

# 2005 7.03 Problem Set 3 ANSWER KEY

Due before 5 PM on WEDNESDAY, October 19, 2005.

Turn answers in to the box outside of 68-120.

PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

**1.** The following sequence is a wild-type gene called *lyeT* that encodes a short protein that is required for a certain bioluminescent species of bacteria to produce light. The sequence given is from the point where transcription starts (called “+1”) to the point where transcription ends (called the “terminator”).

5' -ACTTCGATATGCCTAATATATCGATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA-3'  
3' -TGAAGCTATACGGATTATATAGCTAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT-5'

(a) Which strand (the upper or the lower) is used as the template in transcription?

**lower, because it is the only one of the two that would encode an mRNA with a start codon**

(b) Write out the entire sequence of the mRNA made from this wild-type gene.

**5'- ACUUCGAUAUGCCUAAUAUAUCGAUCGAUCUGUGGGGCCUAGCUAGCUAA  
CCAGAGACGCUACCGA-3' because, if the lower strand is used as the template,  
then the mRNA will look like the upper strand.**

(c) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

**Met Pro Asn Ile Ser Ile Asp Leu Trp Gly Leu Ala Ser (or MPNISIDLWGLAS)**

The following sequence is a mutant version of the above gene (*lyeT*<sup>-</sup>) that is present in a bacterial strain that does not produce light. The sequence given is from the point where transcription starts (called “+1”) to the point where transcription ends (called the “terminator”).

5' -ACTTCGATATGCCTAATATAT**TAG**ATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA-3'  
3' -TGAAGCTATACGGATTATAT**ATC**TAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT-5'

(d) Which strand (the upper or the lower) is used as the template in transcription?

**lower**

(e) Write out the amino acid sequence of any protein that is encoded by this mutated gene.

**Met Pro Asn Ile (or MPNI) because of the nonsense mutation bolded above**

The following sequence is a wild-type gene that encodes a tRNA-ser molecule that recognizes the codon 5'-UCG-3' on all mRNAs in the bacterial cell. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5' -CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCAT**TCG**CTTGCTCATGCTGATACGCGCAACGGT-3'  
3' -GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGT**AGC**GAACGAGTACGACTATGCGCGTTGCCA-5'

(f) Which strand (the upper or the lower) is used as the template in transcription? (Remember that tRNAs are DIRECTLY transcribed from tRNA-encoding genes. There is no mRNA intermediate in the production of a tRNA molecule from a tRNA gene!)

**upper, since it is the only one of the two that would encode a tRNA transcript that would include the proper anticodon 5'-CGA-3' (which can base pair with 5'-UCG-3')**

(g) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

**None, this gene does not code for any protein. This gene encodes a tRNA.**

(h) Put a box around the double-stranded DNA portion of the wild-type tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing at the top of the page.)

The following sequence is a mutant gene that encodes a nonsense-suppressing version of the tRNA-ser gene. This mutation suppresses the effects of *lyeT*<sup>-</sup>. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5' -CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCAT**TAG**CTTGCTCATGCTGATACGCGCAACGGT-3'  
3' -GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGT**ATC**GAACGAGTACGACTATGCGCGTTGCCA-5'

(i) Which strand (the upper or the lower) is used as the template in transcription?

**Upper. Note that this tRNA now has the anticodon 5'-CUA-3', which pairs with the codon in an mRNA of 5'-UAG-3' (which is normally a stop codon but would be read by this mutant tRNA and translated as a serine)**

(j) Put a box around the double-stranded DNA portion of the mutated tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing in the middle of the page.)

(k) Would a strain produce light if it contains the wild-type version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

**yes, because wild-type cells of this species produce light**

(l) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

**no, because a single mutant  $lyeT^-$  strain has the phenotype of inability to make light**

(m) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the mutant version of the *tRNA-ser* gene?

**yes, the mutant *tRNA-ser* would suppress the effects of the amber mutation in the *lyeT* gene. The 5'-UAG-3' in the *lyeT* mRNA would be read as a serine, not as a stop. Thus the double mutant cell (harboring the original *lyeT* mutation and the suppressor mutation) would produce functional LyeT protein, and thus would make light.**

**2.** Wild-type bacteria are capable of a type of movement called “swarming,” in which many bacterial cells bundle together to form rafts that can swim through solid media of a low agar concentration. Someone has given you a mutant strain of bacteria that has the mutant phenotype of being unable to “swarm.” This person tells you that the mutation (*swrM*<sup>-</sup>) which causes this phenotype is either an ochre mutation or an amber mutation. You have another bacterial strain containing a mutant version of a tRNA gene (*Su*<sup>+</sup>) that encodes an ochre suppressor tRNA. This tRNA gene is 60% linked (by cotransduction frequency) to a Tn5 KanR transposon in this strain. This transposon is not linked to *swrM*.

(a) You decide to perform a cotransduction experiment to determine whether the *swrM*<sup>-</sup> mutation is an amber mutation or an ochre mutation. Fill in the blanks in the following paragraph to show what experiment you decide do: **Move *Su*<sup>+</sup> into a *swrM*<sup>-</sup> strain.**

You grow P1 phage on bacteria of the genotype Tn5 KanR Su+.

You use the resulting phage lysate to infect bacteria of the genotype Su- *swrM*<sup>-</sup>.

You select for transductants that can grow on plates containing Kanamycin.

(b) Describe the two possible results you could get from this experiment if you analyzed 1000 transductants. (Include in each answer the predicted number of transductants of each phenotypic class, and the genotypes of the transductants in each class.)

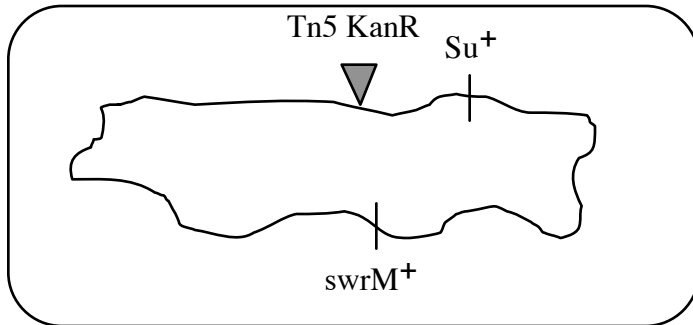
If the *swrM*<sup>-</sup> mutation is an ochre mutation:

**600 can swarm (Tn5 KanR Su+ *swrM*<sup>-</sup>)**

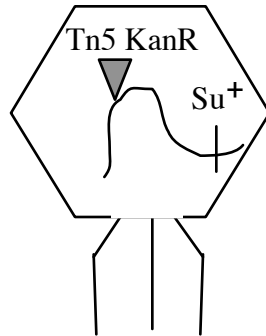
**400 cannot swarm (Tn5 KanR Su- *swrM*<sup>-</sup>)**

**60% cotransduction means that 60% of the time (600/1000), *Su*<sup>+</sup> will be transduced along with the selectable Tn5 transposon. 40% of the time, the Tn5 transposon will enter the cell without *Su*<sup>+</sup> coming in as well.**

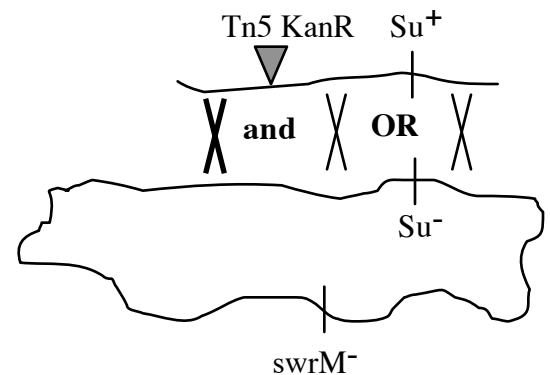
The original cell on which the phage was grown looked like:



When phage infect this cell, replicate, and burst the cell (releasing progeny phage), a small percentage of phage contain bacterial DNA instead of phage DNA. We select for the very small portion of phage that carry the part of the bacterial chromosome carrying Tn5, because we select for transductants on medium containing kanamycin. Thus the transductants we see have been infected with phage carrying a specific piece of the host chromosome:



When these phage infect our recipient strain, this DNA enters the recipient and aligns with homologous sequences in the recipient chromosome. This DNA can integrate into the recipient chromosome by an even number of crossover events. (An odd number of crossovers creates a linear product, and bacteria cannot maintain linear DNA.) One of the two crossovers must be to the left of KanR, because we are selecting for kanamycin resistance, so the KanR gene must have integrated into the recipient genome. 60% of the time, the second crossover will bring Su+ in with KanR. 40% of the time, the second crossover will be to the left of Su+, thus only bringing in KanR.

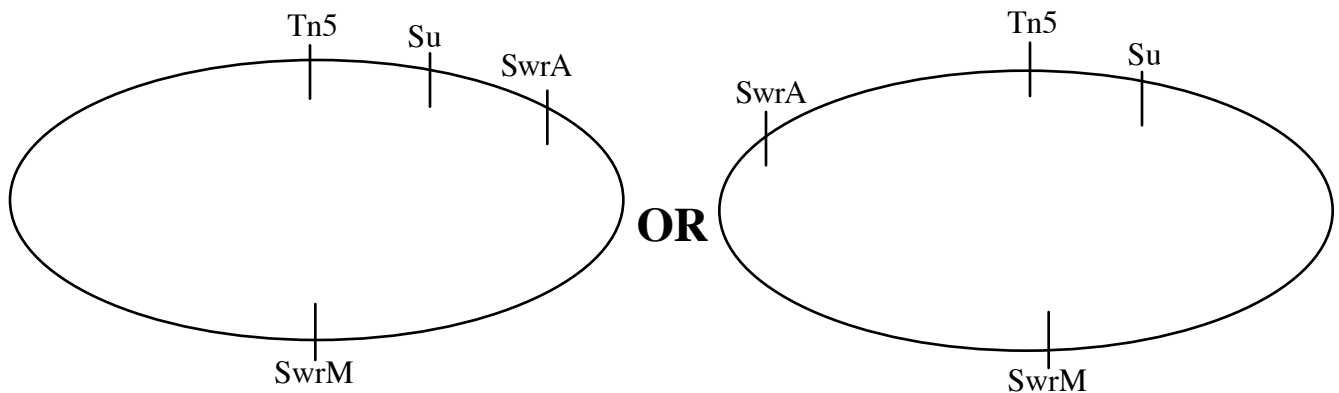


If the *swrM*<sup>-</sup> mutation is an amber mutation:

1000 cannot swarm. We will still see 600 [Tn5 KanR Su<sup>+</sup> swrM<sup>-</sup>] transductants and the 400 [Tn5 KanR Su<sup>-</sup> swrM<sup>-</sup>] transductants, but, in this case, the ochre suppressor cannot suppress the *swrM* mutation so none will swarm. The pictures of the DNA being transduced in would look the same as the above pictures, however.

(c) You find a new gene (*swrA*) in which a specific ochre mutation causes the phenotype of being unable to swarm. This gene is linked to the transposon you used in part (a) with a cotransduction frequency of 30%. Draw all of the possibilities for a map of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the whole chromosome, and the positions and relative order of the Tn insertion, the tRNA locus, the *swrA* locus, and the *swrM* locus.

There are two possible orders:



A higher cotransduction frequency corresponds to tighter genetic linkage, because higher cotransduction frequencies mean that the two markers come together into a new cell more often. Thus they are closer together physically on the chromosome.

Remember that a bacterial cell of most species contains one circular chromosome.

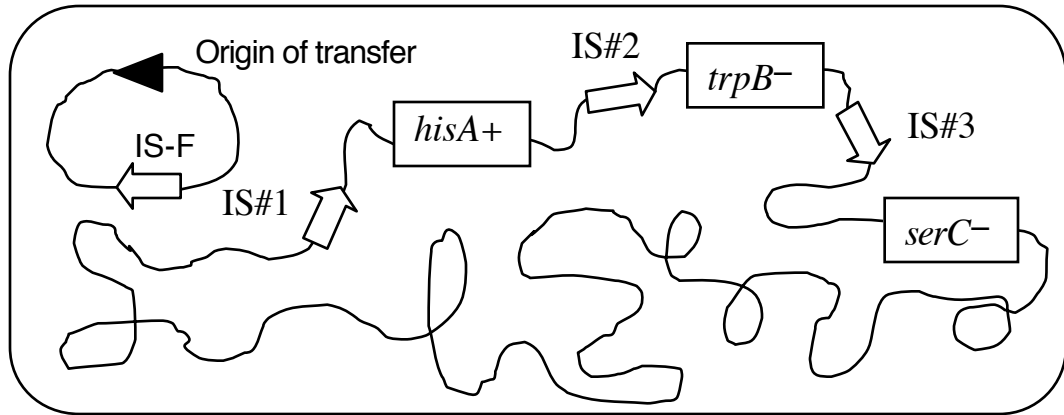
The information that you have with which to make the map is:

1. The tRNA gene is 60% linked (by cotransduction frequency) to a Tn5 KanR transposon in this strain.
2. The transposon is not linked to *swrM*.
3. The *swrA* gene is linked to the transposon with a cotransduction frequency of 30%.

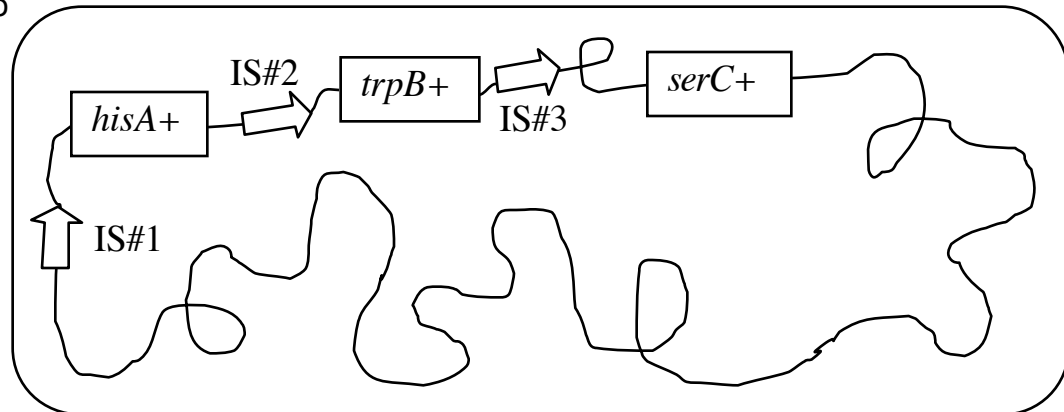
Thus *swrA* must be farther from the transposon than the Su locus. However, these three loci must all be within the same chromosomal region because they are all linked at a cotransduction distance of >0%. *SwrM* must be very far away, because it is not linked to the transposon at all.

**3.** You are studying two strains of *E. coli*. Below are diagrams of the two different strains, showing their chromosomes and the F plasmid (in Strain One, which contains it).

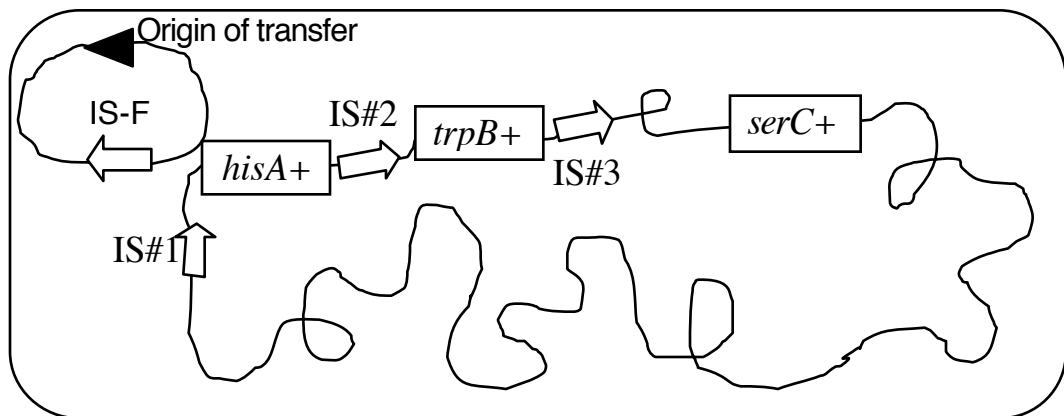
Strain One



Strain Two

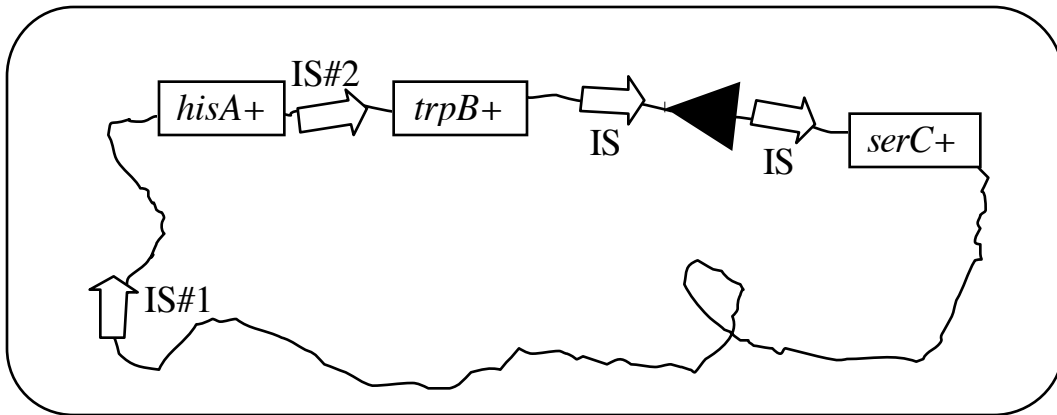


**(a)** Using the notation from above, draw the new strain that would result if Strains One and Two mated together. (Call the resulting strain Strain Three.)

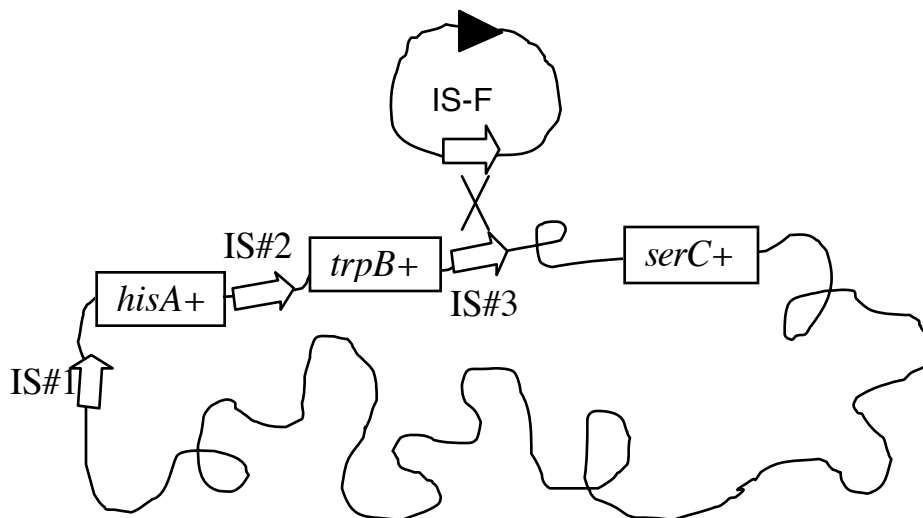


**When an F<sup>+</sup> cell mates to an F<sup>-</sup> cell, the F<sup>+</sup> cell simply transfers the F plasmid to the F<sup>-</sup> cell.**

(b) Using the notation from above, draw the new strain that would result if IS-F and IS#3 recombined together in a cell from Strain Three. (Call the resulting strain Strain Four.)

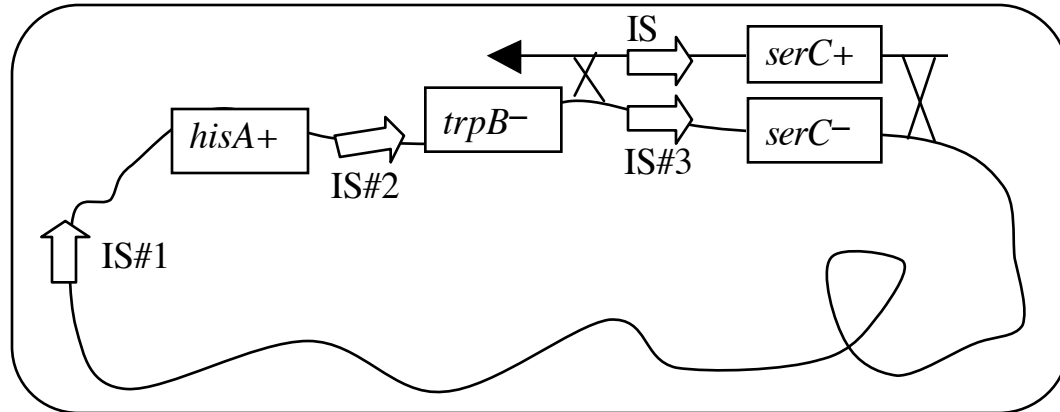


When one recombination event occurs between homologous sequences on two circular pieces of DNA, the result is one large circular piece of DNA. No genetic information is lost or gained in this process. The way to determine the order of the genes on this resulting circular piece of DNA is to draw the two initial DNAs aligned, such that their homologous sequences are lined up with each other. Then, draw a recombination event between them, and trace the resulting product in the shape of a “figure-8.” The aligned pieces of DNA will look like this:



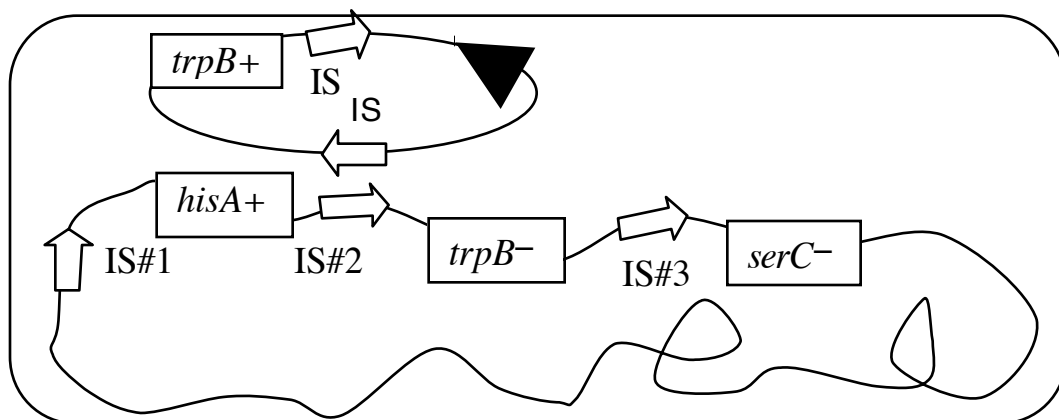
If you trace this structure through the recombination event in the shape of a “figure-8,” the resulting large circle of DNA will be as it is drawn in the cell above.

(c) Using the notation from above, draw the events that would occur in the recipient cell that had been mated into, if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating only for a short time, and you selected for exconjugants on minimal medium that lacks supplemental **serine**. (Call the resulting strain Strain Five.)



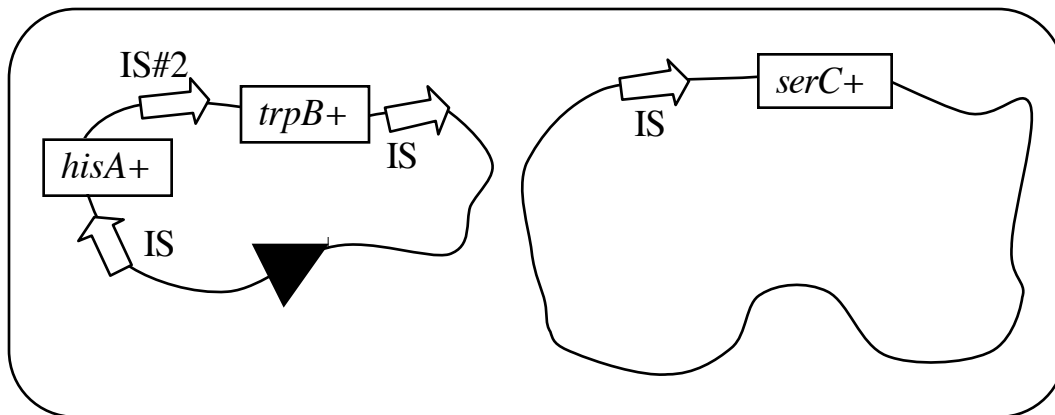
When an Hfr cell mates to an F- cell, the Hfr cell begins to transfer its chromosome into the F- cell, beginning with the origin of transfer, and followed by the first gene behind the blunt end of the origin of transfer symbol (◄). If a short time is allowed for the mating, only the “early markers” will be transferred into the recipient cell. These markers may integrate into the recipient chromosome through a double recombination event between the recipient chromosome and the transferred host chromosome. There must be an even number of crossover events in order for genes from a linear piece of DNA to integrate into a bacterial chromosome, which is circular. If you are selecting for exconjugants on medium that lacks serine, then you are selecting for exconjugants who have acquired the *serC+* gene into their chromosomes. This occurs only after transfer of the genome from the Hfr, and an even number of crossover events flanking the *serC* gene.

(d) Using the notation from above, draw the new strain that would result if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating for only a short time, and you selected for exconjugants on minimal medium that lacks supplemental **tryptophan**. (Call the resulting strain Strain Six.)



The way you can select for F' formation is by mating an Hfr strain to an F- strain, and selecting for a late marker being transferred early. TrpB would normally be the last marker to be transferred by the Hfr (Strain Four). If you end a mating early, then trpB should not be transferred in the way described above in the answer to part (c). The only way trpB can be transferred early is if a recombination event occurred in the donor cell such that two IS sequences in the Hfr chromosome recombined together, forming an F' plasmid. Then that F' would be transferred to Strain 1. F' plasmids are quite small compared to the chromosome, and an entire F' plasmid can be transferred quite quickly to a recipient cell. More about F' plasmids are described in the answer to part (e).

(e) Using the notation from above, draw the new strain that would result if IS#1 recombined with the IS sequence that is farthest away from IS#1 in a cell from Strain Four.



If two IS sequences that flank the origin of transfer in an Hfr chromosome recombine together, an F' plasmid is formed. The region of the Hfr chromosome between the two IS sequences becomes the F' plasmid, and the remaining parts of the chromosome remain in the circular chromosome.

Note that the chromosome (on the right, ~5,000 kb) is much larger than the F' plasmid (on the left, ~100 kb); this drawing is not to scale.

(f) State whether each of the following strains is an F<sup>-</sup> strain, an F' strain, an F<sup>+</sup> strain, or an Hfr strain:

Strain One – F<sup>+</sup> because any strain harboring the F plasmid with no extra bacterial genes inside the plasmid is an F<sup>+</sup> strain

Strain Two – F<sup>-</sup> because any strain harboring no origin of transfer anywhere in the cell is an F<sup>-</sup> strain

Strain Three – F<sup>+</sup>

Strain Four – Hfr because any strain with an origin of transfer in the chromosome is an Hfr that can transfer its own chromosome to other cells

**Strain Five – F- because the homologous recombination events do not bring the origin of transfer into the chromosome. Thus the strain that is formed has exchanged chromosomal markers (serC+ for serC-) but does not have an origin of transfer anywhere in the cell and is thus an F- cell**

**Strain Six – F' because any strain harboring a form of the F plasmid that contains bacterial genes that are normally located on the chromosome is an F' strain**