(4) **1.** Why are strains with tRNA missense suppressors often very sickly but cells with tRNA amber suppressors are healthy?

Missense suppressors will insert the incorrect amino acid at many different sites in many proteins, resulting in nonfunctional proteins, mutant proteins with harmful functions, or improperly folded proteins which are degraded by proteases. Thus, missense suppressors cause extensive damage to cellular proteins. (Note that any given missense suppressor will always insert the same amino acid at the codon(s) that pair with its anticodon.) [2 points]

Amber suppressors only insert incorrect amino acids at UGA codons which -- except in strains with nonsense mutations and then only in the mutant gene(s) --are normally only found at the end of genes. Thus, amber suppressors do not interfer with proper translation within the normal coding sequence of genes. Also, because (i) UGA codons are rarely used as the sole translation stop signal at the end of genes, (ii) the codon-anticodon recognition is influenced by context effects, and (iii) suppression is never 100% efficient because of competition with termination factors, most genes will terminate normally. [2 points]

[Note that this answer describes many different considerations -- I would have accepted a shorter answer that made similar points.]

(4) **2.** Briefly describe two differences between generalized and specialized transduction.

Generalized transduction -- all regions of chromosome transduced, only chromosomal DNA (i.e., no phage DNA) in transducing particles [2 points]

Specialized transduction -- only specific regions of chromosome located near attachment site are transduced, transducing particles carry both chromosomal DNA and phage DNA [2 points]

[Note -- saying that generalized transduction doesn't require integration into the chromosome and specialized transduction requires integration into specific chromosomal sites is simplythe mechanistic reason for the differential packaging, not a separate difference.]

(4) **3.** Briefly describe the differences between a low frequency transducing (LFT) λd*gal* lysate and a high frequency transducing (HFT) λd*gal* lysate of phage λ. [Indicate how the two types of transducing particles are obtained and the relative frequency of transducing particles in each lysate.]

LFT = rare incorrect excision events from a single lysogen produce transducing particles at about 10^{-6} to 10^{-7} of the total phage particles in the lysate [2 points]

HFT = due to simple induction of a dilysogen with one copy of the λd*gal* phage and one copy of the λ helper phage, yielding a lysate with 50% transducing particles. [2 points]

[Note that both LFT and HFT also contain λ phage that are not transducing particles -- for LFT lysates these will be 99.9999% of the total particles in the population and for HFT lysates these will be 50% of the total particles in the population. Also, both events require excision λ from the chromosome. Finally, note that the answer I expected was short but clearly indicated the differences between the two types of lysates.]

(4) **4.** Wild-type P22 cannot transduce plasmid pBR322 but P22 HT can transduce pBR322. Based upon the way these two phage package DNA, propose an explanation for this result.

P22 only transduces DNA with pac or good pseudopac sites while P22 HT recognizes and packages DNA fragments relatively nonspecifically. [2 points]
Thus, the results suggest that pBR322 does not have a pseudopac site so it cannot be transduced by P22, but because P22 HT can package the DNA "nonspecifically", pBR322 can be transduced by P22 HT. [2 points]

5. Following chemical mutagenesis, an *E. coli* mutant was isolated that has two unlinked auxotrophic mutations: a mutation in a *cys* gene (required for cysteine biosynthesis) and a mutation in a *trp* gene (required for tryptophan biosynthesis). The *cys* mutation is polar on downstream genes in the *cys* operon, and the *trp*mutation is polar on downstream genes in the *trp*operon. Cys^+ revertants are found with a frequency of 1 per 10^6 cells. Trp^+ revertants are also found with a frequency of 1 per 10^6 cells. Revertants that are both Cys^+ Trp^+ are found at a frequency of 1 per 10^8 cells.

[**Note this question is <u>almost exactly</u> like a homework question.]**

(2) a.How would you select for Cys^+ revertants only or Trp^+ revertants only? [What kind of medium would you plate the cells on.]

Cys^+ revertants could be isolated by plating the cells on <u>minimal</u> medium <u>with tryptophan but no cysteine</u> [1 point]
Trp^+ revertants could be isolated by plating the cells on <u>minimal</u> medium <u>with cysteine but no tryptophan</u> [1 point]

(2) b. Based upon the frequency of Cys^+ or Trp^+ revertants, what frequency would you have expected to find revertants that are both Cys^+ and Trp^+?

$10^{-6} \times 10^{-6} = 10^{-12}$ (if the two events were independent)

(2) c.The frequency of each of these three types of revertants (Cys^+ Trp^-, Cys^- Trp^+, and Cys^+ Trp^+) is increased about 100-fold if the cells were treated with an intercalating agent like ICR-191. Based upon the these results, what type of mutation most likely caused the Cys^- and Trp^- phenotypes.

Intercalating agents induce frameshift mutations [1 point], so both of the original mutations were probably frameshift mutations. [1 point]

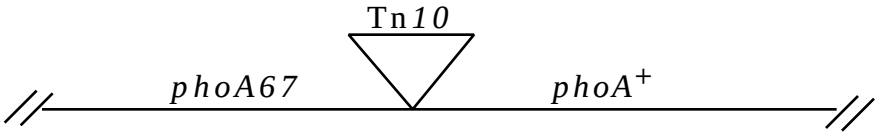
(2) d. How could you directly select for Cys^+ Trp^+ double revertants? [What kind of medium would you plate the cells on.]

Cys^+ Trp^+ revertants could be isolated by plating the cells on <u>minimal</u> medium [1 point]with no cysteine <u>or</u> tryptophan [1 point]

(2) e.What is the most likely explanation for the Cys$^+$ Trp$^+$ revertants. [Be as specific as possible given the data provided.]

Probably due to a tRNA <u>frameshift suppressor</u>. [2 points]
Because (i) both frameshift mutations are simultaneously suppressed, and (ii) the double revertants are induced by intercalating agents which can cause frameshift suppressor mutations also (by inducing frameshift mutations in the DNA corresponding to the tRNA anticodon)

6. *phoA67* is a missense mutation in the *E. coli* alkaline phosphatase gene that has a dominant-negative phenotype -- that is, strains that have a tandem duplication with one copy of the *phoA67* gene and one copy of the *phoA*$^+$ gene are phenotypically PhoA$^-$. [The tandem duplication with Tn*10* at the join-point is shown below.]

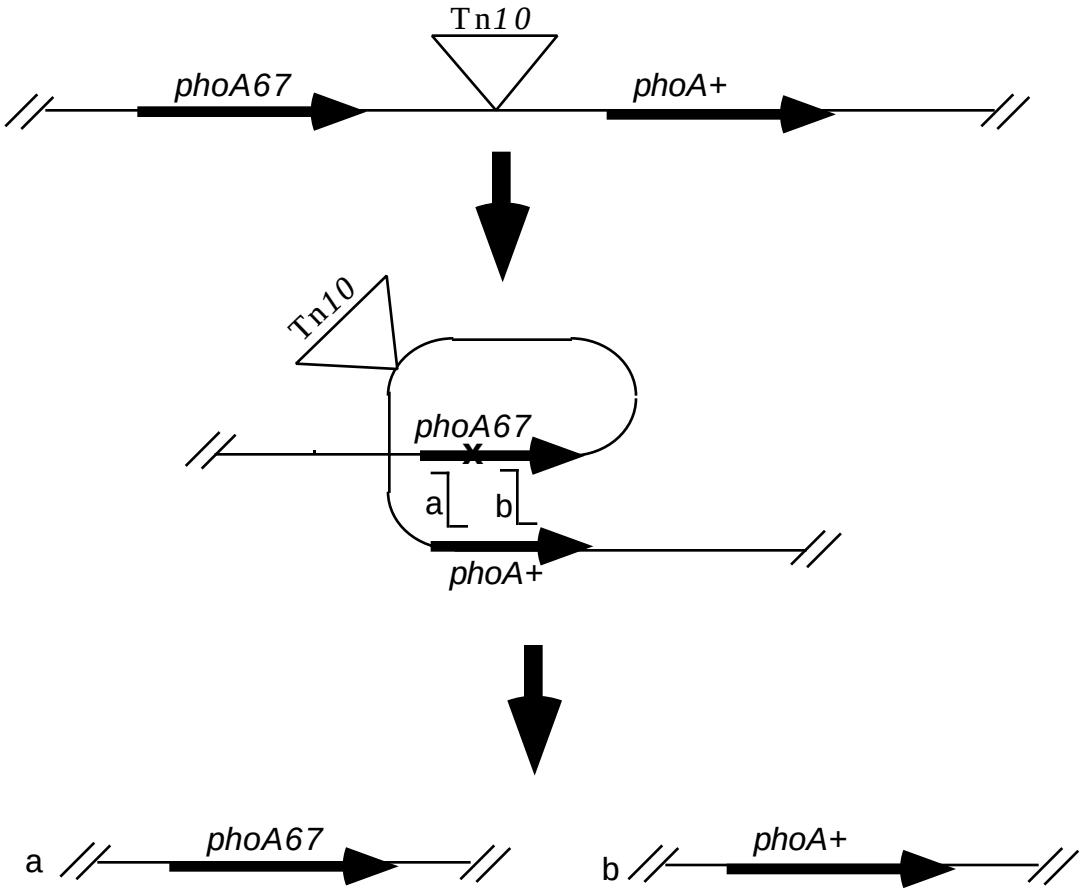


(6) a. Second-site suppressors with a PhoA$^+$ phenotype were isolated in this merodiploid strain. Suggest three potential ways that <u>second-site mutations</u> may restore the PhoA$^+$ phenotype <u>in strains with the tandem duplication</u>.

i. a null mutation in the *phoA67* gene
ii. a second-site suppressor mutation in the *phoA67* gene (an intragenic suppressor)
iii. a second-site suppressor mutation outside of the *phoA67* gene (an intergenic suppressor) -- for example, in the second copy of the *phoA* gene

[2 points per correct answer. A variety of other clever ideas were accepted. A recombination event segregrating the phoA67 allele was not acceptable because the question specified "second-site mutations ... in strains with the tandem duplication.]

(4) b. In the absence of tetracycline, the *phoA67* / *phoA*$^+$ duplication segregrates in 10% of the cells. Two classes of segregrants were obtained. Draw a picture showing how the segregration occurs and indicate the phenotypes of the two types of segregrants.



(1) two classes of segregrants depending upon where X-over occurs
(ii) all segregrants Tet sensitive
(iii) recombination between direct repeats of homologous sequences

(4) c. Segregrants were also isolated from the the *phoA* duplication strains with the second-site suppressor mutations. What would be the phenotype of the two types of segregrants if the suppressor mutation was intragenic (that is, if both mutations were in the same copy of the *phoA* gene)?

I accepted either of the following two answers (but only with a suitable explanation) -- a drawing showing the recombination events and resulting recombinants was also acceptable:

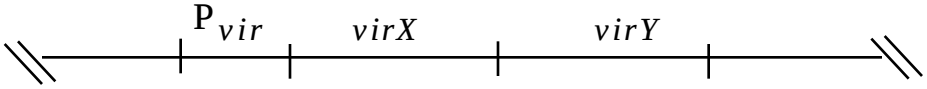
(i) If the two mutations were close together in the same gene, most segregrants would coinherit both intragenic mutations together, so both classes of segregrants would have a PhoA$^+$ phenotype -- one of the segregrants would have the wild-type copy of the *phoA*$^+$ gene and the other segregrant would have the copy of the *phoA* gene with both mutations.

(ii) Three classes of segregrants could be obtained -- simple PhoA$^+$ suppressors which inherited the *phoA*$^+$ gene; PhoA$^+$ suppressors which inherited the two mutations (*phoA67* and the intragenic suppressor) because of a recombination event outside of the *phoA* gene; and *phoA*$^-$ mutants which inherited the *phoA67* allele because of a recombination event between the *phoA67* mutation and the intragenic suppressor mutation. (It is not possible to predict the phenotype of the intragenic suppressor mutation by itself.)

(4) d. What does allele-specific mean and what would you infer about the nature of the suppressor if it was allele-specific?

Allele-specific means that a mutant caused by one mutant allele only interacts with certain other mutants caused by a limited number of alleles (note the interaction is usually between amino acids with other amino acids or amino acids with DNA, not between DNA and DNA). [2 points for this half of answer]
If the *phoA* suppressor mutation was allele-specific, the most likely explanation is that the two mutant alleles directly interact with each other to (at least partially) restore the wild-type phenotype. [2 points for this half of answer]

(4) **7.** Transposon insertion mutations in the *virX* gene decrease the virulence of *Salmonella typhimurium*. in mice. The *virX* gene is located in an operon upstream of the gene*virY*, as shown below.



Does this result prove that the *virX* gene product is involved in virulence? [Explain your answer.]

No! [2 points but not without an explanation!] Transposon insertions are usually polar on downstream genes. Thus, this result cannot distinguish whether the*virX* gene is itself involved in virulence or if the *virY* gene is required for virulence. [2 points]

8. Two deletions in the coding sequence for the *virX* gene were constructed *in vitro*. Both deletions inactivate the *virX* gene product. The predicted transcripts from the wild-type *virX* gene and two *virX* deletion mutants are shown below. [Deleted bases are indicated by brackets. The grouped triplets indicate the correct reading frame of the wild-type transcript.] The virulence of each of the deletion mutants was tested.

```
wild type:     ... GCA  CCC  UUA  GUA  UUU  AAA  AGU ...
deletion #1:   ... GCA [                  ]AAA  AGU ...
deletion #2:   ... GCA  CCC  U[           ]AAA  AGU ...
```

(2) a. What results would you expect for each deletion mutant if the *virX* gene was directly required for virulence? Briefly explain your answer.

Both deletion #1 and deletion #2 would be non-virulent phenotype because no functional *virX* gene product is made. [Note that the question states that both deletions inactivate VirX!]

(4) b. What results would you expect for each deletion mutant if only the *virY*gene was required for virulence? Briefly explain your answer.

Deletion #1 is an in-frame deletion so it would not be polar on the *virY* gene. Even though the mutant *virX* gene product would be inactive, the *virY* gene product would still be expressed so this deletion mutant would be fully virulent. [2 points for this answer]
Deletion #2 would cause a shift in the reading frame and the new reading frame has a UAA stop codon, so it would probably be polar on the *virY* gene. Even though the mutation is within the *virX* gene, this deletion mutant would not express the *virY* gene product and would thus be nonvirulent. [1 points for pointing out deletion is not in frame and 1 point for describing stop codon produced]

9. Phages L5, 29, and TM4 can infect and lyse *Mycobacterium smegmatis*. Phage L5 is a temperate phage (forms turbid plaques) and phage 29 is a lytic phage (forms clear plaques). Gene product 71 ("gp71") from phage L5 encodes the repressor for lysogeny.

M. smegmatis strains that contain a L5 lysogen or a plasmid with gene *71* were infected with different phages as shown in the table below. [+ indicates lysis and - indicates no lysis; * indicates mutant form of gene *71*]

Lysogen or plasmid	**Lysis by phage**			
	ØL5	Ø29	ØTM4	ØL5 *71**
None	+	+	+	+
L5 *71*$^+$	-	-	+	+
L5 *71*(Ts) at 30°C	-	-	+	+
L5 *71*(Ts) at 42°C	+	+	+	+
p[*71*$^+$] single copy	-	-	+	+
p[*71*$^+$] multicopy	-	-	+	-

Answer the following questions based upon what you know about the lysis/lysogeny decision in phage λ.

(3) a. Suggest a simple explanation for the behavior of Ø29 on each of these strains.

Ø29 will grow on the cells that do not express the ØL5 gp71, but not on cells which express gp71. This indicates that gp71 can repress lytic growth of Ø29, so Ø29 is homoimmune with ØL5.

(3) b. Suggest a simple explanation for the behavior of ØTM4 on each of these strains.

ØTM4 will grow lytically on all the strains, whether or not they express ØL5 gp71, so either ØTM4 is heteroimmune with ØL5 or ØTM4 is a lytic phage. (It is not possible to distinguish these alternative explanations from the data given. For example, the data does not indicate whether the ØTM4 plaques were clear or turbid.)

(4) c. Suggest an explanation for the different behavior of gene *71*$^+$ in single vs multicopy plasmids on ØL5 *71**.

2 points for saying that the data indicates that a single copy of *71 is dominant over a single copy *71*$^+$. However, when *71*$^+$ is present in many more copies than *71**, the *71*$^+$ gene is dominant.**
2 points for giving a reasonable explanation of the results. A simple explanation for this is that gp71 must form dimers to function, and that any dimers that contain the mutant gp71* are inactive. When the two genes are present in a one-to-one ratio, the dimers would have the predicted composition: 1/4 of the dimers would be gp71*/gp71* (inactive), 1/2 of the dimers would be gp71*/gp71$^+$ (inactive), and 1/4 of the dimers would be gp71$^+$/gp71$^+$ (active). The low concentration of active dimers may not be able to repress lytic growth of the phage. However, when *71*$^+$ is overproduced, a high concentration of the gp71$^+$/gp71$^+$ active dimers would be made which would inhibit lytic growth of the phage. [A few people got credit for other clever (although less likely) explanations that fit the data.]

10. The mutation *putA900* is a point mutation. The mutation *put-557* is a small deletion within the *put* genes. The mutation *put-544* is a large deletions that includes the entire *put* operon and extends into DNA on both sides of the *put* operon. P22 HT was 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. [-- indicates not tested]

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
put-557	*pyrC*	Pyr^+	3	500		
	pyrD	Pyr^+	0	500		
put-544	*pyrC*	Pyr^+	100	500		
	pyrD	Pyr^+	10	500		

(4) 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* or *put-557*.

The results indicate that the *pyrD* gene is too far from the *put* operon to be packaged into the same P22 transducing particle. [2 points]
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. [2 points]

(4) 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.

Phage P1 carries much more DNA (over twice as much) than P22 [2 points] 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). [2 points]

(2) c. Would you expect the cotransduction frequency be the same or different if the recipient strain contained the deletion instead of the donor? [Explain your answer.]

The amount of DNA that can be transduced depends upon how much can be packaged into a phage head in the donor. Thus, a large deletion in the donor DNA allows more DNA to be co-packaged and may allow cotransduction of genes that are too far apart to be co-packaged from a strain that does not have a large deletion. In contrast, if the donor cannot co-package two genes becaust they are too far apart, it doesn't matter if the recipient has a deletion or not -- the genes will not be cotransudced.

11. Lawes and Maloy collected samples from the South farms on the UI Campus to isolate new phages for enteric bacteria, including *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella aerogenes*. One of the phage obtained, SF1, was isolated from a fresh sample of sheep feces. As a control, phage λ grown on a *E. coli* K12 r^-m^+ strain was also tested on the same strains. The results are shown in the following table. [clear indicates that the phage produced clear plaques on that strain, turbid indicates that the phage produced clear plaques on that strain, and − indicates no plaques were formed]

Phage	Recipient cells					
	E. coli K12		*E. coli* B		*S. typhimurium*	*K. aerogenes*
	$r^- m^+$	$r^+ m^+$	$r^- m^+$	$r^+ m^+$	$r^- m^+$	$r^- m^+$
SF1	clear	−	clear	clear	−	clear
λ	turbid	turbid	turbid	−	−	−

(3) a. What can you infer about the SF1 lifestyle based upon the type of plaques formed? [Briefly explain your reasoning.]

SF1 is probably a lytic phage [1 point] because it always forms clear plaques. [2 points]

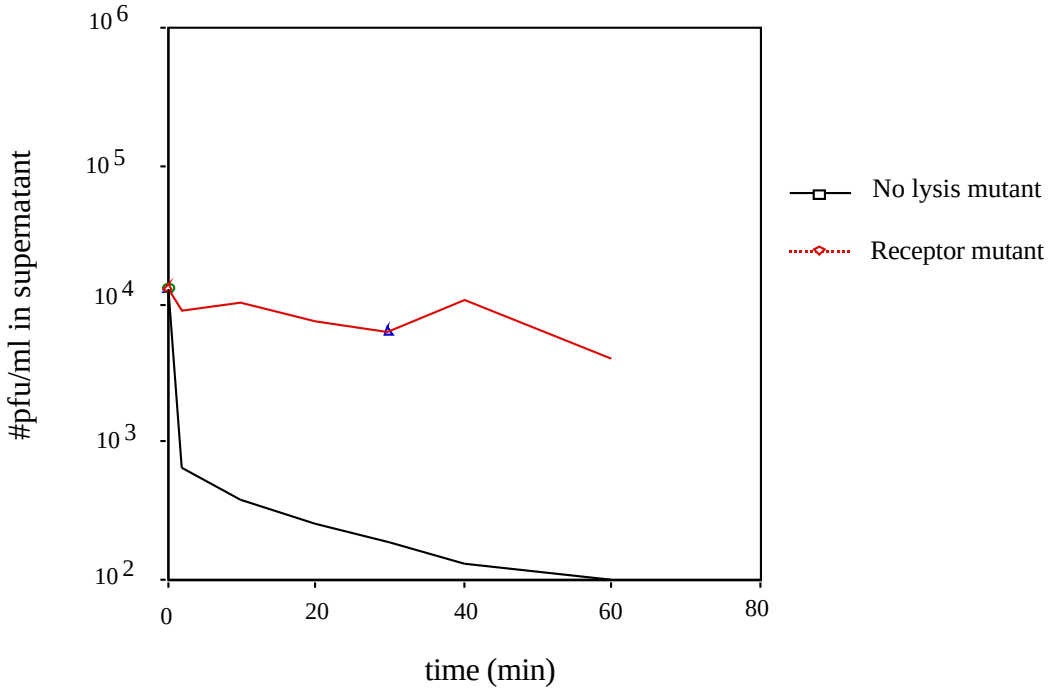
(3) b. From which bacterial strain did phage SF1 probably originate? [Briefly explain your reasoning.]

SF1 probably came from *E. coli* B [1 point] because it will grow in an *E. coli* B r^+ m^+ strain as well as in an *E. coli* B r^- m^+ strain. [2 point]s

(4) c. Give two possible reasons why λ cannot grow on *K. aerogenes* but SF1 can grow on both *E. coli* and *K. aerogenes.*

Either λ may not be able to adsorb to *K. aerogenes* [2 points] or *K. aerogenes* may be missing some host function required for maturation of λ. [2 points]

(4) d. How could a one-step growth curve be used to distinguish between the two possibilities you suggested above? [Show a drawing of the expected results.]



12. A variety of mutations were isolated in the transposon Tn*10* carried on a temperature-sensitive F plasmid that cannot replicate at 42°C. These F(Ts)::Tn*10* mutants were then moved into an *E. coli* host that did not contain Tn*10* or IS*10*, selecting for tetracycline resistance (Tet^r) at 42°C. What would be the predicted effect of the following mutations on the frequency of transposition from the F(Ts)::Tn*10* compared to the wild-type Tn*10*? [Briefly explain each of your answers.]

(3) a. A promoter-up mutation that increases the expression of P_{out}

Increased expression of P_{out} should <u>decrease the frequency of transposition</u> because excess P_{out} <u>mRNA</u> will hybridize to the P_{in} transcript and prevent translation of transposase. [Note for credit you had to specifically indicate that mRNA or transcript is what hybridizes.]

(3) b. A promoter-up mutation that increases the expression of P_{in}

If the increased expression of P_{in} is sufficient, some of the P_{in} transcript will not hybridize with P_{out} <u>mRNA</u> and will thus be available for translation of transposase. Because the frequency of transposition is directly proportional to the amount of transposase made, this should <u>increase the frequency of transposition</u>.

(3) c. A point mutation that prevents base pairing of the P_{in} and P_{out} transcripts

If the P_{in} transcript cannot hybridize with the P_{out} transcript, the P_{in} transcript will remain available for translation of transposase. Because the frequency of transposition is directly proportional to the amount of transposase made, this will result in an increased frequency of transposition.

(3) d. A deletion mutation that removes the outside end of IS*10* L.

Both outside ends of Tn*10* (that is, both the outside end of IS*10*L and IS*10*R) are required for transposition of Tn*10*. Thus, deletion of the outside end of IS*10L* will prevent transposition of Tn*10* (and hence Tet^r as well). However, note that this will not decrease the frequency of transposition of IS*10*R!