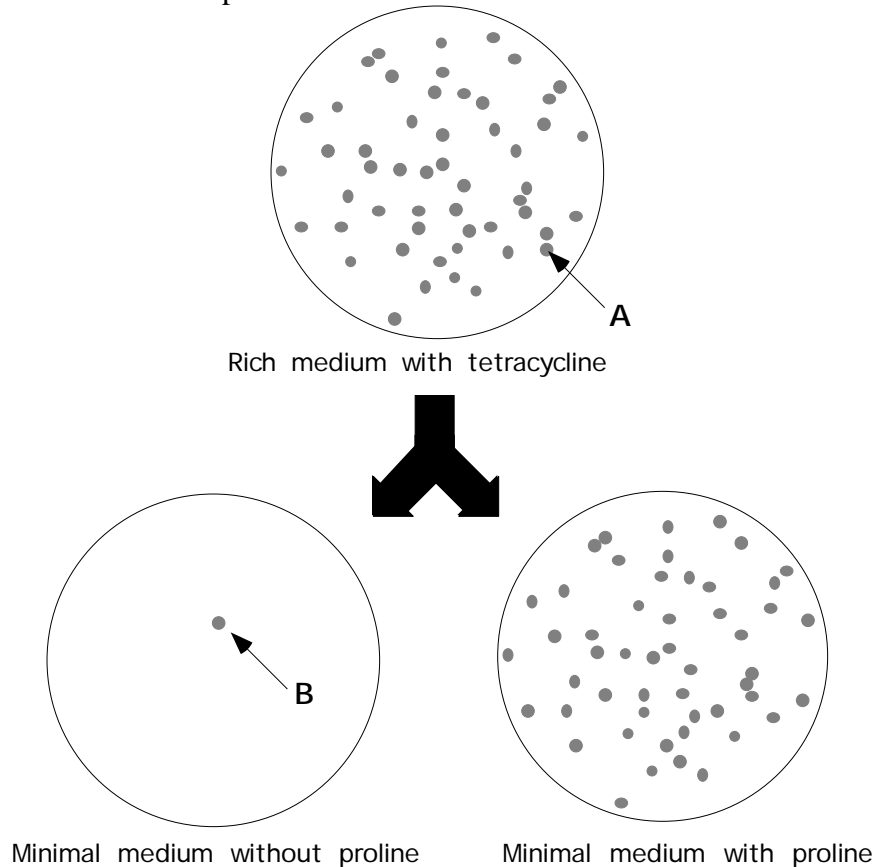


- [10] 1. The *proBA* genes are required for biosynthesis of proline. To isolate a *Tn10* insertion near the *proBA* genes, a strain with a nonsense mutation in the *proB* gene was transduced to Tet<sup>R</sup> with a P22 lysate grown on a random pool of *Tn10* insertions in the *Salmonella* chromosome. The Tet<sup>R</sup> colonies were then replica plated onto minimal medium plus or minus proline. A diagram of the colonies observed on each plate is shown below.



What is the most likely explanation for the phenotype of the colony indicated by the arrow marked A?

ANSWER: This colony is unable to grow on minimal medium either with or without proline, indicating that it is an auxotroph due to inheritance of a *Tn10* insertion that disrupts some other biosynthetic gene.

What are two potential explanations for the phenotype of the colony indicated by the arrow marked B?

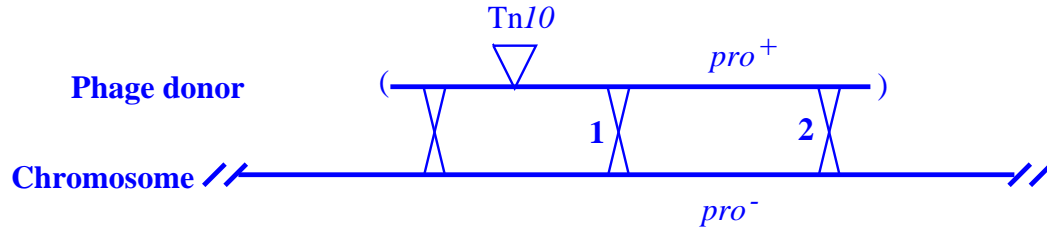
ANSWER: This colony has acquired the ability to grow on medium without proline. This phenotype could be due to either :

- (i) co-transduction of *proB*<sup>+</sup> with a linked *Tn10* insertion (the desired result), or
- (ii) reversion of the amber mutation in the *proB* gene and a *Tn10* insertion elsewhere.

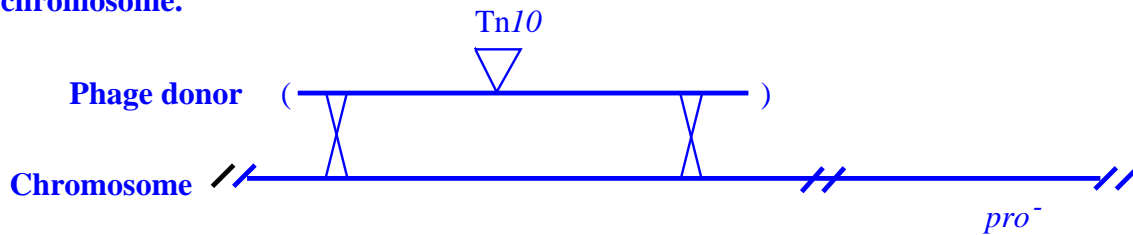
Diagram a genetic experiment you could do to distinguish between the two explanations for colony B. Describe the donor and recipient strains, any selections and screens required, and the media used.

**Backcross:**

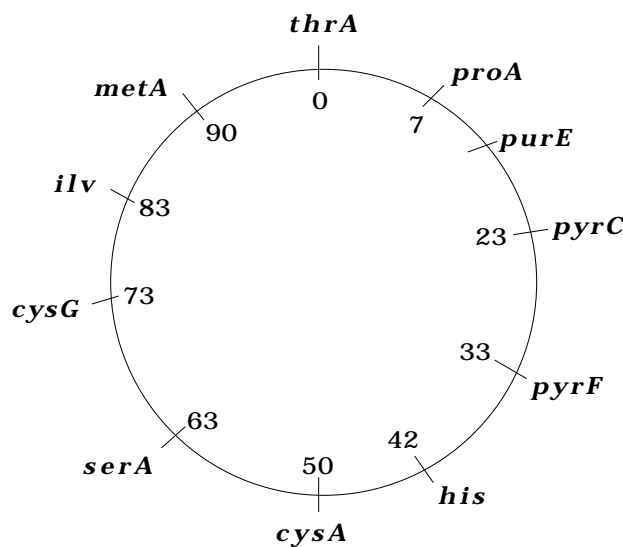
If the *Tn10* is linked to the *proB* gene then a single transducing particle can carry both the *Tn10* and the *proB*<sup>+</sup> gene, so some of the Tet<sup>R</sup> transductants will become Pro<sup>+</sup> (crossover #2) and some will remain Pro<sup>-</sup> (crossover #1).



If the *Tn10* is NOT linked to the *proB* gene then all of the Tet<sup>R</sup> transductants will remain Pro<sup>-</sup> because a transducing particle cannot carry both regions of the chromosome.



- [10] 2. Spontaneous tandem genetic duplications occur frequently in bacteria. Given a collection of *E. coli* strains with *Tn10* insertions in each of the genes on the *E. coli* chromosome shown below, how could you determine if a spontaneous duplication of the 90 min region occurred? Describe the genetic approach you would use and the phenotype expected.



ANSWER: Presumably such a duplication would have resulted in two copies of the *metA*<sup>+</sup> gene. Thus, if you transduced this strain to Tet resistance with a *metA*::*Tn10* insertion all of the transductants would still be Met<sup>+</sup>. To confirm that the transductants contained a *Tn10* insertion in the *metA* gene, you could look for segregants that become Met<sup>-</sup> Tet<sup>R</sup> or Met<sup>+</sup> Tet<sup>S</sup>.

Alternatively, you could confirm the genotype by growing P22 on the duplication strain and transducing a *metA* mutant to (i) MetA<sup>+</sup> (all the transductants should be Tet<sup>S</sup>) and (ii) Tet<sup>R</sup> (all the transductants should be Met<sup>-</sup>). Without this confirmation you would not be sure that the Tn10 insertion actually disrupted the *metA* gene.

[10] 3. Several DNA sequences that look like IS element or transposons were detected in *Brevibacterium*. Based upon their DNA sequence, the potential transposable elements do not encode any known antibiotic resistance or other selectable phenotype. Other than electroporation of plasmids, there is no known gene exchange system for *Brevibacterium*.

a. Briefly describe the difference between an IS element and a transposon.

ANSWER: Both IS elements and Transposons can transpose to new sites on the chromosome in a *recA* independent manner. Both IS elements and transposons produce direct repeats of chromosomal DNA at the insertion site. Both IS elements and transposons usually have indirect repeats at the ends of their sequence. The major difference is thus:

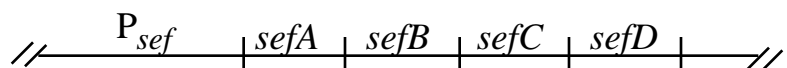
- An IS element only encodes the gene products needed for transposition.
- A Tn encoded the gene products needed for transposition and additional genes (e.g. antibiotic resistance genes).

b. How could you determine if the IS element or transposon was capable of transposition? Describe the approach you would use.

ANSWER: Note that transposition is a rare event (typically about 10<sup>-6</sup> per cell), so testing for transposition requires a selection that will detect those rare cells in the population where a transposition occurred. However, IS elements lack selectable markers, so you would need to look for transposition events that disrupt some selectable marker. For example, the *sacB* gene results in sucrose sensitivity so you could select for insertions that disrupt this gene by demanding resistance to sucrose; the *tetA* gene results in sensitivity to fusaric acid so you could select for insertions that disrupt this gene by demanding growth on plates containing fusaric acid the *putP* gene results in sensitivity to toxic proline analogs so you could select for insertions that disrupt this gene by demanding growth on plates containing azetidine carboxylic acid or dehydroproline; etc. A straightforward approach for selecting for transposition would be to electroporate the strain with a plasmid carrying a gene that you can select against (such as *sacB*, *tetA*, or *putP*). You can select for mutations that disrupt the plasmid encoded gene while simultaneously demanding maintenance of the plasmid by selecting for an antibiotic resistance encoded on the plasmid. To confirm that the mutations are due to an IS element, the mutated plasmid can be purified from the resulting mutants and screened for insertion mutations which would increase the size of the plasmid.

This trick is commonly used to detect transposition events and is called a “transposon trap”.

[10] 4. The *sefA* gene is located in an operon upstream of the *sefBCD* genes, as shown below.



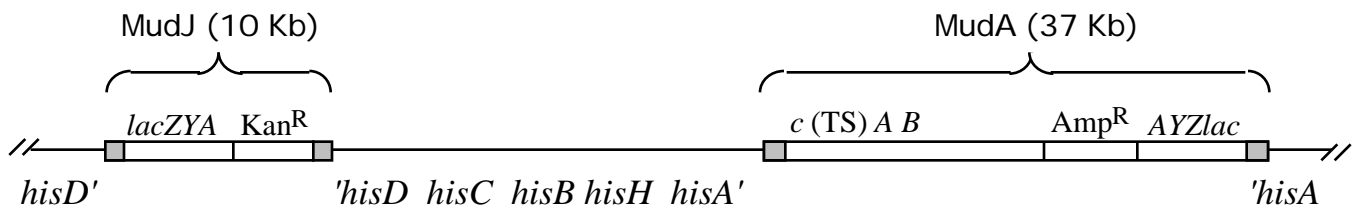
- b. Transposon insertion mutations in the *sefA* gene decrease the virulence of *Salmonella enteritidis* in mice. Does this result prove that the *sefA* gene product is involved in virulence? Briefly explain your answer.

ANSWER: No. Transposon insertions are usually polar and thus the phenotype could be due to the failure to express a downstream gene (*sefB*, *sefC*, or *sefD*). From the results given you cannot determine if the SefA gene product has any role in virulence -- the SefA gene product may not have anything to do with virulence or it may play a critical role in virulence.

- c. How could you confirm these results? Briefly describe the experiment you would do.

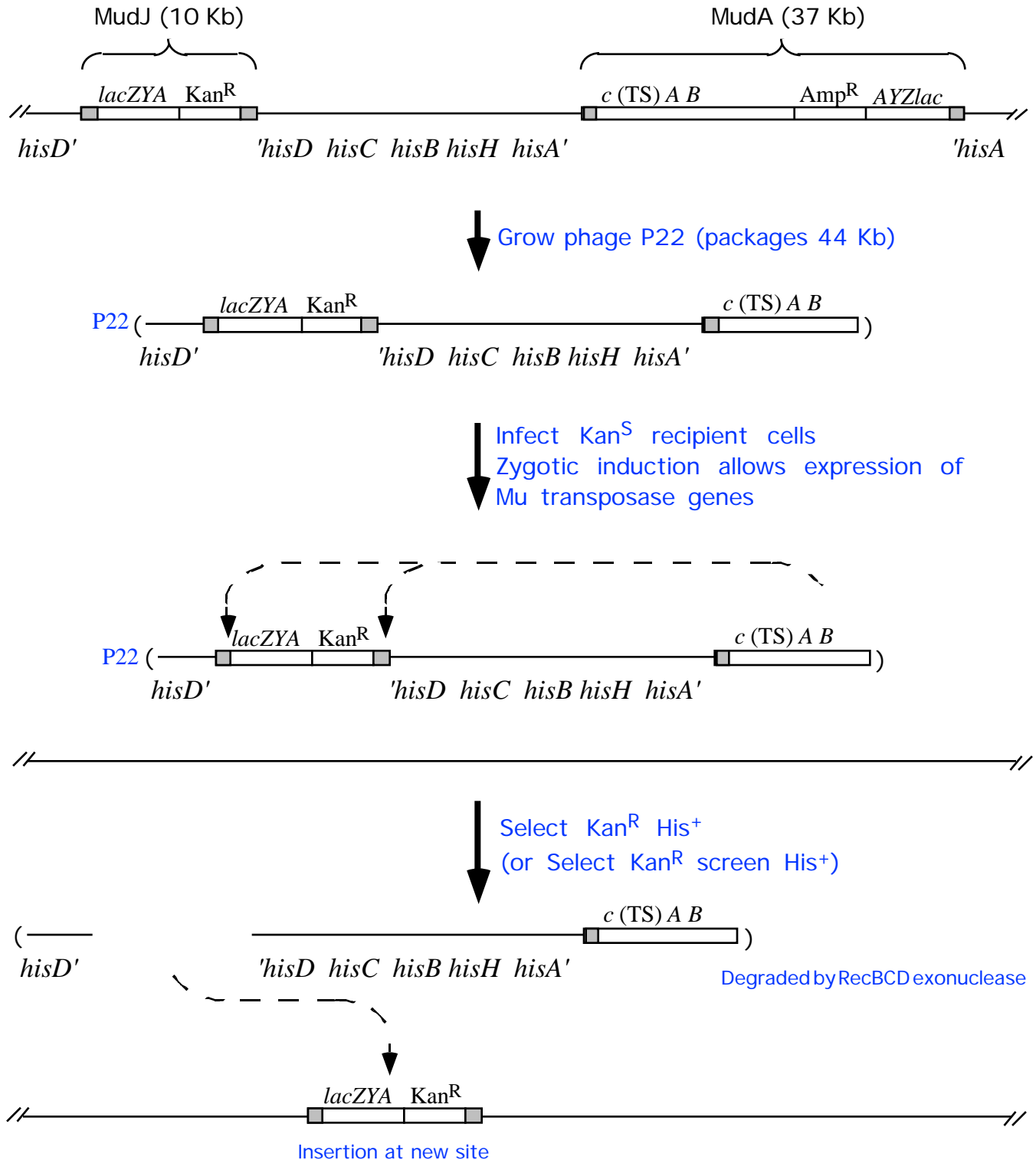
ANSWER: To determine if SefA is required for virulence, you could bring in a plasmid that complements the *sefA* gene. This would result in expression of SefA but none of the downstream genes, hence you would not be able to determine if both *sefA* and the other downstream gene products are required for virulence. Therefore, a better experiment would be to isolate a nonpolar, null mutation in the *sefA* gene – for example, an in frame deletion mutation that removes most of the *sefA* gene would be ideal because no SefA would be produced but all of the downstream gene products would be expressed.

- [10] 5. A phage P22 lysate was grown on the following *Salmonella* strain. Mutations that disrupt the *his* operon are histidine auxotrophs. MudA encodes functional transposase.



- a. Draw a diagram showing how this P22 lysate can be used to isolate a random collection of MudJ insertions in a *Salmonella* recipient. Indicate any selection or screen used and briefly explain the rationale for each step.

ANSWER:



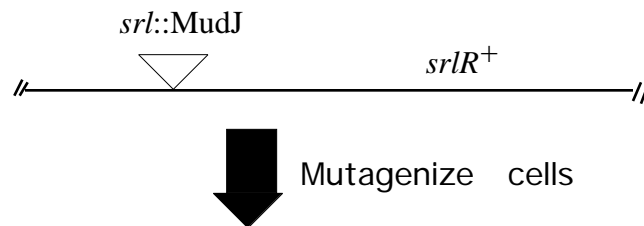
- b. How could you quickly test to determine that the MudJ had transposed to many different sites in the recipients? Indicate any selection or screen used and briefly explain your rationale.  
 ANSWER: A simple way to quickly test for insertions in many different sites would be to replicate plate the *Kan<sup>R</sup>* colonies and screen for auxotrophic mutations (growth on rich medium but not minimal glucose medium). Any auxotrophic mutations could be screened

using crossed pool plates to confirm that they resulted in different auxotrophic requirements. It is also possible to test for mutations that affect different carbon source utilization pathways, but this would require many different types of media to do the initial screen. Note that insertions in different genes will result in different levels of *lacZ* expression, but you can get different levels of *lacZ* expression by insertions within a single gene (for example, insertion at a single site in a gene may result in *lacZ* expression if inserted in the correct orientation and no *lacZ* expression if inserted in the opposite orientation).

[10] 6. The *srl* operon is required for *Salmonella* to grow on sorbitol as a sole carbon source. In the absence of sorbitol a *srl::MudJ(lac, Kan<sup>R</sup>)* operon fusion expresses very low levels of  $\beta$ -galactosidase. However, when sorbitol is added, the *srl::MudJ(lac, Kan<sup>R</sup>)* operon fusion expresses high levels of  $\beta$ -galactosidase.

- a. How could you use this *srl::MudJ(lac, Kan<sup>R</sup>)* fusion to isolate mutations that affect the regulation of the *srl* operon?

ANSWER:



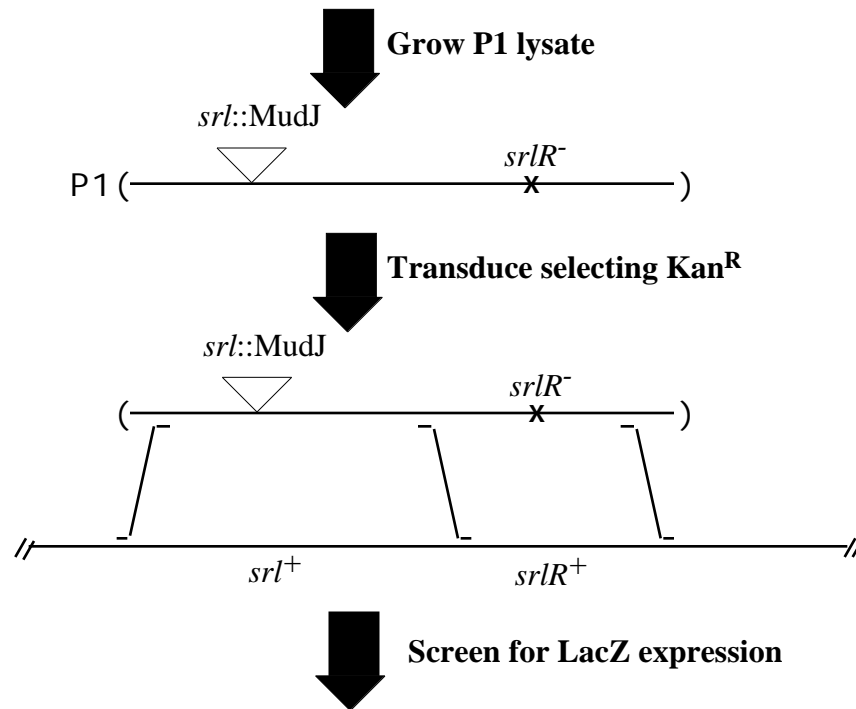
Screen for increased or decreased LacZ expression

- b. If you isolated a mutation in the *srl::MudJ(lac, Kan<sup>R</sup>)* strain that resulted in high constitutive expression of  $\beta$ -galactosidase, what could you conclude about the mechanism of regulation by the wild-type *srl* gene? Briefly explain your answer.

ANSWER: Because MudJ produces operon fusions, these results indicate that you have mutated a regulator that controls transcription of the *srl* operon. Furthermore, if the mutation is due to a null mutation, it suggests that you have inactivated a repressor.

- c. How could you determine if the regulatory mutation is within the *srl* operon or elsewhere on the chromosome? Briefly explain your answer.

ANSWER: Backcross the mutant with the wild-type strain as shown in the figure below.

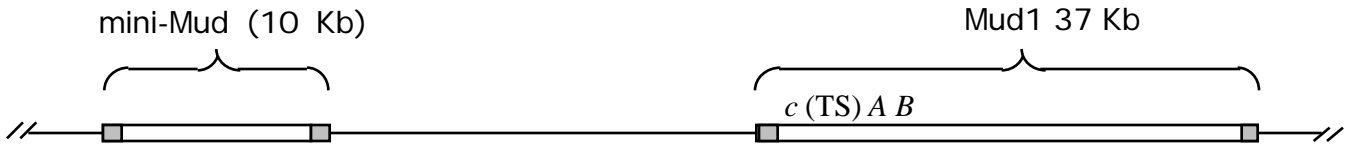


**If regulatory mutation is unlinked, all of the Kan<sup>R</sup> transductants should retain wild-type *srl::MudJ* phenotype. If regulatory mutation is linked then there should be two classes of Kan<sup>R</sup> transductants -- with the wild-type *srl::MudJ* phenotype and some with the mutant phenotype. If the mutation is within the *lacZ* gene then all of the Kan<sup>R</sup> transductants should have the mutant phenotype.**

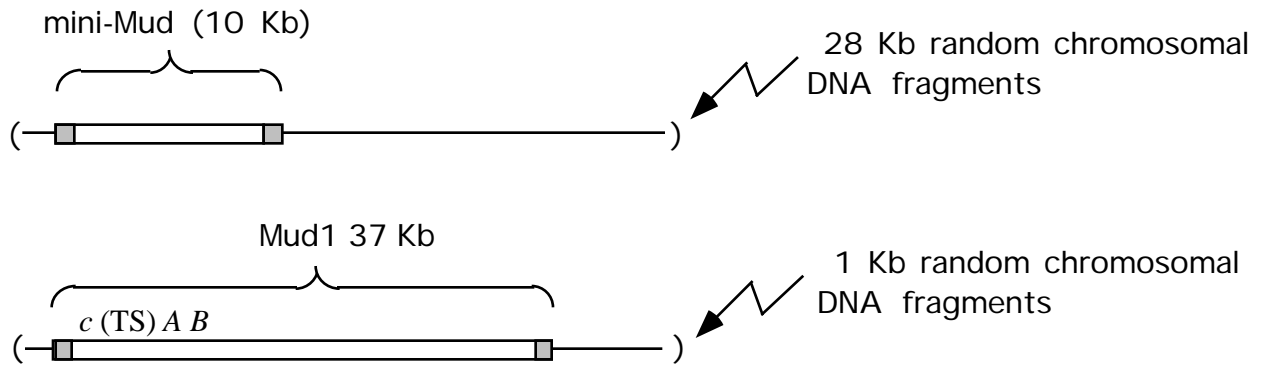
- [10] 7. Some deletion derivatives of Mu are about 10 Kb -- much smaller than the wild-type Mu which is 37 Kb. The “mini-Mu” derivatives can be used for generalized transduction. Given what you know about the organization of Mu and how it reproduces, draw a diagram showing how the mini-Mu phage produce generalized transducing particles.

**ANSWER:**

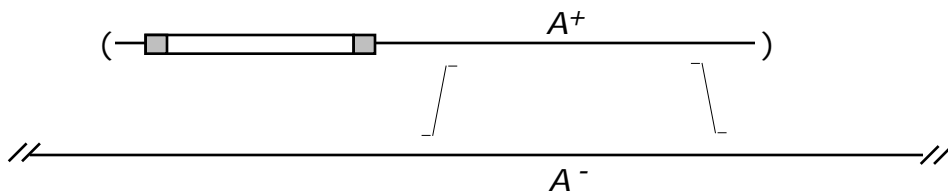
Dilysogen with mini-Mud and Mud1 helper phage  
(to provide essential transposition functions)



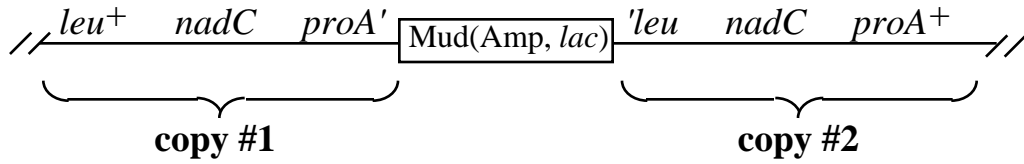
Shift culture to 42°C to induce Mu transposition and packaging into Mu phage particles.  
Both Mud phage will transpose to multiple, random sites in the host chromosome.  
Packaging occurs by a headful mechanism, beginning about 150 bp to the left of MuL and extending into chromosomal DNA sequences beyond MuR.  
Linear DNA is packaged into the phage head



Infect a mutant recipient and select for repair of chromosomal markers. The chromosomal DNA brought into the cell of the Mu phage can transduce markers from any region of the donor chromosome.



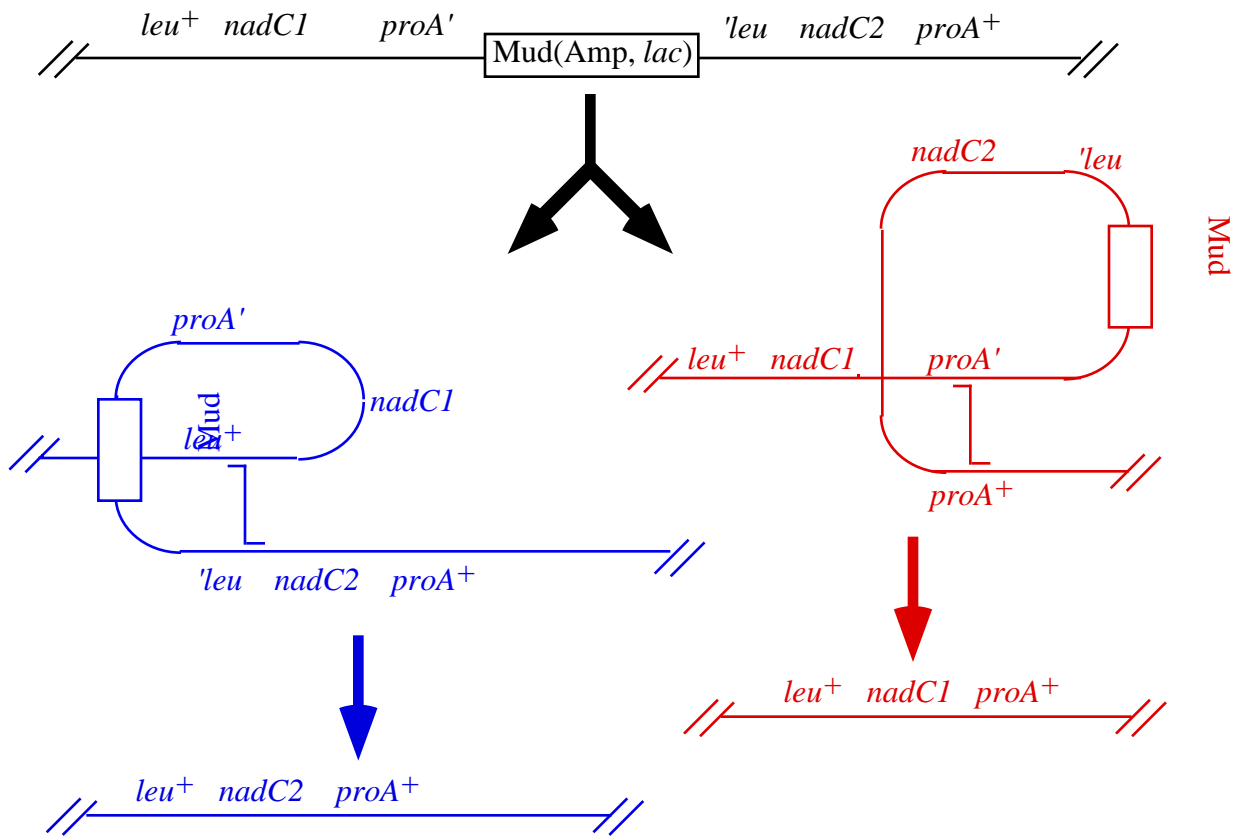
- [10] 8. Chromosomal duplications of the *nadC* gene were constructed in *S. typhimurium*. The resulting merodiploids have one mutant *nadC* allele in copy #1 and a different mutant *nadC* allele in copy #2 with a mini-Mud at the join point, as shown in the figure below.



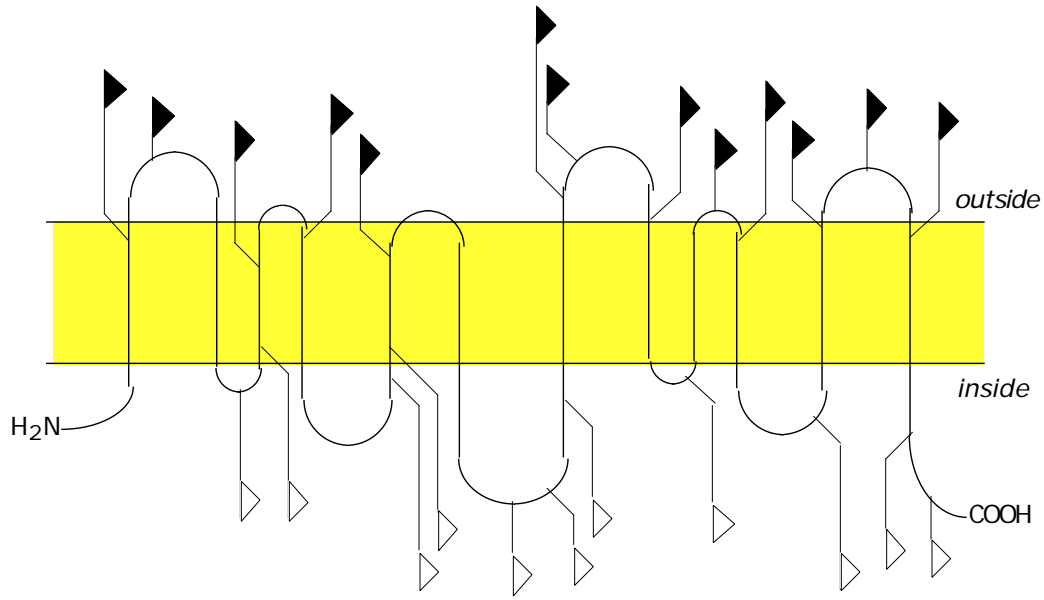
a. Describe two ways that you could prevent segregation of this chromosomal duplication?  
**ANSWER:** Selection for Amp<sup>R</sup> or Lac<sup>+</sup> would avoid loss of the Mud insertion at the join point of the duplication, preventing segregation. Note that selection for Leu<sup>+</sup> and Pro<sup>+</sup> would not prevent segregation of the duplication (see the figure in part b below).

b. Draw a diagram of a recombination event leading to segregation of the chromosomal duplication. Clearly label the genes on the diagram and indicate the position of any cross-overs required.

**ANSWER:**

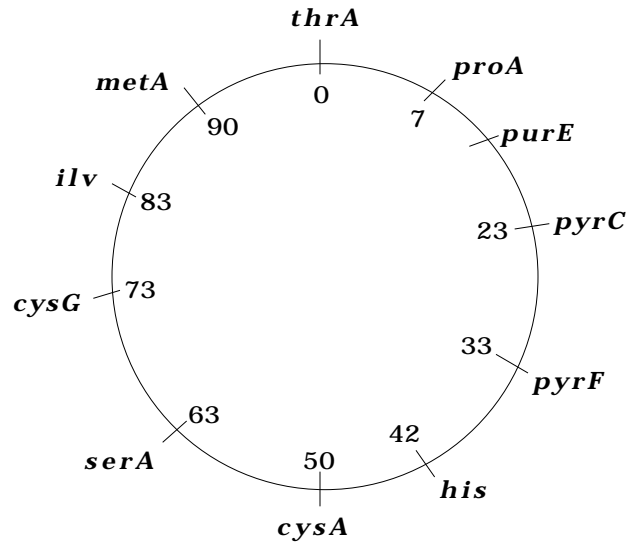


[10] 9. Lactose permease is an integral membrane protein encoded by the *lacY* gene. Computer predictions based upon the hydrophobicity of the amino acid sequence of lactose permease suggested that it spans the membrane 12 times. Tn*phoA* fusions were used to experimentally determine the membrane topology of lactose permease. The results are shown below. [The shaded area represents the membrane bilayer, the curved line that wraps back and forth across the membrane represents the predicted topology of lactose permease, the lines with filled in triangles represent the position of PhoA<sup>+</sup> insertions, and the lines with open triangles represent the position of PhoA<sup>-</sup> insertions.]



- Do these results support the conclusion that there are 12 membrane spanning domains? [Briefly explain your answer.]  
ANSWER: No. Based upon the evidence shown here, it is possible that the entire protein is secreted outside the cell. It is possible to show that the Lac<sup>-</sup> fusions on the inside (as shown in the figure) yield hybrid protein using immunological methods, which would support the model.
- Would you expect to find any PhoA<sup>+</sup> fusions in internal domains of LacY? [Briefly explain your answer.]  
ANSWER: No. Alkaline phosphatase must be outside the cell to allow proper formation of S-S bonds required for the enzymatic activity.
- Would you expect to find any PhoA<sup>-</sup> fusions in external domains of LacY? [Briefly explain your answer.]  
ANSWER: Yes. This is a gene fusion so 2/3 of the insertions would be expected to be in the wrong reading frame.
- What is the advantage of using a transposon vector to construct the *phoA* fusions instead of simply cloning the *phoA* gene into restriction sites within the *lacY* gene?  
ANSWER: The goal is to isolate random fusions at many positions in the gene, so it is easier to construct the fusions via transposition instead of constructing multiple clones (particularly because useful restriction sites will not be present at many positions where you would like the fusion join points).

[10] 10.A map of the *S. typhimurium* chromosome is shown below. It is possible to construct an Hfr at any desired Tn10 insertion in *S. typhimurium* using a F'(Ts) *lac*<sup>+</sup> Tn10.



Using a F'(Ts) *lac*<sup>+</sup> Tn10 donor and any mutant recipient you choose, draw a diagram showing how you could select for an Hfr located at 23 min on the *S. typhimurium* chromosome? [Recall that the *lac* genes are absent from *S. typhimurium* i.]

ANSWER:

To construct the strain carrying the F', mate with F<sup>-</sup> *pyrC*::Tn10 Rif<sup>R</sup> recipient, selecting for Lac<sup>+</sup> Rif<sup>R</sup> at 30°C. (Lac<sup>+</sup> selects for transfer of the F' and Rif<sup>R</sup> counterselects against the donors.) Then select for the Hfr as shown below.

