

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.

Only writing on the **FRONT** of every page will be graded.  
(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:

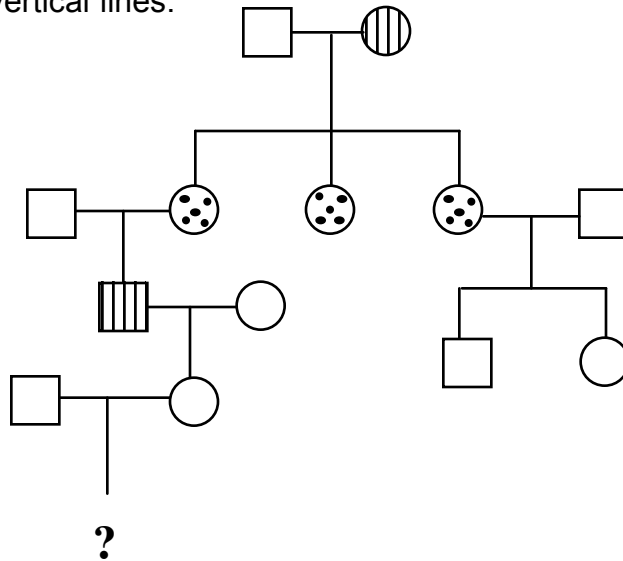
hairless thick:

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):

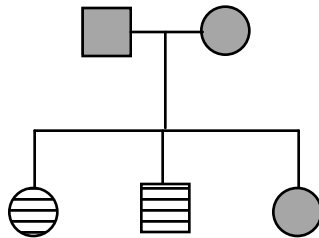
**(b, 6pts)** State whether the two traits in the pedigree are **linked** or **not linked**, or if it is inconclusive given these data.

**(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<sup>-</sup> phenotype of being unable to grow without serine in the medium. The second mutation causes the His<sup>-</sup> phenotype of being unable to grow without histidine in the medium. The third mutation causes a small colony phenotype. Wild-type yeast are Ser<sup>+</sup> His<sup>+</sup> and big. You mate a Ser<sup>-</sup> haploid mutant yeast to a His<sup>-</sup> 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<sup>-</sup> His<sup>+</sup> big  
Ser<sup>-</sup> His<sup>+</sup> small  
Ser<sup>+</sup> His<sup>-</sup> big  
Ser<sup>+</sup> His<sup>-</sup> small                    )  
9 tetrads of this type

Tetrad Type B                    )  
Ser<sup>-</sup> His<sup>+</sup> big  
Ser<sup>-</sup> His<sup>+</sup> big  
Ser<sup>+</sup> His<sup>-</sup> small  
Ser<sup>+</sup> His<sup>-</sup> small                    )  
90 tetrads of this type

Tetrad Type C                    )  
Ser<sup>+</sup> His<sup>-</sup> big  
Ser<sup>-</sup> His<sup>+</sup> small  
Ser<sup>-</sup> His<sup>-</sup> big  
Ser<sup>+</sup> His<sup>+</sup> 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: \_\_\_\_\_

## 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**\_\_\_\_\_

**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<sup>-</sup> mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1<sup>-</sup> mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2<sup>-</sup> mutation is a frameshift mutation that produces a protein product that is 12 kDa.

The first cross: You grow P1 phage on SerC<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> bacteria. (ProA<sup>-</sup> 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<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> SerB1<sup>-</sup> 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: \_\_\_\_\_

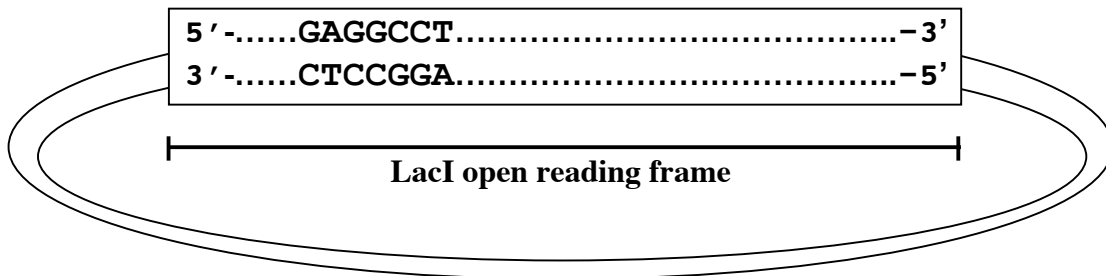
The third cross: You grow P1 phage on SerB1<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> SerB2<sup>-</sup> bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on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

<b>GENOTYPE:</b> <b>Phenotype:</b>	<b>at the ProA locus</b> <b>(+ or -)</b>	<b>at the SerB locus (be sure to include</b> <b>the genotype at SerB1 and SerB2)</b> <b>(+ or -)</b>
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.

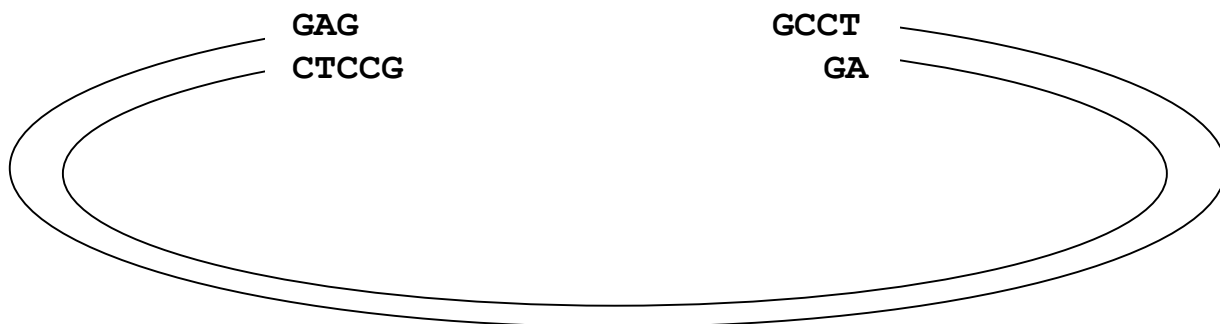
**2. (31 pts)** You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, 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 segment 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* $\Delta$  *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* $\Delta$  *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.

**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.

<u>Genotype</u>	<u>Activity of TolU when:</u>	
	<u>Simple sugars absent</u>	<u>Simple sugars present</u>
A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-
A <sup>-</sup>	-	-
C <sup>-</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	+
A <sup>+</sup> U <sup>-</sup> / F' A <sup>-</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	-	-
C <sup>-</sup> U <sup>+</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>-</sup>	+	+
A <sup>-</sup> B <sup>+</sup> / F' A <sup>+</sup> B <sup>-</sup> C <sup>+</sup> U <sup>+</sup>	+	-
B <sup>-</sup>	+	+
C <sup>-</sup>	+	+
C <sup>-</sup> U <sup>-</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-
A <sup>-</sup> U <sup>+</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-

**(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*<sup>-</sup> *tolC*<sup>-</sup> 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<sup>-</sup>, activator<sup>-</sup>, promoter<sup>-</sup>, operator<sup>-</sup>, super repressor, super activator, dominant negative repressor, dominant negative activator.)

Name: \_\_\_\_\_

## 7.03 Exam Three -- 2005

Name: \_\_\_\_\_

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**Question 1**      **17 pts**\_\_\_\_\_

**Question 2**      **45 pts**\_\_\_\_\_

**Question 3**      **20 pts**\_\_\_\_\_

**Question 4**      **18 pts**\_\_\_\_\_

**TOTAL**      **out of 100**\_\_\_\_\_

Name: \_\_\_\_\_

**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<sup>-</sup> and ProC<sup>-</sup>) that misregulate ProA expression.

You mate a ProB<sup>-</sup> haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB<sup>-</sup> haploid strain to a ProA<sup>-</sup> haploid strain. The resulting diploid expresses ProA properly.

You mate a ProA<sup>-</sup> ProC<sup>-</sup> haploid strain to a ProC<sup>-</sup> haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium.

You mate a ProC<sup>-</sup> haploid strain to a ProA<sup>-</sup> haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB<sup>-</sup> ProC<sup>-</sup> 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<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

**(b, 6pts)** Classify the ProC<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

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

Name: \_\_\_\_\_

**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.

<u>Genotype of mouse</u>	<u>Phenotype of mouse</u>
Wild-type	<i>Stringy</i> expressed only in tail
A <sup>-</sup> / A <sup>-</sup>	<i>Stringy</i> not expressed anywhere
B <sup>-</sup> / B <sup>-</sup>	<i>Stringy</i> expressed in all cells in the body

When you cross mice that are B<sup>-</sup> / B<sup>-</sup> to mice that are deficient in *Stringy*, the resulting mice only have *Stringy* expressed in the tail.

When you cross mice that are B<sup>-</sup> / B<sup>-</sup> to mice that are A<sup>-</sup> / A<sup>-</sup>, and then cross the resulting F<sub>1</sub> mice to each other, you get a genotypic ratio in the F<sub>2</sub> that indicates that the A and B loci segregate independently of each other.

You inject a piece of DNA containing the A<sup>-</sup> allele of the A gene into a fertilized egg produced by the mating of two true-breeding B<sup>-</sup> 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<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

**(b, 6pts)** Classify the B<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

Name: \_\_\_\_\_

**(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.

**(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.

Name: \_\_\_\_\_

**(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).

i)

ii)

iii)

iv)

v)



Name: \_\_\_\_\_

**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:  $\left[ -b \pm \sqrt{b^2 - 4ac} \right] / 2a$

**(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  $q$ .

Name: \_\_\_\_\_

**(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?

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

Name: \_\_\_\_\_KEY\_\_\_\_\_

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**Question 4**      **16 pts**\_\_\_\_\_

**TOTAL**      **out of 100**\_\_\_\_\_

**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

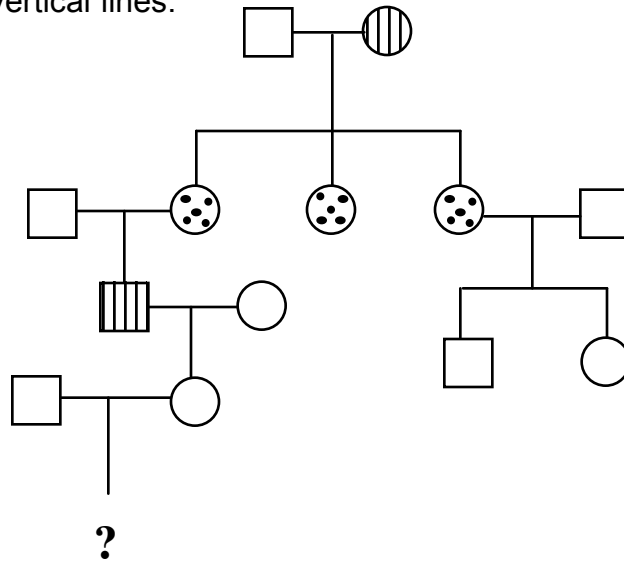
**P generation:** X  $hb^- tl^+$  X  $hb^- tl^+$  X  $hb^+ tl^-$  Y  
**F1:** X  $hb^- tl^+$  X  $hb^+ tl^-$  X  $hb^- tl^-$  Y  
**F2:** X  $hb^- tl^+$  Y OR X  $hb^+ tl^-$  Y  
 hairy thick: **0**  
 hairy thin: **250**  
 hairless thick: **250**  
 hairless thin: **0**

**(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-----  
 hairy thick: **50**  
 hairy thin: **200**  
 hairless thick: **200**  
 hairless thin: **50**

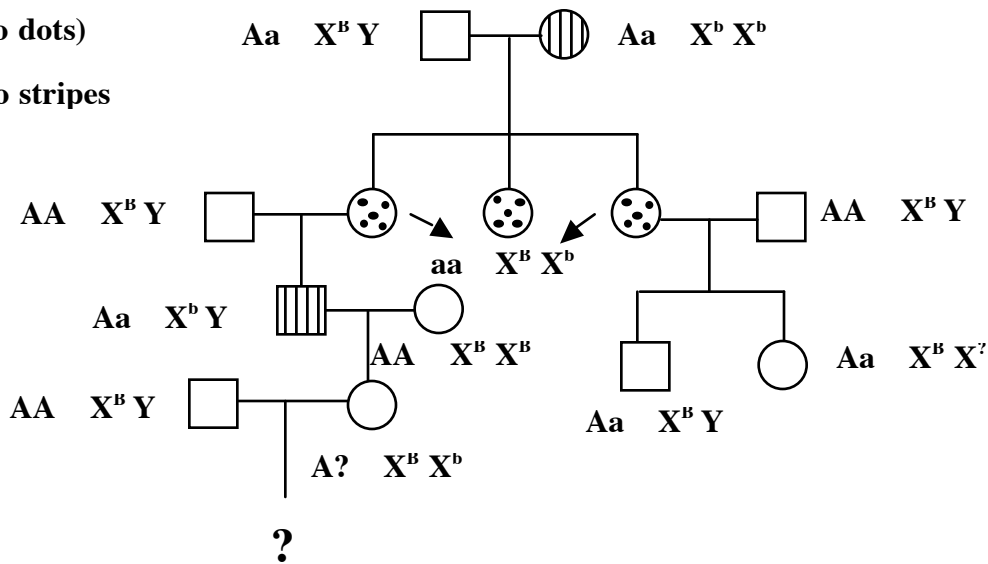
**2. (30 pts)** The following pedigree shows the inheritance two different **rare** traits. Each trait is determined by a different gene. The presence of Trait 1 is indicated by dots, and the presence of Trait 2 is indicated by vertical lines.

(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<sup>b</sup> (X<sup>B</sup> 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.**

**(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 your conclusion had to be consistent with your 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.

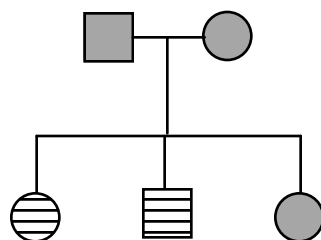
**25%**

**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.)

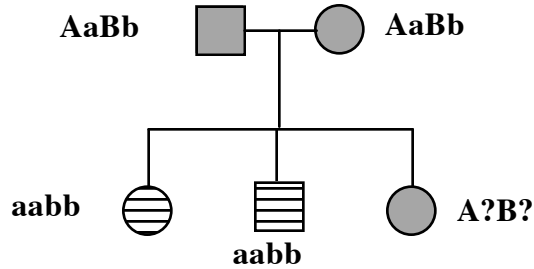


Name: \_\_\_\_\_ KEY \_\_\_\_\_

(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**

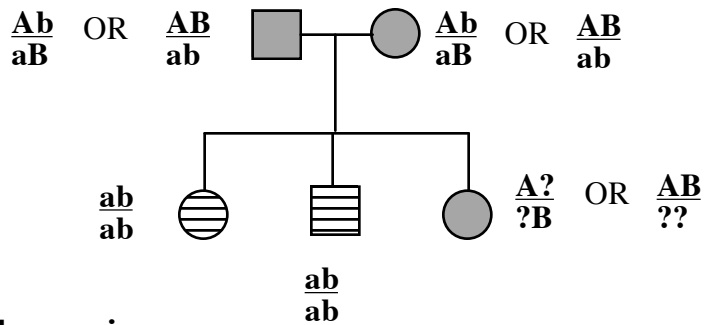
Stripes: a (A is no stripes)  
 Shading: B  
 b is no shading



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 your conclusion had to be consistent with your answer to part D.

**3. (30 pts)** You are studying three mutations in yeast. The first mutation causes the Ser<sup>-</sup> phenotype of being unable to grow without serine in the medium. The second mutation causes the His<sup>-</sup> phenotype of being unable to grow without histidine in the medium. The third mutation causes a small colony phenotype. Wild-type yeast are Ser<sup>+</sup> His<sup>+</sup> and big. You mate a Ser<sup>-</sup> haploid mutant yeast to a His<sup>-</sup> 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 <sup>-</sup> His <sup>+</sup> big	}	9 tetrads of this type
Ser <sup>-</sup> His <sup>+</sup> small		
Ser <sup>+</sup> His <sup>-</sup> big		
Ser <sup>+</sup> His <sup>-</sup> small		

Tetrad Type B

Ser <sup>-</sup> His <sup>+</sup> big	}	90 tetrads of this type
Ser <sup>-</sup> His <sup>+</sup> big		
Ser <sup>+</sup> His <sup>-</sup> small		
Ser <sup>+</sup> His <sup>-</sup> small		

Tetrad Type C

Ser <sup>+</sup> His <sup>-</sup> big	}	1 tetrad of this type
Ser <sup>-</sup> His <sup>+</sup> small		
Ser <sup>-</sup> His <sup>-</sup> big		
Ser <sup>+</sup> His <sup>+</sup> small		

**(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**

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

**(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

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

**(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**

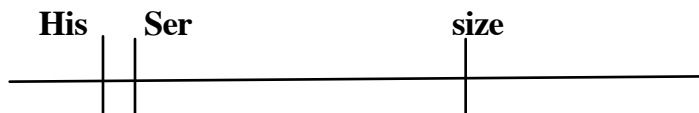
**(f, 4pts)** Are the Ser and His loci linked? If so, what is the genetic distance between them?

**Yes they are, at 0.5 cM**

Use the formula

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

**(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 your map had to be consistent with your answers to parts A-F.**

**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.

$$\frac{400}{20,000} = 0.02$$

**(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)**

$$p(X) = 0.02 \text{ (see part a)}$$

$$p(\text{notX}) = 1 - 0.02 = 0.98$$

$$p(Y|X) = p(\text{1}^{\text{st}} \text{ egg contained "X"}^{\text{A}} \text{ allele}) * p(\text{2}^{\text{nd}} \text{ egg same}) * p(\text{3}^{\text{rd}} \text{ egg same}) \\ = 1/2 * 1/2 * 1/2 = 1/8$$

**(Note that the father donates his Y chromosome and is thus irrelevant.)**

$$p(Y|\text{notX}) = p(\text{1}^{\text{st}} \text{ sperm contained "A"} \text{ allele}) * p(\text{2}^{\text{nd}} \text{ sperm same}) * p(\text{3}^{\text{rd}} \text{ sperm same}) \\ = 1 * 1 * 1 = 1$$

**[Note that the father is wild-type (AA) and this makes the mother irrelevant.]**

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

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

Name: \_\_\_\_\_KEY\_\_\_\_\_

## 7.03 Exam Two -- 2005

Name: \_\_\_\_\_KEY\_\_\_\_\_

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**\_\_\_\_\_

**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<sup>-</sup> mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1<sup>-</sup> mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2<sup>-</sup> mutation is a frameshift mutation that produces a protein product that is 12 kDa.

The first cross: You grow P1 phage on SerC<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> bacteria. (ProA<sup>-</sup> 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).

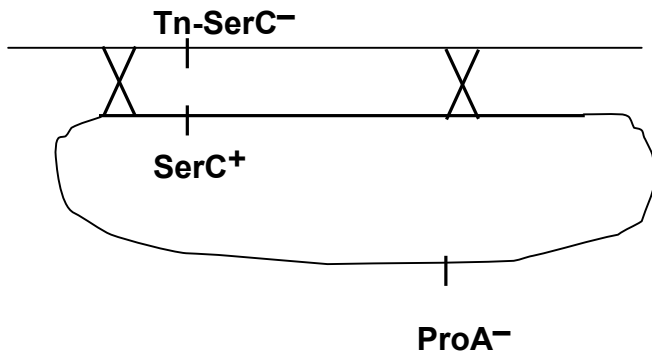
**(a, 6pts)** What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

**0%**

There are two possibilities – either A and C are linked, or they are not.

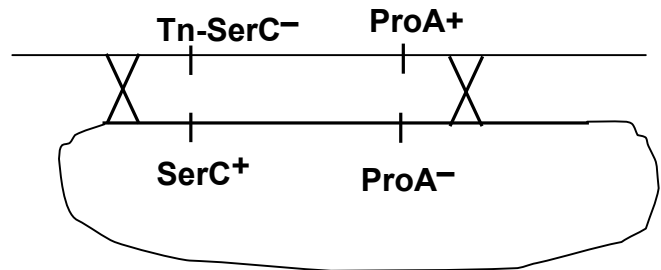
The first cross:

**If A and C are unlinked**



You will never get ProA<sup>+</sup> KanR from this.

**If A and C are linked**



You can get ProA<sup>+</sup> KanR from this.

Given that you never see ProA<sup>+</sup> 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%.

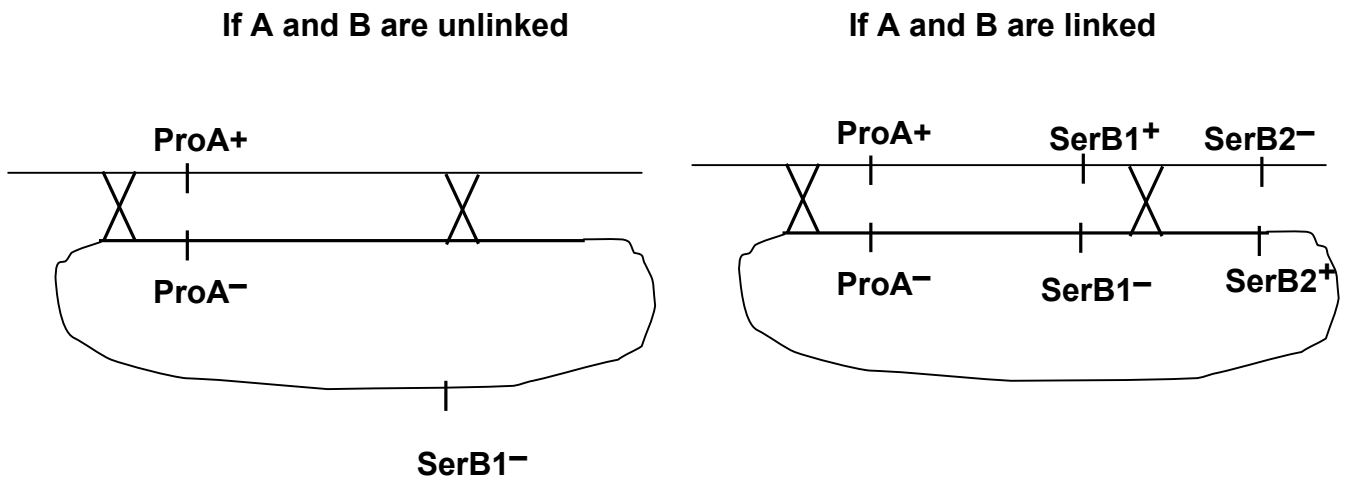
The second cross: You grow P1 phage on SerB2<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> SerB1<sup>-</sup> bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on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



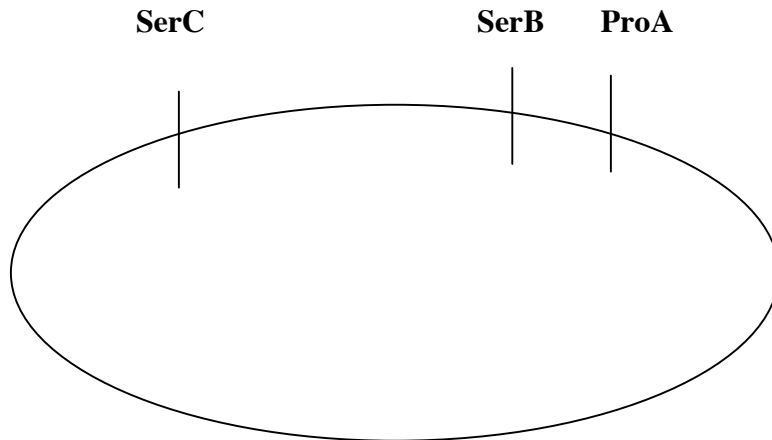
You will never get ProA<sup>+</sup> Ser<sup>+</sup> from this.

You can get ProA<sup>+</sup> SerB<sup>+</sup> from this.

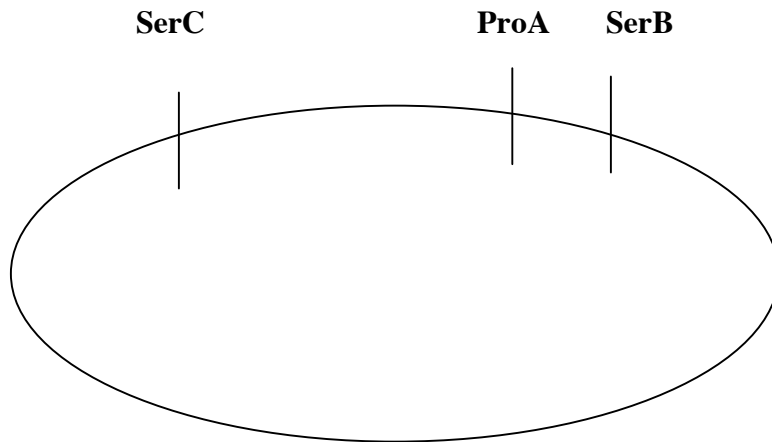
Given that you do see ProA<sup>+</sup> Ser<sup>+</sup> transductants, A and B must be linked.

**(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<sup>5</sup> basepairs), but SerC and SerB are close enough to each other to be linked (see diagram below).



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).



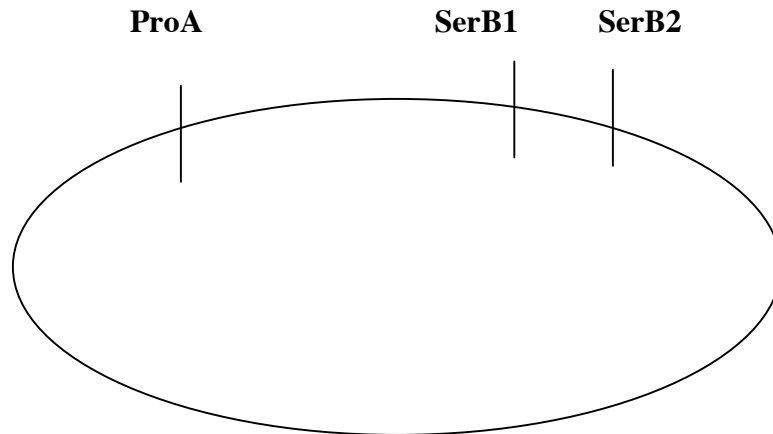
The third cross: You grow P1 phage on SerB1<sup>-</sup> bacteria. You use the resulting phage lysate to infect ProA<sup>-</sup> SerB2<sup>-</sup> bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on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

<b>GENOTYPE:</b> <b>Phenotype:</b>	<b>at the ProA locus</b> <b>(+ or -)</b>	<b>at the SerB locus (be sure to include</b> <b>the genotype at SerB1 and SerB2)</b> <b>(+ or -)</b>
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.



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+.

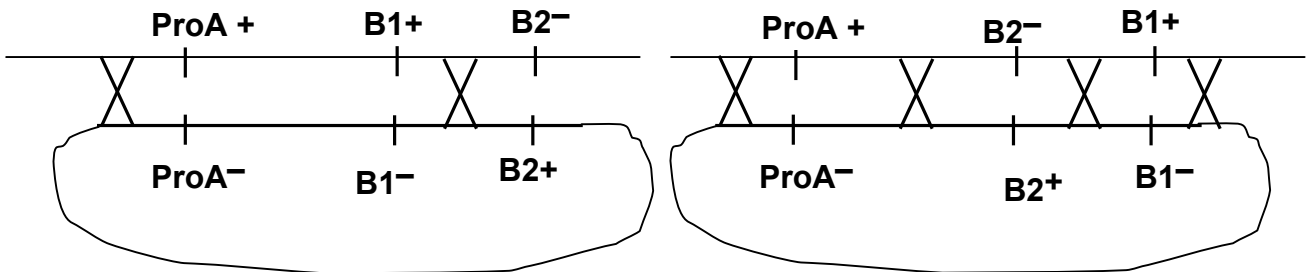
Order One – B1 in middle

Order Two - B2 in middle

The second cross:

Order One

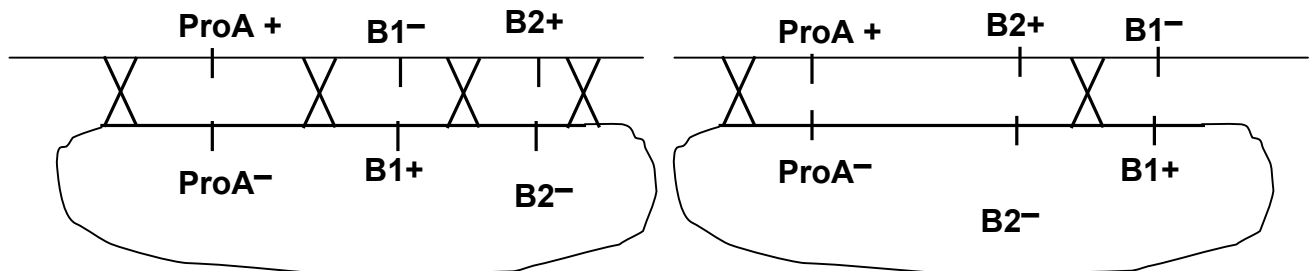
Order Two



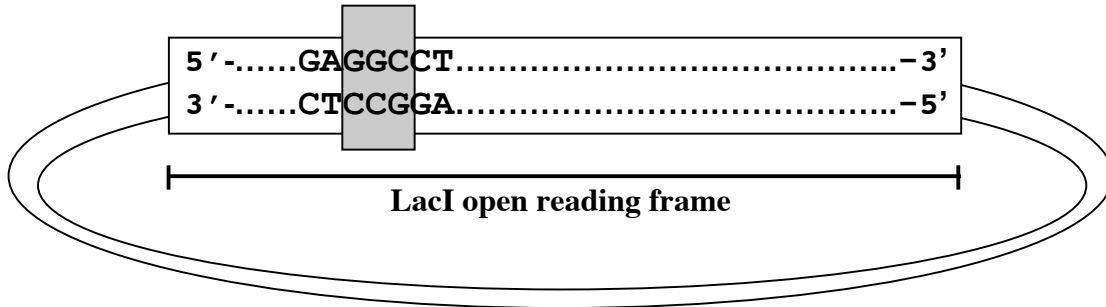
The third cross:

Order One

Order Two



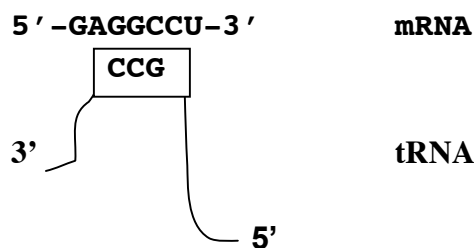
**2. (31 pts)** You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, 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 segment that is highlighted, and label any 5' and 3' ends in your drawing.

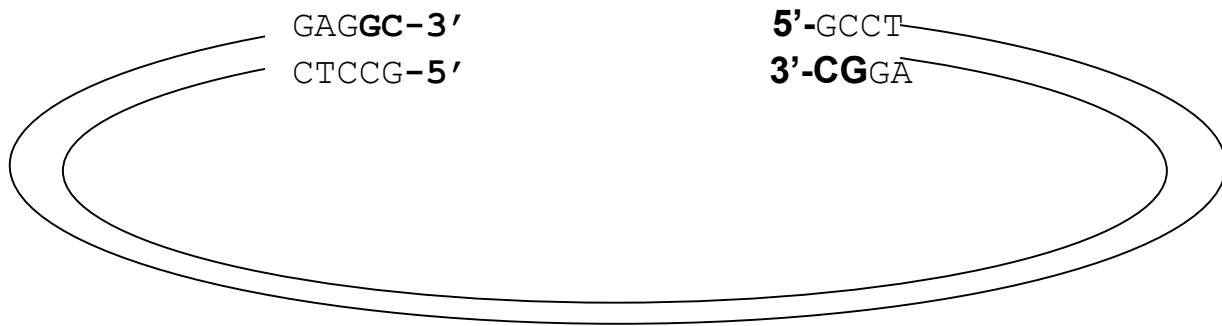
5'-GAGGCCU-3'

**(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.



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 *lacIΔ 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 *lacIΔ 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.

**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*<sup>-</sup>, *tolB*<sup>-</sup>, or *tolC*<sup>-</sup>. TolA, TolB, and TolC are all regulatory components involved in TolU regulation. Below are the phenotypes of different strains that you have constructed.

<u>Genotype</u>	<u>Activity of TolU when:</u>	
	<u>Simple sugars absent</u>	<u>Simple sugars present</u>
1. A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-
2. A <sup>-</sup>	-	-
3. C <sup>-</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	+
4. A <sup>+</sup> U <sup>-</sup> / F' A <sup>-</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	-	-
5. C <sup>-</sup> U <sup>+</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>-</sup>	+	+
6. A <sup>-</sup> B <sup>+</sup> / F' A <sup>+</sup> B <sup>-</sup> C <sup>+</sup> U <sup>+</sup>	+	-
7. B <sup>-</sup>	+	+
8. C <sup>-</sup>	+	+
9. C <sup>-</sup> U <sup>-</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-
10. A <sup>-</sup> U <sup>+</sup> / F' A <sup>+</sup> B <sup>+</sup> C <sup>+</sup> U <sup>+</sup>	+	-

**(a, 7pts)** Classify the *tolA*<sup>-</sup> 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<sup>+</sup> is on a different piece of DNA from U<sup>+</sup>), and A fails the trans test because the trans test strain displays the recessive phenotype (uninducible from A<sup>-</sup> is recessive to inducible from A<sup>+</sup>).**

**(b, 7pts)** Classify the *tolB*<sup>-</sup> mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

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

**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.

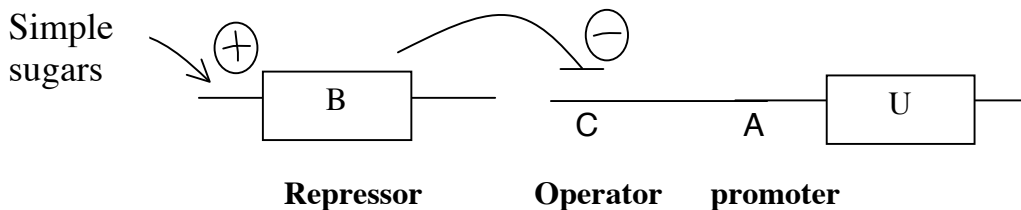
**Cis, constitutive, dominant.** (This means that C is the operator sequence in the U gene.)

**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.

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<sup>-</sup>, activator<sup>-</sup>, promoter<sup>-</sup>, operator<sup>-</sup>, 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

**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<sup>-</sup>. This is because there are two kinds of alleles of activator-encoding genes that give uninducible: dominant negative activator or activator<sup>-</sup>.**

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<sup>-</sup> and ProC<sup>-</sup>) that misregulate ProA expression.

You mate a ProB<sup>-</sup> haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB<sup>-</sup> haploid strain to a ProA<sup>-</sup> haploid strain. The resulting diploid expresses ProA properly.

You mate a ProA<sup>-</sup> ProC<sup>-</sup> haploid strain to a ProC<sup>-</sup> haploid strain. The resulting diploid expresses ProA when proline is present in the growth medium.

You mate a ProC<sup>-</sup> haploid strain to a ProA<sup>-</sup> haploid strain. The resulting diploid expresses ProA properly.

You mate a ProB<sup>-</sup> ProC<sup>-</sup> 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<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

**Trans, recessive, uninducible**

**Trans** – You mate a ProB<sup>-</sup> haploid strain to a ProA<sup>-</sup> 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<sup>-</sup> haploid strain to a wild-type haploid strain. The resulting diploid expresses ProA properly. This means B<sup>-</sup> is recessive.

**Uninducible** – The only mutant phenotypes you see when you sporulate a diploid that contains both the B<sup>-</sup> and C<sup>-</sup> mutations are constitutive and uninducible. C<sup>-</sup> gives constitutive, so B<sup>-</sup> must give uninducible.

**(b, 6pts)** Classify the ProC<sup>-</sup> mutation by its genetic properties (cis vs. trans, constitutive vs. uninducible, dominant vs. recessive).

**Trans, recessive, constitutive**

**Trans** – You mate a ProC<sup>-</sup> haploid strain to a ProA<sup>-</sup> 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<sup>-</sup> haploid strain to a ProA<sup>-</sup> haploid strain. The resulting diploid expresses ProA properly. This means C<sup>-</sup> is recessive.

**Constitutive** – You mate a ProA<sup>-</sup> ProC<sup>-</sup> haploid strain to a ProC<sup>-</sup> 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 expresses ProA even when it is not supposed to (i.e. when the cell already has proline available to it).

(c, 5pts) If you 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<sup>-</sup> C<sup>+</sup> to B<sup>+</sup> C<sup>-</sup>, 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 tetraploid 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 tetraploid resulting from this mating would contain the spores:

GENOTYPE	PHENOTYPE
B <sup>-</sup> C <sup>+</sup>	uninducible
B <sup>+</sup> C <sup>-</sup>	constitutive
B <sup>+</sup> C <sup>+</sup>	inducible
B <sup>-</sup> C <sup>-</sup>	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.

<u>Genotype of mouse</u>	<u>Phenotype of mouse</u>
Wild-type	<i>Stringy</i> 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<sub>2</sub> 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**

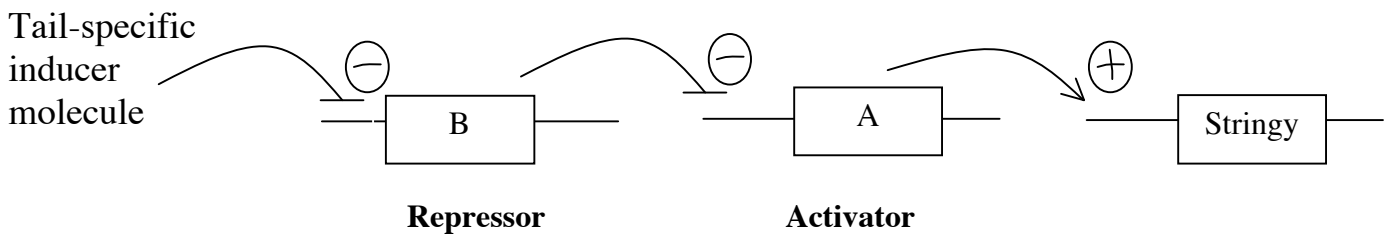
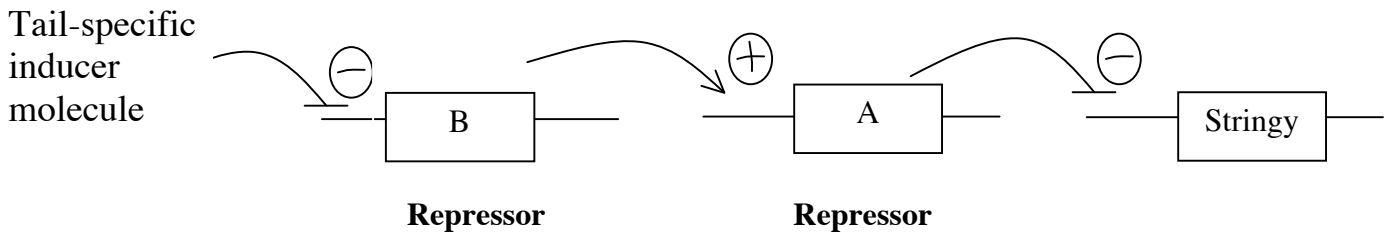
Name: \_\_\_\_\_key\_\_\_\_\_

**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<sup>-</sup>/B<sup>-</sup> mouse with an A<sup>-</sup> 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<sup>-</sup> 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<sup>-</sup>/B<sup>-</sup> mouse with an A<sup>-</sup> 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

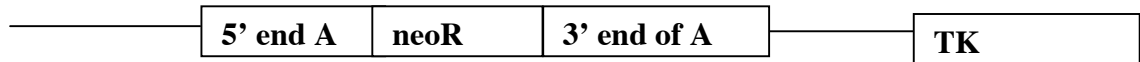
Describe a way to create a genetically modified mouse that would allow you to gain the piece of information you stated in part (d) (and thereby distinguish between your models).

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.

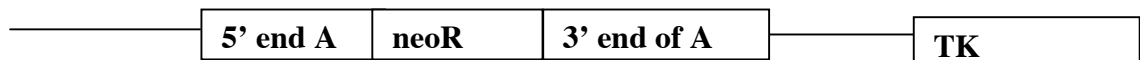
v) If the mice express Stringy everywhere, then the wild-type function of A is to be a repressor (in which case the A<sup>-</sup> allele was a superrepressor). If the mice express Stringy nowhere, then the wild-type function of A is to be an activator (in which case the A<sup>-</sup> allele was a dominant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

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

i) gene targeting

ii)



iii) B<sup>-</sup>/B<sup>-</sup> ES cells

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

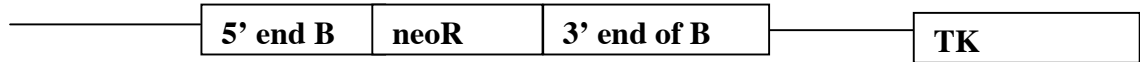
v) If the mice express Stringy nowhere, then the wild-type function of A is to be an activator, AND A is downstream in the pathway.

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.

**POSSIBILITY TWO**

i) gene targeting

ii)



iii)  $A^{-}/A^{-}$  ES cells

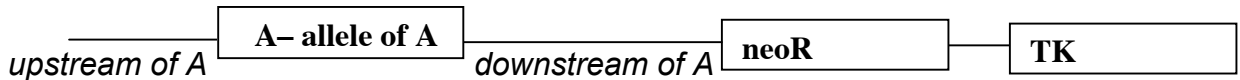
iv) Mate the resulting chimera to  $A^{-}/A^{-}$  mice to get out non-chimeric mice that are  $A^{-}/A^{-}$   $B^{+}/B^{-}$ . Mate two of these mice together and 1/4 of them will be homozygous for the B gene knockout and will be  $A^{-}/A^{-}$ .

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

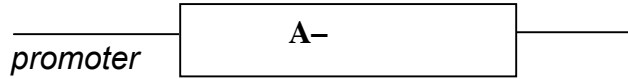
iv) Mate the resulting chimera to  $B^{-}/B^{-}$  mice to get out non-chimeric mice that are  $B^{-}/B^{-}$   $A^{+}/A^{-}$ .

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).

**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:  
(CIRCLE ONE OF THE FOUR)

stay the same

rise

inconclusive

fall

**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\_\_\_\_\_

**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:  $[-b \pm \sqrt{b^2 - 4ac}] / 2a$

**(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:**

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

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

$$S = 90\%$$

$$h = 3\%$$

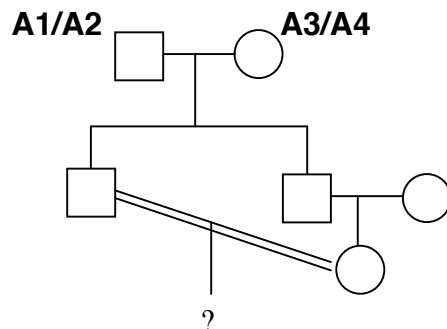
$$\mu = 10^{-6}$$

**Solve for  $q$  using the quadratic equation**

$$q = 0.033 \quad (\text{the other answer from the quadratic is negative})$$

**(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\_\_\_\_\_

$$\text{Chance (child is A1/A1)} = (1/2) * (1/2) * (1/2) * (1/2) * (1/2) = (1/32)$$

$$F = \text{chance (child is A1/A1)} + \text{chance (child is A2/A2)} + \text{chance (child is A3/A3)} + \text{chance (child is A4/A4)}$$

$$F = 4 * (1/32)$$

$$F = 1/8$$

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

For this population to be at steady state:

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

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

$$S = 90\%$$

$$\mu = 10^{-6}$$

$$F = 1/8$$

Solve for q using the quadratic equation

$$q = 5.9 \times 10^{-5} \text{ (the other answer from the quadratic is negative)}$$