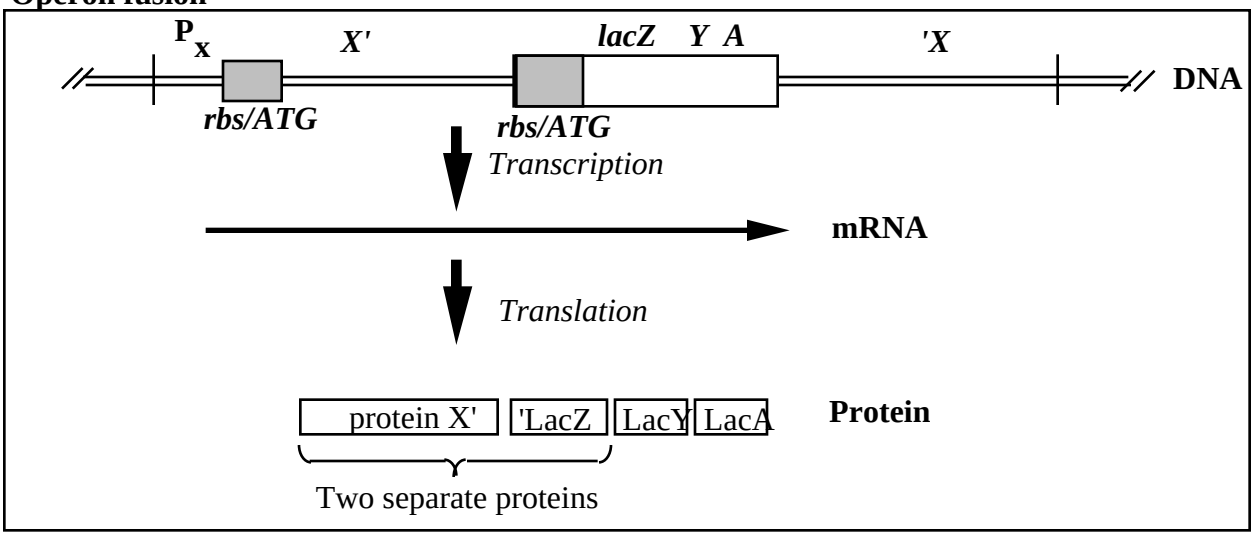
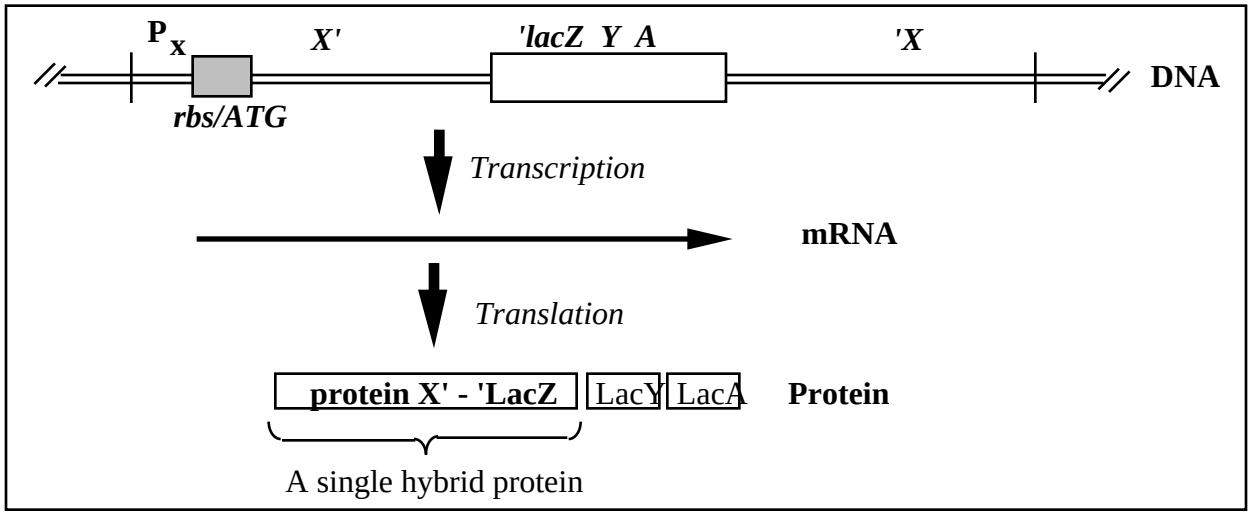
(6) 1. Compare and contrast operon vs gene fusions:

(a) Draw a simple diagram showing each type of fusion with
- transcription and translation start and stop sites
- the mRNA transcript expected
- the expected protein products

Operon fusion



Gene fusion



P_X = Transcriiptional start site

rbs/ATG = Translational start site

(b) **For each type of fusion,** indicate how frequently you would obtain insertions that express the fusion if a transposon derivative (such as Mud) was randomly inserted into a gene.

Operon = 1/2

Gene = 1/2 x 1/3 = 1/6

(6) 2. List 3 different uses of operon or gene fusions. **A few examples include:**

(a) **Study gene expression in vivo**

(b) **Purify fusion proteins**

(c) **Study cell localization**

(d) **In vivo cloning**

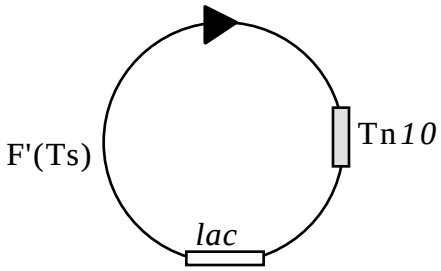
(e) **Portable region of homology for constructing duplications, Hfrs, etc**

(f) **Use as linked marker with selectable phenotype**

(g) **To isolate mutations in genes**

(6) 3. Given following strains, draw a figure showing how you could isolate a Hfr located between the *proA*$^+$ and *proC*$^+$ genes. [Draw out the recombination event, indicating the donor and recipient, and the selection and counterselection used.] **Both chromosomes are Lac$^-$.**

(i) *proB*$^+$ *proA*$^+$ *zha*::Tn*10* *proC*$^+$ Str^S [Note: Tn*10* inserted between proA and proC]
(ii) *proB*$^-$ *proA*$^-$ *proC*$^+$ Str^S / F' (Ts) *lac*$^+$ *zzf*::Tn*10* [Note diagram shown below]



Your answer should show the following.

This procedure requires two steps:

- **(1) Transfer of the F' into the appropriate recipient. This should be done at 30°C to avoid loss of F'. Selection could be Lac$^+$ and counterselection could be Pro$^+$. (Minimal medium with lactose as a carbon source and no proline.) The donor would be the F' strain (ii) and the recipient would be the F$^-$ strain (i).**
- **(2) Selection for the Hfr insertion by homologous recombination between the Tn*10* insertions (selection Lac$^+$ at 42°C). Also note that the Tn*10*s must be in the same orientation for recombination to occur and the recombination should be via a single crossover.**

(6) 4. Once you have constructed the Hfr in the previous question, how could you use it to isolate an F' (Ts) *proB*$^+$ *proA*$^+$. **[Describe the selection and counterselection you would use.]** Use the back of this page.

- **Use Str^R as a counterselection against the donor.**
- **Mate into *proB*$^-$ *proA*$^-$ recipient and select for Pro$^+$ as a late marker <u>or</u>**
- **Mate into *proB*$^-$ *proA*$^-$ *recA* recipient and simply select for Pro$^+$**
- **Medium = minimal medium + glucose (C-source) + streptomycin (NO proline)**

(6) 5. An *E. coli dnaA*(Ts) *lacZ* Str^S strain cannot grow at 42°C because the DnaA protein is required for initiation of DNA replication at *oriC*. A F' *lac*$^+$ is mated into the *dnaA*(Ts) strain at 30°C. If 10^8 cells of the transconjugant are plated on rich medium at 42°C, rare colonies arise. Briefly describe two possible ways the cells may have acquired the ability to grow at 42°C. **This question is directly from the homework.**

(a) **Reversion of *dnaA*(Ts)**

(b) **Integration of F' into chromosome ("integrative suppression")**

(4) 6. Describe a simple genetic approach to distinguish the two possibilities you proposed in the previous question.

Mate potential Hfr with auxotrophic recipients

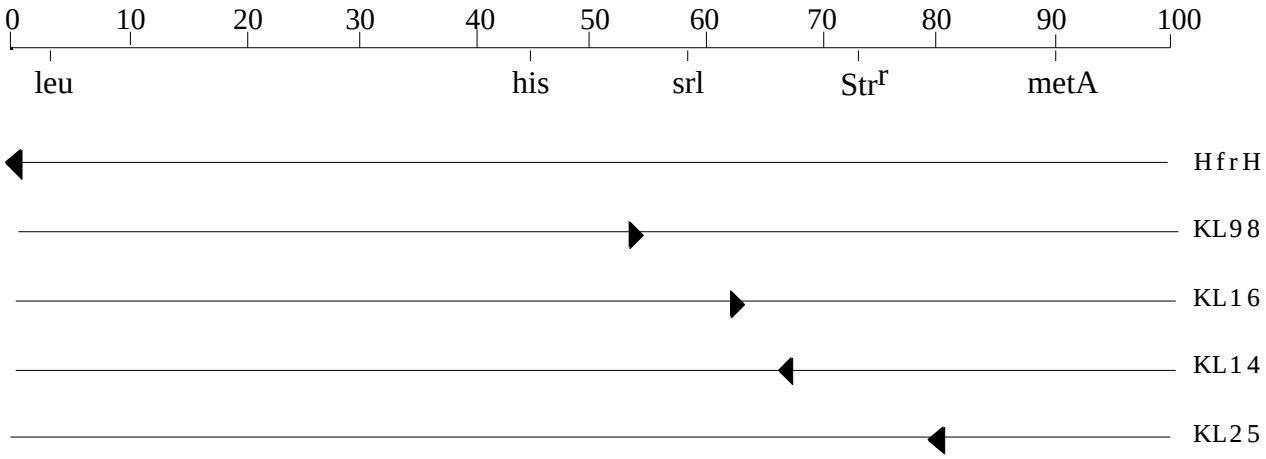
- **If revertant, F' can transfer Lac$^+$ but not chromosomal genes**
- **If Hfr, can transfer chromosomal genes but not Lac$^+$**

(6) 7. Why does the transfer of genes from a Hfr require a functional *recA* gene in the recipient, but transfer of genes on a F' does not? **This question comes directly from PacerForum.**

- **F is recircularized by the one of its own gene products (the TraI protein) if it is completely transferred (because TraI recognizes the oriT site for cutting and for joining)**
- **Hfrs are essentially never fully transferred, so a linear piece of DNA is brought into the recipient and the linear DNA will be degraded if it doesn't recombine with the chromosome.**

(10)8. Several Hfrs from *E. coli* are shown in the figure below with the direction of transfer indicated relative to the map shown at the top of the figure. All of these Hfr strains carry wild-type copies of the genes shown on the map (i.e., *leu*$^+$, *his*$^+$, *srl*$^+$, Str^S, and

metA$^+$). Each of the Hfrs was mixed with a F$^-$ His$^-$ Str^r recipient, and the cells were spread on minimal plates with Glucose as a carbon source and streptomycin. **This question is almost identical to a question from PacerForum -- in fact, the diagram comes from the question posted in PacerForum.**



(a) What is the selection in this experiment? **His$^+$**

(b) What is the counterselection in this experiment? **Str^R**

(c) Rank the five Hfr donors to indicate the relative numbers of transconjugants expected on each mating plate.

Most colonies =	+++++	**KL98**
	++++	**KL16**
	+++	**HfrH**
	++	**KL25**
Least colonies=	+	**KL14**

(10) 9. Roof and Roth isolated a number of Tn*10* insertions and MudJ(*lac*, Kan) operon fusions in the genes required for ethanolamine utilization (*eut*) in *S. typhimurium.* They then constructed double mutants that carried one of the Tn*10* insertions (Tet^r) and one of the MudJ(*lac*, Kan) insertions. The results are shown below. (The numbers shown are allele numbers; + indicates the mutant expresses ß-galactosidase and _indicates the mutant does not express ß-galactosidase.) **This question is very similar to a homework question.**

Tn*10*	MudJ	ß-galactosidase
none	*eut- 5*	+
eut-205	*eut- 5*	+
eut-208	*eut- 5*	−
eut-212	*eut- 5*	−
none	*eut- 6*	+
eut-205	*eut- 6*	+
eut-208	*eut- 6*	+
eut-212	*eut- 6*	−
none	*eut- 19*	+
eut-205	*eut- 19*	+
eut-208	*eut- 19*	−
eut-212	*eut- 19*	−
none	*eut- 38*	+
eut-205	*eut- 38*	−
eut-208	*eut- 38*	−
eut-212	*eut- 38*	−

Based upon these results, draw a simple diagram showing the order of the Tn*10* insertions relative to the MudJ insertions and indicate the direction of transcription. Briefly explain your rationale for this order and orientation.

Rationale = upstream Tn10 will be polar on downstream Mud

Order of mutations with transcription from left to right is shown below:

eut-212 eut-6 eut-208 (eut 5/eut19) eut-20 eut-38

(6) 10. The transposon Tn*phoA* is frequently used to construct gene fusions between secreted proteins and alkaline phosphatase. Briefly describe why *phoA* is used as the reporter gene and why gene fusions are used for this purpose.

- **Alkaline phosphatase is only active if outside of cytoplasm because the cytoplasm is too reduced for formation of necessary S-S bonds.**
- **Gene fusion used because this approach is used to see if hybrid protein carries signals for transfer outside of cell.**

(4) 11. When it is necessary to eliminate one plasmid from a cell, frequently a second plasmid with a different antibiotic resistance is brought into the cell. Relative to the first plasmid, what property(s) would the second plasmid need for this experiment to work?

Both plasmids must be in same Inc group, so selection for second plasmid will "kick" the first plasmid out of the cell.

(6) 12. One way of testing the function of many different amino acid substitutions at a single site in an enzyme requires first converting the corresponding codon in the DNA to TAG by site-directed mutagenesis. **This question comes directly from the "questions to ponder" on a homework handout.**

a. How could the resulting TAG substitution mutant be used to study many different amino acid substitutions at that site?

TAG = UAG in mRNA = amber mutation = nonsense codon

Could move this mutant into different amber suppressor mutants.

Each different suppressor mutant will place a different amino acid at this site.

b. How would you intrepret the results if:

- full enzyme activity resulted?

  The subsituted amino acid does not disrupt function (this does not mean that that residue is not important, only that the substitution used does not disrupt structure or function of the resulting protein!).

- weak or no enzyme activity resulted?

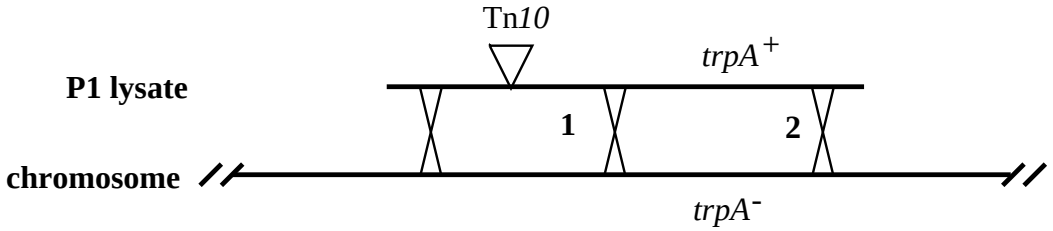
  Either the substituted amino acid disrupts structure or function of the resulting protein <u>or</u>

  the efficiency of suppression may not produce a sufficient amount of functional protein

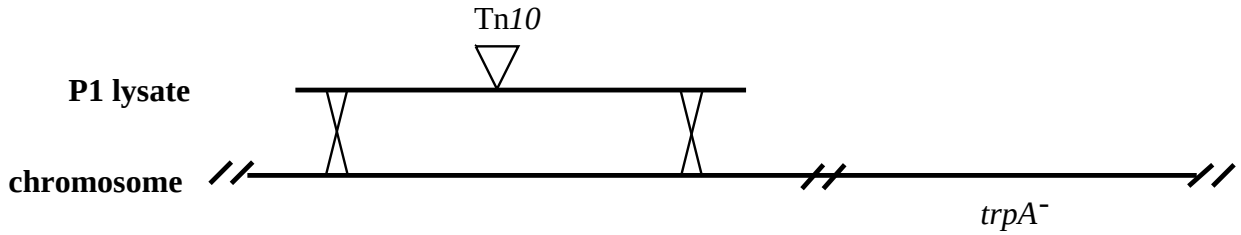
(10) 13. Given a lysate of phage P1 grown on a random pool of random Tn*10* insertions in the wild-type *E. coli* chromosome and a *trpA*$^-$ recipient, how could you isolate a Tn*10* insertion linked to the *trpA* gene? [Show the crosses you would do and indicate the phenotype of each possible class of recombinant obtained. Be sure to indicate how you would confirm that the Tn*10* insertion is linked to the *trpA* gene.] **This question is nearly identical to a homework question.**

The random Tn*10* pool will carry two types of P1 transducing particles: some with Tn*10* near the *trpA* gene and some with Tn*10* somewhere else.

(A) If the Tn*10* is linked to the *trpA* gene then a single transducing particle can carry both the Tn*10* and the *trpA*$^+$ gene, so some of the Tet^r transductants will become Trp$^+$ (crossover #2) and some will remain Trp$^-$ (crossover #1).



(B) If the Tn*10* is NOT linked to the *trpA* gene then all of the Tet^r transductants will remain Trp$^-$ because a transducing particle cannot carry both regions of the chromosome.

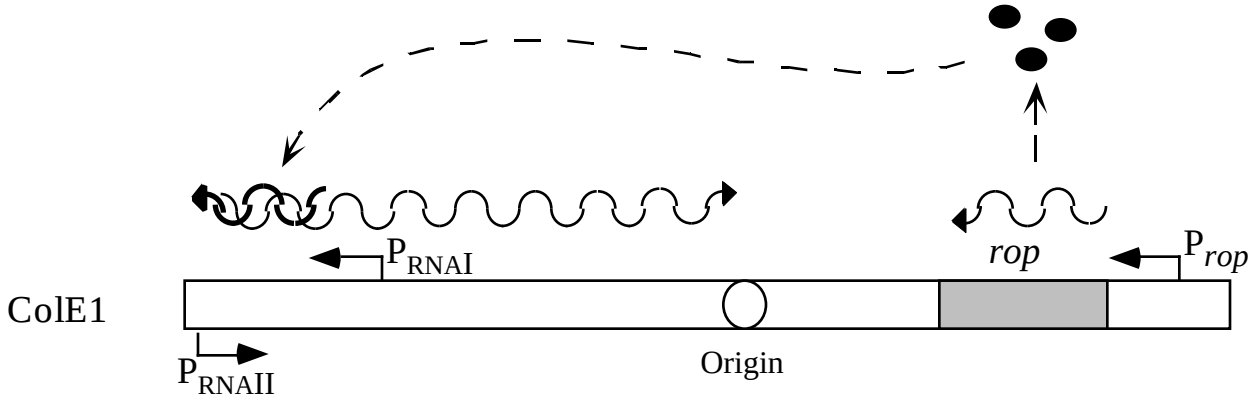


To confirm that the Tn*10* insertion obtained is really linked to the *trpA* gene, you would need to grow phage on the newly isolated Trp+ Tet^R transductant and backcross the original parent *trpA*- mutant, selecting Tet^R and screening for Trp$^-$.

(4) 14. If you have a strain with a Tn*10* insertion linked to the *trpA*$^+$ gene, how could you use this to isolate point mutations in the *trpA* gene? [Draw a figure indicating the donor and recipient and any selection or screen you would use.] **This question asks you to ISOLATE point mutations, not to simply move the Tn10 insertion next to preexisting Trp$^-$ mutations.**

This would involve the following steps: localized mutagenesis of the transducing particles, transduction of a recipient to Tet^R, screening for Trp$^-$.

(10) 15. A cartoon showing the replication control of plasmid ColE1 is shown below. Predict the copy number phenotype of the listed mutants based upon this model for replication control. Briefly explain your rationale.



(a) Predict the phenotype of a mutant that did not make RNAI.
Increased -- more RNA II, more replication, higher copy number

(b) Predict the phenotype of a mutant that did not make RNAII.
Decreased -- no RNA II, no replication, loss of plasmid

(c) Predict the phenotype of a mutant that makes neither RNAI nor RNAII.
Decreased -- no RNA II, no replication, loss of plasmid (loss of RNA II is epistatic to loss of RNA I)

(d) Predict the phenotype of a mutant that did not make Rop protein.
Increased -- decreased stability of RNAI-RNAII hybrid, increased RNAII primer, more replication, higher copy number

(e) Predict the phenotype of a promoter-up mutant of P_{RNAI}.
Decreased -- promoter-up would make more RNAI, more RNAI-RNAII hybrid, less RNAII for replication, lower copy number