2005 7.03 Problem Set 3

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?

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

5'-

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

The following sequence is a mutant version of the above gene (*lyeT*$^{-}$) 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′–ACTTCGATATGCCTAATATATAGATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA–3′
3′–TGAAGCTATACGGATTATATATCTAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT–5′
```

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

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

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′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCATCGCTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGTAGCGAACGAGTACGACTATGCGCGTTGCCA–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!)

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

(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*$^-$. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

```
5′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCATAGCTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGTATCGAACGAGTACGACTATGCGCGTTGCCA–5′
```

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

(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?

(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?

(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?

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*⁻) 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+) 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*⁻ mutation is an amber mutation or an ochre mutation. Fill in the blanks in the following paragraph to show what experiment you decide do:

You grow P1 phage on bacteria of the genotype _____.

You use the resulting phage lysate to infect bacteria of the genotype _____.

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

(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.)

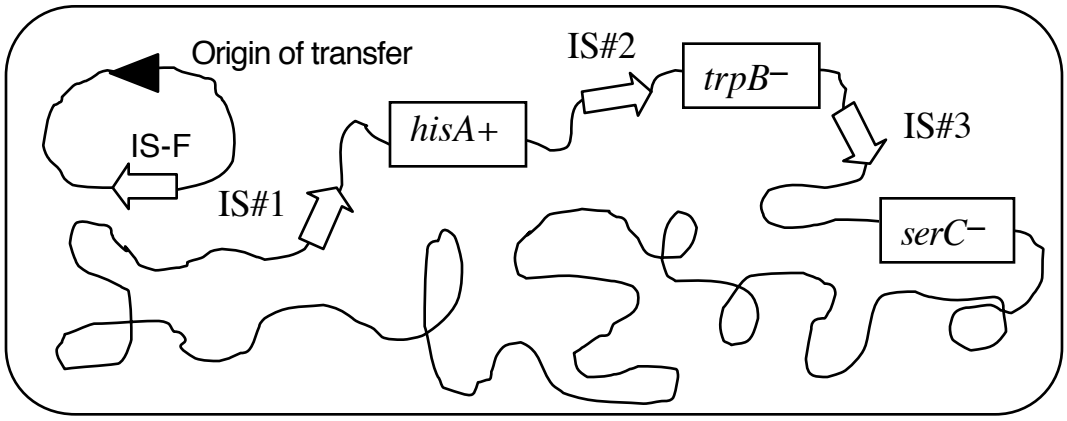
If the *swrM*⁻ mutation is an ochre mutation:

If the *swrM*⁻ mutation is an amber mutation:

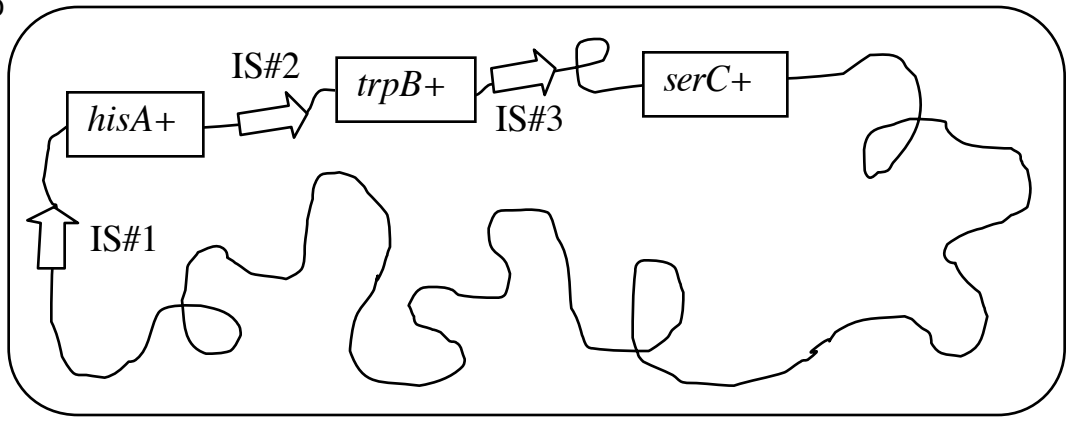
(c) You find a new gene (*swrA*) in which a specific ochre mutation causes the phenotype of being unable to swarm. This gene is linked to the transposon you used in part **(a)** with a cotransduction frequency of 30%. Draw all of the possibilities for a map of the bacterial chromosome that is consistent 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.

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.)

(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.)

(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.)

(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.)

(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.

(f) State whether each of the following strains is an F**−** strain, an F**'** strain, an F**+** strain, or an Hfr strain:

Strain One --

Strain Two --

Strain Three --

Strain Four --

Strain Five –

Strain Six --