Exam Questions from Exam 1 – Basic Genetic Tests, Setting up and Analyzing Crosses, and Genetic Mapping

1. You are studying three autosomal recessive mutations in the fruit fly *Drosophila melanogaster.* Flies that are homozygous for the hb^- mutation are "humpbacked" (wild-type flies are straight-backed). Flies that are homozygous for the bl^- mutation are "blistery-winged" (wild-type flies are smooth-winged). Flies that are homozygous for the st^- mutation are "stubby-legged" (wild-type flies are long-legged).

You mate flies from two true-breeding strains, and the resulting F1 flies are all are straight-backed, smooth-winged, and long-legged. F1 females are then mated to males that are humpbacked, blistery-winged, and stubby-legged. In the F2 generation, among 1000 progeny resulting from this cross, you observe the following phenotypes:

Phenotype	Number
humpbacked, blistery-winged, and stubby-legged	(26 flies)
humpbacked, blistery-winged, and long-legged	(455 flies)
humpbacked, smooth-winged, and long-legged	(24 flies)
straight-backed, blistery-winged, and stubby-legged	(27 flies)
straight-backed, blistery-winged, and long-legged	(4 flies)
straight-backed, smooth-winged, and stubby-legged	(442 flies)
straight-backed, smooth-winged, and long-legged	(22 flies)

(a) The male flies that were bred to the F1 generation in order to produce the F2 generation were humpbacked, blistery-winged, and stubby-legged. On each of their chromosomes, they have the alleles hb^- bl^- st^-. Using this notation, **state the genotype** of each of the two true-breeding parental strains (i.e. the two strains in the **P generation**).

Genotype of one parental strain:

Genotype of the other parental strain:

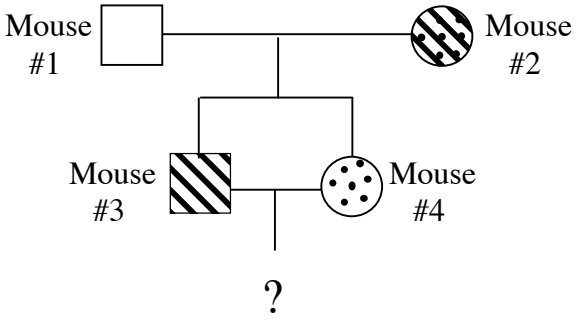
(b) How many flies are found in the class that is the reciprocal class of the humpbacked, blistery-winged, and stubby-legged flies?

(c) What is the genetic distance between the **hb** and **bl** loci? (Label your answer with the proper units.)

(d) What is the genetic distance between the **bl** and **st** loci? (Label your answer with the proper units.)

(e) Draw a genetic map showing the correct order of the **hb**, **bl**, and **st** loci.

2. The following mouse pedigree shows the segregation of two different mutant traits. The mutant trait indicated by the dots is dominant, whereas the mutant trait indicated by the stripes is recessive. Assume 100% penetrance and no new mutations. (Squares = males, circles = females.)



= expressing **dominant** mutant trait, caused by the **"A*" allele**

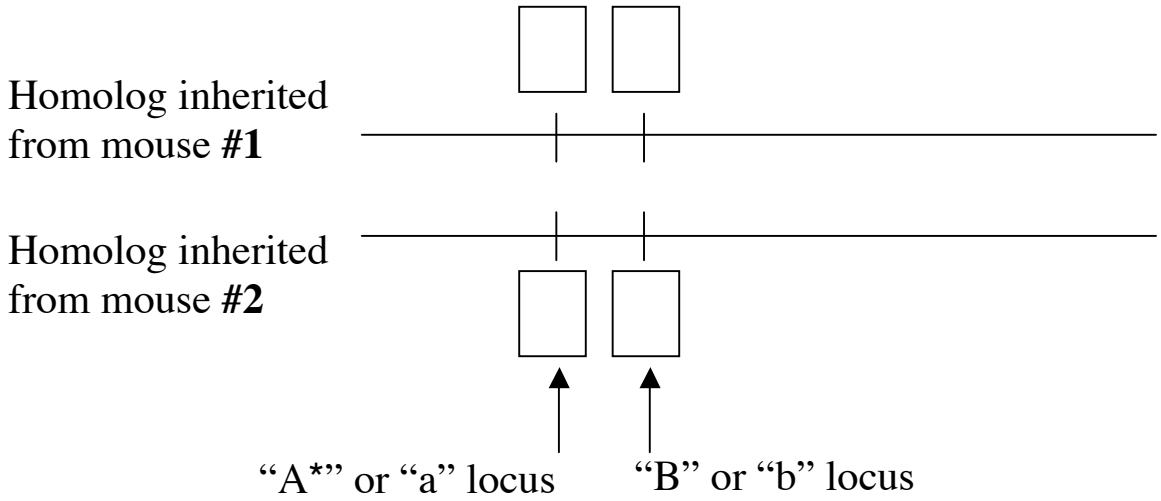
= expressing **recessive** mutant trait, caused by the **"b" allele**

= expressing both mutant traits

(a) Assuming that both mutant traits are due to linked autosomal genes that are 6 cM apart, **fill in** the following chart using the allele notation indicated by the key above. Blocks in the chart that cannot be filled in conclusively should be indicated as "*inconclusive*."
NOTE: One line of the chart is already filled in correctly for you.

	Number of "A*" alleles	Number of "a" alleles	Number of "B" alleles	Number of "b" alleles
Mouse #1				
Mouse #2				
Mouse #3	0	2	0	2
Mouse #4				

(b) Assuming that both mutant traits are due to linked autosomal genes that are 6 cM apart, fill in the boxes with the alleles possessed by mouse #4 on each of the two homologs of this autosome that are depicted in the diagram below.



(c) Assuming that both mutant traits are due to linked autosomal genes that are 6 cM apart, what is the probability that the mouse indicated by a question mark will show **both** mutant traits (the trait encoded by "A*" **and** the trait encoded by "b")?

(d) Assuming that the **recessive** mutant trait is caused by a gene on an autosome and the **dominant** mutant trait is caused by a gene on the X chromosome, **fill in** the following chart using the allele notation indicated by the key above. Blocks in the chart that cannot be filled in conclusively should be indicated as "*inconclusive*."

	X-linked		autosomal	
	Number of "A*" alleles	Number of "a" alleles	Number of "B" alleles	Number of "b" alleles
Mouse #1				
Mouse #2				
Mouse #3				
Mouse #4				

(e) Assuming that the **recessive** mutant trait is caused by a gene on an autosome and the **dominant** mutant trait is caused by a gene on the X chromosome, what is the probability that the mouse indicated by a question mark will show **only** the recessive mutant trait **assuming that the mouse is born female**?

3. You are working with a mutant strain of yeast that is dark tan (wild-type yeast are white). The "dark tan" phenotype of the haploid cells you are working with is caused by two different mutations in the same strain. The two mutations are designated drk1$^-$ and drk2$^-$.

(a) Mating of the **drk1$^-$ drk2$^-$** double mutant to **wild-type** yeast produces diploids that are white. Sporulation of these diploids yields 50 tetrads. 4 of these tetrads (called "Type One") contain four light tan spores. 37 of these tetrads (called "Type Two") contain two dark tan spores and two white spores. 9 of these tetrads (called "Type Three") contain one dark tan spore, two light tan spores, and one white spore. Categorize **each** of the tetrad types as parental ditype (PD), tetratype (TT), or nonparental ditype (NPD).

(b) Are the drk1⁻ and drk2⁻ mutations linked? **If so,** give the distance between them. (Label your answer with the proper units.)

(c) In yeast, 1 cM of genetic distance corresponds to 3,500 base pairs of physical distance. An average yeast gene is about 1,400 base pairs long, and the longest yeast gene is 14,700 base pairs. Keeping this information in mind, you select a "Type Three" tetrad from part **(a)** and mate the two light tan spores from that tetrad to each other. Can you deduce the color of the resulting diploids? **If so**, what color would the diploids be?

Next you isolate a mutant strain of yeast that cannot grow on medium lacking leucine. This strain contains a single mutation you call leu1⁻. The leu1⁻ mutation is near to drk1⁻ on the same chromosome. When the leu1⁻ mutant is mated to wild-type yeast, the resulting diploids cannot grow on medium lacking leucine.

(d) You mate **leu1⁻** yeast to **drk1⁻** yeast and sporulate the resulting diploid. You grow the resulting spores on medium containing leucine. You then test for growth on medium lacking leucine. It is apparent that you have isolated only two types of tetrads, 10 tetrads of Type A and 10 tetrads of Type B. On medium lacking leucine, only two spores from each Type A tetrad can grow; both are light tan in color. Complete the chart below so as to indicate: **How many** spores from each Type B tetrad can grow on medium lacking leucine, **and what color** is each spore that can grow?

	# of spores that can grow on medium lacking leucine	color of each spore that can grow on medium lacking leucine
Type A tetrad	2	both are light tan
Type B tetrad		

(e) What are the genotypes at the leu1 and drk1 loci of each of the two light tan spores from the Type A tetrads that grew on medium lacking leucine?

Genotype of one light tan spore:

Genotype of the other light tan spore:

4. Wild-type humbugs have brown bodies and brown eyes, and are not spotted. You have isolated mutations in three new autosomal humbug genes. The mutation **sp** gives a dominant phenotype of spotted bodies. The mutation **gr** gives a recessive phenotype of green bodies. The mutation **bl** gives the recessive phenotype of black eyes.

You cross two true-breeding mutant strains to produce F1 females heterozygous for **sp**, **gr**, and **bl**. These F1 females are then test-crossed to true-breeding black-eyed, green-bodied non-spotted males. The phenotypes of 3000 progeny are scored as shown below:

Phenotypes			Number of flies in each class
not spotted	black eyes	brown bodies	4
not spotted	brown eyes	green bodies	1347
spotted bodies	black eyes	green bodies	53
spotted bodies	black eyes	brown bodies	1390
spotted bodies	brown eyes	green bodies	2
not spotted	black eyes	green bodies	74
not spotted	brown eyes	brown bodies	61
spotted bodies	brown eyes	brown bodies	70

(a) What are the genotypes of the two true-breeding parents of the F1 females?

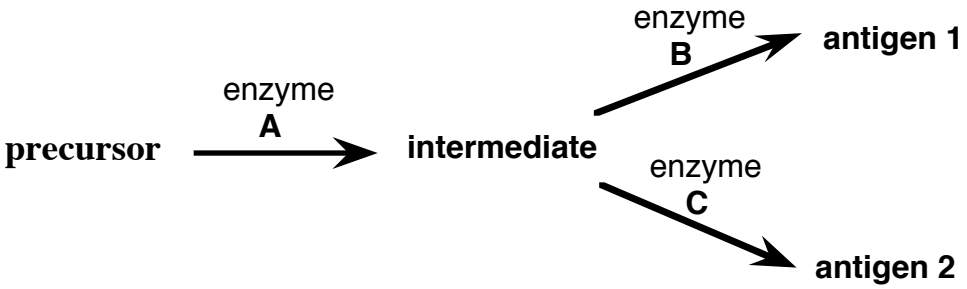
(b) Draw a map showing the order and all pair-wise distances between the **sp**, **gr**, and **bl** genes.

A mutation called eyeless (**ey**) is identified, which gives the autosomal dominant phenotype of having no eyes. You want to map **ey** relative to **bl**, but your colleague claims this can't be done since you obviously can't score the presence of black or brown eyes in an eyeless bug. You don't agree that it can't be done, and you cross a true-breeding single mutant eyeless bug to a true-breeding black-eyed bug. An F1 female that results is then crossed to a true-breeding black-eyed male. The following phenotypes are observed in 100 progeny:

eyeless	51
black-eyed	39
brown-eyed	10

(c) What is the map distance between **bl** and **ey**?

5. Consider two different antigen molecules produced on the surface of blood cells of wild-type mice, according to the biosynthetic pathway below.



Mice homozygous for alleles that block the production of enzyme A (genotype a/a) do not make either antigen 1 or antigen 2. Mice homozygous for defects in the gene encoding enzyme B (genotype b/b) do not make antigen 1. Mice homozygous for defects in the gene encoding enzyme C (genotype c/c) do not make antigen 2. All three of these phenotypes of absences of antigen are autosomal recessive phenotypes.

(a) Two different true-breeding strains of mice have been isolated that do not make either antigen 1 or antigen 2. When an individual from one strain is crossed with an individual from the other strain, all of the F1 mice produce both antigens. Write out the genotypes for both strains. (Use "A," "B," and "C" to designate the wild-type alleles and "a," "b," and "c" to designate the defective alleles of the three genes that encode these enzymes.) Assume that these three genes are unlinked.

(b) Two of the F1 mice are crossed to one another. The possible phenotypes for the F2 progeny are shown below. What proportion of the F2 will be represented by each phenotype on average?

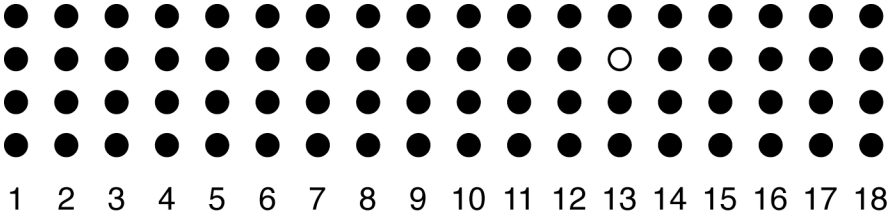
	Fraction of F_2
antigen 1^+, antigen 2^+	
antigen 1^+, antigen 2^-	
antigen 1^-, antigen 2^-	
antigen 1^-, antigen 2^+	

(c) Among the F2 progeny, there will be mice of several different genotypes that are phenotypically antigen 1^- and antigen 2^-. Suppose you wanted to use a test cross in order to test whether a given F2 mouse that does not express either antigen is defective in production of enzyme A. What genotype would you choose for a mouse to be used for such a test cross of the F2 mouse? Describe the possible outcomes of this cross and how you would interpret them.

6. Some yeast mutants with defects in enzymes in the pathway for adenine biosynthesis form red colonies because of the accumulation of an intermediate in the pathway, which is a red pigment.

(a) You have isolated two different red-colored mutants in haploid yeast strains of different mating types, which you call *ade1*$^-$ and *ade2*$^-$. When either the *ade1*$^-$ or *ade2*$^-$ haploid mutant is mated to wild-type haploid yeast, the resulting diploid forms white colonies like those of wild-type yeast. When the *ade1*$^-$ haploid mutant is mated to the *ade2*$^-$ haploid mutant, the resulting diploid makes red colonies. From these observations, describe as much as you can about the *ade1*$^-$ and *ade2*$^-$ mutations and the relationship between them.

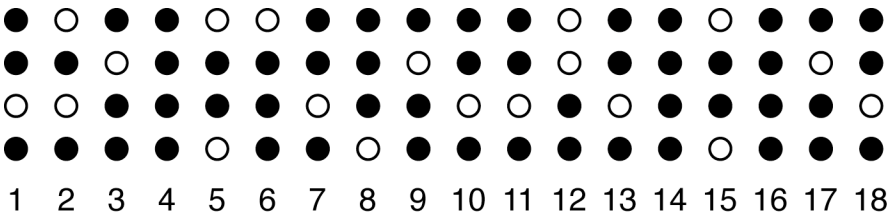
(b) Next, you induce sporulation of the diploid that was formed by mating the *ade1*$^-$ and *ade2*$^-$ haploid strains. From the resulting 18 tetrads shown below, you determine that only one spore clone is white, and the rest are red.



What does this result tell you about the distance between the *ade1* and *ade2* loci?

(c) Next, you isolate a new red-colored mutant, which you call *ade3*$^-$. When the *ade3*$^-$ haploid mutant is mated to wild-type haploid yeast, the resulting diploid is red. You mate the *ade3*$^-$ haploid mutant to an *ade1*$^-$ haploid mutant, and the resulting diploid is red also. What do these results tell you about the *ade3*$^-$ mutant and the relationship between the *ade3*$^-$ and *ade1*$^-$ mutations?

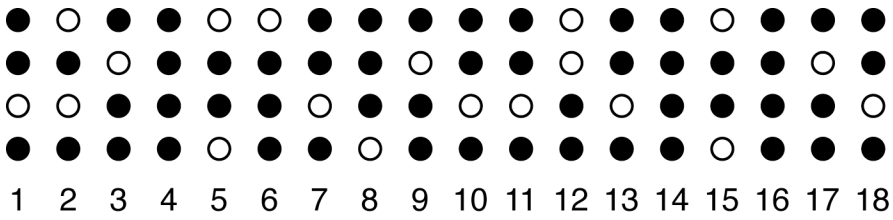
(d) When you induce sporulation of the diploid that was formed by mating the *ade3*$^-$ and *ade1*$^-$ haploid strains, you obtain the results shown below:



Of the 18 dissected tetrads shown, how many tetrads of each type (PD, NPD or TT) are there?

(e) What do the results from this tetrad analysis tell you about the relationship between the *ade3*$^-$ and *ade1*$^-$ mutations? Mention in your answer whether these mutations can be in the same gene.

(f) Suppose that you wanted to do some experiments with an *ade3*$^-$*ade1*$^-$ double mutant haploid yeast strain. Below is the same image from above of the tetrads formed from inducing sporulation of the diploid formed by mating *ade3*$^-$ and *ade1*$^-$ haploid mutants. On this image, **circle each spore clone** that you can be sure is double mutant haploid strain, without any further testing.



(g) You mate an *ade3*$^-$ haploid mutant to an *ade2*$^-$ haploid mutant. You then induce sporulation of the resulting diploid, and dissect 18 tetrads. How many Tetratype (TT) tetrads would you expect to see?

7. You are studying the genetics of a new insect species and have identified three different autosomal recessive traits -- apricot eyes, black body, and curly wings. These phenotypes are caused by alleles in three different genes -- **a**, **b**, and **c** respectively. Wild-type flies have red eyes, brown body, and straight wings, and are genotypically **a+**, **b+**, and **c+**. Two different true-breeding lines are crossed and the F_1 progeny all appear as wild-type. These F_1 progeny are then crossed to individuals from a true-breeding line that has all three recessive traits, and 100 progeny from this cross are analyzed. The phenotypes and numbers are as follows:

Phenotype	Number
wild-type (red eyes, brown body, straight wings)	3
apricot eyes, black body, curly wings	7
apricot eyes, brown body, curly wings	34
red eyes, black body, straight wings	36
red eyes, black body, curly wings	8
apricot eyes, brown body, straight wings	12

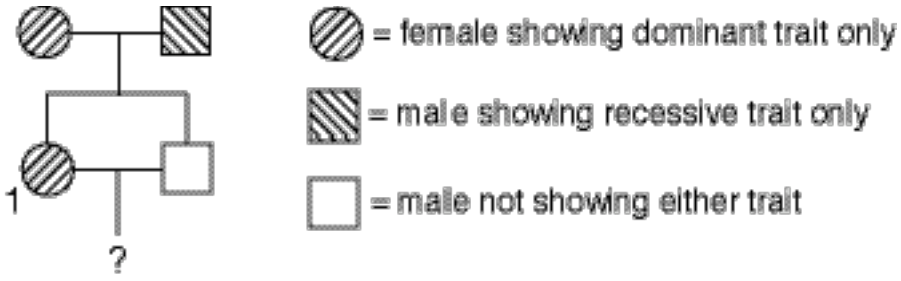
(a) What are the genotypes of the two parental true-breeding lines? Use the notation in the introduction to this question.

(b) Why are there only six phenotypic classes, rather than eight?

(c) Give as much information as you can about the chromosomal positions of the three loci **a**, **b**, and **c**. Include in your answer any relevant map distances in cM.

(d) Given the map distances in part **(c)**, if F_1 insects are crossed to one another, what frequency of the resulting F_2 progeny would have all three recessive traits?

8. The following mouse pedigree shows the segregation of both a dominant and a recessive trait. (Assume all phenotypes are completely penetrant and that no new mutations arise).



(a) What is the genotype of mouse 1 if the two traits are X-linked? For your answer use $\mathbf{X^D}$ to designate the allele for the dominant trait (with $\mathbf{X^d}$ representing the corresponding wild-type allele) and $\mathbf{X^r}$ to designate the allele for the recessive trait (with $\mathbf{X^R}$ representing the corresponding wild-type allele).

(b) If the genes for both traits are 30 cM apart on the X chromosome, what is the probability that a progeny mouse indicated by the **?** will show both traits if she is born **female**?

(c) If the genes for both traits are 30 cM apart on the X chromosome, what is the probability that a progeny mouse indicated by the **?** will show both traits if he is born **male**?

(d) What is the genotype of mouse 1 if the two traits are autosomal? For your answer use **D** to designate the allele for the dominant trait (with **d** representing the corresponding wild-type allele) and **r** to designate the allele for the recessive trait (with **R** representing the corresponding wild-type allele).

(e) If the genes for both traits are 30 cM apart on the same autosome, what is the probability that a progeny mouse indicated by the **?** will show both traits?

9. You have isolated two different mutants of phage λ that make fuzzy plaques, which you name **fz-1⁻** and **fz-2⁻**. These two mutations are in a single gene, the "**fz**" gene. You cross **fz-1⁻** phage with **fz-2⁻**phage by coinfecting *E. coli* with phage of both types. You plate out the resulting phage lysate, and examine 1000 plaques that result from the cross. 15 of these plaques are NOT fuzzy.

(a) What is the distance between the **fz-1** and the **fz-2** loci in map units?

Mutations in the **cI** gene of phage λ give clear plaques, whereas wild-type phage have turbid plaques.

(b) You cross a **cI⁻ fz-1⁻** double mutant to a **fz-2⁻** mutant by coinfecting *E. coli* with both types of mutant phage. You plate the resulting lysate and examine a total of 1000 plaques. Among the 15 plaques that are NOT fuzzy, 12 are clear and 3 are turbid. Draw a genetic map showing the order of the **cI**, **fz-1**, and **fz-2** mutations.

10. You have obtained a strain of *Drosophila*, which is homozygous for the cn^{-} mutation (and thus has cinnabar colored eyes) and is homozygous for the shi-1^{-} mutation (and thus becomes paralyzed at high temperature). You mate this strain to a true-breeding wild-type fly and obtain F1 flies, all of which have the wild type phenotype (red eyes, not paralyzed). F1 females are then mated to males of the starting strain (homozygous cn^{-} and shi-1^{-}). Among 100 progeny from this cross you observe the following phenotypes:

Phenotype	Number
wild-type (not paralyzed, red eyes)	44
paralyzed, cinnabar eyes	41
not paralyzed, cinnabar eyes	7
paralyzed, red eyes	8

(a) From this data, what is the distance between the **cn** and **shi** genes?

(b) You isolate a second allele of the *shibire* gene designated shi-2^{-}, which also causes the recessive phenotype of paralysis at high temperature. Flies from a true-breeding shi-2^{-} strain are crossed to flies from the true-breeding cn^{-}, shi-1^{-} strain described above.
What is the phenotype of the resulting F1 female flies?

F1 females are then mated to males from the true-breeding cn^{-}, shi-1^{-} strain. You collect 10,000 progeny from this cross and note that, although almost all the flies are paralyzed at high temperature, there are 10 that are not paralyzed.

(c) What is the distance between the shi-1 and shi-2 loci?

(d) Among the 10 progeny flies that are not paralyzed that result from the cross described in part **(b)**, 8 have cinnabar eyes and 2 have normal red eyes. On the basis of this information as well as the results from parts **(a)** and **(b)**, draw a genetic map showing the order of the cn, shi-1, and shi-2 loci.

11. You have isolated a new His^- yeast mutant.

(a) When you mate this haploid mutant to a wild-type haploid yeast strain (that is His^+), you find that the resulting diploids are His^+. What does this tell you about the mutant that you isolated?

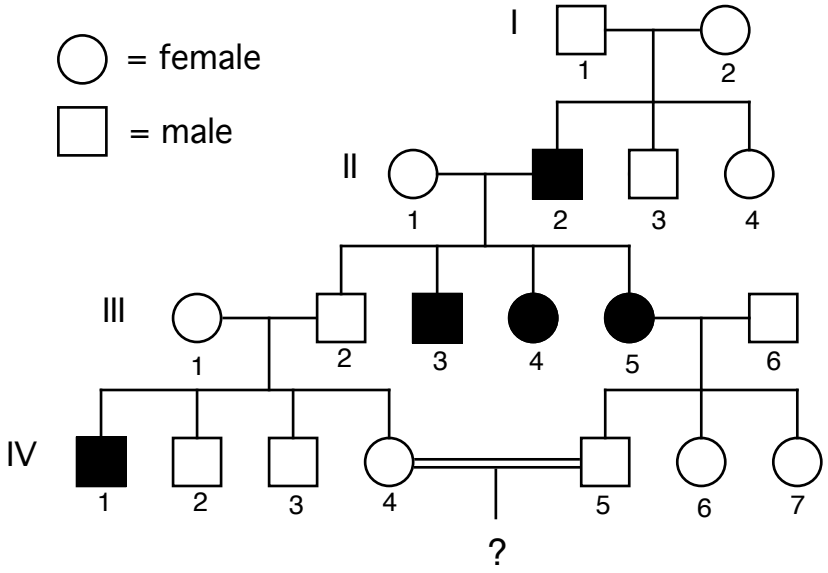
(b) When you induce sporulation in the His^+ diploid from part (**a**), you find that tetrads of three types are produced. From a total of 100 tetrads, the following tetrad types are seen:

Type:	2 His^- : 2 His^+ spores	3 His^- : 1 His^+ spores	4 His^- spores
Number:	65	30	5

What does this result tell you about the original His^- strain? Give any relevant genetic distances (in cM) that you can calculate.

(c) There are a total of 240 His^- spore clones in the 100 tetrads from part (**b**). If you picked two of these His^- clones (of opposite mating type) at random and mated them, what is the probability that the resulting diploid would be His^+? (You may find it helpful to consider the genotypes of the His^- spores in each tetrad type).

12. In the following human pedigree, individuals exhibiting a **common** inherited allergy to milk are shown by shaded-in symbols, and unaffected individuals are shown by unshaded symbols.



(a) Assuming complete penetrance and no new mutations, what is the mode of inheritance of the milk allergy (your choices are: autosomal dominant, autosomal recessive, and X-linked recessive)?

(b) Give the genotypes of the following individuals, using **+** to indicate the allele that does not cause the allergy, and **m** to indicate the allele specifying the milk allergy. In ambiguous cases, indicate all possible genotypes.

	Genotype		Genotype
II-1		**III-2**	
II-2		**III-5**	
II-3			

(c) If cousins **IV-4** and **IV-5** have a child together, what is the probability that the child will have the milk allergy? (Give separate probabilities for sons and daughters if their chances of acquiring the allergy differ.)

13. Wild-type yeast form white colonies. You have isolated two mutants that make red colonies that you call **red3** and **red4**.

(a) When a **red3** haploid mutant is mated to a **red4** haploid mutant of the opposite mating type, the resulting diploid makes white colonies. What does this observation tell us about **red3** and **red4**?

(b) When the diploids from part **(a)** are induced to sporulate, three types of tetrads are found. Type I have 4 red spores. Type II have 1 white spore and 3 red spores. Type III have 2 white spores and 2 red spores

Classify each tetrad type as PD, NPD or TT.

(c) When the number of each tetrad type is tallied, you find that the cross produces 30 Type I tetrads, 16 Type II tetrads, and 4 Type III tetrads.
Are the **red3** and **red4** loci linked? If so, how far apart are they in cM?

(d) One of the Type II tetrads from above is selected for further analysis and you designate the four spore clones **a**, **b**, **c**, and **d**. Clone **a** is white, whereas clones **b**, **c**, and **d** are red. Each clone is mated to either a **red3** haploid mutant or a **red4** haploid mutant, and the color of the resulting diploid is noted.

x **red3** haploid → white diploid
Clone **a** (white)
x **red4** haploid → white diploid

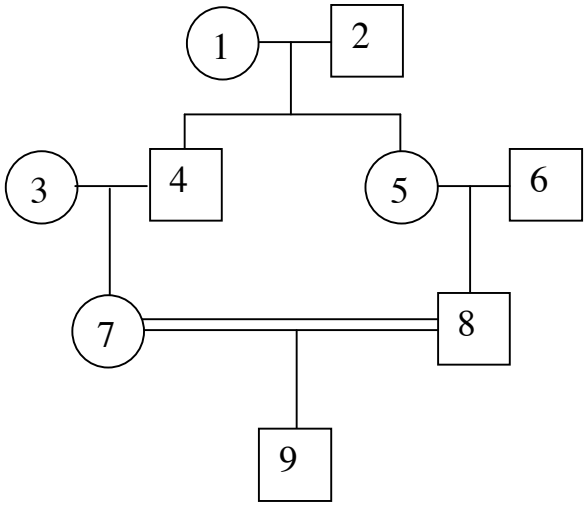
x **red3** haploid → red diploid
Clone **b** (red)
x **red4** haploid → white diploid

x **red3** haploid → red diploid
Clone **c** (red)
x **red4** haploid → red diploid

x **red3** haploid → white diploid
Clone **d** (red)
x **red4** haploid → red diploid

Give the genotypes of each of the four spore clones with respect to **red3** and to **red4**.

14. Consider the following family pedigree where two first cousins have a son. Each individual is numbered for reference in this problem. PLEASE NOTE that, in this pedigree, the phenotypes of the family members are NOT denoted. They will be described in the text of the question instead. In this problem, assume complete penetrance and no new mutations.



(a) Say that female #1 exhibits a rare recessive X-linked trait, and that male #2 does not exhibit the trait. Because the trait is rare, assume that the individuals #3 and #6 neither have nor are carriers of the trait.

What is the probability that male #4 will have the trait?

What is the probability that female #5 will have the trait?

What is the probability that female #7 will have the trait?

What is the probability that male #8 will have the trait?

What is the probability that male #9 will have the trait?

(b) Now say that female #1 exhibits two different rare recessive X-linked traits that are each caused by a single gene. The two genes causing these two traits are found 10 cM apart on the X chromosome. (We will refer to the two traits as trait A and trait B). Male #2 does not exhibit either trait. Assume that individuals #3 and #6 neither have nor are carriers of either trait.

What is the probability that male #**4** will have both traits?

What is the probability that male #**8** will have both traits?

What is the probability that male #**9** will have trait A only?

What is the probability that male #**9** will have both traits?

What is the probability that male #**9** will have neither trait?

15. Consider two autosomal recessive Drosophila mutant phenotypes -- curly-wings (caused by the **cr** allele) and humpback (caused by the **hb-1** allele). The **cr** and **hb** genes are on the same autosome. A wild-type female is crossed to a curly-winged, humpbacked male to produce F_1 flies that all look normal. An F_1 female is then crossed to a curly-winged humpbacked male and 100 progeny from this cross are examined.

Phenotype	Number of flies
Wild-type	41
curly-wings, straight back	12
straight wings, humpback	9
curly-wings, humpback	38

(a) What is the distance between the **cr** and the **hb-1** loci in cM?

Next you isolate a second mutation in a different gene (**hb-2**) that also causes the recessive phenotype of humpback. A female from a true-breeding **hb-2** strain is crossed to a male from a true breeding **cr**, **hb-1** strain. An F_1 female from this cross is then crossed to a true-breeding **cr hb-1 hb-2** male (who has curly wings and a humpback) and 500 progeny are examined.

Phenotype	Number of flies
straight-wings, straight back	5
straight wings, humpback	240
curly-wings, humpback	255

(b) What is the phenotype of the F1 females in this cross?

(c) What is the distance between the **hb-1** and **hb-2** loci in cM?

(d) Draw a genetic map showing the relative order of the **cr**, **hb-1**, and **hb-2** loci.

16. A true-breeding mouse strain exhibits two different rare traits. When a male from this true-breeding strain is crossed to a wild-type female, all of the female F_1 progeny exhibit both traits, whereas all of the male F_1 progeny look wild-type. Assume complete penetrance and no new mutations.

(a) What is the mode of inheritance of the two traits?

(b) The male and female F_1 mice described above are crossed to one another to produce F_2 progeny. Of the male F_2 progeny, 40% have both traits (the rest of the F_2 males either appear wild-type or have only one trait or the other). What fraction of the female F_2 progeny would you expect to have both traits?

(c) What is the map distance (in cM) between the genes for the two traits?

17. You have isolated a yeast mutant that makes small colonies. When you mate your haploid mutant to a haploid wild-type strain, the resulting diploids look like wild-type.

(a) What does this observation tell you about your mutant?

(b) When the diploids from part **(a)** are induced to sporulate, all of the tetrads appear to be PDs. What does this observation tell us about your mutant?

(c) What is the phenotype of each of the four spores from a PD tetrad described in part **(b)**?

(d) You isolate a second haploid mutant that also makes small colonies. When a haploid of one small mutant is mated to a haploid of the other small mutant, the resulting diploids appear normal. What is the relationship between the two “small” mutations?

When the diploids from part **(d)** are induced to sporulate, three types of tetrads are found.

Type I have 4 small spores

Type II have 1 normal and 3 small spores

Type III have 2 normal and 2 small spores

The cross produces 24 type I tetrads, 24 type II tetrads, and 2 type III tetrads.

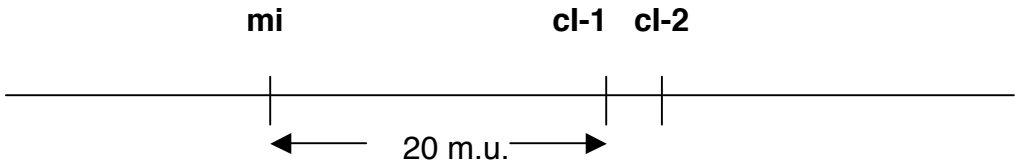
(e) What is the map distance between the two “small” loci?

(f) Give your best estimate for the number of tetrads (out of 50 total) described in part **(d)** that resulted from two crossovers in the interval between the two “small” loci.

18. You have isolated two different mutants of phage lamba in the repressor gene (**cI**); these mutations cause clear plaques rather than the normal turbid plaques. These mutants are called **cI-1⁻** and **cI-2⁻**. You cross **cI-1⁻** phage with **cI-2⁻** phage by coinfecting *E. coli* with phage of both types. Of 1000 plaques that result from the cross, 980 plaques are clear (whereas the rest are turbid).

(a) What is the distance between the **cI-1** and the **cI-2** loci in map units?

Phage mutants that are **mi⁻** are easily detected because they form small plaques. The distance between the **mi** gene and the **cI** gene is about 20 map units. Assume that the genetic order of the loci is as follows:



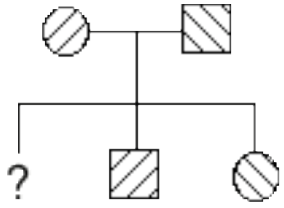
(b) For a cross of a **mi⁻ cI-1⁻** double mutant to a **cI-2⁻** mutant, a total of 1000 plaques are examined. In the table below, fill in the expected number of plaques of each phenotypic type.

Phenotype	**# of Plaques in this class**	**Genotype(s) of Plaques in this class**
Clear, large		
Clear, small		
Turbid, large		
Turbid, small		

19. The genes for two rare human autosomal dominant traits are 10 cM apart (as determined by meiosis in females). In the following pedigrees the traits are indicated as follows. Assume no new mutations arise and complete penetrance in this problem.

 = individual with trait 1  = individual with trait 2  = individual with trait 1 and trait 2

(a) For each of the pedigrees shown below, calculate the probability that the individual designated by "**?**" will have either dominant trait 1, dominant trait 2, or both traits.



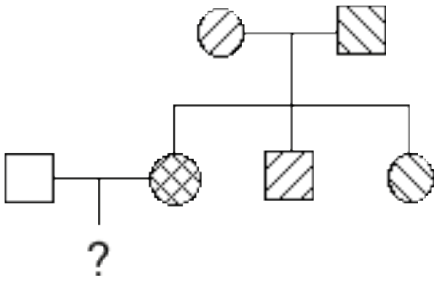
Probability

Dominant trait 1 only

Dominant trait 2 only

Both dominant trait 1 and trait 2

(b)



Probability

Dominant trait 1 only

Dominant trait 2 only

Both dominant trait 1 and trait 2

20. Mutations in the **w** gene on the X chromosome of *Drosophila* give white eyes instead of the normal red. You have isolated both a white-eyed mutation (designated **w-1**) that gives a dominant phenotype, and a white-eyed mutation (designated **w-2**) that gives a recessive phenotype.

(a) A white-eyed male from the **w-1** line is crossed to a wild-type female. What color eyes will the female progeny from this cross have?

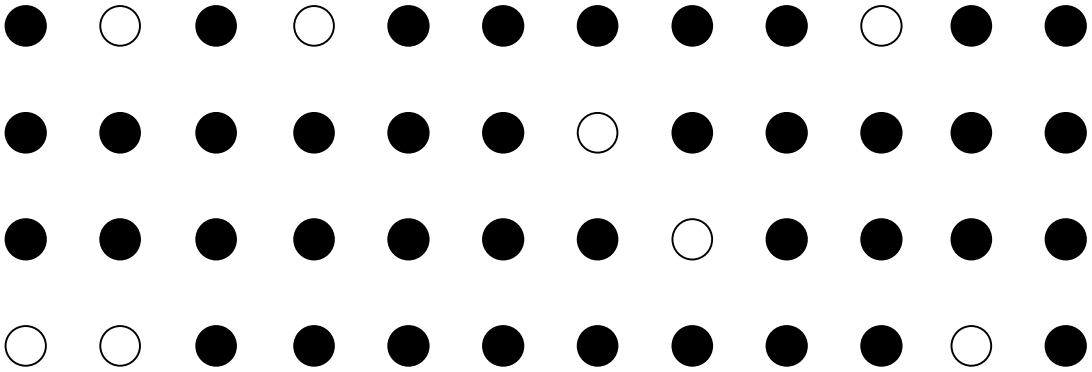
(b) What color eyes will the male progeny from the cross in part **(a)** have?

(c) One of the female progeny from the cross in part (**a)** is mated to a white-eyed male from the **w-2** line. What fraction of the white-eyed progeny from this cross will be female?

(d) A white-eyed female resulting from the cross described in part (**c)** is crossed to a wild-type male. Among 50,000 male progeny produced by this cross, there are 5 that have red eyes. What is the distance between **w-1** and **w-2** in cM?

(e) You have isolated a mutation that is called (**hw**) and gives hairy-wings. The **hw** gene is also on the X chromosome and is linked to the **w** gene. A female fly from a line that is true-breeding for both hairy wings and the **w-2** allele is crossed to a male fly that has normal wings and the **w-1** allele. An F_1 female from this cross is mated to a wild-type male and a very large number of male progeny from this cross are examined. Three of the male progeny have red eyes, and all of these red-eyed males have hairy wings. Draw a genetic map showing the most likely order of **hw**, **w-1**, and **w-2**.

21. Wild-type yeast make white colonies. You have isolated two mutants that make red colonies, which you call **red-1** and **red-2**. A **red1** haploid mutant is crossed to a **red2** haploid mutant. The resulting diploid is induced to sporulate, and twelve resulting tetrads are analyzed as shown below, where a dark circle indicates a red colony, and a white circle indicates a white colony:

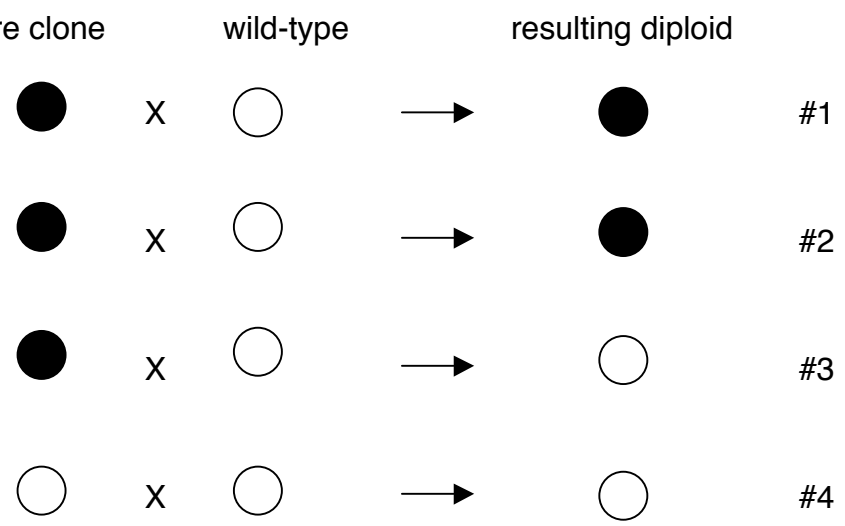


(a) How many tetrads of each type are there?

PD NPD TT

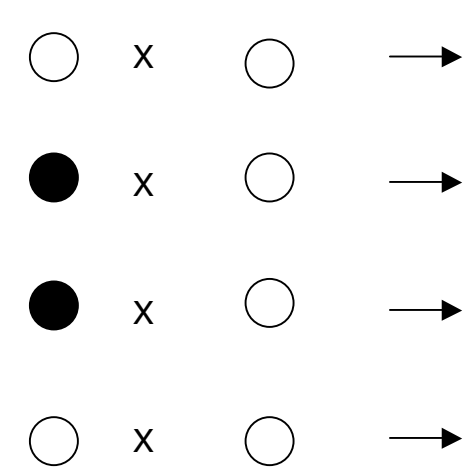
(b) Are the **red1** and **red2** loci linked? If so, how far apart are they in cM?

One of the tetrads from above is selected for further testing. Each of the four spore clones is mated to a wild-type haploid yeast. The phenotypes of the resulting diploids are shown below:



(c) When diploid #3 is induced to sporulate, what will the tetrads look like with respect to red and white phenotypes?

(d) A second tetrad from part **(a)** is chosen, and each of the four spore clones is again mated to a wild-type haploid yeast. In the diagram below, fill in the expected phenotypes of the resulting diploids. State any ambiguities that may exist.



22. Wild-type *Drosophila* have red eyes, and white eyes is an X-linked recessive phenotype caused by a single mutation. A new single mutation that gives the recessive phenotype of apricot colored eyes is isolated. A female from a true-breeding apricot-eyed strain is crossed to a male from a true-breeding white-eyed strain. All of the resulting F1 flies have apricot eyes.

(a) Are the white-eye and apricot-eye mutations in the same gene or in different genes? Explain your answer.

A collection of apricot-eyed F1 females from the cross described above are mated to males from a true-breeding white-eyed strain, and 1000 male progeny are examined. Among these progeny, only 6 flies have normal red eyes.

(b) What is the measured distance between the white-eye and apricot-eye loci in cM?

A new mutation is isolated that causes the recessive eye color “peach.” A female from a true-breeding peach-eyed strain is crossed to a male from a true-breeding white-eyed strain. All of the resulting F1 females have normal red eyes and all of the resulting F1 males have peach eyes.

(c) Is the peach-eye mutation on an autosome or on the X-chromosome? Explain your answer.

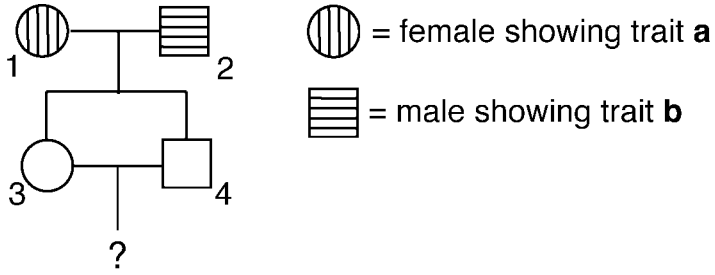
(d) Are the white-eye and peach-eye mutations in the same gene or in different genes? Explain your answer.

A mutation that causes the recessive phenotype of crossveinless wings lies on the X-chromosome. A female from a true-breeding strain with apricot eyes and crossveinless wings is crossed to a male from a single mutant true-breeding strain with white eyes and normal wings. As expected, all of the F1 females from this cross have apricot eyes and normal wings. A large collection of these F1 females are crossed to wild-type males and 10,000 **male** progeny are examined. The observed phenotypes are as follows:

Phenotype		Number
normal wings	white eyes	4,418
crossveinless wings	apricot eyes	4,330
normal wings	apricot eyes	610
crossveinless wings	white eyes	590
normal wings	red eyes	2
crossveinless wings	red eyes	50

(e) Draw a genetic map showing the relative order of the crossveinless, apricot and white loci.

23. The following mouse pedigree shows the segregation of two different autosomal recessive traits. (In this problem, assume that all phenotypes are completely penetrant and no new mutations arise.)

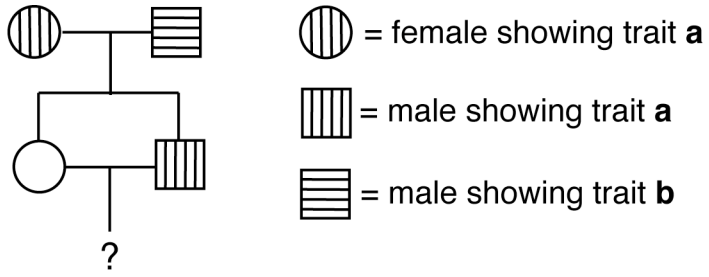


(a) What is the genotype of the mouse designated **3**? (Instructions: Use **A** and **a** to designate the alleles of the gene for trait **a** that give the dominant and recessive phenotypes respectively; and use **B** and **b** to designate the alleles of the gene for trait **b** that give the dominant and recessive phenotypes respectively.)

(b) If the genes for trait **a** and trait **b** are unlinked, what is the probability that a progeny mouse indicated by the "**?**" will show NEITHER recessive trait?

(c) If the genes for trait **a** and trait **b** are 20 cM apart on the same autosomal chromosome, what is the probability that a progeny mouse indicated by the "**?**" will show NEITHER recessive trait?

(d) The pedigree below shows the segregation of two recessive X-linked traits.



If the genes for the two traits are 20 cM apart on the X chromosome, what is the probability that that a **female** progeny mouse indicated by the "**?**" will show NEITHER recessive trait?

24. You have isolated a new mutation of phage λ that makes plaques with rough edges. You call the mutation **r1⁻**. Phage mutants in the repressor gene (**cI⁻**) make clear plaques rather than the normal turbid plaques. You cross a **r1⁻** phage with a **cI⁻** phage by coinfecting *E. coli* with phage of both types. One hundred plaques resulting from the cross are examined and the following phenotypes and numbers are seen:

Plaque Phenotype	Number of Plaques
rough, turbid	44
rough, clear	4
smooth, turbid	6
smooth, clear	46

(a) What is the distance between the **r1** and the **cI** loci in map units?

Next you isolate a second mutation that makes rough plaques that you call **r2⁻**. Note that an **r1⁻ r2⁻** double mutant would be phenotypically rough. When a **r1⁻**, **cI⁻** double mutant phage is crossed to a **r2⁻** mutant phage, the following plaque types and numbers are seen:

Plaque Phenotype	Number of Plaques
rough, turbid	491
rough, clear	499
smooth, turbid	9
smooth, clear	1

(b) What is the distance between the **r1** and **r2** loci in map units?

(c) Draw a genetic map showing the relative order of the **cI**, **r1**, and **r2** loci, as well as the distances that you have determined in parts **(a)** and **(b)**.

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.

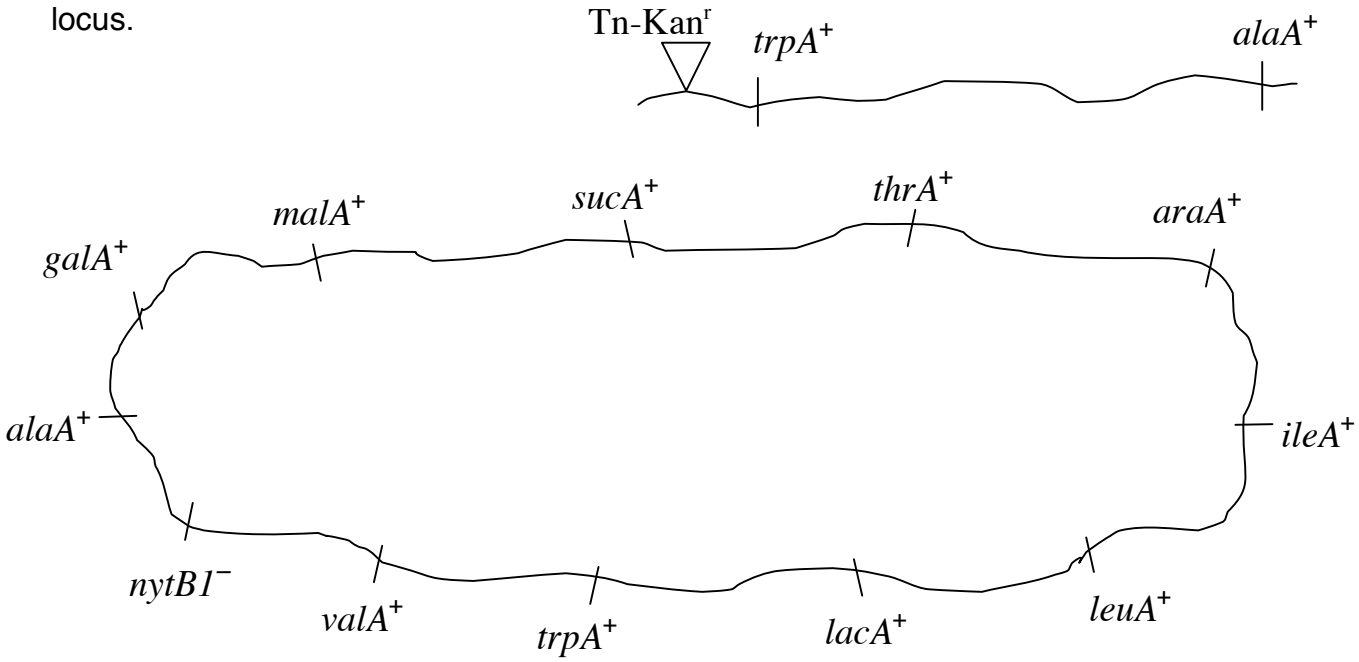
(c) Can you conclude if *nytA1*$^-$ acts in cis or in trans with respect to *nytT*? **If so**, state whether *nytA1*$^-$ acts in cis or in trans, and state what was the most important piece of information (for example, which strain in the table) you used to reach your conclusion.

(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.)

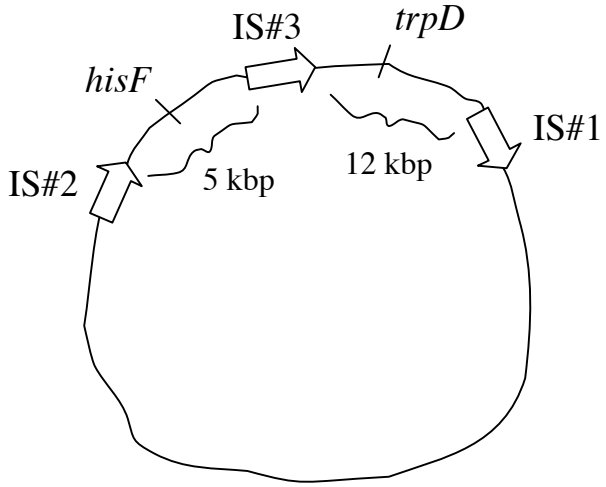
In the **second cross**, you grow P1 phage on a Kan^r strain that contains the transposon insertion and the *nytB1*$^-$ mutation, and use the resulting phage lysate to infect a *nytA1*$^-$ strain. You select for kanamycin resistance (Kan^r), and among 100 Kan^r transductants, you find that only 3 are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

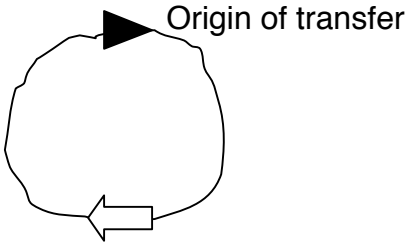
(d) Based on the gene order **that you drew in part (c)**, state the chromosomal genotype of a transductant that must have resulted from a quadruple crossover event between the transduced DNA and the bacterial chromosome of the recipient **in the first cross**. (Be sure to indicate the chromosomal genotype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

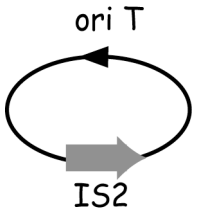
(d) Is the **D** gene most likely to act earlier or later than **B** in the pathway for amylase regulation?

(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.

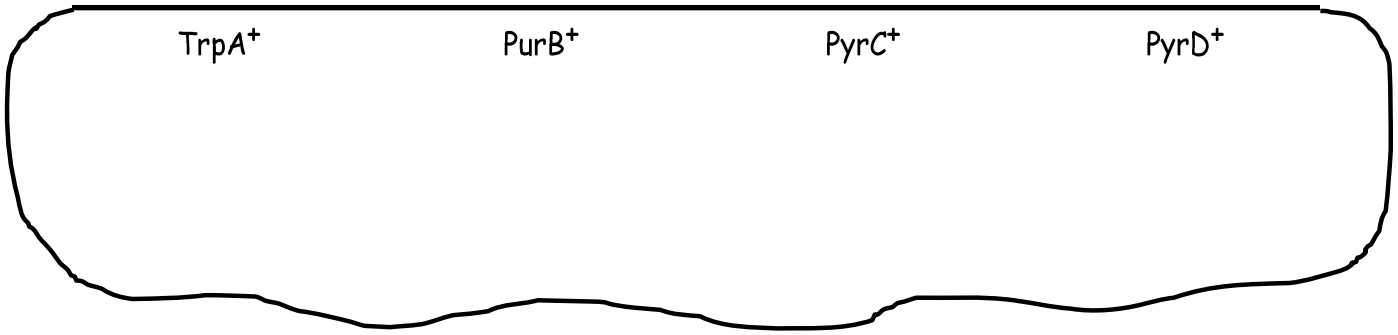
(f) Propose a linear genetic pathway that accounts for the regulation of the amylase gene. Include in your diagram of your genetic pathway model: starch, and the wild-type **A**, **B**, **C**, and **D** genes.

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.

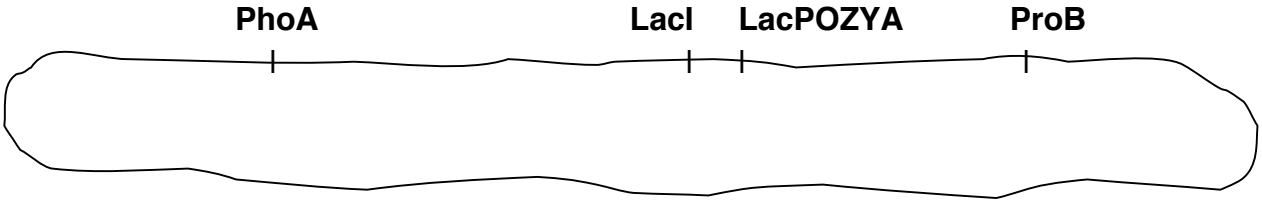
(b) Write out the double-stranded DNA sequence for the anti-codon portion of the gene that encodes $tRNA^{trp}$ (and label the 5' and 3' ends of each DNA strand).

(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 **F^+ $PhoA^+$ Lac^+ $ProB^-$** , and then you isolate a derivative of this strain that, upon mating to an **F^-** recipient strain, can transfer **$PhoA^+$** efficiently but transfers **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 **F^- $PhoA^-$** strain. After 10 minutes of mating, a **$PhoA^+$** exconjugant strain is isolated. Will this new strain itself be able to transfer the **$PhoA^+$** marker to an **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	ß-galactosidase activity +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	maltase activity +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 template 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 ACAT 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.

Exam Questions from Exam 3 – Eukaryotic Gene Regulation, Genome Modifications in Eukaryotes, Population Genetics

1. Consider an autosomal recessive trait that occurs at a frequency of 10^{-6} in a specific human population that is at Hardy-Weinberg equilibrium (ie. random mating is occurring). When answering the following parts, show all of your calculations.

(a) Draw a pedigree below that shows a mating between two relatives that would correspond to an inbreeding coefficient that equals 0.007813. Denote the mating between relatives with a double-bar connecting the two related parents. Start your pedigree with the common pair of ancestors and end your pedigree with the two related parents who are mating.

(b) Now say that all matings in Generation X of the given population are either between unrelated individuals, or have the same inbreeding coefficient as the mating described in part **(a)**. If the incidence of the trait in Generation "X+1" increases to a frequency of $2 X10^{-6}$, what percentage of matings in Generation X must have been between **unrelated** individuals?

(c) Now assume that this autosomal recessive trait causes lethality in childhood. If a constant percentage of matings are between related parents for many generations, would you predict that q would increase OR decrease?

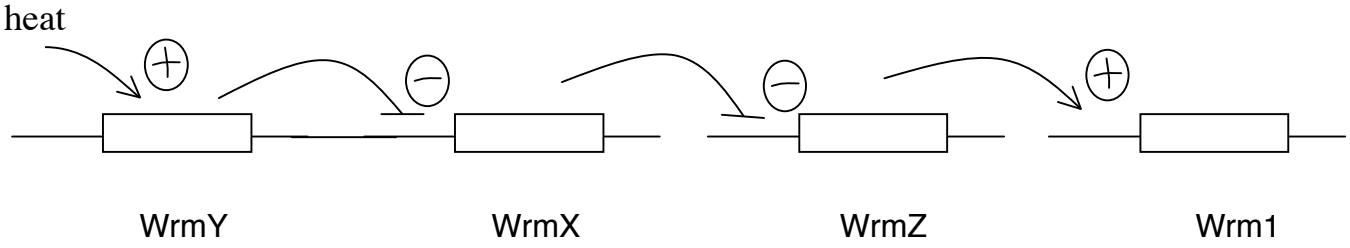
(d) A constant percentage of matings has occurred between related parents for many generations, and yet you find experimentally that q has not changed. Of the three choices below, circle **ALL** that could potentially act against the effect you chose in part **(c)** in order to keep q constant. Explain in one sentence why you chose or did not choose each option.

Choice A: migration

Choice B: heterozygote advantage (Aa over AA)

Choice C: mutation

2. You are studying regulation of the Wrm1 gene, a yeast gene that is expressed in response to heat. You isolate a wrm1::lacZ strain that expresses β-galactosidase when Wrm1 is normally expressed (which is at 36°C but not at 24°C). You use this wrm1::lacZ strain to perform a genetic screen looking for mutants that do not properly regulate expression of Wrm1. In your screen, you isolate a series of mutant strains that either show constitutive or uninducible expression of wrm1::lacZ.
Your results indicate that the following is the correct pathway for regulation of Wrm1 expression. Note that WrmY and WrmX are on the same chromosome, and that WrmX, WrmZ, and Wrm1 are all on different chromosomes.



One of the mutant strains you isolate contains a mutation called WrmX⁻, which is in the **coding region** of WrmX. You mate a WrmX⁻ wrm1::lacZ haploid strain to a wrm1::lacZ haploid strain. The resulting diploids are white on X-gal plates that are incubated at 24°C, and are blue on X-gal plates that are incubated at 36°C.

(a) Classify the WrmX⁻ mutation as constitutive OR uninducible.

(b) Classify the WrmX⁻ mutation as dominant OR recessive.

(c) Classify the WrmX locus as cis-acting OR trans-acting with respect to Wrm1.

You next isolate a mutant strain containing a mutation called WrmY⁻, which is in the **coding region** of WrmY. You mate a WrmY⁻ wrm1::lacZ haploid to a wrm1::lacZ haploid. The resulting diploids are white on X-gal plates, regardless of the temperature at which the plates are incubated.

(d) Classify WrmY⁻ by the type(s) of mutation it could be **with respect to Wrm1**. (Your choices are: repressor –, activator –, UAS–, URS–, super activator, super repressor, dominant negative repressor, dominant negative activator.)

You create diploid yeast by mating WrmX$^-$ WrmY$^-$ wrm1::lacZ haploid yeast to wrm1::lacZ haploid yeast. Sporulation of these diploids yields two types of tetrads, and you correctly conclude (given the number of each type of tetrad) that the WrmX and WrmY loci are linked at a distance of 2.22 cM.

(e) Depicted below are the two types of tetrads that resulted when you sporulated the above diploids. For each type of tetrad, state **how many** you found of that tetrad (out of a total of 90 tetrads), **classify** the tetrad as PD, NPD, or TT, and **color in** all of the spores that would be blue on each of the following Petri plates.

Tetrad Type A
Number of these tetrads out of a total of 90: _____
Classification of these tetrads (PD, NPD, or TT): _____

Color in the spores that would be blue in color when growing on the following plates:

X-gal, 24°C	X-gal, 36°C
○	○
○	○
○	○
○	○

NOTE that the two plates are replicas, so the top spore on the left plate has the same genotype as the top spore on the right plate.

Tetrad Type B
Number of these tetrads out of a total of 90: _____
Classification of these tetrads (PD, NPD, or TT): _____

Color in the spores that would be blue in color when growing on the following plates:

X-gal, 24°C	X-gal, 36°C
○	○
○	○
○	○
○	○

3. The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For the mouse you make**, please state:

i) whether you are using pronuclear injection or gene targeting techniques
ii) what DNA you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg or ES cells
iv) what is the genotype of the fertilized egg or the ES cells you would start with
v) where in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera or not
vii) which additional breeding steps you would do to make the mouse you wanted
viii) two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result

"Non-homologous end joining" is the process by which a DNA sequence gets inserted into a chromosomal region to which it is not homologous. Having a functional copy of the gene "NheJ" is necessary for this process to occur in mice. A mouse with no copies of the NheJ gene is sensitive to irradiation as an adult, but a heterozygote is not sensitive.

You decide to test whether one copy of the *Drosophila* "d-Nhe" gene could fully compensate for the absence of the mouse NheJ gene. You have wild-type homozygous mice (NheJ+/NheJ+), heterozygous mice (NheJ+/NheJ^{-}), and homozygous mutant mice (NheJ^{-}/NheJ^{-}) readily available to you.

4. You are studying how yeast cells grow on the sugar maltose as a carbon source. You find that the sugars maltose and glucose both affect the regulation of the principal enzyme for maltose utilization, which is called maltase. In yeast cells grown without maltose, maltase is not expressed, but maltase is induced when maltose is added to the growth medium. In cells grown in medium that contains both maltose and glucose, maltase is not expressed. You have isolated mutations in three different genes that alter maltase regulation, called **A^{-}**, **B^{-}** and **C^{-}**. All three mutations give recessive phenotypes, and none of the three loci are linked either to maltase or to each other. The maltase expression of wild-type and each of the three mutants are shown below.

	Maltase activity		
	– maltose	+maltose	+maltose & glucose
Wild type	–	+	–
A^-	+	+	–
B^-	–	–	–
C^-	–	+	+

.

(a) For each of the three wild-type genes: state whether it encodes a positive regulator or a negative regulator of maltase, and state whether it affects maltase regulation by maltose or glucose.

A

B

C

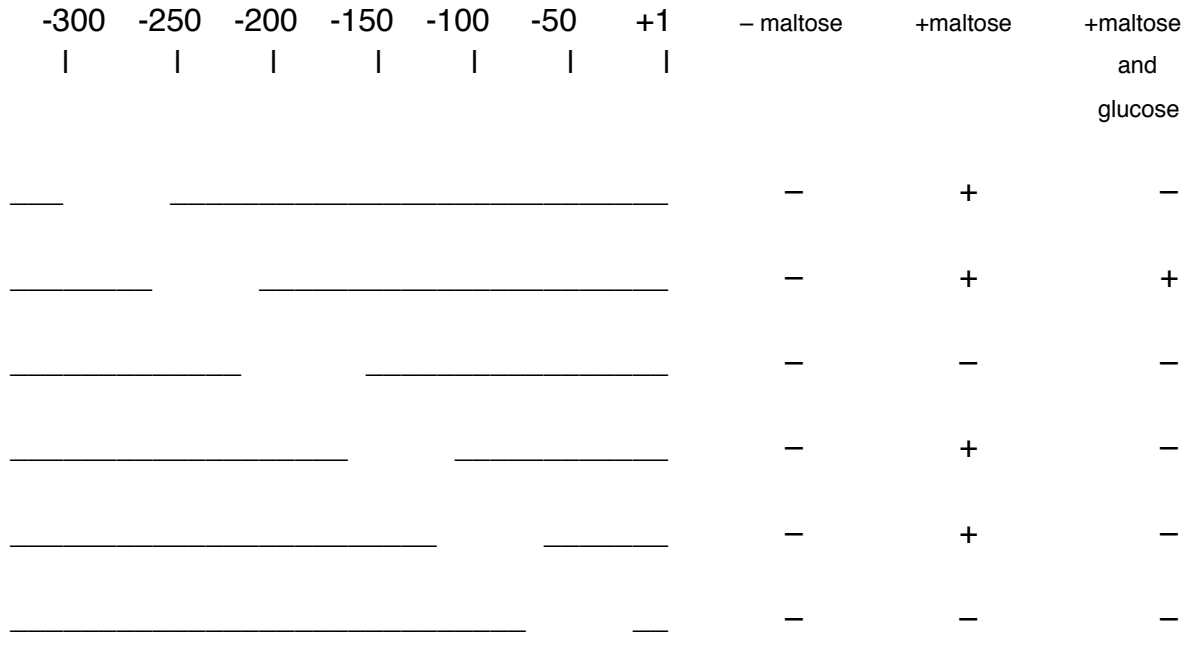
Next you cross an **A^-** haploid mutant to a **B^-** haploid mutant, and induce sporulation of the resulting diploid. After the resulting tetrads are dissected and evaluated for maltase expression in either the presence or absence of maltose, the following tetrad types are observed:

Type 1	Type 2	Type3
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible

(b) What is the phenotype of the **A^- B^-** double mutant haploid yeast strain? Explain how you arrived at your answer.

(c) Draw a genetic pathway showing the interactions between the different regulatory factors encoded by the wild-type **A, B**, and **C** genes. Be sure to include the maltase gene and to indicate where and how the sugars glucose and maltose act.

Next, you construct a set of 50 base-pair deletions within the cis regulatory region of the maltase gene. The ability of each of these deletions to express maltase in cells grown on different sugars is shown below.



(d) The **C** gene encodes a DNA-binding protein. Assuming that the product of gene **C** binds to the cis regulatory region of the maltase gene, to which 50 basepair long region is it most likely to bind?

(e) In general, upstream activation sequences function normally regardless of their spacing relative to the TATA box sequence that binds RNA polymerase. Which of the deletion mutants shown above show this to be true for the upstream activation sequence that responds to the sugar maltose?

5. You have a mutant mouse that displays the phenotype of white fur (instead of the wild-type color for this strain, which is brown fur). You have found that the white mouse is homozygous for a specific mutant alelle in the “whfr” gene. You want to know if being homozygous for this allele is enough to cause the white fur phenotype, or if this mouse is white because of a combination of mutations that it contains. You decide to address this issue using a genetically engineered mouse.

For the mouse you make, please indicate:

1. The method you would use (transgene or gene targeting)
2. What DNA would be introduced into mouse cells
3. The cell type you would introduce the DNA into (fertilized egg or ES cells)
4. The genotype of the cell you would introduce the DNA into
5. The site of integration (i.e. which genomic locus the DNA would enter into)
6. Which additional breeding steps are required to make the mouse you want
7. The possible results of your experiment and how you would interpret each possible result

6. An allele that causes the recessive phenotype of microcephaly has a frequency **q** = 0.0001 in a randomly-mating population in Hardy-Weinberg equilibrium.

(a) What is the expected frequency of microcephalic individuals in this population?

(b) Mutations to produce new alleles for microcephaly occur at frequency of $\boldsymbol{\mu} = 10^{-9}$. If the current frequency of microcephaly was established during human evolution at a time when the fitness of microcephalic individuals was zero but there was a selective advantage for individuals heterozygous for microcephaly, then what was the value of the heterozygous advantage **h**?

(c) Say that, in modern times, the fitness of microcephalics increased to 0.5 and the heteozygous advantage has become zero. Assuming that these conditions hold for many centuries, what will the steady state allele frequency for the allele causing microcephaly eventually become?

7. You are studying a yeast strain that will grow using the sugar raffinose as a carbon source. The gene encoding the raffinase enzyme (Raf1) is expressed when raffinose is present, but it is not expressed when raffinose is absent. To study the regulation of Raf1, you construct a fusion of the Raf1 upstream regulatory sequences to the *E. coli* LacZ reporter gene and place this gene fusion (designated Raf1-LacZ) on an extrachromosomal plasmid. Yeast cells carrying this plasmid express ß-galactosidase only in the presence of raffinose. You use this inducible reporter gene construct to identify two new regulatory mutants designated Raf2$^-$ and Raf3$^-$. The effect of these mutants on expression of the Raf1-LacZ reporter is shown below:

	ß-galactosidase activity	
	+ raffinose	– raffinose
Wild type (Raf1-LacZ)	+	–
Raf2$^-$ (Raf1-LacZ)	+	+
Raf3$^-$ (Raf1-LacZ)	–	–

You extend your analysis of these mutants by constructing three diplods strains (each also carrying the Raf1-LacZ reporter plasmid) with phenotypes shown below:

	ß-galactosidase activity	
	+ raffinose	– raffinose
Raf2$^-$ / Raf2$^+$(Raf1-LacZ)	+	–
Raf3$^-$ / Raf3$^+$(Raf1-LacZ)	+	–
Raf2$^-$ / Raf3$^-$ (Raf1-LacZ)	+	–

(a) What do these results tell you about the relationship between the Raf2$^-$ and Raf3$^-$ mutations? Be as specific as you can about the conclusions that you can draw.

(b) Next, you induce sporulation of a diploid strain produced by crossing a Raf2^{-} (Raf1-LacZ) haploid mutant to a Raf3^{-} (Raf1-LacZ) haploid mutant. Out of a total of 100 resulting tetrads, 70 are Type One, 19 are Type Two, and 11 are Type Three.

Type One	Type Two	Type Three
constitutive	uninducible	regulated
uninducible	uninducible	regulated
uninducible	constitutive	uninducible
regulated	consitutive	uninducible

Does a Raf2^{-} Raf3^{-} double mutant haploid yeast strain show regulated, constitutive, or uninducible expression of the Raf1-LacZ reporter?

(c) On the basis of your answer for part (**b)** and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of Raf1. In your model, include the wild-type Raf1, Raf2 and Raf3 genes. Also show how the sugar raffinose itself might act.

(d) Say that you isolate an allele of the Raf2 gene that gives the dominant phenotype of uninducible expression of Raf1-LacZ expression. This allele (designated Raf2^{u}) lies within the coding sequence of the Raf2 gene. You do a cross between a Raf2^{u} (Raf1-LacZ) haploid mutant and Raf2^{-} (Raf1-LacZ) haploid mutant strain. You then induce sporulation of this diploid. What kind(s) of tetrads would you expect to get and in what frequencies? (Specify the tetrad types in terms of the spore phenotypes with respect to Raf1-LacZ expression, following the format of the list of tetrads shown in part **(b)**.)

8. Suppose that, in an isolated population, there exists a very rare inherited anemia which is autosomal recessive. Assume that selection and mutation rates are negligible.

(a) Given that the frequency of the allele for the anemia is **q**, calculate the probability that a child will be born with the anemia assuming random mating. Express your answer as a function of **q**.

(b) What is the probability (as a function of **q**) that a given individual in the population is a heterozygote? Use the approximation that is valid for a very small **q**.

(c) In this population, marriages between a niece and her biological uncle occur sometimes. Given that the niece in one such marriage is heterozygous for the allele for the anemia, what is the probability that her child will have the anemia?

(d) Given that uncle-niece marriages occur at a frequency of **0.008**, use the answers derived above to calculate the chance that a child born into the population will have the anemia and will have been produced by an uncle-niece marriage. Express your answer as a function of **q**.

(e) If half of the children with the anemia come from uncle-niece marriages and half come from marriages with no obvious inbreeding, what is **q**? If helpful, you may use the approximation that the frequency of random marriages is about one.

9. You are studying the sihZ gene in mice, and you isolate a mutation called “regX” that disrupts proper transcriptional regulation of the sihZ gene. This mutation causes the dominant phenotype of constitutive expression of sihZ. The mutation maps to within a thousand base pairs of the coding sequence of the sihZ gene. You want to know whether regX is a mutation in a non-coding DNA sequence that controls sihZ expression, or whether regX is a mutation in a gene near to sihZ that encodes a regulatory protein for sihZ. You decide to address this issue by creating a genetically engineered mouse.

(a) Would you choose to utilize transgenic or gene-targeting technologies to make your genetically modified mouse?

(b) What exact type of modification to the mouse genome would you make to test your hypothesis?

(c) Draw the DNA construct that you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.

(d) What cell type would you put your DNA construct into? Include your choice of fertilized egg or ES cell, and what the genotype of the cells you would use should be.

(e) Explain what (if any) steps you will need to do to proceed from part **(d)** above to obtaining the final modified mouse you actually want.

(f) Name the <u>two possible</u> phenotypic results you could get from the newly made mice, <u>and</u> the corresponding conclusions you would make based on each result.

10. A early nonsense mutation in the yeast **URA9** gene gives an intermediate level of growth without the nucleotide uracil being provided in the growth medium (Ura+/$^{-}$). From the **ura9^{-}** strain, you isolate a robust Ura+ derivative (strain 1) which you then cross to a haploid wild-type (**URA9+**) strain. You induce sporulation of the resulting diploids, and the tetrads you observe are as follows:

4 Ura+	2 Ura+ : 2 Ura+/$^{-}$	3 Ura+ : 1 Ura+/$^{-}$
101	98	414

(a) What genetic event occurred to give robust growth without uracil in strain 1?

Next, starting with the **ura9^{-}** strain, you isolate a completely Ura^{-} derivative (strain 2) which does not grow without uracil. You cross a haploid strain 2 with a haploid wild-type (**URA9+**) yeast strain. When you induce sporulation of the resulting diploids, the tetrads you observe are as follows:

2 Ura^{-} : 2 Ura+	4 Ura+/$^{-}$	2 Ura+/$^{-}$: 1 Ura^{-} : 1 Ura+
94	97	385

(b) What genetic event occurred to give the Ura- phenotype in strain 2?

(c) Next, you cross a haploid strain 1 to a haploid strain 2, and induce sporulation of the resulting diploids. The first 30 tetrads all show 2 Ura+ : 2 Ura$^-$ segregation. Explain these data with respect to what they tell you about the mutations in strains 1 and 2.

(d) Finally, you look at 10 more tetrads from the cross in part (**c)** and find two that consist of 2 Ura$^+$ spores: 1 Ura+/$^-$ spore: 1 Ura $^-$ spore. Explain these data with respect to what they tell you about the mutations in strains 1 and 2.

11. Trekking in the Himalayas, you discover a "founder generation" of 1000 goats barricaded on all sides by high peaks and massive glaciers. This founder generation consists of 200 **AA** goats, 200 **Aa** goats, and 600 **aa** goats.

(a) What are the frequencies of alleles **A** and **a** in the founder generation?

(b) Is the founder generation in Hardy-Weinberg equilibrium? Show your work.

(c) What is the frequency of the **A** allele in the second generation (that is, in the generation after the founder generation)? (Mating of the founder generation goats is random, fitness does not differ among the three genotypes, and mutation occurs at a negligible rate.)

(d) What are the frequencies of the **AA**, **Aa**, and **aa** genotypes in the second generation?

12. The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For the mouse you make**, please state:

i) whether you are using pronuclear injection or gene targeting techniques
ii) what DNA you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg or ES cells
iv) what is the genotype of the fertilized egg or the ES cells you would start with
v) where in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera or not
vii) which additional breeding steps you would do to make the mouse you wanted
viii) two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result

You have a colony of mutant mice that are unable to smell perfume because they are homozygous for a loss-of-function mutation in the PrfM gene (a gene that you have recently identified and cloned). In that colony, you isolate one mouse with one copy of an unmapped suppressor mutation, SupR; that mouse can now smell perfume. You want to determine whether SupR is an extragenic suppressor mutation or an intragenic suppressor mutation. Do this by creating one type of genetically engineered mouse. (You have wild-type mice, and the mice mentioned above, available to you.)

13. You are studying the regulation of Gln1, a yeast gene involved in glutamine synthesis. Gln1 is not expressed when glutamine is present in the growth medium and is expressed when glutamine is absent. To begin your analysis of regulation, you fuse the promoter region of the Gln1 gene to the LacZ coding sequence and then place this hybrid gene on a yeast plasmid. You find that yeast cells carrying this plasmid (P_{Gln1}–LacZ) only express ß-galactosidase activity when glutamine is absent. You next identify two different mutants that show abnormal regulation of your reporter. You call these mutants $Gln7^-$ and $Gln8^-$. The table below shows the phenotypes of a variety of haploid and diploid yeast strains containing the P_{Gln1}–LacZ reporter. A filled circle indicates a yeast colony that expresses ß-galactosidase activity.

	ß-galactosidase activity	
	– glutamine	+ glutamine
wild type (P_{Gln1}–LacZ)	●	○
$Gln7^-$ (P_{Gln1}–LacZ)	●	●
$Gln7^-$ / $Gln7^+$ (P_{Gln1}–LacZ)	●	○
$Gln8^-$ (P_{Gln1}–LacZ)	○	○
$Gln8^-$ / $Gln8^+$ (P_{Gln1}–LacZ)	●	○

When you mate a $Gln7^-$ (P_{Gln1}–LacZ) mutant to a $Gln8^-$ mutant, the resulting $Gln7^-$ / $Gln8^-$ (P_{Gln1}–LacZ) diploid shows normal ß-galactosidase expression and regulation. After sporulatation, this diploid produces three different tetrad types. Out of a total of 50 tetrads, five are Type 1, thirty-eight are Type 2, and seven are Type 3.

Type 1		Type 2		Type 3	
ß-galactosidase activity		ß-galactosidase activity		ß-galactosidase activity	
– glutamine	+ glutamine	– glutamine	+ glutamine	– glutamine	+ glutamine
●	○	●	●	●	○
●	●	●	●	●	●
●	●	○	○	○	○
●	○	○	○	●	●

(a) Is a $Gln7^-$ / $Gln8^-$ double mutant regulated, constitutive, or uninducible?

(b) Are the $Gln7^-$ and $Gln8^-$ mutations linked? If so, how far apart are they in cM?

(c) In the 50 tetrads you analyze, there are a total of 200 spores. Out of those 200 spores, 17 are $Gln7^{-}$ $Gln8^{-}$ double mutant spores. How many of those 17 came from NPDs?

(d) On the basis of your answer for part **(a)** and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of the Gln1 gene. Your model should include the wild-type Gln7, Gln1, and Gln8 gene products, as well as glutamine.

14. Albinism is a rare condition that is inherited as an autosomal recessive phenotype in many animals, including humans. This phenotype is caused by the body's inability to make melanin, the pigment responsible for most of the black and brown coloration in animals. In a particular population of wild hamsters, albinism occurs in about 1 out of 5500 animals. Assume selection and mutation rates are negligible.

(a) In this population, what is the frequency of the allele responsible for albinism? (Assume Hardy-Weinberg equilibrium for this part of the question.)

(b) What are the inbreeding coefficients for the following hamster matings?

Uncle-niece:

Grandparent-grandchild:

(c) In this population of hamsters, what is the probability that an animal resulting from a 1^{st} cousin mating will be albino?

(d) In this population of hamsters, 1 in every 800 matings is between 1^{st} cousins. (Assume that all other matings are random.) In this population, what fraction of all albino offspring will come from 1^{st} cousin matings?

15. The genetics of the eye disease known as retinitis pigmentosa (RP) are complex in humans, with many dozens of genes implicated. You decide to model this hereditary disease in mice using transgene and/or gene targeting methods.

For both **(a)** & **(b)** indicate:

1. The method you would use (transgene or gene targeting)
2. What DNA would be introduced into mouse cells
3. The cell type you would introduce the DNA into (fertilized egg or ES cells)
4. The genotype of the cell you would introduce the DNA into
5. The site of integration (i.e. which genomic locus the DNA would enter into)
6. Which additional breeding steps are required

(a) You identify a human family in which RP displays autosomal dominant inheritance and is caused by a specific missense mutation in the RP5 gene, on human chromosome #5. Describe how you would create a mouse model of this family's disease. (You have access to a piece of DNA that contains the mutant RP5 gene from an affected member of the family, a piece of DNA that contains the wild-type RP5 gene, and wild-type mice.)

(b) In another human family, you find that RP displays autosomal recessive inheritance and is caused by a loss-of-function mutation in the RP11 gene, on human chromosome #11. Describe how you would create a mouse model of this family's disease. (You have access to a piece of DNA that contains the mutant RP11 gene from an affected member of the family, a piece of DNA that contains the wild-type RP11 gene, and wild-type mice.)

(c) You also obtain a true-breeding strain of mice, called Rpx, which has retinitis pigmentosa. You do not observe retinitis pigmentosa among offspring of Rpx mice mated with wild-type mice. The Rpx mutant has not yet been characterized molecularly, but you suspect that the Rpx strain is mutant in the RP11 gene. Propose an experiment you could do using the mouse model you created in part **(b)** to test this hypothesis, without doing any DNA sequencing or further genetic modifications. Describe the two possible results of your experiment, and how you would interpret each result.

16. To study the regulation of yeast genes that are necessary for the utilization of the sugar sucrose, you construct a fusion of **Suc1** (a gene encoding a sucrose-hydrolyzing enzyme) to the *E. coli* gene for ß-galactosidase. The resulting gene fusion **Suc1-LacZ**, located on an extrachromosomal plasmid, is expressed only when sucrose is provided to the yeast cells. A screen for mutations that affect the regulation of **Suc1-LacZ** has yielded two different mutations that you call **Suc2⁻** and **Suc3⁻**. The table below shows the behavior of the original mutants as well as heterozygous diploids produced by mating the mutants to wild type.

	ß-galactosidase activity	
	– sucrose	+sucrose
Wild type (**Suc1-LacZ**)	–	+
Suc2⁻ (**Suc1-LacZ**)	–	–
Suc2⁻ / Suc2⁺ (**Suc1-LacZ**)	–	+
Suc3⁻ (**Suc1-LacZ**)	+	+
Suc3⁻ / Suc3⁺ (**Suc1-LacZ**)	–	+

(a) When you mate a **Suc2⁻** haploid mutant to a **Suc3⁻** haploid mutant, the resulting **Suc2⁻ / Suc3⁻** diploid shows normal expression and regulation of **Suc1-LacZ**. What does this result tell you about the relationship between the **Suc2⁻** and **Suc3⁻** mutations?

(b) Next, you induce sporulation of the **Suc2⁻ / Suc3⁻** diploid and dissect 50 tetrads. Among the tetrads, 10 are Type One, 5 are Type Two, and 35 are Type Three.

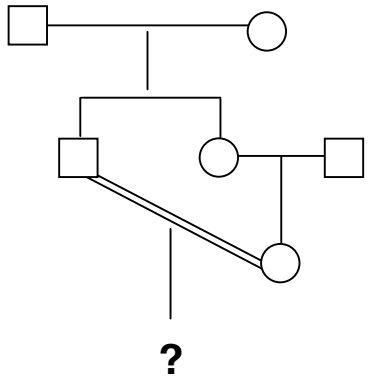
Type One	Type Two	Type Three
constitutive	uninducible	regulated
uninducible	uninducible	constitutive
uninducible	regulated	uninducible
constitutive	regulated	uninducible

Are the **Suc2** and **Suc3** loci linked? Briefly explain your answer.

(c) Is a **Suc2⁻ Suc3⁻** double mutant regulated, constitutive, or uninducible? Briefly explain your answer.

(d) On the basis of your answer for part (**c)** and from the rest of the information given in this problem, diagram a linear genetic pathway to explain the regulation of the **Suc1** gene. For your model, include the wild-type **Suc1**, **Suc2** and **Suc3** genes. Also show how the sugar sucrose itself might act.

17. Diagrammed below is a consanguineous mating of an uncle and niece.



(a) Calculate the inbreeding coefficient for this mating.

(b) Calculate the expected number of genes at which the resulting child will be homozygous by descent. (Assume that there are 30,000 genes in the human genome.)

Now consider a rare disease (fatal in childhood) whose incidence in a random-breeding population is four per million births. In parts **(c)** and **(d)**, calculate the incidence of the disease in the next generation assuming that 1% of all matings are between uncles and nieces (and all other matings are random), given that:

(c) The disease exhibits autosomal dominant inheritance.

(d) The disease exhibits autosomal recessive inheritance.

Let's now return to the purely random-breeding population in which this rare disease (fatal in childhood) has an incidence of four per million births. What rate of mutation (per generation) is required to maintain this incidence, given that:

(e) The disease exhibits autosomal dominant inheritance.

(f) The disease exhibits autosomal recessive inheritance.

Assume now that a new therapy allows many children with the disease to survive, such that affected individuals end up having 80% as many offspring as the population average. After many generations of random mating, a new steady-state balance between mutation and selection is achieved. At this new steady state, what would the incidence of the disease be, given that:

(g) The disease exhibits autosomal dominant inheritance.

(h) The disease exhibits autosomal recessive inheritance.

18. You generate genetically engineered mice that are homozygous for a $P_{amylase}$-LacZ transgene insertion. These mice display a serious heart defect. A reasonable explanation for this observation is that the transgene had randomly inserted into a gene required for heart development or function. This transgene-induced heart defect reminds you of a recessive phenotype associated with a mutation in *small heart* (***sh***), a previously identified locus on mouse chromosome 12. Mice that are homozygous for the **sh** mutation also have serious heart defects.

Propose a breeding experiment to test the hypothesis that the ***sh*** mutation is in the same gene as the transgenic insertion mutation. Describe what two mice you would breed, the potential results of such a breeding, and how you would interpret each potential result.

19. Yeast cells have a set of enzymes that can synthesize the amino acid histidine. You select one of these enzymes, histidinol dehydrogenase, as a reporter gene to study the regulation of the histidine pathway. First, you learn that the **His4** gene is the structural gene for histidinol dehydrogenase, and that recessive **His4⁻** mutations cannot express histidinol dehydrogenase. You also find that the **His4** gene is regulated; it is expressed when histidine is absent from the medium, but is not expressed when histidine is present. These results are summarized below:

	Histidinol dehydrogenase activity	
	+ histidine	– histidine
Wild type	–	+
His4⁻	–	–
His4⁻ / His4⁺	–	+

(a) You isolate a mutant, designated **His10⁻**, which shows constitutive histidinol dehydrogenase expression. A cross of a **His4⁻** haploid mutant to a **His10⁻** haploid mutant gives diploids that show wild-type expression of histidinol dehydrogenase. What does this result allow you to conclude about the **His10⁻** mutant?

(b) When the diploids from part (**a**) are sporulated, the resulting tetrads are of three types with respect to the regulation of histidinol dehydrogenase. Out of a total of 50 tetrads, 30 are Type One, 16 are Type Two, and 4 are Type Three.

Type One	Type Two	Type Three
constitutive	uninducible	uninducible
constitutive	uninducible	uninducible
uninducible	constitutive	regulated
uninducible	regulated	regulated

Are the **His4** and **His10** loci linked? If so, how far apart are they in cM?

(c) Next, you isolate a mutation, designated **His11⁻**, which causes uninducible histidinol dehydrogenase expression. A cross of a **His4⁻** haploid mutant to a **His11⁻** haploid mutant gives diploids that show wild-type expression of histidinol dehydrogenase. On the basis of this result and the results for parts (**a**) and (**b**), diagram two different possible linear genetic pathways that can explain the functions of the **His10** and **His11** gene products in the regulation of **His4**. Be sure to include wild-type **His4**, **His 10**, **His11**, and the amino acid histidine in your answer.

(d) A cross of a **His10⁻** haploid mutant to an **His11⁻** haploid mutant gives diploids that you induce to sporulate. You get the following tetrad types as a result. Out of a total of 50 tetrads, 35 are Type Four, 8 are Type Five, and 7 are Type Six.

Type Four	Type Five	Type Six
uninducible	uninducible	constitutive
uninducible	uninducible	constitutive
constitutive	regulated	uninducible
regulated	regulated	uninducible

Is a **His10⁻ His11⁻** double mutant regulated, constitutive, or uninducible?

(e) On the basis of your answer for part (**d**) and from the rest of the information given in this problem, diagram diagram a linear genetic pathway that can explain the functions of the **His10** and **His11** gene products in the regulation of **His4**. Be sure to include wild-type **His4**, **His 10**, **His11**, and the amino acid histidine in your answer.

20. Consider an autosomal recessive disease in humans that is caused by possessing a specific loss-of-function allele at a single gene locus. Assume complete penetrance and no selection or new mutations.

(a) In population I, the disease has an incidence of 4×10^{-8}. Assuming that mating in the population is random, what is the frequency of the disease allele (**q**)?

(b) What fraction of all matings in population I are between heterozygotes? Show your calculations.

(c) A second population (population II) is also characterized by random mating, but here the disease has an incidence of 10^{-6}. Now suppose that human migration produces a new, mixed population, with 90% of the members of the new population deriving (randomly) from population I and the remaining 10% deriving (randomly) from population II. One generation later, what would the incidence of the disease be in the new, mixed population if mating were random? Show your calculations.

(d) What would the incidence of the disease be in the new, mixed population (from part **c**) if mating were strictly assortative (that is, if individuals originating from population II mated only with other individuals originating from population II and vice versa for population I)? Show your calculations.

(e) Assume that the new, mixed population from part **(c)** has undergone at least one generation of random mating. What is the probability that a child whose parents are first cousins will have the disease?

(f) Now assume that the disease allele in population I differs from (but is in the same gene as) that in population II. Without doing calculations, answer yes or no to the following two questions: Would you modify your response …to question **(c**)?
…to question **(d)**?

(g) Now assume that the disease allele in population I is in a different gene from that in population II. Without doing calculations, answer yes or no to the following two questions: Would you modify your response …to question **(c**)?
…to question **(d)**?

21. You are studying a recessive eye-color mutant phenotype (called *pinkeye*) in the mouse. You have mapped the pinkeye locus down to a small interval that contains two genes, gene A and gene B. The *pinkeye* gene has not yet been defined at a molecular level, but you are confident that either gene A or gene B must be the site of the *pinkeye* mutation. You have pieces of genomic DNA that contain either wild-type gene A or wild-type gene B. In this problem, you will be asked to create two genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered.
For each mouse you make, please state:

i) whether you are using pronuclear injection or gene targeting techniques
ii) what DNA you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg or ES cells
iv) what is the genotype of the fertilized egg or the ES cells you would start with
v) where in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera or not
vii) which additional breeding steps you would do to make the mouse you wanted
viii) two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result

(a) Propose an experiment involving one or more gene-targeting constructs (but no transgenes) that would test whether the **phenotypically defined** *pinkeye* mutation is in the **molecularly defined** gene A.

(b) Propose an experiment involving one or more transgenes (but no gene-targeting constructs) that would test whether the **phenotypically defined** *pinkeye* mutation is in the **molecularly defined** gene A.

22. An autosomal recessive inherited disease with a selective disadvantage of 0.1 occurs at a frequency of 10^{-4} in a randomly mating population.

(a) Say that the allele frequency for the disease was set by a balance between new mutations and selection against the homozygote. In any given generation, what fraction of the disease alleles within the population are new mutations? (You can use approximations that are accurate to within 10%).

(b) Now consider the effect of consanguious matings among members the Aztec royal family. For an autosomal recessive trait that is present in the population at a frequency of 10^{-2} , give the probability that a child with the trait will be produced by the following types of matings. Assume that selection and mutation rates are negligible.

Mating between two unrelated individuals:

Brother-sister mating:

(c) Now consider an X-linked recessive trait that is present at a frequency of 10^{-2}. Give the probability that a child with the trait will be produced by a mating between two unrelated individuals. (You can use approximations that are accurate to within 10%, and you can assume that that the fitness of individuals with the trait is 1.0, the mutation rate is zero, and that the sex of the child is not known.)

Exam Questions from Final Exam – Human Genetics, Nondisjunction, and Cancer, and Cumulative Questions

1. You are working on two different organisms -- the fruit fly *Drosophila* and the yeast *S. cerevisiae*. In each organism, you have isolated two unmapped mutations, C^- and D^-. C^- is a recessive mutation that causes the organism to be four times as large as its normal size. D^- is a recessive mutation that causes the organism to be two times as large as its normal size. Both mutations cause the misregulation of Gene E, to which the C^- and D^- mutations are linked. E^- is also a recessive mutation that causes the organism to be four times as large as its normal size.

Listed below are the genotypes of strains you have made to do different genetic tests. For each strain, briefly describe if and how the strain could be constructed if you were working with yeast or with *Drosophila*.

Your starting materials are: C^- haploid yeast, D^- haploid yeast, E^- haploid yeast, true-breeding C^- *Drosophila*, true-breeding D^- *Drosophila,* and true-breeding E^- *Drosophila*.

The manipulations you can use in your answer are: P1 transductions, matings, conjugations, tetrad analyses, inducing sporulation, transformations, isolating F' strains, and three factor crosses. **For each manipulation**, please state the **genotypes** of the strains you are manipulating.

Also, for each strain, list **all** of the possible genetic tests for which each strain could be used WITHOUT further modifications or matings. (Your choices are: complementation, dominant/recessive, cis, constitutive/uninducible, trans, epistasis.)

(a) genotype of this diploid strain is $C^- D^+ E^+ / C^+ D^+ E^-$
how to make this strain in yeast:

how to make this strain in *Drosophila*:

list **all** of the possible genetic tests for which this strain could be used:

(b) genotype of this haploid strain is C^{-} D^{-} E^{+}
how to make this strain in yeast:

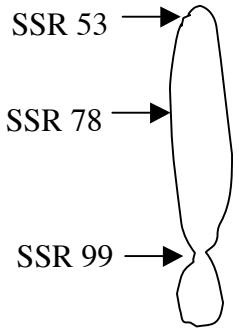
how to make this strain in *Drosophila*:

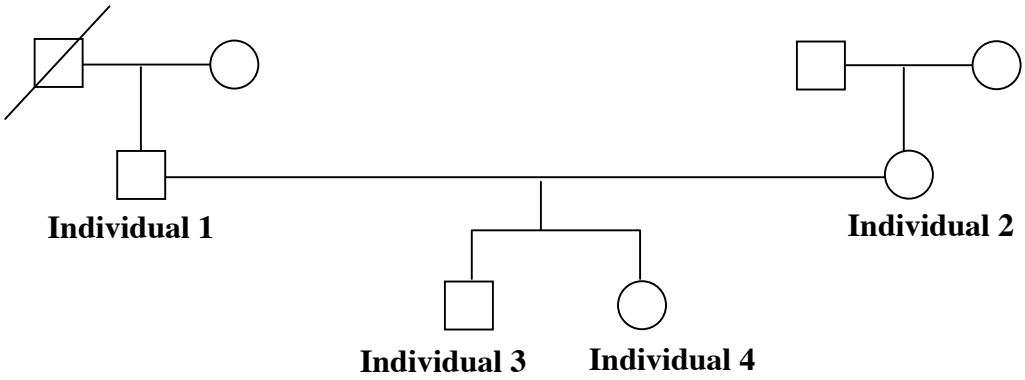
list **all** of the possible genetic tests for which this strain could be used:

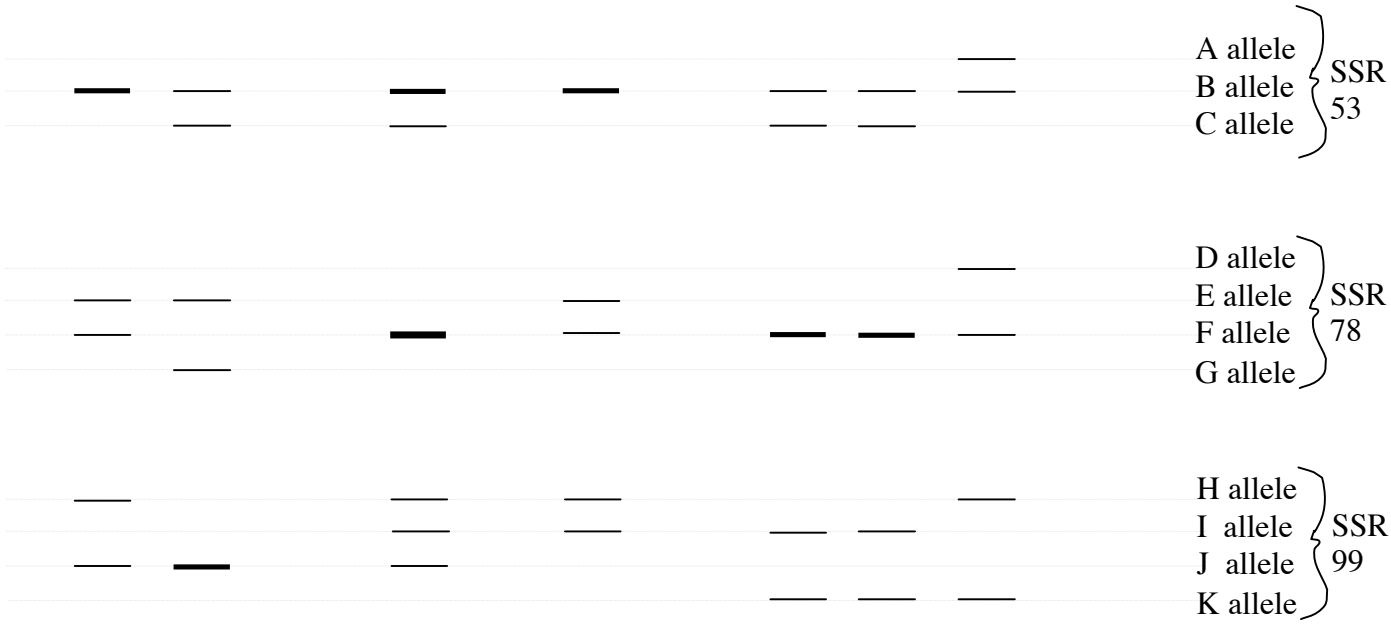
(c) Say you are studying C^{-} and D^{-} in a new species of bacterium which can be manipulated using transposons and P1 phage as *E. coli* can, but cannot maintain F and R factors. Given this, list **all** of the tests you could not perform in this bacterial species.

2. On the next page is a pedigree showing a couple that has a child with trisomy of chromosome 21. The schematic of a gel is also shown on the next page, and this gel reveals the genotypes of each member of the family at three different SSRs found on chromosome 21. A schematic of chromosome 21 is shown below, with the locations of the three SSRs marked. The constriction on the chromosome indicates the centromere.

Schematic of Chromosome 21







	Individual 3	Individual 4
maternally inherited allele(s) at SSR 53		
paternally inherited allele(s) at SSR 53		
maternally inherited allele(s) at SSR 78		
paternally inherited allele(s) at SSR 78		
maternally inherited allele(s) at SSR 99		
paternally inherited allele(s) at SSR 99		

(a) Fill in each row of the chart on the previous page to indicate which alleles each child inherited from each parent.

(b) Draw all phases of each parent (with respect to the three SSRs on chromosome 21) that are possible given everything you know about those parents. Make sure to draw the phases using the proper notation.

Father (individual 1):

Mother (individual 2):

(c) Which child has trisomy 21 (Individual 3 or Individual 4)? **(**Note: Make sure that your chart on page 3 is consistent with that child having trisomy 21.)

(d) During the development of which parent's gametes did the non-disjunction event occur (Individual 1 or Individual 2)?

(e) When in meiosis did non-disjunction occur (meiosis I or meiosis II)?

(f) Draw the following steps in the meiosis that created the gamete that led to the production of the child with trisomy 21 shown in the pedigree. Please label each SSR allele and the centromere on each homolog of chromosome 21. In each drawing, include chromsome 21 and one other chromosome of a different length that undergoes meiotic chromosome segregation normally. Draw these steps only:

i) the cell in metaphase I with its chromosomes lined up showing any crossover events occurring

ii) the two cells in metaphase II with their chromosomes lined up

iii) the four final products of the meiosis (Please indicate the gamete that led to the creation of the child with trisomy 21 with a star.)

3. After extensive genetic linkage studies, you map the locus for the ability to taste or not taste the compound PTC to a 2-centiMorgan (cM) region on human chromosome 7.

You then discover that some PTC non-tasters are homozygous for a 10-kb deletion within the implicated region. The deletion encompasses a gene you call gene Z. Your findings suggest but do not prove that the absence of gene Z results in the inability to taste PTC. You decide to test this hypothesis using a genetically modified mouse. The DNA sequences of the human and mouse Z genes are very similar but not identical. Like people who are PTC tasters, wild-type mice dislike the taste of PTC and won't eat food to which PTC has been added. You have available: 1) genomic DNA pieces with either the human or mouse Z genes and 2) mouse food with and without PTC.

(a) Would you choose to utilize transgenic or gene-targeting technologies to make your genetically modified mouse?

(b) What exact type of modification to the mouse genome would you make to test the hypothesis that the absence of gene Z results in inability to taste PTC? Explain your choice.

(c) Draw the DNA construct that you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.

(d) What cell type would you put your DNA construct into? Include your choice of fertilized egg or ES cell, and what the genotype of the cells you would use should be.

(e) Explain what (if any) steps you will need to do to proceed from part **(d)** above to obtaining the final modified mouse you actually want.

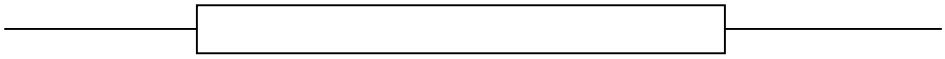
(f) Name the <u>two possible</u> phenotypic results you could get from the newly made mice, <u>and</u> the corresponding conclusions you would make based on each result.

(g) What additional modification would you make to the mouse you just made in order to test the hypothesis that the mouse and human Z genes are functionally interchangeable?

4. You have isolated three mutations in phage λ. One phage mutant is called **sm1⁻** and gives a small plaque phenotype (wild-type phage are **sm1⁺** and give large plaques). Another phage mutant is called **sm2⁻** and gives a small plaque phenotype (wild-type phage are **sm2⁺** and give large plaques). Note that the **sm1⁻** and **sm2⁻** mutations do not suppress each other. The third phage mutant is called **cI⁻** and gives a clear plaque phenotype (wild-type phage are **cI⁺** and give turbid plaques). You cross **sm1⁻** phage to **sm2⁻** phage by coinfecting *E. coli*. When the resulting lysate is plated, you count 2000 of the resulting plaques and find that 15 plaques are large and 1985 plaques are small.

(a) What is the genetic distance between the **sm1⁻** and **sm2⁻** mutations? (Be sure to label your answer with the correct units.)

(b) By using DNA sequencing, you find that **sm1⁻** and **sm2⁻** are mutations in the same open reading frame. The size of the wild-type protein produced from this open reading frame is 55 kDa. The size of the protein product of an **sm1⁻** mutant gene is 44 kDa, and the size of the protein product of an **sm2⁻** mutant gene is 11 kDa. Using the rectangle below to indicate the DNA sequence of this open reading frame, draw in the **relative positions of:** the **sm1⁻** mutation, the promoter, the stop codon, the **sm2⁻** mutation, and the start codon.
(Keep in mind that, on average, one amino acid = 0.11 kDa.)



(c) Express phage λ's recombination rate by stating the number of kilobases of DNA that correspond to 1% recombination for this phage. Show all calculations.

(d) You next cross **cI⁻ sm1⁻** phage to **sm2⁻** phage. When the resulting lysate is plated out and 2000 plaques are examined, you find that you have isolated 15 large plaques (all 15 of which are turbid). Given this new information, draw a map of this phage showing the relative order of the **cI**, **sm1** and **sm2** loci in this box:
(Note: Keep in mind that **sm1⁻** and **sm2⁻** are mutations in the same open reading frame.)

5. A ship carrying 7,000 passengers is about to land on an island that has 33,000 occupants. Each of these two populations is at Hardy-Weinberg equilibrium before the ship's landing, and each population contains an equal number of males and females. Of the 7,000 ship passengers, only 21 are displaying the X-linked recessive trait "huge toes" (and all 21 are male). Of the 33,000 island occupants, only 6 have huge toes (and all 6 are male). When answering the following parts, show all of your calculations.

(a) On the ship before landing, what is your best estimate of the allele frequency for the allele that causes huge toes?

(b) If you select a female child at random from the island (before the ship lands), what would the probability be that she is a carrier of the allele for huge toes?

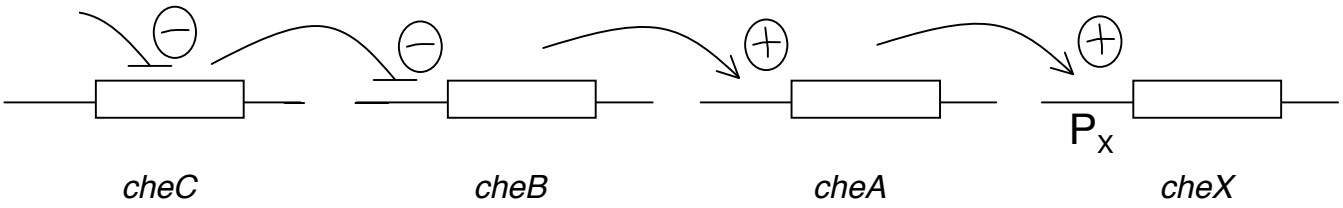
(c) Now the ship lands on the island, and the passengers and island occupants mate together randomly to produce the next generation "G2". What is your best estimate of the allele frequency for the allele that causes huge toes in generation "G2"?

(d) If you select one child from generation "G2" at random, what would the <u>total</u> probability be that it has huge toes? (Take both males and females into account.)

6. You are studying regulation of the *E. coli cheX* gene, a gene that is expressed in response to chemoattractants. *E. coli* expresses the *cheX* gene when chemoattractants are present in the medium, and this allows the bacteria to move towards the chemoattractants. You monitor the expression of the *cheX* gene in order to perform a genetic screen looking for mutants that do not properly regulate expression of *cheX*. In your screen, you isolate a series of mutant strains that either show constitutive or uninducible expression of *cheX*.

From the results of your screen, you deduce the following correct model for regulation of *cheX* expression. Keep in mind that this model is a genetic pathway that should not be interpreted as a molecular model.

chemoattractants

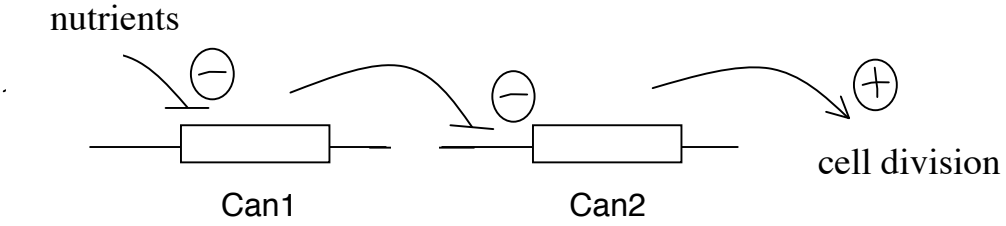


In your screen, you isolate four strains, each of which contains one of the following single mutations:
cheC1, which is in the **coding region** of *cheC*. This mutation gives a recessive phenotype.
cheB2, which is in the **coding region** of *cheB*. This mutation gives a constitutive phenotype.
cheA3, which is in the **coding region** of *cheA*. This mutation gives a recessive phenotype.
P_X^-, which is a deletion of the **promoter sequence** in front of *cheX*

Using this information, fill in the chart below. Fill in each box of the chart below with the word "**yes**" or the word "**no**." Please keep in mind that all sequences not included in the chromosomal genotypes are wild-type. Also please keep in mind that **all sequences not included in the F' plasmid genotypes are ABSENT from that plasmid.**
Genotypes are written in the format: (on the chromosome) / F' (on the F' plasmid).

Genotype of strain	Will *cheX* be expressed when chemoattractants are absent?	Will *cheX* be expressed when chemoattractants are present?
wild-type		
cheC1		
cheB2* / F' P_X^- *cheX+		
cheA3* / F' *cheC1		
cheX+ cheB2 cheA3		
P_X^- cheX+* / F' *cheC1* P_X^+ *cheX+		

7. You are studying cancer progression in mice. Your results indicate that the following is one pathway for how mouse cell division is regulated.



You isolate a mutant strain of mice that contains a mutation (Can1^{-}) in the coding region of Can1. (Note that Can1 is a gene that encodes a monomeric protein). These mutant mice frequently develop cancer as young adults.

(a) Classify the Can1^{-} mutation as causing constitutive OR uninducible cell division.

(b) Classify the Can1^{-} mutation as dominant OR recessive.

(c) Classify the Can1^{+} gene as a proto-oncogene or a tumor suppressor gene.

(d) In a sentence, describe in general what the wild-type function of Can1 is in the cell.

You isolate a mutant strain of mice that contains a mutation (Can2^{-}) in the coding region of Can2. (Note that Can2 is a gene that encodes a monomeric protein). These mutant mice frequently develop cancer as young adults.

(e) Classify the Can2^{-} mutation as causing constitutive OR uninducible cell division.

.

(f) Classify the Can2^{-} mutation as dominant OR recessive.

(g) Classify the Can2^{+} gene as a proto-oncogene or a tumor suppressor gene.

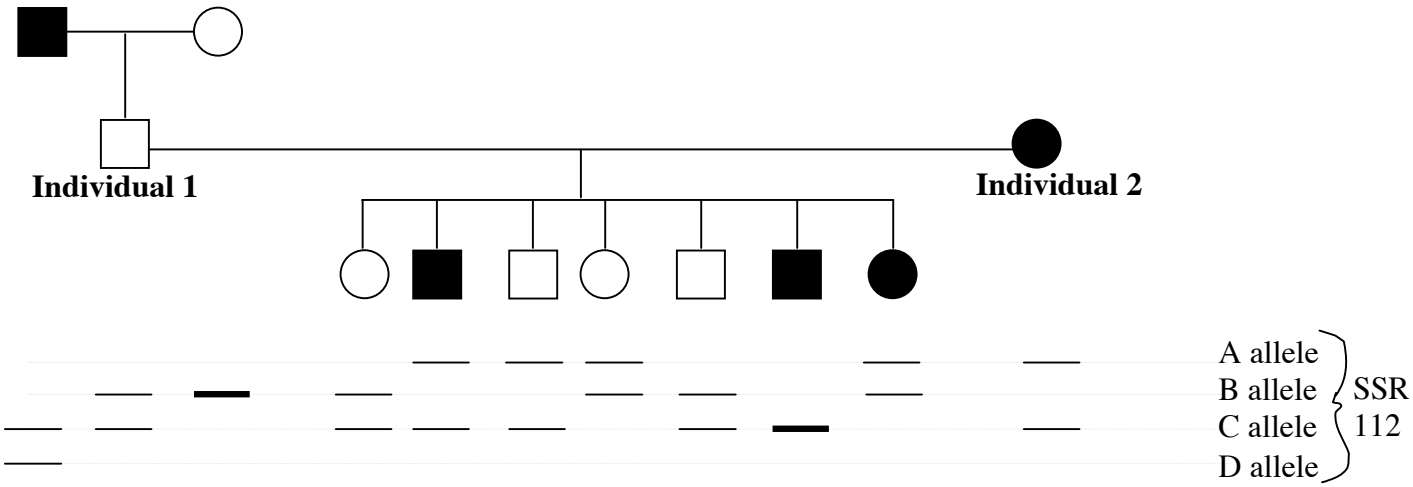
(h) In a sentence, describe in general what the wild-type function of Can2 is in the cell.

(i) You cross a $Can1^{+}/Can1^{-}$ heterozyous mouse to a wild-type mouse. Predict whether or not any of the progeny from this cross would develop cancer in early adulthood. Explain your answer in one or two sentences.

(j) If you analyzed a non-cancerous cell from the $Can1^{+}/Can1^{-}$ parent from part **(i)**, how many wild-type alleles of Can1 would be present?

(k) If you analyzed a cancerous cell from the $Can1^{+}/Can1^{-}$ parent from part **(i)**, how many mutant alleles of Can1 would be present?

8. You are mapping a certain rare disorder that is caused by an allele at the N locus. You suspect that the N locus is linked to SSR112 on human chromosome #17. You analyze the following family for these two loci. Assume complete penetrance and no new mutations. You will fill in the charts below in subsequent parts of the problem.



maternally inherited allele at SSR112							
paternally inherited allele at SSR112							

maternally inherited allele at the N locus							
paternally inherited allele at the N locus							

IF the condition is autosomal recessive [parts **(a)** and **(b)**] (Individual 2 is "nn")

maternally inherited allele at the N locus							
paternally inherited allele at the N locus							

IF the condition is autosomal dominant [parts **(c)** - **(e)**] (Individual 2 is "Nn")

Answer parts **(a)** and **(b)** as if the disorder is autosomal recessive and caused by the "n" allele, so that Individual 2 is "nn."

(a) Fill in the upper four rows of the chart using autosomal recessive inheritance for the disorder. Then answer below: **which parent's** alleles will you follow to correctly calculate a LOD score between the N locus and SSR 112 -- Individual 1 or 2?

(b) Draw all phases of the parent you chose in part **(a)** with respect to SSR 112 and the N locus that are possible given everything you know about that parent. Make sure to draw the phases using the proper notation.

Answer parts **(c)** through **(e)** as if the disorder is autosomal dominant and caused by the "N" allele, so that Individual 2 is "Nn."

(c) Fill in the lower two rows of the chart using autosomal dominant inhertance for the disorder. Then answer below: **which parent's** alleles will you follow to correctly calculate a LOD score between the N locus and SSR 112 -- Individual 1 or 2?

(d) Draw all phases of the parent you chose in part **(c)** with respect to SSR 112 and the N locus that are possible given everything you know about that parent. Make sure to draw the phases using the proper notation.

(e) How many times more likely is it that the data from this family arose because of linkage between the SSR 112 and N loci at $\theta = 0.2$ than because the two loci were unlinked? Show all calculations.

9. You have isolated an *E. coli* mutant that carries both an amber mutation in the HisC gene (HisC-am) and an amber suppressor mutation in a gene encoding a tRNA gene (Su^+). Therefore this strain is phenotypically His^+, meaning that it can grow without the amino acid histidine added to the medium. You obtain a strain which carries the HisC-am mutation and has a Tn5 insertion known to be linked to the HisC gene; this strain is phenotypically His^- and is kanamycin resisistant (Kan^r). The transposon is <u>not</u> in between HisC and the gene encoding the tRNA you are working with.

(a) You grow P1 phage on the HisC-am Tn5 strain and use the resulting phage lysate to infect the HisC-am Su^+ strain, selecting for Kan^r. Among 100 Kan^r transductants, you find that 20 are His^- and 80 are His^+. What distance are you measuring in this experiment, **and** what is that distance numerically?

(b) Next, 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 as well as the HisC-am and Su^+ mutations. You then use this phage lysate to infect a wild-type strain ($HisC^+$ and Su^-) and select for Kan^r. From 100 Kan^r transductants examined, 99 are His^+ and 1 is His^-.

In the second cross, you grow P1 phage on a bacterial strain that carries the Tn5 insertion and is $HisC^+$ and Su^-. You use this phage lysate to infect a bacterial strain with HisC-am and Su^+ mutations, selecting for Kan^r. From 100 Kan^r transductants examined, 90 are His^+ and 10 are His^-.

Draw a genetic map showing the relative positions of the Tn5 insertion, the HisC locus, and the tRNA gene in which the Su+ mutation is located.

10. Mendel's concept of the gene was first applied to a human trait in Archibald Garrod's landmark 1902 paper entitled "The Incidence of Alkaptonuria: A Study in Chemical Individuality." Alkaptonuria is a disease characterized by degenerative arthritis and by urine which turns black upon exposure to air. Because of an enzyme defect, the urine accumulates homogentisic acid, which oxidizes to form a black pigment.

As Garrod reported, and subsequent studies confirmed, 50% of individuals with alkaptonuria in the United Kingdom are offspring of first-cousin marriages. The total incidence of alkaptonuria in the United Kingdom is 1/250,000. Assume that, apart from first-cousin marriages, mating is random, and that that family size is the same in first-cousin matings as it is in random matings. Also assume that all cases of alkaptonuria are caused by the same mutation in one gene, and that mutation rates and selection are negligible.

(a) Is alkaptonuria an autosomal dominant or autosomal recessive disorder? Briefly justify your answer using information from the introduction to this question.

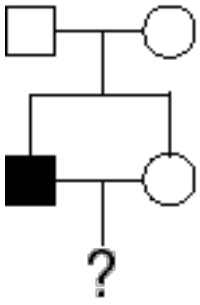
(b) In the United Kingdom, what is the frequency of the allele (call it allele AK) associated with alkaptonuria?

(c) In the United Kingdom, what is the frequency of heterozygotes (AK/+ individuals)?

(d) What is the expected proportion of all autosomal genes at which offspring of first cousins are homozygous by descent?

(e) Based on the data given here, estimate the frequency of first-cousin marriages in the United Kingdom.

11. Consider the following mouse pedigree in which the indicated male exhibits a distinctive rare trait. (Assume complete penetrance and that no new mutations arose in any of the individuals in this problem.)

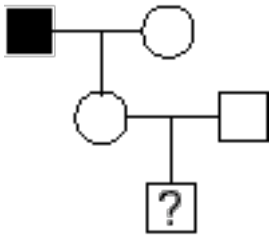


(a) Assuming that the trait is autosomal recessive, calculate the probability that an offspring from the indicated brother-sister mating will exhibit the trait.

(b) Assuming that the trait is X-linked recessive, calculate the probability that a male offspring from the brother-sister mating will exhibit the trait.

(c) Assuming that the trait is X-linked recessive, calculate the probability that a female offspring from the brother-sister mating will exhibit the trait.

(d) Consider the following mouse pedigree in which a male mouse that exhibits two different rare recessive X-linked traits (indicated by the filled symbol) is crossed to a true breeding wild-type female, and a female offspring from this cross is mated to a true-breeding wild-type male.



Given that the genes for the two traits are 10 cM apart on the X chromosome, calculate the probability that a male offspring from the mating will exhibit both traits.

12. The **cI** gene of phage lambda encodes a repressor protein that has a molecular weight of 24 kDa. You have isolated a phage mutant with a defective **cI** gene; this mutant therefore makes clear plaques rather than the normal turbid plaques. But you find that the mutant phage will produce turbid plaques when plated on an *E. coli* strain that contains an amber suppressing mutation in one of its tRNA genes. (The amber codon is UAG.) You find that the repressor protein is 16 kDa when the mutant phage are grown on wild-type *E. coli*, but is 24 kDa when the mutant phage are grown on an amber-suppressing strain of *E. coli*.

(a) What can you deduce about the nature of the **cI** mutation? Be as specific as possible about the kind of base change that caused the mutation and where exactly the mutation lies within the **cI** gene.

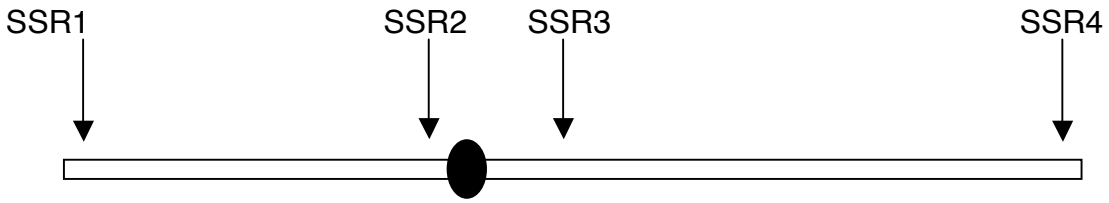
(b) Next you mutagenize the mutant phage described in part (**a)** to isolate a double mutant phage. These double mutant phage still form clear plaques when plated on wild type *E. coli.* However, when you examine the repressor protein produced by the double mutant phage grown on wild-type *E. coli*, you find that the protein is larger than the protein produced by the original single mutant phage. (The protein produced by the original single mutant phage is 16 kDa, whereas the protein produced by the double mutant phage is 17 kDa). Describe what kind of second mutation could give these results, assuming that the second mutation was caused by a single mutational event. Be as specific as possible about the nature of this second mutation.

(c) The codon for tryptophan is 5'-UGG-3'. Write out the DNA base sequence of the segment of the wild-type gene enocding $tRNA^{trp}$ that codes for the anticodon sequence of this tRNA. For your answer, only show the DNA strand that is used as the template for transcription of the $tRNA^{trp}$ molecule from the gene, indicating the 5' and 3' ends of this DNA strand.

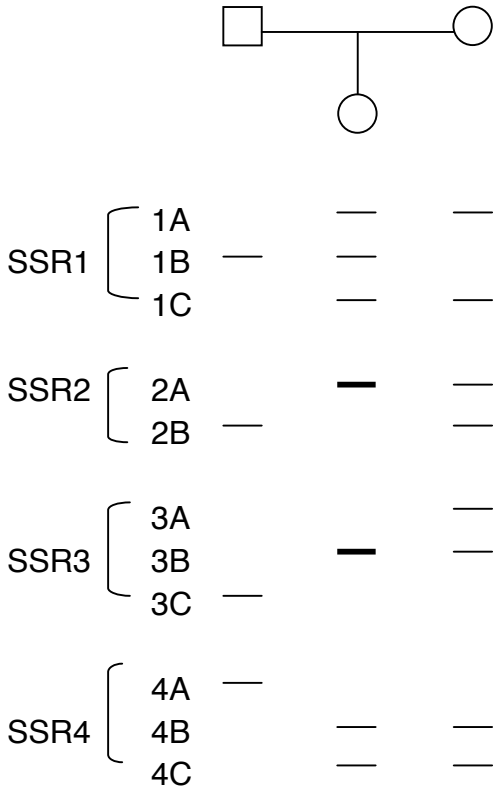
(d) Using the same format as in part **(c)**, write out the DNA base sequence of the same segment of the form of the tRNA gene that produces an amber-suppressing mutant version of $tRNA^{trp}$.

13. Your colleague, who is a medical geneticist, seeks your help in interpreting a patient: an XXY girl.

You prepare genomic DNA samples from the girl and from her parents. You confirm that the stated father is in fact the biological father by testing the family for a large number of autosomal SSRs. You also test the family for a series of SSRs distributed along the X chromosome, as shown below. The oval indicates the centromere.



The schematic of a gel is shown below, and this gel reveals the genotypes of each member of the family at four different SSRs found on the X chromosome:



(a) During the development of which parent's gametes did the non-disjunction event occur?

(b) In which division of meiosis did nondisjunction occur? Briefly explain your answer.

(c) Draw the following steps in the meiosis that created the gamete that led to the production of the XXY child shown in the pedigree. Please label each SSR allele and the centromere on each homolog of the X chromosome. Assume that SSR alleles 1A, 2A, 3B, and 4B are on a single chromosome in the mother's somatic cells. Draw these steps only:

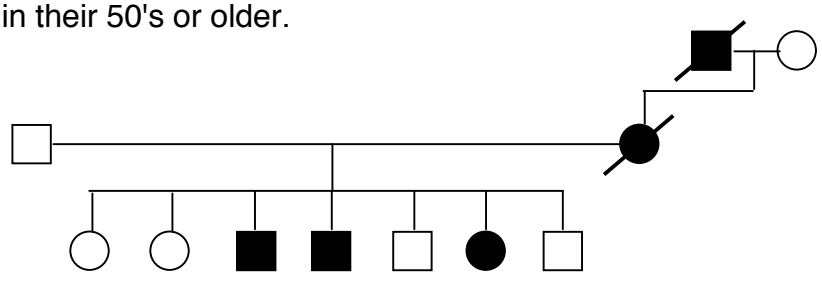
i) the cell in metaphase I with its chromosomes lined up showing any crossover events occurring

ii) the two cells in metaphase II with their chromosomes lined up

iii) the four final products of the meiosis (Please indicate the gamete that led to the creation of the XXY child with a star.)

(d) What might account for this girl having developed as a female despite the presence of a Y chromosome?

14. Your colleague seeks your advice regarding a family in which several individuals (filled circles or squares below) developed colon cancer in their 30's or 40's. All living, unaffected individuals are in their 50's or older.



You speculate that colon cancer in this family might be caused by either:

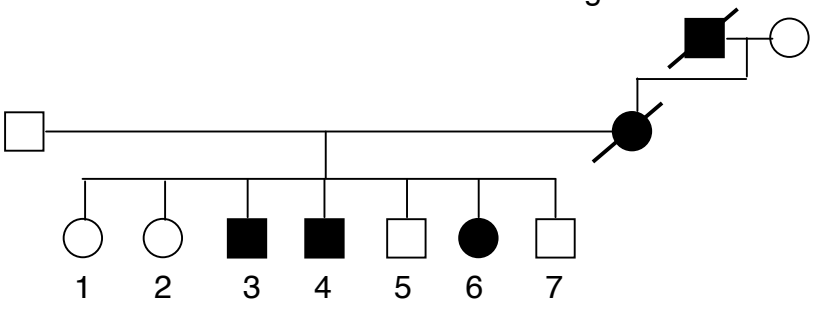
1) germline transmission of a mutation in the APC gene (causing the autosomal dominant disease known as FAP, familial adenomatous polyposis)

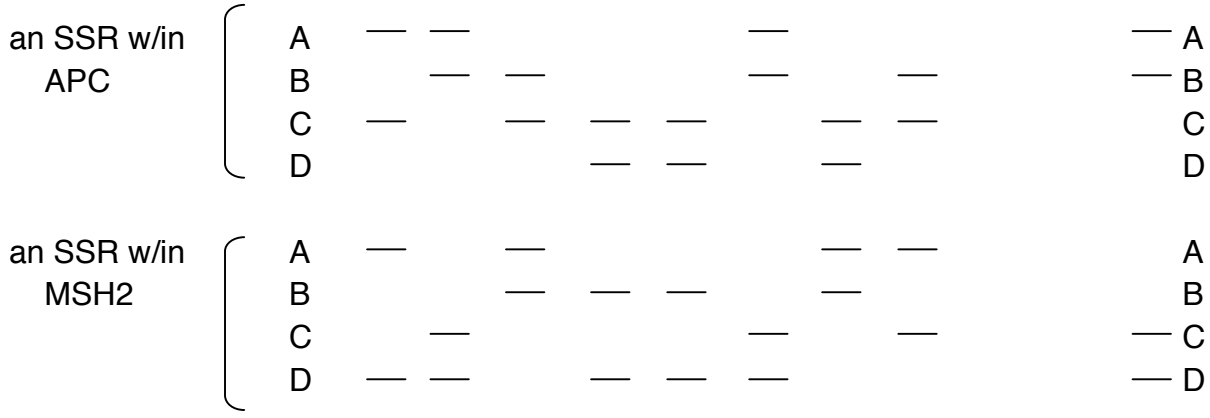
or

2) germline transmission of a mutation in the MSH2 gene involved in DNA mismatch repair (causing the autosomal dominant disease known as HNPCC, hereditary nonpolyposis colon cancer)

(a) You obtain blood DNA samples from all living family members, as well as colon tumor DNA samples from the three living, affected individuals. Your colleague is surprised when you test both the blood and colon tumor DNAs for several SSRs known to show no genetic linkage to either APC or MSH2. How would you use these test results in considering the likelihood of FAP versus HNPCC?

You identify an SSR that is located within an intron of the APC gene, and a second SSR that is located within an intron of the MSH2 gene (which is involved in mismatch repair). You type blood DNA samples for these SSRs and obtain the following results:





You find out that individuals 3 and 4 are twins, but it has not been determined whether they are monozygotic (MZ) or dizygotic (DZ).

(b) Calculate a LOD score for linkage at $\theta = 0$ between the colon cancer locus and the APC locus in this family, assuming that individuals 3 and 4 are DZ twins. Show your calculations.

(c) Does your calculation in part **(b)** change if you assume that individuals 3 and 4 are MZ twins? If so, show how it changes.

(d) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in APC? Briefly justify your answer.

(e) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in MSH2? Briefly justify your answer.

(f) Would your answer to part **(e)** change if you learned that individual 2 had been diagnosed with cancer of the ovary at the age of 35? Briefly explain your answer.

15. Wild-type *E. coli* bacteria are motile (that is, they can swim around). You have isolated a non-motile strain that you designate **mot1⁻**. In order to find a transposon linked to **mot1⁻ ,** you start with a large collection of many *E. coli* strains, each one harboring one different random **Tn5** insertion in an otherwise wild-type genome. (These insertion strains are kanamycin resistant (**Kan^r**) and motile (**mot$^+$**).) 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 **mot1⁻** mutant and select for **Kan^r** transductants. Out of 500 **Kan^r** transductants, 1 is motile (and 499 are non-motile). You designate this motile **Kan^r** transductant "**strain 1**." Next, you grow **P1** phage on **strain 1** and use the resulting phage to infect your original **mot1⁻** strain, selecting for **Kan^r** transductants. Out of 100 **Kan^r** transductants, 70 are motile and 30 are non-motile.

(a) What is the distance between the **Tn5** insertion and **mot1**, expressed as a cotransduction frequency?

(b) Next, you isolate a second non-motile mutant, designated **mot2⁻**. You grow **P1** phage on **strain 1** and use the resulting phage to infect your **mot2⁻** strain. After selection, you isolate 100 **Kan^r** transductants. All of these transductants are non-motile. Based on this result, what conclusion can you draw about the distance between the **mot2** locus and the **Tn5** insertion harbored by **strain 1**?

(c) Finally, you isolate a third non-motile mutation designated **mot3⁻**. You discover that the **mot3** mutation is an allele of the **mot1** gene. To determine the relative order of the **mot3** and **mot1** loci, you set up two different transduction experiments. First, you grow **P1** phage on a derivative of **strain 1** that also carries **mot1⁻** , and then you use the resulting phage to infect your **mot3⁻** strain, selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants 12 are motile and 488 are non-motile. Second, you grow **P1** phage on a derivative of **strain 1** that also carries **mot3⁻** , and use the resulting phage to infect your **mot1⁻** strain, selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants, all are non-motile.

Draw a genetic map showing the relative order of the **Tn5**, **mot1** and **mot3** loci.

16. In order for yeast cells use the amino acid arginine as a nitrogen source, arginine is broken down by the enzyme arginase. You find that the arginase gene is highly transcribed when arginine is present in the medium, but that the arginase gene is not transcribed at all when there is no arginine present. You have identified the gene for the arginase enzyme, which you designate **Arg1**. An allele in this gene (**Arg1⁻**) has the following properties:

Arginase activity

	+ arginine	–arginine
Wild type	–	+
Arg1⁻	–	–
Arg1⁻ / Arg1⁺	–	+

(a) You isolate a mutation, designated **Arg2⁻**, which causes the recessive phenotype of uninducible arginase expression. When you mate an **Arg2⁻** haploid mutant to an **Arg1⁻** haploid mutant, the resulting **Arg2⁻ / Arg1⁻** diploid shows normal arginase expression and regulation. What does this result tell you about the nature of the **Arg2⁻** mutation?

(b) What can you deduce about the normal role of wild-type **Arg2** in arginase regulation?

(c) Next, you isolate a mutation, designated **Arg3⁻**, which causes constitutive arginase expression. You cross an **Arg3⁻** haploid mutant to an **Arg1⁻** haploid mutant, and induce sporulation of the resulting diploid (which shows normal regulation of arginase). This gives the following tetrad types. Out of a total of 50 tetrads, 8 are Type One, 7 are Type Two, and 35 are Type Three.

Type One	Type Two	Type Three
constitutive	uninducible	constitutive
uninducible	uninducible	constitutive
uninducible	regulated	uninducible
regulated	regulated	uninducible

Are the **Arg3** and **Arg1** loci linked? If so, how far apart are they in cM?

(d) You cross an **Arg3⁻** haploid mutant to an **Arg2⁻** haploid mutant, and induce sporulation of the resulting diploid. This gives the following tetrad types. Out of a total of 50 tetrads, 35 are Type Four, 8 are Type Five, and 7 are Type Six.

Type Four	Type Five	Type Six
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible

Does an **Arg3⁻ Arg2⁻** double mutant haploid yeast strain have the phenotype of regulated, constitutive, or uninducible?

(e) Which tetrad type (Four, Five, or Six) contains the most double mutant spores?

(f) On the basis of all of the information given in this problem, diagram a linear genetic pathway to explain the regulation of arginase. For your model, include the wild-type **Arg1**, **Arg2** and **Arg3** genes. Also show how the amino acid arginine itself might act.

17. One in 20,000 human males is an (infertile) XX male (due to a translocation that moves the sex-determination gene Sry onto an X chromosome). If an XX male has a twin, what is the likelihood that the twin is an XX male, assuming that:

(a) The twins are dizygotic.

(b) The twins are monozygotic.

One in 200 individuals in the industrialized world suffers from HNPCC (Hereditary Non-Polyposis Colon Cancer), an autosomal dominant trait. If an individual with HNPCC has a twin, what is the likelihood that the twin has HNPCC, assuming that:

(c) The twins are dizygotic.

(d) The twins are monozygotic.

For mothers 30 years of age, the incidence of trisomy 21 is one per 885 births. Consider an individual (born to healthy 30-year-old parents) who has trisomy 21 due to meiotic non-disjunction in the mother. If the trisomic individual has a twin, what is the likelihood that the twin has trisomy 21, assuming that:

(e) The twins are dizygotic.

(f) The twins are monozygotic.

18. Consider a codominant blood antigen where individuals homozygous for one allele express only antigen M, individuals homozygous for the other antigen express only antigen N, and heterozygous individuals express both N and M antigens. In a study of three populations, you determine the genotype frequencies of individuals that express only M or only N.

(a) Based on this information, fill in the table below, giving the N and M allele frequencies for each population, and state whether each population is in Hardy-Weinberg equilibrium.

	Frequency expressing		Allele frequencies		
Population	**M only**	**N only**	**M**	**N**	**H-W equilib (yes or no)?**
1	0.25	0.25			
2	0.36	0.16			
3	0.01	0.64			

(b) Consider a deleterious allele in a human population which has a selective disadvantage S = 1 in the homozygote and a selective disadvantage S = 0.1 in the heterozygote. The mutation rate is $\mu = 10^{-5}$ for this allele. In a balance between new mutation and selection, what will the steady state allele frequency be? (Your answer can be an estimate accurate to ± 10%. Assume random mating.)

(c) If a different recessive disorder occurs at a frequency of 10^{-3} in the offspring of first cousins in a population, what is the probability that a brother-sister mating from the same population would produce a child with the disorder? (Assume that selection and mutation rates are negligible.)

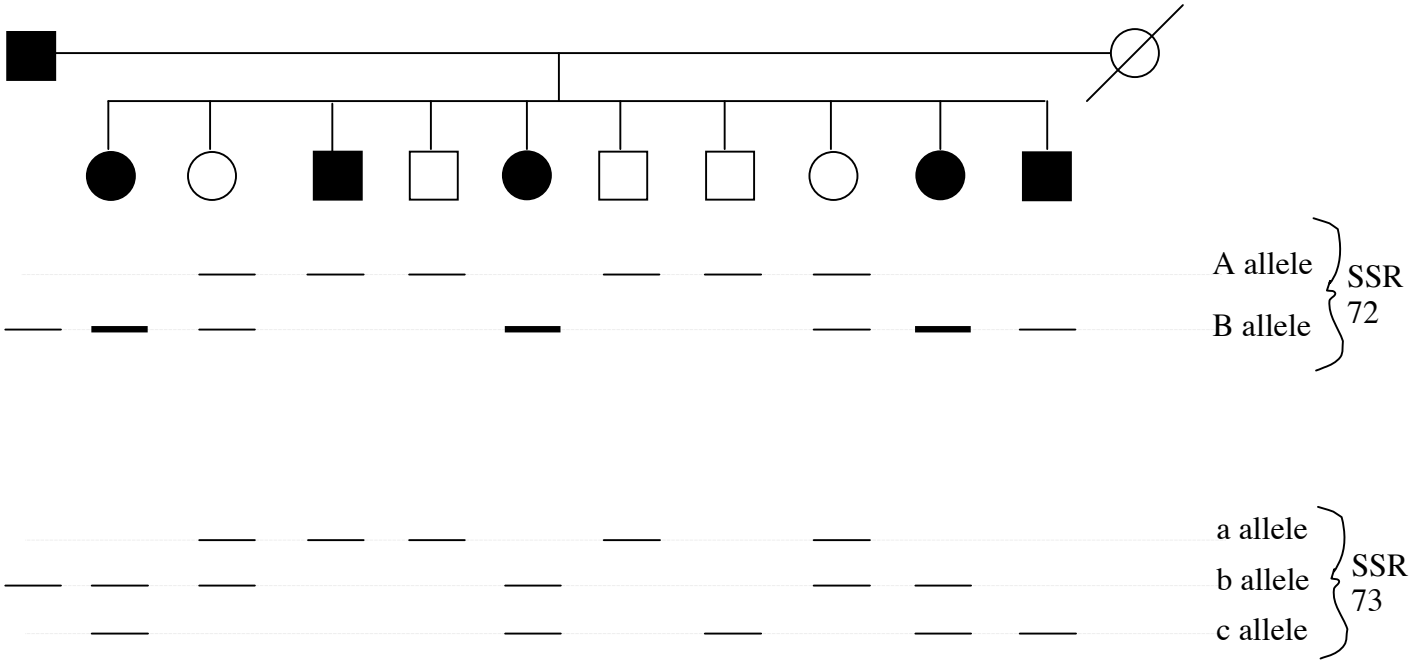
19. HNPCC (Hereditary Non-Polyposis Colon Cancer) shows autosomal dominant inheritance in humans. As discussed in 7.03, some individuals with HNPCC are heterozygous for a loss-of-function mutation in the mismatch repair gene MSH2. These individuals frequently develop cancer of the colon, ovary, uterus, or other organs before age 50.

(a) Which of the following two approaches would yield a better mouse model of HNPCC:
-- random integration of a transgene consisting of a mutant human MSH2 gene (from a human HNPCC patient)
OR
-- knockout of the mouse MSH2 gene?
Briefly justify your answer.

(b) Would you expect mice that are homozygous for the modification you chose in part **(a)** to develop cancer more quickly, more slowly, or at the same rate as mice that are heterozygous for that modification? Briefly justify your answer.

20. Your project is to genetically map the locus for color blindness, an X-linked recessive trait, with respect to SSR markers. Like many X-linked recessive traits, color-blindness is usually found in males. However, the mutant allele frequency is sufficiently high that colorblind females do occur. In this problem, **+** stands for the allele that leads to normal vision, and **clr** stands for the allele associated with color-blindness. Assume complete penetrance and no new mutations.

Here is a family in which some individuals are colorblind:



(a) What is the (deceased) mother's genotype at SSR72?

(b) What is the (deceased) mother's genotype at SSR73?

(c) Diagram all possible phase relationships between the SSR72 and SSR73 alleles in the mother.

(d) Calculate the LOD score for linkage at $\theta = 0.2$ between SSR72 and SSR73 in this family.

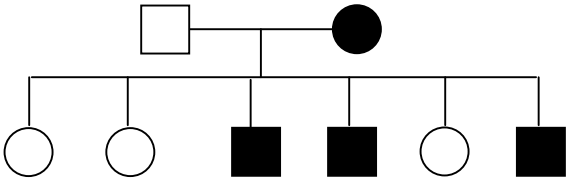
(e) Diagram all possible phase relationships between the alleles at SSR72 and the color blindness locus in the mother.

(f) Calculate a LOD score for linkage at $\theta = 0.1$ between SSR72 and the color blindness locus in this family.

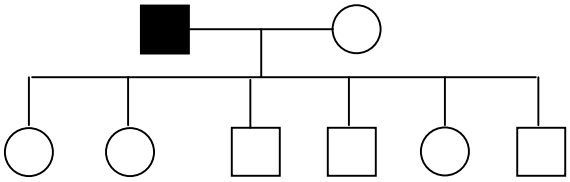
(g) If SSR72, SSR73, and the color blindness locus are all located close together in the same region of the X chromosome, what is their most likely order on the chromosome?

21. The following three crosses involve mice from either true-breeding mutant strains or true-breeding wild-type strains. For this problem, you can assume that a mouse is true-breeding if its parents are not shown in the pedigree. In each case, mice exhibiting the rare mutant traits are indicated by solid symbols. Square symbols designate males and circles designate females. In each case, describe the mode(s) of inheritance that are consistent with the data (your choices are: autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant). Assume that traits are completely penetrant, and that no new mutations have arisen.

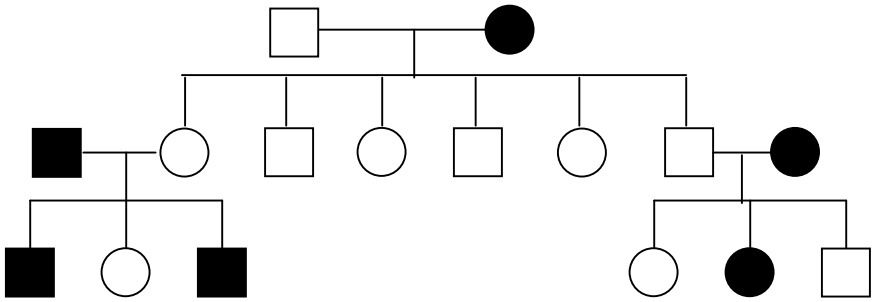
(a)



(b)



(c)



22. *E. coli* can utilize the sugar melibiose after induction of the enzyme melibiase. Melibiase is expressed when bacteria grow in a growth medium that contains melibiose, but not on medium that lacks melibiose. You have isolated a mutation called **mut1** that expresses melibiase constitutively, even on growth medium that lacks melibiose. In order to study melibiase regulation, you isolate an insertion of **Tn5::LacZ** into the melibiase structural gene. (Note that **Tn5** confers kanamycin resistance.) A strain with this insertion shows expression of ß-galactosidase on growth medium that contains melibiose, but not on medium that lacks melibiose.

(a) You grow P1 phage on a bacterial host that carries the **Tn5::LacZ** insertion into the gene encoding melibiase. You then use the resulting phage lysate to infect a **mut1 lacZ**$^{-}$ bacterial strain, selecting for kanamycin resistance. Among the Kan^{r} transductants, 5% give constitutive expression of ß-galactosidase, whereas 95% only express ß-galactosidase when melibiose is present. Is **mut1** linked to the melibiase structural gene and, if so, what is the distance from the **Tn5-LacZ** insertion to **mut1** (in terms of cotransduction frequency)?

(b) You obtain an **F'** plasmid that carries the melibiase structural gene. (This **F'** includes chromosomal sequences that span >100 kbp on either side of the melibiase gene.) You choose a Kan^{r} transductant from part (**a**) that gives constitutive ß-galactosidase expression, and mate the **F'** plasmid into this strain. The resulting merodiploid still gives constitutive expression of ß-galactosidase. What does this observation tell you about the nature of **mut1**?

(c) You further examine the **F'** strain constructed in part (**b**), and find that melibiase expression is regulated normally despite the fact that ß-galactosidase expression is constitutive. State what this observation tells you about the nature of **mut1**.

(d) Draw a linear genetic pathway for the regulation of melibiase, showing the wild-type functions of mut1, the melibiase gene, and the sugar melibiose. Do not invoke more than one unknown trans-acting regulator in your pathway.

(e) How does the molecular defect caused by the **mut1** mutation specifically act to misregulate LacZ gene expression?

23. The sequence of the amber stop codon is $^{5'}$UAG$^{3'}$. One can isolate bacterial strains that carry mutations in genes encoding tRNAs such that they will encode mutant tRNAs that recognize the amber 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 amber (TAG) 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).

(a) 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 amber-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).

24. You are studying the yeast genes needed to metabolize organic phosphates. The key regulated enzyme is phosphatase, which is needed to release inorganic phosphate from organic phosphate compounds in the medium. Phosphatase is not expressed when yeast are grown in growth medium that contains inorganic phosphate, but is induced to high levels when yeast are grown in growth medium with no inorganic phosphate. You have isolated a mutation (which you call **pho4$^-$**) that causes the recessive phenotype of <u>uninducible</u> phosphatase regulation.

	<u>Phosphatase activity</u>	
	+ phosphate	–phosphate
Wild type	–	+
pho4$^-$	–	–
pho4$^-$ / pho4$^+$	–	+

Starting with an uninducible **pho4⁻** strain, you isolate three different double mutant strains (called Suppressor Strains #1, #2, and #3) that restore phosphatase expression (when the yeast are grown on growth medium without phosphate) to these suppressor strains of yeast, even though they each harbor the **pho4⁻** mutation.

(a) Suppressor Strain #1 shows regulated expression of phosphatase. You cross Suppressor Strain #1 haploid yeast to wild-type haploid yeast. Induction of sporulation in the resulting diploid gives the following tetrad types. (Type One is the most abundant class.)

Type One	Type Two	Type Three
regulated	regulated	regulated
regulated	regulated	regulated
regulated	regulated	uninducible
uninducible	regulated	uninducible

What kind of mutation could produce the behavior of Suppressor Strain #1? Be as explicit as possible and explain your reasoning.

(b) Suppressor Strain #2 also shows regulated expression of phosphatase. You cross Suppressor Strain #2 haploid mutant yeast to wild-type haploid yeast. Induction of sporulation in the resulting diploid gives only one tetrad type that is observed, even though you analyze 1000 tetrads.

Type Four
regulated
regulated
regulated
regulated

What kind of mutation could produce the behavior of Suppressor Strain #2? Be as explicit as possible and explain your reasoning.

(c) Suppressor Strain #3 shows constitutive expression of phosphatase. You cross Suppressor Strain #3 haploid yeast to wild-type haploid yeast, and induce sporulation of the resulting diploid (which shows regulated expression of phosphatase). This gives the following three tetrad types. (Type Five is the most abundant class).

Type Five	Type Six	Type Three
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible

Give an explanation for the type of mutation that could produce the behavior of Suppressor Strain #3.

25. Shown below is a hypothetical scheme for the formation of eye pigment in *Drosophila*.

The enzyme encoded by the **Pr** gene converts a purple pigment into the normal red pigment in the eye. The **pr**$^{-}$ allele causes the recessive phenotype of purple eyes. The enzyme encoded by **Bl** gene converts a blue pigment into the purple pigment. The **bl**$^{-}$ allele causes the recessive phenotype of blue eyes. Both the **Pr** and **Bl** genes are on the X chromosome. A male from a true-breeding blue-eyed strain is crossed to a female from a true-breeding purple-eyed strain.

(a) All of the F_1 female progeny from this cross have normal eyes. What colored eyes should the F_1 male progeny have?

(b) An F_1 female fly (with normal eyes) is crossed to a wild-type male, and a large number of male progeny from this cross are examined. Among the male progeny, there are flies with normal red eyes, flies with purple eyes, and flies with blue eyes. You notice that significantly more male progeny have blue eyes than have purple eyes. Give an explanation why this should be the case.

(c) Given that the **Pr** and **Bl** genes are 16 cM apart on the X chromosome, determine the number out of 100 male progeny from the cross in part (**b)** that should have purple eyes, blue eyes, or normal red eyes.

Number

Purple-eyed males:

Blue-eyed males:

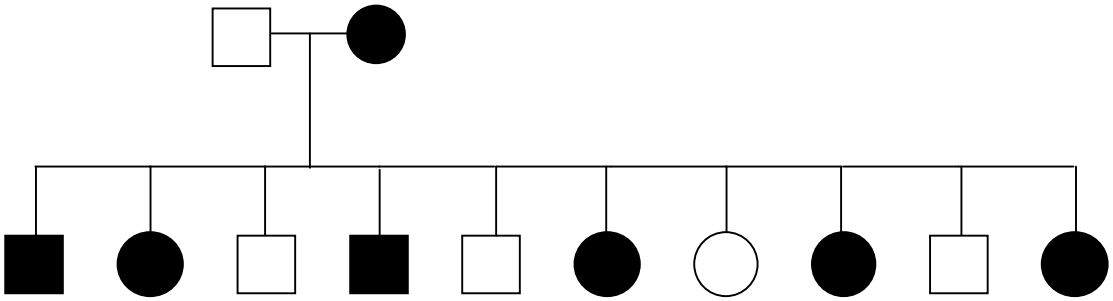
Red-eyed males:

_____

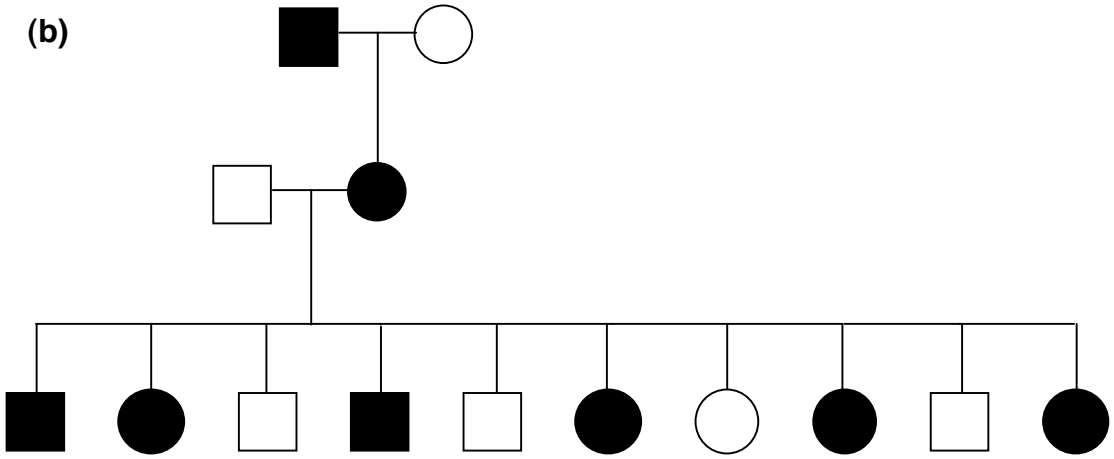
Total = 100

26. When setting out to determine the chromosomal location of a rare human disease gene by genetic linkage analysis (LOD scores), it is useful to calculate the theoretical maximum LOD score that a family of a given size and structure might contribute. For each of the families shown below, calculate, for $\theta = 0$, the theoretically maximum LOD score that could be obtained using an SSR that is genetically inseparable from (shows no recombination with) the disease locus (for a disease that is autosomal dominant). Assume that DNA samples are available for all **living** individuals. Also assume complete penetrance and no new mutations.

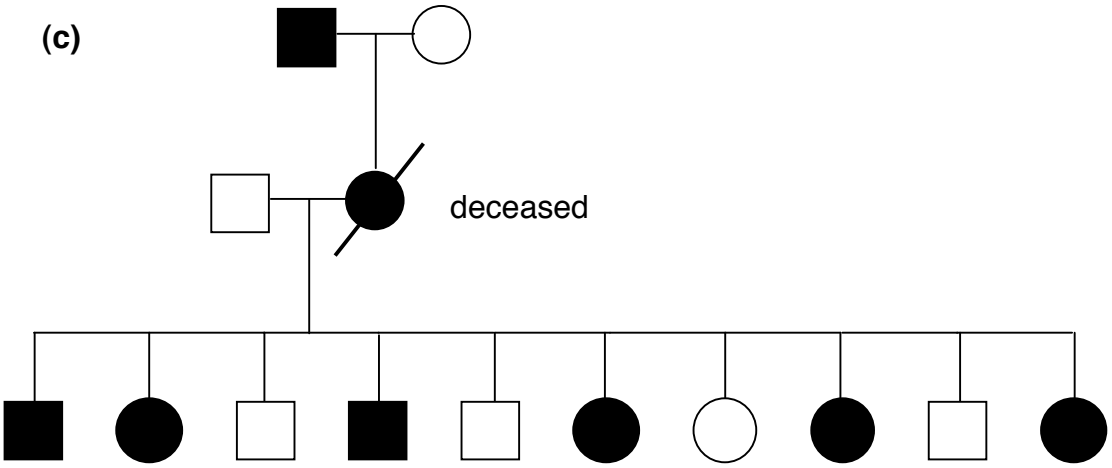
(a)



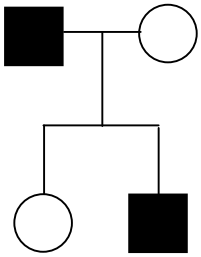
(b)



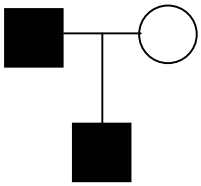
(c)



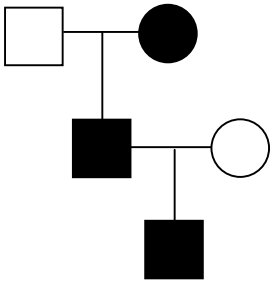
(d)



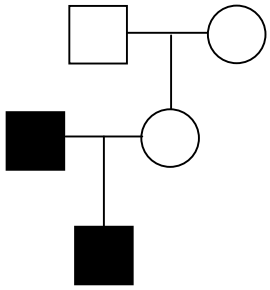
(e)



(f)



(g)



27. You discover a frame-shift mutation in an X-linked gene called SPG in a man who is infertile because of poor sperm production. You postulate that the SPG frameshift mutation is the cause of the man's poor sperm production.

(a) You obtain genomic DNA samples from other men with poor sperm production and sequence their SPG gene. You find that six such men, apparently unrelated, are mutant in SPG. Is it possible that these six men carry the same frameshift mutation found in the first man because of the mutation having been passed through many generations? Explain your answer.

Your classmate discovers an X-linked male-sterile mutant that arose in her mouse colony. She has on hand the mother of this mutant mouse, who gives birth to many sons, all of whom are sterile. Having heard about your studies of the human SPG gene, she discovers that her male-sterile mice are mutant in the mouse SPG gene. You want to know whether the human and mouse SPG genes are functionally interchangeable.

(b) Propose an experiment involving a mouse transgene (but no knockouts and no sequencing) that would test whether the mouse and human SPG genes are functionally interchangeable. When creating engineered mice, the following 8 steps need to be considered. **For the mouse you make**, please state:

i) whether you are using pronuclear injection or gene targeting techniques
ii) what DNA you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg or ES cells
iv) what is the genotype of the fertilized egg or the ES cells you would start with
v) where in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera or not
vii) which additional breeding steps you would do to make the mouse you wanted
viii) two possible phenotypic results you could get from the newly made mice, and the corresponding conclusions you would make based on each result

28. You have isolated two different X-linked mutations in *Drosophila* that affect eye color. Wild-type *Drosophila* have red eyes, whereas flies that carry the **w** mutation have white eyes, and flies that carry the **rng** mutation have orange colored eyes. Both the **w** and **rng** mutations cause recessive phenotypes. (That is, crosses to wild-type of flies from either true-breeding **w** or **rng** strains give F_1 progeny with normal red eyes.)

(a) A male from a true-breeding **w** strain is crossed to a female from a true-breeding **rng** strain. All of the female F_1 progeny from this cross have orange colored eyes. What colored eyes should the male F_1 progeny have?

(b) Are the **w** and **rng** mutations alleles of the same gene, or alleles of different genes? Explain your reasoning.

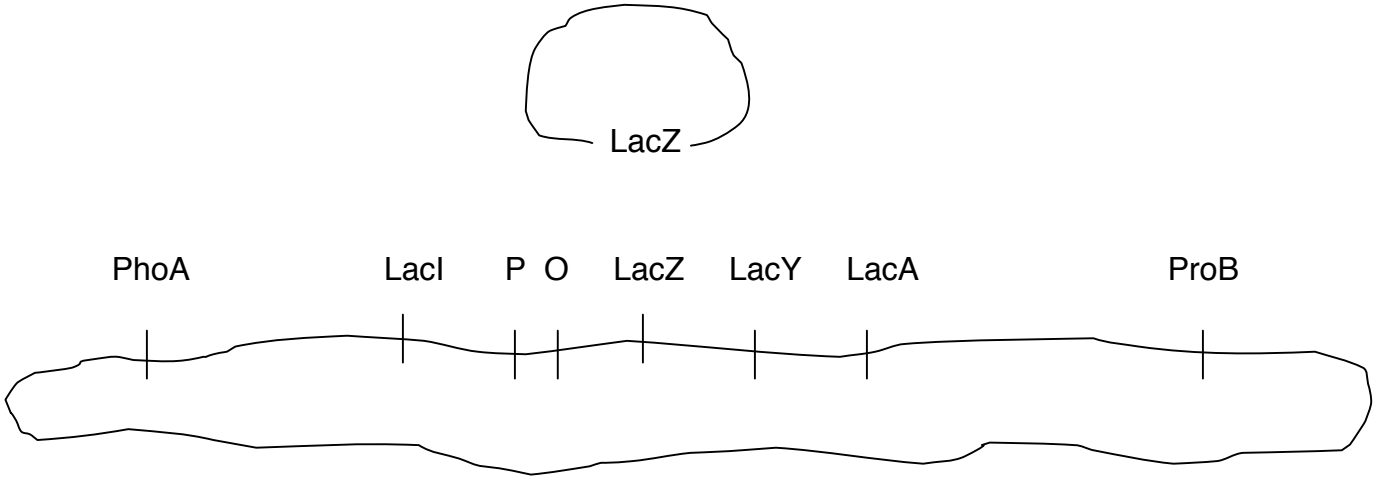
(c) A female F_1 fly (with orange eyes) is crossed to a wild-type male and 1000 male progeny from this cross are examined. Among the male progeny, there are 496 flies with white eyes, 499 flies with orange eyes, and 5 flies with normal red eyes. What is the distance between **w** and **rng** in cM? (Be sure to state any assumptions that you make.)

(d) A mutation that causes short bristles (known as **sh**) is linked to the **w** and **rng** loci. A fly from a true-breeding **sh w** strain (short bristles, white eyes) is crossed to a fly from a true-breeding **rng** strain (long bristles, orange eyes). The female F_1 progeny are then crossed to wild-type males and only males from this cross are examined. The following phenotypic classes and numbers are seen:

Bristle Length	Eye color	Number of MALE flies
Short	white	398
Long	orange	405
Short	orange	90
Long	white	102
Short	red	5

Draw a genetic map showing the relative order of the **sh**, **w**, and **rng** loci.

29. You have constructed an **F'** plasmid that carries the **LacZ** gene. Because the **F'** plasmid carries only the coding sequence of the **LacZ** gene, the **Lac** operon's cis regulatory sequences, and **LacY** and **LacA** genes, are not included on the **F'** plasmid. A diagram of the **F'** plasmid, as well as the host chromosome's **Lac** operon and flanking genes, is shown below.



(a) The **F'** plasmid carrying **LacZ** is transferred into an F- donor strain with a wild-type copy of the **Lac** operon on its chromosome. An **Hfr** is isolated from this strain that can transfer **ProB$^+$** within 10 minutes of mating to a **ProB$^-$** recipient. Draw the origin of transfer on the **F'** plasmid in the diagram above, using the following symbol: ◀ showing the correct orientation for the direction of transfer.

(b) In the space below, draw a diagram showing the organization of the chromosome in the Hfr isolated in part (**a)**. Be sure to show all copies of the **Lac** operon genes and the integrated **F** factor including the orientation of the origin of transfer, and all other markers included in the diagram provided to you above.

(c) Will the **Hfr** isolated in part (**a)** be able to use lactose as a carbon source? Explain why or why not**.**

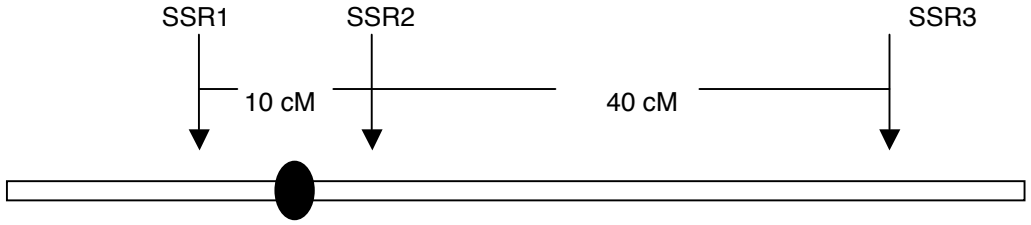
(d) The **Hfr** isolated in part (**a)** is mated to a strain that has a mutation causing the recessive phenotype of constitutive expression of the **Lac** operon. From this mating, you wish to isolate recombinants that show normal **Lac** regulation. Would you expect such recombinants to arise early or late after mating is initiated? Explain briefly.

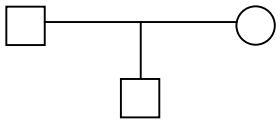
30. The **PyrG** gene is found to lie about 40 kb away from the group of **Lac** genes on the *E. coli* chromosome. You grow P1 phage on a **PyrG$^+$ LacZ$^-$** strain, and then use the resulting phage lysate to infect a **PyrG$^-$ LacI$^-$** strain, selecting for **PyrG$^+$** (the ability to grow without supplemental pyrimidine nucleotides being added to the medium). Among 100 **PyrG$^+$** transductants, 5 show normal regulation of ß-galactosidase, 25 show constitutive expression of ß-galactosidase, and 70 show uninducible expression of ß-galactosidase.

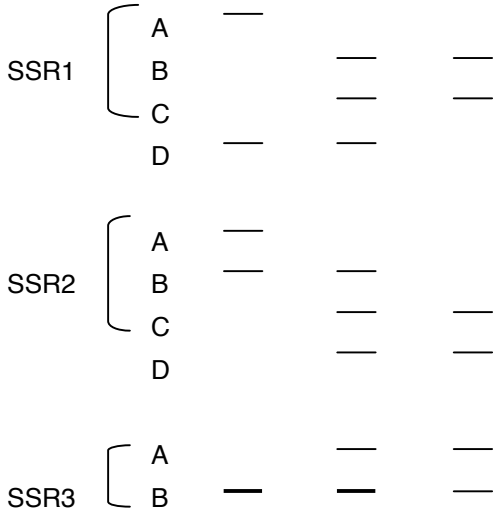
For the reciprocal cross, you grow phage P1 on a **PyrG$^+$ LacI$^-$** strain, and then infect a **PyrG$^-$ LacZ$^-$** strain, selecting for **PyrG$^+$**. Among 100 **PyrG$^+$** transductants, 20 show normal regulation of ß-galactosidase, 50 show constitutive expression of ß-galactosidase, and 30 show uninducible expression of ß-galactosidase.

Draw a diagram of this region of the chromosome that shows where the **PyrG** gene maps relative to **LacZ** and **LacI**.

31. Only a small fraction of human fetuses with trisomy 18 survive to birth, and most of those surviving to birth die in infancy. You prepare DNA samples from umbilical cord blood of a newborn baby with trisomy 18, and from his parents. The schematic of a gel is shown below, and this gel reveals the genotypes of each member of the family at three different SSRs found on chromosome 18. The oval on the diagram of chromosome 18 indicates the centromere.







(a) Did nondisjunction occur before fertilization (during the development of the gametes in the parents) or after fertilization (in the developing embryo)?

In answering the remaining questions, assume that nondisjunction occurred during meiosis.

(b) During the development of which parent's gametes did the non-disjunction event occur?

(c) In which division of meiosis did nondisjunction occur?

(d) Draw the following steps in the meiosis that created the gamete that led to the production of the child with trisomy 18 shown in the pedigree. Please label each SSR allele and the centromere on each homolog of chromosome 18. Assume that SSR alleles 1B, 2C, and 3A are on a single chromosome in the mother's somatic cells. Assume that SSR alleles 1D, 2A, and 3B are on a single chromosome in the father's somatic cells. Draw these steps only:

i) the cell in metaphase I with its chromosomes lined up showing any crossover events occurring

ii) the two cells in metaphase II with their chromosomes lined up

iii) the four final products of the meiosis (Please indicate the gamete that led to the creation of the child with trisomy 18 with a star.)

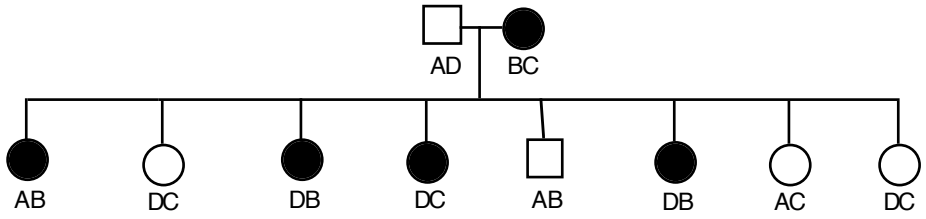
(e) In humans, how many chromatids are normally present in the first polar body?

(f) In humans, how many chromatids are normally present in the second polar body?

(g) In this case of trisomy 18, how many chromatids would have been present in the first polar body?

(h) In this case of trisomy 18, how many chromatids would have been present in the second polar body?

32. In some families, breast cancer displays autosomal dominant inheritance. Here is one such family, with the results of typing for SSR126 (the SSR126 alleles are designated A, B, C, and D):

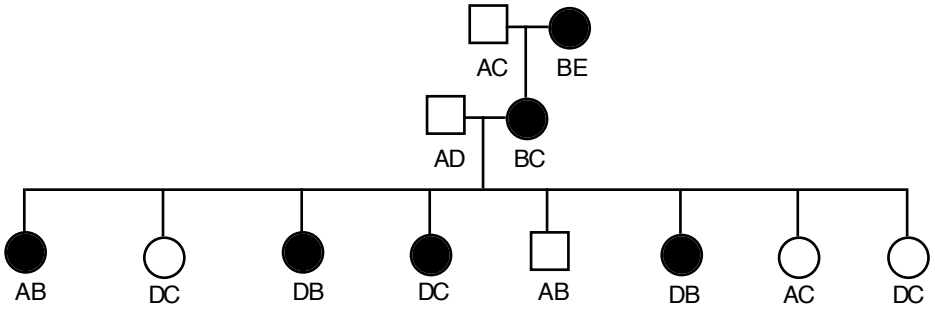


In analyzing this family, we will make three simplifying assumptions:

1) That penetrance is complete in females; all females with the mutation get breast cancer.
2) That males cannot get breast cancer, even if they carry the mutation.
3) That the father is homozygous, as the mutation is rare.

(a) Calculate the LOD score for linkage between breast cancer and SSR126 at $\theta = 0.1$.

One year after your original study, you recover DNA samples from a previous generation and type them for SSR126 (results shown below).



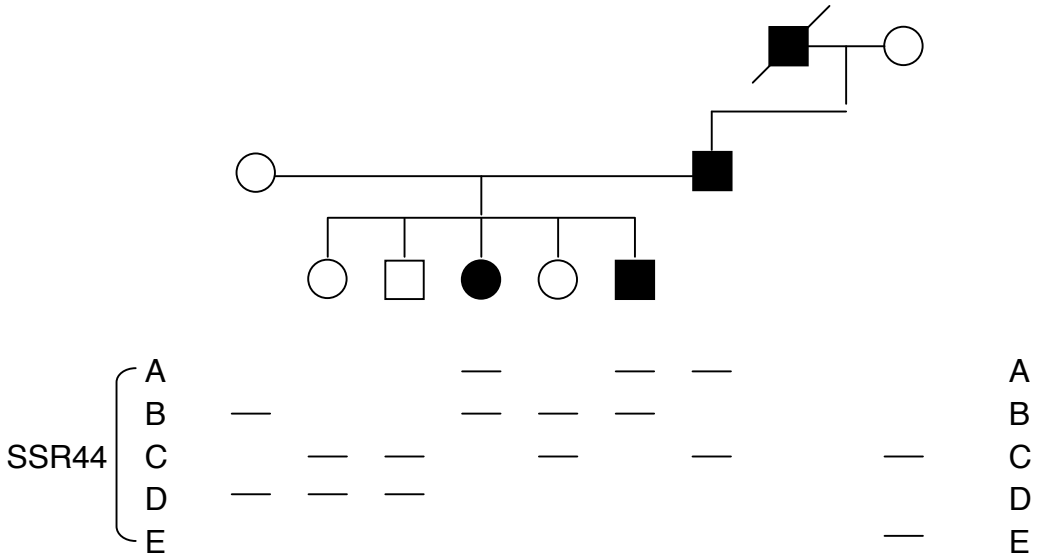
(b) Recalculate this family's LOD score for linkage between breast cancer and SSR126 at $\theta = 0.1$.

(c) No woman in this family developed breast cancer before the age of 37, despite the presence of a predisposing mutation. Why does it take so long for the predisposing mutation to manifest itself? (Focus on the cellular level in your answer. A ONE SENTENCE answer is sufficient.)

(d) You subsequently pinpoint, at the molecular level, the gene whose allele predisposes to breast cancer in this family. You name the gene BRCA, and demonstrate that affected women in this family are heterozygous for a loss-of-function mutation in BRCA. You identify a mouse homolog of the human BRCA gene and generate a mouse model of breast cancer. You create mice that are heterozygous for a loss-of-function mutation in BRCA. You cross these BRCA +/- mice with each other and obtain 185 progeny, 125 of which are BRCA +/-, and 60 of which are +/+. What might explain these breeding results?

33. You are genetically mapping the locus that determines a rare skin disease that shows autosomal dominant inheritance.
Alleles: + (normal) SD (associated with skin disease)

Here is a family in which some individuals are affected. Assume complete penetrance and no new mutations.



(a) Which parent(s) is/are informative with respect to linkage between the skin disease gene and SSR44?

(b) What allele at SSR44 did the affected father inherit from his (deceased) father?

(c) Diagram the phase relationship between the skin disease locus alleles and the SSR44 alleles in the affected father.

(d) Calculate the LOD score for linkage at $\theta = 0.1$ between the skin disease gene and SSR44 in this family.

(e) How many families of this exact type would be needed to achieve a publishable LOD score at $\theta = 0.1$?

34. Consider an autosomal gene at which a rare allele (call it allele **a**) results in homozygotes (**aa**) having only 20% of the number of offspring as average individuals in the population. Assume random mating.

(a) What is the value S for **aa** homozygotes?

Heterozygotes (**Aa**) have a 50.1% chance of surviving an infectious viral disease that afflicts all children in the population. On average, individuals in this population have a 50.0% chance of surviving this infectious disease.

(b) What is the value h associated with allele **a**?

(c) What is the frequency of allele **a** in the population? Show your calculations and state any simplifying assumptions that you make.

(d) What is the frequency of heterozygotes among newborn children?

(e) Suppose that the virus causing the infectious childhood disease is completely eradicated. Estimate the frequency of allele **a** 50 generations later.

35. You have isolated a Tn5 insertion in an otherwise wild-type *E. coli* strain that is linked to the gene encoding the MalT activator protein. Tn5 carries a marker for kanamycin resistance (Kan^R). You grow P1 phage on the strain with the Tn5 insertion and use the resulting phage to infect a $MalT^-$ strain. Among 100 resulting Kan^R transductants, 20 express no maltase activity and 80 express maltase normally. Note that the MalT gene is unlinked to the gene encoding the maltase enzyme MalQ.

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

(b) You grow P1 phage on a $MalT^-$ Kan^R transductant isolated in part **(a)**, and use the resulting phage to infect a $MalT^C$ mutant that is an otherwise wild-type strain. The $MalT^C$ protein binds DNA regardless of whether the inducer maltose is present. The following results are obtained:

Phenotype	number of Kan^R transductants
uninducible	80
constitutive	19
regulated	1

Next you perform the reciprocal cross by growing P1 phage on a $MalT^C$ Kan^R strain carrying the same Tn5 insertion as above. You use the resulting phage to infect a $MalT^-$ mutant that is an otherwise wild-type strain. The following results are obtained:

Phenotype	number of Kan^R transductants
uninducible	20
constitutive	80

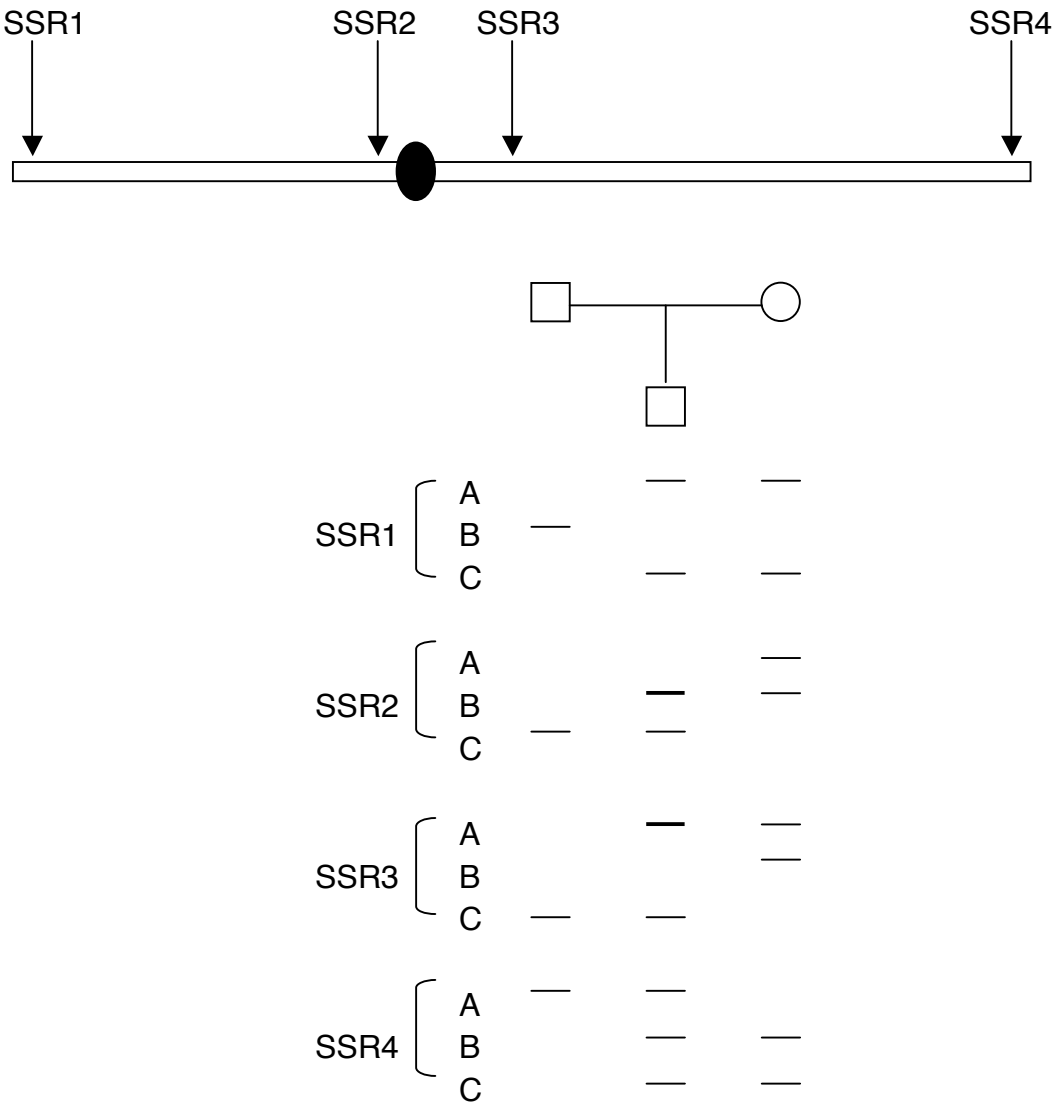
Draw a diagram of the recombination event(s) (that occurred between the transduced DNA and the chromosome) that gave rise to the single regulated transductant from the first cross. Your diagram should clearly show the relative order of Tn5, $MalT^-$, and $MalT^C$ (but cotransduction distances are not necessary).

(c) You have isolated a Tn10 insertion that is linked (50% cotransduction) to a mutant ochre tRNA suppressor allele (Su^+) in an otherwise wild-type *E. coli* strain. The Tn10 insertion carries a marker for tetracycline resistance (Tet^R) and is not linked to MalT. You grow P1 phage on this Tet^R Su^+ strain and use the resulting phage to infect a $MalT^-$ strain. Out of the 100 Tet^R transductants, 50 express maltase normally and 50 express no maltase activity. What type of mutation is $MalT^-$? (Be as specific as possible.)

(d) You grow P1 phage on the Tet^R Su^+ strain from part **(c)** and use the resulting phage to infect a $MalT^-$ $MalT^C$ double mutant. Out of the 100 Tet^R transductants, 50 express maltase constitutively and 50 express no maltase activity. Is the phenotype of a $MalT^-$ $MalT^C$ double mutant regulated, constitutive, or uninducible?

36. You are called by your family physician to provide an expert genetic opinion on an unusual patient: an XXX boy.

You prepare DNA samples from the boy and from his parents. You confirm that the stated father is in fact the biological father by testing the family for a large number of autosomal SSRs. The schematic of a gel is shown on the next page, and this gel reveals the genotypes of each member of the family at four different SSRs found on the X chromosome. The oval on the diagram of the X chromosome indicates the centromere.



(a) During the development of which parent's gametes did the non-disjunction event occur?

(b) In which division of meiosis did nondisjunction occur?

(c) Draw the following steps in the meiosis that created the gamete that led to the production of the XXX child shown in the pedigree. Please label each SSR allele and the centromere on each homolog of the X chromosome. Assume that SSR alleles 1C, 2B, 3A, and 4C are on a single chromosome in the mother's somatic cells. Draw these steps only:

i) the cell in metaphase I with its chromosomes lined up showing any crossover events occurring

ii) the two cells in metaphase II with their chromosomes lined up

iii) the four final products of the meiosis (Please indicate the gamete that led to the creation of the XXX child with a star.)

(d) What might account for this boy's having developed as a male despite the presence of three X chromosomes?

(e) How would you account for the absence in the XXX boy of a paternal allele for SSR1?