Name Answer Key

Student ID#_____

Microbial Genetics,
BIO 410/510
Winter Quarter 2015
Exam III

1.) For a typical lytic phage, describe two types of gene products that you would expect to be expressed early after infection? (2pts)

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.

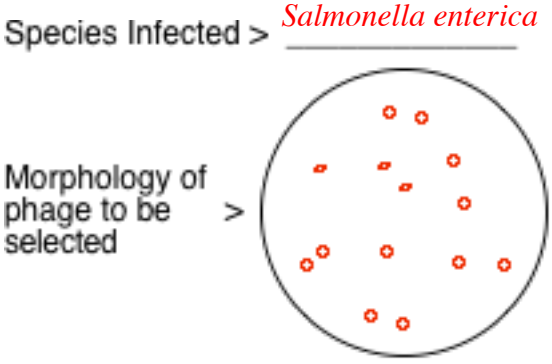
2.) Describe two properties of a phage's reproductive cycle that would make it desirable to use for generalized transduction? (2pts)

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.

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.

3.) 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. (5pts)

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



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.

4.) Name two general classes of mutations that would be expected to produce primarily nonleaky mutations. Indicate why these classes of mutations are often nonleaky. (2pts)

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

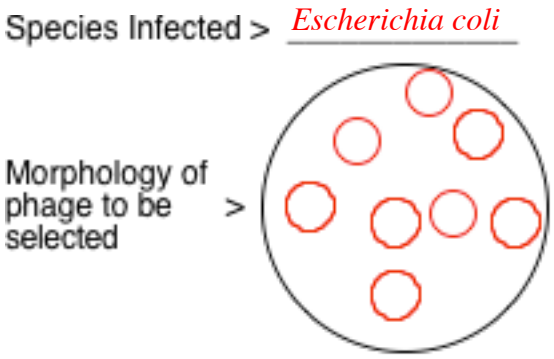
2.) Framshifts tend to cause drastic changes in the protein because they change every amino acid in the protein that is located downstream of the mutation is changed.

5.) Name a general class of mutations that would be expected to generate some leaky mutations in your colleague's 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.

6.) 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. (5pts)

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



7.) 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? (3pts).

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.

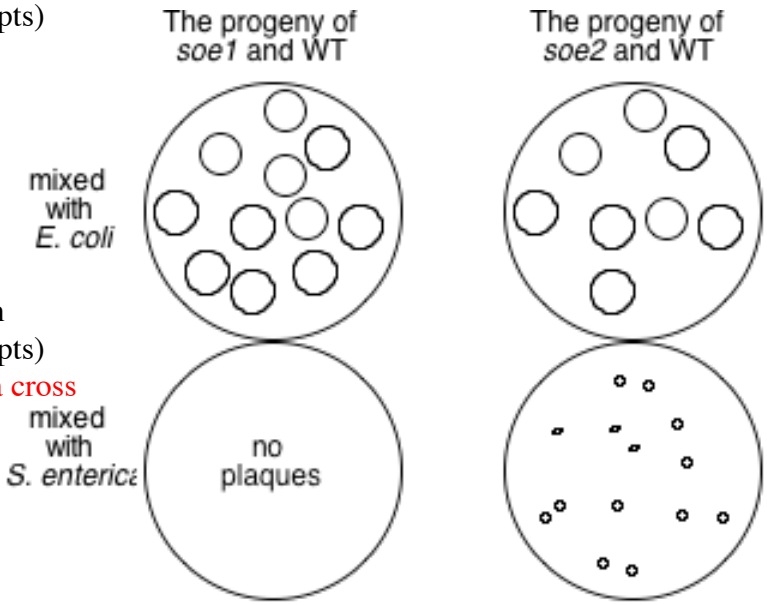
To examine the phenotypes of the progeny, you infect both *E. coli* and *S. enterica* with the progeny, this time using a low MOI and observe the plaques that are produced on each plate. Your results are shown below to the right.

8.) Is *soe1* a true revertant or does it contain a second site suppressor mutation? WHY (4pts)

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

9.) Is *soe2* a true revertant or does it contain a second site suppressor mutation? WHY (4pts)

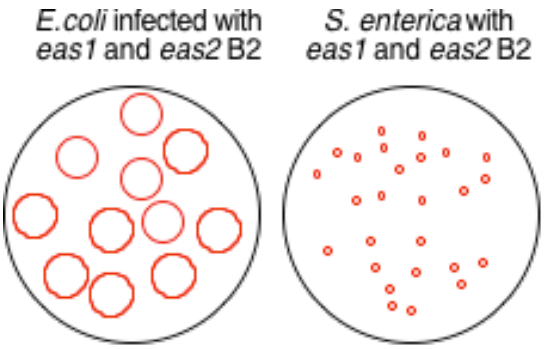
It is a second site suppressors since a cross between a double mutant and wild type phage could produce some recombinants that had either single mutation.



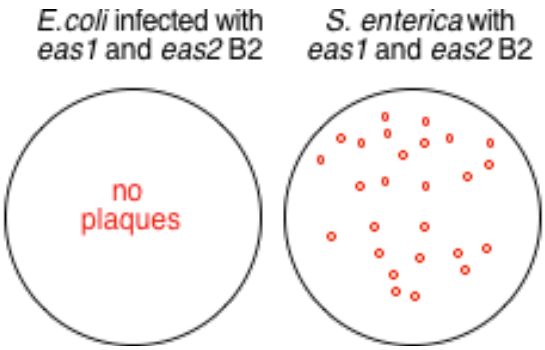
You have isolated ten additional *eas*- mutants. To determine how many genes these two mutants fall into, you decide to use a complementation analysis.

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

10.) 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)

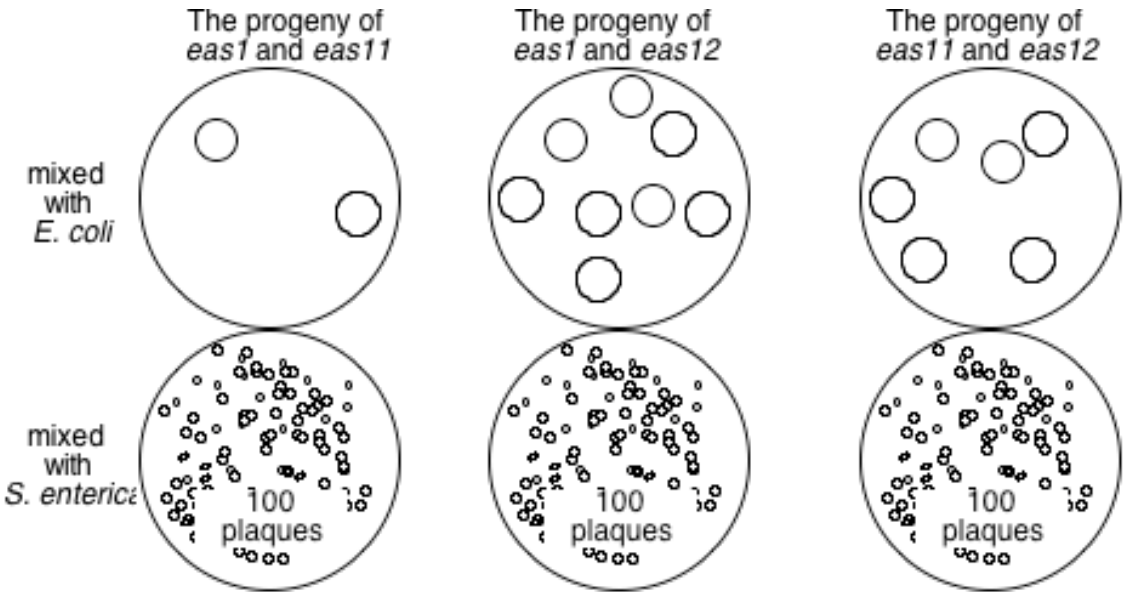


11.) On the plates below, CLEARLY draw how you would expect your plaques to look on each plate if the *eas1* and *eas2* mutations are in the same gene? (4pts)



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.

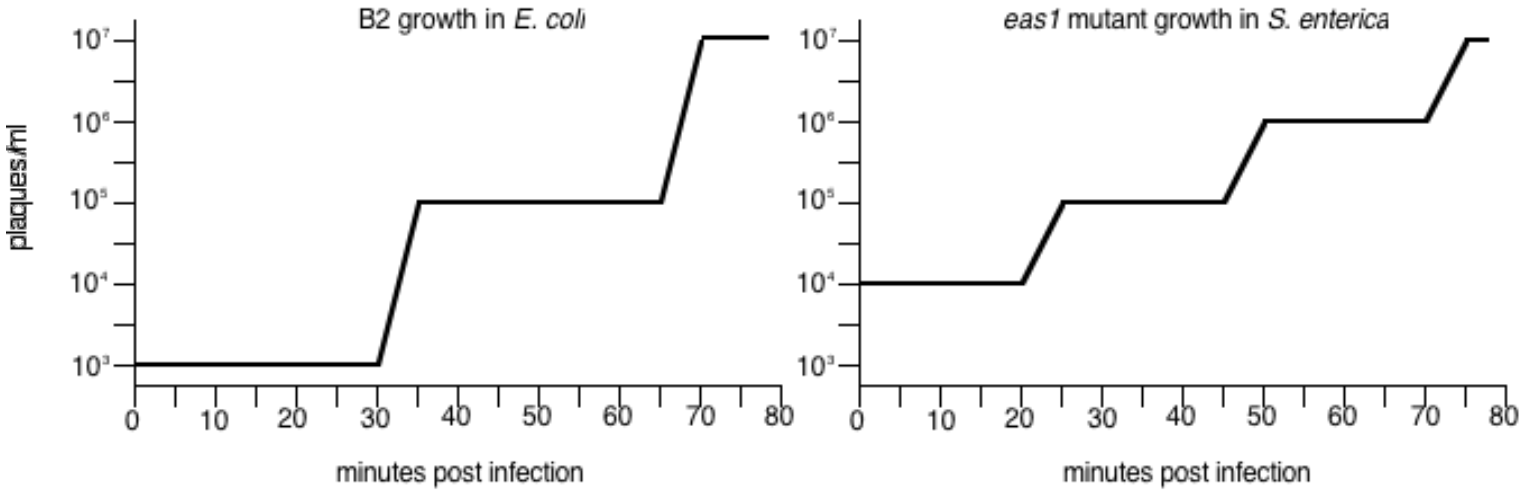


12.) Assuming that you plated these combinations many more times and the ratios remained the same, what is the relative distance and map order between each mutation? Show your work. (8pts)

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.



13.) 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. (4pts)

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

14.) 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 (5pts)

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

15.) 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? (3 pts)

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

16.) 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? (3 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.

Exposure to UV irradiation produces cyclobutane pyrimidine dimers that can block replication and transcription and lead to cell death, mutations, or rearrangements. Most organisms are able to process this type of damage in several different ways. *Neisseria mucosa* has a photolyase, a CPD specific glycoslyase, nucleotide excision repair, and a functional homolog of the UmuC DNA polymerase.

17.) Briefly describe the molecular events that are involved in the repair or tolerance of UV-induced DNA lesions by the following mechanisms (12pts)

a.) Photolyase

The protein binds to the CDP lesions and absorbs light energy which is tranferred to and breaks the cyclobutane bonds, restoring the pyrimidines to their original state by directly reversing the chemical reaction.

b.) CDP glycosylase

1. A glycosylase that recognizes the CDP lesion cleaves the damaged base at the glycosidic bond.
2. An AP endonuclease cleaves the DNA backbone. (if there os not a 3'OH, dRpase is also needed)
3.Polymerase I resynthesizes the damaged region and Ligase seals the nick

c.) Nucleotide excision repair

1. UvrA and UvrB recognize a distortion caused by the lesion in the DNA and bind to the region.
2. UvrC complex makes two incisions 12 base pairs around the lesion containing site.
3. UvrD helicase displaces the damaged fragment
3. Pol I resynthesizes the gap and then ligase seals the nick

d.) UmuC-like DNA polymerases

These polymerases are thought to help the cell tolerate DNA lesions rather than repair them. They are able to polymerize and incorporate nucleotides opposite to the DNA lesion that would otherwise block the normal replicating polymerase Pol III.

The bacterial SOS response upregulates the expression of several repair genes, including *uvrA*, when cells are exposed to DNA damaging agents.

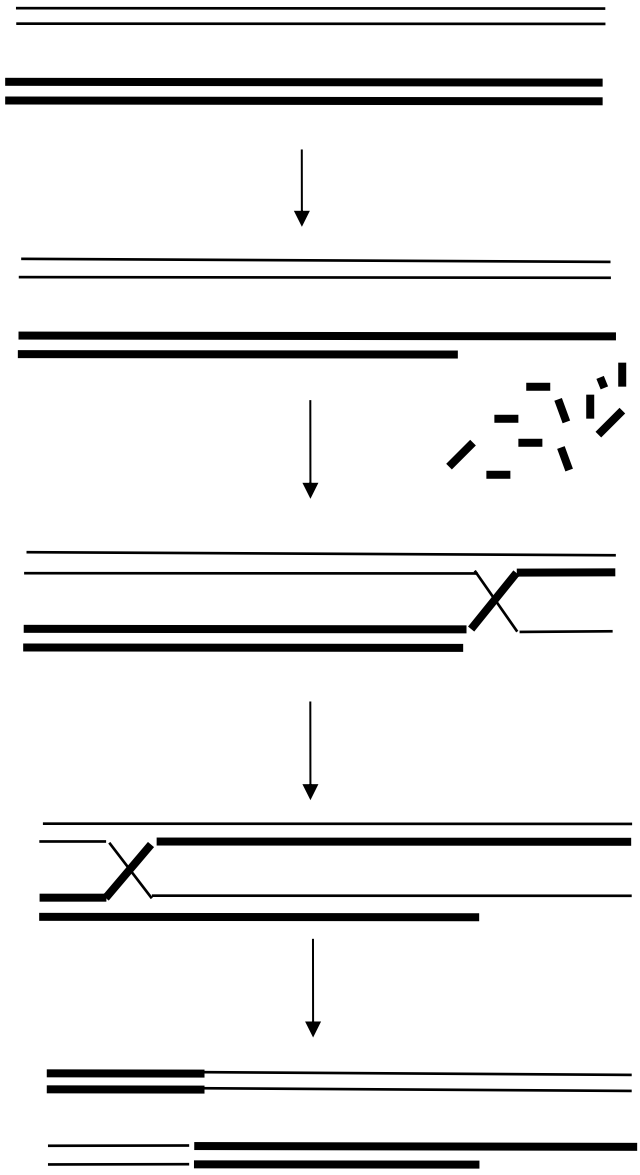
18.) How would the expression level of *uvrA* be expected to change in a mutant that lacks the *lexA* gene product? Why? (4pts)

uvrA expression would increase since LexA normally functions to repress *uvrA* expression. In the absence of LexA repressor, the uvrA gene would be expressed constantly.

19.) How would the expression level of *uvrA* be expected to change in a mutant that lacks the *recA* gene product? Why? (4pts)

uvrA expression would decrease since RecA is required to cleave and inactivate the LexA repressor. In the absence of RecA, the LexA repressor could never be cleaved or inactivated and the *uvrA* gene expression would be constantly repressed.

20.) Name and describe the activity of one gene product in *E.coli* that is associated with performing each step (arrow) shown below. (10pts)



RecBCD helicase nuclease: degrades from a double strand DNA end. At a chi site, RecBCD creates a 3'single strand end by preferentially degrading the 5' strand. This is thought to be involved in the initiation step.

RecA: binds to single stranded DNA and pairs it with homologous duplex DNA. This action catalyzes the strand invasion step of most models.

RuvAB: catalyzes branch migration of Holliday junctions.

RuvC: an endonuclease that cleaves and resolves Holliday junctions.

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 surprise, 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 the following 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 10% are pro$^+$. and 60% are lac$^+$.

21.) What are the two possible orders that these three genes could be in? (5pts)

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

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

22.) You repeat the infection, this time selecting for *lac*$^+$ transductants. Of the *lac*$^+$ transductants, 60% are also *rif*^{R} but none are pro$^+$.

Where does the *rif*^{R} gene map in relation to *pro* and *lac*? (5pts)

lac and *pro* cotransduce less frequently than do *rif* and *pro*. So lac must be further away from pro than rif and therefore

pro----------*rif*----*lac* must be the correct order.