7.02/10.702 Spring 2005

7.02/10.702
Microbial Genetics Exam
Study Questions

ANSWER KEY

Question 1

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

1. Determine titer of cells at an OD550 of 0.25:

$$\textbf{OD550 of 0.25} \times \frac{\textbf{1 x } 10^{8} \textbf{ cfu/mL}}{\textbf{1 OD550}} = \textbf{2.5 x } 10^{7} \textbf{ cfu/ml}$$

2. Determine titer of undiluted cells:

$$\frac{\textbf{4 x } 10^{11} \textbf{ cells}}{\text{L}} \times \frac{\textbf{L}}{\textbf{1000 mL}} = \frac{\textbf{4 x } 10^{8} \textbf{ cells (cfu)}}{\textbf{mL}}$$

3. Calculate dilution factor:

$$\frac{\textbf{4 x } 10^{8} \textbf{ cells (cfu)}}{\textbf{mL}} \times \textbf{ dilution factor} = \frac{\textbf{2.5 x } 10^{7} \textbf{ cfu}}{\textbf{mL}}$$

DF = 0.0625 or 1/16 dilution

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 **62.5** microliters (μL) of culture to **937.5** 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.**

1. **Convert OD550 to cfu/mL:**

$$\textbf{OD550 of 0.25} \times \frac{\textbf{1 x } 10^{8} \textbf{ cfu/mL}}{\textbf{1 OD550}} = \textbf{2.5 x } 10^{7} \textbf{ cfu/mL}$$

2. **Make 1:10,000 dilution:**

$$\textbf{2.5 x } 10^{7} \textbf{ cfu/mL} \times \frac{\textbf{1}}{\textbf{10,000}} = \textbf{2.5 x } 10^{3} \textbf{ cfu/mL}$$

3. **Account for the fact that you only plated 100μL (0.1 mL):**

2.5×10^{3} cfu/mL x 0.1 mL plated = 250 cfu or 250 colonies

Question 1 (continued)

To set up an experiment, you mix 3 ml of undiluted pNK/KBS1 cells (titer of 4×10^{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.**

1. **Define MOI as $\frac{\text{pfu}}{\text{cfu}}$**

2. **Determine # of pfu used in the experiment:**

$$\frac{10^8 \text{ pfu}}{\text{mL}} \times 2 \text{ mL used} = 2 \times 10^8 \text{ pfu}$$

3. **Determine # of cfu used in the experiment:**

$$\frac{4 \times 10^{11} \text{ cells}}{\text{L}} \times \frac{\text{L}}{1000 \text{ mL}} = \frac{4 \times 10^8 \text{ cells (cfu)}}{\text{mL}} \times 3 \text{ mL used} = 1.2 \times 10^9 \text{ cfu}$$

4. **Determine MOI:**

$$\text{MOI} = \frac{\text{pfu}}{\text{cfu}} = \frac{2 \times 10^8 \text{ pfu}}{1.2 \times 10^9 \text{ cfu}} = 0.167$$

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?).

In the P1 transduction experiment, you want one phage carrying bacterial DNA (specifically, carrying your *ara::lacZ* fusion) to infect a recipient cell and for that DNA to recombine into the recipient chromosome. You do NOT want your transductant to then be infected by a second P1 phage, which will most likely contain P1 DNA and will thus kill the cell. At a low MOI (<1), most cells—if infected at all—will only be infected with one phage, ensuring that any transductants obtained will survive.

Question 2

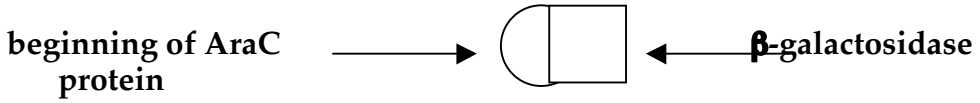
During 7.02 lab, Andrew and Kate isolated an Ara- mutant, and observed dark blue colonies when they patched this mutant on both LB X-gal Kan plates and LB Ara X-gal Kan plates. From this data, they concluded that they had succeeded in creating an *ara::lacZ* translational fusion, mostly likely to the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

*ara*C+-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?)

AraC's role in the cell is to serve as a regulator of the *araBAD* genes (and thus the production of the enzymes involved in the breakdown of arabinose for use as a carbon source). Specifically, without arabinose, it binds and prevents RNAP access to the *araBAD* promoter—thus serving as a negative regulator of *araBAD* transcription. In the presence of arabinose, it recruits RNAP to the promoter, thus serving as a positive regulator of *araBAD* transcription.

In a strain containing an *araC::lacZ* translational fusion, only a portion of the AraC protein is synthesized, and this portion of the AraC protein is not sufficient to recruit RNAP. Without RNAP recruitment, the *araBAD* genes don't get transcribed, and no metabolic enzymes are made. Without these enzymes, no arabinose can be broken down, and the cells are phenotypically Ara-.

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?
NO

Question 2 (continued)

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.

If Andrew and Kate successfully created an *ara::lacZ* translational fusion, then you would expect that the Ara-, KanR, and LacZ+ (constitutive) phenotypes of the original mutant strain would observed in all your transductants.

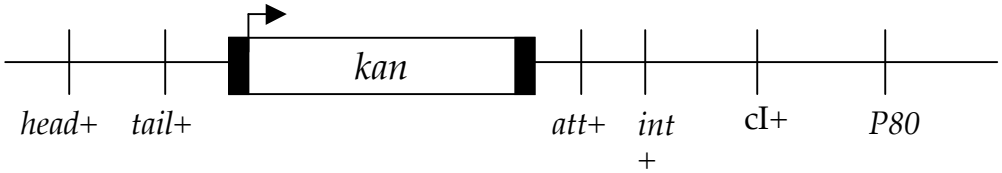
Instead, Andrew and Kate's original strain must have contained two transposons:

Transposon 1 inserted into an *ara* gene, but in the wrong orientation and/or reading frame to get Bgal expression. Movement of this transposon into KBS1 via P1 transduction gives rise to the observed Ara-, KanR, LacZ- transductants. Transposon 2 inserted into a non-*ara* gene, and inserted in the correct orientation and reading frame to create a translational fusion. The gene that the transposon was inserted in is expressed (transcribed and translated) in LB media, and is not regulated by the presence/absence of the sugar arabinose. Movement of this transposon via P1 transduction gives rise to the Ara+, KanR, LacZ+ (constitutive) transductants.

Note that these two transposons must have inserted at least 100 kb apart, as you never obtain a transductant with the phenotype of the original mutant (Ara-, KanR, LacZ+ constitutive). If they were within 100 kb, you might expect some transductants to have this phenotype.

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.

Question 3 (continued)

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.**

NO. This phage contains an amber mutation in a gene required for phage DNA replication, the first step of the lytic life cycle. Since the bacterial strain does not contain an amber suppressor tRNA, no phage replication--and thus no cell lysis—will occur during the infection.

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.**

NONE. To obtain KanR, the phage genome has to integrate into the *att* site of the *E. coli* genome by homologous recombination. As the *att* site is contained in a gene required for swimming—and the recombination event will eliminate the function of the gene containing the *att* site—none of the KanR colonies will be able to swim.

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.**

NONE. In order to become white on a Mac Ara Kan plate, an *ara* gene must be disrupted by either insertion of the transposon or by integration of the entire phage genome. Phage integration will only occur at the *att* site (not in an *ara* gene), and the strain contains no transposase enzyme to allow the transposon to jump into an *ara* gene.

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.**

NONE. The colonies on the Mac Ara Kan plates are all λ702 lysogens (have λ702 integrated into the genome). These lysogens produce the cI repressor protein, which prevents any wild type phage that infect from undertaking the lytic life cycle.

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 **<u>may</u>** contain more than one added antibiotic, amino acid, and/or sugar.

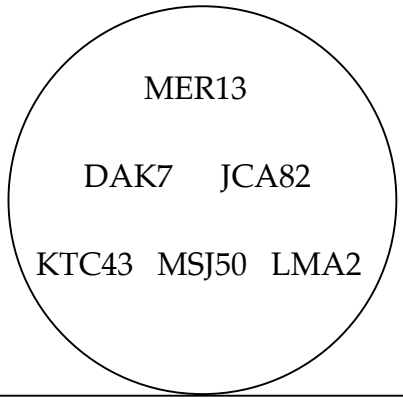
Mystery Plate #	Type of media? **(LB, M9 or Mac)**	Contains Xgal? **(yes or no)**	Must it contain antibiotic(s)? **(yes or no)** If yes, which one(s)? **(Kan, Strep, Tet, Cm)**	Must it contain any added amino acid(s)? **(yes or no)** If yes, which one(s)? **(Phe, Thr, Leu)**	Must it contain any added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

<u>STRAIN LIST</u>
(NOTE: all characteristics which are not noted are wild-type)

<u>strain</u>	**<u>relevant phenotype</u>**
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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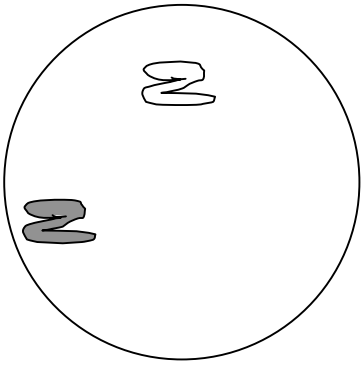
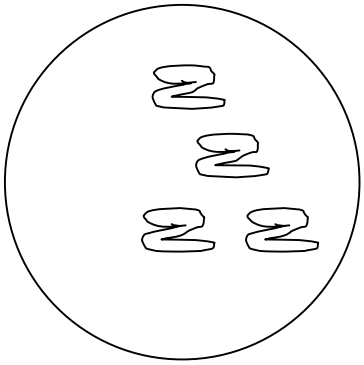
Mystery Plate #1



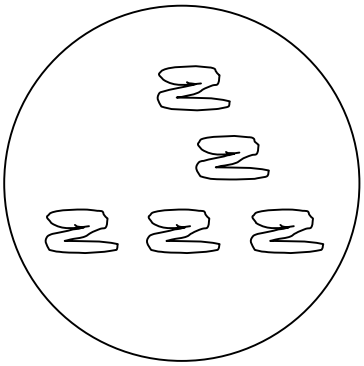
(the agar in plate #1 is clear)

Mystery Plate #2



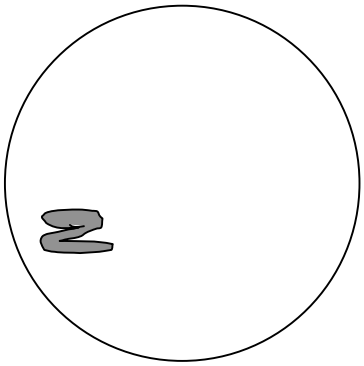
(the agar in plate #2 is red)

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: __False__ The winter sky over Boston is usually ~~blue~~ gray).

NOTE: corrections that make false statements true are noted in **<u>BOLD</u>**

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

__**false**__ b) We used the **<u>LYTIC</u>** life cycle of P1 to generate our P1 transducing lysates.

__**false**__ c) In 7.02 lab, you used M9 Glu Leu plates to screen for colonies that could not **<u>SYNTHESIZE</u>** the amino acid **<u>THREONINE</u>**.

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

__**false**__ e) The term **<u>GENOTYPE</u>** describes the genetic constitution of an organism.

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

__**false**__ g) In a **<u>SCREEN</u>**, both parental cells and mutant cells grow, and can be differentiated from each other by a visible characteristic.

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

__**true**__ 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 in 7.02, and they ask 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?

During P1's life cycle, it chops up the bacterial chromosome and—about 0.1% of the time—packages bacterial DNA into its "head" in place of phage DNA. λ only packages λ DNA into its "head" and thus cannot be used 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	**You only want one phage infection per cell. Multiple infections of a cell may result in a P1 phage lysing a transductant.**
shake phage/cells during 30′ incubation?	YES	**NO**	EITHER	**You are trying to slow down the infection process to prevent multiple rounds of infection; shaking will encourage cell growth and encourage multiple rounds of infection.**
temperature during 30′ incubation	4˚C	**room temp.**	37˚C	**You are trying to slow down the infection process to prevent multiple rounds of infection; high temperatures will encourage cell growth and multiple rounds of infection, so keep at RT.**
cofactor required	Mg+2	**Ca+2**	Na+	**Ca+2 is required for P1 phage binding to the cell surface.**

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.

Disagree. In 30 minutes, only one round of phage infection will occur, and (at low MOI) transductants will not be infected with a second phage. If you let the experiment proceed for 3 hours, multiple rounds of infection will occur, and all the transductants will be lysed by virulent (P1 DNA containing) phage.

Your hallmate is now fully clear about what a powerful tool P1 is, but still has questions about lambda. Help him understand more about lambda 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?

Turbid plaques are a combination of lysed cells (due to lytic infection) and lysogenized cells (due to lysogenic cycle). The plaques look turbid (cloudy) because the lysogenized cells live and form small colonies within the plaque.

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

The plaques were clear because lambda1205 is capable of undergoing the lytic cycle when grown in LE392 cells (and thus lysing cells in a bacterial lawn). There are no lysogens because lambda1205 has a mutation in the phage attachment site.

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?

Clear plaque: no growth
Turbid plaque: some growth (colonies)

No growth with clear plaques because clear plaques contain only phage. Some growth with turbid plaques because the lysogenized cells will survive and form colonies.

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!**

MOI = pfu/cfu

pfu = 10^9 pfu/ml x 0.5ml λ1205 = 5 x 10^8 pfu

$$\textbf{cfu} = 0.021\ OD_{550} \times 25 \times \frac{6.3 \times 10^7\ CFU/ml}{1\ OD_{550}} \times 1ml = 3.3 \times 10^7\ cfu$$

$$\frac{pfu}{cfu} = \frac{5 \times 10^8\ pfu}{3.3 \times 10^7\ cfu} = 15.12$$

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

NO. At this MOI, most cells will be infected with more than one λ1205 phage. This will lead to multiple transposon insertions in the same cell.

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

Question 7 (continued)

c) Based on these data, what is the phenotype 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?).

The phenotype of the Q2W1 mutant is Ara^-, Kan^R, $LacZ^+$ constitutive. We know this because it fails to grow on M9 Ara Leu (Ara^-), grows on LB Kan (Kan^R) and is blue in both the presence and absence of arabinose ($LacZ^+$, constitutive). Since *araC* is the only *ara* gene that is expressed all the time (constitutively), the transposon probably inserted in *araC*.

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?

No. I would expect that the Ara^- strains would be $LacZ^+$, constitutive based on the original characterization; these data show that Ara^- transductants have a $LacZ^+$ inducible phenotype. There is also a class of transductants that is $LacZ^+$ constitutive, but these are Ara^+.

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

The MOI data suggests that the strain may have multiple transposon insertions. This is confirmed in the P1 transduction data, where transductants with two different phenotypes arise from one P1 lysate. A model that is consistent with all the data is as follows:

1) **Two transposons inserted in the *E. coli* genome to create strain Q2W1;**

2) **One transposon inserted in the *araA* or *araB* gene, which results in an Ara-, LacZ+ inducible phenotype;**

Question 7 (continued)

3) **The second transposon inserted in a non-*ara* gene, and this gene is on constitutively (with or without arabinose). This constitutive phenotype masked the "inducible" phenotype caused by the first transposon;**

4) **These two transposons inserted >100 kb away from each other, as the phenotypes caused by each transposon insertion separated during P1 transduction. If they were within 100 kb, you might get transductants that have the same phenotype as the original strain (Q2W1).**

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
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.

Mac Ara Kan plates measure changes in pH, not sugar metabolism directly. We assume that white colonies are "white" because they use amino acids as a carbon source (raising pH), but don't know this for sure (i.e. perhaps they are Ara+, but have a mutation that makes the media more basic!).

The M9 Ara Leu Kan plate confirms that our mutants are Ara-. Arabinose is the sole carbon source on these plates, so Ara- colonies WILL NOT grow, while Ara+ colonies WILL grow.

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.

To have 100% confidence in the phenotypes of your mutants, you need a positive and negative control for each phenotype tested by the plates. The phenotypes tested by these plates are: KanR vs. KanS; Ara+ vs. Ara-; LacZ- vs. LacZ+ (Inducible) vs. LacZ+ constitutive. (Technically, you could tell Thr+ and Thr- as well, but these phenotypes are our focus here.)

Question 8 (continued)

The four strains that allow you to test all these phenotypes are:

BK3 (Ara-, Leu-, LacZ+ inducible, KanR)
H33 (Ara-, Leu-, LacZ+ constitutive, KanR)
KBS1: (Ara+, LacZ-, KanS)
JET3: (Ara+, LacZ-, KanR)

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

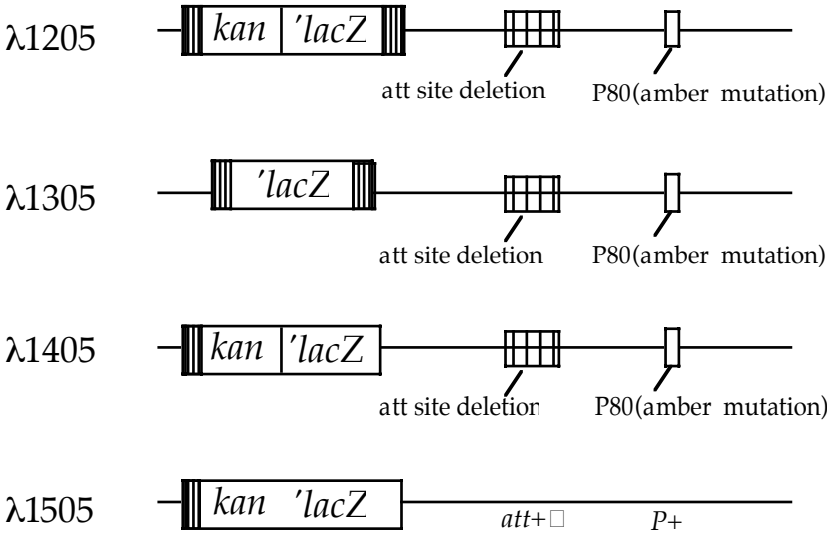
	Mac Ara Kan	LB Xgal Kan	LB Ara Xgal Kan
H33	white	white	white
pNK/KBS1	red	white	white

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?

The Kanamycin (Kan) and Xgal have gone bad. Reasoning for Kan: pNK/KBS1 is a KanS strain, yet grows on Kan-containing plates. Reasoning for Xgal: H33 is LacZ+ constitutive strain. It should therefore be BLUE on both LB Xgal Kan and LB Ara Xgal Kan plates, not WHITE. (Xgal is what is cleaved by the product of *lacZ* to give BLUE.)

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:



Question 9 (continued)

You set up the experiments as she 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	**none**	**KBS1 does not contain transposase, so transposon won't hop into chromosome and make cells KanR. (When cells are plated on Mac Ara Kan plates, they'll die!)**
λ1305	pNK/ KBS1	**none**	**This transposon lacks the gene conferring KanR. Thus, when plated on Mac Ara Kan plates, all cells will die (even if they got a transposon).**
λ1405	pNK/ KBS1	**none**	**This transposon is missing one of the inverted respeats required for transposition (IRs are recognized by transposase). No KanR will be introduced into the cells, and they'll die when plated.**
λ1505	pNK/ KBS1	**less**	**Most cells will be lysed and dead (lack amber mutation that blocks lysis). However, ~10% of phage will enter lysogenic cycle. Cells that have been lysogenized will be KanR and will grow on Mac Ara Kan.**

Question 10

Listed below are seven potential strains (A-G) that could result from the transposon mutagenesis performed in 7.02 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	**NG**	**NG**	**NG**	**NG**	**NG**	**G**	**NG**
M9 Glu Leu Kan	**NG**	**G**	**G**	**G**	**G**	**G**	**NG**
Mac Ara Kan	**NG**	**white**	**white**	**white**	**white**	**red**	**red**
Mac Lac Kan	**NG**	**white**	**white**	**white**	**white**	**white**	**white**
LB Xgal Kan	**NG**	**blue**	**white**	**white**	**white**	**blue**	**white**
LB Ara Xgal Kan	**NG**	**blue**	**white**	**white**	**blue**	**blue**	**white**

Note: NG= no growth; G = growth

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.

This mistake would reduce the number of mutant colonies (KanR) obtained from the transposon mutagenesis. Maltose is required to induced maltose binding protein (maltose receptor), which λ1205 uses to attach to *E. coli* cells. Since λ1205 carries the transposon needed for mutagenesis, low attachment of phage to cells--> low infection-->low mutagenesis frequency.

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

This mistake would reduce the number of KBS1 transductants obtained in the P1 transduction experiment, or eliminate transduction entirely. MC medium contains Ca^{+2} ions, which are a required cofactor for P1 phage attachment; low P1 attachment-->low infection--> low transduction frequency.

c) Sarah titered her λ1205 phage using KBS1 cells.

Sarah would not see any plaques on her titer plates. λ1205 contains an amber suppressor mutation that blocks DNA replication in KBS1 cells. Without DNA replication, no cell lysis can occur—hence no plaques. To titer the phage, Sarah would need to use an amber suppressor host like LE392.

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).

Jenn would expect to get very few transductants in her experiment. At an MOI of 2, a high proportion of cells will be infected by more than one phage. Thus, if a cell was initially infected by a transducing phage and received bacterial DNA, that cell will likely be lysed due to infection by a second, lytic phage. Since lysed cells can't form colonies, Jenn would never see that transductant.

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

Neither strain will grow! Ara- mutants cannot use arabinose as a carbon source, and thus will fail to grow on M9 Ara Leu plates (where arabinose is the sole carbon source). On the other hand, C600 strains are both Leu- and Thr-; this means that they cannot make their own leucine or threonine, and need both of these amino acids provided in the media to grow. Since M9 Glu Leu plates have no threonine, C600 won't grow.

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!**

1. **Determine straight average of the two data sets:**

   (79+83)/2 = 81; (7+10)/2 = 8.5; (1 + 0)/2 = 0.5

2. **Determine weighted average:**

$$\frac{81 + 8.5 + 0.5}{1.11} = 81 \text{ pfus on the plate}$$

****Many people did steps 1/2 in one step by dividing the sum of all the data by 2.22, which is also fine.**

3. **Determine number of pfus in phage/cell mixture:**

$$81 \text{ pfus on plate} \times \frac{1 \text{ ml phage/cell mix}}{0.4 \text{ ml on plate}} = 203 \text{ pfus in mix}$$

4. **Determine titer of original stock:**

$$\frac{203 \text{ pfus in phage/cell mix}}{(0.2 \text{ ml phage in phage cell/mix})(10^{-6} \text{ dilution})} = 1.01 \times 10^{9} \text{ pfu/ml}$$

Question 12 (continued)

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

1. **MOI= $\frac{\text{pfu}}{\text{cfu}}$ = 0.1**

2. **0.1 = $\frac{\text{(0.25 ml λ1205) x (1.01 x } 10^9 \text{ pfu/ml)}}{\text{X cfu}}$**

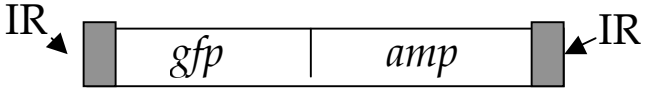
3. **X = 2.5 x 10^9 cfu**

4. **$\frac{\text{2.5 x } 10^9 \text{ cfu}}{\text{5 ml}}$ x $\frac{\text{1 OD550}}{\text{1 x } 10^8 \text{ cfu/ml}}$ = 5 OD550**

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.

Choose two:

1. **transposase: required for transposon to "hop" into DNA**
2. **maltose binding protein (MBP): receptor for λ**
3. **rhamnose metabolic genes: need your starting strain to be "wild type" for the process of interest.**

Question 13 (continued)

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?

Two possibilities:

1. **Mac Rhamnose Amp-◊ look for white/clear colonies**
2. **M9 Rhamnose Amp and M9 Glucose Amp◊ desired mutants would grow on plates with glucose, but not on plates with rhamnose as their sole carbon source.**

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.

Answer: strains 1, 3, and 4.
Reasoning: strains containing a translational *gfp* fusion (that is, insertion of *gfp* behind a promoter in the correct orientation and reading frame) will allow cells to glow green under UV light. These three strains show this phenotype.

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

Answer: strains 3 and 5
Reasoning: These strains fail to grow on M9 Rhamnose Amp (where rhamnose is the sole carbon source) but DO grow on M9 Glucose Amp. This ensures that the defect is specifically in rhamnose metabolism. Because strain 2 can't grow on M9 Glucose Amp either, we cannot say that it is defective in rhamnose metabolism specifically. For example, it may be an amino acid auxotroph or otherwise have difficulty growing on minimal media.

Question 13 (continued)

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

Answer: strains 3 and 4
Reasoning: In these strains, the "green" color is dependent on the presence of the inducer, rhamnose, in the plates. A strain does not have to be Rham- to contain a rhamnose-inducible *gfp* fusion!

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+	28
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)

There are three possible gene orders:

1. ***his*** ***kan*** ***trp***
2. ***his*** ***trp*** ***kan***
3. ***kan*** ***his*** ***trp***

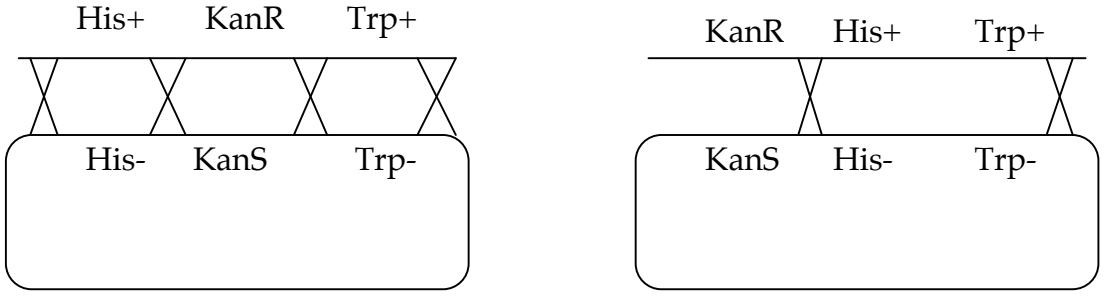
Selecting for His+ and calculating cotransduction frequencies (CF):

CF of His+ and KanR = 108 + 212/1000 = 0.32 (32%)
CF of His+ and Trp+ = 108 + 5/1000 = 0.113 (11.3%)

- **As a high CF indicates that two genes are closer together, this data tells us that his is closer to *kan* than his is to *trp*. Thus, order #2 is eliminated.**

To distinguish between the two remaining gene orders, look at the rare class in each set of data, and determine what type of event was required to generate that rare class:

Rare class (His+ selection) = His+, KanS, Trp+



- **The rare class arises from a quadruple crossover event if the order is *his kan trp* (#1), while it arises from a double crossover event if the order is *kan his trp* (#3). Since rare classes require rare events—and a quadruple crossover is a much rarer event than a double crossover—then gene order #1 is most likely.**

Rare class (KanR selection) = His+, KanR, Trp+

- **Note that this set of data is not particularly informative, as a double crossover event is required to get the "rare" class with either gene order.**

Since KanR marks the *cheA* gene, the order is:

his ***cheA*** ***trp***

NOTE: To get full credit for this type of problem, you need to walk the reader through your reasoning. Simply showing the calculations and gene order is not sufficient!

Question 14 (continued)

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

No. *cheA* is located between the *his* and *trp* genes and *cheB* is located outside the *his* gene. If they were the same gene, they would map to the same location.

You identify a third Che- strain. The Che- phenotype in this strain arises from a mutation in a gene you call *che*C; the *che*C mutation is 100% linked to a gene which confers tetracycline (tet) resistance. You suspect that *che*C 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 *che*C mutations were 100% linked (i.e. they are in the **same gene**)? Explain your answer briefly. (Hint: a diagram may be useful!)

Answer: Your transductants would all be TetS.

Explanation: If *cheA* and *cheC* are 100% linked, then every time a transductant gains KanR, it would LOSE TetR, as diagrammed below: