *eas* mutants, which you decide to call *soe*, tell you about which class of mutation is likely to have occurred in your mutant? WHY? (4pts).

It was probably a frameshift mutation, since these can often revert if another frameshift occurs in the opposite direction to the first. Deletions which remove multiple bases are generally unable to revert.

You would like to determine if your *soe* suppressor mutations are true revertants or second site suppressor mutations. So you chose two suppressors, *soe1* and *soe2*, and you cross these with wildtype B2 phage so than you can then examine the phenotypes of the progeny. To cross the phage, you infect *E. coli* with a mixture of *soe1* phage and wild type B2 phage or *soe2* phage and wild type B2 phage. Then, you allow the co-infected *E.coli* to lyse and collect the progeny.

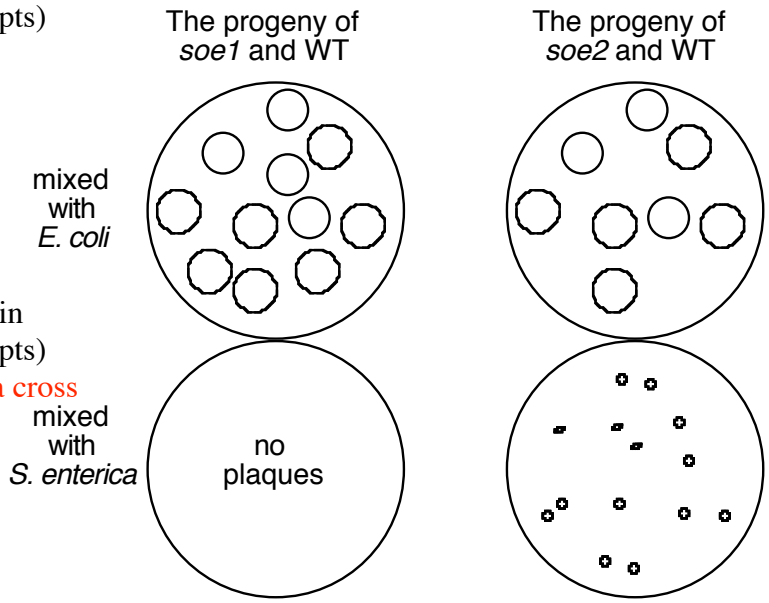
To examine the phenotypes of the progeny, you infect both *E. coli* and *S. enterica* with the progeny, this time using a low MOI and observe the plaques that are produced on each plate. Your results are shown below to the right.

14.) Is *soe1* a true revertant or does it contain a second site suppressor mutation? WHY (5pts)

It is a true revertant. since a cross between two wild type phage can produce only wild type recombinants.

15.) Is *soe2* a true revertant or does it contain a second site suppressor mutation? WHY (5pts)

It is a second site suppressors since a cross between a double mutant and wild type phage could produced some recombinants that had either single mutation.

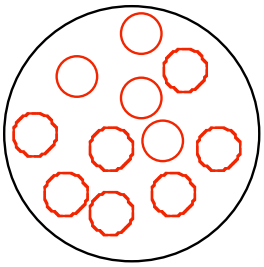


You have isolated ten additional *eas*- mutants. To determine how many genes these two mutants fall into, you decide to use a complementation analysis.

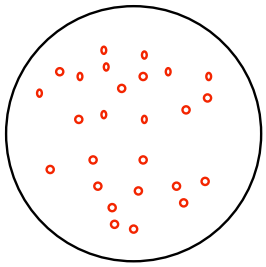
You co-infect either *E. coli* or *S. enterica* with *eas1* B2 and *eas2* B2.

16.) On the plates below, CLEARLY draw how you would expect your plaques to look on each plate if the *eas1* and *eas2* mutations are in different genes? (4pts)

E.coli infected with *eas1* and *eas2* B2

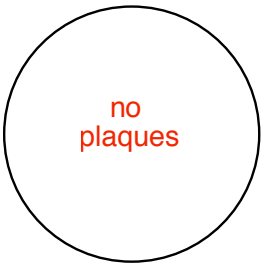


S. enterica with *eas1* and *eas2* B2

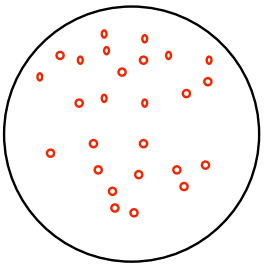


17.) On the plates below, CLEARLY draw how you would expect your plaques to look on each plate if the *eas1* and *eas2* mutations are in the same gene? (4pts)

E.coli infected with *eas1* and *eas2* B2



S. enterica with *eas1* and *eas2* B2



The results of the complementation analysis are shown below. (+) indicates that the two mutations complement each other, (-) indicates that the two mutations do not complement each other.

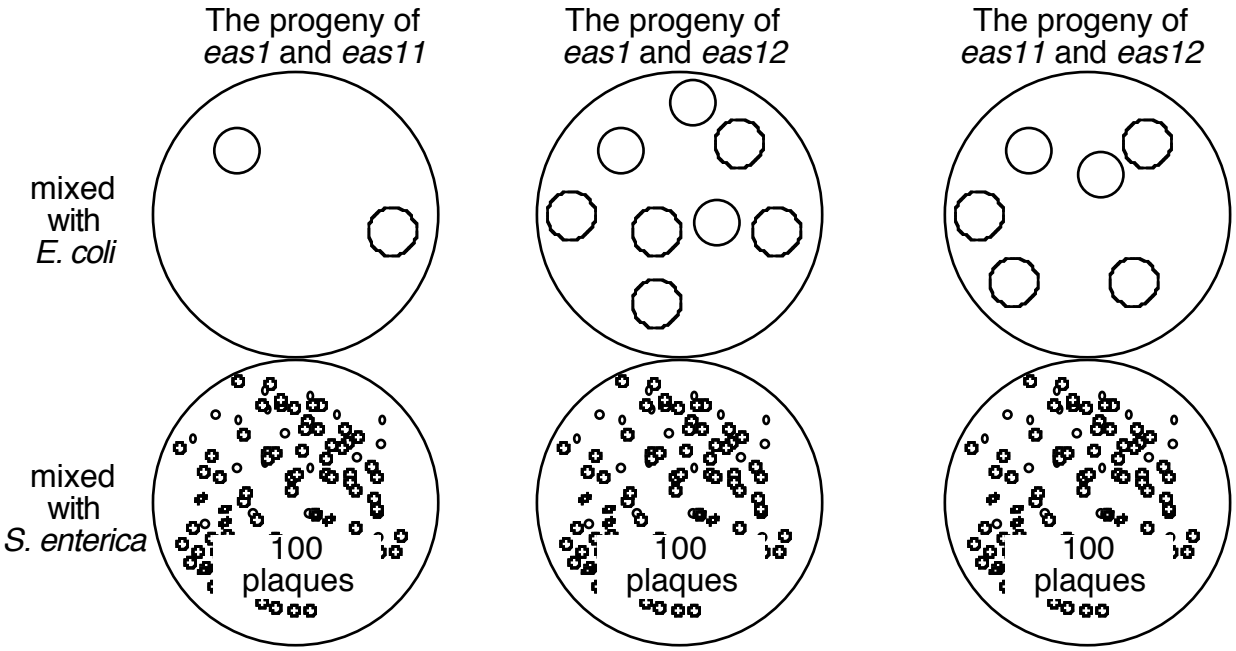
	eas1	*eas2*	*eas3*	*eas4*	*eas5*	*eas6*	*eas7*	*eas8*	*eas9*	*eas10*
eas1	-									
eas2	+	-								
eas3	+	+	-							
eas4	-	+	+	-						
eas5	-	+	+	-	-					
eas6	-	+	+	-	-	-				
eas7	-	+	+	-	-	-	-			
eas8	-	+	+	-	-	-	-	-		
eas9	-	+	+	-	-	-	-	-	-	
eas10	-	+	+	-	-	-	-	-	-	-

18.) Indicate how many genes are represented by these mutations and which mutations are found in the same genes? (6pts)

Gene A: *eas1 eas4 eas5 eas6 eas7 eas8 eas9 eas10*
Gene B: *eas2*
Gene C: *eas3*

You isolate two additional mutations, *eas11* and *eas12,* which appear to be in the same gene as *eas1*. In order to map the order of these 3 mutations, you co-infect *S. enterica* with the following combinations of phage at a high MOI: *eas1* and *eas11*; *eas1* and *eas12*; *eas11* and *eas12*. Then, you allow the co-infected cells to lyse and collect the progeny phage.

To look for recombinants, you use 1ml of each lysate to infect a both a culture of 10^9 *E. coli* and a culture of 10^9 *S. enterica*. You immediately plate these mixtures in top agar at 37C and count the plaques that form. Your results are shown below.

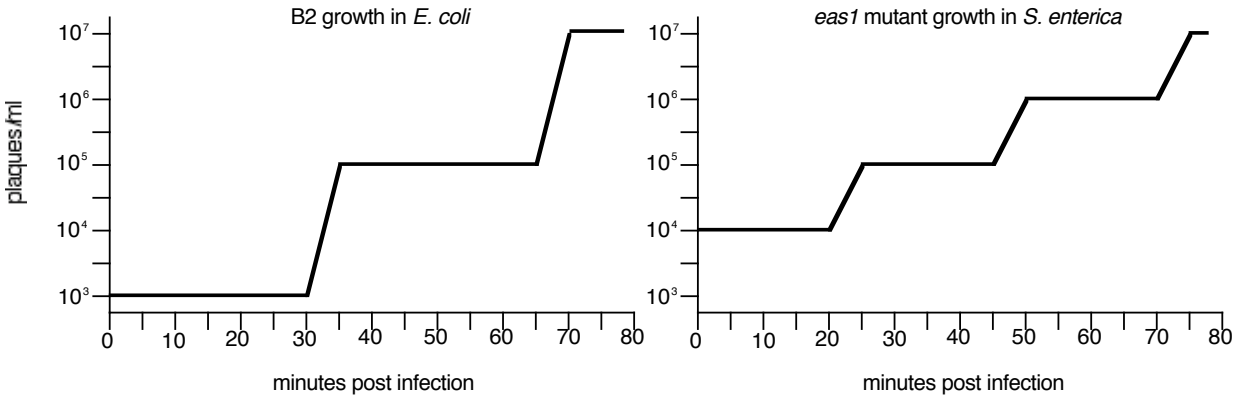


19.) Assuming that you plated these combinations many more times and the ratios remained the same, what is the relative distance and map order between each mutation? Show your work. (10pts)

Recombination Frequency = total recombinant progeny / total progeny
2 wt recombinants X 2 / 100 total phage = 0.04 units between *eas1* and *eas11*
8 wt recombinants X 2 / 100 total phage = 0.16 units between *eas1* and *eas12*
6 wt recombinants X 2 / 100 total phage = 0.12 units between *eas11* and *eas12*

eas12----------0.12 units---------- *eas11*--0.04 units-- *eas1*

You would like to know a little more about the replication of B2 and *eas* mutants in their respective hosts. Using a low MOI, you mix a solution containing either the WT B2 phage with a culture of *E. coli* or the eas1 phage with a culture of *S. enterica*. After 2 minutes (time0), you wash away all the media and uninfected phage. Then, at various time intervals, you plate dilutions of your mixture on a lawn of bacteria and count how many plaques form. The results for each case are shown below.



20.) How long is the lytic reproductive cycle of B2 in *E.coli* and how many bacteriophage are produced each time a phage infects a cell? Show your work. (3pts)
One lytic reproductive cycle takes ~30 minutes. At the start of the cycle, there are 10^3 phage/ml. After one cycle, there are 10^5 phage/ml.

Phage produced per cell infected = (Total phage – Initial phage) after one generation cycle.
= (10^5 phage/ml - 10^3 phage/ml)
= 100 phage / cell infected

21.) How long is the lytic reproductive cycle of *eas1* mutants in *S. enterica* and how many bacteriophage are produced each time a phage infects a cell? Show your work (3pts)
One lytic reproductive cycle takes ~20 minutes.
Phage produced per cell infected = (Total phage – Initial phage) after one generation cycle.
= (10^4 phage/ml - 10^3 phage/ml)
= 10 phage / cell infected

22.) If you were conducting a complementation analysis between two different *eas*- mutants, at what time would you want to plate your culture after coinfection? Why? (4 pts)

Before 30 minutes, because you want to examine the phenotype of the *E.coli* cells that were coinfected.

23.) If you were conducting a recombination analysis between two different *eas*- mutants, at what time would you want to collect the lysate from your culture after coinfection? Why? (4 pts)

After 20 minutes, because you would then want to examine the phenotype of the progeny (potential recombinant) phage that are produced after the phage have replicated and possibly recombined in the *S enterica* cells.