

7.03 Problem Set 3

Due before 5 PM on Thursday, October 16

Hand in answers in recitation section or in the box outside the class

1. (a) The three stop codons are 5'UAG3', 5'UAA3', and 5'UGA3'. Explain why mutagens that specifically induce G•C to A•T mutations will generally produce TAG (amber) and TGA nonsense mutations more frequently than TAA (ochre) mutations.

(b) You are trying to isolate tRNA mutations that will suppress an amber (TAG) mutation. To increase the frequency of such mutations you use a mutagen that produces transition mutations (i.e. C•G to T•A and T•A to C•G base changes). Which tRNA genes could in principle be altered by the mutagen to give the desired suppressor mutation? For your answer give the sequence of the codon normally recognized by the tRNA. In addition, write out the DNA base sequence of the segment of the tRNA gene that codes for the anticodon sequence for both the wild-type and suppressor alleles of the gene. Please show both strands of the DNA and indicate the 5' and 3' ends of each strand indicating which strand is used as the template for transcription of the tRNA molecule.

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.

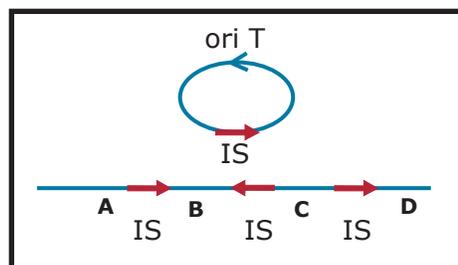


Figure by MIT OCW.

(a) Draw a diagram of the three different Hfrs that can be formed by recombination between the IS on F and each of the IS sequences on the chromosome. For your answer, include the positions of each of the markers (**A**, **B**, **C**, and **D**) and state which of these markers would be transferred early. (You will need to take into account the orientation of the IS and origin of transfer (ori T) on F as well as the orientations of the different IS sequences in the chromosome.)

(b) For each of the three Hfrs in part (a) consider the outcome of further crossovers between IS sequences flanking the integrated F factor (there should be five different relevant IS by IS recombinations for each of the three Hfrs). For each possible recombination event state whether the product is F, an F' or an Hfr. Also provide information about which markers will be transferred on the F's and which markers will be transferred early by the Hfrs.

3. Wild type *E. coli* metabolizes the sugar lactose by expressing the enzyme β -galactosidase. You have isolated a mutant that you call $lac1^-$, which cannot synthesize β -galactosidase and cannot grow on lactose (Lac^-).

(a) You have a wild type (Lac^+) strain carrying a Tn5 insertion known to be near several Lac genes on the *E. coli* chromosome. You grow P1 phage on this strain and use the resulting phage lysate to infect the $lac1^-$ strain, selecting for kanamycin resistance (Kan^r). Among 100 Kan^r transductants, you find that 82 are Lac^- and 18 are Lac^+ . Express the distance between Tn5 and the $lac1^-$ mutation as a cotransduction frequency.

(b) You isolate a second Lac^- mutation, which you designate $lac2^-$. Using the same P1 lysate as in part (a) you infect the $lac2^-$ strain, selecting for Kan^r transductants. In this case, all 100 Kan^r transductants that are examined are Lac^- . What does this result tell you about the relationship between the $lac1^-$ and $lac2^-$ mutations?

(c) Next, you isolate a mutation that constitutively expresses abnormally high levels of β -galactosidase, which you designate lac^C . Preliminary P1 transduction experiments indicate that lac^C is linked to the Tn5 insertion described in part (a). To map lac^C relative to $lac1^-$ you set up two reciprocal crosses. In the first cross you grow P1 on a strain that carries the Tn5 insertion and the $lac1^-$ mutation. You then use this lysate to infect a lac^C mutant and select for Kan^r . From 100 Kan^r transductants examined, 20 are Lac^- , 76 express β -galactosidase constitutively and 4 show normal β -galactosidase expression. In the second cross you grow P1 on a strain that carries the Tn5 insertion and the lac^C mutation. You then use this lysate to infect a $lac1^-$ mutant, and select for Kan^r . From 100 Kan^r transductants examined, 81 are Lac^- and 19 express β -galactosidase constitutively. Draw a genetic map showing the relative positions of the Tn5 insertion and the $lac1^-$ and lac^C mutations. Express any measured distances as cotransduction frequencies.

(d) A third Lac^- strain, called $lac3^-$, is linked to the Tn5 insertion. From a strain carrying the Tn5 insertion and $lac3^-$ mutation you isolate an F' that carries a region of the chromosome that includes both Tn5 and the linked Lac region (You know from mapping experiments that this region includes the sites of both the $lac1^-$ and $lac3^-$ mutations). You introduce this F' into an F^- strain carrying $lac1^-$ by selecting for Kan^r . These merodiploids express β -galactosidase normally. What does this result tell you about the relationship between the $lac3^-$ and $lac1^-$ mutations? If the merodiploid were Lac^- , what could you conclude about the relationship between the $lac3^-$ and $lac1^-$ mutations? (Be sure to consider all the possibilities.)

4. In your study of a new bacterial species you have identified a transducing phage that you call Px. In addition you have worked out methods to make random transposon insertions into the bacterial genome. You have generated two different transposon insertion collections one with 10^5 random Tn5 (Kan^r) insertions and the other with 10^5 random Tn10 (Tet^r) insertions. You grow Px phage on the mixed collection of Tn5 insertions and use the resulting phage lysate to infect the mixed collection of Tn10 insertions. You select 10,000 Kan^r transductants and find that 80 of them are Tet^S. Use this information to estimate the total size of the bacterial genome assuming that both Tn5 and Tn10 insert randomly and that the average size of a fragment recombined into the recipient genome during Px transduction is 55 kbp. (Tn5 is about 5 kbp and Tn10 is 10 kbp.)