Name: KEY
Recitation Section:

7.03 Problem Set 3
Due Friday, March 20, 2015 by 3 PM

Remember to show your work for all questions.

1. You are investigating the genetics of a newly discovered strain of *E. coli*. In order to do this, you infect the bacteria with λ Pam, int⁻ :: Tn5 phage to create knockout mutants. You grow the infected bacteria on kanamycin-containing media in order to select for the bacteria in which transposition occurred. Out of 10000 colonies that grow, you notice that 4 grow in the shape of a star.

a) You know that the genome size of this strain of *E. coli* is 5 Mb. Approximately what size is the gene causing the star-shaped colony phenotype? (Assume that a single gene is the cause, and that a transposon insertion anywhere in the gene would impede its function.)

We assume that the transposons inserted randomly into the genome, so the gene would be 4/10000 = 0.04% of the genome, and therefore ~2000 bp long.

[1 point]

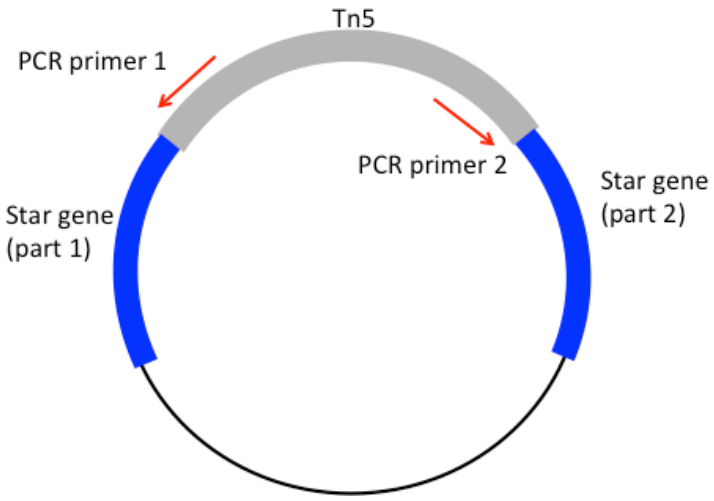
You want to sequence the gene, but first you need to amplify it. In order to do this, you choose one of the star-shaped colonies to sequence the gene from. You plan to use restriction enzymes to cut its DNA and then circularize the DNA fragments and perform PCR.

b) You want the entire gene to be present on the fragment of DNA that contains your transposon in order to sequence it. How many base pairs long does the recognition sequence of the restriction enzyme you use need to be in order for this to be likely?

Since the gene is ~2000 bp, we want the restriction enzyme to cut less often than once every 2000 bp. The smallest recognition site that does this is 6 bp, since it will cut on average once every $4^6 = 4096$ bp.

[1 point]

c) Once you've cut the genomic DNA into fragments, you circularize the DNA fragments and perform PCR to amplify your gene of interest. Draw the circular DNA fragment containing your gene, labeling all genes and regions on the fragment. Also draw the locations of the PCR primers you would use for this amplification. (Be sure to indicate the direction of the PCR primers.)



[1.5 points: 0.5 for each of Tn5, Star gene, and PCR primers]

d) You sequence the PCR product of the gene. Now that you have the sequence of this gene in hand, you decide to investigate the other 3 colonies that showed the star-shaped phenotype. Using modern high-throughput methods, you obtain the RNA and protein content of each of 3 colonies. The first colony contains a truncated version of the protein that the gene codes for; the second colony contains mRNA for the gene, but no protein at all; and the third colony doesn't even contain any mRNA from the gene. For each of these colonies, where is the most likely location in the gene that the transposon was inserted into? Explain your reasoning.

Colony with truncated protein: insertion into the coding sequence, which causes a premature stop codon leading to truncated protein.

Colony with no protein: insertion into the Shine-Dalgarno sequence, which disrupts the sequence and prevents mRNA loading onto the ribosome.

Colony with no mRNA: insertion into the promoter region, which disrupts the ability of the gene to be transcribed.

[0.5 points for each]

2. To learn about genetic screens, you do a mutagenesis screen to create a collection of *E. coli* strains that contain a mutant LacZ gene. You choose to conduct your screen by adding treating your *E. coli* cells with 5-bromouracil, which is a derivative of uracil that can substitute for thymine in DNA. It is able to base pair with both adenine and guanine, leading to conversion of AT base pairs to GC and/or GC to AT during DNA replication.

A functional LacZ gene product will be able to hydrolyze the synthetic compound X-gal to produce a blue pigment yielding a blue colony. LacZ- colonies will remain white.

You screen 10^5 colonies and find 18 white colonies on X-gal plates and designate the mutants LacZ$^-$(A-R).

You decide to sequence the LacZ gene of each of your mutants and one of them, LacZ$^-$J, gives the resulting sequence (beginning at the start site):

5'ATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTAGCCCATTACG
GTCAATCCGCCGTTTGT….. 3'

Bases marked in red differ from the wild-type consensus sequence.

a) What type of mutation would you suspect is causing the LacZ gene malfunction? What is the result of this mutation on the LacZ protein?

   Amber mutation leading to an early stop yielding a truncated protein
   [1 point; ½ ea for early stop, shortened/nonfunctional product]

b) Describe a way to quickly test your hypothesis and interpret possible results.

   Clone LacZ$^-$ J into an *E. coli* strain carrying an amber suppressor mutation (Su+) and grow on plates containing X-gal. If it was due to an amber mutation, the amber suppressor background will lead to blue colonies. If not, the colonies will still be white.
   [1 point; ½ point for experiment, ½ point for interpretation]
   No credit given for techniques not covered in this class

c) You accidentally forget to put an X-gal plate of LacZ$^-$F colonies into the incubator. When you come back to lab after a few days off, you find that all of your LacZ$^-$F colonies have grown up and turned blue while sitting on your bench. What type of mutation does LacZ$^-$F likely carry?

   Temperature sensitive mutation
   [1 point]

You decide to do a suppressor screen with your LacZ$^-$F mutant. This time you use UV to mutagenize and find 9 suppressors, labeled 1-9.

d) How does UV exposure lead to mutagenesis?

Induces TT dimers that initiate an error-prone DNA damage response causing all types of changes.
[1 point; ½ point for causes damage to DNA, ½ point for error prone repair]

e) As a first step to characterize these suppressor strains, you decide to sequence the LacZ gene. What types of suppressors will this allow you to identify?

Back mutations and intragenic suppressors
[1 point; ½ point for each, -½ for also listing extragenic]

3. You are studying virulence factors in an *E. coli* strain. You are interested in genes that produce hemolysins, factors that promote lysis of red blood cells, which can be a cause of pathogenicity. Hemolytic activity can be detected by plating bacteria on blood agar, which contains mammalian red blood cells. Presence of hemolysins in a strain will cause a white clearing around the resulting colonies.

a) You have strain in which one of the 3 hemolysin genes *E. coli* carries, α-hemolysin, is mutated ($hlyA^-$) and cannot cause a white clearing (hly^- phenotype). You also have an Hly^+ strain that carries a Tn5 insertion site near HlyA. You grow P1 phage on this strain and then collect phage lysate that you use to infect the $hlyA^-$ strain. You grow the infected strain on media containing kanamycin to select for kanamycin resistant (Kan^R) tranductants. Out of 200 Kan^R transductants, you isolate 158 that are hly^- and 42 that are Hly^+. What is the distance between the Tn5 site and the hly^- mutation in terms of cotransduction frequency?

In the Hly^+ Kan^r transductants, Hly^+ was co-transduced with Tn5 so the distance between Tn5 and the $hlyA^-$ mutation is:

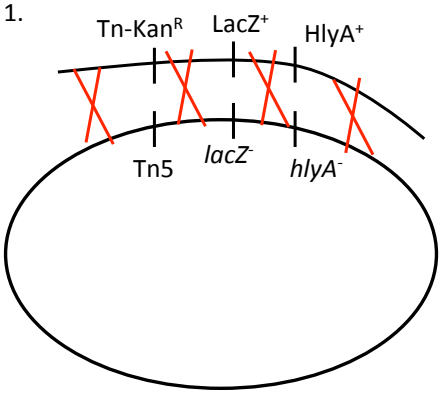
42/200 = 21%
[1 point]

The LacZ gene is also nearby and you wish to determine the order of the three loci (the Tn5 insertion site, HlyA, and LacZ). You repeat the above experiment and grow P1 phage on an $HlyA^+$ $LacZ^+$ but this time use your phage lysate to infect an $hlyA^-$ $lacZ^-$ strain and score for ability to grow on lactose (ability to hydrolyze X-gal to make blue colonies).

b) Consider 2 potential orders: #1: Tn5 – LacZ – HlyA
#2: Tn5 – HlyA – LacZ

(i) Draw the crossover events that will yield Kan^R bacteria that can hydrolyze red blood cells but not grow on lactose.

1.



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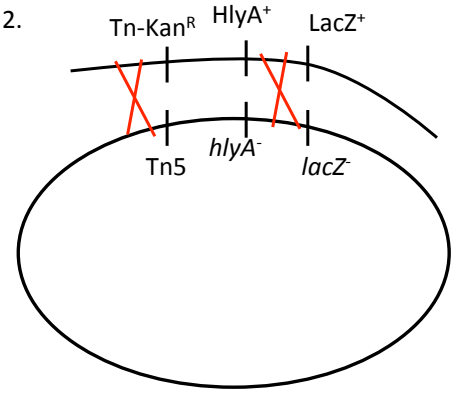
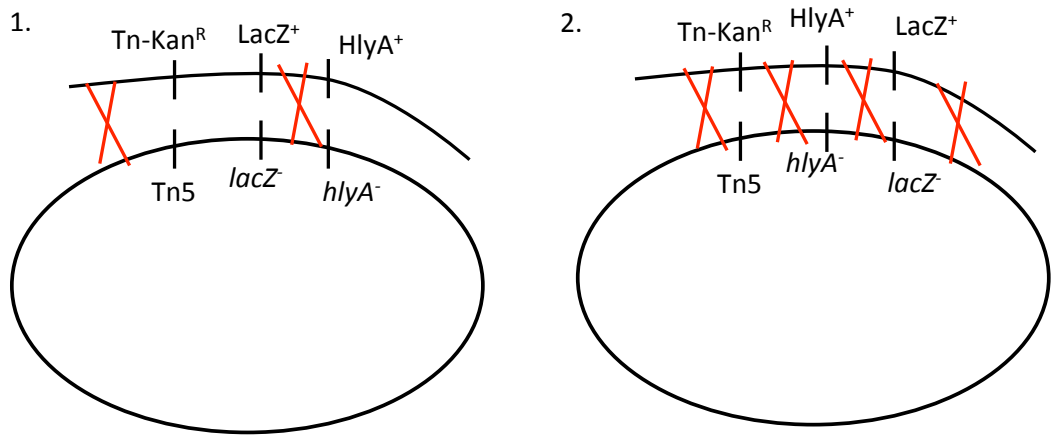


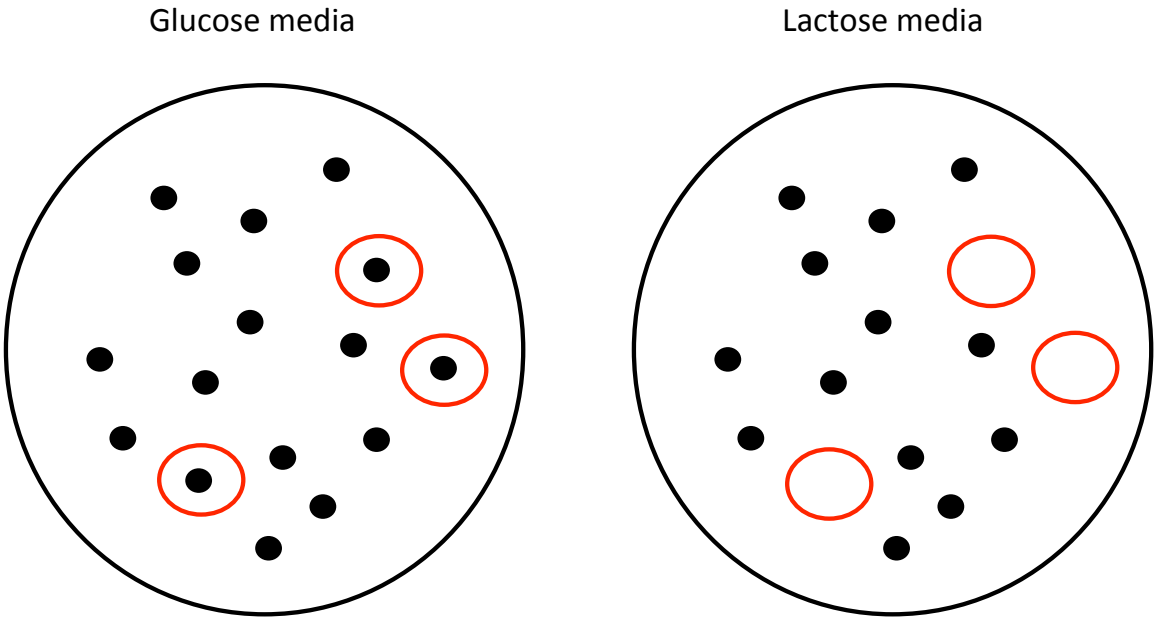
[1 point; ½ point for each correct drawing, no partial credit]

(ii) Draw the crossover events that will yield Kan^R bacteria that cannot hydrolyze red blood cells but can grow on lactose.



[1 point; ½ point for each correct drawing, no partial credit]

c) Your lab ran out of X-gal; how else could you score for the ability to grow on lactose? Draw out how you would identify cells that are lac$^-$.



Replica plate onto lactose media to identify colonies that don't grow on lactose (lac$^-$). Recover these cells from the corresponding colonies on a glucose plate.
[1 point; ½ point for replica plate, ½ point for interpretation]

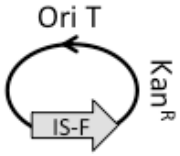
d) Among 1000 Kan^R tranductants scored, you find:

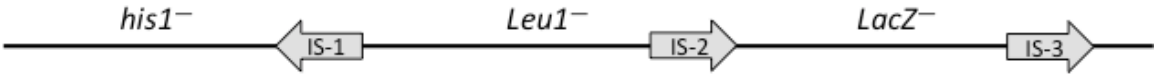
487 can hydrolyze red blood cells and grow on lactose
531 cannot hydrolyze red blood cells and cannot grow on lactose
41 can hydrolyze red blood cells and cannot grow on lactose
3 cannot hydrolyze red blood cells and can grow on lactose

Which of the two orders we've considered is more likely to be correct and why?

Gene order #2: $TnKan^R$ – HlyA – LacZ
The rarest case will be the one containing 4 crossover events
[1 point; ½ point for correct order, ½ point for explanation]

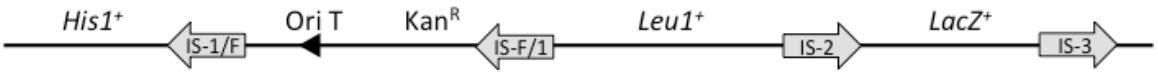
4. You have *E. coli* that contain an F plasmid with an insertion sequence, labeled IS-F in the diagram below. The F plasmid also contains a kanamycin resistance gene. On the *E. coli* chromosome are three copies of the insertion sequence, labeled IS-1, IS-2, and IS-3. In the region containing these three insertion sequences are three genes: *His1*, which is necessary for histidine synthesis; *Leu1*, which is necessary for leucine synthesis; and *LacZ*, which is necessary for lactose hydrolysis. The bacteria are nonfunctional at all three genes. The locations of these three genes relative to the insertion sequences are also shown in the diagram.





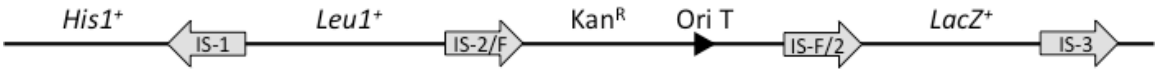
a) The plasmid can integrate into any of the three chromosomal insertion sequences through homologous recombination to form an Hfr bacterium. Draw the Hfr bacteria that result from integration at all three chromosomal insertion sequences. Be sure to show all insertion sequences, genes, and the F plasmid's origin of transfer.

(i) Insertion at IS-1



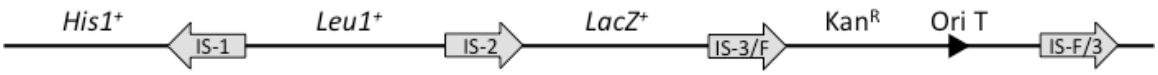
[0.5 points]

(ii) Insertion at IS-2



[0.5 points]

(iii) Insertion at IS-3



[0.5 points]

You take one Hfr strain that you generated and mix it with a strain of *E. coli* that is F^-, *His1*$^-$, *leu1*$^-$, *LacZ*$^-$, susceptible to kanamycin, and resistant to ampicillin. You leave the two stains together for varying amounts of time before replica plating them on several different types of media, all of which contain both kanamycin and ampicillin. You notice that some colonies are *his*$^-$ *leu*$^-$ *Lac*$^+$, some are *his*$^-$ *leu*$^+$ *Lac*$^+$, and some are *his*$^+$ *leu*$^+$ *Lac*$^+$. No other phenotypes are observed.

b) Based on this information, at which insertion sequence did the F plasmid integrate in the Hfr strain? Explain your reasoning.

The plasmid must have inserted at IS-3. Based on the phenotypes, we can determine the order that the nonfunctional genes are being transferred to the F^- strain: first LacZ, then Leu1, and finally His1. From part A, we can see the the genes would be transferred in this order only if the plasmid inserted into IS-3.

[1 point]

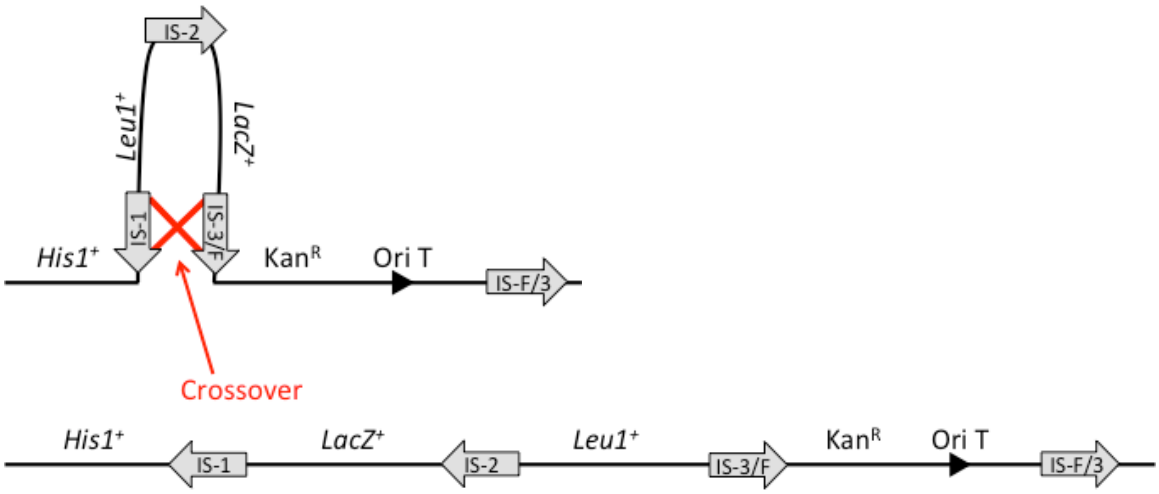
c) In the experiment above, why were all the bacteria grown on media that contained both kanamycin and ampicillin?

We only want bacteria that received DNA from the Hfr to grow so that we can observe the effects of the DNA transfer. The Hfr bacteria themselves are resistant to kanamycin, but are susceptible to ampicillin, so they will not grow on the media. The F^- bacteria that did not receive any DNA from the Hfr are resistant to ampicillin but sensitive to kanamycin, so they also won't grow. Only F^- bacteria that received the Kan^R gene from the Hfr bacteria will be resistant to both ampicillin and kanamycin, so only those will grow.

[0.5 points]

After repeating the experiment in part B several times with the same Hfr strain, you suddenly get a different result in one experiment: the three phenotypes generated are *his*$^-$ *leu*$^+$ *Lac*$^-$, *his*$^-$ *leu*$^+$ *Lac*$^+$, and *his*$^+$ *leu*$^+$ *Lac*$^+$.

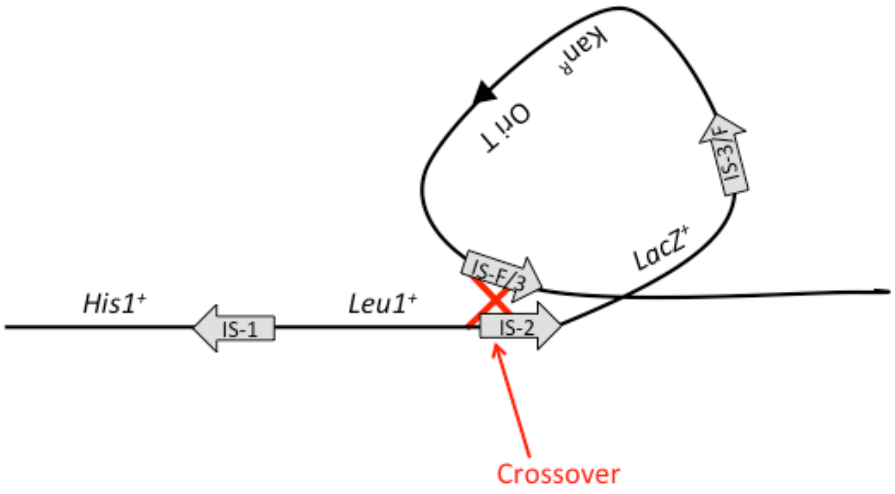
d) What single recombination event in the Hfr strain could have caused this result? Draw both the *E. coli* chromosome during the recombination event and after the recombination event.

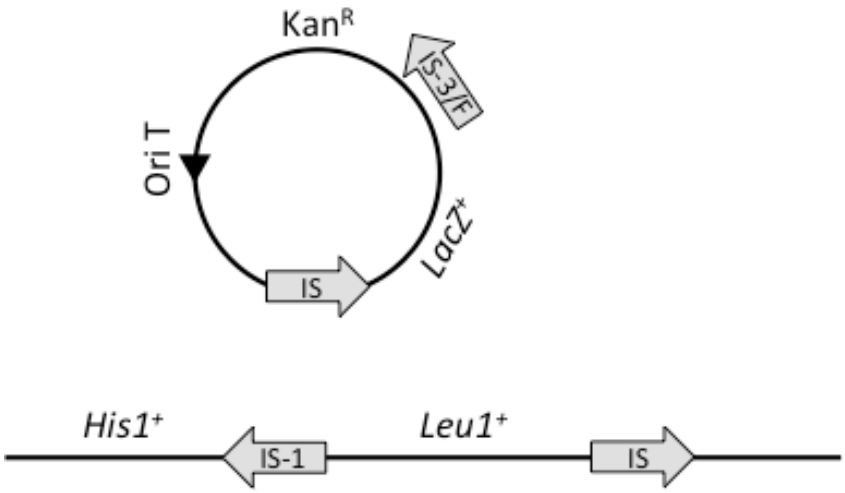


[1 point: 0.5 points for diagram during recombination and 0.5 points for diagram after recombination]

While doing yet another replication of the experiment in part B with the original Hfr strain, you get yet another variant result: no matter how long you allow the Hfr and F^- bacteria to sit together before plating on kanamycin- and ampicillin-containing media, you can only ever grow bacteria that are *his*$^-$ *leu*$^-$ *Lac*$^+$.

e) What single recombination event in the Hfr strain could have caused this result? Draw both the *E. coli* chromosome during the recombination event and after the recombination event.





[1 point: 0.5 points for diagram during recombination and 0.5 points for diagram after recombination]