7.02/10.702 Spring 2005

7.02/10.702
Microbial Genetics Exam Study Questions

These questions—adapted from old exam questions--are meant to help you prepare for the 7.02/10.702 Genetics exam on March 8th, 2005. The exam will likely contain 4 questions based on both lecture and laboratory material. The exam is **CLOSED BOOK** and **CLOSED NOTES**, and will be held in the lecture hall, during normal lecture time.

These study questions are not meant to be exhaustive, but should give you an idea of what topics you should study. We **strongly** urge you to work through these questions **before** looking at the answers, and bring any questions to your Undergrad TA, Grad TA or one of the Instructors.

Answers to these questions will be available on the 7.02/10.702 web site!

Question 1

You are handed an undiluted culture of pNK/KBS1 *E. coli*, and are told that it contains 4 x 10^{11} cells/L. You also know that 1 OD550 of pNK/KBS1 = 1 x 10^{8} cfu/mL.

a) You want to take an OD550 of this culture. By what factor do you need to dilute the cells to ensure an accurate spectrophotometer reading of 0.25? **SHOW YOUR CALCULATIONS.**

b) Complete the following sentence to describe how you would make 1 mL of diluted culture with an OD550 of 0.25:

I would add _____ microliters (μL) of culture to _____ microliters (μL) of dilutant to obtain a final volume of 1 mL.

c) If you made a 1:10,000 dilution of a culture with an OD550 of 0.25, and plated 100 μL of that diluted culture onto an LB plate, how many colonies would grow on the plate? **SHOW YOUR CALCULATIONS.**

To set up an experiment, you mix 3 ml of undiluted pNK/KBS1 cells (titer of 4 x 10^{11} cells/L) with 2 ml of P1 phage with a titer of 10^{8} pfu/mL.

d) Determine the MOI of this experiment. **SHOW YOUR CALCULATIONS.**

e) Circle the experiment that the MOI calculated in part d) is more appropriate for:

making P1 transducing lysates OR P1 transduction

Explain your answer in **two to three sentences** by stating **why** that experiment requires that type of MOI (i.e. what do you want to happen/not happen in the experiment, and how does this kind of MOI ensure that?).

Question 2

During 7.02 lab, Andrew and Kate isolated an Ara- mutant, and observed dark blue colonies when they patched this mutant on both LB X-gal Kan plates and LB Ara X-gal Kan plates. From this data, they concluded that they had succeeded in creating an *ara::lacZ* translational fusion, mostly likely to the *araC* gene.

a) The symbols shown below represent the protein product of the *lacZ* gene and the protein product of the wild type *araC* gene (*araC+*):

lacZ encoded protein

araC+-encoded protein

Draw a diagram of the protein product of an *araC::lacZ* translational fusion, and label the parts of your diagram with the appropriate protein names.

b) Explain **why** a strain that contains an *araC::lacZ* translational fusion is Ara-? (Hint: What is the role of AraC in arabinose metabolism?)

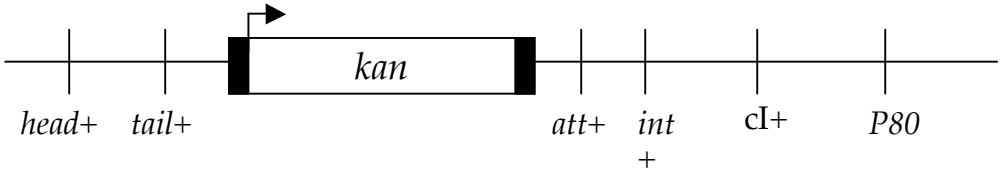
When Andrew and Kate made P1 transducing lysates from their Ara- strain and infected KBS1 (to stabilize their mutation), they obtained two types of transductants. Some transductants were KanR Ara- LacZ-, and others were KanR Ara+ LacZ+(constitutive).

c) Did Andrew and Kate successfully create an *ara::lacZ* translational fusion? _____

d) **Explain** your answer, and Andrew and Kate's experimental result, by describing the location and orientation/reading frame of any transposon insertions that must have existed in their original mutant strain.

Question 3

You are interested in studying the ability of a newly isolated *E. coli* strain to use the sugar arabinose as a carbon source, and decide to perform transposon mutagenesis to identify Ara- mutants. To do this, you infect the *E. coli* strain with λ702, a modified lambda phage containing a version of the Tn10 transposon diagrammed below:



As seen in the diagram, the modified Tn10 transposon consists only of a *kan* gene with its own promoter and start codon flanked by two IS sequences (black bars). The λ702 genome contains wild-type head, tail, integrase (*int*), and cI repressor genes, a functional *att* site, and the transposon. The phage also carries an amber mutation (*P80*) in a gene required for phage DNA replication.

You use λ702 phage to infect an *E. coli* strain that **does not** contain an amber suppressing tRNA, but **does** contain a functional *att* site in a gene required for motility (swimming). The *E. coli* strain contains no plasmids.

You grow the bacteria, mix the bacteria and phage at an appropriate MOI, and allow the infection to proceed. You then plate on Mac Ara Kan plates, and incubate the plates overnight at 37˚C.

a) Did any bacterial cells get lysed by λ702 during the infection described above? **Justify your choice in two sentences or less.**

b) Predict whether all, some, or none of the colonies on the Mac Ara Kan plate will be able to swim. **Justify your choice in two sentences or less.**

c) Predict whether all, some, or none of the colonies on the Mac Ara Kan plate will be white. **Justify your choice in two sentences or less.**

d) Predict whether all, some, or none of the colonies on the Mac Ara Kan plate will be able to be lysed by an infection with **wild-type** λ phage. **Justify your choice in two sentences or less.**

Question 4

On the next page, you will find pictures of four "mystery plates" onto which six bacterial strains have been patched. Use the strain list provided below and the growth/color phenotypes of each bacterial strain to determine the composition of each "mystery plate."

Please answer the questions listed in the table below for each of the "mystery plates." Note that each column heading has a list of **possible** answers to that question, and that a plate **may** contain more than one added antibiotic, amino acid, and/or sugar.

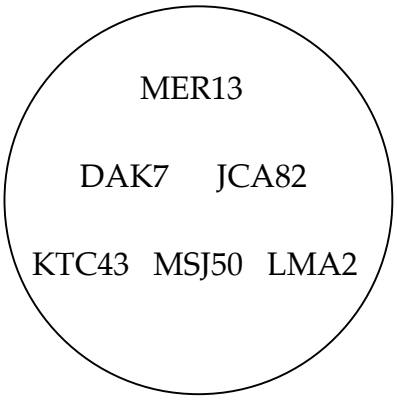
Mystery Plate #	Type of media? **(LB, M9 or Mac)**	Contains Xgal? **(yes or no)**	Must it contain antibiotic(s)? **(yes or no)** If yes, which one(s)? **(Kan, Strep, Tet, Cm)**	Must it contain any added amino acid(s)? **(yes or no)** If yes, which one(s)? **(Phe, Thr, Leu)**	Must it contain any added sugar(s)? **(yes or no)** If yes, which one(s)? **(Ara, Xyl, Lac)**
1					
2		**NO**			
3					
4					

STRAIN LIST
(NOTE: all characteristics which are not noted are wild-type)

strain	**relevant phenotype**
MER13	Lac- KanR CmR Phe-
DAK7	Lac-, LacZ+(inducible) Ara- Xyl- Thr-
JCA82	Lac- KanR StrepR Xyl-
KTC43	Lac- LacZ+(constitutive) TetR CmR
MSJ50	Lac- KanR Leu-
LMA2	Lac- KanR Leu- Thr-

Question 4 (continued)

Each "mystery plate" is patched in the following pattern:



Key to growth/color phenotypes:

 = white patch

 = red patch on MacConkey plate OR blue patch on Xgal plate

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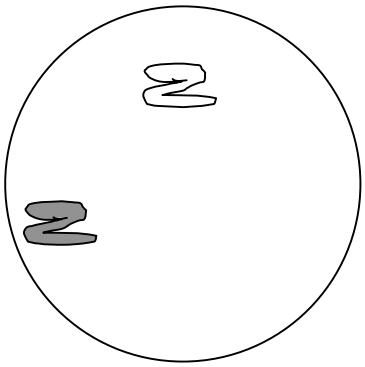
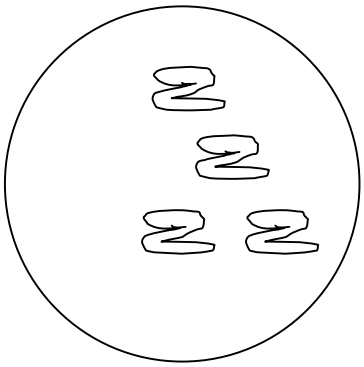
Mystery Plate #1



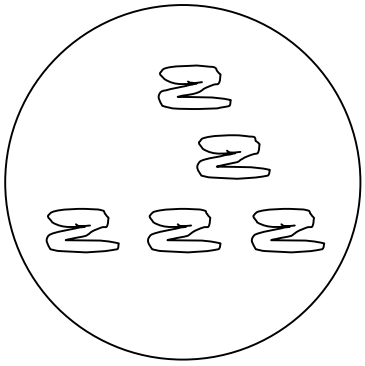
(the agar in plate #1 is clear)

Mystery Plate #2



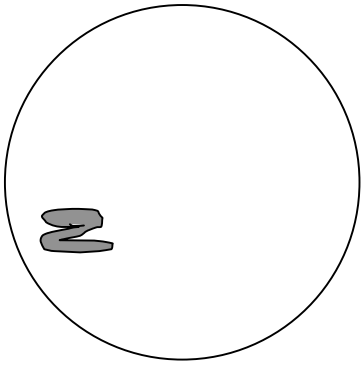
(the agar in plate #2 is red)

Mystery Plate #3



(the agar in plate #3 is clear)

Mystery Plate #4



(the agar in plate #4 is yellow)

Question 5

Please mark whether each of the following statements is true or false.
If a statement is false**, <u>correct it by crossing out and/or substituting words or phrases</u>.**

(For example: <u>__False__</u> The winter sky over Boston is usually ~~blue~~ gray).

_____ a) λ DNA circularizes upon entering the bacterial host via 12 bp, complementary sequences.

_____ b) We used the lysogenic life cycle of P1 to generate our P1 transducing lysates.

_____ c) In 7.02 lab, you used M9 Glu Leu plates to screen for colonies that could not metabolize the amino acid leucine.

_____ d) Repressors are proteins that bind to DNA and turn off transcription of a gene or operon.

_____ e) The term phenotype describes the genetic constitution of an organism.

_____ f) The site on the DNA to which RNA polymerase binds to start transcription is called the promoter.

_____ g) In a selection, both parental cells and mutant cells grow, and can be differentiated from each other by a visible characteristic.

_____ h) Integration of λ DNA into the *E. coli* chromosome during the lysogenic life cycle occurs via homologous recombination.

_____ i) In conservative transposition, the transposon is "cut" out of the donor site and "pasted" in to the recipient site.

Question 6

You are tutoring one of your hallmates, and he asks you to help them work through the differences between lambda phage and P1 phage. Much to the grad TA's dismay, your hallmate thinks that "phage are all the same." Help your friend understand more about P1 by answering the following questions.

(a) When making your transducing lysates, you used P1 phage and not λ phage. What is unique about P1's life cycle (as compared to λ's) that allows it to be useful for transduction experiments?

b) In 7.02 laboratory, you performed **P1 transduction** into KBS1 and C600 strains. For each experimental parameter, circle the correct conditions from the choices given (ONE per parameter!), and explain your reasoning **BRIEFLY.**

Experimental parameter	Circle the correct condition			Reason for your choice?
MOI of this experiment	HIGH	LOW	EITHER	
shake phage/cells during 30′ incubation?	YES	NO	EITHER	
temperature during 30′ incubation	4˚C	room temp.	37˚C	
cofactor required	Mg+2	Ca+2	Na+	

Question 6 (continued)

c) The fire alarm goes off 20 minutes into your 30 minute P1 phage/cell transduction incubation, and you can't continue your experiment for 3 hours!! Your lab partner thinks your experiment will work anyway. Do you agree or disagree? Explain your reasoning.

Your hallmate is now fully clear about what a powerful tool P1 is, but still has questions about λ phage. Help him understand more about λ phage by answering the following questions in **ONE OR TWO SENTENCES**.

d) In lecture, Professor Guarente said that if I infected *E. coli* cells with wild type lambda phage, I would observe cloudy (turbid) plaques. Why are the plaques turbid?

e) When we infected LE392 cells with lambda1205, we got clear plaques. Why were the plaques clear?

f) You decide to inoculate a clear plaque into one tube containing LB media, and a turbid plaque into another tube of LB media. After growing the tubes for an hour at 37˚C, you plate the tubes onto appropriately labeled LB plates. What do you expect to see on the two plates, and why?

Question 7

After successfully completing 7.02, you decide to come back and join the teaching staff as an undergraduate TA. During the Genetics module, one of your student groups needs your help in understanding the results of their transposon mutagenesis and P1 transduction.

They explain to you that they started the transposon mutagenesis by mixing 1 ml of *E. coli* pNK/KBS1 cells with 500 μl of λ1205. To get the titer of the cells, they diluted an aliquot of the *E. coli* pNK/KBS1 cells 1:25, and took the OD_{550}, which they determined to be 0.021. They also titered the λ1205 stock, and found that it contained 10^9 pfu/ml.

a) Assuming that 1 OD550 = 6.3 x 10^7 CFU/ml, calculate the MOI of your students' transposon mutagenesis? **Show your calculations!**

b) Do you think that this MOI is appropriate for transposon mutagenesis? Why or why not?

After selecting and screening for putative Ara- mutants, your students characterize one mutant, Q2W1. They find that the mutant has the following growth and color characteristics

	M9 Ara Leu Kan	Mac Ara Kan	LB Kan	LB Xgal Kan	LB Ara Xgal Kan
Phenotype	NG	G, white	G	G, dark blue	G, dark blue

NG = no growth; G = growth

c) Based on these data, what is the <u>phenotype</u> of the Q2W1 mutant? What gene(s) are likely to have a transposon insertion? Explain your reasoning (i.e. how did you determine the phenotype/genotype?).

Question 7 (continued)

Finally, your students perform P1 transduction using a lysate made from the Q2W1 mutant strain. They infect KBS1 cells with this lysate, and plate the cells on an LB Kan plate. They then patch 20 transductants from the LB Kan plate, and observe the following:

grid #	M9 Ara Leu Kan	Mac Ara Kan	LB Kan	LB Xgal Kan	LB Ara Xgal Kan
1-10	G	G, red	G	G, dark blue	G, dark blue
11-20	NG	G, white	G	G, white	G, dark blue

They also notice that both their "lysate alone" and "cells alone" control plates are clear (i.e. no growth on either).

d) Is the P1 transduction data above consistent with your expectations? Why or why not?

e) Propose a model that is consistent with ALL the data collected by your students.

Question 8

You perform transposon mutagenesis using pNK/KBS1 and λ1205 as in the 7.02 laboratory. You selected and screened for putative Ara- mutants on Mac Ara Kan plates, and then patched to further characterize your strains. You patch your putative Ara- mutants on the following plates:

M9 Ara Leu Kan
M9 Glu Leu Kan
Mac Ara Kan
LB Xgal Kan
LB Ara Xgal Kan

a) Why are white colonies on Mac Ara Kan plates considered only "putative" Ara- mutants? Which plate(s) confirm that they are Ara-? Explain.

b) You have room for only **four** control strains on your plates. Which **four** strains will allow you to interpret the phenotypes of your mutants with 100% confidence? Explain your choices.

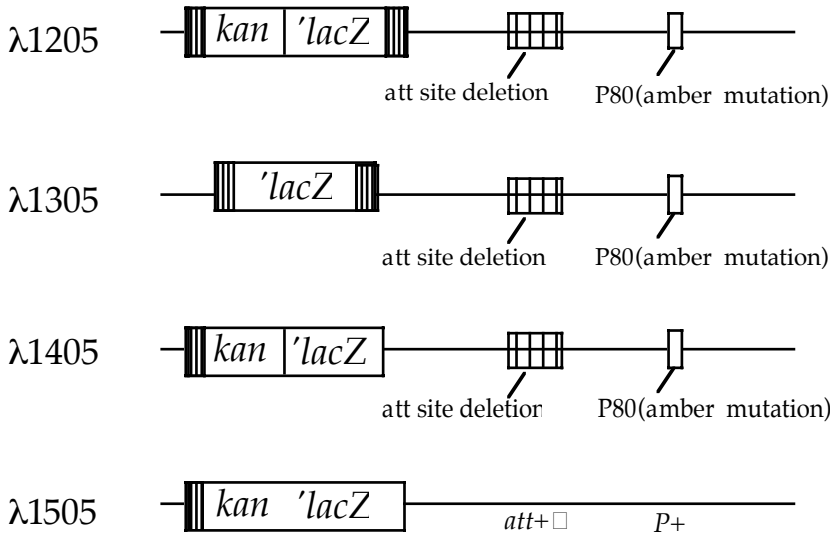
In a different patching experiment (unrelated to parts a and b), you observe the following:

	Mac Ara Kan	LB Xgal Kan	LB Ara Xgal Kan
H33	white	white	white
pNK/KBS1	red	white	white

c) Based on these observations, you suspect that some of the reagents used to make the plates have gone bad. Which reagents do you suspect are bad, and why?

Question 9

Debbie asks you to test several modifications of the transposon mutagenesis experiment you did in the GEN module. She wants you to compare the outcome of each experiment to the outcome of λ1205 infection of pNK/KBS1. Here are diagrams of the four different lambda phage she asks you to try:



You set up the experiments as he asks, and plate the resulting mixture on the same plates you used in 7.02 to select/screen for Ara- transposon insertion mutants.

For each experiment listed below, predict the outcome **in terms of number of colonies expected relative to λ1205 infection of pNK/KBS1**, and explain your prediction briefly.

Phage used	Strain infected	Predicted # of colonies (i.e. none? less? same? more?)	Reason for your prediction
λ1205	KBS1		
λ1305	pNK/ KBS1		
λ1405	pNK/ KBS1		
λ1505	pNK/ KBS1		

Question 10

Listed below are seven potential strains (A-G) that could result from the transposon mutagenesis performed in 7.02/10.702 lab (using strain pNK/KBS1 and lambda1205). On the chart below, **CLEARLY** indicate the growth (G or NG) and/or color phenotypes that you would expect on each plate for each strain.

A. The strain was never infected by lambda1205 (and thus did not receive miniTn10).

B. MiniTn10 inserted into the *araC* gene in the same orientation and reading frame as the *araC* gene is transcribed.

C. MiniTn10 inserted into the promoter of the *araC* gene, blocking *araC* transcription.

D. MiniTn10 inserted into the *araA* gene in the same orientation, but different reading frame, as the *araA* gene is transcribed.

E. MiniTn10 inserted into the *araB* gene in the same orientation and reading frame as the gene is transcribed.

F. MiniTn10 inserts into the gene encoding succinate dehydrogenase (constitutively active promoter, not essential for growth) in the same orientation and reading frame as the gene is transcribed.

G. MiniTn10 inserts into the *thrC* gene (required for threonine biosynthesis) in the same orientation and reading frame as the gene is transcribed. (Note: *thrC* transcription is repressed in the presence of threonine.)

	A	B	C	D	E	F	G
M9 Ara Leu Kan							
M9 Glu Leu Kan							
Mac Ara Kan							
Mac Lac Kan							
LB Xgal Kan							
LB Ara Xgal Kan							

Question 11

Your undergraduate TAs didn't have much success doing the experiments in the Genetics module during Run-Through week. Predict how each mistake affected the results of the experiment described, and explain briefly (one or two sentences max!).

A) Sean forgot to grow his cells overnight in LBMM (LB + maltose + $MgSO_4$) before doing the transposon mutagenesis.

B) During his P1 transduction, Jon resuspended his KBS1 cells in saline instead of MC medium.

C) Sarah titered her λ1205 phage using KBS1 cells.

D) Jenn used an MOI of 2 when infecting KBS1 cells with P1 lysates made from her transposon mutants (i.e. the Day 5 "mutant stabilization" experiment).

E) Mary tried to grow her *ara::lacZ* mutant strain on M9 Ara plates and the C600 strain on M9 Glu Leu plates.

Question 12

Before performing a transposon mutagenesis, you need to titer the λ1205 that you will use in your experiment. To do this, you perform the following experiment:

1. Make 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions of the original λ1205 stock.
2. Mix 0.2 ml of the 10^{-5} phage dilution and 0.8 ml of bacteria in a test tube. Perform this mixture in duplicate (i.e. 2 tubes for the 10^{-5} dilution).
3. Repeat the mixing of phage and bacteria for each of the other three dilutions (also performed in duplicate), and incubate all 8 tubes on your bench for 30 minutes.
4. Take 400 μl from the first 10^{-5} reaction tube and plate the phage/cell mix as in 7.02. Repeat the plating for the other tubes, and grow all 8 plates overnight at 37˚C.
5. Count the plaques that appear on the plate the next morning.

	Number of Plaques on Plate #1	Number of Plaques on Plate #2
10-5 dilution	TNTC	TNTC
10-6 dilution	79	83
10-7 dilution	7	10
10-8 dilution	0	1

a) Use the data above to calculate the titer of the original λ1205 stock. **SHOW ALL CALCULATIONS!**

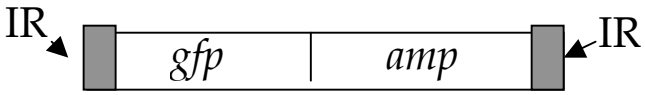
b) To set up your mutagenesis, you mix 5 ml of pNK/KBS1 cells and 0.25 ml of λ1205. In order for this mixture to produce the MOI you selected in part a), to what OD_{550} must you have grown your pNK/KBS1 cells? **SHOW YOUR CALCULATIONS.**

Conversion factor: 1 OD_{550} = 1 x 10^8 cfu/ml

Question 13

You are interested in understanding how the fictional bacterium *R. tannyalis* regulates genes involved in the metabolism (breakdown) of the sugar rhamnose.

You decide to perform transposon mutagenesis to identify mutants defective in rhamnose metabolism. The transposon you use for your mutagenesis--miniTn10-*gfp-amp*—is diagrammed below. The delivery vehicle for miniTn10-*gfp-amp* is λ1207—a modified λ phage that can neither lyse nor lysogenize your starting strain of *R. tannyalis*.



Note: *gfp* encodes GFP, a protein that glows green under UV light; this gene has no promoter or start codon. The *amp* gene encodes resistance to the antibiotic ampicillin, and has its own promoter and start codon.

a) Name **<u>two</u>** proteins that the starting *R. tannyalis* strain must express for your mutagenesis to be successful. Justify your choices.

b) What type of plates would you use to select/screen for putative rhamnose metabolism mutants? What would your desired mutants look like on these plates?

The following table describes the phenotypes of 5 strains isolated from your mutagenesis:

Strain	Growth on LB Amp	Growth on M9 Rhamnose Amp	Growth on M9 Glucose Amp	Color on LB Amp + UV light	Color on LB Rhamnose Amp + UV light
1	+	+	+	green	green
2	+	-	-	white	white
3	+	-	+	white	green
4	+	+	+	white	green
5	+	-	+	white	white

c) Independent of position in the genome, which strain(s) contain translational *gfp* fusions? Explain your reasoning.

Question 13 (continued)

Strain	Growth on LB Amp	Growth on M9 Rhamnose Amp	Growth on M9 Glucose Amp	Color on LB Amp + UV light	Color on LB Rhamnose Amp + UV light
1	+	+	+	green	green
2	+	-	-	white	white
3	+	-	+	white	green
4	+	+	+	white	green
5	+	-	+	white	white

d) Which strain(s) are defective in rhamnose metabolism? Explain your reasoning.

e) Which strain(s) contain rhamnose-inducible *gfp* translational fusions? Explain your reasoning.

Question 14

After 7.02, you join a laboratory that is interested in identifying *E. coli* mutants that are defective in chemotaxis (movement toward a stimulant, such as a sugar).

You mutagenize a wild type *E. coli* strain with the miniTn10 transposon from 7.02, and identify an interesting Che- (chemotaxis) mutant. You stabilize the mutation (which occurs in a gene you call *cheA*) using P1 transduction, and confirm that the KanR and Che- phenotypes are linked.

Your colleagues at another university have identified another *E. coli* Che- mutant (in a gene they call *cheB*). They tell you that *cheB* maps very close to the *his* genes, and can also be cotransduced with the *trp* genes. Using cotransduction, they have deduced the gene order of (and relative spacing and between) *cheB*, *his*, and *trp* to be:

cheB *his* *trp*

To try to determine whether *cheA* and *cheB* are the same gene, you decide to map *cheA* with respect to *his* and *trp*. You perform a P1 transduction experiment using the following strains:

Donor: CheA-, KanR, His+, Trp+

Recipient: CheA+, KanS, His-, Trp-

You obtain the following data:

select for His+ (total= 1000)		**select for KanR (total = 1000)**	
KanR Trp+	108	Trp- His-	390
KanR Trp-	212	Trp+ His-	290
KanS Trp+	5	Trp+ His+	3
KanS Trp-	675	Trp- His+	292

a) Determine the gene order of *cheA*, *his*, and *trp*. **Show all calculations used**, and explain your logic.

Question 14 (continued)

b) Are *cheA* and *cheB* the same gene? Justify your answer briefly.

You identify a third Che- strain. The Che- phenotype in this strain arises from a mutation in a gene you call *cheC*; the *cheC* mutation is 100% linked to a gene which confers tetracycline (tet) resistance. You suspect that *cheC* may be the same gene as *cheA*, and perform the following P1 transduction experiment to test your hypothesis:

Donor: Che-, KanR, TetS

Recipient: Che-, TetR, KanS

You select for KanR transductants, and test each colony for its sensitivity or resistance to tetracycline.

c) What phenotype(s) (TetR or TetS) would these transductants have if the *cheA* and *cheC* mutations were 100% linked (i.e. they are in the **same gene**)? Explain your answer briefly. (Hint: a diagram may be useful!)