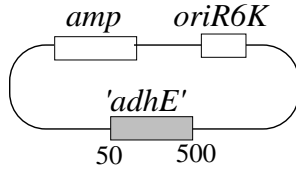
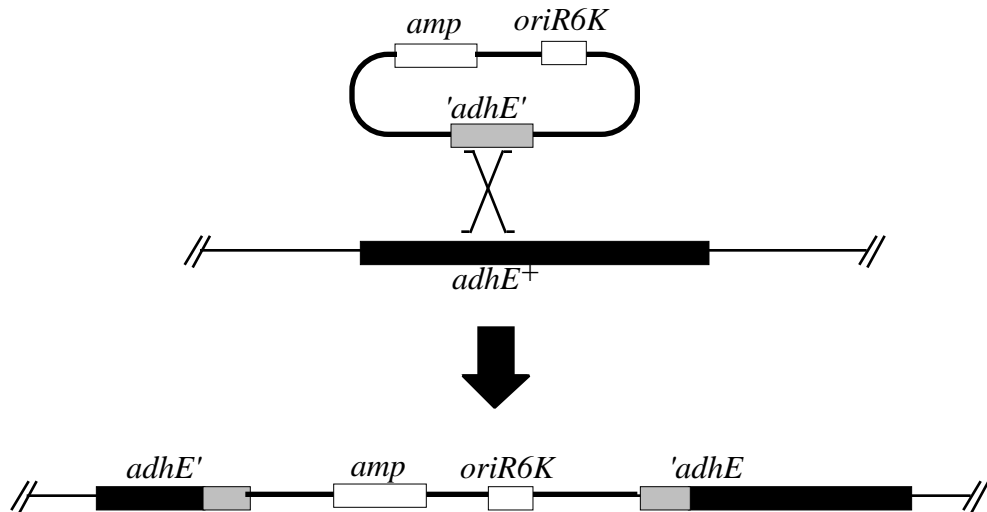


### Exam 3 - Mcbio 316

1. (15) The *adhE* gene is about 2000 bp. Nancy Maulen cloned a fragment corresponding to nucleotide 50 to 500 of the *S. typhimurium adhE* gene into the *pir*-dependent plasmid pGP704. [pGP704 has no homology with the *S. typhimurium* chromosome.]



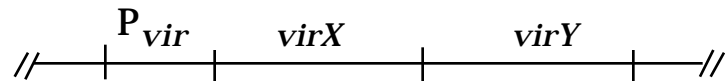
- a. Why is this plasmid *pir*-dependent?  
**ANSWER: The *pir* gene encodes a protein required for plasmid replication.**
- b. What would happen if this plasmid was transformed into a *S. typhimurium adhE*<sup>+</sup> *pir*<sup>+</sup> strain with selection for Amp<sup>R</sup>?  
**ANSWER: The plasmid would replicate independently.**
- c. What is the predicted phenotype of the transformants described in part b above? Why?  
**ANSWER: AdhE<sup>+</sup> Amp<sup>R</sup>**  
**The plasmid would not disrupt the chromosomal *adhE* gene.**
- d. What would happen if this plasmid was transformed into a *S. typhimurium adhE*<sup>+</sup> *pir*<sup>-</sup> strain with selection for Amp<sup>R</sup>?  
**ANSWER: The plasmid would integrate into the chromosome by homologous recombination via the *adhE* homology.**
- e. What would the phenotype of the transformants described in part d above? Draw a diagram to explain your answer.  
**ANSWER: AdhE<sup>-</sup> Amp<sup>R</sup>**



2. (10) Transposon Tn10 encodes resistance to tetracycline. [This is a complete transposon that encodes its own transposase.]

- a. Describe two ways that you could deliver transposon Tn10 to recipient cells to select for transposition events. **ANSWER:**
- **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>.**
  - **Bring the transposon into a recipient cell on a suicide plasmid that cannot replicate in the host — select for Tet<sup>R</sup>.**
- b. List 5 different uses of transposon insertions. **ANSWER:**
- **Mutations in a gene (null mutations with a selectable phenotype)**
  - **Mutations near a gene (linked mutations that facilitate movement of the gene into a new host)**
  - **Localized mutagenesis**
  - **Construction of defined deletions and duplications**
  - **Construction of operon and gene fusions**
  - **Selectable marker to clone a mutant gene or in vivo cloning**

3. (10) The *virX* gene is located in an operon upstream of the *virY* gene, as shown below.

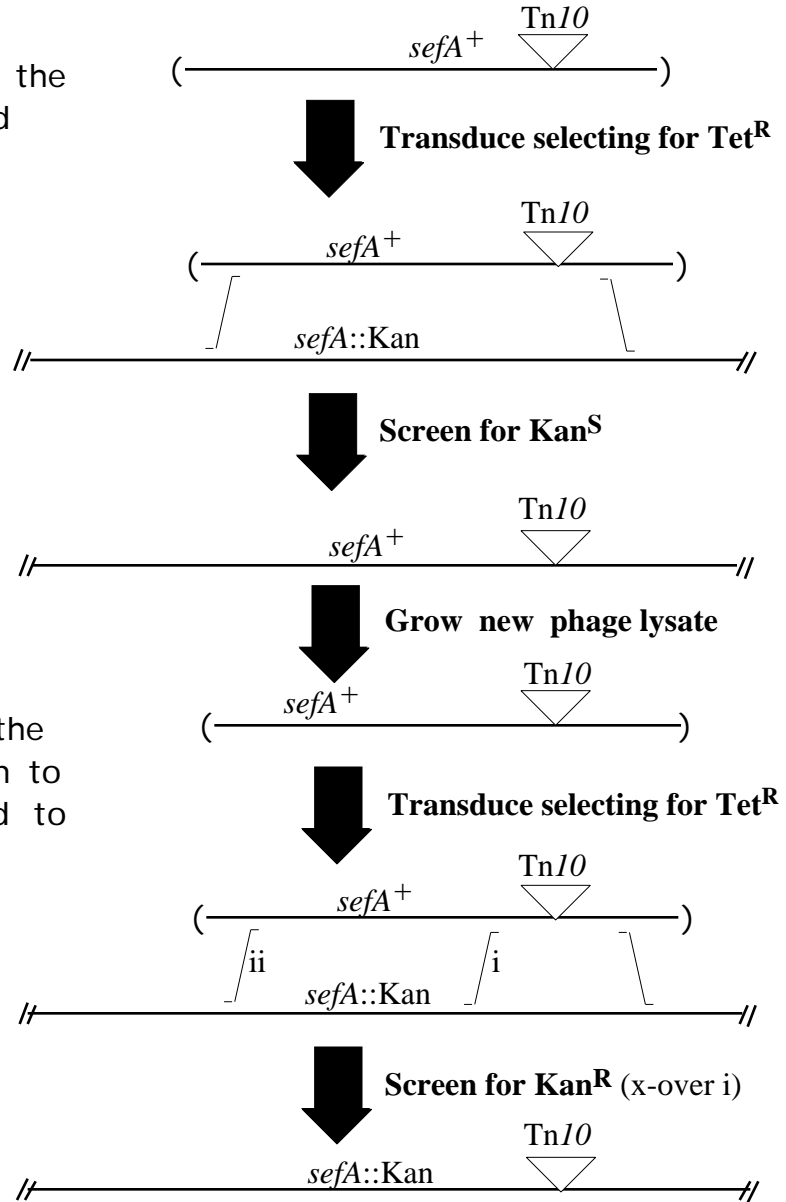


- a. Transposon insertion mutations in the *virX* gene decrease the virulence of *Salmonella typhimurium* in mice. Does this result prove that the *virX* gene product is involved in virulence? [Briefly explain your answer.]  
**ANSWER: No! Transposon insertions are typically polar. The observed phenotype could be due to disruption of the downstream *virY* gene.**
- b. How could you confirm these results? [Briefly describe the experiment you would do.]  
**ANSWER:**
- **Complementation with the *virX*<sup>+</sup> gene**
  - **Isolation of transposon insertions in *virY***
  - **Construction of an in frame deletion in *virX***

4. (10) The *sefA* gene encodes a type of pilus that is unique to *S. enteritidis*. Given a *sefA*<sup>+</sup> strain, a *sefA*::Kan mutant, and a P22 generalized transducing lysate grown on a random pool of Tn10 (tetracycline resistant) transposon insertions:

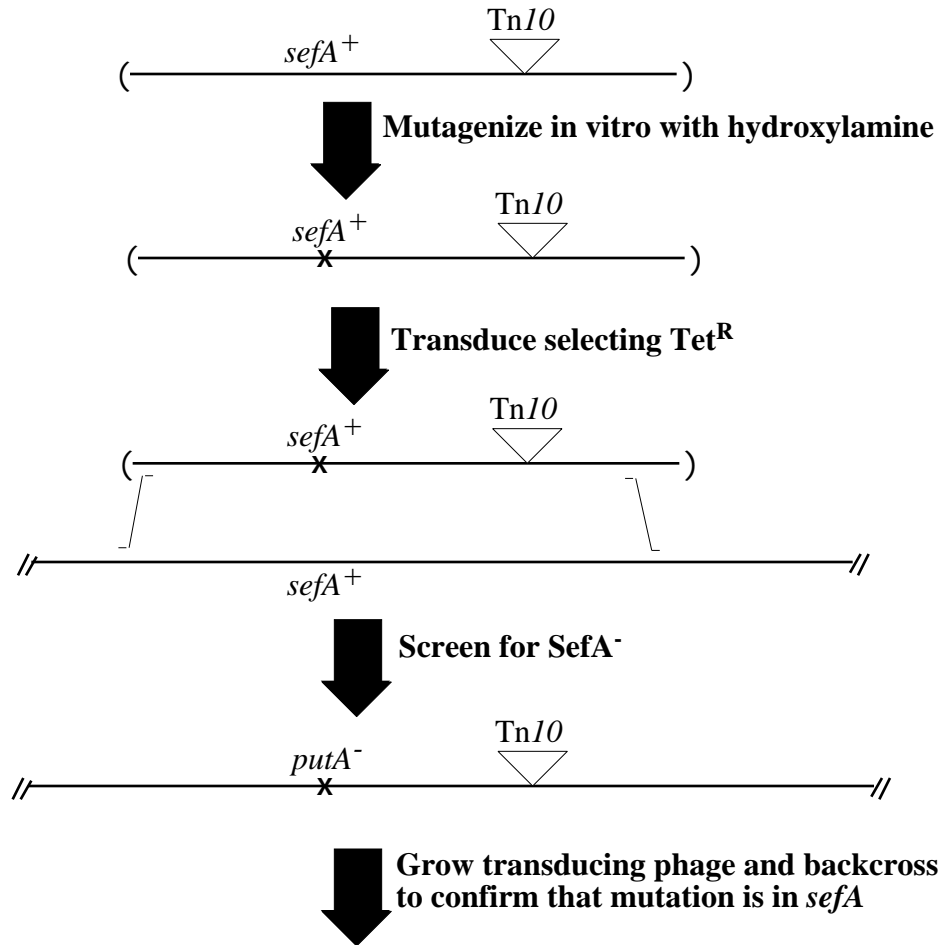
- a. How could you isolate a strain with a Tn10 insertion near the *sefA*::Kan mutation? Draw a diagram showing the donor and recipient DNA with any transposition or recombination events, and indicate the medium you would use for each selection or screen. **ANSWER:**

Step 1 = finding a Tn10 in the random pool that is linked to *sefA*<sup>+</sup>

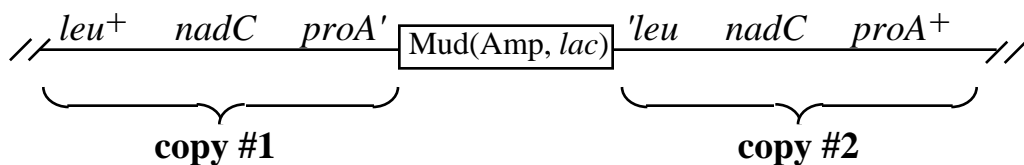


Step 2 = transduction of the linked Tn10 into *sefA*::Kan to isolate an insertion linked to the mutant allele

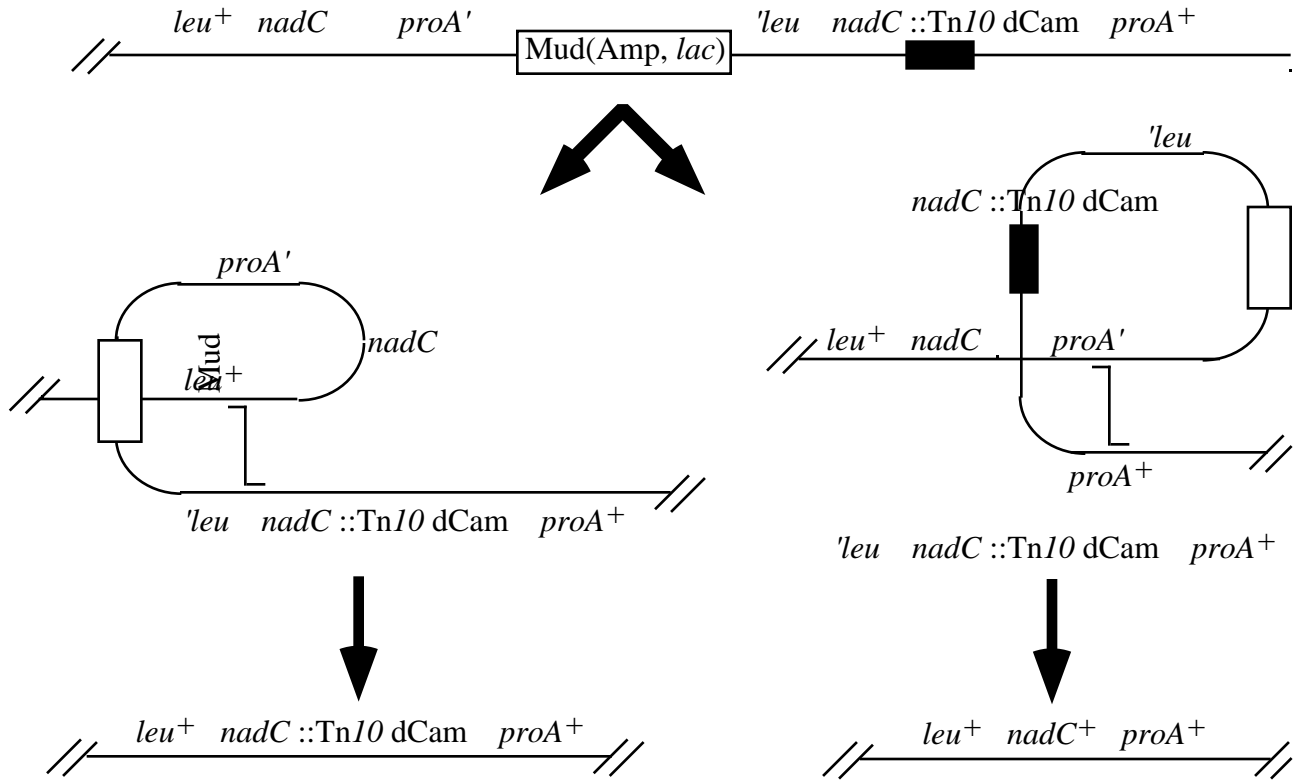
- b. Given a P22 phage lysate grown on a strain with a Tn10 insertion linked to the *sefA*<sup>+</sup> gene, how could you isolate point mutations in the *sefA* gene? Draw a figure indicating the donor and recipient DNA and describe any selections you would use. [You do not need to describe how to screen for *sefA* point mutants.] **ANSWER:**



5. (10) Chromosomal duplications of the *nadC* gene were constructed in *S. typhimurium*. The resulting merodiploids have one mutant *nadC* allele in copy #1 and a different mutant *nadC* allele in copy #2 with a mini-Mud at the join point, as shown in the figure below.



- How could you prevent segregation of this chromosomal duplication?  
**ANSWER: Maintain the duplication of medium with Amp.**
- If one of the copies of *nadC* had a *Tn10dCam* insertion mutation (i.e. *nadC*::*Tn10dCam*), two types of segregants can be obtained. Draw a diagram showing the two classes of recombination events that would result in segregation of the chromosomal duplication. [Clearly label the genes on the diagram, indicate the position of any cross-overs required, and show the two types of segregants obtained.] **ANSWER:**



6. (15) 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  $\beta$ -galactosidase). The results are shown in the following table.

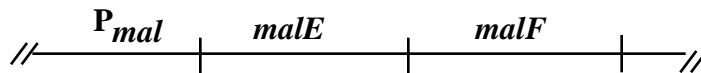
Gene	<i>galK</i> fusion		<i>lacZ</i> fusion	
	Galactose kinase activity		$\beta$ -galactosidase activity	
	- inducer	+ inducer	- inducer	+ inducer
<i>trbF</i>	40	40	400	3400
<i>trbG</i>	5	45	400	3600

- Briefly describe the transcription and translation of a *galK* operon fusion. **ANSWER:**
  - Transcription of the *galK* gene initiates from the upstream *trb* promoter.
  - Translation of the *galK* gene initiates from translational start sites (RBS and ATG) within the *galK* fusion.
- Briefly describe the transcription and translation of a *lacZ* gene fusion. **ANSWER:**
  - Transcription of the *lacZ* gene initiates from the upstream *trb* promoter.
  - Translation of the *lacZ* gene also initiates from translational start sites (RBS and ATG) within the upstream *trb* gene.
- What do the *galK* fusion results indicate about the regulation of the *trbF* and *trbG* genes? **ANSWER:**

**The *trbF* gene is not regulated at the transcriptional level.  
The *trbG* gene is regulated at the transcriptional level.**

- d. What do the *lacZ* fusion results indicate about the regulation of the *trbF* and *trbG* genes? **ANSWER:**  
**The *trbF* gene is regulated at the translational level.  
The *trbG* gene is regulated to the same extent in the gene fusion as in the operon fusion.**
- e. Combining the *galK* and *lacZ* results, briefly compare and contrast the regulation of *trbF* and *trbG* genes. **ANSWER:**  
**The *trbF* gene is regulated at the translational level.  
The *trbG* gene is primarily regulated at the transcriptional level.**

7. (15) *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.



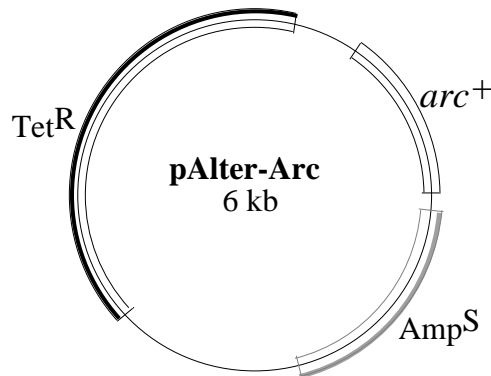
*TnphoA* insertions were isolated in the *malE* and *malF* genes in a host deleted for the chromosomal *phoA* gene. Some *TnphoA* insertions in *malE* and *malF* express alkaline phosphatase activity and some do not express alkaline phosphatase activity.

- a. List 2 reasons why some *malE::TnphoA* insertions would 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::TnphoA* 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::TnphoA* 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.**
- d. It is possible to isolate spontaneous mutants of the *malF::TnphoA* insertion described in part c above that express high levels of alkaline phosphatase enzyme activity. What is a likely way that spontaneous mutants would produce this phenotype?  
**ANSWER: These mutants are probably due to a spontaneous deletion that removes a region of the *malF* gene, resulting in fusion of the PhoA domain to an external domain of the MalF protein.**
- e. Would you expect to find *malE::MudK(lacZ)* insertion that express  $\beta$ -galactosidase activity? Briefly explain your answer.  
**ANSWER: No. The *malE* gene is exported to the periplasm, and export of  $\beta$ -galactosidase is lethal.**

8. (15) The DNA sequence of the *arc* gene from phage P22 is shown below.

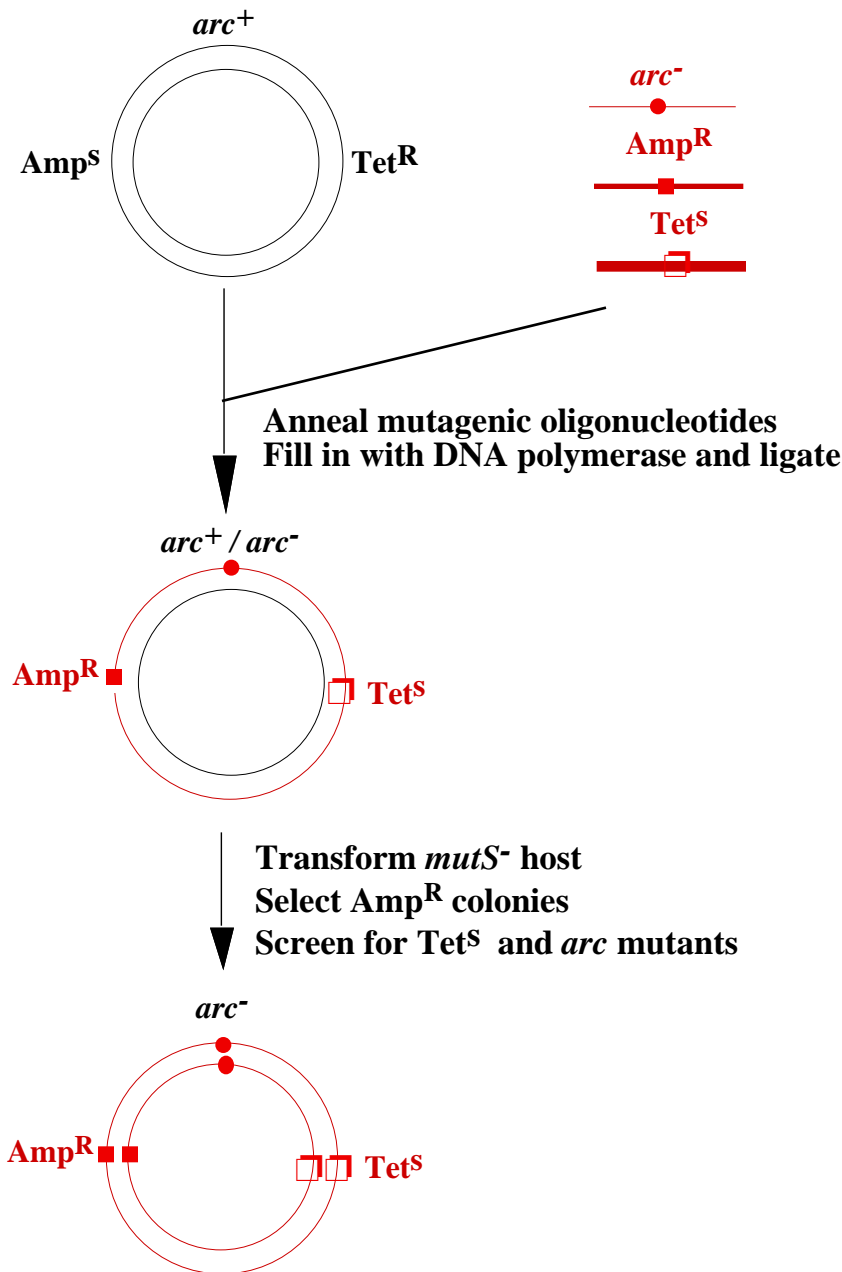
ATG AAA GGA ATG AGC AAA ATG CCG CAG TTC AAT TTG CGG TGG CCT AGA GAA GTA TTG  
 GAT TTG GTA CGC AAG GTA GCG GAA GAG AAT GGT CGG TCT GTT AAT TCT GAG ATT TAT  
 CAG CGA GTA ATG GAA AGC TTT AAG AAG GAA GGG CGC ATT GGC GCG TAA

The *arc*<sup>+</sup> gene was cloned onto the plasmid shown below.



- a. Using any oligonucleotides you chose, indicate how you could construct an amber mutation at the underlined codon using site-directed oligonucleotide mutagenesis [a genetic code table is provided]. Your answer should include:
- the nucleotide sequence of the mutagenic oligonucleotide you would use [although longer oligonucleotides are used experimentally, for simplicity use 6-mers to answer this question] ;
  - a diagram showing the necessary steps of the site-directed mutagenesis.

**ANSWER: I didn't specify which strand the oligonucleotides would anneal to, so you could describe an oligonucleotide that anneals to either the forward or reverse strand. However, the oligonucleotide must: (i) have the correct 5' to 3' polarity indicated; (ii) have T in the DNA, not U; and (iii) have the mutated nucleotide internal to the oligonucleotide, not at the extreme ends. For example, you could use 5' CGTAGT 3' or 5' ACTACG 3'. I would use this mutant oligonucleotide to do site-directed mutagenesis using the MutS method as shown below.**



- b. Using any oligonucleotides you chose, how could you confirm that the phenotype of the resulting mutant is due to the specific site-directed mutation you constructed and not due to some inadvertent secondary mutation acquired during the experiment? [Briefly describe how you would do the experiment and how you would interpret the results.]

**ANSWER: Reversion. The simplest experiment would be to construct the site-directed revertant by reversing the procedure described in part a. [A good reason for taking advantage of the MutS approach using the two opposing antibiotic resistance selections.]**

- c. How could you use this mutant to generate a variety of amino acid substitutions at the mutated codon.

**ANSWER: Simply move the mutant plasmid into a variety of isogenic strains carrying different amber suppressors.**