Name: _____

7.03 Exam One -- 2005

Name: _____

Exam starts at 11:05 am and ends at 11:55 am.

There are 7 pages including this cover page.

Please write your name on each page.

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(You may use the backs, but only as scratch paper.)

Question 1	**24 pts_____**
Question 2	**30 pts_____**
Question 3	**30 pts_____**
Question 4	**16 pts_____**
TOTAL	**out of 100_____**

Name: _____

1. (24 pts) You are studying two recessive mutations in the fruit fly *Drosophila melanogaster.* The hb^- mutation causes flies to have hairy backs (wild-type flies have hairless backs). The tl^- mutation causes flies to have thick legs (wild-type flies have thin legs). You mate females from a true-breeding strain with hairy backs and normal legs to males from a true-breeding strain with normal backs and thick legs. F1 females are then mated to males that have hairy backs and thick legs to produce F2 progeny. If you analyzed 500 **MALE** progeny in the F2 generation, how many flies of each possible phenotypic class would you expect, given that:

(a, 8pts) The two traits are determined by two unlinked autosomal genes

hairy thick:

hairy thin:

hairless thick:

hairless thin:

(b, 8pts) The two traits are determined by two completely linked genes on the X chromosome

hairy thick:

hairy thin:

hairless thick:

hairless thin:

(c, 8pts) The two traits are determined by two autosomal genes that are 20 cM apart

hairy thick:

hairy thin:

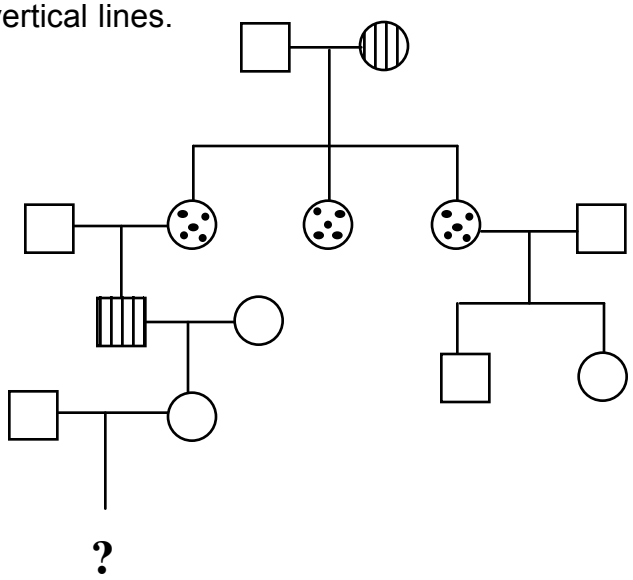
hairless thick:

hairless thin:

Name: _____

2. (30 pts) The following pedigree shows the inheritance two different **rare** traits. Each trait is determined by a different gene. The presence of Trait 1 is indicated by dots, and the presence of Trait 2 is indicated by vertical lines.

(Note that no single individual displays both traits.)



(a, 6pts) What mode(s) of inheritance is/are consistent with each of the traits segregating in this pedigree? (Your choices are: autosomal recessive, autosomal dominant, X-linked dominant, X-linked recessive.) Assume no new mutations and complete penetrance.

Trait 1 (dots):

Trait 2 (vertical lines):

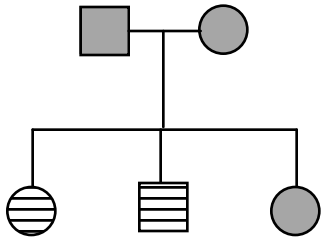
(b, 6pts) State whether the two traits in the pedigree are **linked** or **not linked**, or if it is inconclusive given these data.

(c, 6pts) What is the probability that the child indicated with a question-mark will show at least one of the two traits? Show your work.

Name: _____

Consider this new pedigree, which shows the inheritance of two different rare traits. Each trait is determined by a different gene. The presence of Trait 3 is indicated by horizontal lines, and the presence of Trait 4 is indicated by shading.

(Note that no single individual displays both traits.)



(d, 6pts) What mode(s) of inheritance is/are consistent with each of the traits segregating in this pedigree? (Your choices are: autosomal recessive, autosomal dominant, X-linked dominant, X-linked recessive.) Assume no new mutations and complete penetrance.

Trait 3 (horizontal lines):

Trait 4 (shading):

(e, 6pts) State whether the two traits in the pedigree are **linked** or **not linked**, or if it is inconclusive given these data.

Name: _____

3. (30 pts) You are studying three mutations in yeast. The first mutation causes the Ser⁻ phenotype of being unable to grow without serine in the medium. The second mutation causes the His⁻ phenotype of being unable to grow without histidine in the medium. The third mutation causes a small colony phenotype. Wild-type yeast are Ser⁺ His⁺ and big. You mate a Ser⁻ haploid mutant yeast to a His⁻ small haploid mutant yeast. You induce sporulation of the resulting diploid, and obtain the following tetrad types. (The number of tetrads of each type that you get (out of a total of 100) are shown after the tetrad type.)

Tetrad Type A Ser⁻ His⁺ big Ser⁻ His⁺ small Ser⁺ His⁻ big Ser⁺ His⁻ small	9 tetrads of this type
Tetrad Type B Ser⁻ His⁺ big Ser⁻ His⁺ big Ser⁺ His⁻ small Ser⁺ His⁻ small	90 tetrads of this type
Tetrad Type C Ser⁺ His⁻ big Ser⁻ His⁺ small Ser⁻ His⁻ big Ser⁺ His⁺ small	1 tetrad of this type

(a, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the His and size genes?

TT:

NPD:

PD:

(b, 4pts) Are the His and size loci linked? If so, what is the genetic distance between them?

Name: _____

(c, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the Ser and size genes?

TT:

NPD:

PD:

(d, 4pts) Are the Ser and size loci linked? If so, what is the genetic distance between them?

(e, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the Ser and His genes?

TT:

NPD:

PD:

(f, 4pts) Are the Ser and His loci linked? If so, what is the genetic distance between them?

(g, 6pts) Draw a genetic map showing the correct relative order of the Ser, His, and size loci. If one of the loci is unlinked from the other two, draw it on a separate chromosome.

Name: _____

4. (16 pts) You are studying a recessive trait in a diploid rodent species in which XX organisms are female and XY organisms are male. This trait is determined by a single gene, but you have no idea where in the genome this gene is located. This rodent has 20,000 distinct genes in its genome, 400 of which are found on the X chromosome.

(a, 4pts) Given this information, give your best estimate of the probability that the trait you are studying is X-linked.

(b, 12pts) You mate a female rodent displaying the trait to a wild-type male. You then mate an F1 female to a wild-type male to produce F2 offspring, and analyze only the F2 males. The first three F2 male offspring display the wild-type phenotype.
Given that the first three male F2 offspring show the wild-type phenotype, determine the probability that the trait you are studying is X-linked. Show **all** steps of your work, using clear labels.

Name: _____KEY_____

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TOTAL **out of 100_____**

Name: _____KEY_____

1. (24 pts) You are studying two recessive mutations in the fruit fly *Drosophila melanogaster.* The hb^- mutation causes flies to have hairy backs (wild-type flies have hairless backs). The tl^- mutation causes flies to have thick legs (wild-type flies have thin legs). You mate females from a true-breeding strain with hairy backs and normal legs to males from a true-breeding strain with normal backs and thick legs. F1 females are then mated to males that have hairy backs and thick legs to produce F2 progeny. If you analyzed 500 **MALE** progeny in the F2 generation, how many flies of each possible phenotypic class would you expect, given that:

(a, 8pts) The two traits are determined by two unlinked autosomal genes

P generation: $hb^- hb^- tl^+ tl^+$ X $hb^+ hb^+ tl^- tl^-$
F1: $hb^+ hb^- tl^+ tl^-$ X $hb^- hb^- tl^- tl^-$
F2: $hb^- hb^- tl^+ tl^-$ OR $hb^+ hb^- tl^- tl^-$ OR $hb^- hb^- tl^- tl^-$ OR $hb^+ hb^- tl^+ tl^-$
hairy thick: **125**
hairy thin: **125**
hairless thick: **125**
hairless thin: **125**

(b, 8pts) The two traits are determined by two completely linked genes on the X chromosome

P generation: $X^{hb^- tl^+} X^{hb^- tl^+}$ X $X^{hb^+ tl^-} Y$
F1: $X^{hb^- tl^+} X^{hb^+ tl^-}$ X $X^{hb^- tl^-} Y$
F2: $X^{hb^- tl^+} Y$ OR $X^{hb^+ tl^-} Y$
hairy thick: **0**
hairy thin: **250**
hairless thick: **250**
hairless thin: **0**

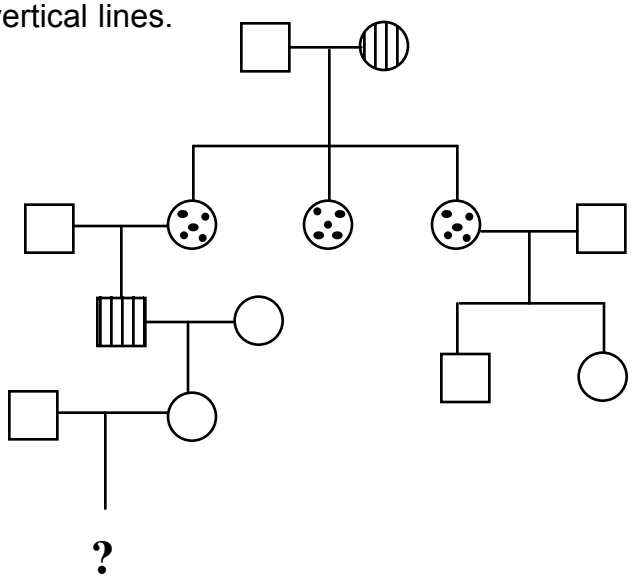
(c, 8pts) The two traits are determined by two autosomal genes that are 20 cM apart

P generation: $hb^- tl^+$ / $hb^- tl^+$ X $hb^+ tl^-$ / $hb^+ tl^-$
F1: $hb^- tl^+$ / $hb^+ tl^-$ X $hb^- tl^-$ / $hb^- tl^-$
F2: $hb^- tl^+$ / $hb^- tl^-$ OR $hb^+ tl^-$ / $hb^- tl^-$ OR $hb^+ tl^+$ / $hb^- tl^-$ OR $hb^- tl^-$ / $hb^- tl^-$
----------parentals---------- ----------recombinants----------
hairy thick: **50**
hairy thin: **200**
hairless thick: **200**
hairless thin: **50**

Name: _____KEY_____

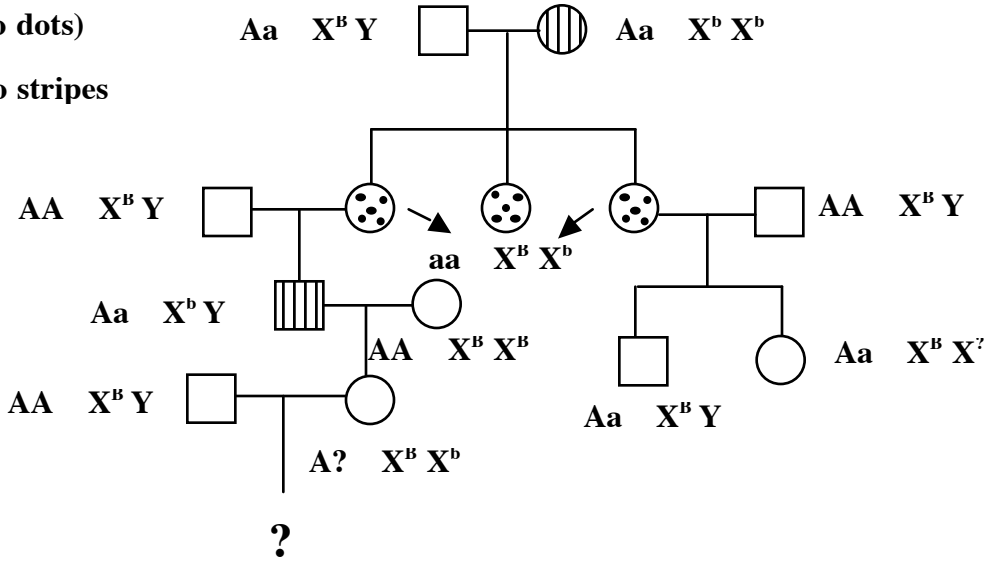
2. (30 pts) The following pedigree shows the inheritance two different **rare** traits. Each trait is determined by a different gene. The presence of Trait 1 is indicated by dots, and the presence of Trait 2 is indicated by vertical lines.

(Note that no single individual displays both traits.)



(a, 6pts) What mode(s) of inheritance is/are consistent with each of the traits segregating in this pedigree? (Your choices are: autosomal recessive, autosomal dominant, X-linked dominant, X-linked recessive.) Assume no new mutations and complete penetrance.

Dots: a (A is no dots)
Stripes: X^b
X^B is no stripes



Trait 1 (dots): **autosomal recessive**
Trait 2 (vertical lines): **X-linked recessive**
Note that the fact that the traits are RARE implies that anyone marrying into the family carries no alleles associated with the trait. This makes autosomal recessive inheritance inconsistent with trait #2.

Name: _____KEY_____

(b, 6pts) State whether the two traits in the pedigree are **linked** or **not linked**, or if it is inconclusive given these data.

Not linked (because one is encoded on the X chromosome and the other is encoded on an autosome; thus the two different genes MUST be on different chromosomes)

Please note that <u>your</u> conclusion had to be consistent with <u>your</u> answer to part A.

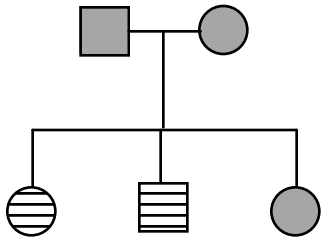
(c, 6pts) What is the probability that the child indicated with a question-mark will show at least one of the two traits? Show your work.

25%

The probability that the child will get Trait 1 is zero because the father is AA.
The probability that the child will get Trait 2 is 25%, because the child first must be a boy for it to show Trait 2 (and the probability of having a boy is 50%), and then, if the child is a boy, he will have a 50% chance of showing Trait 2. This is because the mom is a carrier. Thus the final probability is 50% * 50% = 25%.

Consider this new pedigree, which shows the inheritance of two different rare traits. Each trait is determined by a different gene. The presence of Trait 3 is indicated by horizontal lines, and the presence of Trait 4 is indicated by shading.

(Note that no single individual displays both traits.)

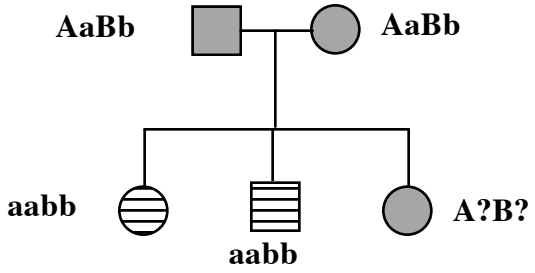


Name: _____KEY_____

(d, 6pts) What mode(s) of inheritance is/are consistent with each of the traits segregating in this pedigree? (Your choices are: autosomal recessive, autosomal dominant, X-linked dominant, X-linked recessive.) Assume no new mutations and complete penetrance.

IF UNLINKED

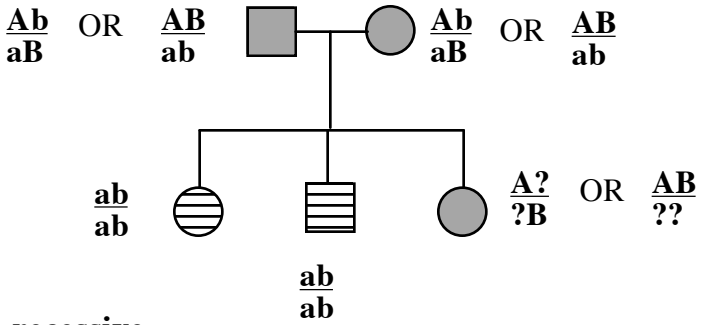
Stripes: a (A is no stripes)
Shading: B
b is no shading



Trait 3 (horizontal lines): **autosomal recessive**
Trait 4 (shading): **autosomal dominant**

IF LINKED

Stripes: a (A is no stripes)
Shading: B
b is no shading



Trait 3 (horizontal lines): **autosomal recessive**
Trait 4 (shading): **autosomal dominant**

(e, 6pts) State whether the two traits in the pedigree are **linked** or **not linked**, or if it is inconclusive given these data.

Inconclusive

Please note that <u>your</u> conclusion had to be consistent with <u>your</u> answer to part D.

Name: _____KEY_____

3. (30 pts) You are studying three mutations in yeast. The first mutation causes the Ser⁻ phenotype of being unable to grow without serine in the medium. The second mutation causes the His⁻ phenotype of being unable to grow without histidine in the medium. The third mutation causes a small colony phenotype. Wild-type yeast are Ser^+ His^+ and big. You mate a Ser⁻ haploid mutant yeast to a His⁻ small haploid mutant yeast. You induce sporulation of the resulting diploid, and obtain the following tetrad types. (The number of tetrads of each type that you get (out of a total of 100) are shown after the tetrad type.)

Tetrad Type A Ser⁻ His^+ big Ser⁻ His^+ small Ser^+ His⁻ big Ser^+ His⁻ small	9 tetrads of this type
Tetrad Type B Ser⁻ His^+ big Ser⁻ His^+ big Ser^+ His⁻ small Ser^+ His⁻ small	90 tetrads of this type
Tetrad Type C Ser^+ His⁻ big Ser⁻ His^+ small Ser⁻ His⁻ big Ser^+ His^+ small	1 tetrad of this type

(a, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the His and size genes?

TT: **Type A**
NPD: **Type C**
PD: **Type B**

(b, 4pts) Are the His and size loci linked? If so, what is the genetic distance between them?

Yes they are, at 7.5 cM
Use the formula

$$\textbf{map distance} = \frac{6\,NPD + TT}{2 \times (\#\ tetrads)} \times 100 = \frac{6(1) + 9}{2(100)} \times 100 = 7.5\ cM$$

Name: _____KEY_____

(c, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the Ser and size genes?

TT: **Type A and Type C**
NPD: **none**
PD: **Type B**

(d, 4pts) Are the Ser and size loci linked? If so, what is the genetic distance between them?

Yes they are, at 5 cM

Use the formula

$$\textbf{map distance} = \frac{6\,\text{NPD} + \text{TT}}{2 \times (\#\ \text{tetrads})} \times 100 = \frac{6(0) + 10}{2(100)} \times 100 = 5\ \text{cM}$$

(e, 4pts) Which Tetrad Types are TTs, NPDs, and PDs with respect to the Ser and His genes?

TT: **Type C**
NPD: **none**
PD: **Type A and Type B**

(f, 4pts) Are the Ser and His loci linked? If so, what is the genetic distance between them?

Yes they are, at 0.5 cM
Use the formula

$$\textbf{map distance} = \frac{6\,\text{NPD} + \text{TT}}{2 \times (\#\ \text{tetrads})} \times 100 = \frac{6(0) + 1}{2(100)} \times 100 = 0.5\ \text{cM}$$

(g, 6pts) Draw a genetic map showing the correct relative order of the Ser, His, and size loci. If one of the loci is unlinked from the other two, draw it on a separate chromosome.

His and Ser are the closest together, both based on the distance calculated and the fact that they only have one TT (and 99 PDs). His and size are the farthest apart, both based on the distance calculated and the fact that they are the only two loci between which you saw double crossovers.
Please note that <u>your</u> map had to be consistent with <u>your</u> answers to parts A-F.

Name: _____KEY_____

4. (16 pts) You are studying a recessive trait in a diploid rodent species in which XX organisms are female and XY organisms are male. This trait is determined by a single gene, but you have no idea where in the genome this gene is located. This rodent has 20,000 distinct genes in its genome, 400 of which are found on the X chromosome.

(a, 4pts) Given this information, give your best estimate of the probability that the trait you are studying is X-linked.

400 / 20,000 = 0.02

(b, 12pts) You mate a female rodent displaying the trait to a wild-type male. You then mate an F1 female to a wild-type male to produce F2 offspring, and analyze only the F2 males. The first three F2 male offspring display the wild-type phenotype.
Given that the first three male F2 offspring show the wild-type phenotype, determine the probability that the trait you are studying is X-linked. Show **all** steps of your work, using clear labels.

X = trait is X-linked
notX = trait is not X-linked and is therefore autosomal
Y = the first three males analyzed are wild-type (note that only males were analyzed, so the gender of the offspring should not be taken into account in your probability calculation)

p(X) = 0.02 (see part a)
p(notX) = 1 – 0.02 = 0.98
p(Y|X) = p(1st egg contained "XA" allele) * p(2nd egg same) * p(3rd egg same)
= 1/2 * 1/2 * 1/2 = 1/8
(Note that the father donates his Y chromosome and is thus irrelevant.)

p(Y|notX) = p(1st sperm contained "A" allele) * p(2nd sperm same) * p(3rd sperm same)
= 1*1*1 = 1
[Note that the father is wild-type (AA) and this makes the mother irrelevant.]

$$p(X/Y) = \frac{p(Y/X) * p(X)}{[p(Y/X) * p(X)] + [p(Y/notX) * p(notX)]}$$

p(X|Y) = 1/393 = 0.25%

Name: _____

7.03 Exam Two -- 2005

Name: _____

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Question 1	**31 pts_____**
Question 2	**31 pts_____**
Question 3	**38 pts_____**
TOTAL	**out of 100_____**

Name: _____

1. (31 pts) You have isolated three bacterial mutants that cannot grow without supplemental serine being included in the growth medium. These three mutations lie in two genes, SerC and SerB. The SerC$^-$ mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1$^-$ mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2$^-$ mutation is a frameshift mutation that produces a protein product that is 12 kDa.

The first cross: You grow P1 phage on SerC$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ bacteria. (ProA$^-$ bacteria have a disruption in the ProA gene, which is required for the bacteria to synthesize their own proline.) You select for KanR transductants. All 200 of the transductants you analyze can grow on plates containing kanamycin and serine and proline, but cannot grow on plates containing kanamycin and serine (but not proline).

(a, 6pts) What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

The second cross: You grow P1 phage on SerB2$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ SerB1$^-$ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on plates lacking serine. The other 67 can only grow on plates containing serine.

(b, 5pts) Are ProA and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

(c, 5pts) Are SerC and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

Name: _____

The third cross: You grow P1 phage on SerB1⁻ bacteria. You use the resulting phage lysate to infect ProA⁻ SerB2⁻ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on plates lacking serine. The other 397 can only grow on plates containing serine.

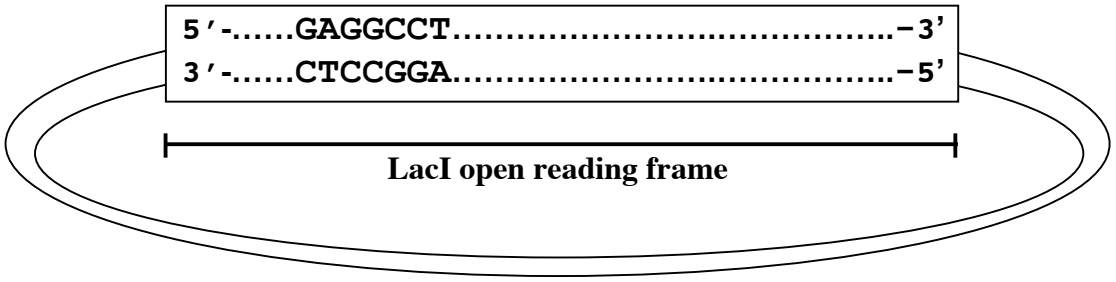
(d, 9pts) In the table below, fill in the genotypes (at the ProA, SerB1, and SerB2 loci) of the different phenotypic classes of transductants obtained from this third cross. Be sure to list **all possible genotypes** in each category.

GENOTYPE: **Phenotype:**	**at the ProA locus (+ or ⁻)**	**at the SerB locus (be sure to include the genotype at SerB1 and SerB2) (+ or ⁻)**
Don't require supplemental serine		
Require supplemental serine		

(e, 6pts) Draw all of the possibilities for a map of the region of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the positions and relative order of the ProA, SerB1, and SerB2 loci.

Name: _____

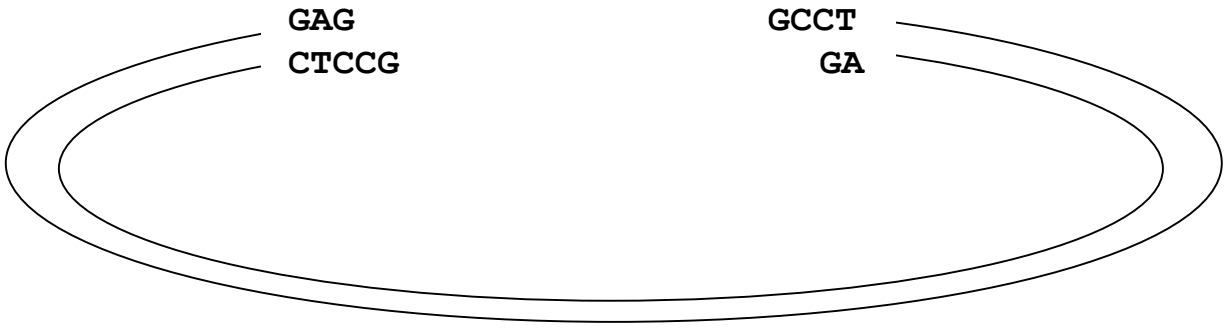
2. (31 pts) You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, the strain carrying the plasmid shows normal inducible expression of beta-galactosidase. A diagram of your plasmid is shown below. For this problem we are going to focus on a highlighted region of DNA sequence present early in the LacI open reading frame.



(a, 5pts) Write out the sequence that would result from transcription of the LacI gene by RNA polymerase, if the lower strand was used as a template. Be sure to give the sequence corresponding to the short segement that is highlighted, and label any 5’ and 3’ ends in your drawing.

(b, 5pts) Label the correct reading frame of this gene, given that a tRNA with the anticodon 5’-GCC-3' is supposed to base-pair with the region of the transcript that you drew in part **(a)**. Label the reading frame in the original plasmid drawing by circling a set of nucleotides that should be read as one codon.

(c, 6pts) The drawing below shows the original plasmid after being cut by a restriction enzyme that recognized the highlighted sequence.



Draw what would result if this cut plasmid were incubated with DNA polymerase in the presence of all four normal nucleotides. DO NOT do the drawing over – simply modify the drawing we gave you. Label any 5’ and 3’ ends in your drawing.

Name: _____

(d, 4pts) You next add DNA ligase to the product you drew in part **(c)**. DNA ligase will reseal the free DNA ends of that product so that a circular molecule reforms. You now transform a *lacI*Δ *E. coli* strain with the new plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase (uninducible, constitutive, or inducible)?

(e, 5pts) In one sentence, explain how the specific molecular change to the LacI gene made in the new plasmid led to the phenotype you predicted above.

(f, 6pts) You now transform a *lacI*Δ *E. coli* strain with the new plasmid that you made in part **(d) <u>and</u>** the original plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase? Explain your answer in one sentence.

Name: _____

3. (38 pts) You are studying the regulation of a bacterial gene (TolU) that encodes an enzyme that is necessary for the bacterium to degrade toluene for use as a carbon source. The tolU gene is only transcribed when simple sugars are not available as a carbon source. You isolate three mutant strains of this bacterium, each of which harbors a single mutation: tolA$^-$, tolB$^-$, or tolC$^-$. TolA, TolB, and TolC are all regulatory components involved in TolU regulation. Below are the phenotypes of different strains that you have constructed.

	Activity of TolU when:	
Genotype	Simple sugars absent	Simple sugars present
A+ B+ C+ U+	+	–
A$^-$	–	–
C$^-$ / **F'** A+ B+ C+ U+	+	+
A+ U$^-$ / **F'** A$^-$ B+ C+ U+	–	–
C$^-$ U+ / **F'** A+ B+ C+ U$^-$	+	+
A$^-$ B+ / **F'** A+ B$^-$ C+ U+	+	–
B$^-$	+	+
C$^-$	+	+
C$^-$ U$^-$ / **F'** A+ B+ C+ U+	+	–
A$^-$ U+ / **F'** A+ B+ C+ U+	+	–

(a, 7pts) Classify the tolA$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(b, 7pts) Classify the tolB$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(c, 7pts) Classify the tolC$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

Name: _____

(d, 8pts) Given your answers to parts **(a)** – **(c)**, draw a genetic pathway that shows the way by which the tolU gene is regulated. Be sure to indicate the wild-type functions of tolU, tolA, tolB, and tolC. Also include a role for simple sugars.

NOTE: Answer all of the remaining parts of this problem based on the model you drew in part **(d)**.

(e, 4pts) What would you predict to be the double mutant phenotype of a tolA$^-$ tolC$^-$ double mutant with respect to tolU expression? (Your choices are: uninducible, constitutive, or regulated.)

(f, 5pts) You isolate an allele at the TolB locus that gives an uninducible phenotype. What kind(s) of mutation could this new allele be with respect to TolU? (Your choices are: repressor$^-$, activator$^-$, promoter$^-$, operator$^-$, super repressor, super activator, dominant negative repressor, dominant negative activator.)

Name: _____KEY_____

7.03 Exam Two -- 2005

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Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)

Question 1	**31 pts_____**
Question 2	**31 pts_____**
Question 3	**38 pts_____**
TOTAL	**out of 100_____**

Name: _____KEY_____

1. (31 pts) You have isolated three bacterial mutants that cannot grow without supplemental serine being included in the growth medium. These three mutations lie in two genes, SerC and SerB. The SerC$^-$ mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1$^-$ mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2$^-$ mutation is a frameshift mutation that produces a protein product that is 12 kDa.

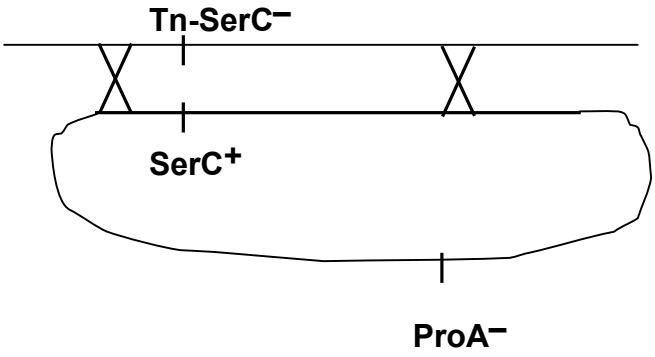
The first cross: You grow P1 phage on SerC$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ bacteria. (ProA$^-$ bacteria have a disruption in the ProA gene, which is required for the bacteria to synthesize their own proline.) You select for KanR transductants. All 200 of the transductants you analyze can grow on plates containing kanamycin and serine and proline, but cannot grow on plates containing kanamycin and serine (but not proline).

(a, 6pts) What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

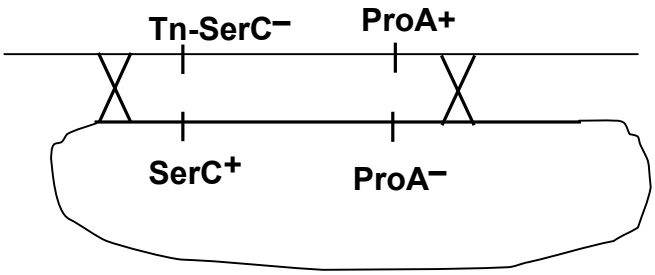
0%

There are two possibilities – either A and C are linked, or they are not.
The first cross:

If A and C are unlinked



If A and C are linked



You will never get ProA+ KanR from this.

You can get ProA+ KanR from this.

Given that you never see ProA+ KanR, the two are unlinked.
This problem asked you to express a distance between ProA and SerC as a cotransduction frequency. The cotransduction frequency between ProA and SerC is 0%.

Name: _____KEY_____

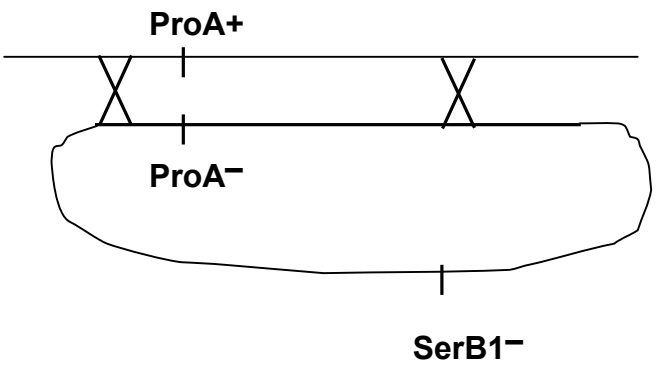
The second cross: You grow P1 phage on SerB2⁻ bacteria. You use the resulting phage lysate to infect ProA⁻ SerB1⁻ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on plates lacking serine. The other 67 can only grow on plates containing serine.

(b, 5pts) Are ProA and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

Definitely.

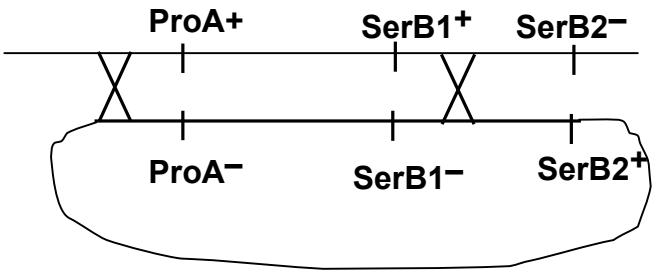
There are two possibilities – either A and B are linked, or they are not.
The second cross:

If A and B are unlinked



You will never get ProA+ Ser+ from this.

If A and B are linked



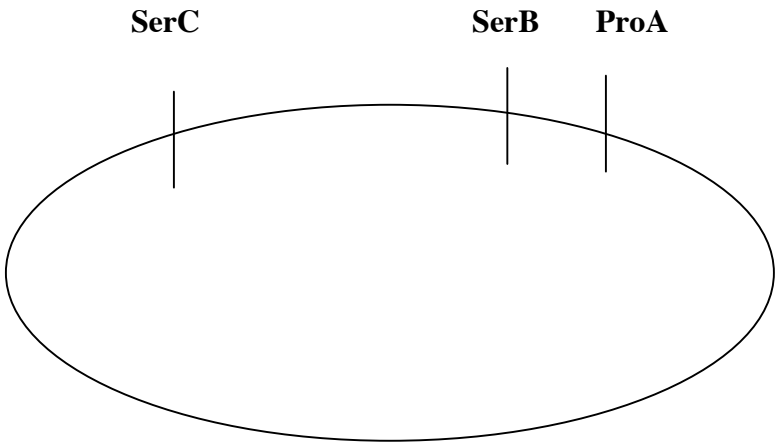
You can get ProA+ SerB+ from this.

Given that you do see ProA+ Ser+ transductants, A and B must be linked.

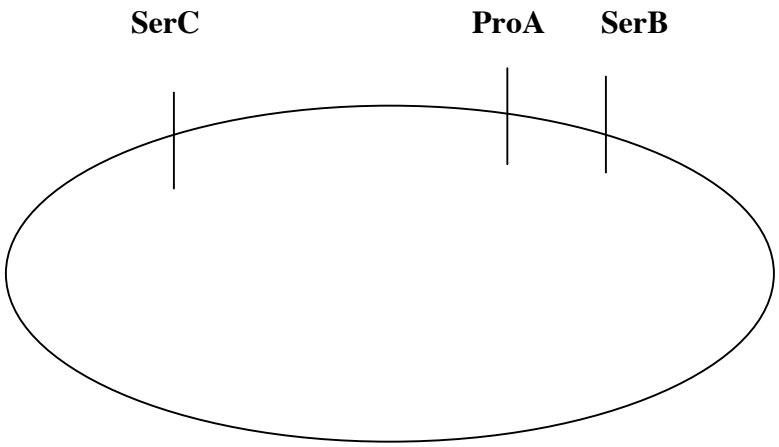
(c, 5pts) Are SerC and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

Maybe. SerC and ProA are unlinked by cotransduction. ProA and SerB are linked by cotransduction. It may be that the map order of these genes is such that SerB is in the middle of SerC and ProA, so SerC and ProA are far enough to be unlinked (more than 10^5 basepairs), but SerC and SerB are close enough to each other to be linked (see diagram below).

Name: _____KEY_____



However it also may be that the map order of these genes is such that ProA is in the middle of SerC and SerB, so SerC and ProA are far enough to be unlinked (more than 10^5 basepairs), and then SerC and SerB are also unlinked because they are even farther from each other than ProA and SerC (see diagram below).



The third cross: You grow P1 phage on SerB1$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ SerB2$^-$ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on plates lacking serine. The other 397 can only grow on plates containing serine.

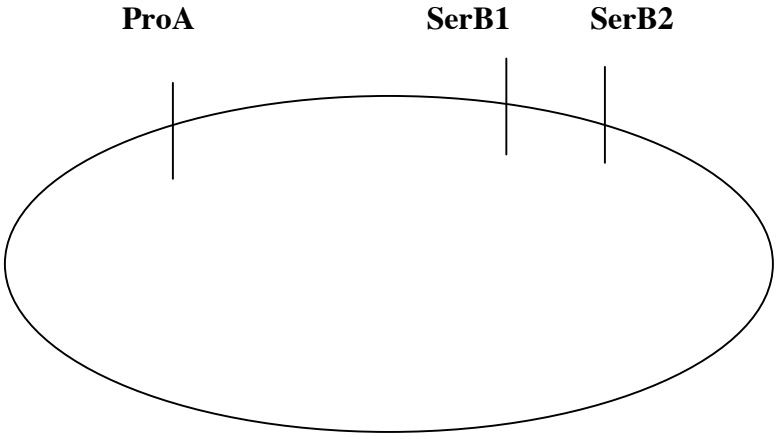
(d, 9pts) In the table below, fill in the genotypes (at the ProA, SerB1, and SerB2 loci) of the different phenotypic classes of transductants obtained from this third cross. Be sure to list **all possible genotypes** in each category.

Name: _____KEY_____

GENOTYPE: **Phenotype:**	**at the ProA locus (+ or ⁻)**	**at the SerB locus (be sure to include the genotype at SerB1 and SerB2) (+ or ⁻)**
Don't require supplemental serine	**+**	**1+ 2+**
Require supplemental serine	**+**	**1+ 2⁻** **1⁻ 2⁻** **1⁻ 2+**

Note that you are selecting for ProA+, so ALL transductants will be ProA+.
Ser+ transductants will only result if both positions in the SerB gene are wild-type.

(e, 6pts) Draw all of the possibilities for a map of the region of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the positions and relative order of the ProA, SerB1, and SerB2 loci.

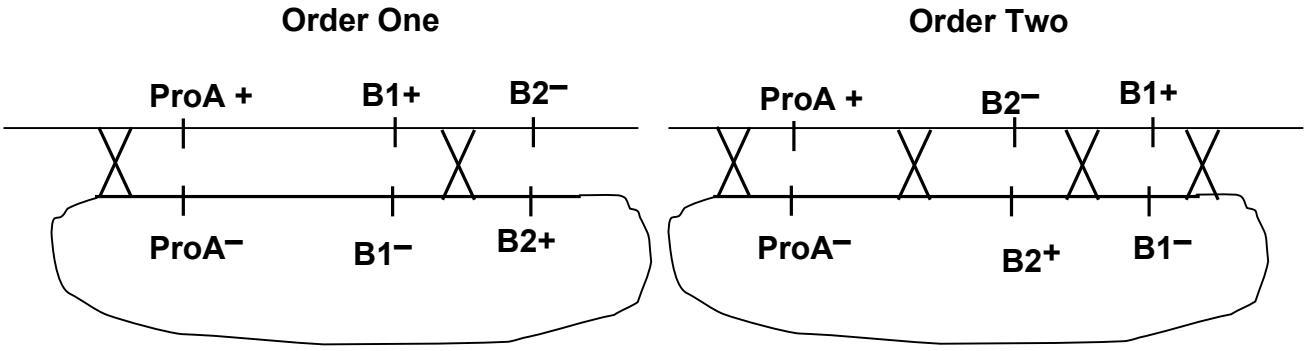


Name: _____KEY_____

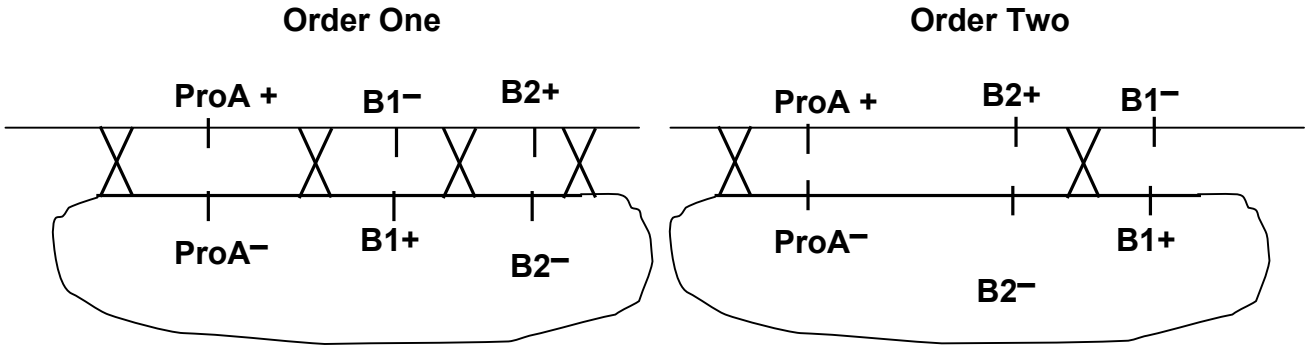
There is only one possible order for 1e. ProA cannot be in the middle, because SerB1 and SerB2 are in the same gene. SerB1 is much more likely to be in the middle because, if B1 is in the middle, you will see a higher frequency of Ser+ transductants in the second cross than in the third cross. (If B2 is in the middle, you would have seen a higher frequency of Ser+ transductants in the third cross than in the second cross). This is because double crossover events are more frequent than quadruple crossover events. Below are drawn the crossovers necessary to create Ser+ transductants. Note that you are selecting for ProA+, so ALL transductants will be ProA+.

Order One – B1 in middle **Order Two - B2 in middle**

The second cross:

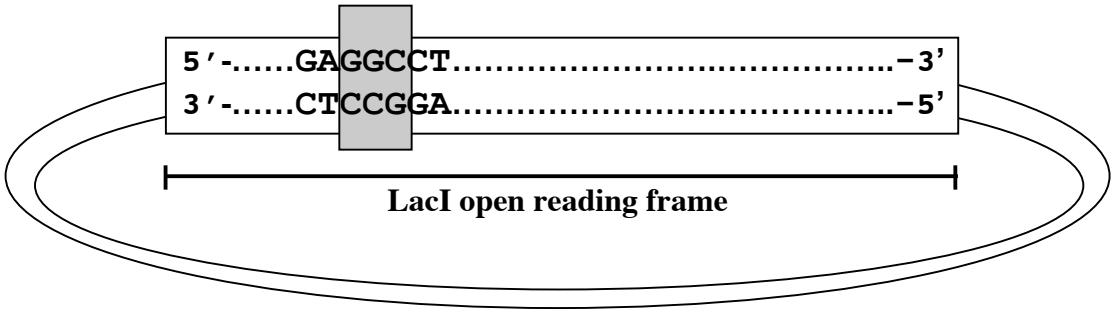


The third cross:



Name: _____KEY_____

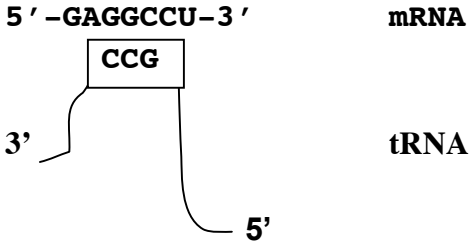
2. (31 pts) You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, the strain carrying the plasmid shows normal inducible expression of beta-galactosidase. A diagram of your plasmid is shown below. For this problem we are going to focus on a highlighted region of DNA sequence present early in the LacI open reading frame.



(a, 5pts) Write out the sequence that would result from transcription of the LacI gene by RNA polymerase, if the lower strand was used as a template. Be sure to give the sequence corresponding to the short segement that is highlighted, and label any 5’ and 3’ ends in your drawing.

5’-GAGGCCU-3’

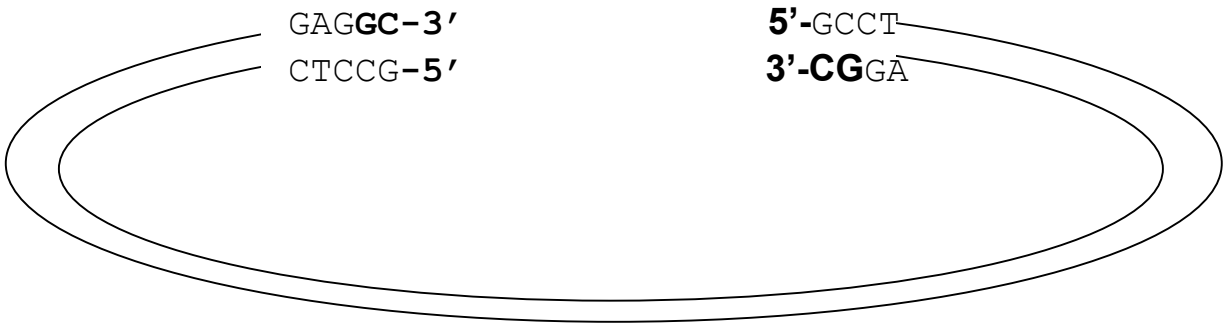
(b, 5pts) Label the correct reading frame of this gene, given that a tRNA with the anticodon 5’-GCC-3' is supposed to base-pair with the region of the transcript that you drew in part **(a)**. Label the reading frame in the original plasmid drawing by circling a set of nucleotides that should be read as one codon.



See drawing above. Note that, if you did not label the reading frame in the original plasmid drawing (and instead labeled the reading frame in the mRNA you drew in part a), you did not receive full credit.

(c, 6pts) The drawing below shows the original plasmid after being cut by a restriction enzyme that recognized the highlighted sequence.

Name: _____KEY_____



Draw what would result if this cut plasmid were incubated with DNA polymerase in the presence of all four normal nucleotides. DO NOT do the drawing over – simply modify the drawing we gave you. Label any 5’ and 3’ ends in your drawing.

See drawing above. DNA polymerase can do replication, but only of sequences for which there is a template (i.e. you can't fill in other nucleotides in the middle because there is no template there.) Note that you did not receive credit unless you labeled the 5' and 3' ENDS of the DNA molecule. The ends are located at the two opposite sides of the linear product you created in part c).

(d, 4pts) You next add DNA ligase to the product you drew in part **(c)**. DNA ligase will reseal the free DNA ends of that product so that a circular molecule reforms. You now transform a *lacI*Δ *E. coli* strain with the new plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase (uninducible, constitutive, or inducible)?

Constitutive.

(e, 5pts) In one sentence, explain how the specific molecular change to the LacI gene made in the new plasmid led to the phenotype you predicted above.

The mutation made in lacI from religating the plasmid is a +2 frameshift mutation (a 2 base pair insertion). This means that the entire frame of LacI (from early on in the gene) is shifted off from the original frame. Thus the protein created from this mutated version of the lacI gene would be non-functional because none of the subsequent codons would be read correctly. LacI is a repressor of LacZ, so losing function in LacI would lead to constitutive expression of LacZ.

(f, 6pts) You now transform a *lacI*Δ *E. coli* strain with the new plasmid that you made in part **(d) and** the original plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase? Explain your answer in one sentence.

Name: _____KEY_____

Inducible. The mutation made in lacI from religating the plasmid would be recessive, because it is a loss-of-function mutation that destroys the function of LacI. Thus, if you had a cell with wild-type LacI and non-functional LacI, you would see the wild-type phenotype.

3. (38 pts) You are studying the regulation of a bacterial gene (TolU) that encodes an enzyme that is necessary for the bacterium to degrade toluene for use as a carbon source. The tolU gene is only transcribed when simple sugars are not available as a carbon source. You isolate three mutant strains of this bacterium, each of which harbors a single mutation: tolA$^-$, tolB$^-$, or tolC$^-$. TolA, TolB, and TolC are all regulatory components involved in TolU regulation. Below are the phenotypes of different strains that you have constructed.

	Activity of TolU when:	
Genotype	Simple sugars absent	Simple sugars present
1. A+ B+ C+ U+	+	–
2. A$^-$	–	–
3. C$^-$ / **F'** A+ B+ C+ U+	+	+
4. A+ U$^-$ / **F'** A$^-$ B+ C+ U+	–	–
5. C$^-$ U+ / **F'** A+ B+ C+ U$^-$	+	+
6. A$^-$ B+ / **F'** A+ B$^-$ C+ U+	+	–
7. B$^-$	+	+
8. C$^-$	+	+
9. C$^-$ U$^-$ / **F'** A+ B+ C+ U+	+	–
10. A$^-$ U+ / **F'** A+ B+ C+ U+	+	–

(a, 7pts) Classify the tolA$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

Cis, uninducible, recessive. (This means that A is the promoter sequence in the U gene.)
Uninducible – Strain 2
Recessive – Strain 10
Cis – Strain 4 is the trans test (because A+ is on a different piece of DNA from U+), and A fails the trans test because the trans test strain displays the recessive phenotype (uninducible from A$^-$ is recessive to inducible from A+).

(b, 7pts) Classify the tolB$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

Trans, constitutive, recessive. (This means that B is a repressor.)

Name: _____KEY_____

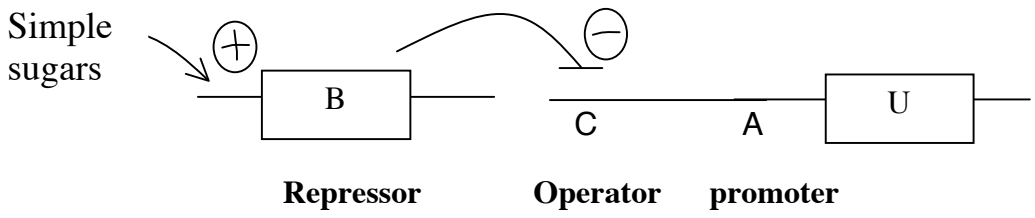
Constitutive – Strain 7
Recessive – Strain 6. Strain 6 is a complementation test for A and B. This strain shows the wild-type phenotype, which can only occur if A and B are both recessive and in different genes.
Trans – Strain 6. Strain 6 is a complementation test for A and B. This strain shows the wild-type phenotype, which can only occur if A and B are both recessive and in different genes. The fact that A is cis to U means that A and U are in the same gene. The fact that B is in a different gene than A means that B is not in the same gene as U. All cis sequences are in the same gene as the reporter, so B must be trans.

(c, 7pts) Classify the tolC$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

Cis, constitutive, dominant. (This means that C is the operator sequence in the U gene.)
Constitutive – Strain 8
Dominant – Strain 3
Cis – Strain 9 is the trans test (because C$^-$ is on a different piece of DNA from U+), and C fails the trans test because the trans test strain displays the recessive phenotype (inducible from C+ is recessive to constitutive from C$^-$).

(d, 8pts) Given your answers to parts **(a)** – **(c)**, draw a genetic pathway that shows the way by which the tolU gene is regulated. Be sure to indicate the wild-type functions of tolU, tolA, tolB, and tolC. Also include a role for simple sugars.

If you answered parts a – c correctly, then the model is:



Note that operators and promoters are cis-acting sequences. Cis-acting sequences must be linked to the reporter gene (that is, physically linked). Thus you must have drawn A and C physically linked to U to get full credit. Operator and promoter sequences are part of the reporter gene. They are sequences that do not get transcribed or translated, but are instead control sequences that are found before the transcription start site of the reporter gene.

Name: _____KEY_____

Also, to get full credit:
-- The net effect of B must have been negative, because B is a repressor.
-- The net effect of sugars must have been negative, because sugars inhibit expression of the U gene.
-- The order in your pathway must have been:
Signal (sugars) then B (the trans-acting regulator) then U (the reporter gene).
-- The wild-type functions of the elements in your pathway must have been obvious from your drawing, or clearly labeled and drawn consistently with the label you gave.

PLEASE NOTE that we cannot interpret what you mean if you draw a blocking arrow labeled with a plus sign, and we cannot interpret what you mean if you draw a pointed arrow labeled with a negative sign. Such arrows send us mixed signals.

If you did not answer parts a-c correctly, then the model that you drew had to be consistent with what the predicted wild-type functions of A, B, and C would have been, had your answers to a-c been correct. Thus, if you determined that C was trans, you had to draw C as trans-acting in your model (for example). Note that there are NO cis uninducible dominant mutations, and NO cis constitutive recessive mutations, so if you gave such an answer to parts a, b, or c, then there is nothing that you could have drawn in your model that would have been consistent with the properties that you determined of the mutant.

NOTE: Answer all of the remaining parts of this problem based on the model you drew in part **(d)**.

(e, 4pts) What would you predict to be the double mutant phenotype of a tolA$^-$ tolC$^-$ double mutant with respect to tolU expression? (Your choices are: uninducible, constitutive, or regulated.)

If you drew A as the promoter and C as the operator in your model (which is correct), then the correct answer is UNINDUCIBLE, because the U gene would have no promoter and thus could never be expressed.
If you drew A and C as trans-acting regulators (which is incorrect), then the correct answer to part e would be the phenotype of mutating the downstream regulator (whichever one you drew closer to U, either A or C).

(f, 5pts) You isolate an allele at the TolB locus that gives an uninducible phenotype. What kind(s) of mutation could this new allele be with respect to TolU? (Your choices are: repressor$^-$, activator$^-$, promoter$^-$, operator$^-$, super repressor, super activator, dominant negative repressor, dominant negative activator.)

If you drew B as being a net repressor in your model (which is correct), then the only correct answer is SUPER-REPRESSOR. Drawing B as a repressor in your model tells us that the wild-type function of B is to be a repressor. Therefore, the B gene encodes a

Name: _____KEY_____

repressor. Therefore any allele of the B gene must be a repressor allele. The only allele of a repressor-encoding gene that gives the uninducible phenotype is the super-repressor allele.

If you drew B as being a net activator in your model (which is incorrect), then the correct answer to part f) is dominant negative activator or activator⁻. This is because there are two kinds of alleles of activator-encoding genes that give uninducible: dominant negative activator or activator⁻.

Name: _____

7.03 Exam Two -- 2005

Name: _____

Exam starts at 11:05 am and ends at 11:55 am.

There are 7 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)

Question 1	**31 pts_____**
Question 2	**31 pts_____**
Question 3	**38 pts_____**
TOTAL	**out of 100_____**

Name: _____

1. (31 pts) You have isolated three bacterial mutants that cannot grow without supplemental serine being included in the growth medium. These three mutations lie in two genes, SerC and SerB. The SerC$^-$ mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1$^-$ mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2$^-$ mutation is a frameshift mutation that produces a protein product that is 12 kDa.

The first cross: You grow P1 phage on SerC$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ bacteria. (ProA$^-$ bacteria have a disruption in the ProA gene, which is required for the bacteria to synthesize their own proline.) You select for KanR transductants. All 200 of the transductants you analyze can grow on plates containing kanamycin and serine and proline, but cannot grow on plates containing kanamycin and serine (but not proline).

(a, 6pts) What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

The second cross: You grow P1 phage on SerB2$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ SerB1$^-$ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on plates lacking serine. The other 67 can only grow on plates containing serine.

(b, 5pts) Are ProA and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

(c, 5pts) Are SerC and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

Name: _____

The third cross: You grow P1 phage on SerB1⁻ bacteria. You use the resulting phage lysate to infect ProA⁻ SerB2⁻ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on plates lacking serine. The other 397 can only grow on plates containing serine.

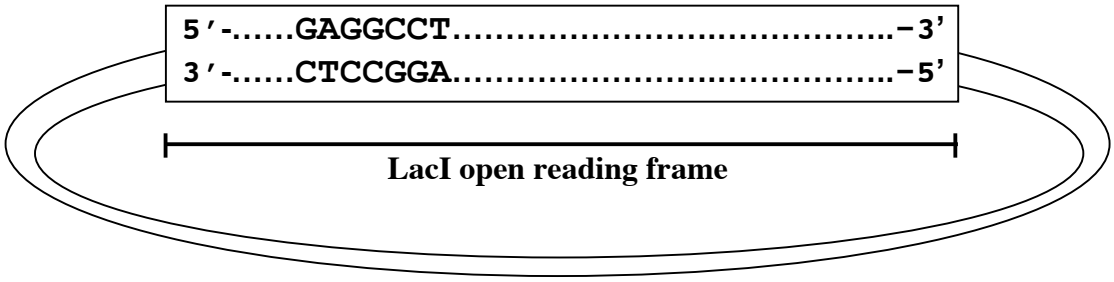
(d, 9pts) In the table below, fill in the genotypes (at the ProA, SerB1, and SerB2 loci) of the different phenotypic classes of transductants obtained from this third cross. Be sure to list **all possible genotypes** in each category.

GENOTYPE: **Phenotype:**	**at the ProA locus (+ or ⁻)**	**at the SerB locus (be sure to include the genotype at SerB1 and SerB2) (+ or ⁻)**
Don't require supplemental serine		
Require supplemental serine		

(e, 6pts) Draw all of the possibilities for a map of the region of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the positions and relative order of the ProA, SerB1, and SerB2 loci.

Name: _____

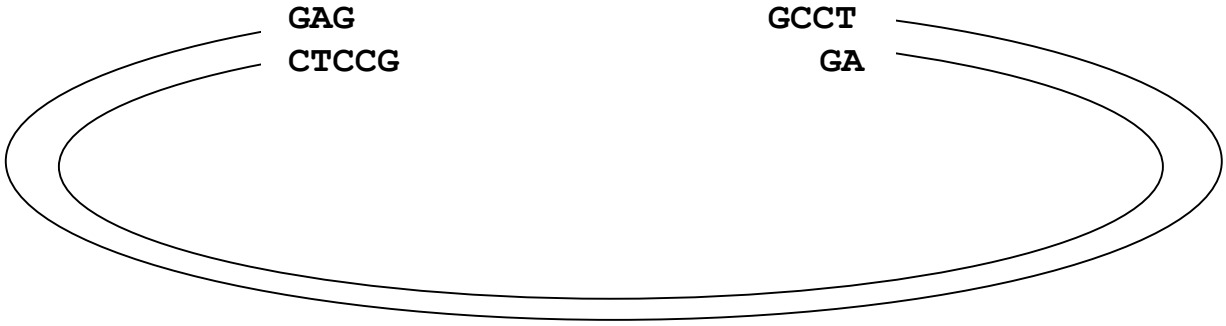
2. (31 pts) You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, the strain carrying the plasmid shows normal inducible expression of beta-galactosidase. A diagram of your plasmid is shown below. For this problem we are going to focus on a highlighted region of DNA sequence present early in the LacI open reading frame.



(a, 5pts) Write out the sequence that would result from transcription of the LacI gene by RNA polymerase, if the lower strand was used as a template. Be sure to give the sequence corresponding to the short segement that is highlighted, and label any 5’ and 3’ ends in your drawing.

(b, 5pts) Label the correct reading frame of this gene, given that a tRNA with the anticodon 5’-GCC-3' is supposed to base-pair with the region of the transcript that you drew in part **(a)**. Label the reading frame in the original plasmid drawing by circling a set of nucleotides that should be read as one codon.

(c, 6pts) The drawing below shows the original plasmid after being cut by a restriction enzyme that recognized the highlighted sequence.



Draw what would result if this cut plasmid were incubated with DNA polymerase in the presence of all four normal nucleotides. DO NOT do the drawing over – simply modify the drawing we gave you. Label any 5’ and 3’ ends in your drawing.

Name: _____

(d, 4pts) You next add DNA ligase to the product you drew in part **(c)**. DNA ligase will reseal the free DNA ends of that product so that a circular molecule reforms. You now transform a *lacI*Δ *E. coli* strain with the new plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase (uninducible, constitutive, or inducible)?

(e, 5pts) In one sentence, explain how the specific molecular change to the LacI gene made in the new plasmid led to the phenotype you predicted above.

(f, 6pts) You now transform a *lacI*Δ *E. coli* strain with the new plasmid that you made in part **(d) <u>and</u>** the original plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase? Explain your answer in one sentence.

Name: _____

3. (38 pts) You are studying the regulation of a bacterial gene (TolU) that encodes an enzyme that is necessary for the bacterium to degrade toluene for use as a carbon source. The tolU gene is only transcribed when simple sugars are not available as a carbon source. You isolate three mutant strains of this bacterium, each of which harbors a single mutation: tolA$^-$, tolB$^-$, or tolC$^-$. TolA, TolB, and TolC are all regulatory components involved in TolU regulation. Below are the phenotypes of different strains that you have constructed.

	Activity of TolU when:	
Genotype	Simple sugars absent	Simple sugars present
A+ B+ C+ U+	+	–
A$^-$	–	–
C$^-$ / **F'** A+ B+ C+ U+	+	+
A+ U$^-$ / **F'** A$^-$ B+ C+ U+	–	–
C$^-$ U+ / **F'** A+ B+ C+ U$^-$	+	+
A$^-$ B+ / **F'** A+ B$^-$ C+ U+	+	–
B$^-$	+	+
C$^-$	+	+
C$^-$ U$^-$ / **F'** A+ B+ C+ U+	+	–
A$^-$ U+ / **F'** A+ B+ C+ U+	+	–

(a, 7pts) Classify the tolA$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(b, 7pts) Classify the tolB$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(c, 7pts) Classify the tolC$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

Name: _____

(d, 8pts) Given your answers to parts **(a)** – **(c)**, draw a genetic pathway that shows the way by which the tolU gene is regulated. Be sure to indicate the wild-type functions of tolU, tolA, tolB, and tolC. Also include a role for simple sugars.

NOTE: Answer all of the remaining parts of this problem based on the model you drew in part **(d)**.

(e, 4pts) What would you predict to be the double mutant phenotype of a tolA$^-$ tolC$^-$ double mutant with respect to tolU expression? (Your choices are: uninducible, constitutive, or regulated.)

(f, 5pts) You isolate an allele at the TolB locus that gives an uninducible phenotype. What kind(s) of mutation could this new allele be with respect to TolU? (Your choices are: repressor$^-$, activator$^-$, promoter$^-$, operator$^-$, super repressor, super activator, dominant negative repressor, dominant negative activator.)

Name: _____key_____

7.03 Exam Three -- 2005 KEY

Name: _____

Exam starts at 11:05 am and ends at 11:55 am.

There are 8 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)

Question 1	**17 pts_____**
Question 2	**45 pts_____**
Question 3	**20 pts_____**
Question 4	**18 pts_____**
TOTAL	**out of 100_____**

Name: _____key_____

1. (17 pts) You are studying the expression of the yeast gene ProA that is necessary for the synthesis of the amino acid proline. ProA is normally expressed only when the cell is lacking supplemental proline in the growth medium. You isolate two haploid yeast strains (ProB$^-$ and ProC$^-$) that misregulate ProA expression.

You mate a ProB$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly.

You mate a ProA$^-$ ProC$^-$ haploid strain to a ProC$^-$ haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium.

You mate a ProC$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB$^-$ ProC$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly. You induce sporulation of this diploid, and examine 40 tetrads. 30 (of those 40) each contain: two spores that do not express ProA when proline is absent from the growth medium, one spore that expresses ProA when proline is present in the growth medium, and one spore that expresses ProA properly.

(a, 6pts) Classify the ProB$^-$ mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, uninducible
Trans – You mate a ProB$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly. This means that the A and B mutations complement each other and are thus in different genes. This means that B must act in trans to A.
Recessive -- You mate a ProB$^-$ haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly. This means B– is recessive.
Uninducible – The only mutant phenotypes you see when you sporulate a diploid that contains both the B– and C– mutations are constitutive and uninducible. C– gives constitutive, so B– must give uninducible.

(b, 6pts) Classify the ProC$^-$ mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, constitutive

Trans – You mate a ProC$^-$ haploid strain to a ProA$^-$ haploid strain. The resulting diploid expresses ProA properly. This means that the A and C mutations

Name: _____key_____

complement each other and are thus in different genes. This means that C must act in trans to A.
Recessive -- You mate a ProC⁻ haploid strain to a ProA⁻ haploid strain. The resulting diploid expresses ProA properly. This means C– is recessive.
Constitutive – You mate a ProA⁻ ProC⁻ haploid strain to a ProC⁻ haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium. This means that a cell that has a functional copy of A but has no functional copies of C expreses ProA even when it is not supposed to (i.e. when the cell already has proline available to it).

(c, 5pts) If you a drew a linear pathway showing the regulation of ProA, which function would you place closer to ProA: ProB or ProC?

ProB

When you sporulate a diploid that was produced from a mating of B– C+ to B+ C–, you see mostly tetratypes. You know they are tetratypes because the types of spores do not come in pairs. (There are three types of spores.)
Each tetratype contains: two spores that do not express ProA when proline is absent from the growth medium (uninducible), one spore that expresses ProA when proline is present in the growth medium (constitutive), and one spore that expresses ProA properly (inducible). A tetratype resulting from this mating would contain the spores:

GENOTYPE	**PHENOTYPE**
B– C+	**uninducible**
B+ C–	**constitutive**
B+ C+	**inducible**
B– C–	**NOT KNOWN PREVIOUSLY**

The spore of unknown phenotype must be the double mutant, and it shows the single mutant phenotype of B (uninducible), so B must be more downstream in the pathway (i.e. closer to the reporter gene).

2. (45 pts) You are studying the transcriptional regulation of a mouse gene called *Stringy*. This gene is normally only expressed in tail cells due to the presence of a tail-specific inducer molecule in these cells. You have isolated two true-breeding mutant strains of mice that do not spatially regulate the expression of the *Stringy* gene properly. The strains of mice that you have, and their corresponding phenotypes, are listed in the table below.

Genotype of mouse	Phenotype of mouse
Wild-type	*Stringy* expressed only in tail

Name: _____key_____

A^- / A^-	*Stringy* not expressed anywhere
B^- / B^-	*Stringy* expressed in all cells in the body

When you cross mice that are B^- / B^- to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail.
When you cross mice that are B^- / B^- to mice that are A^- / A^-, and then cross the resulting F1 mice to each other, you get a genotypic ratio in the F_2 that indicates that the A and B loci segregate independently of each other.

You inject a piece of DNA containing the A^- allele of the A gene into a fertilized egg produced by the mating of two true-breeding B^- mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born does not express *Stringy* in any cells in its body.

(a, 6pts) Classify the A^- mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, dominant, uninducible

Trans, dominant, and epistatic to B -- You inject a piece of DNA containing the A^- allele of the A gene into a fertilized egg produced by the mating of two true-breeding B^- mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born does not express *Stringy* in any cells in its body. The phenotype you are seeing in this A+/A+/A– B–/B– mouse is the phenotype of A–, not the phenotype of B– (which is constitutive). This tells you three things:
1) A– is dominant. This mouse has 3 copies of A and two of them are wild-type, and yet you see the mutant phenotype. Thus A– must be dominant.
2) A– can affect Stringy in trans. A– must have randomly integrated into the mouse genome, and yet it can influence Stringy expression. This means that A is capable of acting on Stringy from a distance.
3) A is downstream of B. This mouse is a double mutant : A– and B–/B–. The phenotype shown is that of A–. Thus A must be downstream of B in the pathway.

Uninducible – Line #2 of the chart

(b, 6pts) Classify the B^- mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Trans, recessive, constitutive

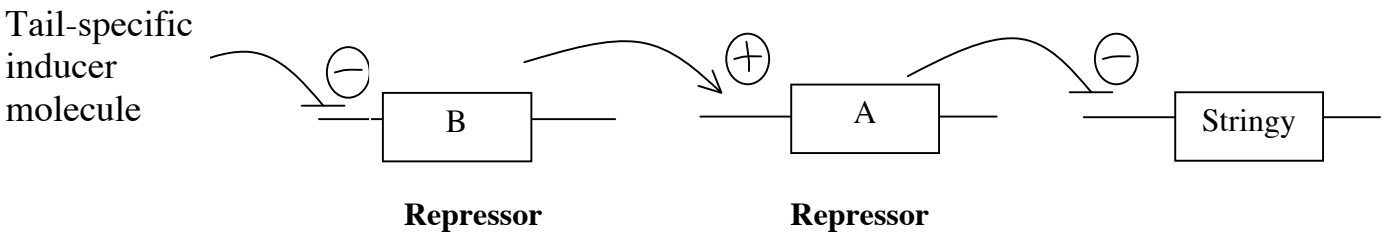
Name: _____key_____

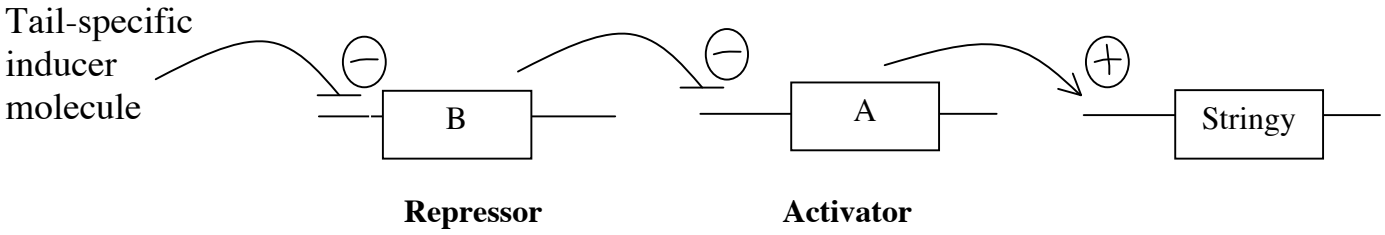
Trans -- When you cross mice that are B⁻ / B⁻ to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail. This means that B and Stringy complement each other, so B and Stringy must be in different genes. Thus B must act in trans on Stringy.

Recessive -- When you cross mice that are B⁻ / B⁻ to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail. This means that B– is recessive.

Constitutive – Line #3 of the chart

(c, 12pts) Draw TWO different linear genetic pathways that are consistent with your answers to parts **(a)** and **(b)**. Be sure to indicate the wild-type A, B, and *Stringy* genes in your model, and also include the tail-specific inducer molecule.





B must have a net negative effect because B is trans and, when you take B function away, you get constitutive expression of Stringy.

A's net effect is unknown, because the only mutation you have in A is a dominant mutation, and you cannot determine wild-type function from a dominant mutation.

The tail-specific molecule is the signal to start the pathway, and it must have a net positive effect because it is an inducer molecule.

In any gene regulation pathway, the reporter gene is always at the end (because it is the output), and the signal is always at the beginning (because it is the input).

Name: _____key_____

We did an epistasis test when we made a transgenic mouse that was a B–/B– mouse with an A– transgene. A is dominant, so this mouse is a double mutant mouse that is mutant both in the A function and in the B function. Whichever phenotype this mouse displays is the phenotype of the single mutation in the more downstream gene in the pathway. This mouse shows uninducible expression of Stringy, so A is the most downstream gene.

(d, 6pts) Clearly state which one piece of information you would need to know in order to determine which of the models you drew in part **(c)** was correct.

You would need to know the wild-type function of the A gene. The A locus operates in trans, and thus it must either encode an activator or a repressor. However you do not know which one it encodes because the only mutation you have in A gives a dominant phenotype, and dominant mutations cannot be used to determine wild-type function. Given that you do not know the wild-type function of A, you do not know whether the mutant allele A– is a superrepressor allele or a dominant negative activator allele.

Most people wrote here that you needed an epistasis test, but in fact we already gave you an epistasis test when we made a transgenic mouse that was a B–/B– mouse with an A– transgene. A is dominant, so this mouse is a double mutant mouse that is mutant both in the A function and in the B function. Whichever phenotype this mouse displays is the phenotype of the single mutation in the more downstream gene in the pathway.

(e, 15pts) You want to distinguish between the two models listed in part **(c)**. You could do this by creating a genetically engineered mouse. **For the mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) what is the **genotype** of the fertilized egg or the ES cells you would start with
iv) which **additional breeding** steps you would do to make the mouse you wanted
v) **two possible** phenotypic results you could get from the newly made mice,
and the corresponding conclusion you would make for each result

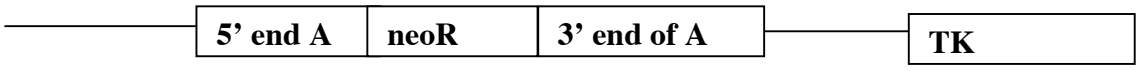
Describe a way to create a genetically modified mouse that would allow you to gain the piece of information you stated in part **(d)** (and thereby distinguish between your models).

Name: _____key_____

IF YOU GOT PART d) RIGHT, YOU NEED TO DETERMINE THE WILD-TYPE FUNCTION OF A:

i) gene targeting

ii)



iii) wild-type ES cells

iv) Mate the resulting chimera to wild-type to get out non-chimeric heterozygotes. Mate two heterozygotes together and 1/4 of them will be homozygous for the A gene knockout.

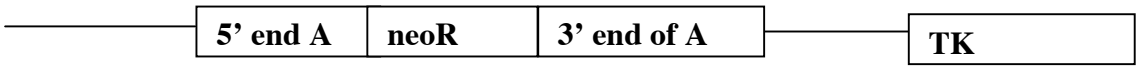
v) If the mice express Stringy everywhere, then the wild-type function of A is to be a repressor (in which case the A– allele was a superrepressor). If the mice express Stringy nowhere, then the wild-type function of A is to be an activator (in which case the A– allele was a dominant negative activator).

IF YOU GOT PART d) WRONG, YOU PROBABLY TRIED TO DO AN EPISTASIS TEST HERE, in which case you could have tried 4 different experiments:

<u>POSSIBILITY ONE</u> (best option of 4 b/c it also tells you the wt function of A)

i) gene targeting

ii)



iii) B–/B– ES cells

iv) Mate the resulting chimera to B–/B– mice to get out non-chimeric mice that are B–/B– A+/A^{KO}. Mate two of these mice together and 1/4 of them will be homozygous for the A gene knockout and will be B–/B–.

v) If the mice express Stringy nowhere, then the wild-type function of A is to be an activator, AND A is downstream in the pathway.

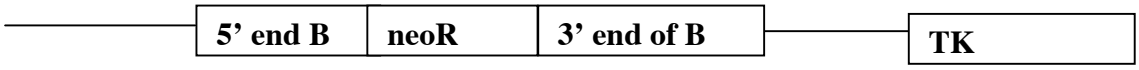
If the mice express Stringy everywhere, you can't really conclude anything because you don't yet know the loss-of-function phenotype of A. A could be a repressor, and if it were, this result would not give you order in the pathway because both single mutant phenotypes would be constitutive.

Name: _____key_____

POSSIBILITY TWO

i) gene targeting

ii)



iii) A–/A– ES cells

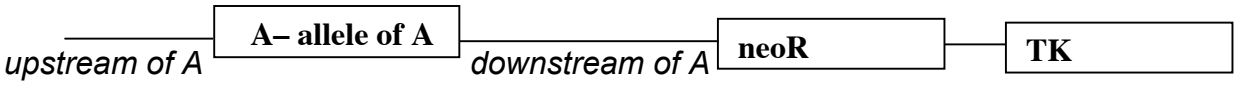
iv) Mate the resulting chimera to A–/A– mice to get out non-chimeric mice that are A–/A– B+/B–. Mate two of these mice together and 1/4 of them will be homozygous for the B gene knockout and will be A–/A–.

v) A–/A– mice are going to have uninducible expression of Stringy no matter what, because we basically made this mouse for you already in the introduction to this question. The only difference between your experiment and ours was that you ended up with an A–/A– mouse, and we ended up with an A–/A+/A+ mouse. However the result of the experiment would be the same. Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

POSSIBILITY THREE

i) gene targeting

ii)



iii) B–/B– ES cells

iv) Mate the resulting chimera to B–/B– mice to get out non-chimeric mice that are B–/B– A+/A–.

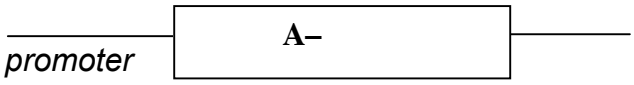
v) A– mice are going to have uninducible expression of Stringy no matter what. We basically did this experiment for you in the introduction to this question (except we added a copy of A– using pronuclear injection instead of gene targeting). Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

Name: _____key_____

POSSIBILITY FOUR

i) pronuclear injection

ii)



iii) B–/B– egg

iv) no breeding is necessary but you could have bred the transgene to homozygosity if you wanted to

v) We actually did this experiment for you in the introduction to this question. Thus your conclusion should have been that you know you would get the uninducible result (because that is what we told you happened in the introduction).

3. (20 pts) For each situation below, predict whether the frequency of the ALLELE ("q") associated with the trait/disorder in consideration will **stay the same**, **rise**, or **fall**. If you cannot conclude, choose "***inconclusive***."

For parts **(a)**, **(b)** and **(c)**, assume that the mutation rate is zero.

(a, 5pts) There is a population in which a rare autosomal recessive trait (with S = 0, h = 0) exists. This population always mates randomly. All of a sudden, heterozygotes obtain a selective advantage.

The allele frequency "q" will: stay the same (rise) inconclusive fall
(CIRCLE ONE OF THE FOUR)

Heterozygotes are being selected for, and this is the only force acting upon q. The allele frequency q would therefore rise.

(b, 5pts) There is a population in which a rare autosomal recessive disorder (with **S = 1**, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding.

Name: _____key_____

The allele frequency "q" will: stay the same rise
(CIRCLE ONE OF THE FOUR)
inconclusive (fall)

Inbreeding increases the proportion of homozygotes in the population. Everytime a homozygote "aa" is created, those two alleles are removed from the population because the "aa" organism dies (S = 100%). Thus "a" alleles are constantly being removed from the population.

(c, 5pts) There is a population in which a rare autosomal **dominant** trait (with S = 0, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding.

The allele frequency "q" will: (stay the same) rise
(CIRCLE ONE OF THE FOUR)
inconclusive fall

Inbreeding does not affect autosomal dominant or X-linked traits in any way. This is because dominant traits only need to be inherited from one parent, and rare X-linked traits only affect males and are thus only inherited from the mother.

(d, 5pts) There is a population in which a rare autosomal recessive disorder (with **S = 1**, h = 0) exists. All of a sudden, this population goes from mating randomly to participating in some amount of inbreeding. Simultaneously, the mutation rate rises from being negligible to a rate that would now affect allele frequency.

The allele frequency "q" will: stay the same rise
(CIRCLE ONE OF THE FOUR)
(inconclusive) fall

Part "b" tells you that the combination of S=100% and inbreeding makes q go down, but mutation rates make q go up. It is unclear how large mu is, and how many people are inbreeding (and what relation these people are to each other), so you cannot conclude what the relative strength of these two forces are with respect to each other.

Name: _____key_____

4. (18 pts) Consider a gene in which mutations occur at a rate of 10^{-6}. Mutations in this gene will cause an autosomal recessive disease. Homozygotes for the allele associated with the disease have a fitness which is 10% that of those not carrying that allele. SHOW ALL OF YOUR WORK, indicate all equations you use, and use clear labels.

Note: If you need the quadratic formula, it is: $[-b +/- \sqrt{(b^2 - 4ac)}] / 2a$

(a, 6pts) Assume that, for many generations, this population has been at steady state because of a balance between mutation, selection for heterozygotes, and selection against affected individuals. Assume heterozygotes have a fitness which is 103% that of those not carrying the allele associated with the trait. Assume random mating. Calculate the steady-state value of q.

For this population to be at steady state:

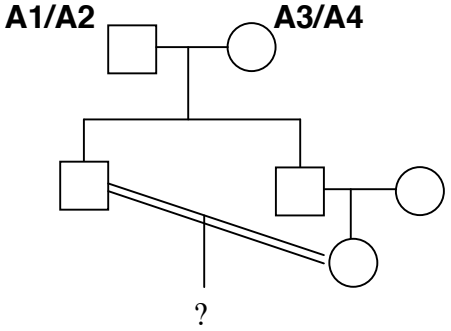
$$\Delta q_{\text{sel against homozygotes}} + \Delta q_{\text{sel for heterozygotes}} + \Delta q_{\text{mut}} = 0$$

$$-Sq^2 + hq + \mu = 0$$

S = 90%
h = 3%
mu = 10^{-6}
Solve for q using the quadratic equation

q = 0.033 (the other answer from the quadratic is negative)

(b, 5pts) Now assume that, for many generations, this population has been at steady state because of a balance between mutation, inbreeding, and selection against affected individuals (i.e. there is NO heterozygote advantage in this population). For a very long time, 15% of all children have been products of uncle-niece matings (and the remaining 85% have been products of random matings).
What is F equal to for an uncle-niece mating?



Name: _____key_____

Chance (child is A1/A1) = (1/2) * (1/2) * (1/2) * (1/2) * (1/2) = (1/32)

F = chance (child is A1/A1) + chance (child is A2/A2) + chance (child is A3/A3) + chance (child is A4/A4)

F = 4 * (1/32)

F = 1/8

(c, 7pts) Calculate the steady-state value of q for the situation described in part **(b)**.

For this population to be at steady state:

$$\Delta q_{\text{sel against homozygs from random mating}} + \Delta q_{\text{sel against homozygs from inbreeding}} + \Delta q_{\text{mut}} = 0$$

$$(-Sq^2) * 85\% \quad + \quad (-SFq) * 15\% \quad + \quad \mu \quad = 0$$

S = 90%
mu = 10^{-6}
F = 1/8
Solve for q using the quadratic equation

q = 5.9 X 10^{-5} (the other answer from the quadratic is negative)

Name: _____

7.03 Final Exam -- 2005

Name: _____

The exam starts at 9 am and ends at 12 pm.

There are 18 pages including this cover page.

Please write your name on each page.

Only writing on the front of every page will be graded.

Question 1	**24 pts_____**
Question 2	**26 pts_____**
Question 3	**20 pts_____**
Question 4	**24 pts_____**
Question 5	**24 pts_____**
Question 6	**22 pts_____**
Question 7	**34 pts_____**
Question 8	**26 pts_____**

TOTAL out of 200_____

Name: _____

1. (24 pts) You are studying three autosomal mutations in flies. Each of these three mutations lies in a different gene. All three genes lie on the same autosome. The wn1⁻ mutation is recessive and causes the phenotype of short wings (wild-type flies have long wings). The wn2⁻ mutation is recessive and also causes the phenotype of short wings. The ey⁻ mutation is dominant and causes the phenotype of small eyes (wild-type flies have big eyes). You cross true-breeding wn1⁻ wn2⁻ short-winged females to true-breeding ey⁻ males to obtain an F1 generation. You then cross female F1 flies to true-breeding wn1⁻ wn2⁻ big-eyed males. You analyze the resulting progeny, and find that there are flies in the progeny from all four phenotypic classes:

Short wings Small eyes
Long wings Small eyes
Short wings Large eyes
Long wings Large eyes

For parts **(a) – (c)**, write out complete genotype(s) and phenotype(s) of the flies we ask for. By complete genotype, we mean the genotype at all loci discussed in the problem. By complete phenotype, we mean the phenotype at all traits discussed in the problem. If there are multiple answers, write ALL POSSIBLE answers.
Use "**+**" to indicate wild-type alleles.

(a, 6pts) Write out complete genotype(s) and phenotype(s) of both parents.

	Phenotype	**Genotype**
P generation mother		
P generation father		

(b, 6pts) Write out complete genotype(s) and phenotype(s) of F1 flies.

	Phenotype	**Genotype**
F1 generation (mother of F2)		
Father to whom you cross the F1 mother		

Name: _____

(c, 8pts) Write out complete genotype(s) of the different F2 flies.

Phenotype	**Genotype**
Short wings Small eyes	
Long wings Small eyes	
Short wings Large eyes	
Long wings Large eyes	

(d, 4pts) Remember from class that, oddly enough, male flies do not undergo recombination during meiosis. You cross true-breeding $wn1^{-}$ $wn2^{-}$ short-winged females to true-breeding ey^{-} males to obtain an F1 generation. You then cross male F1 flies to true-breeding $wn1^{-}$ $wn2^{-}$ big-eyed females. If you analyze 2000 resulting progeny, predict the number of the following kinds of flies that you will get:

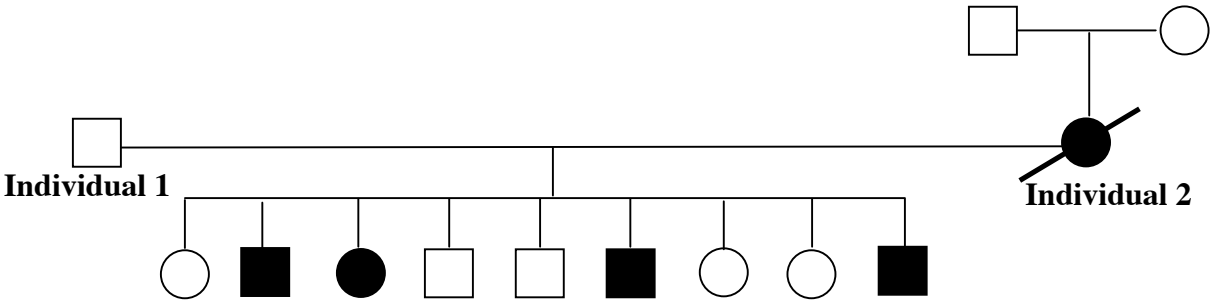
Phenotype	Number of flies
Short wings Small eyes	
Long wings Small eyes	
Short wings Large eyes	
Long wings Large eyes	

Name: _____

2. (26 pts) You are studying a new species of primate that is diploid, and has four pairs of autosomes. You have found a rare autosomal recessive disease that is lethal in old age, and is prevalent in a primate family living in the wild. The mother (Individual 2) has already died from this disease. You want to find the genetic locus responsible for this disease, and decide to use SSR mapping to do so. Your first step is to determine which chromosome the locus responsible for the disease is located on. You have access to blood samples of all living members of the family, and you use these blood samples to genotype each living member of the family at four SSRs:

SSR12, on chromosome 1
SSR13, on chromosome 2
SSR14, on chromosome 3
SSR17, on chromosome 4

The pedigree of the primate family, and the SSRs possessed by each family member, are shown in the chart below. Assume complete penetrance and no new mutations.



SSR													
12	AB	AA	AA	AB	BB	AB	AA	BB	AA	BB	AB		AB
13	BC	AC	BB	BB	AC	AC	BB	AC	BC	BB	AA		BB
14	AC	AB	BC	BC	AB	AB	AB	BC	BC	AB	BC		AB
17	AB	AB	AB	AB	AB	BB	BB	AB	BB	BB	AB		BC

Name: _____

(a, 4pts) Fill in the empty column of the chart, which indicates the deceased mother's genotypes at each of the four SSRs. Indicate any ambiguous alleles with a question-mark (**?**).

(b, 4pts) If you want to determine the LOD score for this family for the locus responsible for the disease and one SSR, which parent(s) would be relevant (Individual One, Individual Two, or both)?

(c, 4pts) Do you know the phase of the parent(s) you listed in part **(b)**?

For parts **(d)** and **(e)**, calculate the LOD score at $\theta = 0.1$ for this family for the locus responsible for the disease and each of the following SSRs. For each LOD score, clearly write the expression you used to calculate the LOD score.

(d, 7pts) the locus responsible for the disease and SSR12.

(e, 7pts) the locus responsible for the disease and SSR13.

Name: _____

3. (20 pts) Hemophilia is an inherited bleeding disorder. People with hemophilia lack the ability to clot because of an absence in their blood of clotting factors, which are proteins necessary for clotting. There are several types of hemophilia; one common form is hemophilia A. Hemophilia A is an X-linked recessive disorder possessed by people who lack any functional clotting factor VIII. You want to create a genetically engineered mouse model for hemophilia A. Mice have a homolog of the human gene encoding clotting factor VIII; the mouse homolog also lies on the X chromosome. You are interested to see whether a mouse lacking functional clotting factor VIII will show the same phenotype as a human lacking functional clotting factor VIII.

(a, 8pts) You want to create a genetically engineered mouse model for hemophilia A. **For the mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) what is the **genotype** of the fertilized egg or the ES cells you would start with
iv) which **additional breeding** steps you would do to make the mouse you wanted
v) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusion you would make for each result

i)

ii)

iii)

iv)

v)

Name: _____

(b, 4pts) You find that you are successful in creating a mouse model of hemophilia A. However, you are unable to keep any of the mice with hemophilia A alive, because even the smallest movement gives them injuries that are lethal. How are you going to maintain your genetically engineered strain of mice, given that mice with the disease have an S equal to 100%?

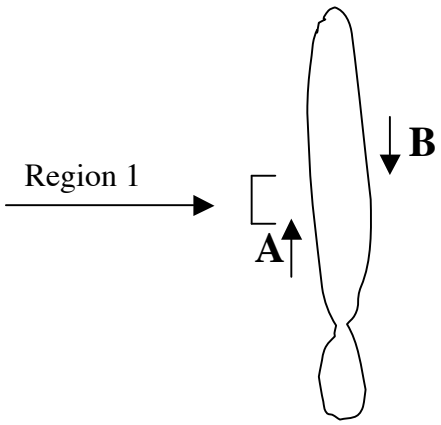
(c, 4pts) Which aspect of the creation of your strain of genetically engineered mice would demonstrate that the gene for clotting factor VIII is "haplosufficient" (i.e. NOT haplo**in**sufficient)? Explain your answer in one sentence.

(d, 4pts) You want to test whether clotting factor VIII, which is mutated in people with hemophilia A, physically interacts with clotting factor IX, which is mutated in people with hemophilia B. Which technique that we have discussed would you use to test this?

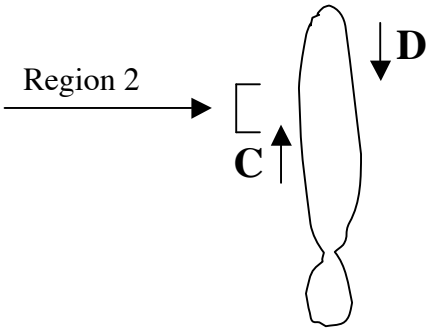
Name: _____

4. (24 pts) You are a human geneticist studying cancer. You have four cell types that have been derived from four different tumors (each from a different patient with a different type of cancer). You have designed a PCR-based assay to detect large chromosomal abnormalities such as deletions, duplications, inversions, and translocations. It turns out that each of your cell types has a different one of these abnormalities affecting either one or both of the following chromosomal regions (Regions 1 & 2). In each cell type, this chromosomal abnormality contributes to the development of the cancer in these cells. In the diagram below, the small arrows indicate PCR primers you will be using in your assay. Note that Regions 1 and 2 are not the same size (i.e. they are not drawn to scale in the drawing).

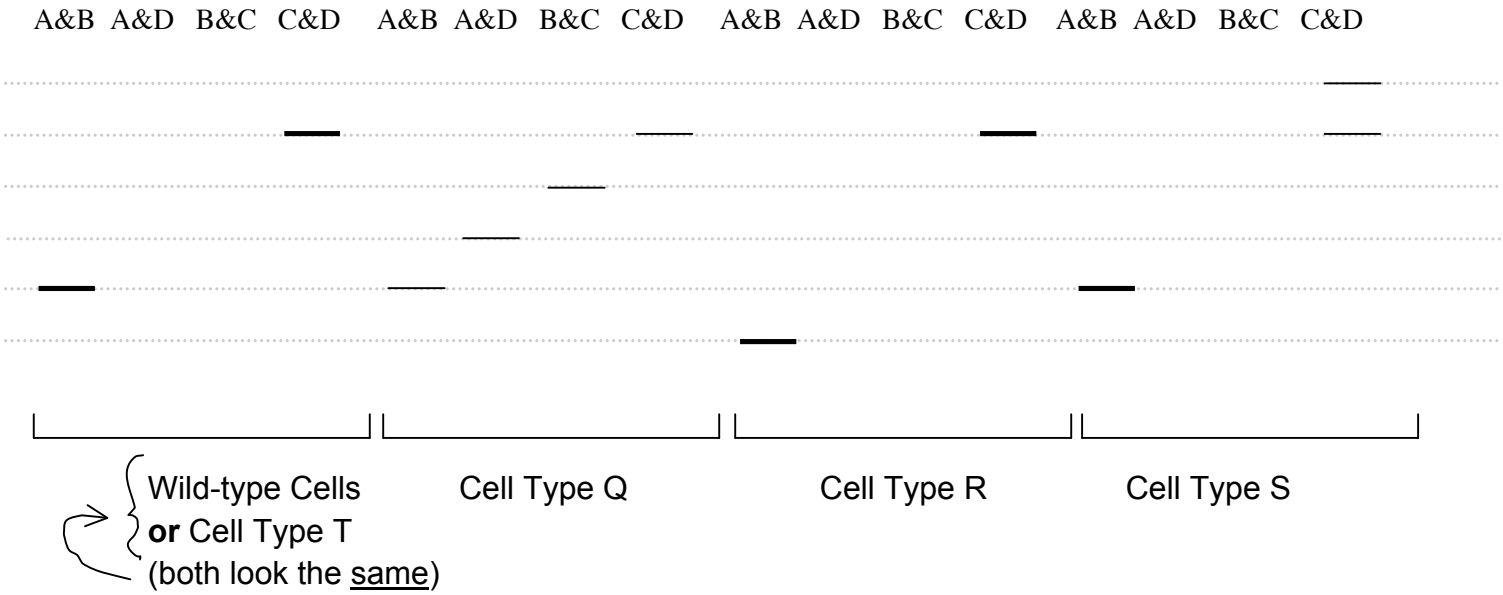
Schematic of Chromosome 3



Schematic of Chromosome 7



You do PCR using four different pairs of primers (in four separate reactions) on each of the four cell lines, and wild-type cells. The primers used are listed at the top of each lane in the gel.



Name: _____

(a, 10pts) State which type of chromosomal abnormality is present in each cell type, and whether you think it is present in a heterozygous or homozygous state. If you cannot conclude, write "*inconclusive*."

	Type of rearrangement	**Heterozygous or homozygous**
Cell Type Q		
Cell Type R		
Cell Type S		
Cell Type T		

(b, 3pts) Do you think that Cell Type R's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

(c, 3pts) Do you think that Cell Type S's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

Name: _____

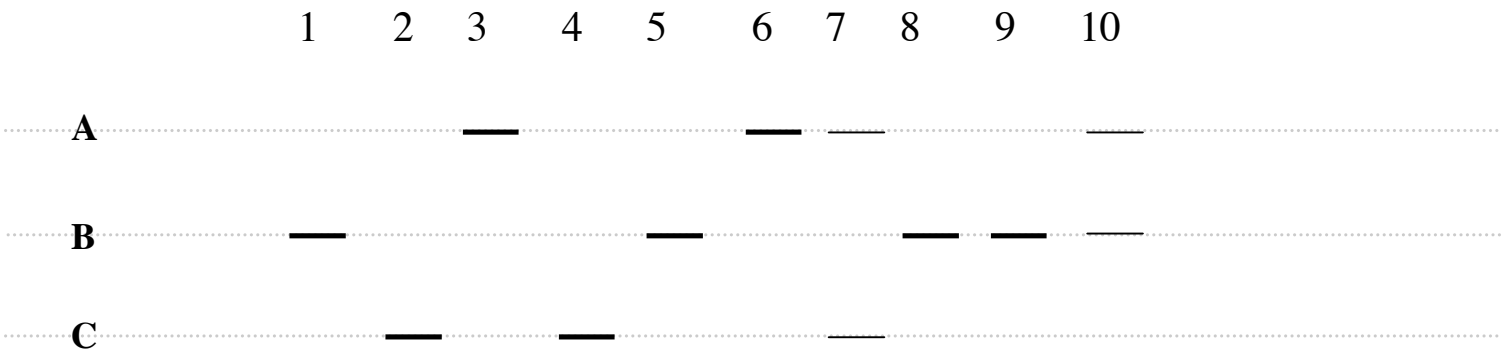
(d, 8pts) The general type of chromosomal abnormality found in Cell Type Q can cause cancer by either affecting an oncogene or a tumor suppressor gene. Give an example of how the general type of chromosomal abnormality found in Cell Type Q could affect each type of gene, and thereby lead to cancer.

Oncogene:

Tumor Suppressor Gene:

5. (24 pts) There are 10 people living on an island. You take their blood samples and genotype them for a specific autosomal SSR that is in a non-coding region of the genome, and that has no functional effect. During your studies, assume that no mutations occur at this SSR locus, and that no people move to or leave the island. Below is a gel indicating the results of your genotypic analysis.

Individuals from Generation One:

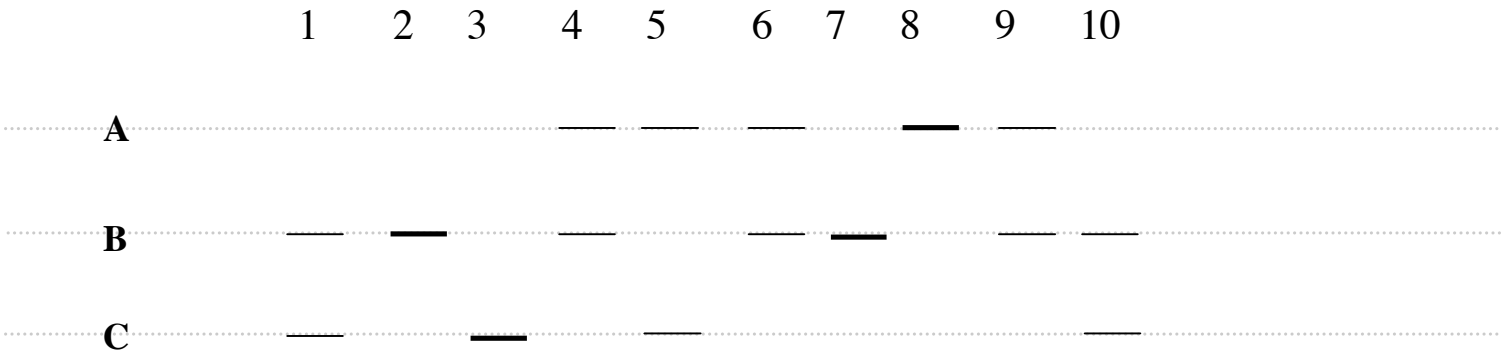


Name: _____

(a, 6pts) What are the frequencies of each possible genotype in Generation One?

The ten individuals then split into 5 couples randomly, and each have two children to create a second generation of ten people. Below is a gel indicating the results of your genotypic analysis on this next generation.

Individuals from Generation Two:



(b, 6pts) What are the frequencies of each allele in Generation Two?

(c, 4pts) What fraction of all **B** alleles are found in heterozygotes in Generation Two?

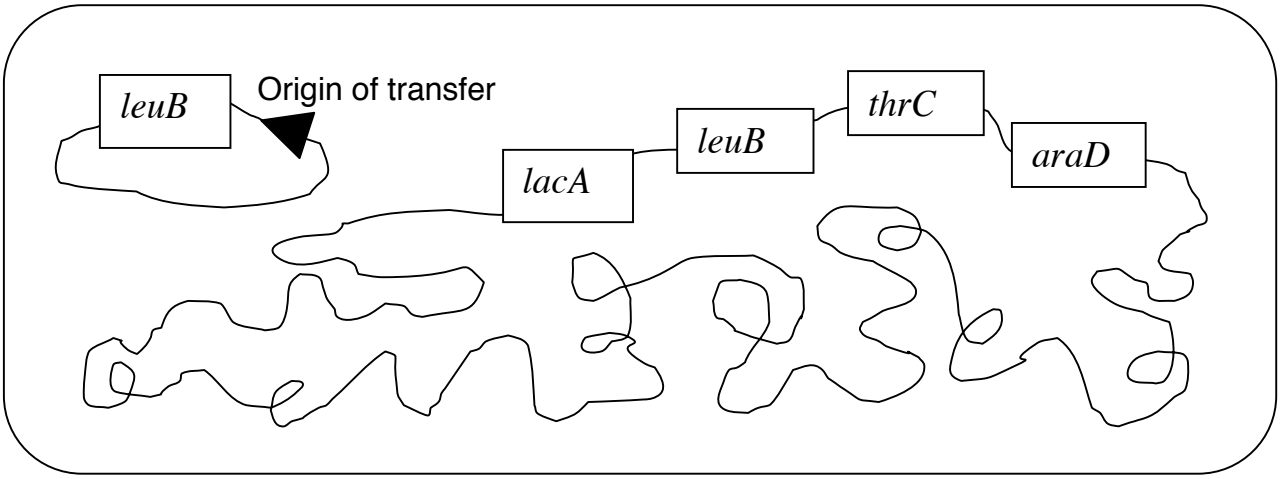
Name: _____

(d, 8pts) Does Generation Two have genotypic frequencies that are consistent with the island population being at Hardy-Weinberg equilibrium? Use chi-square analysis to support your answer. For the chi square test you do, give the observed and expected **number** of individuals, the degrees of freedom, and your calculated value for χ^2. Finally, give your conclusion given the test results.

p value:	.995	.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
df = 1	.000	.000	.016	.46	2.7	3.8	5.0	6.6	7.9
df = 2	.01	.05	.21	1.4	4.6	6.0	7.4	9.2	10.6
df = 3	.07	.22	.58	2.4	6.3	7.8	9.3	11.3	12.8

Name: _____

6. (22 pts) You are studying a merodiploid strain of *E. coli* that contains a form of the F factor. Below is a diagram of all of the genetic material in the strain. The size of the chromosome is about 50 times larger than that of the extrachromosomal element.



(a, 2pts) Is the strain drawn above an F+ strain, an Hfr strain, an F' strain, or an F⁻ strain?

(b, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain drawn above.

Gene Name	How transferred?
lacA	
leuB	
thrC	
araD	

(c, 2pts) Now assume that a single homologous recombination event occurs between the extrachromosomal element and the chromosome. Is the resulting strain an F+ strain, an Hfr strain, an F' strain, or an F⁻ strain?

Name: _____

(d, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain discussed in part **(c)**.

Gene Name	How transferred?
lacA	
leuB	
thrC	
araD	

(e, 10pts) Circle all of the following methods that could be used to make a merodiploid bacterial strain if you started with a strain that is an F^- strain. The possibilities are:

mating it with an Hfr strain

mating it with an F+ strain

mating it with an F' strain

transducing it with wild-type P1 phage

infecting it with wild-type phage lambda

transforming it with a wild-type R factor

7. (34 pts) Below is a segment of the messenger RNA produced from the *C. elegans* wild-type **lin-14** gene. This segment (shown below) is perfectly complementary to a segment of the microRNA produced from the wild-type *lin-4* gene.

wild-type **lin-14** mRNA = 5'–…CUCAGGGAAC…–3'

(a, 2pts) Write out as much of the sequence of the *lin-4* RNA as you can predict, using the format of the drawing above.

Name: _____

(b, 4pts) Write out as much of the sequence of the double-stranded *lin-4* gene as you can predict, using the format of the drawing above. Clearly label the strand that is used as a template during transcription.

You isolate a point mutation in **lin-14** that causes increased function of **lin-14**. The phenotype of a **lin-14** (gf) worm is that the L1 stage is repeated over and over again during development. The sequence of the gain-of-function mutant **lin-14** RNA is:

mutant **lin-14** (gf) mRNA = 5'–…CUCAAGGAAC…–3'

You do a genetic screen in order to isolate suppressor mutations that return development back to wild-type in **lin-14** (gf) worms. You isolate a single strain (which is homozygous for both **lin-14** (gf) and a suppressor mutation "sup*****") in your screen. This strain contains an extragenic suppressor mutation that is a point mutation.

(c, 4pts) Write out exactly which sequence change has been acquired by your suppressed strain, and state where the sequence change is located in the genome.

(d, 8pts) You make the following strains of *C. elegans*. Which developmental phenotype (mutant or wild-type) would these strains show? If mutant, list the exact mutant phenotype you would see. (In this chart, "wt" = wild-type.)

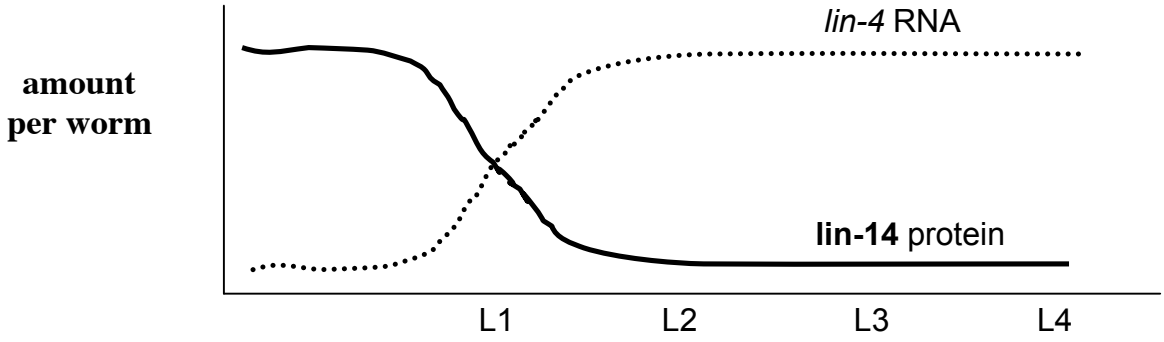
Strain Genotype	Developmental Phenotype
lin-14 (gf) **/ lin-14** (gf) sup***** / wt	
lin-14+ **/ lin-14**+ sup***** / sup*****	
lin-14+ **/ lin-14**+ sup***** / wt	

Name: _____

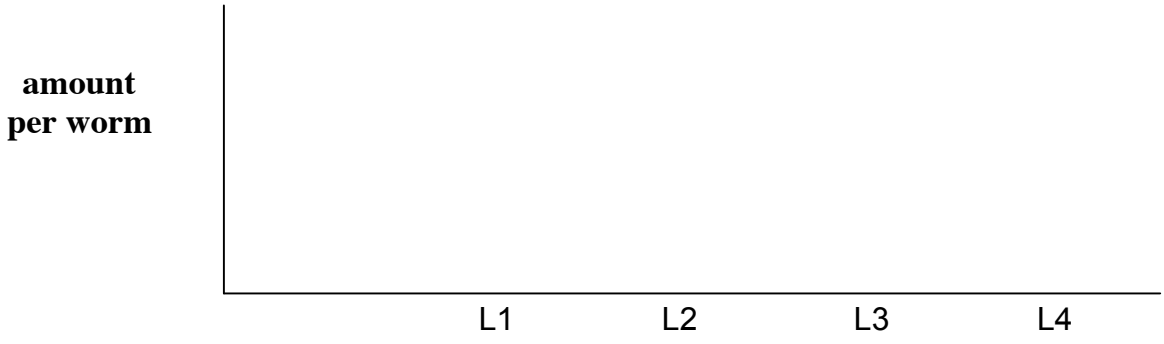
(e, 3pts) You do an experiment to test the levels of the **lin-14** RNA throughout the L1, L2, L3, and L4 larval stages. Which technique would you use to do this experiment?

(f, 2pts) Write the sequence of the probe you would use in the experiment from **(e)**.

(g, 3pts) Your experiment tells you that **lin-14** expression is **not** regulated at the transcriptional level. In class, the following slide was shown that denotes levels of the *lin-4* RNA and the **lin-14** protein during the four developmental stages of the worm. Add one line to the diagram that shows **lin-14** RNA levels during the development of a wild-type worm.



(h, 8pts) Below, draw in the *lin-4* RNA levels, **lin-14** protein levels, and **lin-14** RNA levels that would exist in your original single mutant worm with the **lin-14** (gf) mutation in it.



Name: _____

8. (26 pts) You have discovered a new restriction-modification gene pair in a bacterial species. One gene of the pair, rstR, encodes a restriction enzyme, and the other gene (which is adjacent to rstR in the genome), rstM, encodes a modification enzyme. You have two mutant strains of bacteria. Strain One is an rstR$^-$ strain that contains an ochre mutation early in the rstR coding sequence. Strain Two is an rstM$^-$ strain that contains an amber mutation early in the rstM coding sequence. Strain Two also contains: 1) a nonsense-suppressing allele of a tRNA gene that is unlinked to the rstM locus, **and** 2) a Tn5 KanR transposon linked to the rstM locus with a cotransduction frequency of 80%.

(a, 6pts) Strain Two actually **must** contain a nonsense-suppressing allele of a tRNA gene. Why do you think that is?

(b, 3pts) Give your best estimate of the distance between the transposon insertion and rstR (expressed as a cotransduction frequency).

(c, 3pts) Give your best estimate of the distance between rstM and rstR (expressed as a cotransduction frequency).

(d, 3pts) Do you think that rstM is cis-acting or trans-acting?

Name: _____

(e, 5pts) You want to determine the relative order of the transposon insertion, the rstM locus, and the rstR locus. You grow P1 phage on Strain Two and use the resulting phage lysate to infect Strain One. You select for transductants using kanamycin, and obtain 20 transductants that can grow. Estimate the number of KanR transductants that would be of each of the following genotypic classes.

Genotype	**Number**
rstM+ rstR+	
rstM$^-$ rstR$^-$	
rstM$^-$ rstR+	
rstM+ rstR$^-$	

(f, 6pts) Draw all of the possibilities for a map of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the whole chromosome, and the positions and relative order of the Tn insertion, the rstM locus, the tRNA gene, and the rstR locus.

Name: _____KEY_____

7.03 Final Exam -- 2005 KEY

Name: _____KEY_____

The exam starts at 9 am and ends at 12 pm.

There are 18 pages including this cover page.

Please write your name on each page.

Only writing on the front of every page will be graded.

Question 1	**24 pts_____**
Question 2	**26 pts_____**
Question 3	**20 pts_____**
Question 4	**24 pts_____**
Question 5	**24 pts_____**
Question 6	**22 pts_____**
Question 7	**34 pts_____**
Question 8	**26 pts_____**

TOTAL out of 200_____

Name: _____KEY_____

1. (24 pts) You are studying three autosomal mutations in flies. Each of these three mutations lies in a different gene. All three genes lie on the same autosome. The wn1⁻ mutation is recessive and causes the phenotype of short wings (wild-type flies have long wings). The wn2⁻ mutation is recessive and also causes the phenotype of short wings. The ey⁻ mutation is dominant and causes the phenotype of small eyes (wild-type flies have big eyes). You cross true-breeding wn1⁻ wn2⁻ short-winged females to true-breeding ey⁻ males to obtain an F1 generation. You then cross female F1 flies to true-breeding wn1⁻ wn2⁻ big-eyed males. You analyze the resulting progeny, and find that there are flies in the progeny from all four phenotypic classes:

Short wings Small eyes
Long wings Small eyes
Short wings Large eyes
Long wings Large eyes

For parts **(a) – (c)**, write out complete genotype(s) and phenotype(s) of the flies we ask for. By complete genotype, we mean the genotype at all loci discussed in the problem. By complete phenotype, we mean the phenotype at all traits discussed in the problem. If there are multiple answers, write ALL POSSIBLE answers.
Use "**+**" to indicate wild-type alleles.

(a, 6pts) Write out complete genotype(s) and phenotype(s) of both parents.

	Phenotype	**Genotype**
P generation mother	**Short wings, big eyes**	**wn1⁻ wn2⁻ ey+** **wn1⁻ wn2⁻ ey+**
P generation father	**Long wings, small eyes**	**wn1+ wn2+ ey⁻** **wn1+ wn2+ ey=**

(b, 6pts) Write out complete genotype(s) and phenotype(s) of F1 flies.

	Phenotype	**Genotype**
F1 generation (mother of F2)	**Long wings, small eyes**	**wn1+ wn2+ ey⁻** **wn1⁻ wn2⁻ ey+**
Father to whom you cross the F1 mother	**Short wings, big eyes**	**wn1⁻ wn2⁻ ey+** **wn1⁻ wn2⁻ ey+**

Name: _____KEY_____

(c, 8pts) Write out complete genotype(s) of the different F2 flies.

Phenotype	Genotype
Short wings Small eyes	$\frac{wn1^-\ wn2^-\ ey^-}{wn1^-\ wn2^-\ ey+}$ **OR** $\frac{wn1^-\ wn2+\ ey^-}{wn1^-\ wn2^-\ ey+}$ **OR** $\frac{wn1+\ wn2^-\ ey^-}{wn1^-\ wn2^-\ ey+}$
Long wings Small eyes	$\frac{wn1+\ wn2+\ ey^-}{wn1^-\ wn2^-\ ey+}$
Short wings Large eyes	$\frac{wn1^-\ wn2^-\ ey+}{wn1^-\ wn2^-\ ey+}$ **OR** $\frac{wn1^-\ wn2+\ ey+}{wn1^-\ wn2^-\ ey+}$ **OR** $\frac{wn1+\ wn2^-\ ey+}{wn1^-\ wn2^-\ ey+}$
Long wings Large eyes	$\frac{wn1+\ wn2+\ ey+}{wn1^-\ wn2^-\ ey+}$

(d, 4pts) Remember from class that, oddly enough, male flies do not undergo recombination during meiosis. You cross true-breeding wn1⁻ wn2⁻ short-winged females to true-breeding ey⁻ males to obtain an F1 generation. You then cross male F1 flies to true-breeding wn1⁻ wn2⁻ big-eyed females. If you analyze 2000 resulting progeny, predict the number of the following kinds of flies that you will get:

Phenotype		Number of flies
Short wings	Small eyes	**0**
Long wings	Small eyes	**1000**
Short wings	Large eyes	**1000**
Long wings	Large eyes	**0**

You cross an F1 male $\frac{wn1+\ wn2+\ ey^-}{wn1^-\ wn2^-\ ey+}$ **to a female** $\frac{wn1^-\ wn2^-\ ey^+}{wn1^-\ wn2^-\ ey+}$

There are no recombinants, so the male either gives the top chromosome or the bottom chromosome, and the female always gives wn1⁻ wn2⁻ ey+. Thus all F2 flies are either

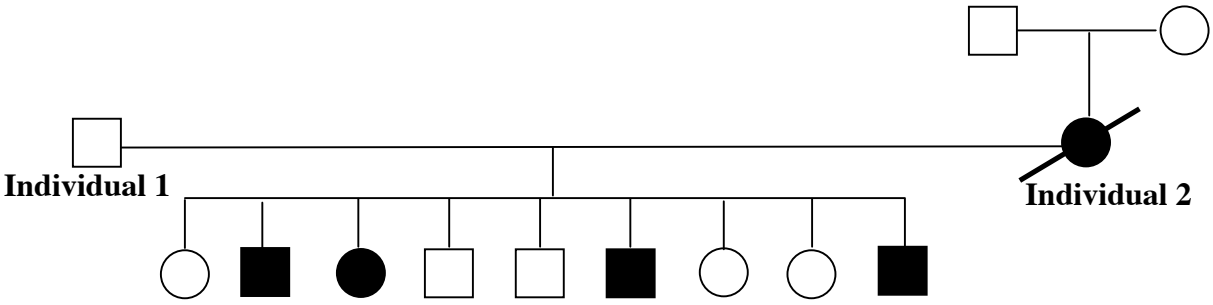
$\frac{wn1+\ wn2+\ ey^-}{wn1^-\ wn2^-\ ey+}$ **OR** $\frac{wn1^-\ wn2^-\ ey^+}{wn1^-\ wn2^-\ ey+}$

Name: _____KEY_____

2. (26 pts) You are studying a new species of primate that is diploid, and has four pairs of autosomes. You have found a rare autosomal recessive disease that is lethal in old age, and is prevalent in a primate family living in the wild. The mother (Individual 2) has already died from this disease. You want to find the genetic locus responsible for this disease, and decide to use SSR mapping to do so. Your first step is to determine which chromosome the locus responsible for the disease is located on. You have access to blood samples of all living members of the family, and you use these blood samples to genotype each living member of the family at four SSRs:

SSR12, on chromosome 1
SSR13, on chromosome 2
SSR14, on chromosome 3
SSR17, on chromosome 4

The pedigree of the primate family, and the SSRs possessed by each family member, are shown in the chart below. Assume complete penetrance and no new mutations.



SSR													
12	AB	AA	AA	AB	BB	AB	AA	BB	AA	BB	AB	**AB**	AB
13	BC	AC	BB	BB	AC	AC	BB	AC	BC	BB	AA	**AB**	BB
14	AC	AB	BC	BC	AB	AB	AB	BC	BC	AB	BC	**B?**	AB
17	AB	AB	AB	AB	AB	BB	BB	AB	BB	BB	AB	**B?**	BC

Name: _____KEY_____

(a, 4pts) Fill in the empty column of the chart, which indicates the deceased mother's genotypes at each of the four SSRs. Indicate any ambiguous alleles with a question-mark (**?**).

(b, 4pts) If you want to determine the LOD score for this family for the locus responsible for the disease and one SSR, which parent(s) would be relevant (Individual One, Individual Two, or both)?

Individual One, because he is heterozygous at all loci involved.

(c, 4pts) Do you know the phase of the parent(s) you listed in part **(b)**?

No, because you do not know his parents.

For parts **(d)** and **(e)**, calculate the LOD score at $\theta = 0.1$ for this family for the locus responsible for the disease and each of the following SSRs. For each LOD score, clearly write the expression you used to calculate the LOD score.

(d, 7pts) the locus responsible for the disease and SSR12.

$$\log \frac{(1/2)\,(0.45)^3\,(0.05)^4 + (1/2)\,(0.05)^3\,(0.45)^4}{(0.25)^7} = -1.33$$

Since you do not know the genotypes of the parents of Individual One, there are two possible phases for Individual One. We will call + the allele that does not confer the diease, and – the allele that does confer the disease.

Phase One	Phase Two
–B from father = parental	**–B from father = recombinant**
+A from father = parental	**+A from father = recombinant**
–A from father = recombinant	**–A from father = parental**
+B from father = recombinant	**+B from father = parental**

The first child received +A
The second child received –A
The third child received – and ???
The fourth child received +B
The fifth child received + and ???
The sixth child received –A
The seventh child received +B
The eighth child received +A
The ninth child received –B

Name: _____KEY_____

****You cannot tell whether children three and five are recombinants or parentals, because you cannot tell which allele was given to these children by Individual One. Thus these meioses are not informative, and cannot be included in the analysis.****

For phase one, there are three parental children and four recombinant children.
For phase two, there are four parental children and three recombinant children.

Since theta = 0.1, this means you'd expect 10% recombinants and 90% parentals.
The chances of Individual One creating each type of gamete are:

Phase One	Phase Two
–B from father = 45%	**–B from father = 5%**
+A from father = 45%	**+A from father = 5%**
–A from father = 5%	**–A from father = 45%**
+B from father = 5%	**+B from father = 45%**

For the denominator of the odds ratio, you assume that the SSR and the disease locus are NOT linked. UN-linkage corresponds to theta = 0.5, because UN-linkage gives 50% parentals and 50% recombinants.

The LOD score is the log of the ratio of the odds (linkage over unlinkage):

$$\log \frac{(1/2)\,(0.45)^3\,(0.05)^4 + (1/2)\,(0.05)^3\,(0.45)^4}{(0.25)^7}$$

(e, 7pts) the locus responsible for the disease and SSR13.

$$\log \frac{(1/2)\,(0.45)^9\,(0.05)^0 + (1/2)\,(0.05)^9\,(0.45)^0}{(0.25)^9}$$

Since you do not know the genotypes of the parents of Individual One, there are two possible phases for Individual One. We will call + the allele that does not confer the diease, and – the allele that does confer the disease.

Phase One	Phase Two
–B from father = parental	**–B from father = recombinant**
+C from father = parental	**+C from father = recombinant**
–C from father = recombinant	**–C from father = parental**
+B from father = recombinant	**+B from father = parental**

Name: _____KEY_____

The first child received +C
The second child received –B
The third child received –B
The fourth child received +C
The fifth child received +C
The sixth child received –B
The seventh child received +C
The eighth child received +C
The ninth child received –B

For phase one, there are nine parental children.
For phase two, there are nine recombinant children.

Since theta = 0.1, this means you'd expect 10% recombinants and 90% parentals.
The chances of Individual One creating each type of gamete are:

Phase One	Phase Two
–B from father = 45%	**–B from father = 5%**
+C from father = 45%	**+C from father = 5%**
–C from father = 5%	**–C from father = 45%**
+B from father = 5%	**+B from father = 45%**

For the denominator of the odds ratio, you assume that the SSR and the disease locus are NOT linked. UN-linkage corresponds to theta = 0.5, because UN-linkage gives 50% parentals and 50% recombinants.

The LOD score is the log of the ratio of the odds (linkage over unlinkage):

$$\log \frac{(1/2)\,(0.45)^9\,(0.05)^0 + (1/2)\,(0.05)^9\,(0.45)^0}{(0.25)^9}$$

3. (20 pts) Hemophilia is an inherited bleeding disorder. People with hemophilia lack the ability to clot because of an absence in their blood of clotting factors, which are proteins necessary for clotting. There are several types of hemophilia; one common form is hemophilia A. Hemophilia A is an X-linked recessive disorder possessed by people who lack any functional clotting factor VIII. You want to create a genetically engineered mouse model for hemophilia A. Mice have a homolog of the human gene encoding clotting factor VIII; the mouse homolog also lies on the X chromosome. You are interested to see whether a mouse lacking functional clotting factor VIII will show the same phenotype as a human lacking functional clotting factor VIII.

Name: _____KEY_____

(a, 8pts) You want to create a genetically engineered mouse model for hemophilia A. **For the mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) what is the **genotype** of the fertilized egg or the ES cells you would start with
iv) which **additional breeding** steps you would do to make the mouse you wanted
v) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusion you would make for each result

i) gene targeting, because you are trying to remove information from the genome

ii) the gene that encodes clotting factor VIII, but with a drug resistance gene inserted in the middle of it

iii) wild-type ES cells

iv) The mouse that would be born would be a heterozygous chimera. You should take this mouse and breed it to wild-type mice to generate some heterozygous females that are not chimeras. You can then mate this heterozygous female to a wild-type male, and half of her sons would be the mice that you want.

v) If half of the sons of a heterozygous female are hemophiliacs, then lacking clotting factor VIII has the same phenotype in mice as it does in humans. If all of the sons of a heterozygous female are able to clot, then lacking clotting factor VIII plays a different role in mice than it does in humans.

(b, 4pts) You find that you are successful in creating a mouse model of hemophilia A. However, you are unable to keep any of the mice with hemophilia A alive, because even the smallest movement gives them injuries that are lethal. How are you going to maintain your genetically engineered strain of mice, given that mice with the disease have an S equal to 100%?

You can maintain this strain because females are diploid for the X chromosome, so you can maintain heterozygous females (who will not have hemophilia), but any time you want to generate hemophiliacs, you can simply mate the heterozygous female to a wild-type male, and half of her sons will have hemophilia.

Name: _____KEY_____

(c, 4pts) Which aspect of the creation of your strain of genetically engineered mice would demonstrate that the gene for clotting factor VIII is "haplosufficient" (i.e. NOT haplo**in**sufficient)? Explain your answer in one sentence.

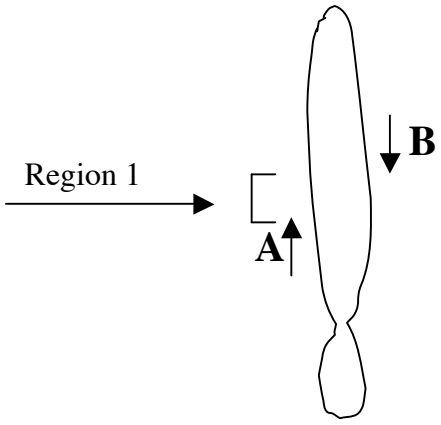
The fact that heterozygous females are not hemophiliacs means that this gene is haplosufficient. HaploINsufficiency refers to genes that are necessary in two functional copies in order to give a wild-type phenotype. Most genes are haplosufficient, so a deletion in one copy of the gene will not cause a phenotype.

(d, 4pts) You want to test whether clotting factor VIII, which is mutated in people with hemophilia A, physically interacts with clotting factor IX, which is mutated in people with hemophilia B. Which technique that we have discussed would you use to test this?

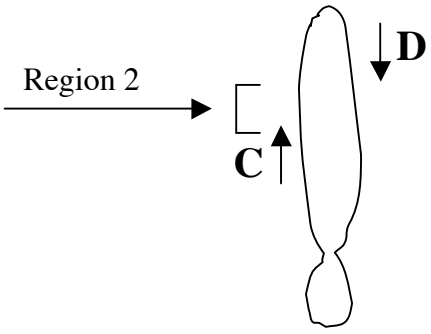
The two-hybrid assay. This technique allows you to test whether any two proteins of interest physically interact with one another.

4. (24 pts) You are a human geneticist studying cancer. You have four cell types that have been derived from four different tumors (each from a different patient with a different type of cancer). You have designed a PCR-based assay to detect large chromosomal abnormalities such as deletions, duplications, inversions, and translocations. It turns out that each of your cell types has a different one of these abnormalities affecting either one or both of the following chromosomal regions (Regions 1 & 2). In each cell type, this chromosomal abnormality contributes to the development of the cancer in these cells. In the diagram below, the small arrows indicate PCR primers you will be using in your assay. Note that Regions 1 and 2 are not the same size (i.e. they are not drawn to scale in the drawing).

Schematic of Chromosome 3

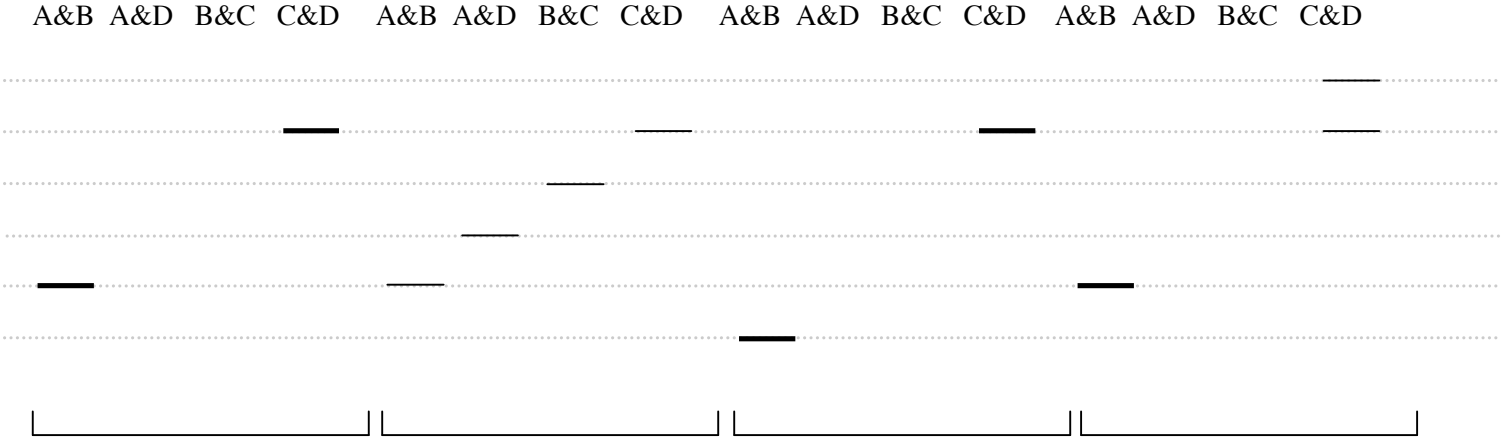


Schematic of Chromosome 7



Name: _____KEY_____

You do PCR using four different pairs of primers (in four separate reactions) on each of the four cell lines, and wild-type cells. The primers used are listed at the top of each lane in the gel.



Wild-type Cells **or** Cell Type T (both look the same)

Cell Type Q

Cell Type R

Cell Type S

(a, 10pts) State which type of chromosomal abnormality is present in each cell type, and whether you think it is present in a heterozygous or homozygous state. If you cannot conclude, write "*inconclusive*."

	Type of rearrangement	**Heterozygous or homozygous**
Cell Type Q	**Translocation**	**Heterozygous**
Cell Type R	**Deletion**	**Homozygous**
Cell Type S	**Duplication**	**Heterozygous**
Cell Type T	**Inversion**	**inconclusive**

Q is a translocation because both chromosomes (3 and 7) are affected. It is heterozygous because one band for chromosome 3 and one band for chromosome 7 show the wild-type patterns.
R is a deletion because one of the PCR products becomes smaller than it is supposed to be. It is homozygous because the organism's two homologous chromosomes both give the smaller sized fragment using primers A&B.

Name: _____KEY_____

S is a duplication because one of the PCR products becomes larger than it is supposed to be. It is heterozygous because one band in the last lane of the gel still shows the wild-type pattern, even though the other band in the last lane of the gel stays the correct size.
T is an inversion because an inversion within the boundaries set by the PCR primers will not affect the size of the PCR product. One cannot tell whether it is homozygous or heterozygous because the original chromosome and the inverted chromosome look the same in this PCR-based assay.

(b, 3pts) Do you think that Cell Type R's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

A tumor suppressor gene, because deletions remove information from the chromosome, so they are typically loss of function alleles. Loss of function alleles of tumor suppressor genes lead to cancer, but loss of function alleles of oncogenes do not lead to cancer.

(c, 3pts) Do you think that Cell Type S's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

An oncogene, because duplications add information to the genome, so they are typically gain of function or increased function alleles. Gain of function alleles of oncogenes lead to cancer, but gain of function alleles of tumor suppressor genes do not lead to cancer.

(d, 8pts) The general type of chromosomal abnormality found in Cell Type Q can cause cancer by either affecting an oncogene or a tumor suppressor gene. Give an example of how the general type of chromosomal abnormality found in Cell Type Q could affect each type of gene, and thereby lead to cancer.

Oncogene:

A translocation can lead to the placement of a strong promoter (normally in front of a different gene) in front of an oncogene. This increases expression of an oncogene, which leads to cancer.

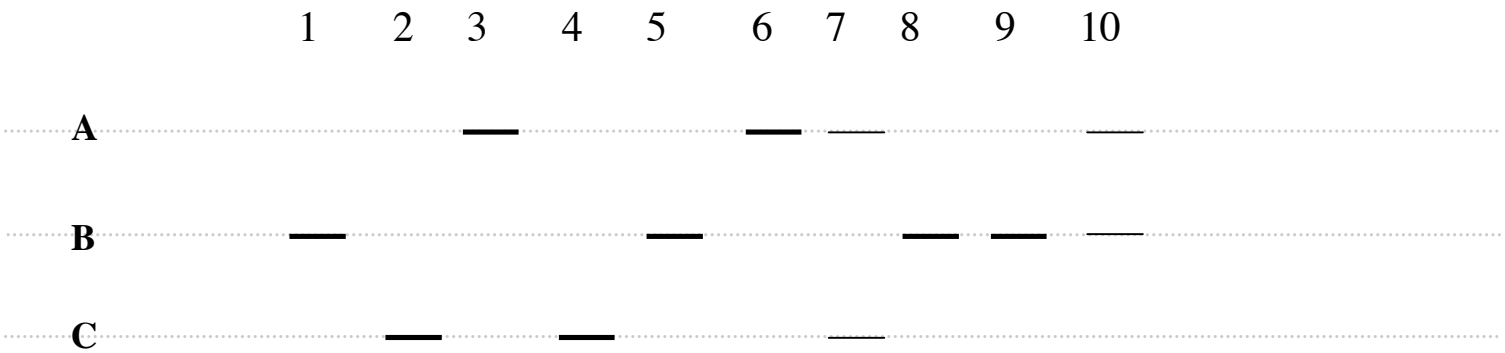
Tumor Suppressor Gene:

If the translocation breakpoint is within a tumor suppressor gene, that gene would be torn apart, and thus inactivated. If the translocation were homozygous, then both copies of the tumor suppressor gene would be inactivated, and this would lead to cancer.

Name: _____KEY_____

5. (24 pts) There are 10 people living on an island. You take their blood samples and genotype them for a specific autosomal SSR that is in a non-coding region of the genome, and that has no functional effect. During your studies, assume that no mutations occur at this SSR locus, and that no people move to or leave the island. Below is a gel indicating the results of your genotypic analysis.

Individuals from Generation One:

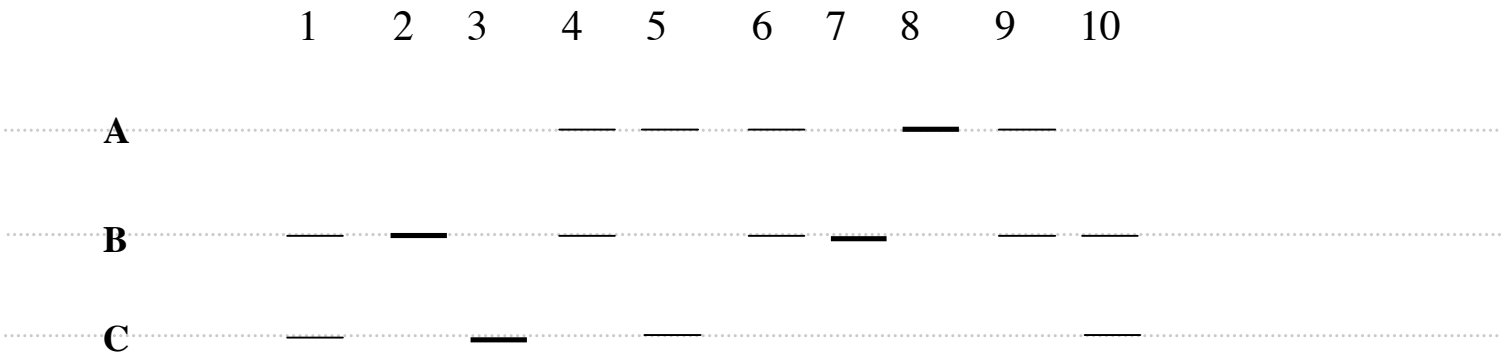


(a, 6pts) What are the frequencies of each possible genotype in Generation One?

f(AA) = 2/10
f(AB) = 1/10
f(AC) = 1/10
f(BC) = 0/10
f(BB) = 4/10
f(CC) = 2/10

The ten individuals then split into 5 couples randomly, and each have two children to create a second generation of ten people. Below is a gel indicating the results of your genotypic analysis on this next generation.

Individuals from Generation Two:



Name: _____KEY_____

(b, 6pts) What are the frequencies of each allele in Generation Two?

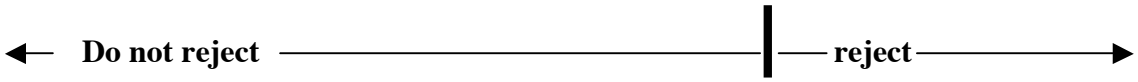
f(A) = 6/20
f(B) = 9/20
f(C) = 5/20

(c, 4pts) What fraction of all **B** alleles are found in heterozygotes in Generation Two?

5/9. There are 9 total B alleles in the population of 20 alleles, and 5 of those are found in heterozygotes.

(d, 8pts) Does Generation Two have genotypic frequencies that are consistent with the island population being at Hardy-Weinberg equilibrium? Use chi-square analysis to support your answer. For the chi square test you do, give the observed and expected **number** of individuals, the degrees of freedom, and your calculated value for χ^2. Finally, give your conclusion given the test results.

p value:	.995	.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
df = 1	.000	.000	.016	.46	2.7	3.8	5.0	6.6	7.9
df = 2	.01	.05	.21	1.4	4.6	6.0	7.4	9.2	10.6
df = 3	.07	.22	.58	2.4	6.3	7.8	9.3	11.3	12.8



Generation One and Two both have the same allele frequencies:
f(A) = 6/20 = p = 0.3
f(B) = 9/20 = q = 0.45
f(C) = 5/20 = r = 0.25

For Generation Two to be at Hardy-Weinberg equilibrium, it would have to have the observed numbers of individuals of each genotype that match the expected number of individuals of each genotype that are predicted by the equations:
f(AA) = p^2 = 0.3 * 0.3 = 0.09
f(AB) = 2pq = 2 * 0.3 * 0.45 = 0.27
f(AC) = 2pr = 2 * 0.3 * 0.25 = 0.15
f(BC) = 2qr = 2 * 0.45 * 0.25 = 0.225
f(BB) = q^2 = 0.45 * 0.45 = 0.2025
f(CC) = r^2 = 0.25 * 0.25 = 0.0625

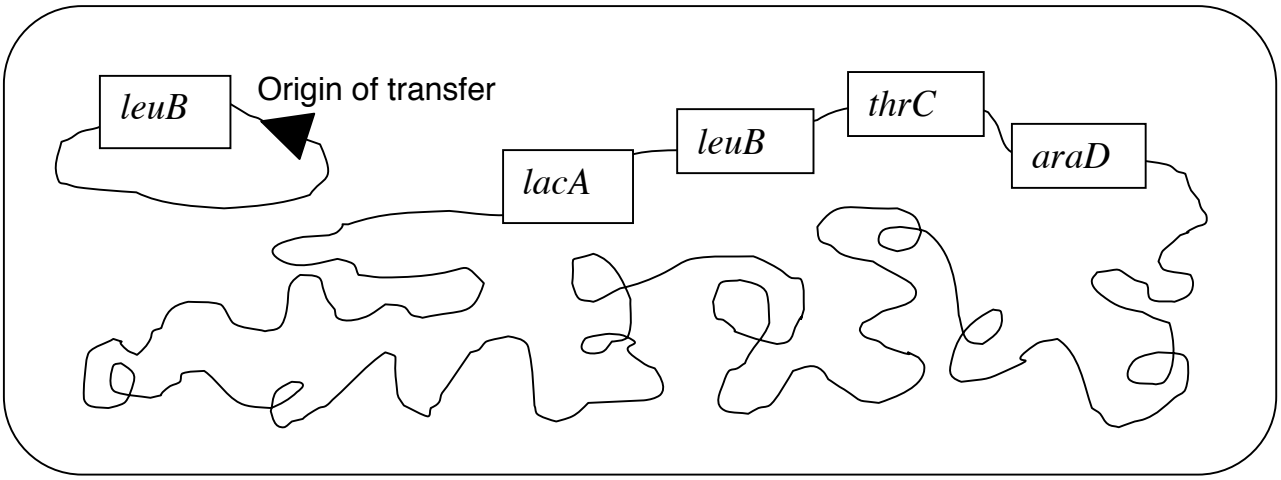
Name: _____KEY_____

Since there are 10 total individuals in Generation Two, you can multiply the frequency of each expected genotype by 10 to get the number of individuals of each expected genotype.

Genotype	Expected number	Observed Number	$\frac{(O-E)^2}{E}$
AA	**0.9**	**1**	**0.0111**
AB	**2.7**	**3**	**0.0333**
AC	**1.5**	**1**	**0.1666**
BC	**2.25**	**2**	**0.0277**
BB	**2.025**	**2**	**0.0003**
CC	**0.625**	**1**	**0.225**

To get the chi-squared value, you sum the numbers in the last column to get = 0.464.
The degrees of freedom = # classes – 1 = 5.
The chi-squared value you get is so small that, even if you had one df, you would still not be able to reject your hypothesis that Generation Two is at Hardy Weinberg equilibrium.

6. (22 pts) You are studying a merodiploid strain of *E. coli* that contains a form of the F factor. Below is a diagram of all of the genetic material in the strain. The size of the chromosome is about 50 times larger than that of the extrachromosomal element.



(a, 2pts) Is the strain drawn above an F+ strain, an Hfr strain, an F' strain, or an F⁻ strain?

An F' strain, because it contains a form of the F plasmid that harbors an extra gene that is normally found on the bacterial chromosome.

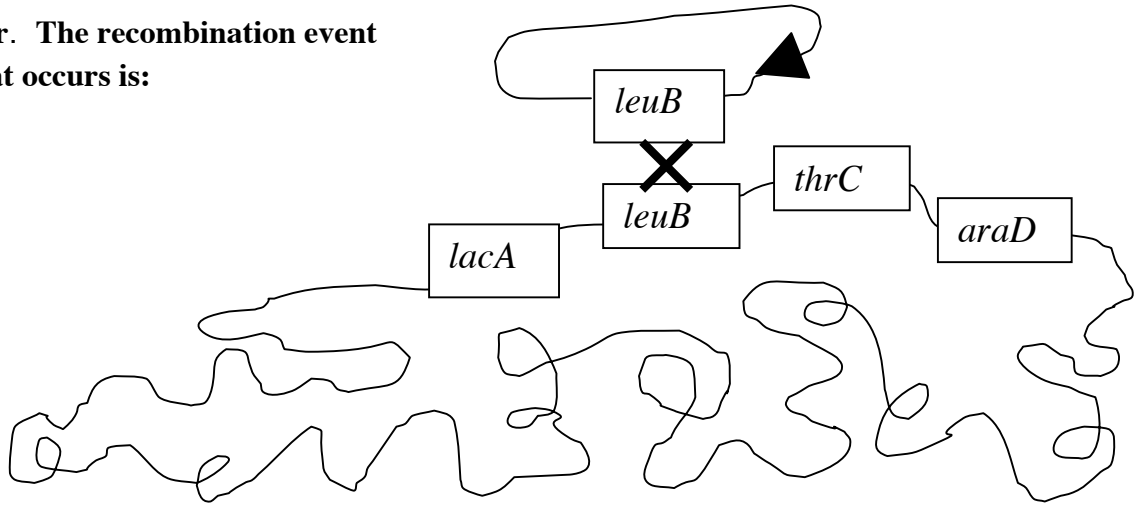
Name: _____KEY_____

(b, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain drawn above.

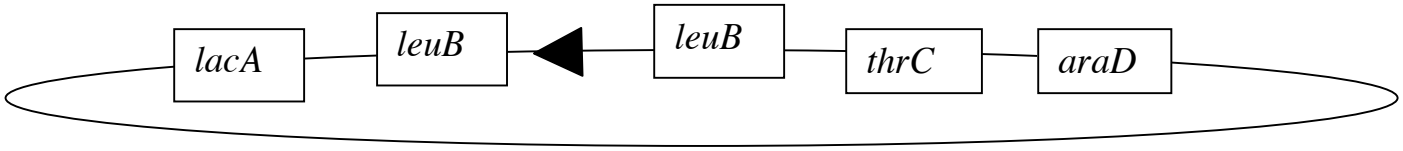
Gene Name	How transferred?
lacA	**Never (because the only copy of lacA is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)**
leuB	**Early (because a copy of leuB is on the very small F' plasmid that can be entirely transferred to a new cell very quickly and efficiently)**
thrC	**Never (because the only copy of thrC is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)**
araD	**Never (because the only copy of araD is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)**

(c, 2pts) Now assume that a single homologous recombination event occurs between the extrachromosomal element and the chromosome. Is the resulting strain an F+ strain, an Hfr strain, an F' strain, or an F– strain?

Hfr. The recombination event that occurs is:



And the product is:



This is an Hfr because the origin of transfer is now on the bacterial chromosome itself.

Name: _____KEY_____

(d, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain discussed in part **(c)**.

Gene Name	How transferred?
lacA	**Late, because, in the Hfr that is formed, lacA is the second to last gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.**
leuB	**Early, because, in the Hfr that is formed, leuB is the first gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.**
thrC	**Early, because, in the Hfr that is formed, thrC is the second gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.**
araD	**Early, because, in the Hfr that is formed, araD is the third gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.**

(e, 10pts) Circle all of the following methods that could be used to make a merodiploid bacterial strain if you started with a strain that is an F^- strain. The possibilities are:

mating it with an Hfr strain

mating it with an F+ strain

mating it with an F' strain

transducing it with wild-type P1 phage

infecting it with wild-type phage lambda

transforming it with a wild-type R factor

Mating it with an Hfr strain gives an F- strain.
Mating it with an F+ strain gives an F+ strain.
Mating it with an F' strain gives an F' strain, which is a merodiploid strain because the bacterial chromosome has one copy of every bacterial gene, and the F' plasmid has one copy of a few bacterial genes on it too. Thus the bacterial strain is diploid for any bacterial genes carried on the F' plasmid.
Transducing it with wild-type P1 phage leads to lysis.
Infecting it with wild-type phage lambda leads to lysis.
Transforming it with a wild-type R factor leads to the strain acquiring antibiotic resistance, but wild-type R factors do not contain any genes that are found on the unmodified bacterial chromosome, so the resulting strain is not a merodiploid.

Name: _____KEY_____

7. (34 pts) Below is a segment of the messenger RNA produced from the *C. elegans* wild-type **lin-14** gene. This segment (shown below) is perfectly complementary to a segment of the microRNA produced from the wild-type *lin-4* gene.

wild-type **lin-14** mRNA = 5'–…CUCAGGGAAC…–3'

(a, 2pts) Write out as much of the sequence of the *lin-4* RNA as you can predict, using the format of the drawing above.

***lin-4* RNA is a microRNA that is perfectly complementary to this region of the** lin-14 **mRNA. Thus the *lin-4* RNA sequence would be 5'- GUUCCCUGAG -3'**.

(b, 4pts) Write out as much of the sequence of the double-stranded *lin-4* gene as you can predict, using the format of the drawing above. Clearly label the strand that is used as a template during transcription.

The *lin-4* RNA sequence is 5'- GUUCCCUGAG -3' so the *lin-4* gene would be:
5′–…GTTCCCTGAG…–3′
3′—…CAAGGGACTC…—5′ and the bottom is the strand used as a template

You isolate a point mutation in **lin-14** that causes increased function of **lin-14**. The phenotype of a **lin-14** (gf) worm is that the L1 stage is repeated over and over again during development. The sequence of the gain-of-function mutant **lin-14** RNA is:

mutant **lin-14** (gf) mRNA = 5'–…CUCAAGGAAC…–3'

You do a genetic screen in order to isolate suppressor mutations that return development back to wild-type in **lin-14** (gf) worms. You isolate a single strain (which is homozygous for both **lin-14** (gf) and a suppressor mutation "sup*****") in your screen. This strain contains an extragenic suppressor mutation that is a point mutation.

(c, 4pts) Write out exactly which sequence change has been acquired by your suppressed strain, and state where the sequence change is located in the genome.

When the sequence of lin-14 **changes in the region that is complementary to *lin-4*,** lin-14 **becomes impervious to regulation by *lin-4*. This means that the levels of** lin-14 **protein will no longer drop when the levels of the *lin-4* microRNA begin to rise. A way to suppress this would be to mutate *lin-4* so that it regains perfect complementarity to** lin-14 **and thus regains the ability to regulate** lin-14**. Thus the change would be in the *lin-4* gene, such that it now has the sequence:**

Name: _____KEY_____

5′–…GTTCCTTGAG…–3′
3′—…CAAGGAACTC…—5′
This gene will produce a *lin-4* microRNA that can bind to and regulate the mutated lin-14 **that already exists in your original single mutant strain.**

(d, 8pts) You make the following strains of *C. elegans*. Which developmental phenotype (mutant or wild-type) would these strains show? If mutant, list the exact mutant phenotype you would see. (In this chart, "wt" = wild-type.)

Strain Genotype	Developmental Phenotype
lin-14 (gf) **/ lin-14** (gf) sup***** / wt	**Wild-type, because** lin-14 **is mutated so that it cannot be regulated by wild-type *lin-4*, but there is mutant *lin-4* present that has the ability to regulate the mutant** lin-14.
lin-14+ / lin-14+ sup***** / sup*****	**L1 stage repeated over and over, because** lin-14 **is wild-type so it can only be regulated by wild-type *lin-4*, but there is no wild-type *lin-4* present**. **Thus** lin-14 **has increased function, and the phenotype of worms with too much** lin-14 **function is that the L1 larval stage is repeated over and over.**
lin-14+ / lin-14+ sup***** / wt	**Wild-type, because** lin-14 **is wild-type so it can be regulated by wild-type *lin-4*, and there is wild-type *lin-4* present**.

(e, 3pts) You do an experiment to test the levels of the **lin-14** RNA throughout the L1, L2, L3, and L4 larval stages. Which technique would you use to do this experiment?

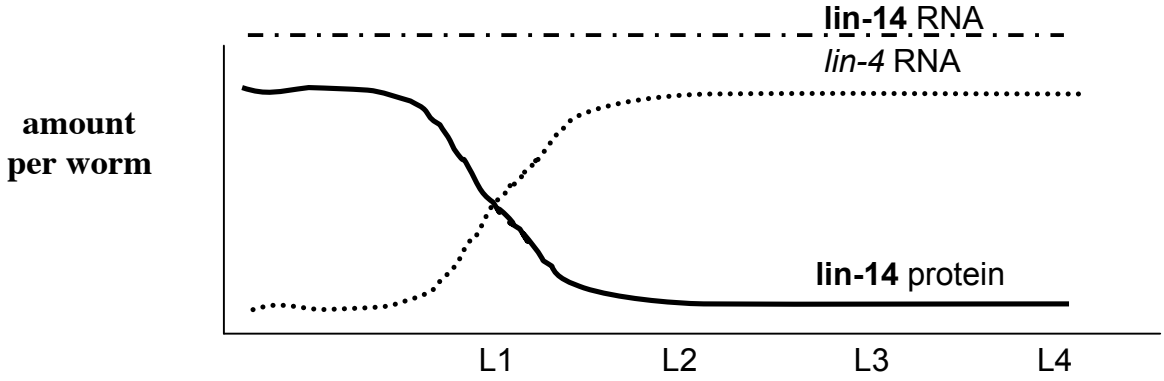
Northern blot. **You would isolate RNA from the four different larval stages, run them out on a denaturing agarose gel, transfer the RNA in the gel to a membrane, and probe the membrane with a probe that is complementary to the** lin-14 **RNA.**

(f, 2pts) Write the sequence of the probe you would use in the experiment from **(e)**.

5'- GUUCCCUGAG -3' or 5'- GTTCCCTGAG -3'. One could use a DNA or an RNA probe in a Northern. Your probe should be perfectly complementary to the lin-14 **mRNA whose levels you are measuring.**

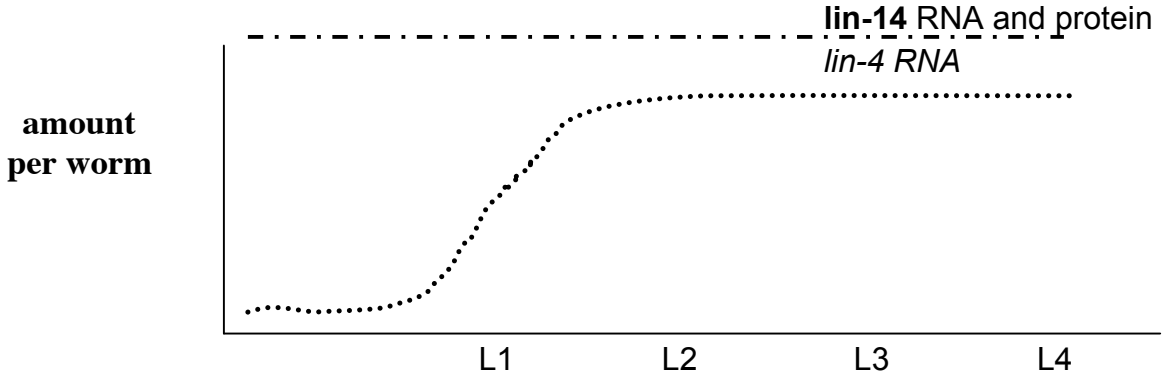
Name: _____KEY_____

(g, 3pts) Your experiment tells you that **lin-14** expression is **not** regulated at the transcriptional level. In class, the following slide was shown that denotes levels of the *lin-4* RNA and the **lin-14** protein during the four developmental stages of the worm. Add one line to the diagram that shows **lin-14** RNA levels during the development of a wild-type worm.



The lin-14 **gene is not regulated at the level of transcription, which means that a constant amount of RNA is being produced from this gene at all times.**

(h, 8pts) Below, draw in the *lin-4* RNA levels, **lin-14** protein levels, and **lin-14** RNA levels that would exist in your original single mutant worm with the **lin-14** (gf) mutation in it.



The lin-14 **gene is not regulated at the level of transcription, which means that a constant amount of RNA is being produced from this gene at all times. The production of** lin-14 **protein from** lin-14 **RNA is normally inhibited whenever *lin-4* RNA is being made. However the original single mutant strain has a mutation in** lin-14 **such that it becomes insensitive to regulation by *lin-4*. Thus the** lin-14 **protein will be unaffected by the absence or presence of *lin-4* RNA in this strain.**

Name: _____KEY_____

8. (26 pts) You have discovered a new restriction-modification gene pair in a bacterial species. One gene of the pair, rstR, encodes a restriction enzyme, and the other gene (which is adjacent to rstR in the genome), rstM, encodes a modification enzyme. You have two mutant strains of bacteria. Strain One is an rstR$^-$ strain that contains an ochre mutation early in the rstR coding sequence. Strain Two is an rstM$^-$ strain that contains an amber mutation early in the rstM coding sequence. Strain Two also contains: 1) a nonsense-suppressing allele of a tRNA gene that is unlinked to the rstM locus, **and** 2) a Tn5 KanR transposon linked to the rstM locus with a cotransduction frequency of 80%.

(a, 6pts) Strain Two actually **must** contain a nonsense-suppressing allele of a tRNA gene. Why do you think that is?

Strain Two has a genotype of rstM- rstR+. This strain produces functional restriction enzyme from the rstR+ allele, but non-functional methylase modifying enzyme from the rstM- allele. This strain's restriction enzyme would chew up its own DNA, thereby destroying itself, unless it produced functional methylase to methylate the DNA sites usually recognized by the restriction enzyme to prevent cutting. Thus the nonsense-suppressing allele of the tRNA gene must exist to suppress the inability of an rstM- rstR+ strain to produce functional methylase.

(b, 3pts) Give your best estimate of the distance between the transposon insertion and rstR (expressed as a cotransduction frequency).

80%. rstR and rstM are right next to each other in the genome, so the distance between the transposon and rstM should be the same as (or very close to) the distance between the transposon and rstR.

(c, 3pts) Give your best estimate of the distance between rstM and rstR (expressed as a cotransduction frequency).

1000%. rstR and rstM are right next to each other in the genome, so they should essentially always move together into a new cell by cotransduction.

(d, 3pts) Do you think that rstM is cis-acting or trans-acting?

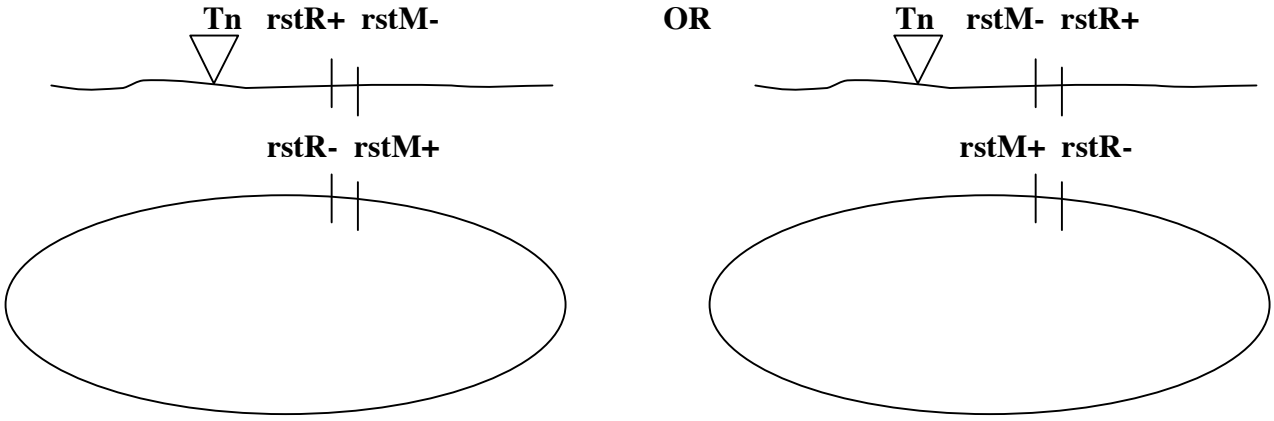
Trans-acting. rstM encodes a protein that can float around the cell and work on its substrate (DNA), which it methylates. Cis-acting sequences are DNA elements that do not encode free-floating gene products.

Name: _____KEY_____

(e, 5pts) You want to determine the relative order of the transposon insertion, the rstM locus, and the rstR locus. You grow P1 phage on Strain Two and use the resulting phage lysate to infect Strain One. You select for transductants using kanamycin, and obtain 20 transductants that can grow. Estimate the number of KanR transductants that would be of each of the following genotypic classes.

Genotype	Number
rstM+ rstR+	**0**
rstM$^-$ rstR$^-$	**0**
rstM$^-$ rstR+	**0**
rstM+ rstR$^-$	**20**

The transduction experiment that you are doing is pictured below:



Either way, rstR and rstM are so close together that it is unlikely to get a crossover in between them. The transposon and the rst genes will be cotransduced 80% of the time, and the other 20% of the time, the transposon will come in without the rst genes. Thus, for either experiment, 80% of the cotransductants will be rstR+ rstM-, and 20% will be rstR- and rstM+. However, rstR+ rstM- cells cannot live without a nonsense-suppressing allele of a tRNA gene. You are growing phage on the strain that has this nonsense-suppressing allele, but the tRNA gene is unlinked to the rst genes and thus will never come along into the new strain. Thus the rstR+ rstM- transductants will die (the restriction enzymes in these cells will chew up the genome of these cells), and you will be left with only rstR- rstM+ cells.

Name: _____KEY_____

(f, 6pts) Draw all of the possibilities for a map of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the whole chromosome, and the positions and relative order of the Tn insertion, the rstM locus, the tRNA gene, and the rstR locus.

You do not know the order of rstR and rstM, but you do know that they are right next to each other. You know that the transposon is linked to them at a distance of 80% cotransduction, and that the tRNA gene is unlinked to them. Thus there are two possible orders: