Name **KEY**

7.02
MICROBIAL GENETICS
EXAM
March 9th, 2005

EXAM GRADING KEY

Question 1 (20 points)

Please mark whether each of the following statements is true or false. If a statement is false, **correct the statement by crossing out and/or substituting word(s) or phrase(s).**

(For example: **__False__** The winter sky over Boston is usually ~~blue~~ **gray**).

(2 pts) **___false____** a) Bacteria are reproducing at their maximal growth rate during the ~~stationary~~ **log or exponential** phase of the growth curve.

(2 pts) **___false____** b) Two biological processes (studied/performed in the GEN module) that require homologous recombination are generalized transduction and ~~transposition~~ **specialized transduction**.

(1 pt) **____true____** c) The three mechanisms of transposition discussed by Professor Guarente in lecture were conservative, replicative, and RNA-mediated

(3 pts) **___false____** d) Each end of a transposon has an ~~operator site~~ **inverted repeat (or "end")** that is recognized by the enzyme ~~RNA polymerase~~ **transposase**.

(1 pt) **___true____** e) An Ara- mutant is unable to breakdown (metabolize) the sugar arabinose.

(1 pt) **___true____** g) In lab, we used a low MOI for our transposon mutagenesis to avoid having multiple transposon insertions in the bacterial chromosome.

(3 pts) **___false____** h) Because wild type lambda is a ~~lytic~~ **lysogenic** phage, infection of bacterial cells with this phage will lead to the formation of ~~clear plaques~~ **"cloudy" or "turbid"**.

(2 pts) **____false____** i) The movement of DNA from one bacterial strain to another via ~~lambda~~ **P1** phage is called generalized transduction.

OR

i) The movement of DNA from one bacterial strain to another via lambda phage is called ~~generalized~~ **specialized** transduction.

Question 1 (continued)

false j) If the Ara- and KanR phenotypes are genetically linked in the donor strain, transductants which are Ara- will ~~never~~ **always** be KanR.

false k) ~~100%~~ **1/6** of miniTn10-induced Ara- mutants will be phenotypically both KanR and LacZ+.

true l) The titer of a bacterial culture is given in colony forming units (cfu) per milliliter (mL).

Grading note: 1 point was given for correctly indicating "true" or "false" and 1 point for each correction that was made. Answers different from those given were evaluated on a case by case basis.

Question 2 (17 points)

You receive a set of four unmarked strains (1-4), patch them on the plates shown below, and obtain the data shown in the table below:

	A	B	C	D	E	F	G	H	I	J	L
	M9 Glu	M9 Glu Leu	M9 Ara Leu	Mac Ara	Mac Lac	LB Xgal	LB Ara Xgal	LB Kan	LB Cm	LB Tet	plaques or colonies formed after infection with λ1205
Strain 1	NG	G	NG	G, white	G, white	dark blue	dark blue	G	G	NG	
Strain 2	NG	G	G	G, red	G, white	white	white	NG	G	G	KanR colonies
Strain 3	G	G	NG	G, white	G, white	white	white	G	NG	NG	
Strain 4	NG	NG	NG	G, red	G, white	white	white	NG	NG	NG	plaques

Question 2 (continued)

a) Using the data provided in the table above, list the **phenotypes** of each strain.

Strain	Phenotypes
1	**Leu-, Ara-, Lac-, LacZ+ (constitutive), KanR, CmR, TetS** **(4 pts)**
2	**Leu-, Ara+, Lac-, LacZ-, KanS, CmR, TetR** **(3.5 pts)**
3	**Leu+, Ara-, Lac-, LacZ-, KanR, CmS, TetS** **(3.5 pts)**
4	**unknown auxotrophy, Ara+, Lac-, LacZ-, KanS, CmS, TetS** **(4 pts, 1 for "unknown auxotrophy")**

b) Strains 1-4 correspond to four strains used in the Microbial Genetics module. Identify each strain **by name** in the blanks provided. **(2 points total)**

(Reminder: the strains used were pNK/KBS1, KBS1, LE392, BK3, EJ1, C600, H33, JET3)
Strain 1: **H33** Strain 2: **pNK/KBS1** Strain 3: **EJ1** Strain 4: **LE392**

Grading note: Because there was some confusion about whether students needed to learn the phenotypes of different strains, everyone received one point here (for strain 1 and 3). Strain 2 and 4 could be determined from the data provided, and were worth 0.5 points each.

Question 3 (21 points)

You are interested in studying the regulation of genes involved in the biosynthesis of the amino acid tyrosine in *E. coli*. Thus, you decide to perform transposon mutagenesis using λ1205 as in the 7.02/10.702 lab. Your goal in this experiment is to create a *tyr::lacZ* translational fusion.

(6 pts) a) Given the goal of your experiment, name **three** characteristics that the starting strain for your mutagenesis **must** have, and **why** this characteristic is necessary? (Note: the "starting strain" is the strain that you will infect with λ1205.)

Here are six answers that were accepted (1 point for answer, 1 point for reasoning)

Characteristic	Why is this characteristic necessary?
pNK (source of transposase)	**Transposase is required for miniTn10 to hop from the λ1205 genome into the chromosome**

Question 3 (continued)

KanS	**Insertion of miniTn10 confers KanR, which allows <u>selection</u> of cells with a transposon insertion; thus, starting strain cannot already be KanR**
LacZ- (0.5 for Lac-)	**You want to use *lacZ* as a reporter gene to tell you about *tyr* regulation (<u>screening</u> for LacZ+), so your starting strain cannot make its own Bgalactosidase (*lacZ* gene product)**
Tyr+	**Your starting strain must be capable of performing the function you wish to mutate (here, biosynthesis of tyrosine) so you can <u>screen</u> for mutants, which will become Tyr-.**
Expresses maltose binding protein (MBP)	**MBP serves as the receptor for λ1205 on the *E. coli* cell surface; without it, λ1205 cannot bind to the cell and deliver the miniTn10 transposon**
Does NOT express the amber suppressor tRNA	**If the strain expressed the amber suppressor tRNA, λ1205 would be capable of lysing the bacterial cell—which would not be acceptable if you are trying to isolate transposon-induced mutants.**

Note that "maltose" and "Mg+2" are conditions of the media in which the cells are grown, not the cells themselves.

(1 pt) b) What type of plate will you use to **<u>select</u>** for strains that have received a transposon insertion anywhere in the chromosome? **rich media + Kan**

Note that KanR is a phenotype, not a plate! (-0.5 if said KanR)

(2 pts) c) What two plates will you use to **<u>screen</u>** for strains that have received a transposon insertion in a gene involved in tyrosine biosynthesis?

M9 Glu (Kan) and **M9 Glu Tyr (Kan)**

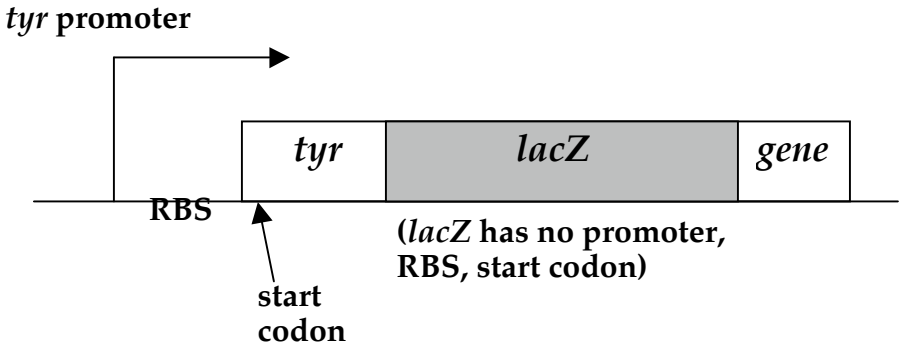
- **<u>no credit</u> if your plates did not contain a carbon source (like Glu or Ara)**
- **many people wanted to use Xgal plates here, which screens for LacZ phenotypes. You are looking for Tyr+ or Tyr- here**

(2 pts) d) What will be the phenotypes of a Tyr- strain on the plates you chose in part c)?

A Tyr- strain <u>will not grow</u> on the M9 Glu (Kan) plate but <u>will grow</u> on the M9 Glu Tyr (Kan) plate.

Question 3 (continued)

(6 pts) e) **Diagram** (draw a picture of) a *tyr::lacZ* translational fusion at the **DNA** level. Be sure to include and label any relevant DNA sites (e.g. promoter, start codon, ribosome binding site) and gene name(s).



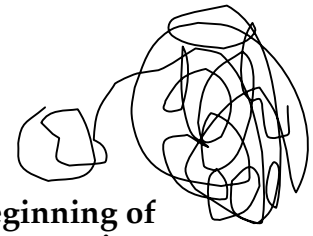
Grader was looking for the following:

- ***lacZ* inserted into the middle of a *tyr* gene**
- ***tyr* promoter "upstream" of the start of the *tyr* gene**
- **RBS between the promoter and the start of the *tyr* gene**
- **Start codon at the beginning of *tyr* gene**
- **NO promoter, RBS, start codon on *lacZ* gene**
- ***lacZ* in correct orientation and next to the "start" of the tyr gene**

(4 pts) f) Diagram the **RNA** and **protein** products that will be transcribed and translated from the DNA you drew in part e). Be sure to label your drawings with gene and protein name(s)!

RNA:

Protein:



β-galactosidase

Grader was looking for the following:

- **RNA has both *tyr* and *lacZ* genes named**
- **Protein is a fusion between *tyr* and *lacZ* protein products**
- **Protein names are correct (partial Tyr and β-galactosidase)**

Notes:

- **The protein will not continue with the rest of the Tyr protein, as Bgal has a stop codon**
- **The fusion will not include the KanR protein, as this will be encoded on a different mRNA and has its own translational signals**

Question 4 (25 points)

You are interested in studying the *E. coli* genes involved in the utilization of the fictional sugar imaginose (*ima* genes). You decide to identify Ima- mutants by transposon mutagenesis using λ1205.

To prepare for transposon mutagenesis, you titer your λ1205 phage stock using the following protocol:

1. Grow overnight culture of KBS1 cells in LB + $CaCl_2$.
2. Mix 0.9 mL cells with 0.3 mL of properly diluted phage and incubate for 30 minutes on the bench.
3. Plate using the "top agar" protocol used in 7.02/10.702 lab.

After incubating your plates overnight at 37°C, you find **no plaques** on your plates!

(10.5 pts) a) In the space provided, **identify** the **three** mistakes that were made in setting up the λ1205 titering experiment. **Explain** why each mistake resulted in "no plaques," and state how you would **correct** each error in future experiments.

"Protocol mistake"	Why did this mistake result in "no plaques"?	Correction?
used KBS1 cells (0.5 points)	**KBS1 cells do not contain the amber suppressor tRNA required to allow λ1205 to lyse cells (2 points)**	**use LE392 cells (1 point)**
grew cells overnight in LB + $CaCl_2$ (0.5 points)	**λ1205 requires Mg^{+2} as a cofactor for infection, not Ca^{+2} (2 points)**	**Grow cells overnight in the presence of $MgCl_2$ (LBMM) (1 point)**
failed to grow cells in the presence of maltose (0.5 points)	**λ1205 uses the maltose binding protein as a receptor for entering *E. coli* cells (2 points) (2 points)**	**Grow cells overnight in the presence of maltose (LBMM) (1 point)**

After correcting your λ1205 titering protocol, you determine that your λ1205 phage stock has a titer of 5×10^8 pfu/mL.

You also have a culture of pNK/KBS1 cells for use in your mutagenesis experiment. A 1:75 dilution of this pNK/KBS1 culture has an OD_{550} of 0.534, and you have previously determined that 1 $OD_{550} = 1 \times 10^8$ cfu/mL.

Question 4 (continued)

(6.5 pts) b) What **volume** of the λ1205 phage stock should be added to 0.5 mL of undiluted pNK/KBS1 cells to give an M.O.I. of 0.2? In your answer, be sure to **define MOI** and **SHOW ALL CALCULATIONS**.

MOI = $\frac{\#\ pfu}{\#\ cfu}$

MOI = 0.2 = $\frac{(mL\ of\ phage)(pfu/mL)}{(mL\ of\ cells)\ (cfu/mL)}$

cfu/mL of undiluted cells = $(0.534\ OD_{550})\left(\frac{1 \times 10^8\ cfu/ml}{OD_{550}}\right)(75) = 4 \times 10^9$ cfu/mL

0.2 = $\frac{(mL\ of\ phage)(5 \times 10^8\ pfu/mL)}{(0.5\ mL\ of\ cells)(4 \times 10^9\ cfu/mL)}$

mL of phage = $\frac{(0.2\ pfu/cfu)(0.5\ mL\ of\ cells)(4 \times 10^9\ cfu/mL)}{(5 \times 10^8\ pfu/mL)} = 0.8$ mL

Grading notes:
- **2 points for defining MOI**
- **1 point for setting up calculation correctly (mL phage x titer, etc.)**
- **3 points for correctly determining cell titer for use in calculation**
- **0.5 points for correct volume**

You identify putative Ima- mutants as white colonies on Mac Ima Kan plates, then screen these mutants on the appropriate plates to determine if you have created any *ima::lacZ* translational fusions. You find one mutant (Strain X) with the following phenotypes:

M9 Imaginose	M9 Glucose	LB Xgal	LB Xgal + imaginose
no growth	growth	white	blue

(5 pts) c) Based on what you know about the regulation of other operons encoding genes for sugar metabolism, explain **how/where** the transposon has inserted and **why** Strain X is only blue on LB Xgal + imaginose plates.

The transposon has inserted into a gene for imaginose metabolism (2 points) in the correct orientation and reading frame to give a LacZ+ strain (1.5 points). Since strain X is only blue on LB Xgal + imaginose plates, the gene interrupted by the transposon must be imaginose-inducible (1.5 points); thus, Bgal is only produced in the presence of the inducer imaginose.

Question 4 (continued)

You treat Strain X with EMS, a chemical that causes single base changes in DNA. You recover an interesting mutant, Strain Y, which contains a **loss-of-function mutation** in the **regulator** of the *ima* utilization operon. Strain Y has the following phenotypes:

M9 Imaginose	M9 Glucose	LB Xgal	LB Xgal + imaginose
no growth	growth	white	white

(3 pts) d) Do the phenotypes of Strain Y indicate that the operon for imaginose utilization is under negative or positive control? Explain your answer briefly.

Positive control (1 point). The regulator mutant causes the reporter to be "OFF" in both the presence and absence of the inducer imaginose (1 point). This suggests that the regulator is normally required to turn "ON" transcription of imaginose inducible genes (1 point).

(Note: if it was negatively regulated, a loss of function mutation in the regulator would lead to an "always ON" (constitutive) phenotype.)

Question 5 (12 points)

You have identified a mutant that has the KanR gene from miniTn10 linked to a gene involved in the utilization of the sugar maltose (*mal::kanR*). You would like to map the *mal* gene is relation to two nearby loci, *phe* and *gal*.

To do your mapping experiment, you grow P1 phage on your mutant donor strain, and use the resulting P1 lysate to infect an appropriate recipient strain. The phenotypes of the donor and recipient strains are shown below:

Donor: Mal-(KanR), Phe-, Gal+
Recipient: Mal+(KanS), Phe+, Gal-

You select for Gal+ transductants, and screen these for their Kan and Phe phenotypes. You obtain the following data:

Phenotype	Number of transductants
Gal+, KanS, Phe+	490
Gal+, KanR, Phe+	10
Gal+, KanS, Phe-	720
Gal+, KanR, Phe-	80

Question 5 (continued)

(6 pts) a) What are the cotransduction frequencies of Gal and Kan? Of Gal and Phe? **SHOW YOUR CALCULATIONS.**

CTF of Gal+ and KanR = $\frac{\text{\# Gal+ and KanR}}{\text{total \# of Gal +}} = \frac{10 + 80}{1300} = 6.9\%$

CTF of Gal+ and Phe- = $\frac{\text{\# of Gal + and Phe-}}{\text{total \# of Gal +}} = \frac{720 + 80}{1300}$ 61.5%

People generally lost points here for a few reasons:

1. **Not showing work (what numbers did you add together?)**
2. **Using Phe+ instead of Phe- in calculation (need to look at DONOR markers)**

(2 pts) b) Which of the two loci (*phe* or *mal*) is closest to *gal*? Explain your answer in one sentence.

***phe* is closest to *gal*, as the cotransduction frequency of Gal and Phe is higher than that of Gal and Kan. Higher cotransduction frequency = genes are closer together.**

c) What is the most likely gene order of the *mal*, *gal*, and *phe* genes? Explain your reasoning (diagrams may be useful here!).

UNGRADED
Because of a mistake in the design of the transduction experiment, you cannot actually determine unequivocally which of the two remaining gene orders is correct. An explanation of why this is so is attached at the end of this key. Thus, we decided not to grade this part of the problem, and thus problem 5 was graded out of 12 points instead of 17 points.

In addition to being KanR, Phe-, and Gal+, the donor strain is also Ara+, whereas the recipient strain is Ara-. When you patch the Gal+ transductants on Mac Ara plates, you find that **ALL** the transductants are Ara-.

(4 pts) d) What does this result tell you about the distance (in kb) between the *gal* and *ara* genes on the *E. coli* chromosome? Explain your answer **briefly**.

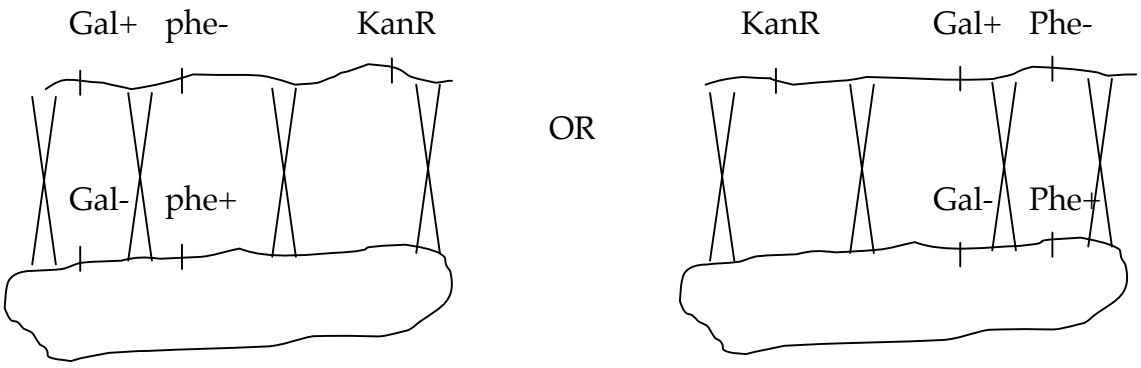
If all the Gal+ recipients are also Ara-, then that means that Ara+ is NEVER cotransduced with Gal+ from the donor into the recipient (CTF = 0%) (1 point). Thus, these two genes must be far enough away so that they can never be packaged in the same P1 phage head (1 point) a distance of at least 100 kb (will also accept 50 kb, as that is what Professor Guarente said in lecture) (2 points).

Note: MANY people said that the two genes were 100% linked (and thus always cotransduced). If this were the case, then ALL the Gal+ transductants would have the same Ara phenotype as the DONOR, which is Ara+. This is NOT observed.

Explanation of Problem 5, part c)

You have already determined from part b) that phe is closest to gal. Thus the only two possible gene orders are:

Thus the transduction that you are doing is either:



Drawn in above in each diagram are the four possible locations where crossovers could occur. The crossovers will be referred to from left to right as crossovers #1, #2, #3, and #4. Because you are selecting for Gal+ transductants, the only transductants you will see are ones that had a crossover event on each side of Gal+. Thus, even though all possible double crossovers (#1 and #2, #1 and #3, #1 and #4, #2 and #3, #2 and #4, #3 and #4) and the quadruple crossover could have occurred, the only ones you would get out of your selection for each order are:

#1 and #2 gives KanS phe+	#1 and #3 gives KanR phe+
#1 and #3 gives KanS phe-	#1 and #4 gives KanR phe-
#1 and #4 gives KanR phe-	#2 and #4 gives KanS phe-
quadruple crossover gives KanR phe+	#2 and #3 gives KanS phe+
the rarest class would be KanR phe+	all of these classes are double crossovers so it is not possible to predict
(this would be consistent with the data)	which double crossover would be the most common and which would be the rarest because crossover frequency is not linearly proportional to distance

(explanation continued on the next page)

Explanation of Problem 5, part c) (continued)

That having been said, if you had to wager a guess for the order on the right about which crossovers would be rare and which would be common, a good guess would be that crossover #3 would be rare, because it occurs in between two genes that are close together. Also, it would be a good guess that crossover #1 would be rare, because KanR is so far away that it might not even always be on the same piece of DNA as the other two markers and thus might not even have the chance to happen. Thus, a reasonable prediction would be that any double crossover that involves #1 and #3 would be the rarest, and any double crossover that does not involve either crossovers #1 or #3 would be the most common. Thus the rarest double crossover could be #1 and #3, which yields KanR phe+, which is indeed the rarest class in our data. The most common double crossover could be #2 and #4, which yields KanS phe-, which is indeed the most common class in our data. Thus we cannot state that this order is inconsistent with our data.