7.03 Problem Set 3

Due before 5 PM on Wednesday, October 18

Hand in answers in recitation section or in the box outside of 68-120

1. The following DNA sequence fragment comes from the middle of a bacterial gene. To start the analysis of this coding sequence you will first need to find the open reading frame (note that you do not know the orientation of the gene).

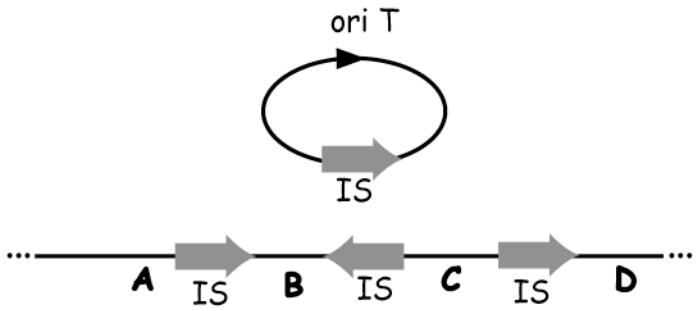
5' CTCGGCTAATATCGATCGCTAGTGTCATAGCTCTCGGGTAATGACGATCACGA 3'

a) Within this segment of DNA note all of the possible nonsense mutations that can be produced by a single base change by a mutagen that causes only transition mutations (G•C to A•T *or* A•T to G•C).

b) Within this segment of DNA note all of the possible single-base change nonsense mutations that can be produced by a single-base transversion mutation.

c) Consider the gene for $tRNA^{trp}$. Write out the double-stranded DNA segment of this gene that codes for the anticodon of $tRNA^{trp}$ (be sure to label 5' and 3' ends). Write out all of the possible mutations that can convert $tRNA^{trp}$ to a nonsense suppressing tRNA. For each mutation indicate whether it is a transition or transversion and which kind of nonsense mutation will be suppressed.

2. The diagram below shows the F factor and a portion of the *E. coli* chromosome that has three different insertion sequences (IS) of the same type as is carried on F. Assume that you have available a variety of strains with mutations in the genetic markers A, B, C, D.



a) Describe with as much detail as you can how you would use this F^+ strain to isolate an F' factor that carries the B marker. For your answer diagram any relevant intermediate strains as well as the final F' factor. For your answer please show all of the markers as well as the position and orientation of each IS sequence and the origin of transfer (ori T).

3. Wild type *E. coli* can utilize the sugar galactose and is therefore phenotypically Gal$^+$. You have isolated a mutant that you call *gal1*$^-$, which cannot grow on galactose (Gal$^-$).

a) You have a wild type (Gal$^+$) strain carrying a Tn5 insertion. You grow P1 phage on this strain and use the resulting phage lysate to infect the *gal1*$^-$ strain, selecting for kanamycin resistance (Kan^r). Among 100 Kan^r transductants, you find that 75 are Gal$^+$ and 25 are Gal$^-$. What does this result tell you about the relationship between the *gal1*$^-$ mutation and the Tn5 insertion?

b) You grew P1 phage on one of the Gal$^-$ Kan^r transductants isolated in part (**a**) and then used these phage to transduce a wild-type strain. What fraction of the Kan^r transductants would be Gal$^+$?

c) You isolate a second Gal$^-$ mutation, which you designate *gal2*$^-$. Using the same P1 lysate as in part (**a**) you infect the *gal2*$^-$ strain, selecting for Kan^r transductants. In this case, none of the 100 Kan^r transductants are Gal$^+$. What does this result tell you about the relationship between the *gal1*$^-$ and *gal2*$^-$ mutations?

c) Next, you isolate a third Gal$^-$ strain, called *gal3*$^-$. Preliminary P1 transduction experiments indicate that *gal3*$^-$ is linked to the Tn5 insertion described in part (**a**). To map *gal3*$^-$ relative to *gal1*$^-$ you set up two reciprocal crosses. In the first cross you grow P1 on a strain that carries the Tn5 insertion and the *gal1*$^-$ mutation. You then use this lysate to infect a *gal3*$^-$ mutant and select for Kan^r. From 100 Kan^r transductants examined, 85 are Gal$^-$ and 15 are Gal$^+$. In the second cross you grow P1 on a strain that carries the Tn5 insertion and the *gal3*$^-$ mutation. You then use this lysate to infect a *gal1*$^-$ mutant, and select for Kan^r. From 100 Kan^r transductants examined, 98 are Gal$^-$and 2 are Gal$^+$. Draw a genetic map showing the relative positions of the Tn5 insertion and the *gal1*$^-$ and *gal3*$^-$ mutations. Express any measured distances as co-transduction frequencies.

d) Explain why it is necessary to carry out two reciprocal three-factor crosses in part (**c**) in order to determine the relative positions of the *gal1*$^-$ and *gal3*$^-$ mutations.

4. An F^- $HisA^-$ *E. coli* strain can be converted to His^+ by a variety of different genetic manipulations including: transduction with a P1 phage lysate grown on a $HisA^+$ strain, mating to an Hfr strain that carries $HisA^+$ on the chromosome, or mating to a strain with $HisA^+$ on an F' factor. You are given a variety of $HisA^-$ strains with unknown genetic properties. You subject each strain to a variety of genetic tests to diagnose how it may have been altered. Based on the outcome of these tests, deduce which genetic capabilities have been altered then propose a specific type of mutation or genetic alteration that might give rise to these properties. (This question is intended to stretch your thinking about bacterial genetics somewhat beyond what has been explicitly covered in lecture. Possibilities you should consider include acquisition of various kinds of mutations or extra chromosomal elements. For some strains more than one mechanism is possible.)

a) Strain 1 can be converted to His^+ by conjugation with either a $HisA^+$ Hfr or an F' $HisA^+$ strain, but cannot be converted to His^+ by P1 transduction.

b) Strain 2 can be converted to His^+ by conjugation with an F' $HisA^+$ strain, but cannot be converted to His^+ by P1 transduction or by conjugation with a $HisA^+$ Hfr.

c) Strain 3 can be converted to His^+ by P1 transduction, but cannot be converted to His^+ by conjugation with either a $HisA^+$ Hfr or an F' $HisA^+$ strain.

d) Strain 4 can be converted to His^+ by P1 transduction or by conjugation with a $HisA^+$ Hfr, but cannot be converted to His^+ by congugation with an F' $HisA^+$ strain.

e) Strain 5 cannot be converted to His^+ by P1 transduction or by conjugation with either a $HisA^+$ Hfr or an F' $HisA^+$ strain.