

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 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).

The first step is to determine the open reading frame (ORF). There are 6 possibilities: 2 orientations (left to right or right to left) and 3 reading frames (start from the first, second, or third base pair). The correct ORF is the one without any stop codons, since this DNA is in the middle of a bacterial gene. There is only one ORF that fits the requirement, and it reads from right to left, starting from the second nucleotide.

So the coding DNA (that will end up resembling the mRNA) strand reads:

5' T CGT GAT CGT CAT TAC CCG AGA GCT ATG ACA CTA GCG ATC GAT ATT AGC
CGA G 3'

Transition mutations are mutations that change one purine-pyrimidine pair to the other purine-pyrimidine pair. There is only one single base transition nonsense mutation and it is highlighted by **(C)**.

5' T CGT GAT CGT CAT TAC CCG AGA GCT ATG ACA CTA GCG ATC GAT ATT AGC
(C)GA G 3'

You can find the potential nonsense mutations by mutating each stop codon's base pair, as follows. Then search for the mutated stop codons in the sequence (Highlighted in yellow)

Stop Codon	Mutate 1 st nt	Mutate 2 nd nt	Mutate 3 rd nt
TAA	TGA	TAG	CAA
TAG	CAG	TGG	TAA
TGA	CGA	TAA	TGG

1b) 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.

Transversion mutations change a purine to pyrimidine and vice versa. There are two nonsense transversion mutations, and they are highlighted by [].

The first mutation can change from a C → A or G. The second mutation is a mutation of A → T.

5' TCGT GAT CGT CAT TA[C] CCG [A]GA GCT ATG ACA CTA GCG ATC GAT ATT AGC CGA G 3'

You can also find these potential nonsense mutations by mutating each stop codon's base pair. (The ones crossed out are degenerative of a previous mutation.)

Stop Codon	Mutate 1 st nt	Mutate 2 nd nt	Mutate 3 rd nt
TAA	AAA	TTA	TAT
	GAA	TCA	TAC
TAG	AAG	TTG	TAT
	GAG	TCG	TAG
TGA	AGA	TTA	TGT
	GGA	TCA	TGC

1c) 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.

Trp codon: 5' UGG 3'

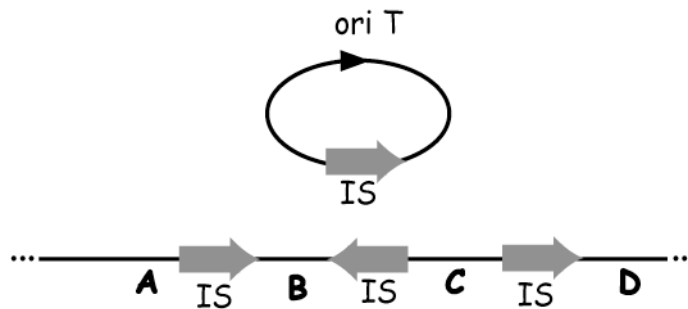
Trp anticodon: 3' ACC 5'

Gene for the anticodon of tRNA^{trp} : 5' TGG 3' → template strand
3' ACC 5' → coding strand

Possible mutations:

1. Mutate first G·C → A·T (transition) get a UAG (amber) nonsense mutation suppressed
2. Mutate second G·C → A·T (transition) get a UGA (opal) nonsense mutation suppressed
3. Mutate both G·C → A·T (two transitions, double mutations rare) get a UAA (ochre) nonsense mutation 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).

(The numbers refer to the diagram on the next page)

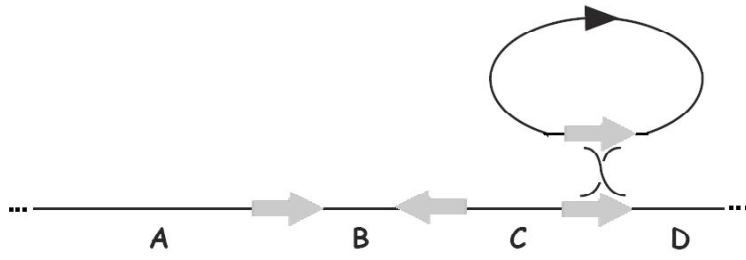
First, a crossover must happen between the IS on the F factor and the IS between markers C and D on the *E. coli* chromosome (1.)

This results in an Hfr strain that can transfer marker D early and efficiently, but transfers marker B very late (2.). Note that this is the only crossover event that results in an Hfr strain with these properties. These properties can be screened for by conjugating the Hfr strain to D^- and B^- strains, respectively.

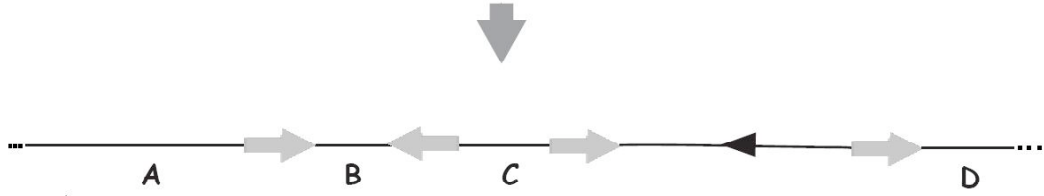
Within the identified Hfr strain, the *E. coli* chromosome can "loop around" such that a crossover event occurs between the "first" and "last" IS sequences of interest (3.).

If this event occurs, the resulting strain will contain an F' factor carrying the B marker (as well as the C marker) (4.). Note that this F' factor can now transfer B and C early and efficiently. Thus, identification of a strain from a population of the Hfr strain in (2.) that can now transfer B efficiently will allow us to isolate the desired F' factor.

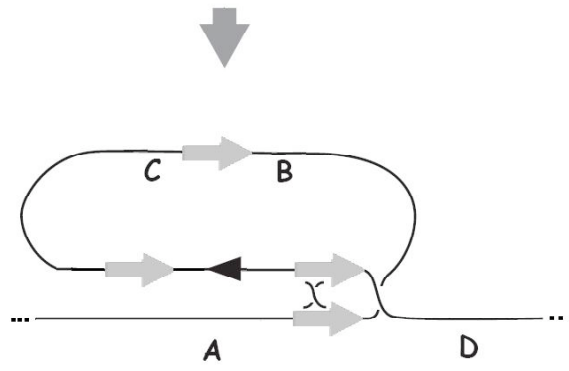
1.)



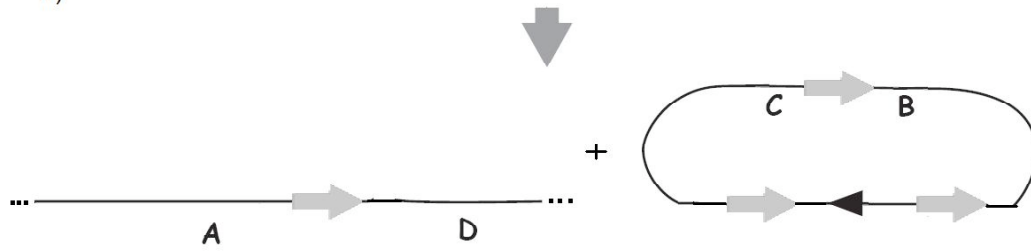
2.)



3.)



4.)



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?

The *gal1*⁻ mutation and the Tn5 insertion can be considered linked due to the 75% co-transduction frequency.

The donor strain is Gal⁺ (with Tn5 insertion) and the recipient strain is Gal⁻. Therefore, in the Gal⁺ Kan^r transductants, *gal1*⁺ was co-transduced with Tn5. This indicates that *gal1* and Tn5 are within 10⁵ base pairs apart and are linked. The distance between Tn5 and the *gal1*⁻ mutation is:

$$(75/100) * 100\% = 75\%.$$

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⁺?

0.25 - The donor strain is Gal⁻ (with Tn5 insertion) and the recipient strain is Gal⁺ (WT). Thus, Gal⁺ Kan^r transductants result when Tn5 and *gal1*⁻ are not co-transduced. Since the probability of co-transduction of *gal1*⁻ and Tn5 is 0.75 (from part a), then:

$$\begin{aligned} P(\textit{gal1}^- \text{ and Tn5 not co-transduced}) &= 1 - P(\textit{gal1}^- \text{ and Tn5 co-transduced}) \\ &= 1 - 0.75 \\ &= 0.25 \end{aligned}$$

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?

gal1⁻ and *gal2*⁻ are unlinked and in different genes (0% co-transduction frequency, more than 10⁵ bp apart)

The donor strain is Gal⁺ and the recipient strain is Gal⁻. Since we do not see any Kan^r Gal⁺ transductants, we can conclude that Tn5 and *gal2* were never co-transduced. This indicates that the distance between *gal2*⁻ and Tn5 is at least one phage head apart (10⁵ bp). We also know from part (a) that Tn5 and *gal1* are linked because they have a co-transduction frequency of 75%. Thus, we can conclude that *gal1* and *gal2* are greater than 10⁵ bp from each other, unlinked, and in two different genes.

With regard to the relative order, if we knew that Tn5 was between *gal1* and *gal2* we could conclusively state that *gal1* and *gal2* are also greater than one phage head apart. However, since Tn5 and *gal1* are 75% co-transduced (meaning closely linked), we can probably still conclude that *gal1* and *gal2* are greater than one phage head apart.

d) 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.

Order: Tn5----*gal3*----*gal1*. The only distance determinable is Tn5 to *gal1*, which is 75% (from part a). The best way to solve this type of problem is to draw out the two crosses. We will consider only two possible orders, instead of three, because the order where Tn5 is in the middle is impractical for three-factor co-transduction experiments.

There are two reciprocal crosses and two possible orders, thus there are four diagrams to draw: (1+ and 1- represent WT *gal1*+ and mutant *gal1*-; 3+ and 3- represent WT *gal3*+ and mutant *gal3*-)

	Order #1 (Tn5, <i>gal1</i> , <i>gal3</i>)	Order #2 (Tn5, <i>gal3</i> , <i>gal1</i>)
Cross #1 (Tn5, 1- infect 3-) 15 Gal+	<pre> -----Tn5-----1-----3+----- X X X X -----1+-----3----- </pre>	<pre> -----Tn5-----3+-----1----- X X -----3-----1+----- </pre>
Cross #2 (Tn5, 3- infect 1-) 2 Gal+	<pre> -----Tn5-----1+-----3----- X X -----1-----3+----- </pre>	<pre> -----Tn5-----3-----1+----- X X X X -----3+-----1----- </pre>

The data given allow us to determine which of the two possible orders is correct. As in any three-factor cross, we determine the order by looking for the rarest class. In this case, the rarest class is Gal+, and the genotype is Tn5, 1+, 3+.

From these data, we see that cross #1 produced 15 Gal+, while cross #2 produced only 2 Gal+. This is the key observation that allows us to determine order.

Looking back at the diagram, each X represents a crossover that is needed to make a Tn5, 1+ 3+ genotype.

If we assume that order #1 is correct, then we would get more Gal+ transductants in cross #2 than cross #1 because only two crossovers are required as opposed to four. If we assume that order #2 is correct, then we would get more Gal+ transductants from cross #1. The data show that cross #1 produces more Gal+ transductants, thus order #2 is correct:

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-----Tn5-----gal3-----gal1-----
|-----75%-----|
                    
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The distance between Tn5 and *gal3* cannot be determined. Though we see that we get 15 Gal⁺ from a Tn5 and *gal3*⁺ co-transduction, the 15 does not represent all possible Tn5 and *gal3* co-transductions. The 15 only represents a crossing over somewhere to the left of Tn5 and the other crossing over event happening between *gal3* and *gal1* to generate the Gal⁺ phenotype. The co-transduction frequency of Tn5 and *gal3* must represent the crossing over events that happen to the left of Tn5 and anywhere to the right of *gal3*.

The distance between *gal3* and *gal1* also is not determinable, even if we could solve the distance between Tn5 and *gal3*. This is because we selected for kanamycin resistant transductants, therefore all data are relative to the Tn5 marker. Co-transduction frequencies are not additive.

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

We need both crosses to determine which cross is less frequent, since both a double crossover and a quadruple crossover can result in the same Gal⁺ phenotype.

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.)

4a) 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.

A large deletion mutation may not be complemented by the 100 kb DNA fragment carried by the phage

The mutations could be in genes that are greater than 100kb apart.

The mutant *E. coli* strain could be resistant to phage P1.

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.

This may result from a large deletion mutation, so there is no recombination due to the absence of homologous DNA in the mutant chromosome.

This may result from a mutation in the cell's homologous recombination system, e.g. in gene *RecA*.

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.

This may result from mutations that disrupt histidine biosynthesis and render the mutant *E. coli* strain conjugation⁻ (i.e. unable to recognize the mating pilus).

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 conjugation with an F' $HisA^+$ strain.

This may result from a mutation that:

Results in a dominant *his*⁻ allele (intragenic)

Cannot re-circularize or stably maintain the F plasmid, rapidly degrades the F plasmid, or covalently modifies the plasmid such that gene expression is prevented.

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

This may result from a mutation resulting in a constitutive trans-acting (extragenic) repressor (or super-repressor) of histidine biosynthesis.