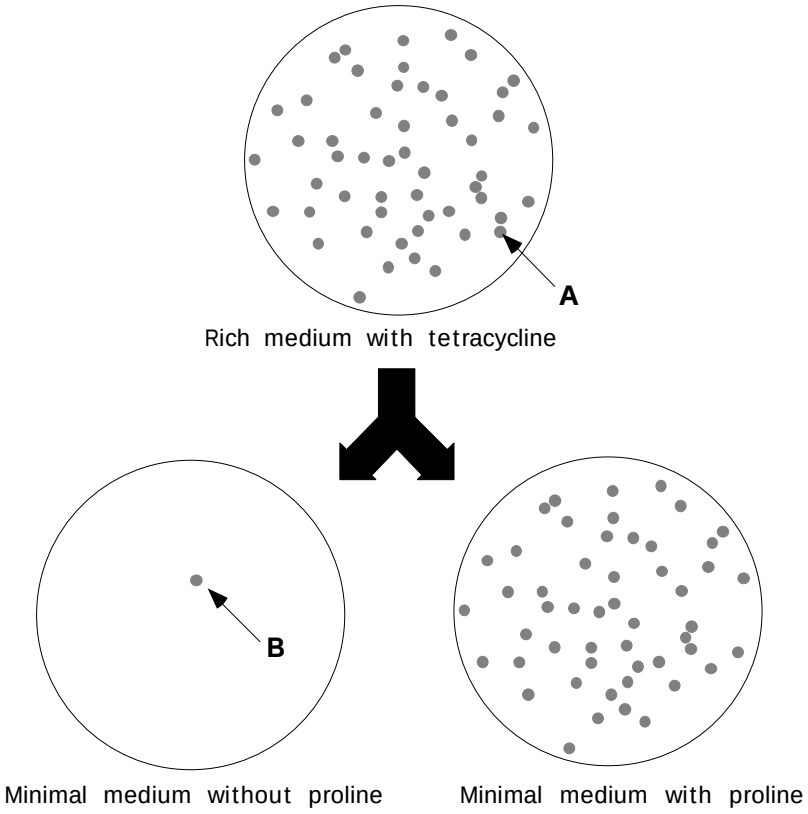
The *proBA* genes are required for biosynthesis of proline. To isolate a Tn*10* insertion near the *proBA* genes, a strain with a nonsense mutation in the *proB* gene was transduced to Tet^R with a P22 lysate grown on a random pool of Tn*10* insertions in the *Salmonella* chromosome. The Tet^R colonies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



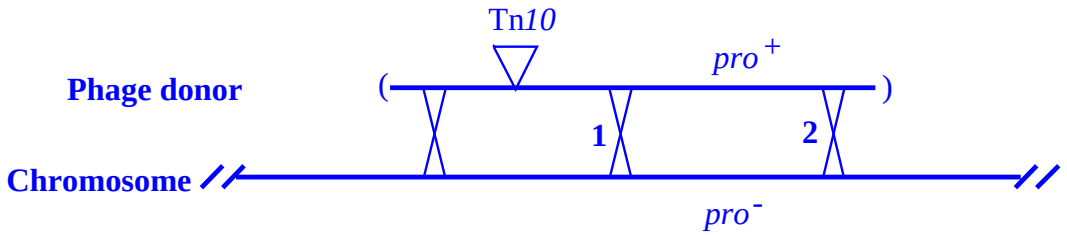
a. What is the most likely explanation for the phenotype of the colony indicated by the arrow marked A?
ANSWER: This colony fails to grow on minimal medium with proline so it is probably due to a Tn10 insertion in some other auxotrophic marker (i.e. a gene required for the biosynthesis of some essential metabolite).

b. What are <u>two</u> potential explanations for the phenotype of the colony indicated by the arrow marked B?
ANSWER: This colony could be either due to (i) transduction of *proB*$^+$ with a linked Tn10 insertion, or (ii) the simultaneous acquisition of a random Tn10 insertion and an independent *proB*$^+$ reversion.

c. 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 Tn*10* is linked to the *proB* gene then a single transducing particle can carry both the Tn*10* and the *proB*$^+$ gene, so some of the Tet^R transductants will become Pro$^+$ (crossover #2) and some will remain Pro$^-$ (crossover #1).



If the Tn*10* is NOT linked to the *proB* gene then all of the Tet^R transductants will remain Pro$^-$ because a transducing particle cannot carry both regions of the chromosome.