

- (15) 1. Transposons provide useful tools for genetic analysis. List 5 different uses of transposon insertions.

ANSWER: Many answers are possible, however, if multiple items on the list were not clearly “different uses” they were not given points. (3 points per accepted item)

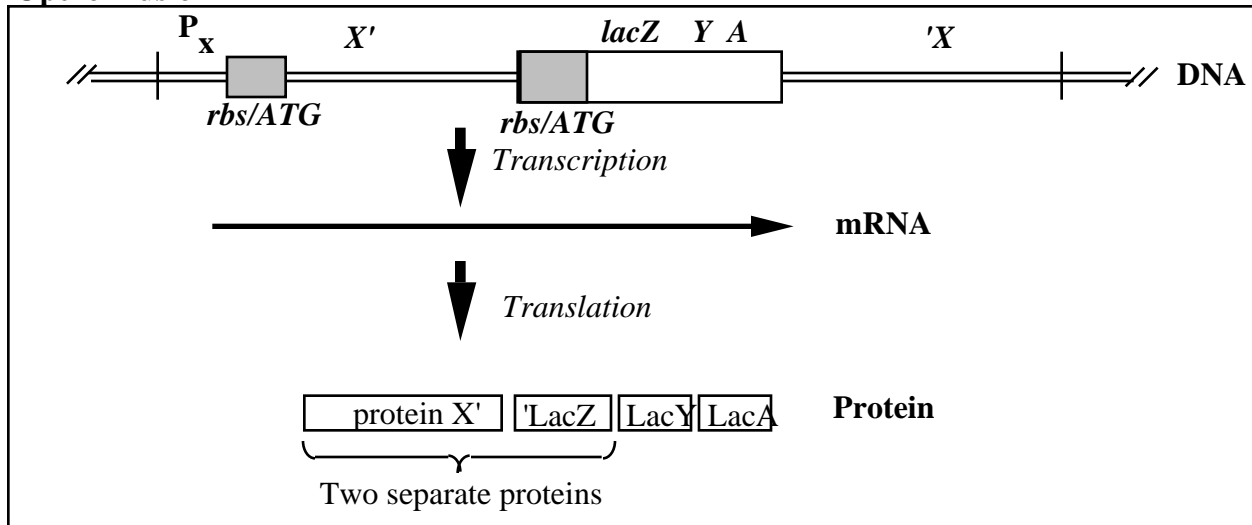
- Transposons can be found inserted at a large number of sites on the bacterial chromosome. (In principle, it is possible to find transposon insertions in or near any gene of interest.)
- Interrupted genes suffer complete loss of function. (Transposon-generated mutations are considered to be null alleles in all but rare exceptional cases.)
- The phenotype of the insertion mutation is completely linked to drug-resistance in genetic crosses. (It is possible to transfer mutations into new strain backgrounds simply by selecting for drug-resistance.)
- Insertion mutants can be recovered at high frequency after low-level “mutagenesis”. (Strains with multiple mutations are rare, and backcrossing allows one to establish that the mutant phenotype is due exclusively to the transposon insertion.)
- Insertion mutations revert by precise excision with concomitant loss of drug resistance. (This generally occurs at very low frequency; with some transposons, such as bacteriophage Mu, it is never observed.)
- Insertions in operons are strongly polar. (There are special-case exceptions to this rule.) Thus, transposon insertions can be used to determine whether genes are in an operon.
- Transposons can generate deletions nearby. (This provides a convenient method for isolating deletions, and even determining genetic map position.)
- Transposons can provide a portable region of homology. (Insertions can be used to construct deletions or duplications with defined endpoints, or can serve as sites of recombinational integration of other genetic elements.)
- Insertions behave as point mutations in fine-structure genetic mapping. (This is true only when the insertion serves as the recipient in a cross.)
- Insertions can be obtained which are *near* but not *within* a gene of interest. (Such insertions are useful for constructing deletions, and for genetic mapping.)
- Special transposons can be used to construct operon or gene fusions.

- (10) 2. Compare and contrast operon vs gene fusions.

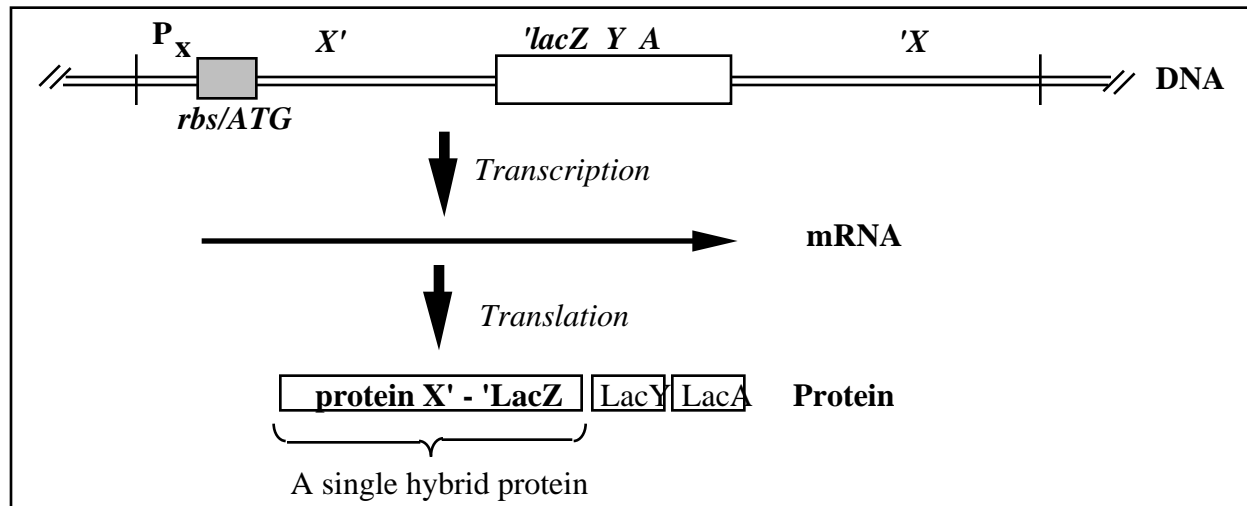
- a. Draw a simple diagram showing for each type of fusion: (i) the transcription and translation start and stop sites; (ii) the mRNA transcript expected; and (iii) the protein products expected.

ANSWER: See figures drawn below. Your answer must clearly indicate EACH of the items described above for each type of fusion.

Operon fusion



Gene fusion



P_x = Transcriptional start site

rbs/ATG = Translational start site

- b. For each type of fusion, indicate how frequently you would obtain insertions that express the reporter gene product if a transposon derivative (such as *Mud*) was randomly inserted into a gene. [Briefly explain your answer.]

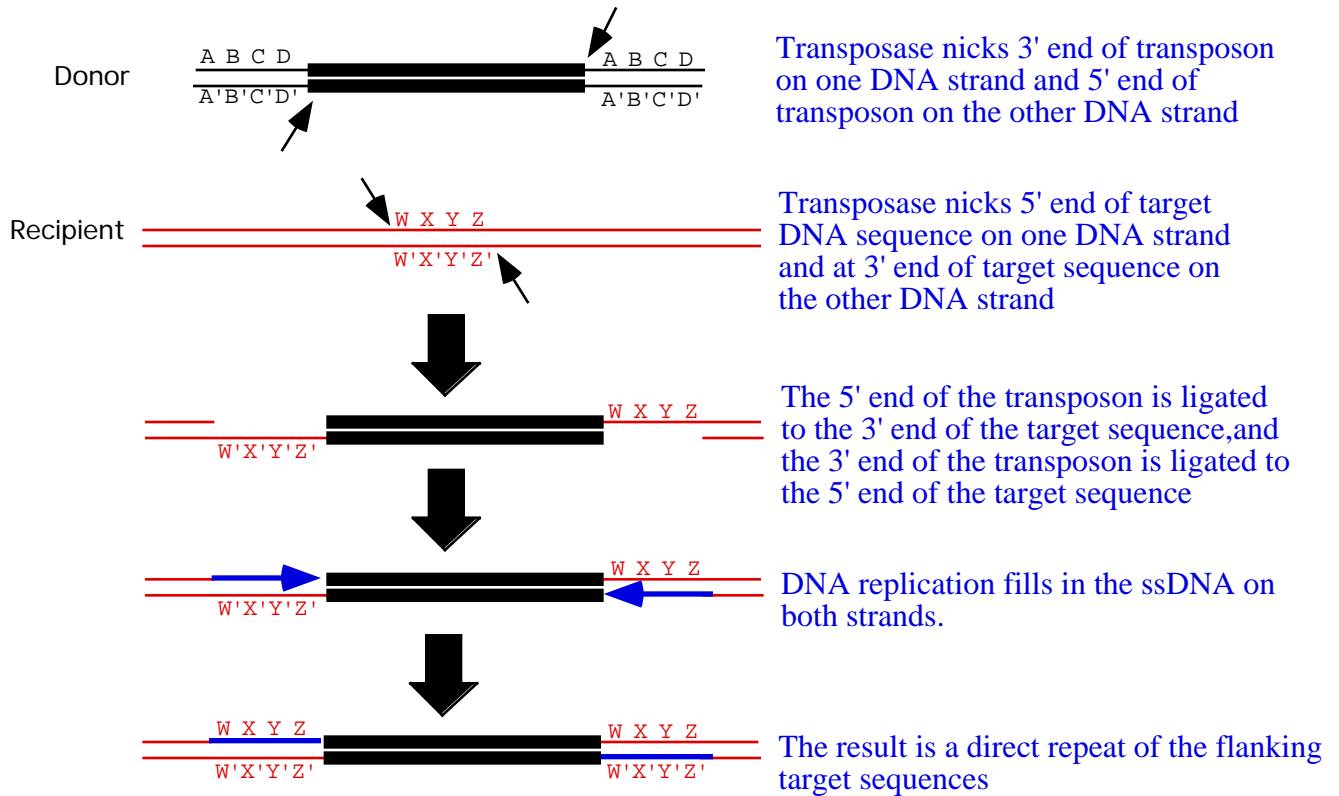
ANSWER: Your answer must include both the math and the explanation.

Operon fusions = 1/2 (two possible orientations)

Gene fusions = 1/2 x 1/3 = 1/6 (two possible orientations and 3 possible reading frames)

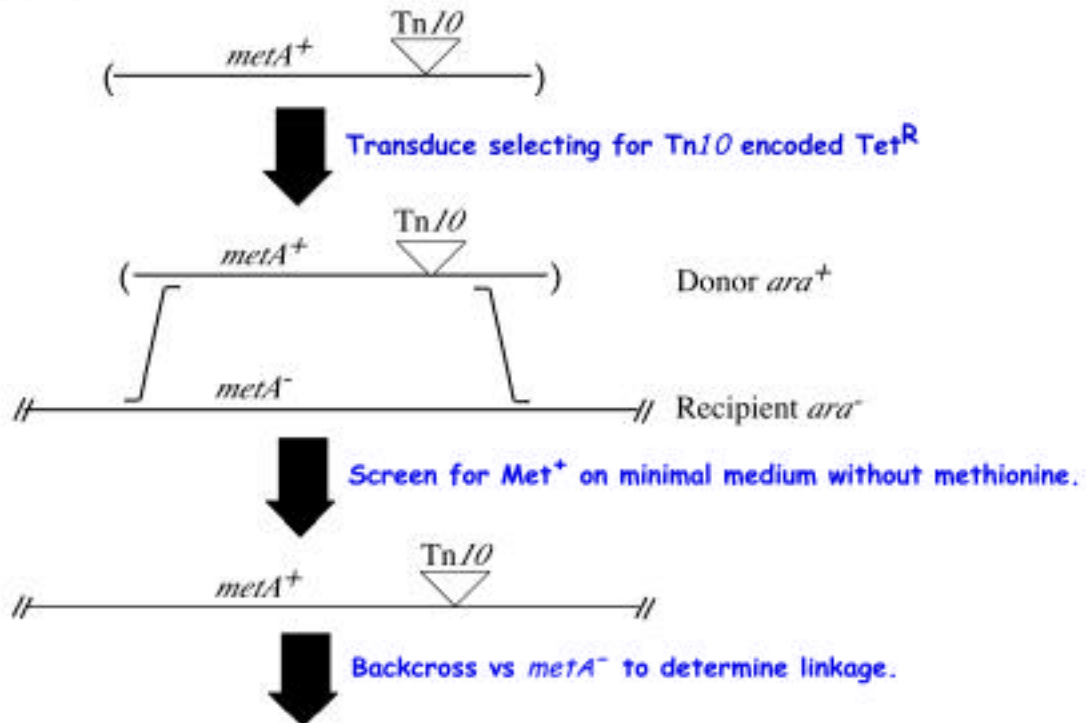
- (5) 3. Transposon insertions are flanked by a short direct repeat (usually 5-10 bp) of the target DNA sequence. Draw a diagram to indicate how these direct repeats are formed.

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



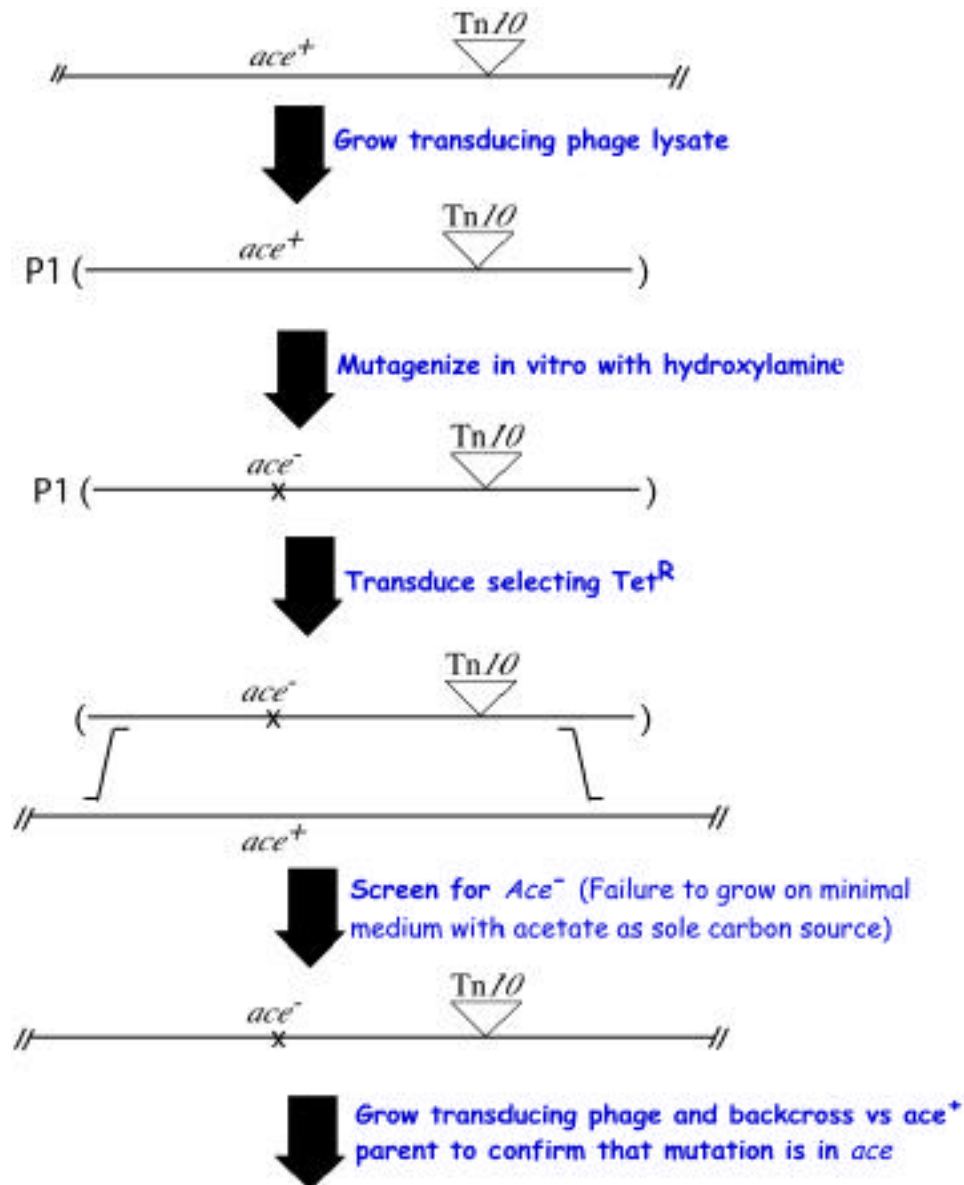
- (15) 4. The *metA* gene is required for the biosynthesis of methionine. Transposon *Tn10* encodes Tet^R.
- a. Given a mutant with a base substitution mutation in the *metA* gene **and** a P1 generalized transducing lysate grown on a random pool of *Tn10* insertions, how could you isolate a *Tn10* near (but not in) the *metA*⁺ gene? Draw a diagram showing how you would do the experiment and indicate the medium you would use for each selection or screen.

ANSWER: A generalized transducing lysate with random Tn10 insertions will include insertions at many chromosomal sites, including insertions adjacent to the *metA*⁺ operon.



- b. The *aceA* gene is required for the utilization of acetate as a sole carbon source. The *aceA* gene is closely linked to the *metA* gene. Given a strain with a Tn10 insertion near the *metA*⁺ gene, how could you isolate point mutations in the *aceA* gene? Draw a diagram showing how you would do the experiment and indicate the medium you would use for each selection or screen.

ANSWER: See figure below.

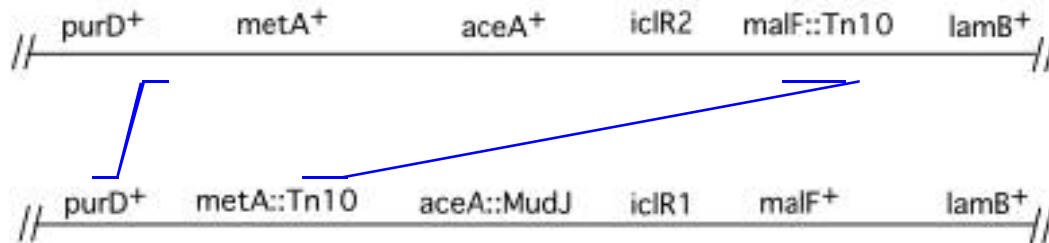


(20) 5. The genotypes of two *Salmonella* strains are shown in the figure below. $Tn10$ encodes Tet^R , and $MudJ$ forms $Kan^R lacZ$ operon fusions. The $purD$ gene is required for biosynthesis of purines, the $metA$ gene is required for biosynthesis of methionine, the $aceA$ gene is required for utilization of acetate as a sole carbon source, the $iclR$ gene encodes a regulator of $aceA$, the $malF$ gene is required for utilization of maltose as a sole carbon source, and the $lamB$ gene is required for growth on maltodextrins as a sole carbon source.

- a. Draw a diagram showing how you could use the following two strains to select for a duplication of the region of the chromosome between the $metA$ and $malF$ genes. Show all crossovers and indicate what phenotypes you would select for.

ANSWER: See cross-overs drawn in figure below. Note that the directions of the cross-overs matters - if the crossovers are drawn in the opposite way they will result in a deletion instead of a duplication. The duplication can be obtained by selecting for growth on minimal medium with maltose as a sole carbon source and tetracycline: this

selects for Tet^R (tetracycline ensures that you haven't lost the Tn10 insertion), Met⁺ (absence of methionine in the medium ensures that you have one copy of the *met*⁺ gene), and Mal⁺ (maltose as sole carbon source ensures that you have one copy of the *mal*⁺ gene).

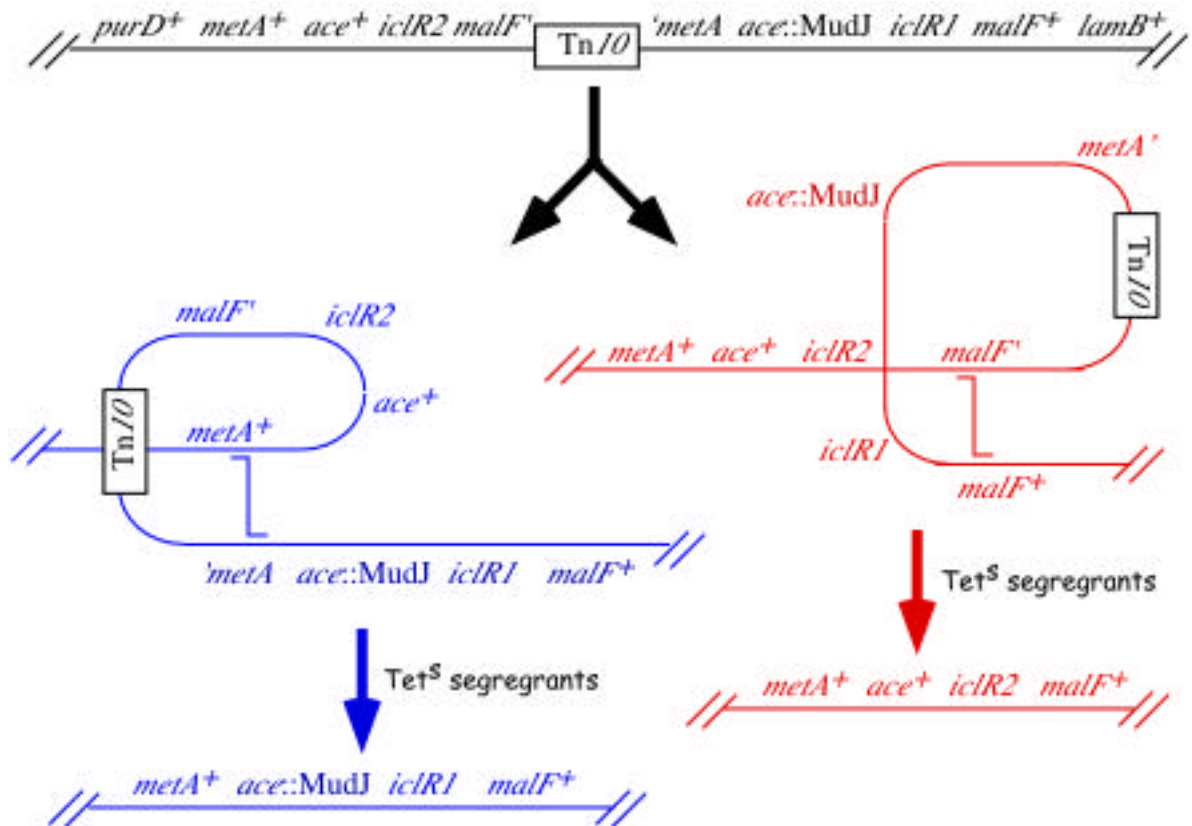


- b. In wild-type strains expression of the *aceA* gene is induced by growth on acetate. The *iclR* gene product regulates expression of the *aceA* gene. What can you infer about the mechanism of *IcIR* regulation if the duplication strain expresses *lacZ* constitutively? Briefly explain your answer.

ANSWER: It is probably a TRANSCRIPTIONAL REPRESSOR because a duplication that is defective for both copies of *iclR* (as shown by the alleles in the figure) results in constitutive expression of *lac* from the OPERON fusion.

- c. Draw a diagram showing how you could demonstrate genetically that you had actually constructed the desired duplication. Indicate the expected phenotypes. [Use the back of this page if you need more space.]

ANSWER: The best test is segregation. Segregants can arise by homologous recombination between the regions of homology on opposite sides of the Tn10 join point. The recombination will result in loss of Tn10 and hence Tetracycline sensitivity. A simple duplication will result in segregants that have the *ace* and *iclR* alleles present in the parental strain. Note that in the two examples shown below, recombination is between one of the markers closest to the join point, however, the recombination could occur between any homologous sequences (*ace* x *ace*, *iclR* x *iclR*, etc).

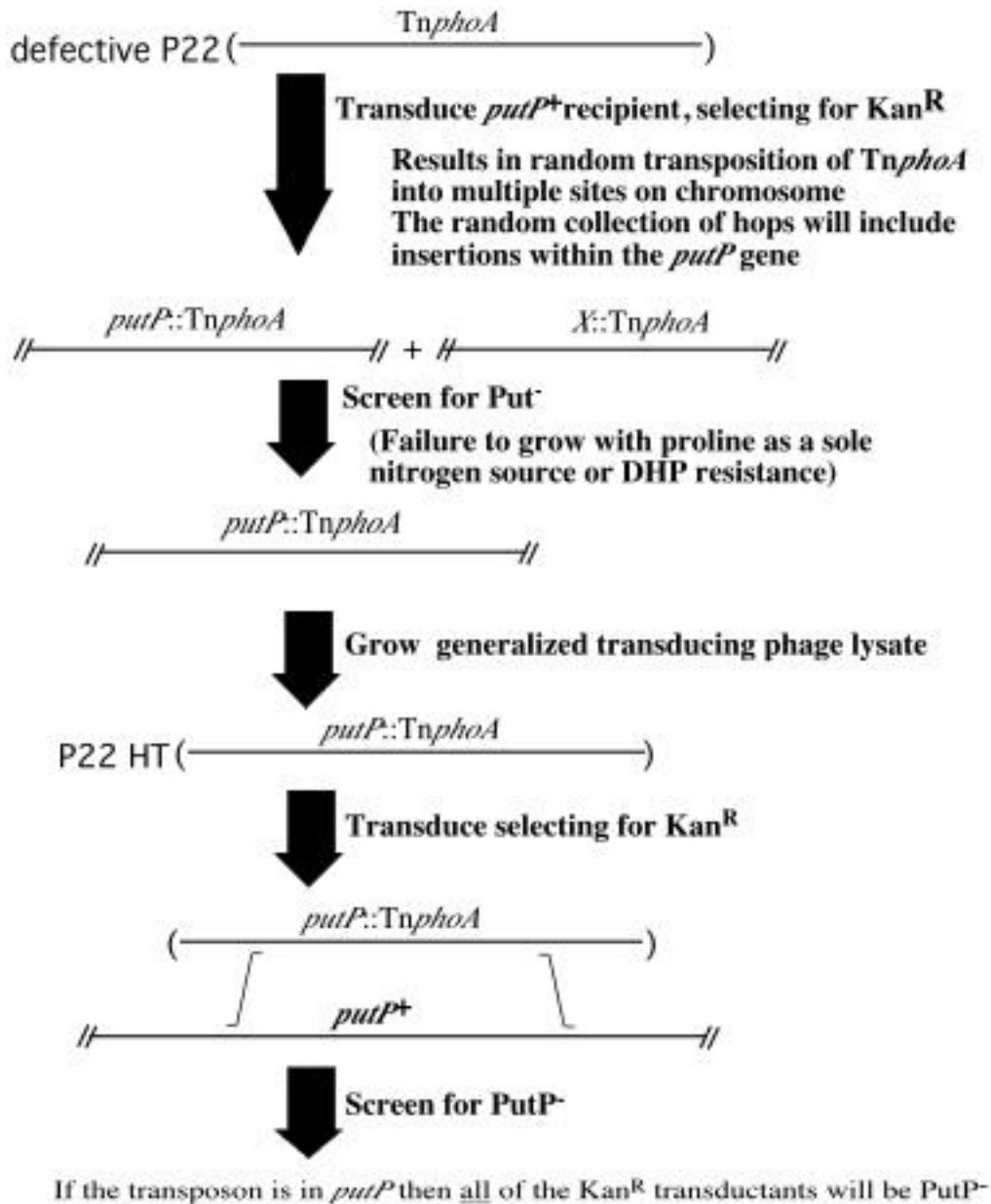


(15) 6. The *putP* gene is an integral membrane protein required for transport of proline in *Salmonella*. Null mutations in *putP* prevent the cell from growing on medium with proline as a sole carbon or nitrogen source, and make the cell resistant to the toxic proline analog, dehydroproline.

TnphoA encodes resistance to kanamycin (Kan^R). Wild-type *Salmonella* lacks the *phoA* gene.

- a. A derivative of P22 is available that is defective for lysogeny and for phage replication and carries *TnphoA*. Draw a diagram showing how you could use this phage to isolate *putP::TnphoA* insertion mutations in a wild-type *Salmonella* strain. [Indicate any selections or screens required to detect the desired mutants.]

ANSWER: see figure below.



- b. Some of the *TnphoA* insertions in *putP* form blue colonies on plates with XP indicating that they are PhoA⁺, and some of the insertions in *putP* form white colonies on plates with XP indicating that they are PhoA⁻. List **three** reasons why some of the *putP::TnphoA* insertions might 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 *putP::TnphoA* insertions did not express alkaline phosphatase enzyme activity but a cell extract from this mutant reacted with anti-[alkaline phosphatase] antibody. What is the most 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.

- (10) 7. Ken Zahn made an interesting observation concerning overexpression of the phage *int* gene. He made a plasmid construct that placed the *int* gene downstream of a very powerful regulatable promoter and supplied a strong ribosome binding site for the mRNA transcript encoding *int*. When *int* expression was turned on from this construct, protein synthesis was inhibited and the cells ultimately died. Zahn showed that the cell death was not due to toxicity of Int protein because the deleterious effects of *int* overexpression could be reversed if he supplied a plasmid that expressed an arginyl tRNA gene that makes a tRNA that decodes the AGA codon.

He also noticed an interesting pattern of usage of arginine codons in the Int protein as shown in the table below.

Codon	Number of codons in <i>int</i>	Frequency of usage in <i>E. coli</i> proteins
CGU	2	0.42
CGC	6	0.37
CGA	5	0.05
CGG	1	0.08
AGA	10	0.04
AGG	9	0.03

- a. From the information above, Zahn proposed a model for the inhibition of protein synthesis and eventual cell death due to expression of Int in this derivative. What do the results suggest about the mechanism of overexpression toxicity?

ANSWER: The high concentration of *int* mRNA sequesters the low abundance tRNA that decodes the AGA (and probably AGG) codon, hence the AGA codons in other mRNAs are not translated. As a result, essential proteins are not made and the cell dies.

- b. Zahn was eventually able to alleviate the overexpression effect. Describe a series of genetic experiments that would overcome the overexpression effect. Indicate any mutations you would make and why.

ANSWER: Site-directed mutagenesis was used to change the rare arginine codons AGA and AGG to the abundant arginine codons CGU or CGC.

- (10) 8. You have isolated a new gene from *Salmonella*. After obtaining the DNA sequence of the gene and performing a GenBank search you find that the gene has homology to known Type III topoisomerases from a variety of bacteria. It has a tyrosine residue that is conserved in all known Type III topoisomerases. One reaction that Type III topoisomerases perform is relaxation of negative supercoils in plasmid DNA. The reaction requires a nick mediated by a tyrosine residue in the enzyme to form a phosphotyrosyl intermediate with the DNA. This allows the DNA to rotate 360° which results in loss of a negative supercoil. The DNA is then ligated by the enzyme to form a new phosphodiester bond in the DNA. Subsequently the

enzyme is released from the DNA.

- a. Assume that you have a biochemical assay available for the enzyme. Draw a diagram showing a genetic experiment you could do to determine if the enzyme is a Type III topoisomerase.

ANSWER: Diagram should show site-directed mutagenesis to change Tyr to Phe or Ala (can use any of the site-directed mutagenesis approaches discussed in class).

- b. Describe a genetic experiment that could help you purify a large amount of the protein for biochemical studies.

ANSWER: Add a His tag on the N- or C-terminus of the protein.

- c. What is one possible problem with the experiment described in part b?

ANSWER: The His tag could decrease protein activity.

Bonus question (4 points)

List the features needed for a good, high level expression cloning vector.

ANSWER: Plasmid would need a strong promoter, inducible expression mediated by a regulator (activator or repressor) and a cognate DNA-binding site (operator), a good translation initiation site (rbs and ATG), an antibiotic resistant selection, and a multiple cloning site to facilitate insertion of a DNA fragment downstream of the promoter.