Problem set questions from Exam 2 Unit – Mutations, Bacterial Genetics, and Bacterial Gene Regulation

Central Dogma, Mutagens and Mutations

1. The three stop codons in the genetic code are 5'UAG3', 5'UAA3', and 5'UGA3'.

(a) The sequence of the ochre stop codon is $^{5'}$UAA$^{3'}$. One can isolate bacterial strains that carry mutations in genes encoding tRNAs such that they will encode mutant tRNAs that recognize the ochre stop codon (as opposed to wild-type tRNAs, which recognize one of the 61 non-stop codons). You are trying to isolate single mutations in tRNA genes that will suppress an ochre mutation. To increase the frequency of such mutations, you use a mutagen that produces transition mutations (i.e. C•G to T•A and T•A to C•G base changes). Which tRNA genes could in principle be altered by the mutagen to give the desired suppressor mutation?

(b) For each answer you gave in part **(a)**, write out the DNA sequence of the part of the wild-type gene encoding the wild-type tRNA that encodes for the anti-codon portion of that tRNA. (Label the 5' and 3' ends of both DNA strands, and indicate which strand is used as the *template* during transcription of the tRNA).

(c) For each answer you gave in part **(a)**, write out the DNA sequence of the part of the mutant gene encoding the ochre-suppressing mutant tRNA that encodes for the anti-codon portion of that tRNA. (Label the 5' and 3' ends of both DNA strands, and indicate which strand is used as the *template* during transcription of the tRNA).

2. You are studying an *E. coli* gene that encodes an enzyme of interest to you. You have isolated a mutant allele of this gene in which an amber nonsense mutation lies in the middle of the gene. This mutant allele leads to production of a truncated form of the enzyme.

(a) By placing a +1 frameshift mutation a short distance before the amber mutation and a –1 frameshift mutation a short distance after the amber mutation, you create a triple mutant form of the gene that restores expression of a functional full-length enzyme. Explain how this could be.

(b) You isolate a second mutant allele of this gene that has a +1 frameshift mutation inserted near the middle of the gene. This allele produces non-functional enzyme. You isolate intragenic suppressors of the +1 frameshift mutation, thereby acquiring double mutant forms of the gene that now do again produce functional enzyme. You find that a number of these intragenic suppressor mutations are different –1 frameshift mutations a short distance *after* the +1 frameshift mutation. But to your surprise, you find that –1 frameshift mutations placed a short distance *before* the +1 frameshift mutation lead to expression of a truncated enzyme. Propose a mechanism to explain these results.

(c) Organisms that live in environments exposed to light often have a relatively low proportion of A•T base pairs in their DNA. (This is probably because neighboring thymine residues are particularly sensitive to reaction to UV light.) The genes in such organisms tend to be biased for codons that contain G•C rather than A•T base pairs. As it turns out, it is much easier to predict where the coding sequences (open reading frames) are in organisms with a low A•T content in their DNA. To see why this is so, calculate the probability of finding an open reading frame that is 50 codons long by chance in a genome containing equal frequencies of A•T and G•C base pairs. Compare this to the frequency of finding an open reading frame of the same length in a genome that is made up of 40% A•T and 60% G•C base pairs.

3. One way to isolate nonsense suppressor mutations in tRNA genes is to select for the simultaneous suppression of the mutant phenotype of a strain carrying nonsense mutations in two different genes. This selection works to isolate nonsense-suppressing mutant alleles of tRNA genes, because it is extremely unusual to get intragenic suppressor mutations in the two different genes mutated in the original strain at the same time. The *E. coli* **his1** and **his2** genes are required for synthesis of the amino acid histidine, and strains harboring mutations in either gene will not grow unless histidine is provided in the growth medium.

If you wanted to isolate a nonsense suppressor mutation in a gene encoding a tRNA by the method described above, explain why it would be a bad idea to start with an original strain that has an amber mutation (TAG) in the **his1** gene and an ochre mutation (TAA) in the **his2** gene.

4. Consider a phage gene that encodes the enzyme lysozyme. The lysozyme protein has a molecular mass of 60 kDa (kilodaltons). You have isolated a small collection of mutants in the lysozyme gene that fail to produce a functional lysozyme enzyme. Taking advantage of the fact that you can detect the lysozyme protein from an extract of phage infected cells, you determine the size of the lysozyme proteins expressed by each mutant.

Wild type	60 kDa
Mutant 1	60 kDa
Mutant 2	15 kDa
Mutant 3	20 kDa
Mutant 4	50 kDa

(a) In genetic crosses, you find that Mutant 1 and Mutant 2 lie very close to one another. List the types of nucleotide sequence changes that could have produced Mutant 1, and the types of changes that could have produced Mutant 2.

(b) When Mutant 2 phage are crossed to Mutant 3 phage (by coinfecting *E. coli* with these two different types of mutant phage), only 2 out of 1000 of the progeny phage in the resulting phage lysate make wild-type lysozyme. What frequency of wild-type progeny would you expect from a cross of Mutant 3 phage with Mutant 4 phage?

(c) Frameshift mutations can be classified as follows: addition of one base = +1, deletion of one base = –1, addition of two bases = +2, deletion of two bases = –2, etc. Within the lysozyme gene, you find a +1 frameshift mutation that can be combined with a nearby –1 frameshift to produce a double mutant that makes functional lysozyme. But when a +2 frameshift and a –2 frameshift at the same positions as the +1 and –1 frameshifts are combined, you find that functional lysozyme is not produced. Explain.

(d) Assume that Mutant 2 contains a +1 frameshift in the lysozyme gene, and that Mutant 3 phage contains a –1 frameshift in the lysozyme gene. Without knowing anything else about the sequence of the lysozyme gene, calculate the probability that full-length lysozyme will be produced from a double mutant form of the lysozyme gene that contains both the +1 and the –1 frameshifts. (For your calculation assume the average mass of an amino acid to be 110 daltons. Also please note that, by full-length lysozyme, we mean a protein of 60 kDa that may or may not be functional lysozyme protein.)

5. You are trying to isolate mutations in the gene encoding $tRNA^{trp}$ that will produce mutant forms of this tRNA that will recognize a stop codon, as opposed to the normal trp codon.

(a) Write out the RNA sequence of the anticodon segment of wild-type $tRNA^{trp}$. Be sure to note the 5' and 3' ends. Also remember that in RNA, U (uracil) takes the place of T (thymine).

(b) Write out the DNA base pairs from the gene encoding $tRNA^{trp}$ that code for the anticodon segment of wild-type $tRNA^{trp}$. Show both DNA strands, each with the 5' and 3' ends labeled.

(c) The mutagen hydroxylamine will deaminate cytosine in DNA to produce uracil. Upon DNA replication of a DNA strand containing a uracil, DNA polymerase places A (adenine) across from uracil. Thus, hydroxylamine converts a GC basepair to an AT basepair. You use hydroxylamine to generate single, double, and triple mutations in the gene encoding $tRNA^{trp}$. Write out the mutant forms of the $tRNA^{trp}$ gene that can be produced via hydroxylamine mutagenesis. Show both DNA strands, each with the 5' and 3' ends labeled.

(d) Write out the RNA sequence of the anticodon segment of mutant $tRNA^{trp}$ molecules that could be produced from the mutant genes you have drawn in part **(c)**. Be sure to note the 5' and 3' ends. Also remember that in RNA, U (uracil) takes the place of T (thymine).

(e) Which, if any, of the three nonsense codons could in principle be recognized by a mutant tRNA produced from a <u>single</u> hydroxylamine-generated mutation in the $tRNA^{trp}$gene?

Transposons and Cotransduction Mapping in bacteria (Moving DNA between bacterial cells by transduction [using phage])

1. You have isolated two *E. coli* mutants in the PyrF gene, called PyrF-1 and PyrF-2. These mutants require a pyrimidine nucleotide (such as uracil) to be added to the medium in order to grow; this phenotype is referred to as Pyr$^-$.

In order to work with these mutants, you wish to isolate a **Tn5** insertion that is linked to the PyrF locus. To do this, you start with a collection of 1000 different *E. coli* strains, each harboring one random **Tn5** insertion in an otherwise wild-type *E. coli* genome. (These insertion strains are all Kan^r and Pyr+.) You grow **P1** phage on the entire mixed collection of **Tn5** insertion strains and then infect a PyrF-1 mutant and select for Kan^r transductants. Most of the Kan^r transductants remain Pyr$^-$, but one out of 1500 is Pyr+.

(a) What do you know about the position of the **Tn5** insertion in this rare Pyr+ transductant? What is the maximum distance away that it could be from the PyrF-1 locus?

Next you grow **P1** phage on the Kan^r Pyr+ transductant isolated above, and then use the resulting phage lysate to infect the PyrF-1 mutant bacterial strain. You select for Kan^r transductants and then test these transductants for the ability to grow without pyrimidine added to the growth medium. You find that 30 out of 100 Kan^r transductants are Pyr+.

(b) Give the distance between the **Tn5** insertion and PyrF-1, expressed as a cotransduction frequency.

The **Tn5** insertion shows about the same linkage to PyrF-2 as to PyrF-1. You construct a strain that has both the **Tn5** insertion and PyrF-1 and another strain that has both the **Tn5** insertion and PyrF-2. Using these strains you perform two reciprocal crosses.
In the first cross, **P1** phage is grown on the **Tn5** PyrF-1 strain, and the resulting phage lysate is used to infect a PyrF-2 strain. In this transduction experiment, 7 out of 1000 Kan^r transductants are Pyr+.
In the reciprocal cross, **P1** phage is grown on the **Tn5** PyrF-2 strain, and the resulting phage lysate is used to infect a PyrF-1 strain. In this experiment, 50 out of 1000 Kan^r transductants are Pyr+.

(c) Draw a map showing the relative order of the **Tn5** insertion, PyrF-1 and PyrF-2.

2. The *E. coli* **ser1** gene is required for synthesis of the amino acid serine, and strains harboring mutations in this gene will not grow unless serine is provided in the growth medium. Say that you have a strain with an amber mutation in the **ser 1** gene. After mutagenesis with the mutagenic chemical EMS, you select for double-mutants that revert back to having a **ser$^+$** phenotype (the ability to grow on medium without supplemental serine). All of your double mutant strains had the original amber mutation in **ser1**, but then each double mutant has an additional second mutation somewhere in the genome. In some double mutant strains, this second mutation might be in the **ser1** gene. In other double mutant strains, this second mutation might be in a gene encoding a tRNA, and the mutation might cause the production of an amber-suppressing mutant form of a tRNA.

(a) Explain why it would be very unlikely to acquire an intragenic suppressor mutation in the **ser1** gene that is at a site in the gene that is different from the site of the original mutant amber codon.

In order to distinguish between your isolation of intragenic suppressor mutations and extragenic suppressor mutations, you decide to isolate a **Tn5** insertion linked to the **ser1** gene. To do this, you start with a collection of 1000 different *E. coli* strains. Each *E. coli* strain contains one random **Tn5** insertion in an otherwise wild-type *E. coli* chromosome. These insertion strains are all kanamycin resistant (Kan^r) and **ser$^+$**. You grow **P1** phage on a mixture of the entire collection of 1000 **Tn5** insertion strains, and then use the resulting phage lysate to infect a **ser1$^-$** mutant strain. You select for Kan^r transductants. Most of the Kan^r transductants are **ser$^-$**, but one out of 1300 transductants is **ser$^+$**.

(b) Next you grow **P1** phage on this **ser $^+$** Kan^r transductant isolated above, and use the resulting lysate to infect the original **ser1$^-$** mutant. After selecting for Kanamycin resistance, you test these Kan^r transductants for their ability to grow in the absence of serine. You find that among 100 Kan^r transductants, 60 are **ser$^-$** and 40 are **ser$^+$**.

Give the distance between the **Tn5** insertion and **ser1**, expressed as a cotransduction frequency.

(c) Next you choose one of the **ser$^+$** double mutants that you isolated in the introduction to this question, in order to test for the presence of a suppressor mutation. To do this, you grow **P1** phage on one of the **ser$^-$** Kan^r transductants isolated in part (**b**). You then use the resulting phage lysate to infect the **ser$^+$** double mutant strain, and select for Kan^r transductants. Out of 100 Kan^r transductants, all are **ser$^+$**. What does this result tell you about the whether the **ser$^+$** double mutant strain contained an intragenic suppressor mutation or an extragenic suppressor mutation? Explain your logic.

(d) Next you choose a second **ser$^+$** double mutant strain, in order to test for the presence of a suppressor mutation. You use the same **P1** phage lysate generated in part **(c)** to infect the second **ser$^+$** double mutant strain, and select for Kan^r transductants. Out of 100 Kan^r transductants, 45 are **ser$^-$** and 55 are **ser$^+$**. What does this result tell you about the whether the **ser$^+$** double mutant strain contained an intragenic suppressor mutation or an extragenic suppressor mutation?

(e) Is the cotransduction frequency obtained in part **(d)** consistent with the cotransduction frequency obtained in part (**b**)? Explain.

3. In a transduction experiment, phage P1 is grown on a bacterial host of genotype A$^+$ B$^+$ C$^+$ and the resulting lysate is used to infect a recipient strain of genotype A$^-$ B$^-$ C$^-$. Transductants are obtained by selecting for the A$^+$ phenotype.
The genes are in an order such that B is in the middle, and the distance between A and B is greater than the distance between B and C. Based on this information, state whether each of the following statements is true or false, and why.

(a) If none of the A$^+$ transductants were also C$^+$, then the distance between A and C would be greater than about 100 kbp.

(b) The cotransduction distance between A and B can be obtained from the fraction of A$^+$ transductants that are A$^+$ B$^+$ C$^+$ and A$^+$ B$^+$ C$^-$.

(c) It is possible that the cotransduction distance between A and C could be 0%.

(d) The number of A$^+$ transductants that are B$^-$ and C$^+$ will be much greater than the number of A$^+$ transductants that are B$^+$ and C$^-$.

4. You have used mutagenesis with the chemical EMS to isolate four different *E. coli* mutants that will not grow unless the amino acid histidine is provided in the growth medium. You label these mutants **his1⁻**, **his2⁻**, **his3⁻**, and **his4⁻**.

(a) In order to test for linkage between **his1⁻** and the other three **his⁻** mutants, you set out to isolate a **Tn5** insertion linked to the **his1⁻** mutant. To do this, you start with a collection of 1000 different *E. coli* strains, each of which contains one random **Tn5** insertion in the otherwise wild-type *E. coli* genome. (These insertion strains are all kanamycin resistant (Kan^r) and **his⁺**.) You grow **P1** phage on a mixture of the entire collection of **Tn5** insertion strains, and then use the resulting phage lysate to infect the **his1⁻** mutant. You select for Kan^r transductants. Most of the Kan^r transductants are **his⁻**, but one out of 2000 is **his⁺**.

Explain how this **his⁺** transductant arose.

(b) Next you grow **P1** phage on the **his⁺** transductant isolated in part **(a)** above, and use the resulting phage lysate to infect the original **his1⁻** mutant. After selecting for Kan^r transductants, you test these transductants for their ability to grow in the absence of histidine supplemented in the growth medium. You find that among 100 Kan^r transductants, 20 are **his⁻** and 80 are **his⁺**.

Give the distance between the **Tn5** insertion and **his1**, expressed as a cotransduction frequency.

(c) The same **P1** phage lysate generated in part **(b)** above is used to infect either a **his2⁻** mutant or a **his3⁻** mutant, and Kan^r transductants are isolated. For the infections of the **his2⁻** and the **his3⁻** mutants, none of the Kan^r transductants are **his⁺** (even though you examine hundreds of Kan^r transductants from each transduction experiment). What does this tell you about the relationship between the **his1⁻** mutation and the **his2⁻** and **his3⁻** mutations, and why?

(d) How would you determine whether the **his2⁻** and **his3⁻** mutations are likely to be alleles of the same gene?

(e) The **P1** phage lysate generated in part **(b)** is used to infect a **his4⁻**mutant, and Kan^r transductants are isolated. Among 100 Kan^r transductants examined, 21 are **his⁻** and 79 are **his⁺**. What does this tell you about the relationship between the **his1⁻** and the **his4⁻** mutations, and why?

(f) Using the procedure outlined above, you construct one strain that has both the **Tn5** insertion and **his1⁻**, and another strain that has both the **Tn5** insertion and **his4⁻**. Using these strains you perform two reciprocal crosses.
In the first cross, **P1** phage is grown on the **Tn5 his1⁻** strain, and the resulting phage lysate is used to infect a **his4⁻** strain. In this transduction experiment, 10 out of 500 Kan^r transductants are **his⁺**. In the reciprocal cross, **P1** phage is grown on the **Tn5 his4⁻** strain, and the resulting phage lysate is used to infect a **his1⁻** strain. In this experiment, 1 out of 500 Kan^r transductants are **his⁺**.

Draw a map showing the relative order of the **Tn5** insertion, **his1⁻** and **his4⁻**.

(g) Are the **his1⁻** and **his4⁻** mutations two different alleles of the same gene, or alleles of two different genes?

5. Wild-type *E. coli* have flagella that allow them to swim towards nutrient sources. Non-motile mutants are easily detected because wild-type *E. coli* makes colonies with diffuse edges when plated on soft agar, whereas nonmotile mutants make compact colonies. You have isolated two non-motile mutants that you call **mot1⁻** and **mot2⁻**.

(a) At this stage, why is it not feasible to carry out crosses between **mot1⁻** and **mot2⁻** bacteria by using **P1** phage transduction to try to determine whether the **mot1** and **mot2** loci are linked?

In order to remedy the problem outlined in part **(a)**, you set out to isolate a **Tn5** insertion linked to one of the **mot** loci. To do this, you start with a collection of 1000 different bacterial strains, each one of which harbors a single random **Tn5** insertion in an otherwise wild-type *E. coli* genome. (These insertion strains are all kanamycin resistant (Kan^r) and motile (mot⁺).) You grow **P1** phage on the entire mixed collection of **Tn5** insertion strains, and then use the resulting phage lysate to infect the **mot1⁻** mutant and select for Kan^r transductants. Most of the Kan^r transductants remain non-motile, but one out of 3000 is motile.

Next you grow **P1** phage on the motile transductant isolated above, and then use the resulting phage lysate to infect the **mot1⁻** mutant bacteria. You select for Kan^r transductants and then test these transductants for motility. You find that 60 out of 100 Kan^r transductants are motile.

(b) Give the distance between the **Tn5** insertion and **mot1**, expressed as a cotransduction frequency.

(c) By sequencing the bacterial DNA neighboring the transposon insertion, you determine that the **Tn5** insertion lies about 50 kbp from the **PhoS** gene (which is required for phosphate sensing). A headful of DNA for phage **P1** is 100 kbp. In transduction experiments, you find that the **Tn5** insertion and the **PhoS** gene show 50% cotransduction, but **PhoS** and **mot1** are not linked by P1 phage transduction. Explain.

You find that the **Tn5** insertion shows about the same linkage to **mot2** as to **mot1**, and that mot2 and PhoS are not linked. You construct a strain that has both the **Tn5** insertion and **mot1⁻** and another strain that has both the **Tn5** insertion and **mot2⁻**. Using these strains, you perform two reciprocal crosses.
In the first cross, **P1** phage is grown on the **Tn5 mot1⁻** strain, and the resulting phage lysate is used to infect a **mot2⁻** strain. In this transduction experiment, all 1000 Kan^r transductants are non-motile.
In the reciprocal cross, **P1** phage is grown on the **Tn5 mot2⁻** strain, and the resulting phage lysate is used to infect a **mot1⁻** strain. In this experiment, 920 out of 1000 Kan^r transductants are non-motile.

(d) Draw a map showing the relative order of the **Tn5** insertion, **mot1, mot2**, and PhoS.

F plasmids, Hfrs, F' plasmids (Moving DNA between bacterial cells by conjugation [using mating])

1. The region of the *E. coli* chromosome surrounding the Lac operon contains the markers PhoA — Lac I – LacZ,Y,A — ProB, in that order. Lac and PhoA are about 50 kb apart, and Lac and ProB are about 80 kb apart. You have isolated an F' plasmid from an F+ $LacZ^-$ strain that contains the entire Lac region but does not contain either PhoA or ProB. In order to identify different Hfr strains that come from this F' strain, you mate 100 individual colonies of a $PhoA^+$ Lac^+ $ProB^+$ / F' $LacZ^-$ strain to an F^- $PhoA^-$ $LacZ^-$ $ProB^-$ recipient. You screen the exconjugates for $PhoA^+$ or $ProB^+$. Out of the 100 exconjugant colonies tested, 3 have the ability to transfer $ProB^+$ efficiently, but none have the ability to transfer $PhoA^+$ efficiently.

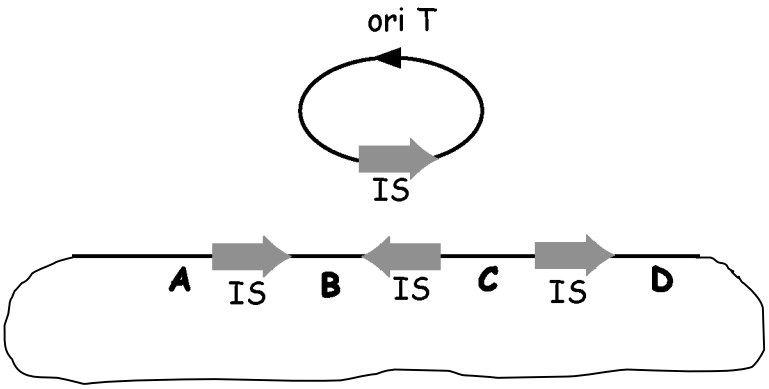
(a) Draw the structure of the F' plasmid contained in your $PhoA^+$ Lac^+ $ProB^+$ / F' $LacZ^-$ strain, showing the orientation of the origin of transfer relative to the orientation of the Lac operon.

(b) You next move the F' plasmid drawn in part **(a)** into a strain that is F^- $PhoA^+$ $LacO^c$ $ProB^+$. Because of the $LacO^c$ mutation, this new F' strain expresses ß-galactosidase constitutively. From this strain, you isolate a rare derivative that shows normal inducible regulation of ß-galactosidase. Will this derivative transfer the inducible Lac operon early or late?

(c) To explain your answer to part **(b)**, diagram the recombination event occurring between the F' plasmid and the bacterial chromosome that gives the normally regulated ß-galactosidase. Make sure to indicate the position of all of the bacterial genes mentioned in this problem, and the origin of transfer.

(d) If you were interested in studying the effects of a newly isolated lac mutation on the regulation of LacZ, what kinds of genetic tests could the F' plasmid from part **(a)** be used for? (Your choices are: cis/trans test, epistasis test, constitutive/uninducible test, complementation test.)

2. The diagram below shows the F factor plasmid, and a portion of the *E. coli* chromosome that contains three different insertion sequences (IS) of the same type as that which is carried on the F plasmid. The position of four genes on the *E. coli* chromosome (**A**, **B**, **C**, **D**) is shown. Note that the drawing is not to scale.



(a) Draw a diagram of the chromosomes of three different Hfrs that can be formed by recombination between the IS sequence on the F plasmid and an IS sequence on the bacterial chromosome. For your answer, include the positions of each of the chromosomal genes (**A**, **B**, **C**, and **D**). Also include the correct orientations of the IS sequence and origin of transfer (ori T) from the F plasmid, as well as the orientations of the IS sequences in the bacterial chromosome.

(b) For each of the three Hfrs you have drawn in part **(a)**, state which of the four bacterial genes (**A**, **B**, **C**, and **D**) would be transferred early to an F- bacterial donor cell.

(c) For each to the three Hfrs in part **(a)**, consider the outcome of further crossovers between IS sequences flanking the integrated F factor that could result in the formation of F' plasmids. For each of the three Hfrs, draw all possible F' plasmids that can be formed from such further crossover events.

(d) For each of the F' plasmids you have drawn in part **(c)**, state which of the four bacterial genes (**A**, **B**, **C**, and **D**) would be transferred to an F- bacterial donor cell.

3. Transposons are not only useful as portable genetic markers, they can also serve as portable regions of homology for recombination. In this problem we will see how a **Tn5** insertion can be used to construct an **F'** plasmid with desired characteristics. These methods rely on the use of a special **F** factor that carries an insertion of **Tn5** (this factor is designated **F::Tn5**).

(a) You start with an *E. coli* strain containing a **Tn5** insertion that is linked to one gene, **mot1**, that is required for bacterial swimming (motility), and is linked to a second gene, **PhoS**, that is required for phosphate sensing. Note that **mot1** and **PhoS** are not linked to each other by cotransduction.
You introduce **F::Tn5** into this strain. An **Hfr** can then be isolated by selection for a strain that can transfer **PhoS** early in a mating experiment. Given that the **Hfr** arose by homologous recombination between the **Tn5** on the **F** factor and the **Tn5** on the chromosome, draw a diagram showing the recombination event between **F::Tn5** and the chromosome that would form this Hfr. For your answer, include the location of **PhoS** and **mot1** as well as the relative orientations of the two **Tn5** elements and the origin of transfer on **F::Tn5**.

(b) Draw out the resulting Hfr, showing all of the elements you showed in part **(a)**.

4. You are given a double mutant *E. coli* strain that you know contains an F' plasmid that carries the Lac genes, but you don't know precisely which alleles of these Lac genes are on the chromosome of the strain, or on the F' contained in the strain.

(a) First you test the Lac phenotype of the strain and find that it expresses ß-galactosidase constitutively. Next you set up a mating of your strain to an F^- strain that has a chromosomal deletion of the Lac genes -- you find that the strains that received the F' Lac expresses ß-galactosidase in a normally-regulated fashion. Given these observations, propose what the two lac mutations in this original strain were, and whether they were on the chromosome or on the F' plasmid. List all possibilities.

(b) An F' plasmid carrying a segment of chromosomal DNA can occasionally recombine with the homologous chromosomal sequences to produce an Hfr strain. Starting with the F' strain that you were given originally in the introduction to this problem, you isolate a number of derivatives that have become Hfrs. You can deduce the structure of these different Hfrs by mating each Hfr strain to an F^- strain that has a chromosomal deletion of the Lac genes, and then testing the resulting strains for the properties of the Lac operon that are transferred at early times after mating.
By performing this test, you find that you have three isolated different Hfr strains from your original F' strain:
-- one Hfr strain transfers a Lac operon that expresses ß-galactosidase constitutively early
-- another Hfr strain transfers a Lac operon that gives uninducible expression early
-- and another Hfr strain transfers a Lac operon that shows normal regulation early.
Use this information to narrow down any ambiguities about the lac mutations you proposed to be in the original strain from part **(a)**.

(c) On the basis of these results, draw a map of the F' plasmid that existed in your original F' strain. On your diagram, show the direction of the origin of transfer relative to the arrangement of Lac genes.

(d) For each of the three Hfrs, show the F' factor recombining with the chromosome in the way that it must have done to create each Hfr strain. (Hint: to solve this tricky problem, it will help to draw out both possible orientations and then determine the behavior of all possible recombination events between the F' plasmid and the chromosome.)

5. You are studying an interesting phenotype in a bacterial species related to *E. coli*, which is that some mutants of this species make dry, crusty looking colonies, instead of wild-type shiny colonies.

You use transposon mutagenesis to isolate two sets of mutants dry-looking colonies. In order to generate the first set of mutants, you wish to isolate a **Tn5** insertion that lands inside a gene important for maintaining the wild-type shiny colony phenotype. Such a transposon insertion will disrupt the function of the gene into which it lands, causing a dry colony morphology. To do this, you start with a collection of 4000 different random **Tn5** insertions in an otherwise wild type *E. coli* strain. These insertion strains are all Kan^r , and all of them give shiny colonies except for three, numbered 1-3, which give dry colonies.

In order to generate the second set of mutants, you wish to isolate a **Tn10** insertion that lands inside a gene important for maintaining the wild-type shiny colony phenotype. Such a transposon insertion will disrupt the function of the gene into which it lands, causing a dry colony morphology. To do this, you start with a collection of 4000 different random **Tn10** insertions in an otherwise wild type *E. coli* strain. These insertion strains are all Tet^r , and all of them give shiny colonies except for three, numbered 4-6, which give dry colonies.

You grow P1 phage on each of the three strains carrying the Tn5 insertions, and then you use the resulting phage lysates for transduction of the Tn5 marker into each of the three Tn10 insertion mutants by selecting for Kan^r after infection of the recipient strain. For example, you would grow P1 phage on donor strain #1, and use the resulting phage lysate to infect the recipient strain #4. You would select for transductants by selecting for kanamycin resistance. You could then see how many of your Kan^r transductants are still Tet^r (as was the original donor strain before transduction). The results of this set of experiments are as follows.

Donor strain	Recipient strain	% Kan^r Tet^s	% Kan^r Tet^r
1::Tn5	4::Tn10	100%	0%
1::Tn5	5::Tn10	10%	90%
1::Tn5	6::Tn10	24%	76%
2::Tn5	4::Tn10	2%	98%
2::Tn5	5::Tn10	100%	0%
2::Tn5	6::Tn10	100%	0%
3::Tn5	4::Tn10	15%	85%
3::Tn5	5::Tn10	100%	0%
3::Tn5	6::Tn10	100%	0%

(a) For each of the six transposon insertions, state which of the other five insertions are closely linked to it. (For example, you might decide that the transposon insertion in strain #1 is closely linked only to the transposon insertion in strain #4, but not to the transposon insertions in strains 2,3,5, and 6.)

(b) What are the distances between each set of insertions that are linked to each other, expressed as cotransduction frequencies? (For example, if you happen to decide that the transposon insertion in strain #1 is linked to the transposon insertion in strain #4, what is the cotransduction distance between those two transposon insertions?)

(c) As it turns out, the set of Tn5 insertions (strains 1-3) were generated in an Hfr strain. In mating experiments, you find that although the 1::Tn5 Hfr strain transfers Kan^r late, even after a brief mating time it is possible to isolate a few Kan^r exconjugants. Why can you isolate a few Kan^r exconjugants early in a mating?

You choose for further study two of these $Kanr^r$ exconjugate strains that were isolated after a brief mating time to an F- Tet^S Kan^S shiny strain. (For reference, you call these strains a and b.) You mate strains a and b with strains 4-6 (which are dry and crusty), and selecting for both Kan^r and Tet^r. You then examine whether the exconjugants resulting from this mating have dry and crusty colonies, or have wild-type shiny colonies.

Donor strain	Recipient strain	Kan^r Tet^r exconjugant morphology
a	4::Tn10	dry
a	5::Tn10	dry
a	6::Tn10	wild-type
b	4::Tn10	dry
b	5::Tn10	wild-type
b	6::Tn10	wild-type

(d) Propose a mechanism to explain why the wild-type colony morphology is restored in some of these matings.

(e) Explain why the results for matings with strains a and b differ. Your answer should include diagrams of the form of F factor that exists in strain a, and the form of the F factor that exists in strain b. In your diagram, include any origin of transfers or transposon insertions present on the F factors.

(f) Did the Tn5 and Tn10 insertions cause loss-of-function or gain-of-function mutations?

(g) Did the Tn5 and Tn10 insertions cause dominant or recessive dry colony phenotypes?

Regulation of lactose metabolism enzymes in bacteria

1. You have isolated five new *E. coli* mutants that do not properly regulate the expression of genes in the Lac operon. You designate these mutants Lac1$^-$, Lac2$^-$, Lac3$^-$, Lac4$^-$, and Lac5$^-$. Based on the results of the genetic tests outlined below, deduce the nature of each mutation (cis vs. trans, dominant vs. recessive, constitutive vs. uninducible). Then state which mutation (out of all the different kinds of Lac mutations we learned about in class) is most likely to be represented by Lac1$^-$, Lac2$^-$, Lac3$^-$, Lac4$^-$, and Lac5$^-$.

(a)

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac 1$^-$ Lac Z$^-$ / F' Lac $^+$	–	+
Lac 1$^-$	+	+
Lac 1$^-$ / F' LacZ$^-$	+	+
Lac 1$^-$ / F' Lac $^+$	+	+

(b)

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac 2$^-$	–	–
Lac 2$^-$ / F' Lac $^+$	–	–
Lac 2$^-$ / F' LacZ$^-$	–	–
Lac 2$^-$ Lac Z$^-$ / F' Lac $^+$	–	–

(c)

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac 3$^-$	+	+
Lac 3$^-$ / F' LacZ$^-$	–	+
Lac 3$^-$ / F' Lac $^+$	–	+
Lac 3$^-$ Lac Z$^-$ / F' Lac $^+$	–	+

(d)

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac 4$^-$	+	+
Lac 4$^-$ / F' Lac $^+$	+	+
Lac 4$^-$ Lac Z$^-$ / F' Lac $^+$	+	+
Lac 4$^-$ / F' LacZ$^-$	+	+

(e)

	ß-galactosidase activity	
	– IPTG	+ IPTG
Lac 5^-	–	–
Lac 5^- / F' LacZ^-	–	–
Lac 5^- Lac Z^- / F' Lac $^+$	–	+
Lac 5^- / F' Lac $^+$	–	+

2. You have isolated an *E. coli* strain harboring a **Tn5** insertion that is linked to the **Lac** operon. Since the **Tn5** insertion does not perturb **Lac** gene expression, the site of insertion can be assumed not to be within the **Lac** I-ZYA region itself, but you have no other information about the location of this transposon insertion.

(a) You grow phage **P1** phage on a bacterial strain that has the **Tn5** insertion and a **LacI$^-$** mutation. The resulting phage lysate is used to infect a **LacZ$^-$** mutant, and transductants are selected by plating on kanamycin plates. Among the Kan^r transductants, the patterns of LacZ expression are that 50% are properly regulated (by IPTG), 10% are constitutive, and 40% are uninducible. On the basis of this information, draw a genetic map of the position of the **Tn5** insertion relative to **LacI** and **LacZ**.

(b) You will now use the same **Tn5** insertion to determine the order of two different **LacI$^-$** mutations. Phage **P1** is grown on a bacterial strain with the **Tn5** insertion and the **LacI-1$^-$** mutation. The resulting phage lysate is then used to infect a **LacI-2$^-$** strain, and Kan^r transductants are selected. Among 500 Kan^r transductants, 5 are regulated by IPTG and the rest are constitutive.

In a second transduction experiment, phage **P1** is grown on a strain with the **Tn5** insertion and the **LacI-2$^-$** mutation. The resulting phage lysate is then used to infect a **LacI-1$^-$** strain, and Kan^r transductants are selected. Among 500 Kan^r transductants, all are constitutive. Draw a map showing the relative order of the **Tn5** insertion and the **LacI-1** and **LacI-2** loci.

3. For the following merodiploid *E. coli* strains, determine the level ß-galactosidase expression in either the presence or absence of 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**. Finally, assume that the presence of **Lac I^{-d}** protein will fully prevent all other forms of the repressor in the same cell from binding to DNA.

lac I^{-d} Z^{+} / F' lac I^{+} Z^{-}

lac O^{+} Z^{-} / F' lac O^{c} Z^{+}

lac I^{+} Z^{-} Y^{-} / F' lac I^{s} Z^{+} Y^{+}

lac I^{+} O^{c} Z^{+} / F' lac I^{-d} O^{+} Z^{+}

lac I^{+} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{+} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}

lac I^{-d} O^{c} Z^{-} / F' lac I^{s} O^{+} Z^{+}

4. You have isolated two mutations in the *E. coli* Lac operon that cause constitutive expression of the LacZYA genes. You designate these mutants $Lac1^{-}$ and $Lac2^{-}$. Making use of an F' plasmid that carries a version of the entire Lac region with the LacY gene mutated, you construct strains that you test for both ß-galactosidase activity and Lac permease activity, with results shown below. In the notation below, any gene not indicated is wild-type, if present.

	ß-galactosidase activity		Lac permease activity	
	– IPTG	+ IPTG	– IPTG	+ IPTG
$Lac\ 1^{-}$ Lac Z^{-} / F' LacY $^{-}$	–	+	+	+
$Lac\ 2^{-}$ Lac Z^{-} / F' LacY $^{-}$	+	+	+	+

Classify each mutation as dominant or recessive and as cis- or trans-acting, giving the experimental result that allows you to arrive at each conclusion. Finally, deduce what type of Lac mutation that you learned about in class best fits the properties of $Lac\ 1^{-}$ and of $Lac\ 2^{-}$.

5. Wild-type *E. coli* metabolizes the sugar lactose by expressing the enzyme ß-galactosidase. You have isolated a mutant that you call *lac1*$^{-}$, which cannot synthesize ß-galactosidase and cannot grow on lactose as the only carbon source (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 found 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 100 Kan^{r} transductants, you find that 82 are Lac^{-} and 18 are Lac^{+}. Express the distance between Tn5 and the *lac1* locus as a cotransduction frequency.

(b) You have a wild type (Lac^{+}) strain carrying a Tn10 insertion known to be near to but not within the group of Lac genes found 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 tetracycline resistance (Tet^{r}). Among 100 Tet^{r} transductants, you find that 100 are Lac^{-}. Express the distance between Tn5 and the *lac1* locus as a cotransduction frequency.

(c) Next, you isolate a mutation that constitutively expresses abnormally high levels of ß-galactosidase, which you designate *lac2*^{c}. Preliminary P1 transduction experiments indicate that *lac2*^{c} is linked to the Tn5 insertion described in part **(a)**. 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 strain that carries the Tn5 insertion from part **(a)** and the *lac1*$^{-}$ mutation. You then use this lysate to infect a *lac2*^{c} mutant, and select for Kan^{r}. From 100 Kan^{r} transductants examined, 20 are Lac^{-}, 76 express ß-galactosidase constitutively and 4 show normal ß-galactosidase expression.

In the second cross, you grow P1 phage on a strain that carries the Tn5 insertion from part **(a)** and the *lac2*^{c} mutation. You then use this lysate to infect a *lac1*$^{-}$ mutant, and select for Kan^{r}. From 100 Kan^{r} transductants examined, 81 are Lac^{-} and 19 express ß-galactosidase constitutively.

Draw a genetic map showing the relative positions of the Tn5 insertion from part **(a)** and the *lac1* and *lac2* loci.

(d) A third Lac⁻ strain, called *lac3*⁻, is linked to the Tn5 insertion. From a strain carrying the Tn5 insertion from part **(a)** and *lac3*⁻ mutation, you isolate an F' plasmid that carries a region of the chromosome that includes both Tn5 and the linked Lac region. (You know from mapping experiments that this region includes both the *lac1* and *lac3* loci.) You introduce this F' plasmid into an F⁻ donor strain carrying *lac1*⁻ by selecting for Kan^r. These merodiploids express ß-galactosidase normally. What two things does this result tell you about the *lac3*⁻ and *lac1*⁻ mutations?

(e) If the merodiploid strain described in part **(d)** had been Lac⁻, what could you have concluded about the *lac3*⁻ and *lac1*⁻ mutations? (Be sure to consider all the possibilities.)

Characterizing novel pathways that control the expression of bacterial genes

1. You are studying a new strain of *E. coli* that can utilize the disaccharide melibiose very efficiently. You find that utilization of this sugar depends on the enzyme melibiase, which is encoded by the gene Mel1. Mel1 is not expressed unless melibiose is present in the growth medium.

(a) You have isolated a mutation that causes constitutive melibiase activity, which you designate MelA$^-$. P1 phage mapping experiments using a Tn5 insertion linked to Mel1 show that MelA$^-$ is not linked to Mel1. Moreover you find that, when a mutant tRNA gene encoding an amber suppressor tRNA is introduced into a MelA$^-$ mutant, normal melibiase regulation is restored. Classify the MelA$^-$ mutation in terms of its basic genetic properties (dominant vs recessive, cis vs trans, constitutive vs uninducible), explaining the rationale behind your conclusions. Based on these properties, make a proposal for the type of regulatory function encoded by wild-type MelA.

(b) Next you isolate a mutation, designated MelB$^-$, which gives uninducible melibiase activity. Mapping experiments show that MelB is linked to Mel1. Using an F' factor that carries the chromosomal region surrounding Mel1, you perform the following genetic tests:

	melibiase activity	
	– melibiose	+ melibiose
wild type (Mel1$^+$)	–	+
Mel1$^-$	–	–
MelB$^-$	–	–
MelB$^-$ / F' Mel$^+$	–	+
Mel1$^-$ / F' Mel$^+$	–	+
MelB$^-$ / F' Mel1$^-$	–	+

Describe the basic genetic properties of the MelB$^-$ mutation (dominant vs recessive, cis vs trans, constitutive vs uninducible), explaining the rationale for your conclusions. Based on these properties, make a proposal for the type of regulatory function encoded by wild-type MelB.

(c) Diagram two possible models for regulatory pathways for Mel1 that can explain the behavior of the MelA$^-$ and MelB$^-$ mutations. For each model include a role for the inducer melibiose, and wild-type Mel1, MelA, and MelB.

(d) You next construct a MelA⁻ MelB⁻ double mutant, which gives the following behavior:

	melibiase activity	
	– melibiose	+ melibiose
MelA⁻ MelB⁻	–	–

Which of your two models is consistent with this new data?

(e) Next, you isolate a third mutant, MelC⁻, which gives constitutive melibiase expression. The MelC⁻ mutation is closely linked to Mel1 and MelB⁻. Genetic tests of the MelC⁻ mutation yield the following:

	melibiase activity	
	– melibiose	+ melibiose
MelC⁻	+	+
MelC⁻ / F' Mel⁺	+	+
MelC⁻ Mel1⁻/ F' Mel⁺	+	+
MelC⁻ / F' Mel1⁻	+	+

Describe the basic genetic properties of the MelC⁻ mutation (dominant vs recessive, cis vs trans, constitutive vs uninducible), explaining the rationale for your conclusions. Based on these properties, make a proposal for the type of regulatory function encoded by wild-type MelC.

(f) A MelB⁻ MelC⁻ double mutant shows uninducible melibiase activity. Assuming that MelC⁻ mutations affect the same gene as MelB⁻ mutations, propose two different models for the regulation of Mel1. Each answer should include a diagram showing the entire pathway for Mel1 regulation, indicating the function of wild-type Mel1, MelA, MelB, MelC,and the sugar melibiose.

(g) What type of mutation could MelC⁻ be with respect to Mel1? (Your choices are: repressor⁻, activator⁻, promoter⁻, operator⁻, dominant negative repressor, dominant negative activator, super-repressor, super-activator.)

2. You are studying the ability of a bacterial strain to use urea as a nitrogen source. You have identified the structural gene for urease, which you designate UreA. You find that normally urease is not expressed, but that urease is induced when urea is present in the growth medium.

(a) You isolate a mutant that gives constitutive expression of urease that you designate ure1$^-$. Through the use of cotransduction experiments with a transposon linked to UreA, you find that the ure1 locus is closely linked to UreA. Propose three different models to explain the regulation of UreA; each model should include wild-type Ure1 and UreA, and the inducer urea. (One model will require invoking one unknown trans-acting regulator.)

(b) You construct a plasmid that contains the wild-type UreA gene and its surrounding chromosomal sequences. (Assume that the wild-type version of the DNA sequence mutated in ure1$^-$ is included on the plasmid.) When the plasmid is introduced into a ure1$^-$ mutant strain, the resulting merodiploid expresses urease constitutively. Which models from part **(a)** still hold given this new data?

(c) Next you construct a double mutant that contains both the ure1$^-$ mutation and a ureA$^-$ mutation (this strain does not express urease). When the plasmid described above is introduced into the double mutant strain, you find that the resulting merodiploid only expresses urease when urea is present in the medium. What is the wild-type function of Ure1 with respect to UreA regulation?

(d) Using transposon mutagenesis, you isolate a second mutation, which you call ure2::Tn. This transposon insertion causes the constitutive expression of urease. The site of transposon insertion (which you call Ure2) is unlinked to the UreA gene. Bearing in mind that transposon insertions usually inactivate the gene in which they land, propose a wild-type function for Ure2 with respect to UreA regulation.

(e) You isolate a new mutation that gives uninducible urease expression of urease, which you call ure3$^-$. The ure3 locus is unlinked to UreA and unlinked to Ure2. You construct a ure1$^-$ ure3$^-$ double mutant and a ure2::Tn ure3$^-$ double mutant, and find that both strains express urease constitutively. Propose two models for UreA regulation that include a role for urea in controlling urease regulation, and the wild-type UreA, Ure1, Ure2, and Ure 3 genes.

(f) Now assume that, in contrast to what is stated in part **(e)**, ure3– and ure2::Tn are alleles of the same gene. Propose a different model for UreA regulation that includes a role for urea in controlling urease regulation, and the wild-type UreA,

3. Genes (such as the **Lac** and **Mal** genes) whose function is to metabolize compounds that can be used as energy (such as the sugars lactose and maltose) are often regulated in the sense that they are induced by the presence of substrate compounds. On the other hand, genes (such as the **his** and **ser** genes) involved in the biosynthesis of compounds needed by the cell (such as the amino acids histidine and serine) are often repressed by the presence of the compound that is produced by the biosynthetic pathway.

Consider a biosynthetic gene **X** that is regulated by the product (compound **Y**) in the sense that **X** is not transcribed when **Y** is present but is transcribed when **Y** is absent. You have identified two regulatory genes, **A** and **B**, that are both unlinked to gene **X**. Loss-of-function mutations in gene **A** cause constitutive synthesis of gene **X** (even in the presence of **Y**), whereas loss-of-function mutations in gene **B** cause gene **X** to be uninducible (even in the absence of **Y**).

(a) Draw two different possible regulatory models showing the interactions among the wild-type genes **A**, **B**, and **X**. For your model, use the symbol $\rightarrow$ to designate an activating interaction and the symbol $-|$ to designate an inhibitory interaction. For your models, show explicitly how compound **Y** would genetically interact with the appropriate protein to give the observed regulatory behavior.

(b) For each model from part **(a)**, describe the expected behavior of an $\mathbf{A^-\ B^-}$ double mutant.

4. You are studying the regulation of a new set of *E. coli* genes that are required to utilize the amino acid lysine as a source of nitrogen. The first enzyme in this lysine-utilization pathway is encoded by the gene LutA. Because the enzymatic activity of this LutA enzyme is difficult to assay, you decide to study its regulation by constructing a fusion of the LutA promoter (with associated cis-acting regulatory elements) to the LacZ gene. This gene fusion (designated P_{LutA}–LacZ) is placed on a plasmid that can be transformed into *E. coli* cells. In *E. coli* (with no endogenous LacZ gene), the reporter gene construct shows the following regulation in response to lysine in the medium.

	ß-galactosidase activity	
	– lysine	+lysine
wild type (P_{LutA}–LacZ)	–	+

You have isolated two mutations that affect the regulation of P_{LutA}–LacZ, which you designate Lut1$^-$ and Lut2$^-$. Both mutations are located in the same region of the chromosome. You have an F' factor that carries this entire chromosomal region, designated F' Lut$^+$. Genetic tests with the mutants yield the following results:

	ß-galactosidase activity	
	– lysine	+lysine
Lut1$^-$ (P_{LutA}–LacZ)	+	+
Lut2$^-$ (P_{LutA}–LacZ)	–	–
Lut1$^-$ (P_{LutA}–LacZ)/ F' Lut$^+$	–	+
Lut2$^-$ (P_{LutA}–LacZ)/ F' Lut$^+$	–	+

(a) Classify the Lut1$^-$ and Lut2$^-$ mutations in terms of their basic genetic properties (constitutive vs uninducible, dominant vs recessive, cis- vs trans-acting). Explain the rationale behind your conclusions. Based on these properties, make a proposal for the wild-type regulatory functions of the Lut1 and Lut2 genes.

(b) Give two possible models for regulatory pathways for LutA that can explain the behavior of the Lut1$^-$ and Lut2$^-$ mutations. For each model you diagram, include the wild-type LutA, Lut1, and Lut2 gene products. Also be sure to include in your models a role for the amino acid lysine.

(c) Next, you construct a Lut1$^-$ Lut2$^-$ double mutant, which gives the following behavior:

	ß-galactosidase activity	
	– lysine	+lysine
Lut1$^-$ Lut2$^-$ (P_{LutA}–LacZ)	+	+

Which of your two models from part **(b)** is consistent with this new data?

(d) You isolate a third mutant, Lut3$^-$ which has the following behavior:

	ß-galactosidase activity	
	– lysine	+lysine
Lut3$^-$ (P_{LutA}–LacZ)	–	–
Lut3$^-$ (P_{LutA}–LacZ)/ F' Lut$^+$	–	–

As above, classify the Lut3$^-$ mutation in terms of its basic genetic properties (constitutive vs uninducible, dominant vs recessive, cis- vs trans-acting). Explain the rationale behind your conclusions. Based on these properties, make a proposal for the wild-type regulatory function of the Lut3 gene.

(e) P1 transduction crosses reveal that the Lut3 locus is not linked to the Lut1 locus. Additionally, you find that the Lut1$^-$ Lut3$^-$ double mutant has the following behavior:

	ß-galactosidase activity	
	– lysine	+lysine
Lut1$^-$ Lut3$^-$ (P_{LutA}–LacZ)	–	–

Give two models for the regulatory pathway for LutA that are consistent with all of the data in this problem. In the model you diagram, include the wild-type LutA, Lut1, Lut2, and Lut3 gene products. Also be sure to include in your model a role for the amino acid lysine.