

Name _____ **KEY** _____ Bench Letter _____ UTA _____

7.02 Microbial Genetics in Lab Quiz

Fall, 2001

September 28, 2001
ANSWER KEY

This quiz contains 4 questions worth a total of 48 points.

**Be sure to write your name, Bench letter and Undergraduate TA's
(UTA) name on EACH page (2 points)**

GOOD LUCK!

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1. (12 points)

What was the purpose of the following reagents in the Genetics module? A complete answer will **BRIEFLY** describe when it was used in the module (what experiment) and what it is/how it works/what it does.

LB, Arabinose, X-gal, Kanamycin (LB Ara Xgal Kan) Plates:

These plates were used to test whether our *ara::lacZ* fusions created by transposon mutagenesis are inducible by the presence of arabinose. Arabinose is required to induce transcription of the *araBAD* operon. X-gal is a chromogenic substrate for the product of the *lacZ* gene (β -galactosidase); only $LacZ^+$ colonies are blue on these plates. Kanamycin in the plates selects for the presence of the transposon. Arabinose-inducible *ara::lacZ* fusions will be blue on this plate, and white on LB X-gal Kan plates, while arabinose-constitutive *ara::lacZ* fusions will be blue on both LB X-gal Kan and LB Ara X-gal Kan plates.

Phage lambda 1205:

λ 1205 was used to deliver the miniTn10 transposon into pNK/BW140 cells during transposon mutagenesis. λ 1205 is defective in lysis of *E. coli* because of an amber mutation in a DNA replication gene. It is also incapable of lysogeny because of a mutation in the *attP* (attachment) site (NOT because of the amber mutation!!). These characteristics make λ 1205 a good delivery vehicle for the transposon.

Bacterial strain C600:

C600 was used for mapping the *ara::lacZ* fusion with respect to two other genes in the *E. coli* genome, *leu* and *thr*. The C600 “recipient strain” has mutations in the *leu* and *thr* genes (Leu^- , Thr^-), and is also Kan^S (Ara^+), while the “donor strain” (your mutant) is Leu^+ , Thr^+ , Kan^R (and presumably Ara^-). Calculation of cotransduction frequencies between the genetic markers allows you to determine the likely gene order.

2. (12 points)

A wild-type *E. coli* strain was grown in minimal medium containing glucose and lactose as carbon sources. Over time you followed the growth of the culture and obtained the growth curve indicated below. Describe the *lac* operon promoter/operator at t1 and t3, including the relevant repressors and activators regulating this promoter. (Diagrams would be useful here!)

1. When glucose and lactose are present (t1) cells will utilize glucose and the *lac* operon will not be induced. Thus, no β -galactosidase protein is made. Lactose will be utilized by the cell (i.e. the *lac* operon will be transcribed) only after glucose has been used up (t3). This phenomenon is called catabolite repression.

2. In the presence of glucose and lactose (t1), the *lac* operon is not transcribed for two reasons:

- a. Lactose cannot enter the cell. Thus, the *lac* repressor LacI will bind to the promoter region of the *lac* operon and prevent binding of the *E. coli* transcription machinery (RNAP).**
- b. CAP cannot bind to the promoter, as cyclic AMP levels are low due to high levels of glucose present in the medium.**

3. At t3, all the glucose in the medium will have been used up, the *lac* operon will be transcribed and β -galactosidase will be produced. In the presence of lactose, the *lac* operon is transcribed for two reasons:

- a. The *lac* repressor LacI cannot bind to the promoter, as allolactose binds to LacI and prevents it from binding to the operator site.**
- b. Due to the lack of glucose, cyclic AMP levels are high, allowing CAP to bind to the promoter. This is necessary for recruiting the *E. coli* transcriptional machinery to the promoter. These two events allow the transcription of the *lacZ* gene, which encodes the enzyme β -galactosidase.**

PLEASE NOTE: A DRAWING OF THE *lac* PROMOTER AT T1 AND T3 WAS ALSO AN ACCEPTABLE ANSWER.

3. (12 points)

a) You have reason to believe that *E. coli* can metabolize imaginose, a sugar you recently discovered. Based on what you learned in 7.02 describe a strategy (in flow chart form) to identify the genes involved in metabolizing this sugar.

In order to identify mutants involved in the metabolism of imaginose, I would begin by mutagenizing the cells with transposon mutagenesis. (Other strategies of mutagenesis would work, but each has its own challenges.)

***E. coli* BW140(Lac-)/plasmid pNK cells (Gene encoding transposase is found on plasmid pNK and is inducible by IPTG)**



Infect with λ 1205 carrying mini-Tn10 transposon (λ 1205 is a defective phage that serves as a delivery vehicle for mini-Tn10; mini-Tn10 contains Kan^R gene and *lacZ*)



Select for mutants that have received a transposon on plates containing kanamycin



Screen for mutants that can no longer utilize imaginose as a result of transposon insertion.

The screen and selection can be done on MacConkey Imaginose Kan plates. I will look for mutants that are no longer able to ferment Imaginose. These colonies will be white because they are metabolizing amino acids in the media, which raises pH, and turns the pH sensitive dye white.

(You could also patch colonies on M9 Glucose and M9 Imaginose plates and look for growth. Cells that grow on M9 Glucose and not on M9 Imaginose are Imaginose-. However, it is very important that you don't plate your initial transposon mutagenesis mixture directly on M9 Imaginose - You will select against the mutant you are interested in identifying.)

(Identifying the regulation of genes in response to the presence of imaginose through *lacZ* activity is not necessary at this point, as it doesn't directly indicate disruption of a gene involved in imaginose metabolism.)

b) How would you test whether the genes you identified are induced in the presence of imaginose?

In order to determine whether the expression of my mutants was inducible by imaginose, I would assay the activity of β -galactosidase on X-gal, which is encoded by the '*lacZ*' gene in our transposon. If '*lacZ*' has inserted in the correct orientation and reading frame in the gene affected in my mutant, it will be transcribed and translated, and β -

galactosidase activity will correlate with transcription from my mutant gene. I would take my white mutants and patch on LB X-gal Kan plates and LB X-gal Imaginose Kan plates. I expect that mutants that are inducible by Imaginose will produce white colonies on LB X-gal Kan plates (in absence of imaginose) but will produce blue colonies in the presence of imaginose.

4. (12 points)

You want to perform a mutagenesis using the miniTn10 transposon. Debbie has given you a suspension of *E. coli* BW140/pNK cells that contains 10^8 colony forming units/ml. Debbie also gave you a λ 1205 stock with a titer of 10^9 pfu/ml.

The 7.02 staff has determined that the transposon mutagenesis works best if you do the following:

1. Use an MOI of 0.3
2. Plate 0.2 ml of cells per plate
3. Plate 10^5 cells per plate

a) By what factor will you need to dilute the cells? (Show all calculations!)

1. You want to plate 105 cells/plate, and 0.2 ml of cells per plate. You have a cell stock with a titer of 10^8 cells/ml

2. Therefore, the titer of your plated cells must be: 10^5 cells/0.2 ml \rightarrow 5×10^5 cells/ml

3. $\frac{10^8 \text{ cells/ml}}{5 \times 10^5 \text{ cells/ml}} \rightarrow 2 \times 10^2$

4. Therefore, you must dilute your cell stock 1/200 (5×10^{-3})

b) By what factor will you need to dilute the phage? (Show all calculations!)

1. You want an MOI of 0.3

4. You have pfu = 10^9

2. MOI = $\frac{\text{pfu}}{\text{cfu}} = 0.3$; cfu = 5×10^5 (from part a)

5. $\frac{10^9 \text{ pfu}}{1.5 \times 10^5 \text{ pfu}} = 6.7 \times 10^3$

3. pfu = $0.3 \times (5 \times 10^5) = 1.5 \times 10^5$

6. Therefore, you must dilute your phage 1/6700, or 1.5×10^{-4}

c) By using an MOI of 0.3 a high percentage of cells will receive no phage. Why did Debbie nevertheless insist that you use a low MOI rather than a high one, i.e. MOI=10?

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If the MOI is too high, a high percentage of *E. coli* will receive more than one phage, and thus more than one transposon. It will be hard to impossible to determine which transposon was responsible for the phenotype observed.