

NAME: _____

MICR/MBMB 460

Fall, 2008

Exam II

1. (12 pts) P1 phage is used in a generalized transduction experiment with the following *E. coli* strains:

Donor: *metA*⁻ *galK*⁺ *valD*⁺

Recipient: *metA*⁺ *galK*⁻ *valD*⁻

The results were:

<u>Met</u>	<u>Gal</u>	<u>Val</u>	<u>No. of colonies</u>
+	+	+	17
+	-	+	541
-	+	+	227
-	-	+	385

a) What are the cotransducing frequencies between the selected marker and both unselected markers?

b) What are the corresponding distances between the selected marker and the unselected markers? Hint: P1 packages about 2' of DNA.

c) Draw a map of the *E. coli* chromosome in this region, using the proper units. Remember to determine the correct order of the genes.

2. (2 pts; circle the correct answer) Generation of a transductant from a linear piece of transferred DNA requires an odd / even number of crossover events.

3. (2 pts each; **14 pts** total) For each of the following items, match the item to the type of regulation the item BEST represents. You may use the choices on the right more than once.

- | | |
|---|----------------------------------|
| a) _____ Interaction of <i>hok</i> and <i>sok</i> RNAs | A) Transcriptional activation |
| b) _____ Transcript from P _{RE} | B) Transcriptional repression |
| c) _____ CIII interaction with CII | C) Negative autoregulation |
| d) _____ Q functioning properly | D) Positive autoregulation |
| e) _____ Pcf interacting with P' | E) Antitermination |
| f) _____ Xre binding to O ₃ and O ₄ | F) Translational regulation |
| g) _____ RstC interacting with RstR | G) Post-translational regulation |

4. (**2 pts**; circle the correct answers) The lambda repressor is considered a repressor that negatively autoregulates itself when it is bound to O_R1 / O_R2 / O_R3 and an activator that positively autoregulates itself when it is bound to O_R1 / O_R2 / O_R3.

5. (**4 pts**) You are studying a mutant lambda phage that excises its DNA from the *E. coli* chromosome immediately after integration. Thus a lysogen is never created even though the mutant lambda goes through all three phases of the lysogenic cycle. You have sequenced all of the promoter and protein-encoding regions of the lambda DNA and no mutations were observed. What is one explanation for your observations?

6. (**6 pts**) Indicate if the following statements regarding transformation are True (T) or False (F).

- a) Transformation requires that both the donor and recipient be alive _____
- b) The donor must be competent _____
- c) If the transferred DNA is a replicon its fate is either restriction or recombination _____

7. (4 pts) You are studying a mutant lambda lysogen that cannot be induced. You have determined that the host RecA* protein is functioning normally. In addition, you have also ascertained that CI can be cleaved. You have sequenced the P_L and P_R lambda promoters and both are normal. Nonetheless, this mutant lambda never enters the delayed early stage and, consequently, always dies along with the damaged host. What is one possible explanation for your observations?

8. (4 pts) During our discussion of phage encoded toxins we covered the effect induction has on toxin dosage. Besides transcription of the toxin genes during induction, illustrate the other effect induction has in a simple drawing below.

9. (4 pts) DRAW the O_R region in lambda and where Cro would be bound if Cro concentrations were high.

10. (4 pts) In the bacteriophage P1 plasmid addiction system, what would be the most likely consequence of a mutation in *phd* that rendered the Phd protein resistant to proteolytic degradation?

11. (20 pts) You are characterizing a mutant phenotype, *speedy*, of a virulent phage in which the plaque size is twice that of the wild type (presumably because the mutant *speedy* phage are able to complete an infection cycle much more quickly than normal). To determine how many genes control the *speedy* phenotype, you perform a cis-trans test with several *speedy* point mutants.

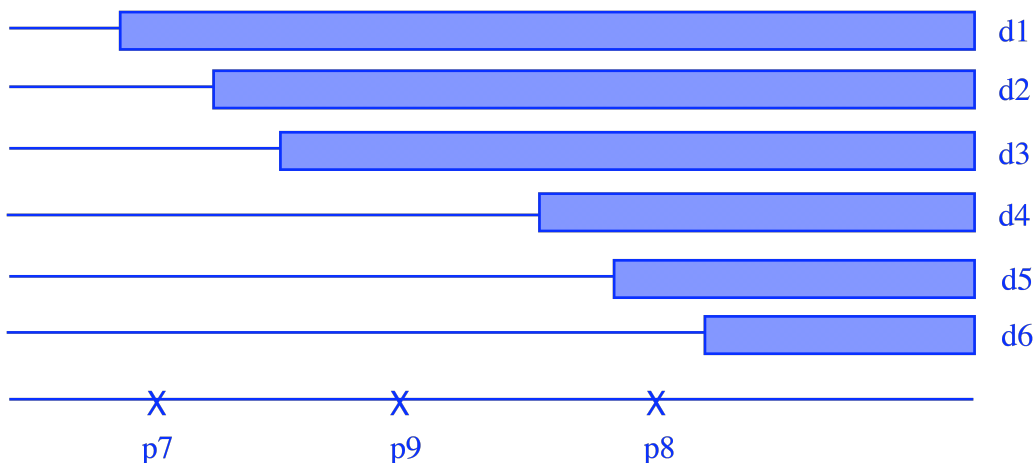
	p1	p2	p3	p4	p5	p6
p1	-	+	+	+	+	+
p2	+	-	+	-	-	+
p3	+	+	-	+	+	-
p4	+	-	+	-	-	+
p5	+	-	+	-	-	+
p6	+	+	-	+	+	-

a) How many genes control the *speedy* phenotype?

b) Identify which point mutations are located in the same gene.

c) You now perform deletion mapping on three more point mutants, p7, p8 and p9, using the six deletion mutants shown below. Given that the point mutations are located as shown on the line map, fill in the table with the results you expect from the deletion mapping experiment.

	d1	d2	d3	d4	d5	d6
p7						
p8						
p9						



d) To obtain a fine-resolution map of three different point mutants (p10, p11 and p12), you perform phage genetic crosses and score the results as shown.

Cross	# Wild-Type	Total # Phage	% Recombination
p10 X p11	56	467	
p10 X p12	23	769	
p11 X p12	54	599	

Fill in the table by determining the percent recombination for each cross.

Draw a map of these three point mutations, indicating order and distance.

12. (4 pts) A *V. cholera* lysogen containing a CTX prophage that cannot be induced (no progeny phage particles produced) has been isolated. Analysis of the late genes for phage particle morphogenesis indicates no mutations. Experiments also indicate that RecA* can cleave the RstR protein and that the EspD secretory system of *V. cholera* is functional. What are **two** possible reasons why this prophage cannot be induced? **Limit your answer to four sentences or less.**

13. (4 pts) A *E. coli* H-19B lysogen has been isolated that has a mutation in the *fur* gene that renders the corresponding protein an active repressor regardless of the presence of its corepressor. How would this mutation affect the level of shiga toxin produced by the stable lysogen? How would the level of toxin differ if the lysogen were inside the intestine versus in the soil? **Limit your answer to three sentences or less.**

14. (4 pts) What are two of the four methods discussed in class used to determine if a gene or region of DNA has been horizontally transferred?

15. **Essay (12 pts)** In essay format, answer the following questions in **four sentences or less per question**.

a) What are the similarities of lambda and PBSX induction?

b) Describe the role of antiterminators in both lambda and PBSX.

c) Compare and contrast the propagation of lambda and PBSX using the terms lysis, lysogeny, vertical transmission, and horizontal transmission.