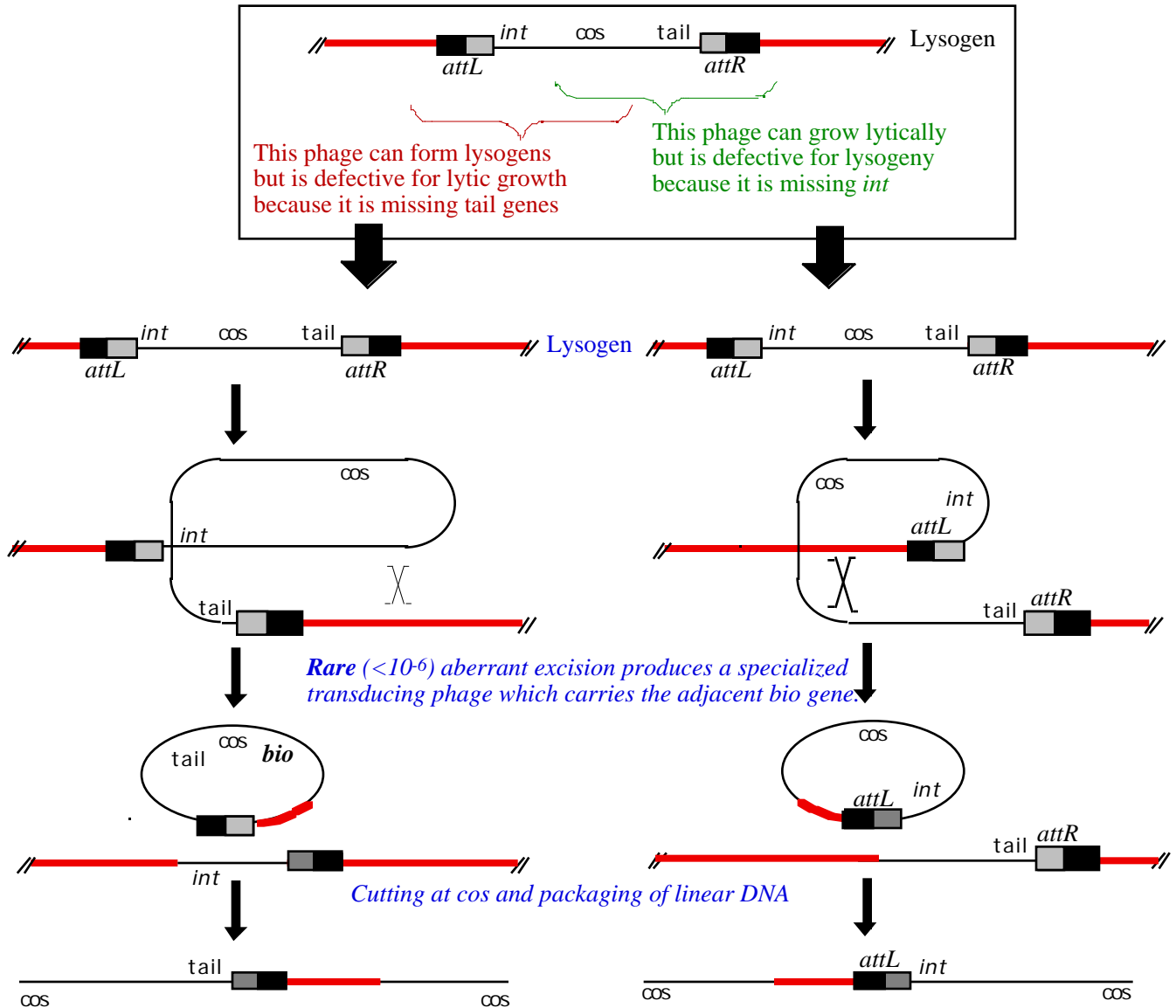


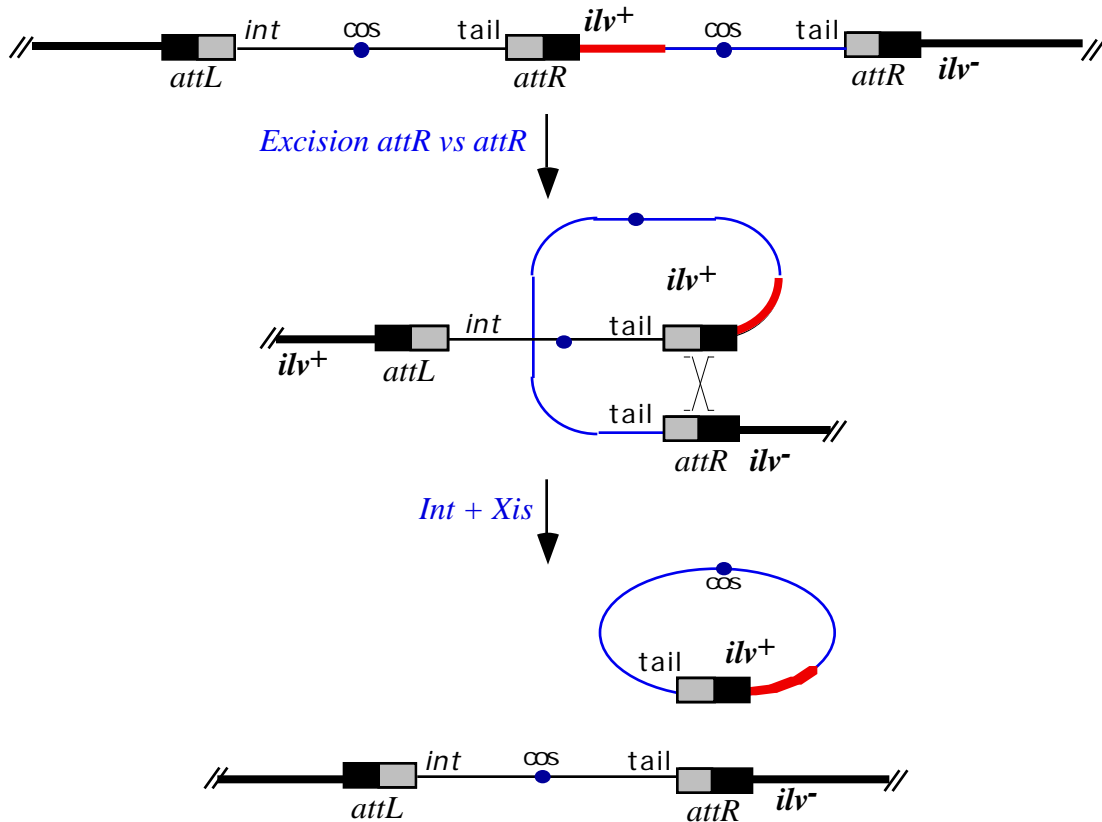
(10) 1. There is an attachment site for the temperate phage SPβ adjacent to the *ilvA* gene on the *Bacillus subtilis* chromosome. SPβ specialized transducing phage can be isolated that carry *ilvA*⁺ but are not defective for lytic growth. [Answer the following questions assuming SPβ is organized like phage .]

a. Draw a diagram which shows why some specialized transducing particles that are defective for lytic growth and other specialized transducing phage are not defective for lytic growth?
ANSWER: The drawing in the box was a sufficient answer. Some people added the mechanistic details shown below the box.



b. Given a LFT SPβ *ilvA*⁺ specialized transducing lysate, draw a diagram showing how a HFT lysate containing *ilvA* specialized transducing particles could be formed. [Indicate the expected frequencies for each type of phage produced.]

ANSWER: See diagram below. Note that you did not need to draw the formation of the LFT.



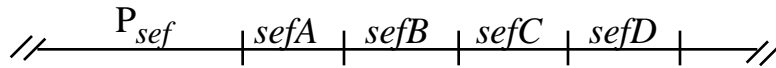
Excision via recombination between *att* sites due to inactivation of repressor and expression of phage integrase + excisionase, results in induction of both phage and subsequent phage reproduction and cell lysis -- 50% of the resulting phage will be specialized transducing phage and 50% of the phage will be wild-type phage

- (10) 2. Mutants of two *E. coli* strains were infected with two different phage. 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 turbid plaques on that strain, and "-" indicates no plaques were formed]

Phage	Recipient cells			
	<i>E. coli</i> K-12		<i>E. coli</i> B	
	<i>r⁻ m⁺</i>	<i>r⁺ m⁺</i>	<i>r⁻ m⁺</i>	<i>r⁺ m⁺</i>
P1 _{vir}	clear	-	clear	clear
80	turbid	turbid	turbid	-

- From which bacterial strain did phage P1_{vir} probably originate? Explain your answer.
ANSWER: *E. coli* B because it is not restricted in the *E. coli* B *r⁺* recipient
- From which bacterial strain did phage 80 probably originate? Explain your answer.
ANSWER: *E. coli* K-12 because it is not restricted in the *E. coli* K-12 *r⁺* recipient

- (10) 3. The *sefA* gene is located in an operon upstream of the *sefBCD* genes, as shown below.



a. Transposon insertion mutations in the *sefA* gene decrease the virulence of *Salmonella enteritidis* in mice. Does this result prove that the *sefA* gene product is involved in virulence? Briefly explain your answer.

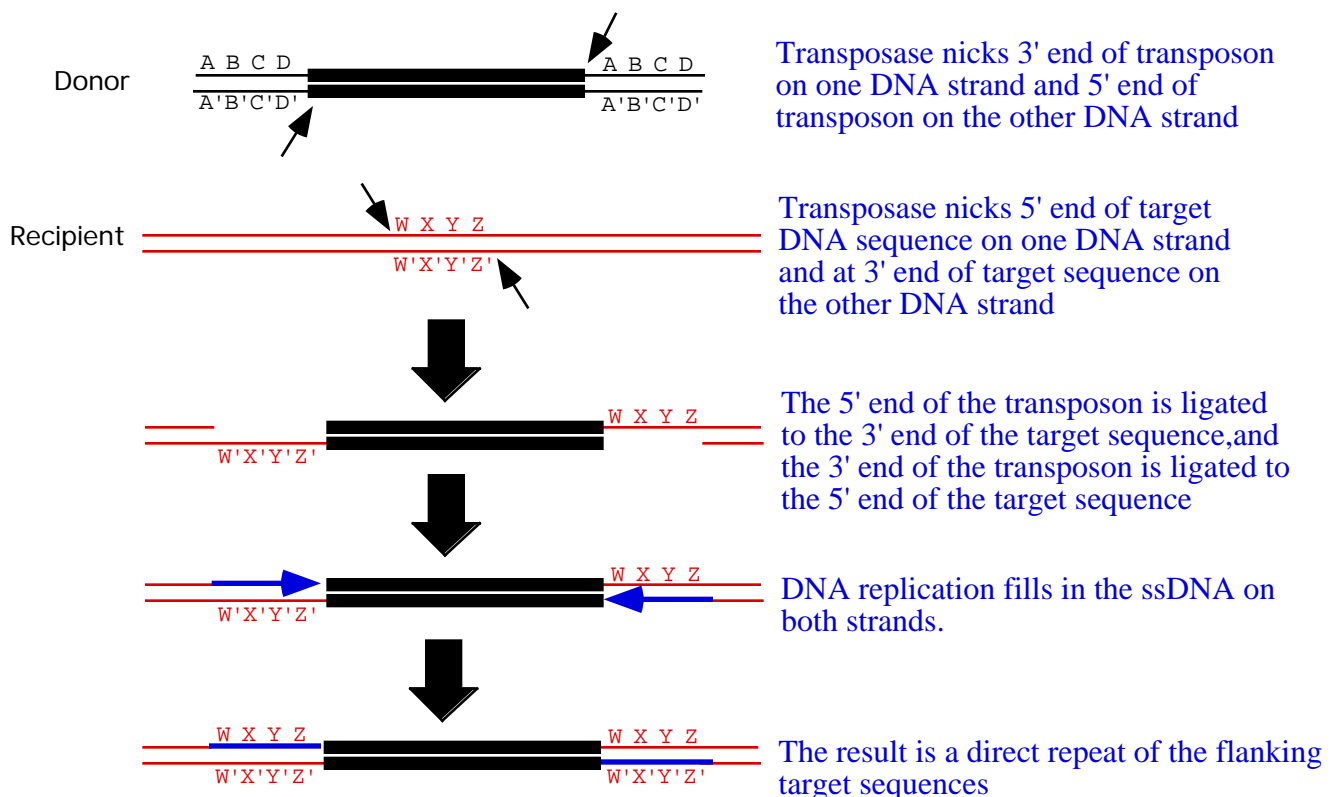
ANSWER: No. Transposons are usually polar, so the phenotype could be due to the failure to express the downstream *sefB*, *sefC*, or *sefD* gene products.

b. How could you determine whether the observed phenotype is due to the *sefA* gene product?

ANSWER: Complementation with *sefA*⁺. Another acceptable answer would be construction of a nonpolar mutation in *sefA* or testing a transposon insertion in *sefB* (which would prevent expression of *sefBCD*).

(5) 4. Transposon insertions are flanked by a short direct repeat (usually 5-10 bp) of the target DNA sequence. Draw a diagram to indicate why.

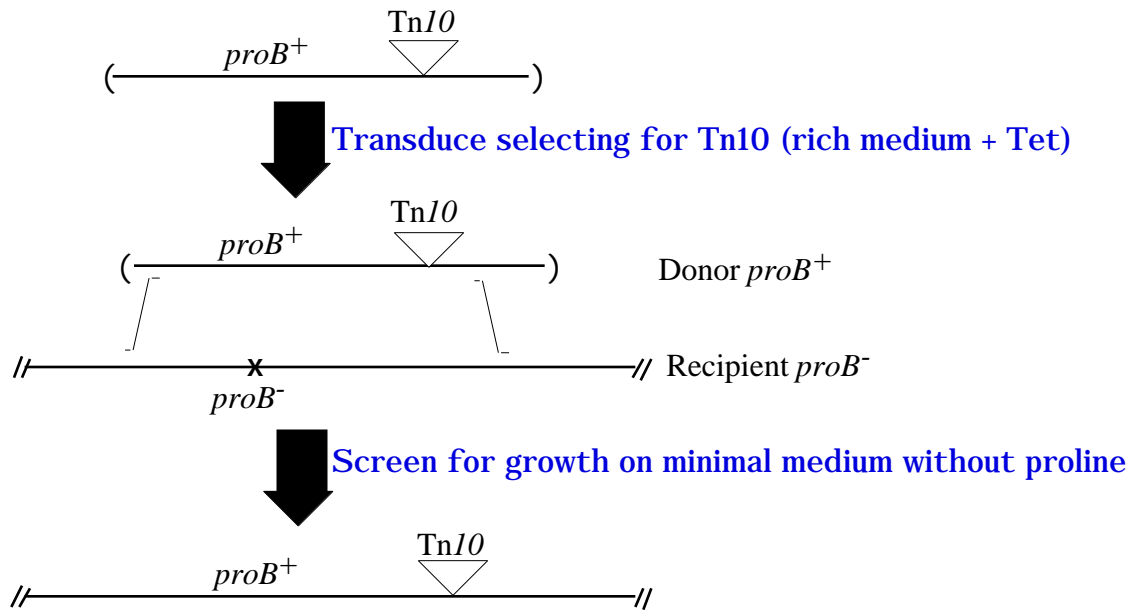
ANSWER: See diagram below. Note that you must indicate the sites of nicking and joining, and mention that DNA pol fills in the gaps.



(15) 5. The *proB* gene is required for the biosynthesis of proline. Transposon Tn10 encodes Tet^R.

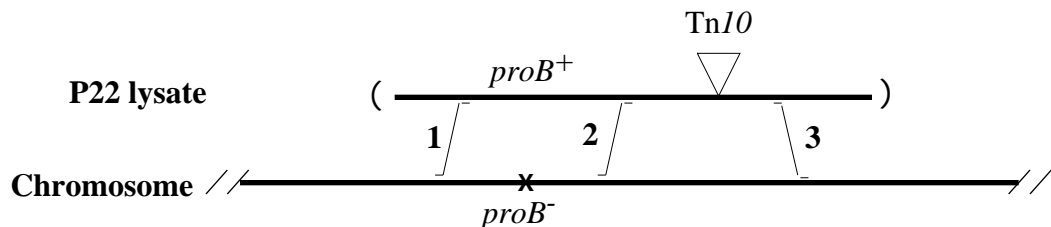
a. Given a *proB* mutant and a P22 generalized transducing lysate grown on a random pool of Tn10 insertions, how could you isolate a Tn10 near (but not in) the *proB*⁺ gene? Draw a diagram showing how you would do the experiment and indicate the medium you would

use for each selection or screen. **ANSWER:**



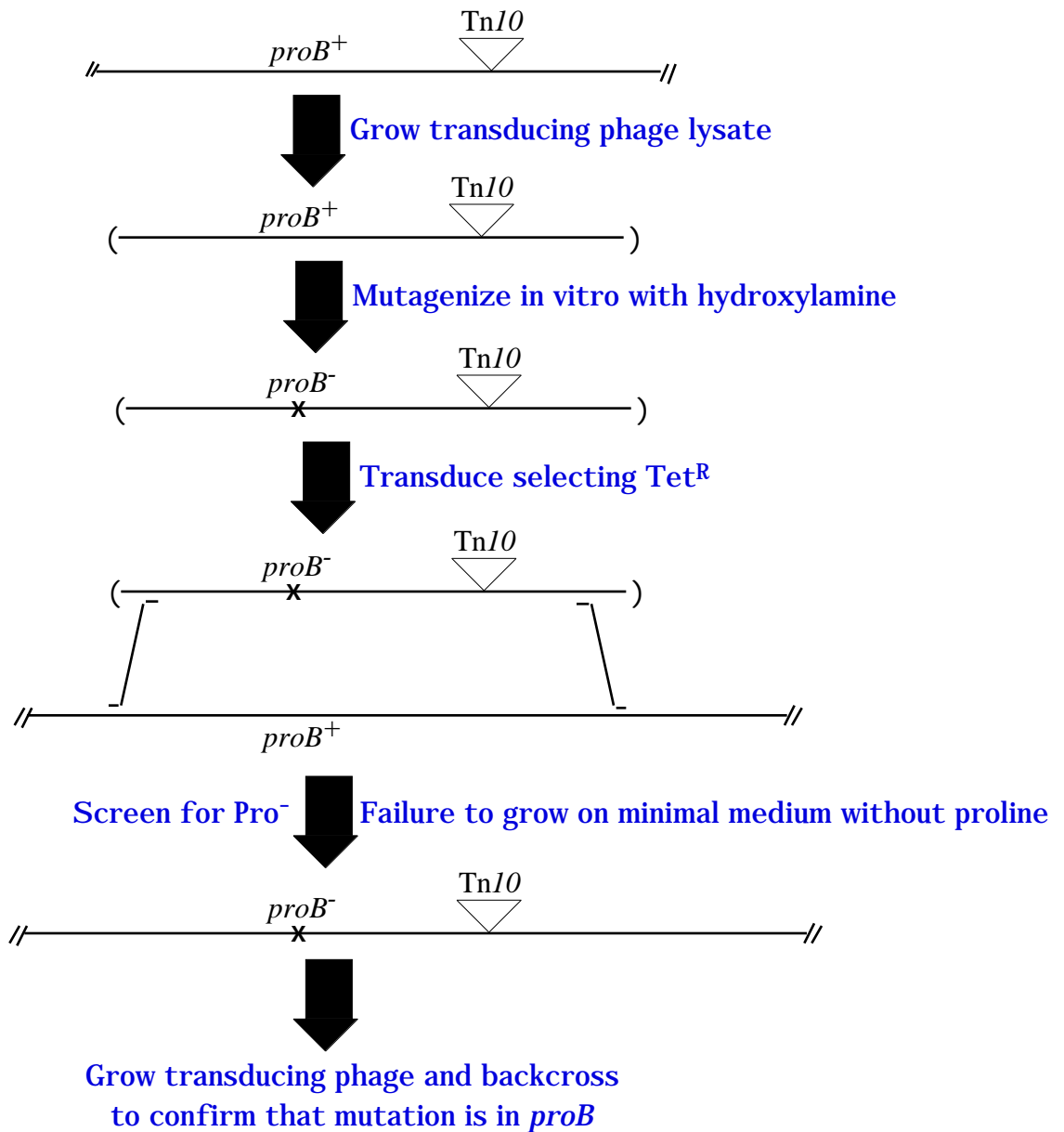
- b. Given a strain with a Tn10 insertion near the proB^+ gene and a strain with a proB62 allele, how could you obtain a Tn10 insertion near the proB62 gene? Draw a diagram showing how you would do the experiment and indicate the medium you would use for each selection or screen. **ANSWER: Note $\text{proB62} = \text{proB}^-$.**

Backcross Some of the Tet^R transductants will become Pro^- (crossovers #2 x 3).



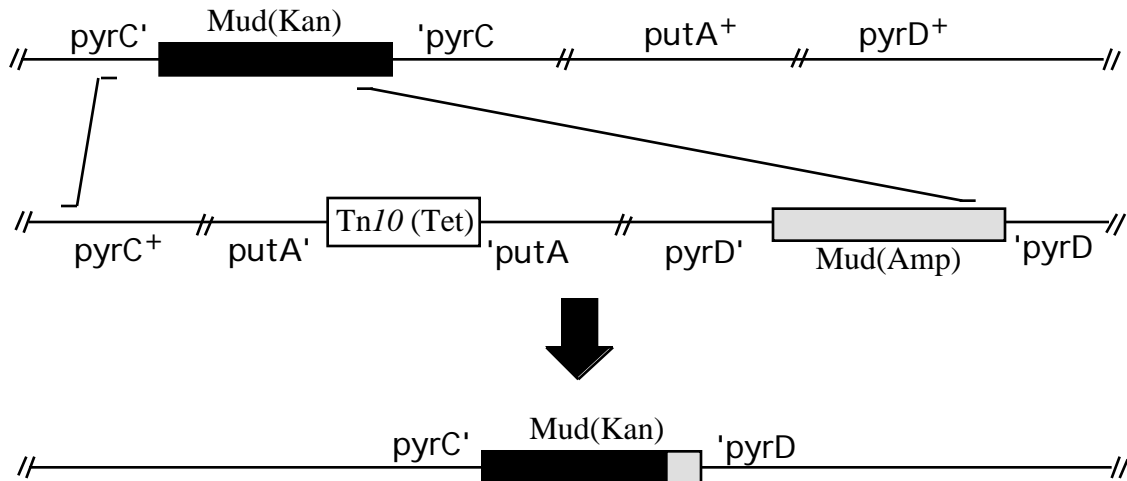
Select Tet^R (rich medium + Tet) then screen for Pro^- (minimal medium without proline)

- c. How could you use a Tn10 insertion near the proB^+ gene to isolate new point mutations in the proB gene? Draw a figure indicating the donor and recipient and any selection or screen you would use. **ANSWER:**



[10] 6. Given the two *Salmonella typhimurium* strains shown in the figure below

- a. Draw a diagram showing how you could select for a chromosomal deletion that removed the DNA between the *pyrC* and *pyrD* genes **ANSWER: See cross-overs in diagram.**



The deletion will be Tet^S PutA⁻

- b. How could you demonstrate genetically that you had actually constructed the desired deletion.

ANSWER: Deletion mapping -- check to see if the mutant can repair putA point mutations . If the desired deletion was constructed it should be unable to repair any putA mutations.

- [5] 7. When *hisD*::Tn5(Kan) was transduced into a naïve recipient (i.e. a cell that does not already have a copy of Tn5), 50 of the 100 resulting Kan^R colonies were unable to grow on minimal medium without histidine, and the other 50 colonies grew on minimal medium without histidine. In contrast, when *hisD*::Tn5(Kan) was transduced into a recipient with a *put*::Tn5(Cam) insertion, every one of the 100 Kan^R colonies tested was unable to grow on minimal medium without histidine. Explain these results.

ANSWER: Note that the His⁻ insertions arise via homologous recombination while the His⁺ insertions arise via transposition. If the strain already has a resident copy of Tn5, then the frequency of transposition of an incoming Tn5 is prevented, indicating tht the resident Tn5 makes a "repressor" that turns off transposition functions on the incoming copy.

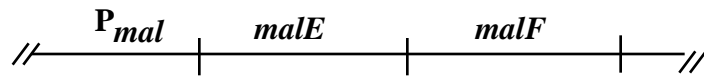
- [5] 8. To determine how the conjugal genes from IncP plasmids are regulated, two types of fusion mutations were constructed: operon fusions with the *galK* gene (encoding galactose kinase) and gene fusions with the *lacZ* gene (encoding β-galactosidase). The expression of each fusion was assayed with or without induction. The results are shown in the following table.

Gene	<i>galK</i> operon fusion Galactose kinase activity		<i>lacZ</i> gene fusion β-galactosidase activity	
	- inducer	+ inducer	- inducer	+ inducer
<i>trbF</i>	40	40	40	400
<i>trbG</i>	5	45	50	450

What do the results indicate about the regulation of the *trbF* and *trbG* genes? Briefly explain your answer.

ANSWER: *trbF* is regulated at the translational level because regulation is only observed with the gene fusion; *trbG* is regulated at the transcriptional level because the same fold regulation is observed for the operon and gene fusions.

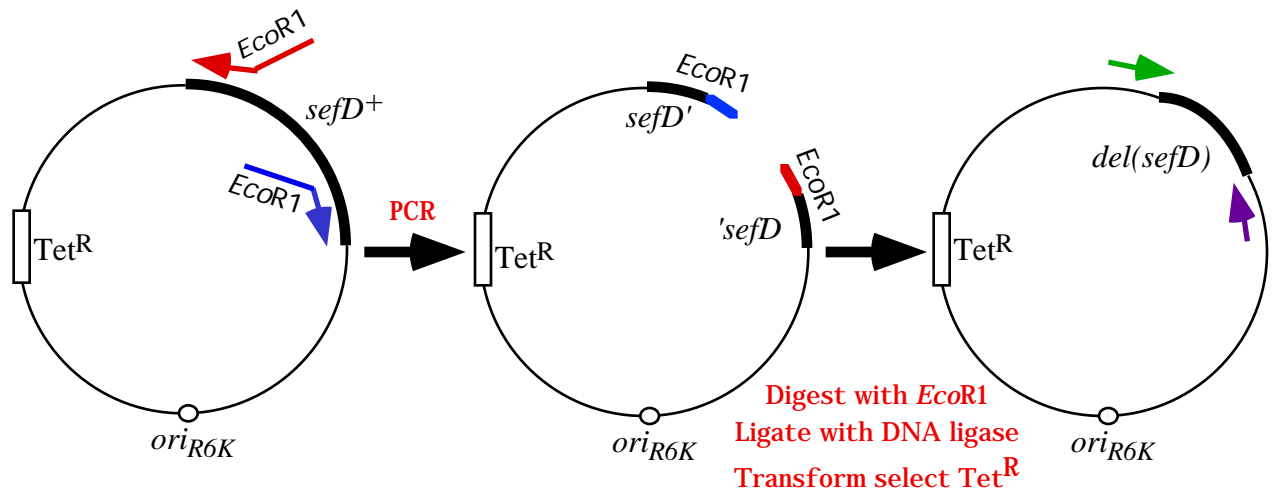
- [10] 9. *E. coli* can use maltose as a carbon source. The *malE* and *malF* genes are required for transport of maltose into the cell. The ***malE* gene encodes a periplasmic protein** and the ***malF* gene encodes a cytoplasmic membrane protein.**



TnphoA gene fusions were isolated in the *malE* and *malF* genes in a host deleted for the chromosomal *phoA* gene. Some *TnphoA* insertions in *malE* and *malF* express alkaline phosphatase activity and some do not express alkaline phosphatase activity.

- a. List 2 reasons why some *malE*::*TnphoA* insertions might not express alkaline phosphatase activity.
ANSWER: Either inserted in the wrong orientation or wrong reading frame. (Note that this protein is exported into the periplasm, so *TnphoA* insertions will be exported into an environment where S-S bonds can form.)
- b. One of the *malF*::*TnphoA* insertions did not express alkaline phosphatase enzyme activity but reacted with antibody against alkaline phosphatase. What is the most likely explanation for this phenotype?
ANSWER: The join-point between the integral membrane protein and the *phoA* fusion places the *phoA* protein on the inside of the cytoplasmic membrane -- thus, the *PhoA* protein is made but it is unable to properly form S-S bonds and dimerize, so it remains inactive.

- [10] 10. The *sefD* gene encodes a subunit of the SEF fimbriae from *Salmonella enteritidis*. The *sefD* gene was cloned onto a multicopy plasmid as shown below. How could you use PCR to construct a derivative of this plasmid clone with a deletion in *sefD* and with an *EcoRI* site at the join point? Indicate the location and DNA strand where any primers you use would hybridize on the original clone. **ANSWER: See figure below.**



Deletion removes sequence between 3' ends of PCR primers and leaves EcoRI site at the join point of the deletion

- (10) 11. Derivatives of green fluorescent protein (GFP) that have different colors have been obtained by site-directed mutagenesis. Yellow fluorescent protein (YFP) results from a Thr to Tyr substitution mutation.
- Even if an efficient screen was available, it is unlikely that this mutant would have been obtained via random mutagenesis. Why?
ANSWER: The Thr to Tyr substitution requires three bp substitutions (even in the simplest scenario, a minimal of two bp substitutions would be required for a Thr to Tyr substitution). If these are independent events, at a typical frequency of 10^{-6} per cell per generation, the frequency you would expect the three independent events to occur would be $10^{-6} \times 10^{-6} \times 10^{-6} = 10^{-18}$.
 - Given a copy of the *gfp* gene cloned onto a plasmid, and the sequence of this region of the *gfp* gene from nucleotide 601-620 shown below, how could you use site-directed mutagenesis to construct a Thr to Tyr substitution mutation? Draw the sequence of the primer you would use and describe how the mutagenesis procedure used would "enrich" for the desired recombinants. [A genetic code table is attached to the end of this exam.]

5' CTGTCCACACAATCTGCCCT 3'

ANSWER: Recall that the codon table shows mRNA codons but DNA primers substitute T for U. See figure below for example of how to construct the mutant using the dut ung approach. (Note that if the primer had U residues, it would be destroyed in the *dut⁺ ung⁺* recipient as well as the original template.)

Single stranded template from *dut⁻ ung⁻* host contains dU in DNA
 (Note that ssDNA can be obtained by simply denaturing dsDNA)

