

NAME: _____

MICR/MBMB 460

Fall, 2007

Exam II

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

Donor: *alaB*⁻ *bioC*⁺ *pheE*⁺

Recipient: *alaB*⁺ *bioC*⁻ *pheE*⁻

The results were:

<u><i>alaB</i></u>	<u><i>bioC</i></u>	<u><i>pheE</i></u>	<u>No. of colonies</u>
-	-	+	56
-	+	+	317
+	-	+	738
+	+	+	354

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

B) Give the formula for calculating map distance from cotransducing frequency.

C) What are the corresponding distances between the selected marker and the unselected markers?

D) 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 each; **12 pts** total) For each of the following items, match the item to the type of regulation the item BEST represents.

- | | |
|---|----------------------------------|
| a) _____ pCI binding to O_R2 | A) Transcriptional activation |
| b) _____ Cro binding to O_R1 | B) Transcriptional repression |
| c) _____ P_{anti-Q} product interacting with P_R to t_R3 transcript | C) Negative autoregulation |
| d) _____ pN functioning properly | D) Positive autoregulation |
| e) _____ sib region on the P_L to t_L2 transcript | E) Antitermination |
| f) _____ pCI interacting with P_{RE} | F) Antisense RNA |
| | G) mRNA degradation |
| | H) Post-translational regulation |

3. (**2 pts**) DRAW the O_R region in lambda and where pCI would be bound if pCI concentrations were low.

4. (**6 pts**) Identify the following items as having to do with transformation (TF), conjugation (C), and/or transduction (TD). NOTE: There may be more than one process involved per item!

_____ Improper excision from the bacterial chromosome

_____ Donor is alive

_____ Recombination may be required after the transfer event

5. (**8 pts**) State whether the following processes typically involve homologous recombination (HR) or illegitimate recombination (IR).

_____ Lambda integration

_____ Generalized transduction

_____ Production of a specialized transducing particle

_____ Production of an F'

6. (6 pts) You conduct a conjugation experiment in the lab between an F⁺ *E. coli* strain (the donor) and an F⁻ recipient. Unfortunately, you only know that the donor is F⁺; you don't know whether the donor is actually F⁺, Hfr or F'. The donor has the phenotype Arg⁺ Tyr⁺ Kan^S while the recipient is Arg⁻ Tyr⁻ Kan^R. Following the conjugation event, you isolate many Arg⁻ Tyr⁺ Kan^R cells and some cells that are Arg⁺ Tyr⁺ Kan^R.

a) The only explanation for these results is that your donor strain is F⁺ / Hfr / F'. (Circle only one)

b) How many of the exconjugants do you expect to become F⁺?

b) DRAW the location of the F factor in the donor relative to the Arg⁺, Tyr⁺ and Kan^R genes.

7. (4 pts)

a) You conduct a U-tube experiment with two bacterial strains and discover that genetic transfer still occurs even if you filter out whole cells. What genetic transfer event cannot be responsible?

b) You now use a filter in your U-tube that filters out whole cells and phage particles, and no genetic transfer is evident. What genetic transfer event must be taking place?

8. (6 pts) You are studying a lambda mutant that can never be induced. You have sequenced all of the protein-coding genes in the lambda genome and they are all normal. In addition, you know that LexA is cleaved after the cell is exposed to UV light, so the RecA* activity also seems to be normal. Give one possible reason why this lambda mutant can't be induced. (Hint: You only sequenced the protein-coding genes of the lambda lysogen.)

9. (6 pts) In two sentences each, explain the likely consequences of a lambda mutation that rendered *pcII* non-functional:

a) in a healthy *E. coli* cell.

b) in an unhealthy *E. coli* cell.

10. (4 pts) DRAW the translational regulation of the P_R to t_R3 transcript.

11. (4 pts) Only one lambda protein is produced in a lysogenic cell, but this protein makes the cell immune to superinfection by another lambda phage. Name the protein and explain how this protein prevents any new lambda DNA entering the lysogenic cell from being expressed.

12. (6 points) You are studying two separate RNA molecules from the same bacterial strain. One of these is a mRNA that is encoded by the bacterial genome and produces oxidoreductase when translated. The other RNA is not a mRNA and its gene is carried by an F' plasmid. Following conjugation of this F⁺ strain to an F⁻ strain, you notice that production of the oxidoreductase protein is greatly reduced in the recipient. However, the amount of oxidoreductase mRNA produced is the same before and after the conjugation event. You run the conjugation experiment several more times and always get the same result: less oxidoreductase activity after conjugation than before conjugation. In 10 words or less, give a possible explanation for the role of the RNA produced by the F' plasmid.

14. (24 pts) ESSAY FORMAT. You have isolated a lambda phage that contains a conditional *cro* mutation. When the host is grown at 37°C, the Cro protein functions normally. However, when the host is grown at 42°C, a mutant Cro protein is produced that has equal affinities for O_R1, O_R2 and O_R3, even at very low mutant Cro concentrations. Describe in detail what will happen if this lambda phage is allowed to infect a healthy bacterial cell at 37°C and the temperature is shifted to 42°C after delayed early infection has begun. In your description, include all of the following: *N* (pN), *cro* (Cro), *Q* (pQ), *cI* (repressor), *cII* (pcII), *cIII* (pcIII), *nut*, *qut*, P_R, P_L, P_R2, P_{RE}, P_{RM}, t_L1, t_L2, t_R1, t_R2, t_R3, t_R4, t_R5, O_L, O_R1, O_R2, O_R3.