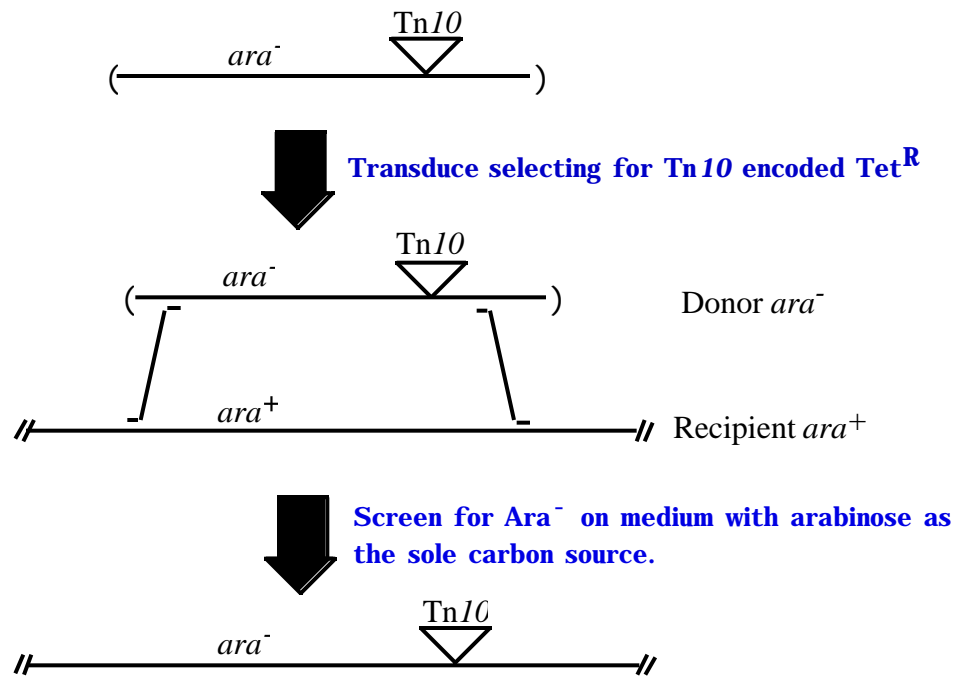


- (10) 1. Transposon Tn5 has inverted repeats at each end of the transposon. The same inverted repeats are found at the end of every Tn5 insertion.
- Briefly describe the important features of these inverted repeats and their function.  
**ANSWER: The inverted repeats encode transposase and the ends of the inverted repeats provide binding sites for transposase that are essential for transposition.**
  - Short direct repeats are observed flanking each Tn5 insertion. The sequence of these direct repeats is different for each Tn5 insertion. Briefly explain how these direct repeats are formed and why they are different for each Tn5 insertion.  
**ANSWER: The direct repeats are host DNA from the transposition target site that are generated by staggered cuts which are subsequently filled in by DNA replication.**
- (5) 2. Transposon Tn10 encodes resistance to tetracycline. This is a complete transposon that encodes its own transposase. Briefly describe two ways that you could deliver Tn10 to recipient cells that would select for transposition events.  
**ANSWER: There are many ways this question could be answered. Two are listed below.**  
**(1) Bring the transposon into a recipient cell on a defective phage that cannot replicate in the host or lysogenize the host – select for Tet<sup>R</sup>.**  
**(2) Bring the transposon into a recipient cell on a suicide plasmid that cannot replicate in the host – select for Tet<sup>R</sup>.**
- (10) 3. The *araBAD* operon is required for growth on arabinose as a sole carbon source. A random collection of Tn10 insertions was isolated in an *araBAD*<sup>-</sup> mutant background. Given a lysate of phage P1 grown on this random pool of Tn10 insertions and an *ara*<sup>+</sup> recipient, how could you isolate a Tn10 insertion linked to the *ara*<sup>-</sup> mutation? Draw a figure showing the crosses you would do and indicate how you would select or screen for the desired mutants.  
**ANSWER:**

A generalized transducing lysate with random Tn10 insertions will include insertions at many chromosomal sites, including insertions adjacent to the *ara* operon

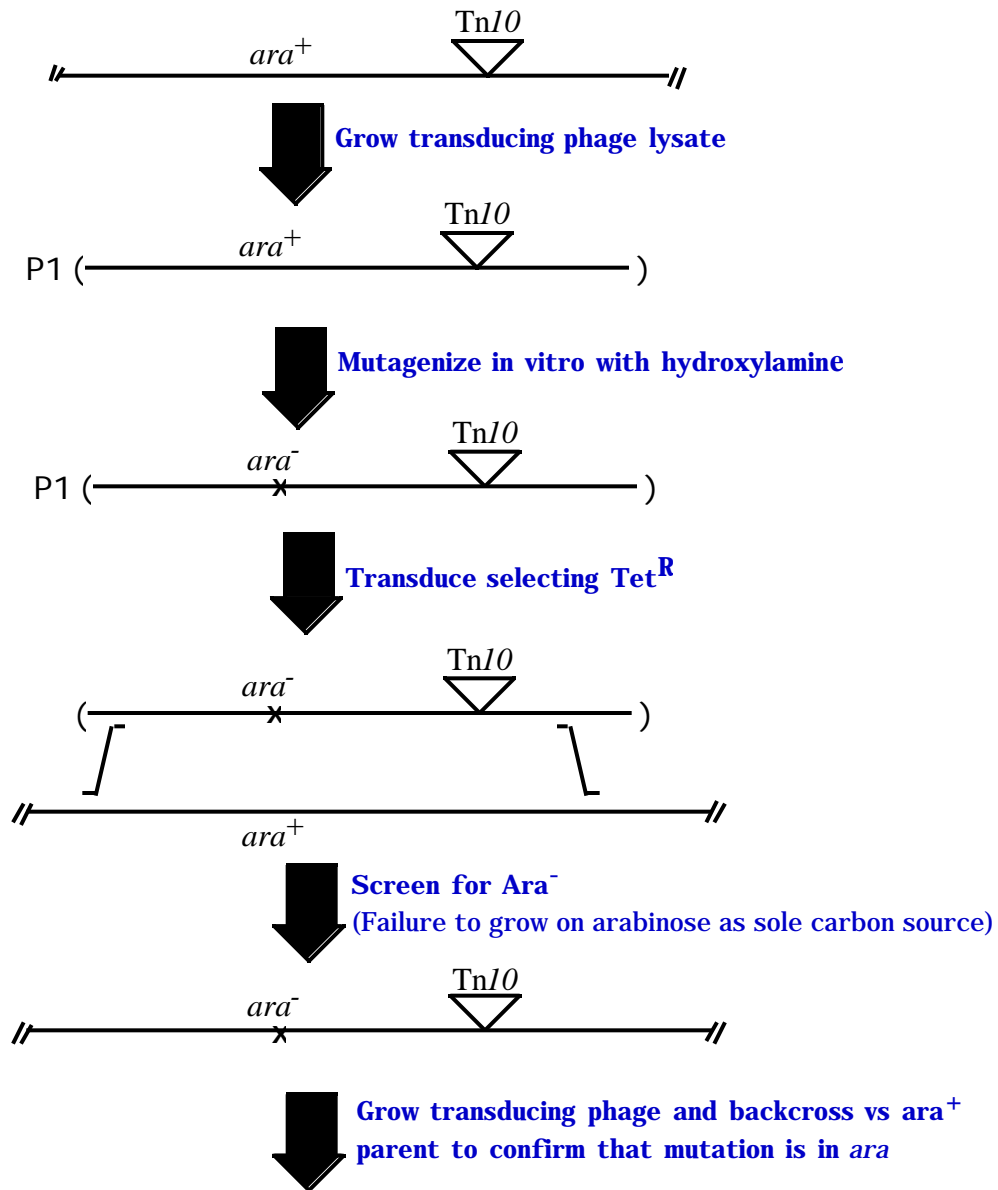


The results should be confirmed by backcrossing the Ara<sup>+</sup> Tet<sup>R</sup> transductants against the *ara*<sup>-</sup> recipient.

(10) 4. Given a Tn10 insertion that is cotransducible with the wild-type *araBAD*.

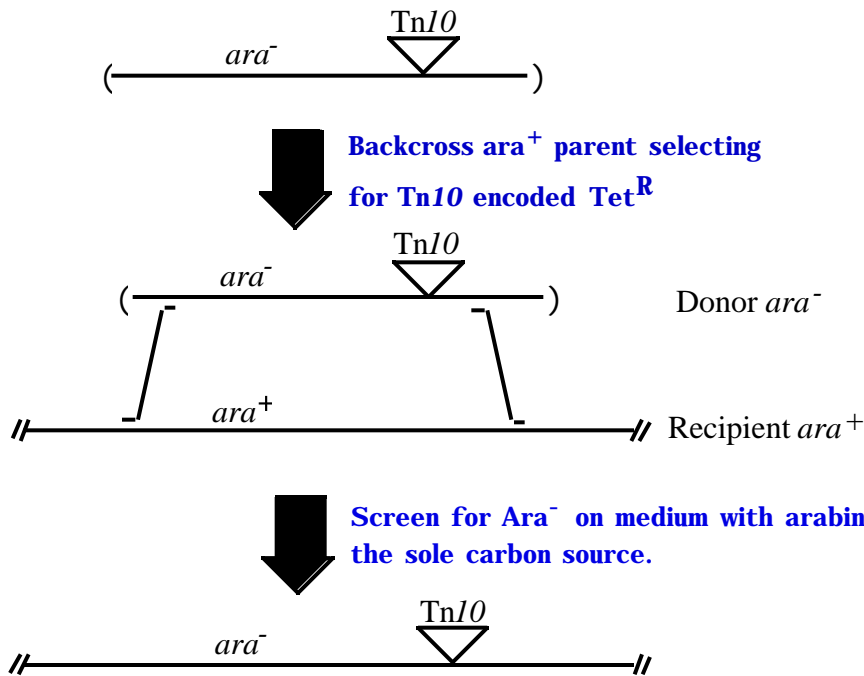
- How could you take advantage of this insertion to isolate point mutations in *araBAD*? Draw a figure indicating the donor and recipient strains and any selection or screen you would use.

**ANSWER:**

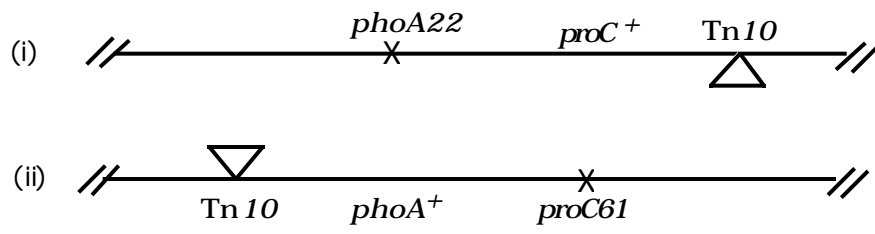


- c. How would you demonstrate the mutants are in the *araBAD* operon? Briefly describe the donor and recipient strains and any selection or screen you would use.

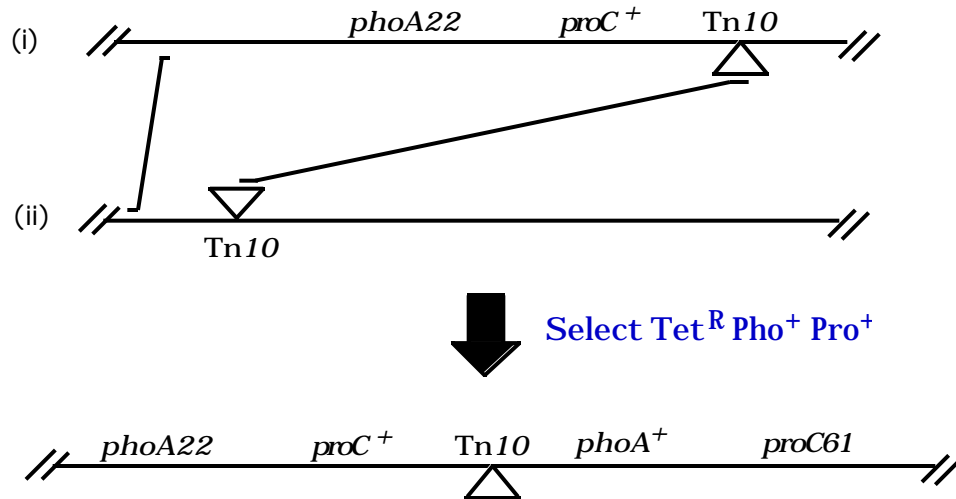
**ANSWER:** Backcross as described in the final arrow above and shown below.



- (15) 5. The allele *phoA22* is a missense mutation that has a PhoA<sup>-</sup> phenotype (inability to use X-P). The allele *proC61* is a missense mutation that has a Pro<sup>-</sup> phenotype (auxotrophy for proline).
- a. Given the following two strains, how could you construct a strain that has a tandem duplication with one copy of the *phoA22* allele and one copy of the *phoA*<sup>+</sup> allele. Draw a diagram showing the recombination events and indicate how you would select or screen for the tandem duplication.



**ANSWER:** Transduce as shown below.

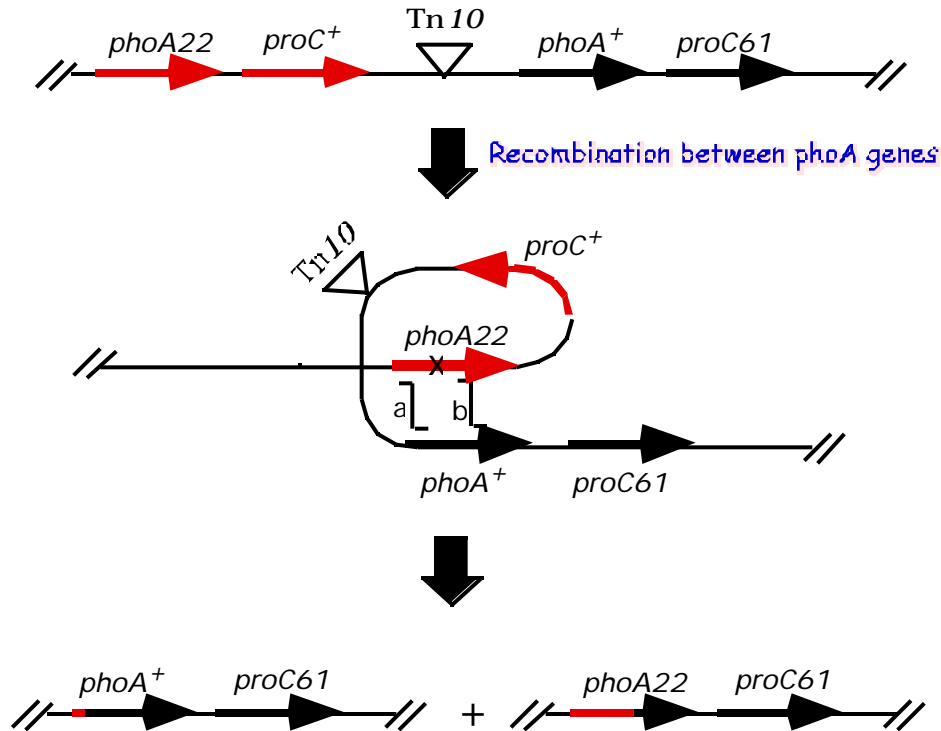


b. How could you prevent segregation of this duplication?

**ANSWER:** Either maintain selection for PhoA<sup>+</sup> (growth with organic phosphate as sole P-source) or Pro<sup>+</sup> (growth on minimal medium without proline) or select for Tet<sup>R</sup> (growth on medium with tetracycline). Moving a *recA* mutation into the duplication strain will also work.

c. In the absence of selection, the duplication segregates at a frequency of about 1% per generation. Two classes of *proC61* segregants are obtained. Draw a picture showing how the segregation occurs and indicate the phenotypes of the two types of *proC61* segregants.

**ANSWER:** Two classes of *phoA* segregants are obtained depending upon where the cross-over occurs (cross-over "a" yields *phoA*<sup>+</sup> and cross-over "b" yields *phoA* mutants); all segregants lose the Tn10 at the join point of the duplication, thus both classes of segregants are Tet sensitive; note that recombination occurs between direct repeats of the homologous sequences. See figure below.



(10) 6. Operon fusions and gene fusions have distinct properties that have unique uses.

a. Briefly describe 2 specific uses of operon fusions. [Each answer must describe a unique use.]

**ANSWER: The answer should describe a use of operon fusions per se, not simply transposons.**

**Some examples include:**

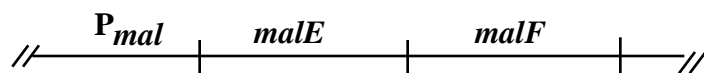
- Study transcriptional regulation
- Determine the direction of transcription
- Determine operon structure by testing for polarity of upstream mutations
- Isolate regulatory mutations by selection for altered expression of the reporter gene

b. Briefly describe 2 specific uses of gene fusions. [Each answer must describe a unique use.]

**ANSWER: The answer should describe a use of gene fusions per se, not simply transposons or operon fusions. Some examples include:**

- Study translational regulation (or its absence by comparison with operon fusion results)
- Purify fusion proteins
- Determine topology of membrane proteins
- Assay for export of secreted proteins

(15) 7. The *malEF* operon is shown below. The *malE* gene encodes a periplasmic protein and the *malF* gene encodes a cytoplasmic membrane protein.



*TnphoA* forms gene fusions to alkaline phosphatase. *TnphoA* insertions 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 of the *malE*::Tn*phoA* insertions might not express alkaline phosphatase activity.

**ANSWER:**

- **Insertions may be in the wrong orientation**
- **Insertions may be in the wrong reading frame**

- b. List 3 reasons why some *malF*::Tn*phoA* insertions would not express alkaline phosphatase activity.

**ANSWER:**

- **Insertions may be in the wrong orientation**
- **Insertions may be in the wrong reading frame**
- **Insertions may lack export signals**

- c. One of the *malF*::Tn*phoA* insertions does not express alkaline phosphatase enzyme activity but reacts with antibody against alkaline phosphatase. What is a likely explanation for this phenotype?

**ANSWER: The gene fusion probably results in a hybrid protein but the PhoA domain is not exported so it remains inactive. Antibody can detect the presence of the inactive protein in the cell.**

- (15) 8. Site-directed mutagenesis was done to determine if a "helix-turn-helix" motif in the PutA protein is required for DNA-binding

- a. Given the following sequence, what oligonucleotide could you use to obtain a double mutant that changes both val ala and asp ala? Specify the 5' end of your oligonucleotide. [A genetic code table attached to the back of the exam.]

**ANSWER: There are several possible oligos that would yield the desired mutations. One example is shown below:**

3' CGA TAC TAA **CGG** **CGG** AGA AGG CGA 5'

5' GCT ATG ATT <b>GTC</b> <b>GAC</b> TCT TCC GCG 3'
ala met ile <b>val</b> <b>asp</b> ser ser ala

5' GCT ATG ATT **GCC** **GCC** TCT TCC GCT 3'

ala met ile **ala** **ala** ser ser ala

- b. Briefly describe how the *dut ung* procedure enhances isolation of the desired recombinants.

**ANSWER:**

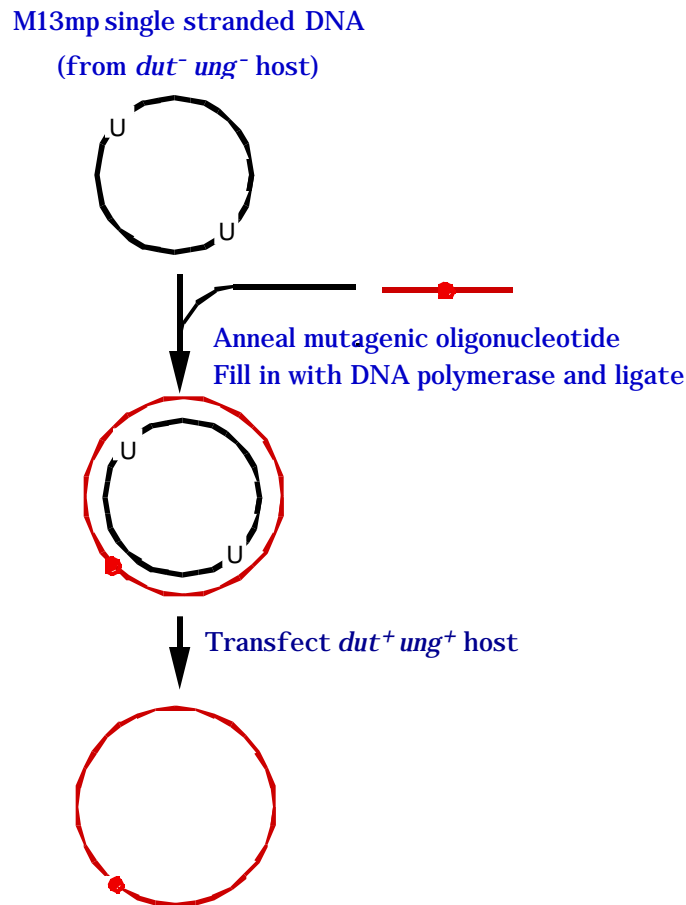
**(i) The DNA template is obtained from a *dut ung* mutant of *E. coli*. The *dut* gene encodes dUTPase which normally degrades dUTP. An elevated concentration of dUTP accumulates in *dut* strains, resulting in incorporation of U in place T at some positions during DNA replication. The *ung* gene encodes uracil N-glycosylase which normally removes U from DNA. Thus, in the double mutant U is occasionally incorporated into DNA and this error is not repaired**

(ii) A mutagenic oligonucleotide is annealed to a ssDNA template obtained from the *dut ung* strain. This oligonucleotide serves as a primer for in vitro DNA replication. The in vitro DNA replication reaction contains DNA polymerase, dATP, dTTP, dGTP, and dCTP but no dUTP, so no U is incorporated into the newly synthesized strand of DNA. After synthesis of the second strand of DNA is completed, the ends are covalently joined by DNA ligase. The resulting dsDNA consists of the template strand which contains U residues, and the newly synthesized strand which contains the mutant bases present in the oligonucleotide primer and does not contain any U residues.

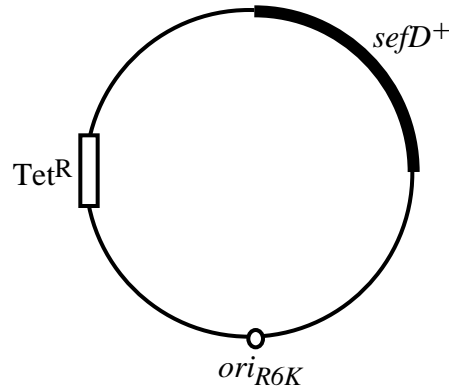
(iii) The dsDNA is then transformed into an *ung<sup>+</sup>* recipient cell. The uracil N-glycosylase recognizes the U residues in the DNA, and excises the U leaving apyrimidinic (AP) sites in the template strand. Presence of AP sites makes the DNA strand biologically inactive because it cannot be replicated by DNA polymerase and the DNA is cut at the AP sites by specific endonucleases. Hence, when the dsDNA is introduced into the *ung<sup>+</sup>* recipient, only the mutant strand will be replicated.

c. How would you use the *dut ung* method to construct the double mutant? Draw a diagram indicating the steps of this method.

ANSWER:

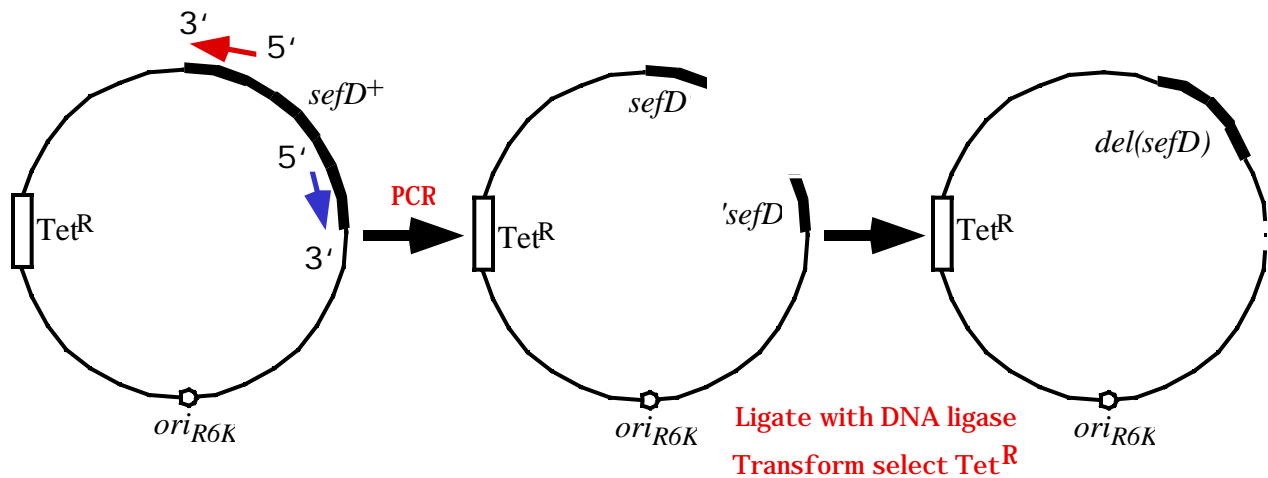


(10) 9. A clone carrying the *sefD* gene is shown below.



- a. How could you use PCR to construct a deletion in *sefD*? Indicate where any primers you would use and where they hybridize on the clone. You do not need to indicate the oligonucleotide sequence, only the relative positions where any oligonucleotides would anneal and the 5'-3' orientation of the oligonucleotides. [Note that *ori*<sub>R6K</sub> is a -dependent origin of replication.]

**ANSWER:** You could construct the desired deletion by PCR with the primers shown in blue and red in the figure below. Your answer should indicate the 5' and 3' end of the primers. NOTE that DNA replication occurs in the 5' to 3' direction.

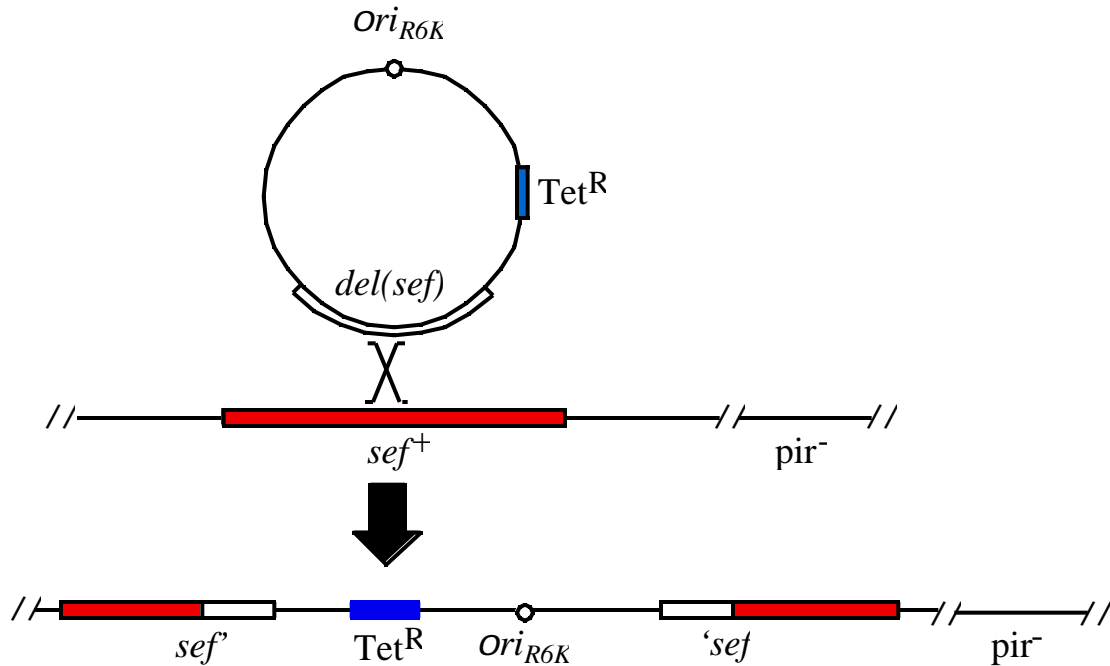


**Deletion removes sequence between 3' ends of PCR primers**

- b. How could you move this deletion mutation onto the chromosome? Draw a diagram showing what you would do and indicating any important properties of the recipient strain.

**ANSWER:** Your answer should show how the deletion mutation can be recombined onto the chromosome to remove the wild-type allele. The answer must show the regions which recombine, appropriate cross-overs, and any selections or screens used. Two approaches are feasible:

- Integration of the suicide plasmid via homology with sequences adjacent to *sefD* but located outside of the deleted region (taking advantage of a *pir* minus host that prevents replication of the *ori*<sub>R6K</sub> plasmid) to obtain a chromosomal duplication.



- Alternatively use of the lambda red system to integrate a linear DNA fragment obtained by cleavage of the plasmid or PCR of the region adjacent to *sefD*.

(5) **EXTRA CREDIT** — Briefly describe 5 uses of mutations.

**ANSWER:** There are many possible answers but the answer must provide a concrete, specific example of how a particular mutation can be used in a general way. A few examples are listed below:

1. To determine the function of a gene by inference from a loss of function phenotype
2. To map a gene to determine its location with respect to adjacent genes and to facilitate the ability to move the mutation into other strains
3. To identify gene products that interact (e.g. by isolating interaction suppressors)
4. To identify new pathways that can fulfill a particular biochemical role (e.g. by isolating bypass suppressors)
5. To modulate the expression of a gene product - either increasing or decreasing the expression of a gene product (e.g. by isolating regulatory mutations)
6. To determine the order of functions of a group of gene products that act in a biochemical pathway (e.g. by epistasis analysis or TS/CS shift experiments)
7. To test the role of specific amino acid or nucleotide residues in a gene product
8. To modify a gene product so it has a new function or catalyzes production of a novel product (e.g. altered function mutations)