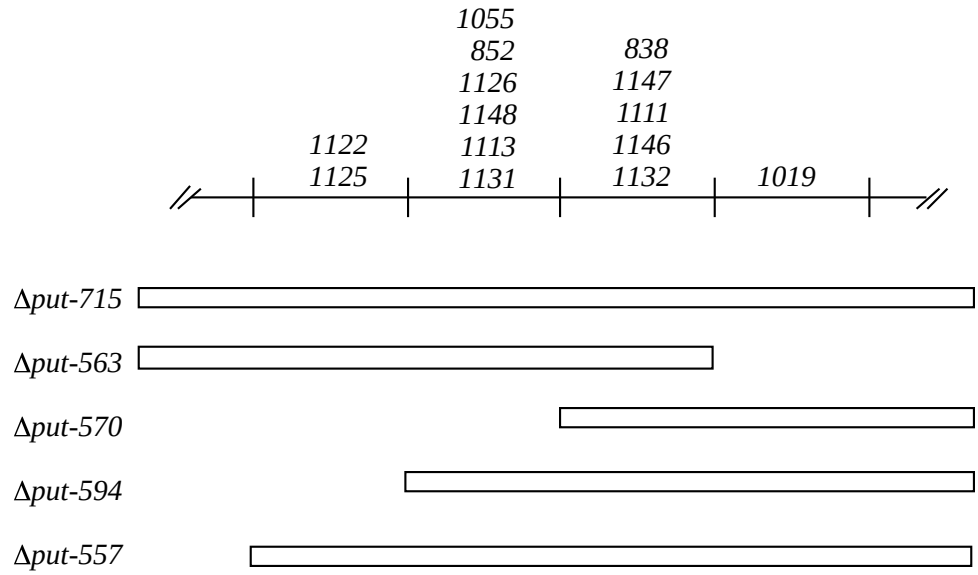
Mcbio 316: Exam 2 **ANSWER KEY**
Spring 2000

(10) **1.** Proteins encoded by the *put* operon allow cells to use proline as a sole carbon or nitrogen source. A deletion map of part of the *put* operon is shown below. The open bar indicates the region deleted in the indicated mutant. The numbers above the line represent allele numbers of *put* mutants that map within the four deletion intervals shown.



a. A new Put$^-$ mutant was isolated that <u>can</u> revert to Put$^+$ but <u>cannot</u> repair any of these deletions. What can you infer about the type of mutation and where it is located?
ANSWER: The new mutant can revert so it is probably NOT a deletion (i.e. it is probably a point mutant). The new mutant cannot repair any of the deletions so it most likely lies within the region spanned by every deletion (that is, the interval including mutations 838, 1147, etc).

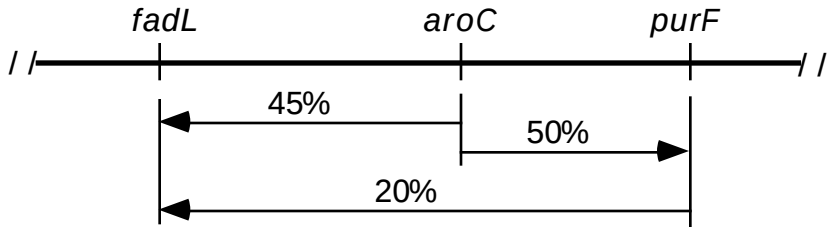
b. A second Put$^-$ mutant was isolated that <u>cannot</u> revert to Put$^+$ and <u>cannot</u> repair any of these deletions. What can you infer about the type of mutation and where it is located?
ANSWER: The new mutant can<u>not</u> revert so probably <u>is</u> a deletion (i.e. it is probably not a point mutant). The new mutant cannot repair any of the deletions so it probably includes the region spanned by every deletion (that is, the interval including mutations 838, 1147, etc) -- but it may remove additional DNA as well.

c. How could you use genetic mapping to determine what part of the *put* operon was affected in the Put$^-$ mutant described in b? [What donor(s) and recipient(s) would you use and how you would select for recombinants.]
ANSWER: Check for recombination with other point mutations located in each of the different deletion intervals, selecting for repair of the Put- phenotype. If the mutation is a relatively large deletion, it will probably be unable to repair multiple point mutations (you could use the new mutant either as donor <u>or</u> recipient in these experiments but it is usually a good idea to use the deletion mutant as a recipient because deletions do not undergo true reversion).

(10) **2.** Two-factor crosses were done to map several mutations. The results are shown below.

Donor	Recipient	Selected marker	Recombinants	Number obtained
fadL purF$^+$	*fadL*$^+$ *purF*	*purF*$^+$	*fadL*	20
			fadL$^+$	80
fadL aroC$^+$	*fadL*$^+$ *aroC*	*aroC*$^+$	*fadL*	45
			fadL$^+$	55
aroC$^+$ *purF*	*aroC purF*$^+$	*aroC*$^+$	*purF*	50
			purF$^+$	50

Draw a linkage map of the *fadL*, *purF*, and *aroC* genes showing cotransduction frequencies with arrows pointing toward the unselected marker.

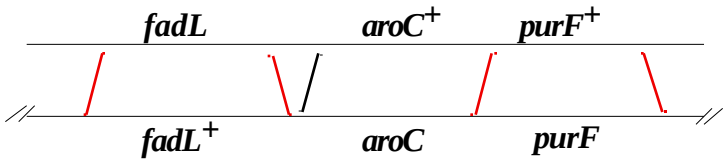


(10) **3.** To confirm the gene order determined from two-factor crosses, the following three factor crosses were done.

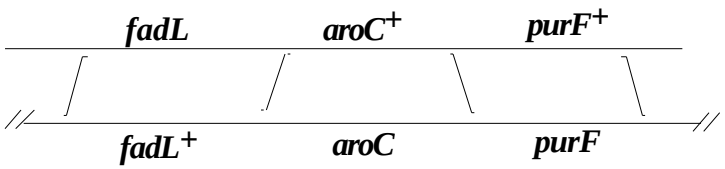
Donor	*fadL*	*purF*$^+$	*aroC*$^+$
Recipient	*fadL*$^+$	*purF*	*aroC*

Selected phenotype	Recombinants	Number obtained
PurF$^+$	*fadL*$^+$ *aroC*$^+$	183
	fadL *aroC*$^+$	129
	fadL$^+$ *aroC*	238
	fadL *aroC*	17
AroC$^+$	*fadL*$^+$ *purF*$^+$	305
	fadL *purF*$^+$	246
	fadL$^+$ *purF*	328
	fadL *purF*	266

From these data, determine the order of the *fadL*, *purF*, and *aroC* genes. For each of the three-factor crosses, show a drawing of the recombination event(s) that lead to your conclusion.



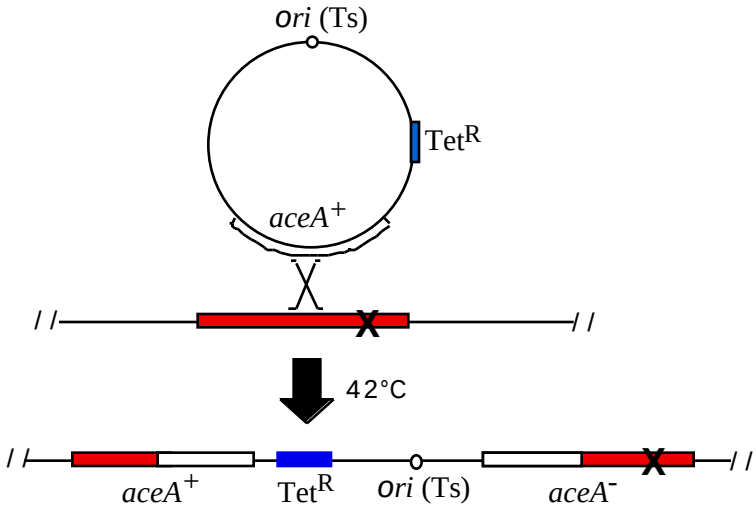
Rarest class of recombinants will require four crossovers. Rarest class of recombinants was *aroC fadL*, thus *aroC* is the middle gene.



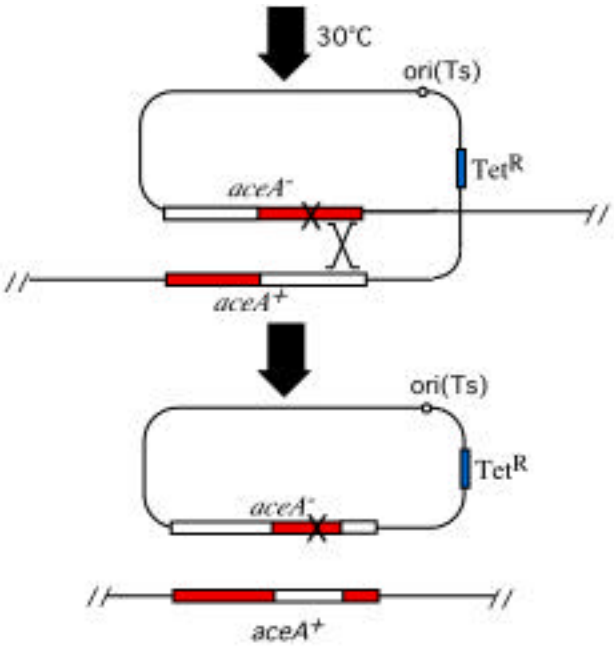
No rare class of recombinants were obtained, thus the selected marker (*aroC*) is the middle gene.

(10) **4.** Integration of a cloned gene on suicide plasmid is a useful trick for constructing a tandem chromosomal duplication. Given a low copy number plasmid with a temperature sensitive origin of replication, tetracycline resistance, and a complete *aceA*$^+$ gene, and a recipient with a mutation in the *aceA* gene:

a. How could you construct a chromosomal duplication for complementation analysis? [Draw a diagram showing the crossover and resulting chromosomal duplication. Indicate the media and growth conditions you would use to select for the chromosomal duplication.]

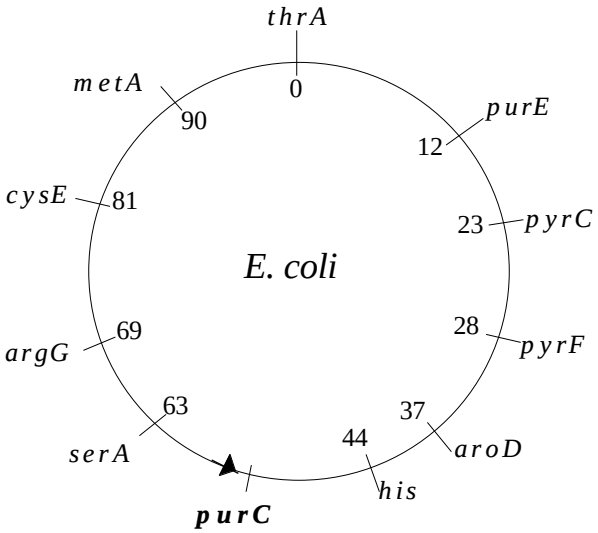


b. How would you obtain segregrants with a single copy of the *aceA*$^+$ gene on the chromosome? [Draw a diagram showing the crossover and resulting recombinants.]

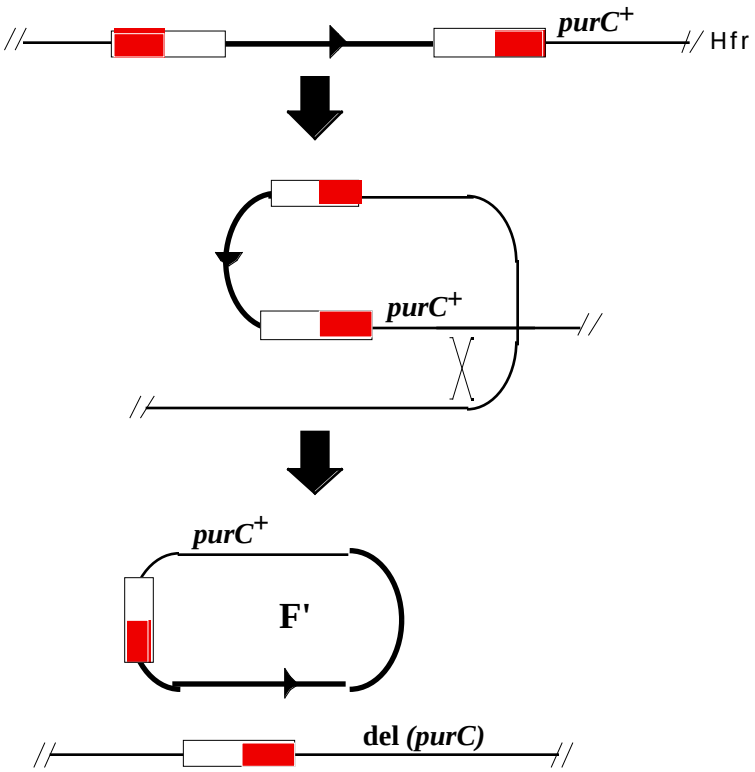


c. How could you maintain two copies of the *aceA* gene in a cell but prevent recombination between them? [Describe the genetic background and growth conditions you would use.]
ANSWER: Bring the plasmid into a *recA* mutant host on medium with Tet at 30°C.

(10) **5.** An *E. coli* strain KL98 contains an F-plasmid integrated adjacent to the *purC*$^+$ gene (required for biosynthesis of purines) with *oriT* oriented as shown by the arrow in the figure below. Answer the following questions using only donors or recipients with wild-type or mutant alleles of the genes shown on the map below.



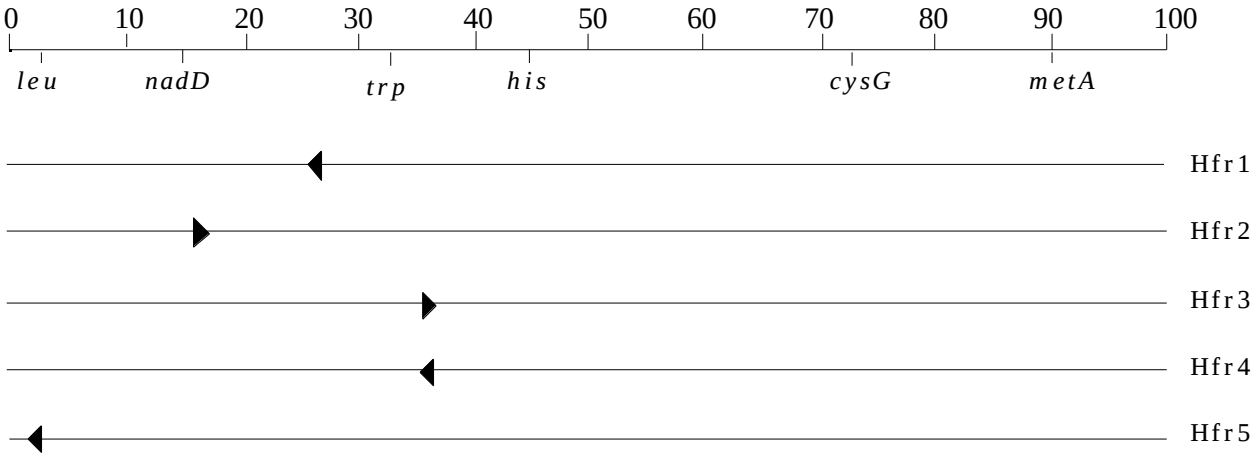
a. Draw a diagram showing how an F' carrying the *purC*$^+$ gene could be formed.



b. At what frequency would you expect to find cells carrying F' *purC*$^+$ in a population of KL98 cells? **ANSWER: about 10^{-6}**

c. How would you select for exconjugants with the desired F' *purC*$^+$? [Indicate the phenotypes of donor and recipients, and the selection and counterselection you would use.]
ANSWER: Donor = purC$^+$ serA$^-$; Recipient = purC$^-$ serA$^+$;
Selection = PurC$^+$; Counterselection = SerA$^+$

(10) **6.** *Salmonella* is able to use proline as a sole nitrogen source (Put^+). A mutant was isolated that is unable to use proline as a nitrogen source (Put^-). Other than the Put^- phenotype, the mutant was wild-type. To determine where the mutation mapped, it was mated with five different Hfr donor strains. In addition to the Hfr insertion, the donor strains each have a *metA* mutation which makes them methionine auxotrophs. The map position and orientation of each Hfr donor is shown in the figure below.



Note: Donor strain Met^- Put^+ and recipient strain is Met^+ Put^-

a. What medium would you use to select against the recipient cells in this experiment?
ANSWER: Minimal medium with Proline as a sole nitrogen source (Put^+ Met^+)

b. What medium would you use to counterselect against the donor cells in this experiment?
ANSWER: Minimal medium without methionine

c. The results are shown in the following table. Based upon these results, what is the most likely map position of the *put*$^+$ mutation? [Indicate the map position relative to the Hfr origins.]

Donor strain	Put^- recombinants
Hfr 1	0
Hfr 2	0
Hfr 3	500
Hfr 4	0
Hfr 5	450

ANSWER: The region between about 15 min and 25 min on the figure above (between the tip of the arrowheads of Hfr1 and Hfr2).

(10) **7.** It is possible to "cure" a strain of the plasmid pLAFR which encodes resistance to tetracycline (Tet^R) by mating in a second plasmid pPH1JI which encodes resistance to gentamicin (Gen^R).

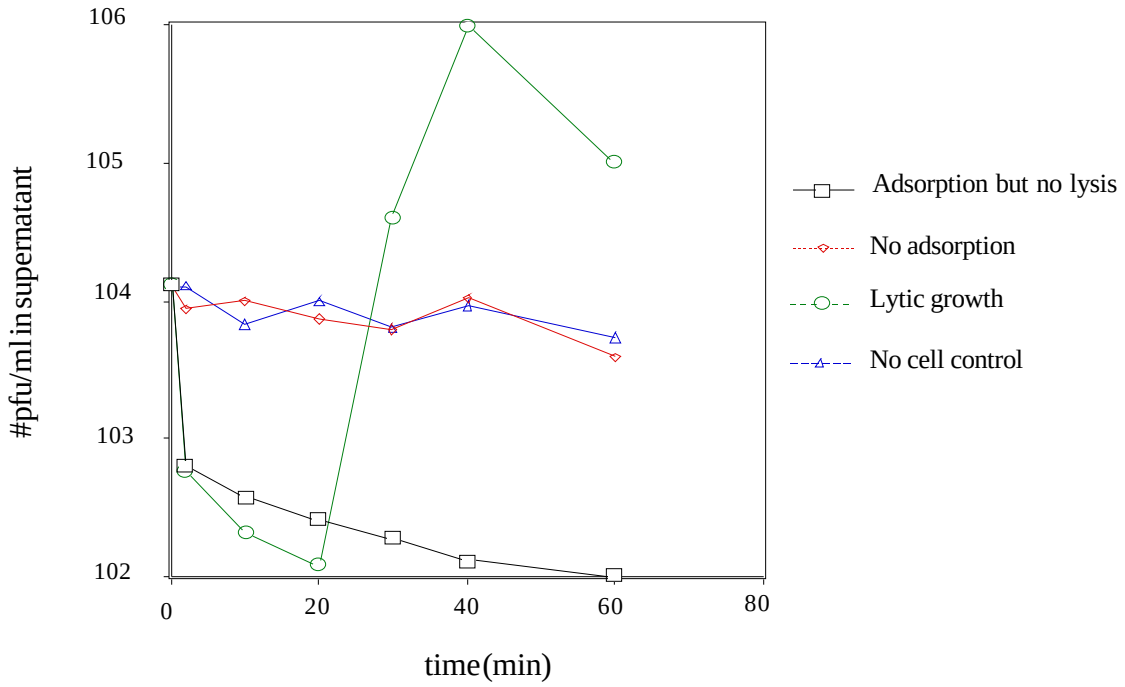
a. What does this suggest about the properties of these two plasmids? [Briefly explain your answer.]
ANSWER: The two plasmids are probably incompatible, so only one of the plasmids can be stably maintained in the cell. Hence, selection for the second plasmid results in loss in the first plasmid.

b. Would this trick work if the only selectable marker on pPH1JI was Tet^R? [Briefly explain your answer.]
ANSWER: No. Because the recipient cell is already Tet^R, there is no selection for inheritance of the second plasmid. Hence, very few cells would probably be transformed and even if they were you would not be able to distinguish them from the untransformed cells.

(10) **8**. Phage λ is unable to grow lytically on wild-type *Salmonella typhimurium.*

a. Suggest two possible reasons for these results.
ANSWER: Either (i) lambda may not be able to adsorb to *S. typhimurium* or (ii) *S. typhimurium* may be missing some host function required for maturation of lambda (i.e. lambda would adsorb to the cells but would not lyse them).

b. How would a one-step growth curve distinguish between these two possibilities? [Draw a diagram showing a one-step growth curve with the expected results for each possibility.]



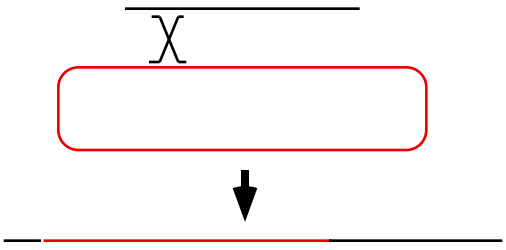
(10) **9.** The mutation *putA900* is a point mutation. The mutation *put-544* is a large deletion that includes the entire *put* operon and extends into DNA on both sides of the *put* operon. Phage P22 HT and phage P1 were grown on each of these mutant strains and used to transduce recipients with a *pyrC* or *pyrD* mutation, selecting for Pyr^+. The results are shown below.

Donor	Recipient	Selected phenotype	Cotransduction of *put*⁻ from phage P22 donor		Cotransduction of *put*⁻ from phage P1 donor	
			# Put^-	# Total	# Put^-	# Total
putA900	*pyrC*	Pyr^+	4	500	256	500
	pyrD	Pyr^+	0	500	20	500
Δ*put-544*	*pyrC*	Pyr^+	100	500		
	pyrD	Pyr^+	10	500		

a. Explain why cotransduction between the *pyrD* and *put* genes was observed when the P22 donor contained *put-544* but not when the P22 donor contained *putA900*.
ANSWER: The results indicate that the pyrD gene is too far from the put operon to be packaged into the same P22 transducing particle. However, if the donor has a large deletion, more of the adjacent DNA can be packaged into the transducing particle -- in this case, both the pyrD gene and the put-544 mutation.

b. Explain the difference in the observed cotransduction frequency when P1 is used as the transducing phage compared to when P22 is used as the transducing phage.
ANSWER: Phage P1 carries much more DNA (over twice as much) than P22 so these results indicate that although the pyrD and put genes cannot be co-packaged in a single P22 transducing particle (about 44 Kb), they can be co-packaged in a single P1 transducing particle (about 110 Kb).

c. What would happen if a P22 transducing fragment recombined with a chromosomal gene via a single crossover? **ANSWER: The chromosome would be linearized and degraded by exonucleases, resulting in cell death. See figure below.**



(10) **10**. Three temperate phage were isolated that infect *Salmonella*. To determine if these phage were related to phage P22, two strains of bacteria were infected.

DB21 = *S. typhimurium* strain which is nonlysogenic
DB21(P22) = *S. typhimurium* strain which is lysogenic for phage P22

The results are shown in the following table where (+) indicates lysis and (-) indicates no lysis. Answer the following questions based upon what you know about λ lysogeny.

Phage	Lysis of DB21	Lysis of DB21(P22)
A1	–	–
A2	+	+
A8	+	–

Note that the three phage (A1, A2, and A8) are each infecting the same pair of bacterial strains.

a. Suggest an explanation for each of the six results.
ANSWER:
- **Phage A1 cannot lyse DB21 or the P22 lysogen of DB21, indicating that it cannot adsorb to or cannot complete replication and morphogenesis in these strains (one possible reason could be that phage A1 always froms a lysogen in these strains, but you would need to explain why this is true in DB21 derivatives and not other *Salmonella* strains -- remember that this phage was "isolated" as a *Salmonella* phage. [Recall that Hfl is a host gene product, not a phage gene product.]**
- **Phage A2 can lyse both DB21 and DB21(P22) lysogens, indicating that P22 does not repress growth ofA2 and, hence, A2 must be heteroimmune with phage P22.**
- **Phage A8 can lyse DB21, but cannot lyse DB21(P22) indicating that it is homoimmune with P22.**

b. What causes two phage to be homoimmune? [Be specific.]
ANSWER: When a homoimmune phage infects the lysogen, the repressor produced in the lysogen binds to operator sites that control gene expression of the incoming phage and prevents lytic growth. The repressor responsible for homoimmunity is the "major repressor", equivalent to the cI protein of phage λ.

c. What result would you expect if the DB21(P22) was infected with P22*virB3* which has a mutation that inactivates O_L and O_R?
ANSWER: The "major repressor" produced by the P22 prophage would be unable to bind to O_L and O_R and thus RNAP would bind to P_L and P_R and transcription would initiate from these sites. The inability to repress transcription from P_L and P_R would result in lysis.