Exam Questions from Exam 2 – Mutations, Bacterial Genetics, and Bacterial Gene Regulation

1. Drawn below is part of a wild-type gene. The DNA sequence shown encodes the last amino acids of a protein that is normally 380 amino acids long. The bracketed codon indicates the correct reading frame of this gene. The lower strand of the gene is used as the template during the transcription of mRNA from this gene.

```
  ⌐  ⌐
...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3′
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5′
```

(a) In the copy of the sequence drawn below, circle one base pair that you could change to make a mutant form of the gene that produces a protein that is now 381 amino acids long. Indicate the identity of one new base pair that could take its place.

```
...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3′
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5′
```

(b) In the copy of the sequence drawn below, draw a slash between two base pairs where you could add one extra base pair in order to make a single mutant form of the gene that produces a protein that is 373 amino acids long. Indicate the identity of the one new base pair you are adding.

```
...GCTAAGTATTGCTCAAGATTAGGATGATAAATAACTGG-3′
...CGATTCATAACGAGTTCTAATCCTACTATTTATTGACC-5′
```

(c) Multiple mutant suppressor tRNAs could suppress the early termination defect in part **(b)** by allowing a longer protein to be produced from that mutant form of the gene. Make a list of **all** of the tRNA genes that could be mutated to produce such mutant suppressor tRNAs if each tRNA gene contained a **single base** substitution. (Use the notation: “ala-tRNA.”)

2. You are studying the regulation of a bacterial gene called *nytT*, which is expressed only when the bacterial strain is grown in the dark. You isolate two mutations, *nytA1*$^-$ and *nytB1*$^-$, which affect the regulation of *nytT*.

	Genotype	Is *nytT* expressed in the dark?	Is *nytT* expressed in the light?
Strain 1	*nytA*$^+$ *nytB*$^+$ *nytT*$^+$ (wild type)	yes	no
Strain 2	*nytA1*$^-$ *nytB*$^+$ *nytT*$^+$	no	no
Strain 3	*nytA*$^+$ *nytB1*$^-$ *nytT*$^+$	no	no
Strain 4	*nytA*$^+$ *nytB*$^+$ *nytT*$^+$ / F' *nytA1*$^-$	yes	no
Strain 5	*nytA*$^+$ *nytB*$^+$ *nytT*$^+$/ F' *nytB1*$^-$	yes	no

You grow P1 phage on an otherwise wild-type strain that contains a transposon insertion carrying a gene that confers tetracycline resistance. The transposon insertion in this strain is linked to the *nytT* locus with a cotransduction frequency of 85%, and this insertion does not alter normal *nytT* regulation. You use the resulting lysate to infect a *nytA1*$^-$ strain, and select for tetracycline resistance. None of the 30 Tet^r cotransductants you examine express the *nytT* gene under any conditions. You obtain the same results when you use the same P1 lysate to infect a *nytB1*$^-$ recipient strain.

(a) Can you conclude if *nytA1*$^-$ is constitutive or uninducible? **If so**, state whether *nytA1*$^-$ is constitutive or uninducible, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

(b) Can you conclude if *nytA1*$^-$ is dominant or recessive? **If so**, state whether *nytA1*$^-$ is dominant or recessive, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

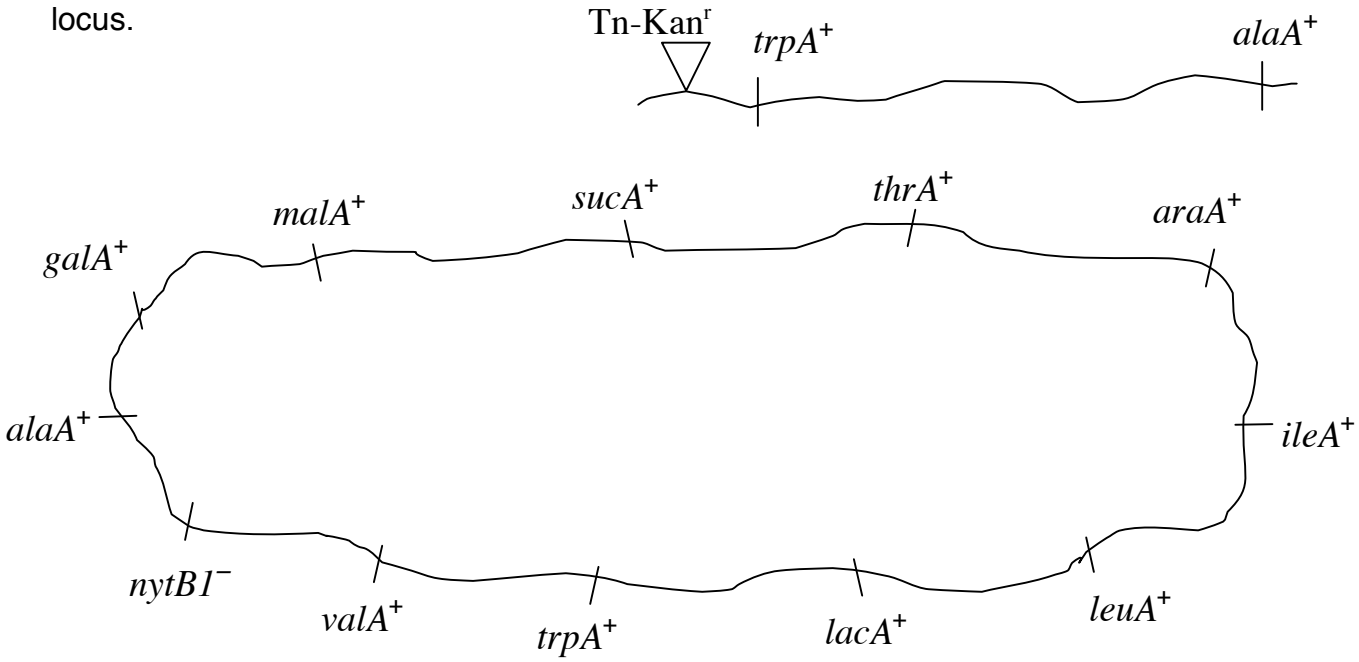
(c) Can you conclude if *nytA1*$^-$ acts in cis or in trans with respect to *nytT*? **If so**, state whether *nytA1*$^-$ acts in cis or in trans, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

(d) Diagram **three possible models** for regulatory pathways for *nytT* that can explain the behavior of the *nytA1*$^-$ and *nytB1*$^-$ mutations. (Please diagram only linear pathways in which each gene is controlled by no more than one regulator. Please do not include any steps that invoke unknown players.) For each model, include **only** the following: wild-type *nytA, nytB,* and *nytT,* and "bright light."

3. After you perform the experiments from Question #2, you decide to continue studying the regulation of the bacterial gene *nytT*, which is expressed only when the bacterial strain is grown in the dark. You decide to map the two mutations, *nytA1*$^-$ and *nytB1*$^-$, which you isolated in Question #2. Please refer to the table in the introduction to Question #2 for information about how these mutations affect the regulation of *nytT*.

You find that the *nytA* and *nytB* loci are linked using P1 cotransduction experiments. You isolate a transposon insertion that carries a gene encoding kanamycin resistance. This transposon insertion is near to, but not between, the *nytA* and *nytB* loci.

(a) You grow P1 phage on an otherwise wild-type strain that contains the transposon insertion carrying kanamycin resistance. You use the resulting lysate to infect a *nytB1*$^-$ strain, and select for kanamycin resistance. Drawn below are the *E. coli* chromosome and the DNA transduced by P1 during this cotransduction experiment. (Please note that these drawings are not to scale.) **Redraw** the DNA transduced by P1 so that it lines up with the homologous region of the *E. coli* chromosome. Then **draw in** the recombination events necessary to achieve the cotransduction of Tn-Kan^r and the *nytB* locus.



(b) In the transduction experiment described in part **(a)**, out of a total of 50 Kan^r cotransductants, 15 can express the *nytT* gene in the dark and 35 cannot. **Express the distance** between the transposon and the *nytB* locus as a cotransduction frequency.

To map the *nytA* and *nytB* loci, you set up two reciprocal crosses:
In the **first cross**, you grow P1 phage on a Kan^r strain that contains the transposon insertion and the $nytA1^-$ mutation, and use the resulting phage lysate to infect a $nytB1^-$ strain. You select for kanamycin resistance (Kan^r), and among 100 Kan^r transductants, you find that only 13 are able to express *nytT*. (All 13 show normal *nytT* regulation.)

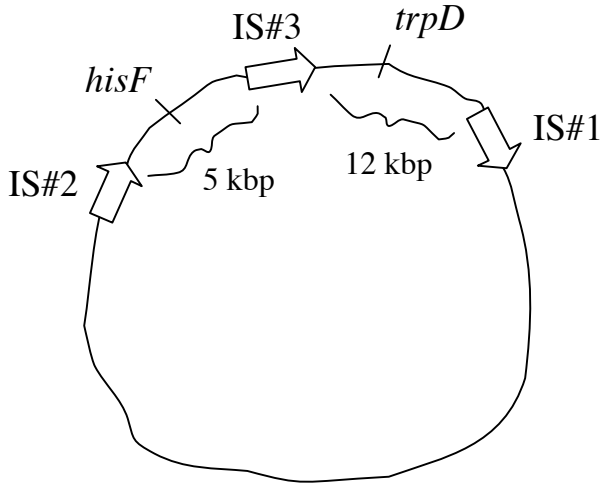
In the **second cross**, you grow P1 phage on a Kan^r strain that contains the transposon insertion and the $nytB1^-$ mutation, and use the resulting phage lysate to infect a $nytA1^-$ strain. You select for kanamycin resistance (Kan^r), and among 100 Kan^r transductants, you find that only 3 are able to express *nytT*. (All 3 show normal *nytT* regulation.)

(c) Draw a genetic map showing the correct relative positions of the transposon insertion (Tn-Kan^r) and the *nytA* and *nytB* loci in this box:

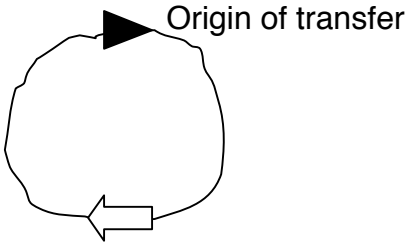
(d) Based on the gene order **that you drew in part (c)**, state the chromosomal genotype of a transductant that must have resulted from a quadruple crossover event between the transduced DNA and the bacterial chromosome of the recipient **in the first cross**. (Be sure to indicate the chromosomal genotype at both the *nytA* and *nytB* loci.)

(e) Based on the gene order **that you drew in part (c)**, which of the following is the most reasonable distance between Tn-Kan^r and the *nytA* locus, as expressed as a cotransduction frequency? (**Your choices are**: 20%, 30%, OR 40%.)

4. You are studying a strain of *E. coli* whose total genome size is **4,639 kilobase pairs** (kbp). The chromosome of this *E. coli* strain is diagrammed below, and its three insertion sequences are indicated. Note that this drawing is **not to scale**.
NOTE: Assume that the size of each insertion sequence is 1 kbp.



You are utilizing a form of the F factor that is **95 kbp** in length. This F factor has a single IS sequence and a single origin of transfer, as indicated:



Fill in the chart on the next page, which considers cells containing the above chromosome and F factor.

Fill in the chart below. Two boxes have been done for you.
NOTE: Assume each cell described in Column 1 contains only what is listed -- NO OTHER recombination events have occurred in each cell besides those listed.

Type of cell	What is the size of the circular *E. coli* chromosome in the cell?	What is the size of the extrachromosomal circle of DNA in the cell?	Can *hisF* be transferred efficiently, inefficiently, OR never?	Can *trpD* be transferred efficiently, inefficiently, OR never?
An F^- bacterial cell	4,639 kbp	0 kbp (there isn't one)		
An F^+ bacterial cell				
An Hfr cell (**named "Hfr A"**) resulting from recombination between IS#4 and IS#3				
A cell resulting from recombination between IS#2 and IS#1 in "Hfr A"				

5. You have isolated a mutation in the Lac I gene; this mutation causes constitutive LacZYA gene expression. DNA sequencing reveals that the mutant form of the LacI gene has an amber mutation in about the middle of the Lac I coding sequence. However, you find that when you introduce an amber-suppressing mutant allele of the gene encoding $tRNA^{trp}$ into the strain carrying the Lac I mutation, the strain still expresses LacZYA genes constitutively. Propose **two different** explanations for why the amber-suppressing mutant allele of the gene encoding $tRNA^{trp}$ fails to suppress this particular amber mutation.

6. You have isolated an *E. coli* mutant which you call Lac1$^-$. This mutant cannot grow on the sugar lactose as the only carbon source. (Such a phenotype is called Lac$^-$.)

(a) You have a wild-type (Lac$^+$) strain carrying a Tn5 insertion known to be near to but not within the group of 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 50 Kan^r transductants, you find that 10 are Lac$^-$ and 40 are Lac$^+$. Express the distance between Tn5 and the Lac1 locus as a cotransduction frequency.

(b) You isolate a second Lac$^-$ mutation, which you designate Lac2$^-$. To map the Lac2 locus relative to the Lac1 locus, you set up two reciprocal crosses. In the first cross, you grow P1 phage on a bacterial strain that carries the Tn5 insertion described in part (**a**) and the Lac2$^-$ mutation. You then use this resulting phage lysate to infect a Lac1$^-$ mutant bacterial strain, and select for Kan^r. From 100 Kan^r transductants examined, 96 are Lac$^-$ and 4 are Lac$^+$.
In the second cross, you grow P1 phage on a bacterial strain that carries the Tn5 insertion and the Lac1$^-$ mutation. You then use this resulting phage lysate to infect a Lac2$^-$ bacterial mutant, and select for Kan^r. From 100 Kan^r transductants examined, all are Lac$^-$. Draw a genetic map showing the relative positions of the Tn5 insertion and the Lac1 and Lac2 loci.

(c) Further analysis of the Lac1$^-$ mutation reveals that the Lac1$^-$ mutant does not express ß-galactosidase (even in the presence of IPTG) but expresses permease normally. Of the Lac mutations that we learned about in class, name the one type of single mutation that best explains the properties of Lac1$^-$.

(d) Further analysis of the Lac2$^-$ mutation reveals that the Lac2$^-$ mutant expresses NEITHER ß-galactosidase or permease (even in the presence of IPTG). Of the Lac mutations that we learned about in class, name the two types of mutations that best explain the properties of Lac2$^-$.

7. You have identified a new strain of *E. coli* that can grow on starch. The starch-degrading enzyme "amylase" is made only at low levels under normal growth conditions, but when starch is added to the *E. coli* culture, the levels of amylase enzyme increase 100-fold. You isolate three mutants that affect amylase synthesis. The mutant **A**$^-$ is in the structural gene for amylase and prevents the synthesis of amylase enzyme. Both the **B**$^-$ and **C**$^-$ mutations, which occur in loci that are linked to **A**, give expression of amylase even in the absence of starch. The table below gives the amylase enzyme activities for a set of strains in either the presence or absence of the inducer starch.

	Amylase activity in enzyme units	
	– starch	+ starch
$A^+ B^+ C^+$	1	100
$A^- B^+ C^+$	0	0
$A^+ B^- C^+$	100	100
$A^+ B^+ C^-$	100	100
$A^- B^+ C^+$ / **F'** $A^+ B^+ C^+$	1	100
$A^+ B^- C^+$ / **F'** $A^+ B^+ C^+$	100	200
$A^+ B^+ C^-$ / **F'** $A^+ B^+ C^+$	2	200
$A^+ B^- C^+$ / **F'** $A^- B^+ C^+$	100	100
$A^- B^- C^+$ / **F'** $A^+ B^+ C^+$	1	100
$A^+ B^+ C^-$ / **F'** $A^- B^+ C^+$	1	100
$A^- B^+ C^-$ / **F'** $A^+ B^+ C^+$	1	100

(a) Describe the genetic properties of the **B**$^{-}$ mutation (cis vs trans, dominant vs recessive, constitutive vs uninducible), and propose a molecular function for the regulatory component that is encoded by the wild-type **B** locus.

(b) Describe the genetic properties of the **C**$^{-}$ mutation (cis vs trans, dominant vs recessive, constitutive vs uninducible), and propose a molecular function for the regulatory component that is encoded by the wild-type **C** locus.

You isolate a new mutation (**D**$^{-}$) that alters amylase expression. In P1 transduction experiments, it is found that the **D** is not linked to **A**, **B**, or **C**. The properties of some strains with the **D**$^{-}$ mutation are shown below.

(**NOTE** that F' D^{+} does not carry the amylase gene.)

	Amylase activity in enzyme units	
	– starch	+ starch
D^{+}	1	100
D^{-}	1	1
D^{-}/ **F'** D^{+}	1	100
D^{-} A^{-}	0	0
D^{-} B^{-}	100	100
D^{-} C^{-}	100	100

(c) Describe the genetic properties of the **D**$^{-}$ mutation (cis vs trans, dominant vs recessive, constitutive vs uninducible), and propose a molecular function for the regulatory component that is encoded by the wild-type **D** locus.

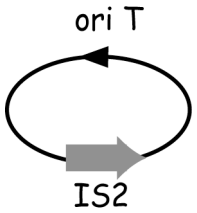
(d) Is the **D** gene most likely to act earlier or later than **B** in the pathway for amylase regulation?

(e) Is the **D** gene most likely to act earlier or later than **C** in the pathway for amylase regulation?

By performing biochemical experiments, you find that the protein product of the gene that is affected by the $\mathbf{D^-}$ mutation binds to starch and can also bind to DNA at a site near to the **A**, **B**, and **C** loci.

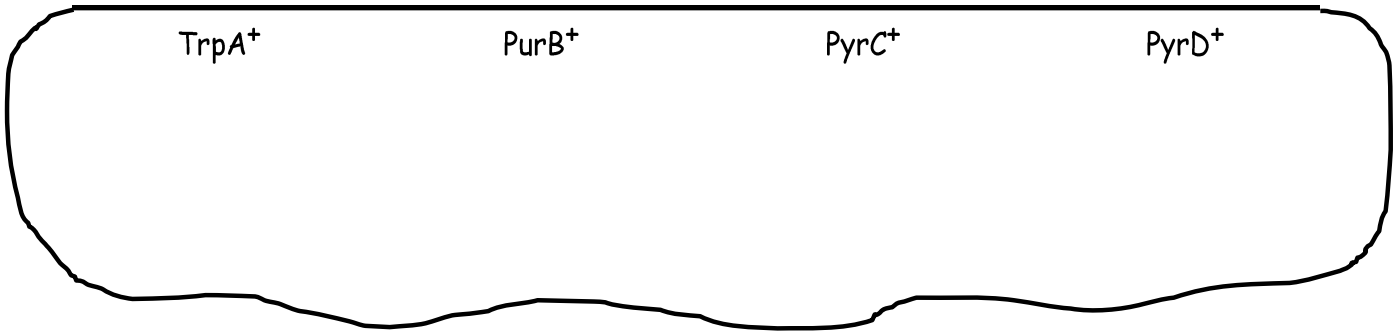
(f) Propose a linear genetic pathway that accounts for the regulation of the amylase gene. Include in your diagram of your genetic pathway model: starch, and the wild-type **A**, **B**, **C**, and **D** genes.

8. Below is a diagram of the F factor showing the direction of the origin of transfer (ori T) and an **IS2 element** carried on this F plasmid.



From a wild-type F^+ strain, you isolate an Hfr strain that transfers $PyrD^+$ early and efficiently, but does *not* transfer the neighboring markers $PyrC^+$, $PurB^+$ and $TrpA^+$ until very late (after 90 minutes of a mating reaction).

(a) On the map of the *E. coli* chromosome shown below, draw in an **IS2 element** (represented by an arrow to show proper orientation) that existed in the chromosome of the F+ bacterium that could have recombined with the **IS2 element** on the F plasmid to produce the Hfr described above.



(b) You mate the Hfr strain isolated in part (**a**) to an F$^-$ PyrC$^-$ donor strain, and, after a brief (~10 minute) mating you isolate a rare PyrC$^+$ recipient strain. In subsequent matings, the newly isolated PyrC$^+$ F' strain can transfer PyrC$^+$ and PurB$^+$ early and efficiently, but cannot transfer either PyrD$^+$ or TrpA$^+$. Following the format of the drawings used in this problem, draw the chromosome of this F' strain you have created. Include any of the following that are applicable: IS2 insertional sequences, oriT, TrpA+/$^-$, PurB+/$^-$, PyrC+/$^-$, PyrD+/$^-$.

(c) Following the format of the drawings used in this problem, draw the form the F plasmid that is contained within this F' strain you have created. Include any of the following that are applicable: IS2 insertional sequences, oriT, TrpA+/$^-$, PurB+/$^-$, PyrC+/$^-$, PyrD+/$^-$.

(d) You have isolated a new PurB$^-$ allele that causes the phenotype of the inability to grow unless purine nucleotides are added to the medium. However, you find that when the F' isolated in part (**b**) is mated into this PurB$^-$ strain, the resulting recipients bearing the F' remain unable to grow in the absence of added purine nucleotides. Propose an explanation for this finding.

9. The Mot genes of *E. coli* are required for motility (swimming) of these bacteria. You have isolated a non-motile mutant that you designate **Mot1$^-$**. You grow P1 phage on an otherwise wild-type bacterial strain that carries a **Tn5** insertion that is linked to one of the Mot genes. You then use the resulting phage lysate to infect a **Mot1$^-$** strain, and select for kanamycin resistance. From 50 transductants isolated by selecting for Kan^r , you find that 35 are motile and 15 are non-motile.

(a) What is the distance between the **Tn5** insertion and the **Mot1** locus (expressed as a cotransduction frequency)?

(b) You grow P1 phage on one of the non-motile Kan^r transductants (**Tn5 Mot1$^-$**) isolated above. You then use the resulting phage lysate to infect a second non-motile strain that carries a mutation designated **Mot2$^-$**. A total of 200 Kan^r transductants are isolated, and NONE are motile. Does this result tell you whether the **Mot1** and **Mot2** loci are linked? Explain why or why not.

(c) Next, you grow P1 phage on a strain that carries both the **Tn5** insertion and the **Mot2$^-$** mutation. When the resulting phage lysate is used to infect a strain that carries the **Mot1$^-$** mutation, you find that 5 out of 200 Kan^r transductants are motile. Based on this result, as well as the results from parts (**a**) and (**b**), draw a map showing the relative order of the **Tn5** insertion and the **Mot1** and **Mot2** loci. (Note that you have since discovered that **Mot1** and **Mot2** are two different alleles of the same gene.)

(d) You can detect the protein products of the Mot genes. You observe that one of these proteins is 58 kDa in a wild-type strain but is 40 kDa in a **Mot1$^-$** mutant and 30 kDa in a **Mot2$^-$** mutant. Given this information, draw a diagram of the Mot1/2 gene, showing the direction of transcription of this Mot gene relative to the position of the **Tn5** insertion.

(e) You introduce a mutant version of a tRNA^{ser} gene into the **Mot1$^-$** mutant strain. This mutant tRNA^{ser} allele is called **Su$^+$**, and it encodes an amber-suppressing mutant form of the tRNA^{ser} gene. The Mot protein in this **Mot1$^-$ Su$^+$** double mutant strain is now 58 kDa. What specific kind of mutation is **Mot1$^-$**?

(f) The sequence of the amber stop codon is $^{5'}$UAG$^{3'}$. Write out the DNA sequence of the portion of the mutant gene that encodes the anti-codon segment of a mutant amber-suppressing tRNA^{ser} molecule. (Label the 5' and 3' ends of both strands and indicate which is the strand used as a template during transcription of the tRNA).

10. Raffinose is a sugar that requires the lactose permease (the LacY gene product) to enter an *E. coli* cell. However, raffinose does not act as an inducer for the Lac operon (as lactose does). Wild-type (Lac^+) *E. coli* can not grow on raffinose as the only carbon source, because without the presence of lactose, there is not enough Lac permease expression induced to take up raffinose.

In the following experiments, you will be using the ProA gene as a selectable marker, much like you would use a transposon insertion. The ProA gene is linked to the Lac operon with a cotransduction frequency of about 60%. Beginning with an *E. coli* strain that is $ProA^-$ Lac^+, you isolate a collection of ten different mutants that can now grow on raffinose as the only carbon source. You show that each of the ten mutations is linked to the Lac operon / ProA region of the chromosome.

(a) What three possible types of Lac operon mutations that we learned about in class could you have isolated, given that you did a screen for mutants that gained the ability to grow on raffinose as the only carbon source?

(b) You introduce an F' $ProA^+$ Lac^+ plasmid into each of your mutant strains by selecting for the Pro^+ phenotype (ability to grow without the amino acid proline added to the growth medium). You find that all of the resulting merodiploids are no longer able to grow on raffinose as the only carbon source. Using this information, narrow down your choices from part (**a**) – which Lac operon mutations might you have isolated?

(c) You now redo your original genetic screen from the introduction to this question, but using a different starting strain. Using an *E. coli* strain with $ProA^-$ Lac^+ on the chromosome that carries an F' Pro^+ Lac^+, you isolate a new collection of mutants that can grow on raffinose as the only carbon source. Using this information, narrow down your choices from part (**a**) – which Lac operon mutations might you have isolated in this new screen?

(d) You mate one of the mutant strains from part (**c**) to a strain that is $ProA^-$ Lac^+ and does not contain the F factor. You select for the Pro+ phenotype in order to ensure that the F' factor was transferred. The resulting merodiploids that you isolate are *not* able to grow on raffinose as the only carbon source. Where was the original mutation that allowed growth on raffinose located – on the F' plasmid or on the bacterial chromosome of the mutant strains from part (**c**)?

11. You are studying the regulation of ubiquinone synthesis in bacteria. The Ubi1 gene encodes a key enzyme in the pathway for ubiquinone synthesis. In order to study the regulation of the Ubi1 gene transcription, you construct a reporter gene construct by inserting the LacZ gene into the coding sequence for the Ubi1 gene (this hybrid gene is designated P_{ubi1}–LacZ). You find that ß-galactosidase is expressed at a high level when ubiquinone is *absent* from the growth medium, but ß-galactosidase is not expressed when ubiquinone is *present*. You find a mutation designated A^-, which gives constitutive ß-galactosidase expression from the P_{ubi1}–LacZ reporter gene construct. Moreover, you find that A^- is closely linked to the Ubi1 gene. You have an F' plasmid that carries the Ubi1 gene along with its neighboring genes and regulatory sites. Using the F' plasmid, you carry out the following genetic tests:

	ß-galactosidase activity	
	–ubiquinone	+ubiquinone
$A^+ P_{ubi1}$–LacZ	+	–
$A^- P_{ubi1}$–LacZ	+	+
$A^- P_{ubi1}$–LacZ / F' A^+ $Ubi1^+$	+	–
$A^+ P_{ubi1}$–LacZ / F' A^- $Ubi1^+$	+	–

(a) Characterize the A^- mutation based on its genetic properties (dominant vs. recessive, cis-acting vs. trans-acting). Also propose a function for the regulatory component that is encoded by the wild-type A gene.

Next, you isolate a second regulatory mutation designated B^- that causes constitutive expression of ß-galactosidase from the P_{ubi1}–LacZ promoter fusion. You find that the B^- mutation is *not* linked to the Ubi1 gene. An F' plasmid is isolated that carries the region of a wild-type bacterial chromosome that is proximal to the B locus. Genetic tests reveal the following properties:

	ß-galactosidase activity	
	–ubiquinone	+ubiquinone
$B^+ P_{ubi1}$–LacZ	+	–
$B^- P_{ubi1}$–LacZ	+	+
$B^- P_{ubi1}$–LacZ / F' B^+	+	–

(b) Draw *two* different linear regulatory pathways showing the possible relationships between the two different regulatory factors encoded by A and B. For your answer, be sure to include the wild-type Ubi1 gene, A gene, B gene, and the small molecule ubiquinone.

(c) Why can't you use the A^- and B^- mutations you have isolated to distinguish between the two models you proposed in part **(b)**?

Next, you isolate an allele of the B gene that you call B^S. B^S causes uninducible expression of P_{ubi1}–LacZ. The genotype and phenotype of strains carrying the B^C mutation are as follows:

Genotype	Phenotype
B^S P_{ubi1}–LacZ / F' B^+	uninducible
B^S A^- P_{ubi1}–LacZ	constitutive

(d) Draw out the model from part (**b**) that is consistent with these new results.

(e) How might the B^S mutation alter the function of the B protein to give uninducible expression of the Ubi1 gene?

12. You have isolated a **Tn5** insertion in an otherwise wild-type *E. coli* strain that you think may be linked to the **Lac** operon. You grow **P1** phage on the strain with the **Tn5** insertion and use the resulting phage lysate to infect a **LacI⁻** bacterial strain. Among the resulting Kan^r transductants, 30% have constitutive Lac expression and 70% are regulated normally.

(a) What is the distance between LacI and the Tn5 insertion expressed as a cotranduction frequency?

(b) Next, you want to map the **Tn5** insertion described in part **(a)** relative to two different **LacI⁻** mutations (**LacI-1⁻** and **LacI-2⁻**). To do this you perform two reciprocal crosses. In the first cross, you grow P1 phage on a bacterial host that has the **Tn5** insertion and **LacI-1⁻**. The resulting phage lysate is then used to infect a **LacI-2⁻** strain. Among the Kan^r transductants, 99% are constitutive and 1% are regulated normally. For the second cross, you grow P1 phage on a bacterial host that has the **Tn5** insertion and the **LacI-2⁻** mutation. The resulting phage lysate is then used to infect a **LacI-1⁻** strain. In this experiment, all of the Kan^r transductants are constitutive.

Draw a genetic map showing the relative order of **Tn5**, **LacI-1** and **LacI-2**.

(c) Say that you wanted to isolate a **LacI-1⁻ LacI-2⁻** double mutant. Which cross from part **(b)** would be a better starting point to search for the desired double mutant?

(d) For each of the crosses (that is, the first and the second), there is a transductant class that you know is the result of a quadruple crossover. Give the phenotype (with respect to beta-galactosidase expression) of the quadruple crossover class from the first cross.

13. The codon for tryptophan is $^{5'}UGG^{3'}$.

(a) Write out the RNA sequence of the anti-codon portion of $tRNA^{trp}$, with the 5' and 3' ends of the RNA labeled.

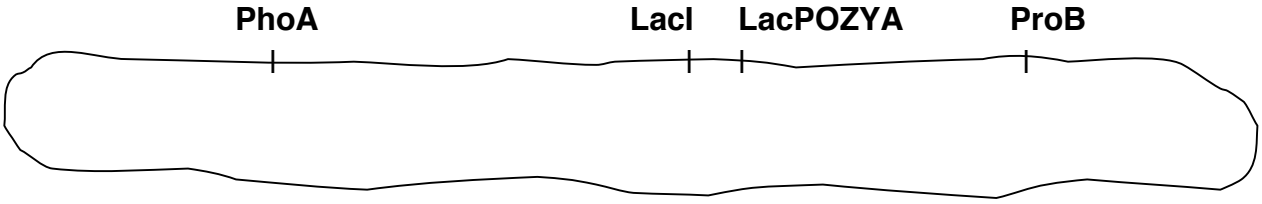
(b) Write out the double-stranded DNA sequence for the anti-codon portion of the gene that encodes $tRNA^{trp}$ (and label the 5' and 3' ends of each DNA strand).

(c) The sequence of the amber stop codon is $^{5'}UAG^{3'}$. You can isolate a mutant version of the gene encoding $tRNA^{trp}$ that encodes an amber-suppressing mutant version of $tRNA^{trp}$. Write out the RNA sequence of the anti-codon portion of this mutant amber-suppressing tRNA (and label the 5' and 3' ends of the RNA).

(d) You have a mutagen that can chemically modify the base guanine so that it can form base pairs with thymine. Thus this mutagen causes GC base pairs to become changed to AT base pairs. Will treatment of *E. coli* with this mutagen increase the probability of generating amber-suppressing mutant alleles of the gene encoding $tRNA^{trp}$?

(e) The sequence of the ochre stop codon is $^{5'}UAA^{3'}$. Which is more probable – mutating the gene encoding $tRNA^{trp}$ to become an ochre-suppressor or mutating it to become an amber-suppressor?

14. The region of the *E. coli* chromosome near the **Lac** operon is diagramed below:



You start with a strain that is $\mathbf{F^+\ PhoA^+\ Lac^+\ ProB^-}$, and then you isolate a derivative of this strain that, upon mating to an $\mathbf{F^-}$ recipient strain, can transfer $\mathbf{PhoA^+}$ efficiently but transfers $\mathbf{Lac^+}$ much less efficiently and only after long mating times.

(a) Draw a diagram of the **Hfr** that you have isolated showing where the **F** plasmid has inserted into the chromosome and the direction of the origin of transfer (using the symbol ◀).

(b) The **Hfr** described above is mated to an $\mathbf{F^-\ PhoA^-}$ strain. After 10 minutes of mating, a $\mathbf{PhoA^+}$ exconjugant strain is isolated. Will this new strain itself be able to transfer the $\mathbf{PhoA^+}$ marker to an $\mathbf{F^-\ PhoA^-}$ recipient strain? Explain why or why not.

Now you would like to introduce a **LacO^c** mutation into the **Lac** operon carried by the **Hfr** strain drawn in part **(a)**. To do this, you grow phage P1 on a **ProB$^+$ LacO^c** host, and then use the resulting phage lysate to infect the **Hfr** strain described above (genotype: **Hfr PhoA$^+$ Lac$^+$ ProB$^-$**), selecting for **ProB$^+$**.

(c) Describe a specific test that you could use to find strains that carry **LacO^c** among the **ProB$^+$** transductants.

(d) Given that **ProB** and **LacO** show linkage of 60% by cotransduction, how many **LacO^c** strains would you expect to find among 10 **ProB$^+$** transductants?

(e) From the transductant isolated in part (**c)** (genotype: **Hfr PhoA$^+$ LacO^c ProB$^+$**), you isolate an **F'** plasmid that can transfer both **LacO^c** and **ProB$^+$** early and efficiently. This **F'** strain is mated to an **F$^-$ LacZ$^-$ ProB$^-$** recipient to produce a strain with the following genotype: **PhoA$^+$ LacZ$^-$ ProB$^-$ / F' LacO^c ProB$^+$**. This strain shows constitutive **Lac** expression, but you are able to isolate a rare derivative of this strain that shows normal inducible **Lac** regulation. Draw a diagram showing how the strain with normal inducible **Lac** regulation could be produced. Your answer should show both the chromosome and **F'** plasmid in the starting strain (**PhoA$^+$ LacZ$^-$ ProB$^-$ / F' LacO^c ProB$^+$**), clearly indicating all relevant genetic loci. Any homologous recombination events should be indicated, as should the direction of the origin of transfer.

(f) Would the final strain in part **(e)** (that has inducible **Lac** regulation) be an **F$^-$**, **F$^+$**, **Hfr**, or **F'** bacterial strain?

15. For each of the two following subparts (one for the lac operon and one for the mal operon), predict the number of units of enzyme activity that will be displayed by a strain of the given genotype, grown under the given conditions.

(a) For the following merodiploid strains, determine the level ß-galactosidase expression in either the presence or absence of the inducer IPTG. Assume that, when no repressor is bound to DNA, 100 units of ß-galactosidase activity are produced from each functional copy of the **LacZ** gene. Assume that, when repressor is fully bound to DNA, only 1 unit of enzyme is produced for each functional copy of **LacZ**. The presence of **Lac I^{-d}** protein will fully prevent any other forms of the repressor in the same cell from binding to DNA. The **Lac I^{s}** protein binds to DNA but not to the inducer.

	ß-galactosidase activity –IPTG	+IPTG
Lac O^+ Z^+ / F' Lac O^c Z^-	_____	_____
Lac I^+ O^+ Z^+ Y^- / F' Lac I^- O^+ Z^+ Y^+	_____	_____
Lac I^+ O^c Z^+ / F' Lac I^- O^+ Z^+	_____	_____
Lac I^{-d} O^c Z^+ / F' Lac I^s P^- O^+ Z^+	_____	_____

(b) For the following merodiploid strains, determine the level maltase activity in either the presence or absence of the inducer maltose. Assume that, when the activator (**MalT**) is bound to DNA, 100 units of maltase activity are produced from each functional copy of the **MalQ** gene. Assume that, when no activator is bound to DNA, only 1 unit of enzyme is produced for each functional copy of **MalQ**. The **$MalT^C$** protein binds DNA regardless of whether maltose is present.

	maltase activity –maltose	+maltose
$MalT^-$ Q^+ / F' $MalT^+$ Q^-	_____	_____
$MalT^C$ Q^+ / F' $MalT^+$ Q^-	_____	_____
$MalT^C$ Q^- / F' $MalT^-$ Q^+	_____	_____

16. You are studying the regulation of methanol utilization in bacteria. Methanol oxidase, encoded by the **Mox** gene, is the key enzyme in the methanol utilization pathway. Methanol oxidase is expressed at high levels when methanol is present in the growth medium, but methanol oxidase is not expressed when methanol is absent. You find a mutation designated $\mathbf{A^-}$, which gives constitutive **Mox** expression and is closely linked to the **Mox** gene. You have $\mathbf{Mox^-}$ and $\mathbf{A^-}$ mutations as well as an **F'** plasmid that carries the **Mox** gene along with neighboring genes and regulatory sites. You carry out the following genetic tests:

	Methanol oxidase activity	
	– methanol	+ methanol
A^+ Mox^+	–	+
A^- Mox^+	+	+
A^- Mox^+ / **F'** A^+ Mox^+	–	+
A^- Mox^+ / **F'** A^+ Mox^-	–	+
A^- Mox^- / **F'** A^+ Mox^+	–	+

(a) Give as complete a description as you can of the properties of the $\mathbf{A^-}$ mutation (cis vs. trans, dominant vs. recessive, constitutive vs. uninducible), and propose a molecular function for the regulatory component that is encoded by the wild-type **A** gene.

Next, you isolate two regulatory mutations that are not linked to **Mox** but that are very closely linked to each other. You call these mutations $\mathbf{B1^-}$ and $\mathbf{B2^-}$. An **F'** plasmid is isolated that carries the region of the chromosome where the **B** mutations lie. Genetic tests reveal the following properties:

	Methanol oxidase activity	
	– methanol	+ methanol
$B1^-$ Mox^+	+	+
$B2^-$ Mox^+	–	–
$B1^-$ Mox^+ / **F'** B^+	–	+
$B2^-$ Mox^+ / **F'** B^+	–	–

(b) Why can't you use a complementation test to determine whether the **B1⁻** and **B2⁻** mutations lie in the same gene?

(c) Assuming that the **B1⁻** and **B2⁻** mutations are in fact in the same gene, propose a molecular function for the regulatory component encoded by the wild-type **B** gene.

(d) Describe how the **B1⁻** and **B2⁻** mutations affect the regulatory function encoded by the **B** gene, being as specific as possible.

(e) Draw two different linear genetic pathways showing the possible relationships between the two different regulatory factors encoded by the wild-type **A** and **B** genes. For your answer, be sure to include the **Mox** gene and to indicate where and how methanol is acting.

(f) To distinguish the two models from part **(e)**, you construct an **A⁻ B2⁻** double mutant. Why is it better to choose the **B2⁻** rather than the **B1⁻** allele for this double mutant epistasis test?

You find that the **A⁻ B2⁻** double mutant has the following behavior:

	Methanol oxidase activity	
	– methanol	+ methanol
A^- $B2^-$ Mox^+	–	–

(g) Draw a final linear genetic pathway showing the interactions between the different regulatory factors encoded by the wild-type **A** and **B** genes. Be sure to include the **Mox** gene and to indicate where and how methanol acts.

17. Phage T4 expresses an enzyme lysozyme, which enables the phage to lyse infected bacterial cells. Mutations in the lysozyme gene can prevent T4 from forming plaques on a lawn of *E. coli* bacteria. You have isolated two T4 single mutants that cannot make plaques on wild-type (**Su$^-$**) bacteria, but that can make plaques on an *E. coli* strain carrying a mutant form of a tRNA gene that encodes a UGA nonsense suppressor tRNA (**Su$^+$**).

(a) The two phage single mutants are coinfected into a **Su$^+$** host bacterial strain so that each bacterial cell receives at least one phage of each type. The resulting phage lysate produced from this coinfection will form 10^7 plaques/ml when plated on a **Su$^+$** bacterial host, but will only form 5 x10^4 plaques/ml when plated on a **Su$^-$** bacterial host. What is the distance between the sites of the two phage lysozyme mutations, in map units?

(b) The size of the normal phage lysozyme protein is 45 kDa. One of the single mutants makes a lysozyme fragment that is 20 kDa, while the other makes a fragment that is 31 kDa. Using 0.11 kDa as the average mass of an amino acid, and knowing that the total genetic length of the phage T4 chromosome is 400 map units, estimate the physical length of phage T4 DNA in base pairs.

(c) Suppose that both T4 phage single mutants (which can grow on an *E. coli* strain carrying a UGA nonsense suppressor) were generated by a mutagen that causes C•G to T•A mutations. Using the genetic code table, determine the possible codon(s) in the wild-type T4 lysozyme gene that could have been mutated to produce the phage mutants. (For each of your answers, show both strands of the wild-type DNA segment that would encode the codon that is the site of one of the single mutations in the lysozyme gene. Indicate the 5' and 3' ends of each strand, and indicate which strand is used as the <u>template</u> in transcription to produce lysozyme mRNA.)

18. You have isolated a **Tn5** insertion in an otherwise wild-type *E. coli* strain; this transposon is near to but not within the group of lac genes on the *E. coli* chromosome. You grow **P1** phage on the *E. coli* strain with the **Tn5** insertion, and use the resulting phage lysate to infect a **LacZ$^-$** *E. coli* strain. You select for Kanamycin resistance. Among the resulting Kan^r transductants, 40% have no ß-galactosidase activity and 60% express ß-galactosidase normally.

(a) What is the distance between the **Tn5** insertion and **LacZ**, expressed as a cotransduction frequency?

You grow **P1** phage on one of the Kan^r transductants isolated in part **(a)** that is **LacZ$^-$**. You use the resulting phage lysate to infect a **LacI$^-$** mutant *E. coli* strain, and then isolate 1,000 Kan^r transductants. For each transductant, you assay both ß-galactosidase activity (**LacZ**) and Lac permease activity (**LacY**) in the presence or absence of inducer.

(b) In the table below fill in the **Lac** genotypes (at the **LacZ**, **LacY**, and **LacI** loci) of the different classes of transductants.

Number of transuctants	ß-galactosidase	permease	Genotype
578	uninducible	regulated	_____
400	constitutive	constitutive	_____
20	uninducible	constitutive	_____
2	regulated	regulated	_____

(c) What is the distance between the **Tn5** insertion and the **LacI** gene, expressed as a cotransduction frequency?

(d) Draw a genetic map showing the relative order of **Tn5**, **LacZ**, and **LacI**.

19. An enzyme that you are interested in from *E. coli* is regulated by the following scheme:
Protein **A** is a transcriptional repressor of the gene encoding your enzyme, and protein **B** is a transcriptional repressor of the gene encoding **A**. **B** is active as a repressor only when it is bound to the inducer molecule. When the inducer is absent, **B** will not bind to its operator sequence, so **A** will be expressed, and the transcription of the gene encoding your enzyme will be repressed.

(a) Diagram this pathway as you have learned to diagram genetic pathways in class. Be sure to include the inducer, and the wild-type genes that encode your enzyme, **A**, and **B**.

(b) An allele of the **B** gene ($\mathbf{B^*}$) is isolated that binds to DNA and represses regardless of whether inducer is present or not. A deletion of the operator site in front of the **A** gene ($\mathbf{O^-_A}$) is isolated that will not bind the **B** repressor. In the table below, indicate for each strain whether the enzyme will be synthesized with or without inducer (using the format "yes" or "no").

	– inducer	+ inducer
$\mathbf{B^*}$		
$\mathbf{O^-_A}$		
$\mathbf{B^* O^-_A}$		

(c) An allele of the **A** gene is isolated that prevents the **A** repressor from binding to DNA and, in a heterozygous merodiploid strain, will also actively prevent wild-type **A** protein from binding DNA. This allele is called $\mathbf{A^*}$. Indicate in the table below where the enzyme will be synthesized (using the format "yes" or "no").

	– inducer	+ inducer
$\mathbf{A^*}$		
$\mathbf{A^*/ F' A^+}$		
$\mathbf{O^+_A\ A^*/ F' O^-_A\ A^+}$		

20. In order to study regulation of starch degradation in *E. coli*, you isolate a **Tn5::LacZ** insertion in the gene for the starch-degrading enzyme amylase. This insertion disrupts the gene encoding amylase, and also inserts the reporter gene into this gene, such that ß-galactosidase is now only expressed when starch is present in the growth medium. You isolate two mutations (**sta1⁻** and **sta2⁻**) that cause altered regulation of the **Tn5::LacZ** reporter. The **sta1** locus is unlinked to the **Tn5::LacZ** insertion, and the **sta1⁻** mutation causes the recessive phenotype of uninducible ß-galactosidase expression. The **sta2** locus is linked to the **Tn5::LacZ** insertion (90% cotransduction), and the **sta2⁻** mutation causes the recessive phenotype of constitutive ß-galactosidase expression. You put an **F' sta2+** plasmid into your transposon-containing **sta2⁻** strain, and find that this new merodiploid strain gives regulated reporter gene expression.

In a transduction experiment, you grow **P1** phage on a strain carrying the **Tn5::LacZ** insertion and the **sta2⁻** mutation. You use the resulting phage lysate to infect a **sta1⁻** mutant (which does not carry the **Tn5::LacZ** insertion). Some of the resulting Kan^r transductants express ß-galactosidase constitutively, and some have uninducible expression. Construct a model to explain amylase regulation that is consistent with all of this information. In your model, include starch itself, and the wild-type **Sta1**, **Sta2**, and amylase genes.

21. The following sequence (and some of the encoded amino acids) lies within the coding sequence of a wild-type *E. coli* gene, "gene X":

```
...CTC TCT TTC ATG ACT AGG GGG GGG TAA GCT AA...
...leu ser phe met...
```

A mutant *E. coli* strain is isolated that has an additional A residue giving the sequence:

...CTC TCT TTC ATG AC**A**T AGG GGG GGG TAA GCT AA...

Describe a possible suppressor mutation (that is not simply the back mutation) that might revert the defect of the mutation shown above. Choose an **INTRAGENIC** suppressor for parts **(a)** – **(c)**. Have the suppressor mutation affect only 1 nucleotide.

(a) Show the exact DNA sequence of the segment of the mutant gene that causes the suppression. State which gene this change would take place in.

(b) Give the amino acid sequence that would be encoded by the mutant "gene X" sequence in a strain which contains both the original mutation and the suppressor mutation.

(c) Mention what stipulations would be necessary for this double mutant strain to still produce fully functional protein product.

Describe a possible suppressor mutation (that is not simply the back mutation) that might revert the defect of the mutation shown above. Choose an **EXTRAGENIC** suppressor for parts **(d)** – **(f)**. Have the suppressor mutation affect only 1 nucleotide.

(d) Show the exact DNA sequence of the segment of the mutant gene that causes the suppression. State which gene this change would take place in.

(e) Give the amino acid sequence that would be encoded by the mutant "gene X" sequence in a strain which contains both the original mutation and the suppressor mutation.

(f) Mention what stipulations would be necessary for this double mutant strain to still produce fully functional protein product.