Exams

Fall 2000

7.03 Exam 1

Name: _____

Section: **TA:**

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

There are seven pages including this cover page
Please write your name on each page.

Also...

- Look over the entire exam so you don't spend too much time on hard questions leaving easy questions unanswered.

- Check your answers to make sure that they make sense.

- To help us give partial credit, show your work and state any assumptions that you make.

Question 1	**35 points**
Question 2	**35 points**
Question 3	**30 points**

Name:

1. **(a** 4 pts.) Wild type yeast form white colonies. You have isolated two mutants that make red colonies that you call **red1⁻** and **red2⁻**. When a **red1⁻** mutant is mated to a **red2⁻** mutant of the opposite mating type the resulting diploid makes white colonies. What does this observation tell us about **red1⁻** and **red2⁻**?

(b 9 pts.) When the diploids from part **(a)** are sporulated, three types of tetrads are found.

Type I have 4 red colonies

Type II have 1 white and 3 red colonies

Type III have 2 white and 2 red colonies

Classify each tetrad type as PD, NPD or T.

Type I Type II Type III

(c 10 pts.) When the number of each tetrad type is tallied you find that the cross produces 30 Type I tetrads, 16 Type II tetrads, and 4 Type III tetrads.

Are the **red1⁻** and **red2⁻** mutations linked? If so, how far apart are they in cM?

Name:

(d 12 pts.) One of the Type II tetrads from above is selected for further analysis and you designate the four spore clones **a**, **b**, **c**, and **d**. Clone **a** is white, whereas clones **b**, **c**, and **d** are red. Each clone is mated to either a **red1**$^{-}$ mutant or a **red2**$^{-}$ mutant and the color of the resulting diploid is noted.

Clone **a** (white)
- x **red1**$^{-}$ → white diploid
- x **red2**$^{-}$ → white diploid

Clone **b** (red)
- x **red1**$^{-}$ → red diploid
- x **red2**$^{-}$ → white diploid

Clone **c** (red)
- x **red1**$^{-}$ → red diploid
- x **red2**$^{-}$ → red diploid

Clone **d** (red)
- x **red1**$^{-}$ → white diploid
- x **red2**$^{-}$ → red diploid

Give the genotypes of each of the four spore clones

Clone **a**:

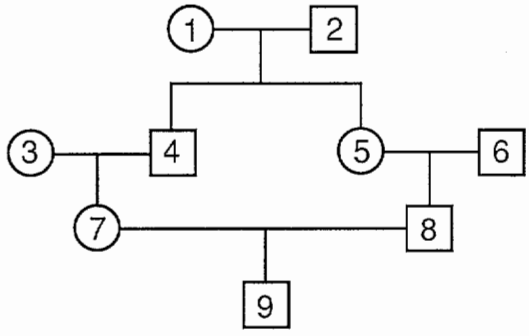
Clone **b**:

Clone **c**:

Clone **d**:

Name:

2. Consider the following pedigree where two first cousins have a son. Each individual is numbered for reference in this problem.



(a 15 pts.) Say that female 1 is affected by a rare recessive X-linked trait and that male 2 does not have the trait. Assume that individuals 3 and 6 neither have nor are carriers of the trait.

What is the probability that male **4** will be affected by the trait?

What is the probability that female **5** will be affected by the trait?

What is the probability that female **7** will be affected by the trait?

What is the probability that male **8** will be affected by the trait?

What is the probability that male **9** will be affected by the trait?

Name:

(b 20 pts.) Now say that female 1 is affected by two different rare recessive X-linked traits that are 10 cM apart (we will refer to them as trait 1 and trait 2). Male 2 does not exhibit either trait. Assume that individuals 3 and 6 neither have nor are carriers of either trait.

What is the probability that male **4** will be affected by both trait 1 and trait 2?

What is the probability that male **8** will be affected by both trait 1 and trait 2?

What is the probability that male **9** will be affected by trait 1 only?

What is the probability that male **9** will be affected by both trait 1 and trait 2?

What is the probability that male **9** will be affected by neither trait 1 nor trait 2?

Name:

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

Phenotype	Number
wild type	43
curly-wings	11
humpback	9
curly-wings, humpback	37

(a 10 pts.**)** What is the distance between the **cr** and the **hb-1** mutations in cM?

Next you isolate a second recessive humpback mutation (**hb-2**). A female from a true breeding **hb-2** strain is crossed to a male from a true breeding **cr**, **hb-1** strain. An F_1 female from this cross is then crossed to a male from a true breeding **cr**, **hb-1** strain and 500 progeny are examined.

Phenotype	Number
curly-wings	5
humpback	240
curly-wings, humpback	255

(b 10 pts.**)** What is the distance between the **hb-1** and **hb-2** mutations in cM?

Name:

(c 10 pts.) Draw a genetic map showing the relative order of the **cr**, **hb-1**, and **hb-2** mutations as well as the distances that you have determined.

7.03 Exam 1

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Question 1	35 points
Question 2	35 points
Question 3	30 points

Name:

1. (**a** 4 pts.) Wild type yeast form white colonies. You have isolated two mutants that make red colonies that you call **red1**$^-$ and **red2**$^-$. When a **red1**$^-$ mutant is mated to a **red2**$^-$ mutant of the opposite mating type the resulting diploid makes white colonies. What does this observation tell us about **red1**$^-$ and **red2**$^-$?

red1 & red2 complement and are therefore in different genes

(**b** 9 pts.) When the diploids from part (**a**) are sporulated, three types of tetrads are found.

Type I have 4 red colonies

Type II have 1 white and 3 red colonies

Type III have 2 white and 2 red colonies

Classify each tetrad type as PD, NPD or T.

Type I	Type II	Type III
PD	T	NPD

(**c** 10 pts.) When the number of each tetrad type is tallied you find that the cross produces 30 Type I tetrads, 16 Type II tetrads, and 4 Type III tetrads.

Are the **red1**$^-$ and **red2**$^-$ mutations linked? If so, how far apart are they in cM?

Yes they are linked. PD >> NPD so use

$$\text{Distance} = 100\,\frac{(T + 6NPD)}{2\Sigma}$$

$$= \frac{100\,(16 + 6(4))}{100} = 40 \text{ cM}$$

Name:

(d 12 pts.) One of the Type II tetrads from above is selected for further analysis and you designate the four spore clones **a**, **b**, **c**, and **d**. Clone **a** is white, whereas clones **b**, **c**, and **d** are red. Each clone is mated to either a **red1$^-$** mutant or a **red2$^-$** mutant and the color of the resulting diploid is noted.

Clone **a** (white)
- x **red1$^-$** → white diploid
- x **red2$^-$** → white diploid

Clone **b** (red)
- x **red1$^-$** → red diploid
- x **red2$^-$** → white diploid

Clone **c** (red)
- x **red1$^-$** → red diploid
- x **red2$^-$** → red diploid

Clone **d** (red)
- x **red1$^-$** → white diploid
- x **red2$^-$** → red diploid

Give the genotypes of each of the four spore clones

Clone **a**: RED1$^+$ RED2$^+$

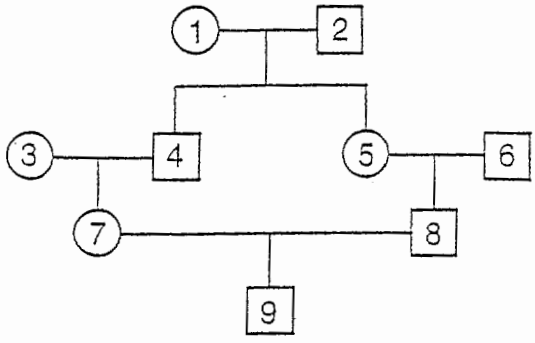
Clone **b**: red1$^-$ RED2$^+$

Clone **c**: red1$^-$ red2$^-$

Clone **d**: RED1$^+$ red2$^-$

Name:

2. Consider the following pedigree where two first cousins have a son. Each individual is numbered for reference in this problem.



(a 15 pts.) Say that female 1 is affected by a rare recessive X-linked trait and that male 2 does not have the trait. Assume that individuals 3 and 6 neither have nor are carriers of the trait.

What is the probability that male **4** will be affected by the trait?

P = 1

What is the probability that female **5** will be affected by the trait?

P = 0

What is the probability that female **7** will be affected by the trait?

P = 0

What is the probability that male **8** will be affected by the trait?

P = 1/2

What is the probability that male **9** will be affected by the trait?

P = 1/2

Name:

(b 20 pts.) Now say that female 1 is affected by two different rare recessive X-linked traits that are 10 cM apart (we will refer to them as trait 1 and trait 2). Male 2 does not exhibit either trait. Assume that individuals 3 and 6 neither have nor are carriers of either trait.

What is the probability that male 4 will be affected by both trait 1 and trait 2?

$P = 1$

What is the probability that male 8 will be affected by both trait 1 and trait 2?

$P = (0.9)(0.5) = 0.45$

What is the probability that male 9 will be affected by trait 1 only?

$P = (0.1)(0.5) = 0.05$

What is the probability that male 9 will be affected by both trait 1 and trait 2?

~~22~~ $P = (0.9)(0.5) = 0.45$

What is the probability that male 9 will be affected by neither trait 1 nor trait 2?

$P = (0.9)(0.5) = 0.45$

Name:

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

Phenotype	Number
wild type	43
curly-wings	11
humpback	9
curly-wings, humpback	37

(**a** 10 pts.) What is the distance between the **cr** and the **hb-1** mutations in cM?

$$\text{distance} = \frac{\text{recombinants}}{\text{total}} \times 100 = \left(\frac{20}{100}\right)100 = 20 \text{ cM}$$

Next you isolate a second recessive humpback mutation (**hb-2**). A female from a true breeding **hb-2** strain is crossed to a male from a true breeding **cr**, **hb-1** strain.. An F_1 female from this cross is then crossed to a male from a true breeding **cr**, **hb-1** strain and 500 progeny are examined.

Phenotype	Number
curly-wings	5
humpback	240
curly-wings, humpback	255

(**b** 10 pts.) What is the distance between the **hb-1** and **hb-2** mutations in cM?

must count hidden recombinants

$$\text{distance} = \frac{5 \times 2}{500}(100) = 2 \text{ cM}$$

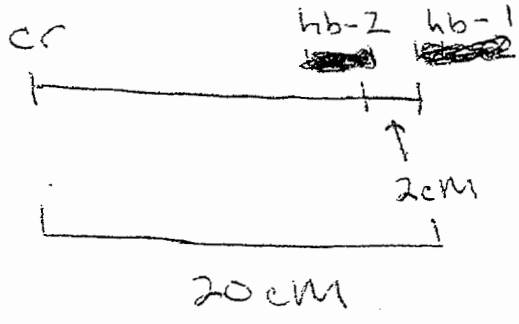
Name:

(c 10 pts.) Draw a genetic map showing the relative order of the **cr**, **hb-1**, and **hb-2** mutations as well as the distances that you have determined.

wild type is the rarest class ⇒ double cross-over

parental: $\frac{cr \quad hb\text{-}1 \quad +}{+ \quad + \quad hb\text{-}2}$

⇒ hb-2 must be in the middle



7.03 Exam 2

Name: _____

Section: _____ **TA:** _____

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

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Question 1	**26 points**
Question 2	**25 points**
Question 3	**24 points**
Question 4	**25 points**

1st position (5′ end) ↓	2nd position U	C	A	G	3rd position (3′ end) ↓
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Name:

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

(a 6 pts.**)** The two mutants are infected together into a **Su$^+$** host so that each cell receives at least one phage of each type. The resulting phage form 10^7 plaques/ml when plated on a **Su$^+$** host, but will only form 5×10^4 plaques/ml when plated on a **Su$^-$** host. What is the distance between the two mutations in map units?

(b 8 pts.**)** The size of the normal lysozyme protein is 45 kDa. One of the mutants makes a lysozyme fragment that is 22 kDa, while the other makes a fragment that is 33 kDa. Using 0.11 kDa as the average mass of an amino acid and knowing that the total genetic length of the phage T4 chromosome is 400 map units, estimate the physical length of phage T4 DNA in base pairs.

(c 12 pts.**)** Suppose that one of the T4 mutants (which can only grow on an *E. coli* strain carrying a UGA nonsense suppressor) was generated by a mutagen that causes transition mutations (C•G to T•A or T•A to C•G). Using the genetic code (on the front of the exam) determine which codon(s) in wild type T4 could have been altered by a single transversion mutation to produce the phage mutant. For your answer, show both strands of the DNA, indicate the 5' and 3' ends of each strand, and indicate which strand is used as the template in transcription to produce lysozyme mRNA.

Name:

2. **(a** 7 pts.**)** You have isolated a **Tn5** insertion in an otherwise wild type *E. coli* strain that you think may be linked to the **Lac** operon. You grow **P1** phage on the strain with the **Tn5** insertion and use the resulting phage to infect a **LacI**$^-$ strain. Among the resulting Kan^r transductants, 30% have constitutive **Lac** expression and 70% are regulated normally. What is the distance between **LacI** and the **Tn5** insertion expressed as a cotransduction frequency?

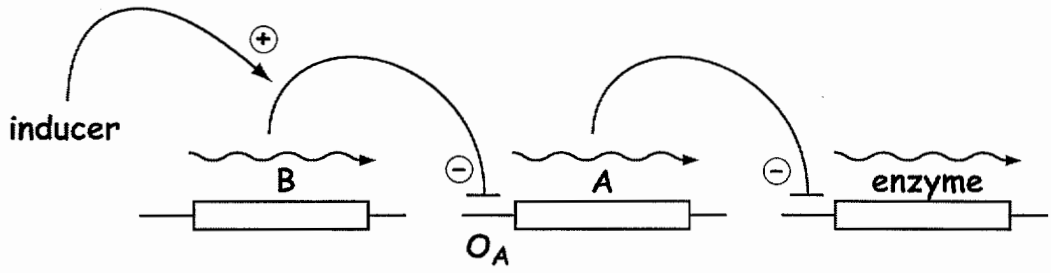
(b 12 pts.**)** Next, you want to map the **Tn5** insertion described in part **(a)** relative to two different **LacI**$^-$ mutations (**LacI-1**$^-$ and **LacI-2**$^-$). To do this you perform two reciprocal crosses. In cross 1 you grow **P1** phage on a host that has the **Tn5** insertion and **LacI-1**$^-$. The resulting phage are then used to infect a **LacI-2**$^-$. Among the Kan^r transductants, 99% are constitutive and 1% are regulated normally. For cross 2, you grow **P1** phage on a host that has the **Tn5** insertion and the **LacI-2**$^-$ mutation. The resulting phage are then used to infect a **LacI-1**$^-$ strain. In this experiment, all of the Kan^r transductants are constitutive.

Draw a genetic map showing the relative order of **Tn5**, **LacI-1**$^-$ and **LacI-2**$^-$. Also give any relevant distances (obtained from either the two-factor cross in (**a**) or the three factor crosses in (**b**)) expressed as cotransduction frequencies.

(c 6 pts.**)** Say that you wanted to isolate a **LacI-1**$^-$ and **LacI-2**$^-$ double mutant. Which cross from part **(b)** would be a better starting point to search for the desired double mutant and why?

Name:

3. An enzyme in *E. coli* is regulated by the following scheme:



A is a transcriptional repressor of the enzyme and **B** is a transcriptional repressor of the gene for **A**. **B** is active as a repressor only when it is bound to the inducer molecule. When the inducer is absent, **B** does not bind to its operator, **A** is expressed, and enzyme synthesis is repressed.

(a 12 pts.) An allele of the **B** gene ($\mathbf{B^s}$) is isolated that binds to DNA and represses regardless of whether inducer is present or not. An allele of the operator site of the **A** gene ($\mathbf{O^c_A}$) is isolated that will not bind the **B** repressor. In the table below indicate for each strain whether the enzyme will be synthesized, with or without inducer (use + or –).

	– inducer	+ inducer
$\mathbf{B^s}$		
$\mathbf{O^c_A}$		
$\mathbf{B^s\ O^c_A}$		

(b 12 pts.) An allele of the **A** gene is isolated that disrupts the ability of the **A** repressor to bind DNA. In a heterozygous merodiploid this allele will also interfere with the ability of wild type **A** protein to bind DNA. The allele is therefore called $\mathbf{A^{-d}}$. Indicate in the table below when the enzyme will be synthesized.

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

Name:

4. To study regulation of starch utilization in *E. coli* you isolate a **Tn5::LacZ** insertion in the gene for the starch degrading enzyme amylase. This insertion only expresses ß-galactosidase when starch is present in the medium. You isolate two mutations that cause altered regulation of the **Tn5::LacZ** reporter. The **sta1⁻** mutation is unlinked to the **Tn5::LacZ** insertion and has uninducible ß-galactosidase expression. The **sta2⁻** mutation is linked to the **Tn5::LacZ** insertion (50% cotransduction) and expresses ß-galactosidase constitutively. By constructing the appropriate merodiploid you determine that the **sta2⁻** mutation is recessive.

(a 15 pts.**)** Construct two models for amylase regulation that explains all of the properties of the **sta1⁻** and **sta2⁻** mutations**.** For your models, represent the relevant gene products as **Sta1** and **Sta2**, include the amylase gene, and be sure to indicate where and how the inducer starch acts.

(b 10 pts.**)** In a transduction experiment you grow phage **P1** on a strain carrying the **Tn5::LacZ** insertion and the **sta2⁻** mutation. You use the resulting phage to infect a **sta1⁻** mutant (which does not carry the **Tn5::LacZ** insertion). About half of the resulting Kan^r transductants constitutively express ß-galactosidase and half have uninducible expression. What does this observation tell you about the phenotype of a **sta1⁻ sta2⁻** double mutant? Which of your models is supported by this observation?

7.03 Exam 2

Name: Key

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U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Name: KEY

1. Phage T4 expresses an enzyme lysozyme, which enables phage to lyse infected cells. Mutations in the lysozyme gene can prevent T4 from forming a plaques on a lawn of *E. coli*. You have isolated two T4 mutants that can not make plaques on wild type (**Su⁻**), but that can make plaques on an *E. coli* strain carrying a UGA nonsense suppressor (**Su⁺**).

(a 6 pts.) The two mutants are infected together into a **Su⁺** host so that each cell receives at least one phage of each type. The resulting phage form 10^7 plaques/ml when plated on a **Su⁺** host, but will only form 5×10^4 plaques/ml when plated on a **Su⁻** host. What is the distance between the two mutations in map units?

$$\text{map distance} = \frac{\text{recombinants}}{\text{total}} \qquad \frac{2(5\times10^4)}{10^7} = 1.0 \text{ map units}$$

(b 8 pts.) The size of the normal lysozyme protein is 45 kDa. One of the mutants makes a lysozyme fragment that is 22 kDa, while the other makes a fragment that is 33 kDa. Using 0.11 kDa as the average mass of an amino acid and knowing that the total genetic length of the phage T4 chromosome is 400 map units, estimate the physical length of phage T4 DNA in base pairs.

$$\frac{33\text{kDa} - 22\text{kDa}}{1 \text{ map unit}} = 11 \text{ kDa/map unit} \qquad 11 \text{ kDa/m.u.} \times \frac{1 \text{ a.a.}}{.11 \text{ kDa}} = 100 \frac{\text{aa}}{\text{map unit}}$$

$$100 \frac{\text{aa}}{\text{m.u.}} \times 3 \text{ bp/aa} = 300 \text{ bp/m.u.}$$

$$300 \text{ bp/m.u.} \times 400 \text{ m.u.} = 120{,}000 \text{ bp}$$

(c 12 pts.) Suppose that one of the T4 mutants (which can only grow on an *E. coli* strain carrying a UGA nonsense suppressor) was generated by a mutagen that causes transition mutations (C•G to T•A or T•A to C•G). Using the genetic code (on the front of the exam) determine which codon(s) in wild type T4 could have been altered by a single transversion mutation to produce the phage mutant. For your answer, show both strands of the DNA, indicate the 5' and 3' ends of each strand, and indicate which strand is used as the template in transcription to produce lysozyme mRNA.

5'-UGA-3'

DNA 5'-CGA-3'
template → 3'-GCT-5'
↓
CGA mRNA codon
↓
Arg

5' TAA 3'
3' ATT 5'
↓
UAA
↓
Stop
Not an option because a mutation would be

DNA 5' TGG 3'
3' ACC 5' ← template
↓
UGG mRNA
↓
Trp

Name: KEY

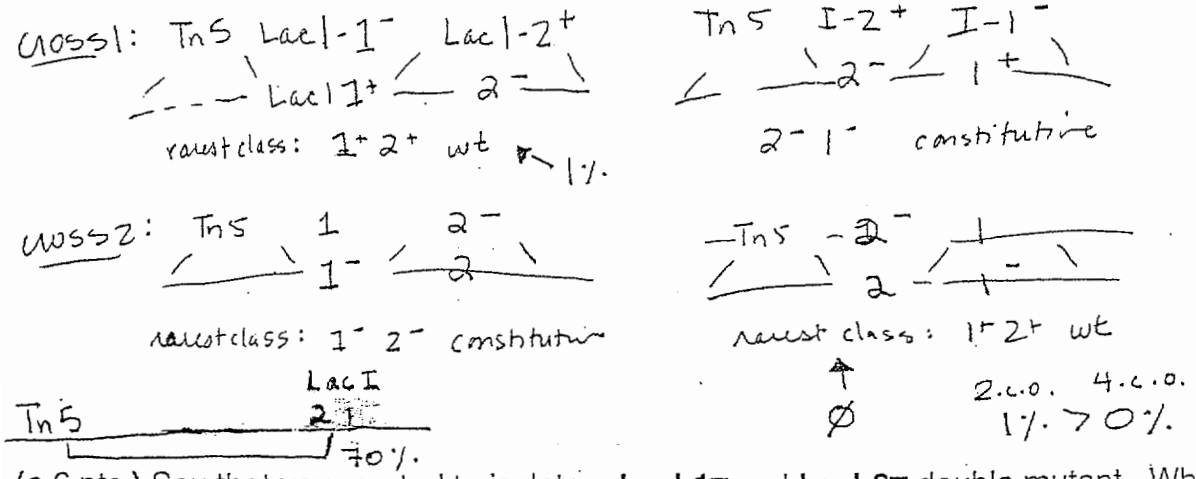
2. (a 7 pts.) You have isolated a **Tn5** insertion in an otherwise wild type *E. coli* strain that you think may be linked to the **Lac** operon. You grow **P1** phage on the strain with the **Tn5** insertion and use the resulting phage to infect a **LacI⁻** strain. Among the resulting Kan^r transductants, 30% have constitutive **Lac** expression and 70% are regulated normally. What is the distance between **LacI** and the **Tn5** insertion expressed as a cotransduction frequency?

Tn5 LacI$^+$ ⟶ LacI$^-$
donor (regulated) recipient (constitutive)

70% cotransduction of LacI$^+$ + Tn5

(b 12 pts.) Next, you want to map the **Tn5** insertion described in part (a) relative to two different **LacI⁻** mutations (**LacI-1⁻** and **LacI-2⁻**). To do this you perform two reciprocal crosses. In cross 1 you grow **P1** phage on a host that has the **Tn5** insertion and **LacI-1⁻**. The resulting phage are then used to infect a **LacI-2⁻**. Among the Kan^r transductants, 99% are constitutive and 1% are regulated normally. For cross 2, you grow **P1** phage on a host that has the **Tn5** insertion and the **LacI-2⁻** mutation. The resulting phage are then used to infect a **LacI-1⁻** strain. In this experiment, all of the Kan^r transductants are constitutive.

Draw a genetic map showing the relative order of **Tn5**, **LacI-1⁻** and **LacI-2⁻**. Also give any relevant distances (obtained from either the two-factor cross in (a) or the three factor crosses in (b)) expressed as cotransduction frequencies.

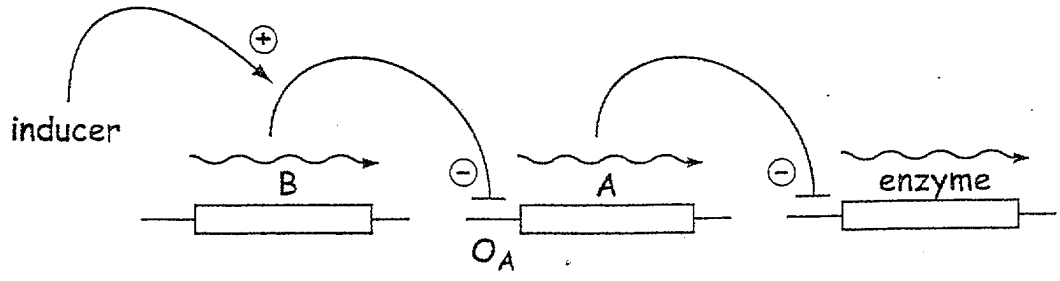


(c 6 pts.) Say that you wanted to isolate a **LacI-1⁻** and **LacI-2⁻** double mutant. Which cross from part (b) would be a better starting point to search for the desired double mutant and why?

Cross 2 would be the better choice because there would be approx. 1% frequency of getting the double mutant, which requires 2 crossovers in cross 2. but 4 crossovers in cross 1.

Name: KEY

3. An enzyme in *E. coli* is regulated by the following scheme:



A is a transcriptional repressor of the enzyme and **B** is a transcriptional repressor of the gene for **A**. **B** is active as a repressor only when it is bound to the inducer molecule. When the inducer is absent, **B** does not bind to its operator, **A** is expressed, and enzyme synthesis is repressed.

(a 12 pts.) An allele of the **B** gene (B^s) is isolated that binds to DNA and represses regardless of whether inducer is present or not. An allele of the operator site of the **A** gene (O^c_A) is isolated that will not bind the **B** repressor. In the table below indicate for each strain whether the enzyme will be synthesized, with or without inducer (use + or –).

	– inducer	+ inducer
B^s	+	+
O^c_A	–	–
$B^s\ O^c_A$	–	–

(b 12 pts.) An allele of the **A** gene is isolated that disrupts the ability of the **A** repressor to bind DNA. In a heterozygous merodiploid this allele will also interfere with the ability of wild type **A** protein to bind DNA. The allele is therefore called A^{-d}. Indicate in the table below when the enzyme will be synthesized.

	– inducer	+ inducer
A^{-d}	+	+
A^{-d} / F' A^+	+	+
$O^+_A\ A^{-d}$ / F' $O^c_A\ A^+$	+	–

constitutive →

↑ inducer turns off transcription of A^{-d}

Name: KEY

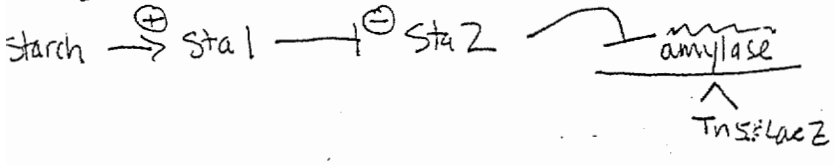
4. To study regulation of starch utilization in *E. coli* you isolate a **Tn5::LacZ** insertion in the gene for the starch degrading enzyme amylase. This insertion only expresses ß-galactosidase when starch is present in the medium. You isolate two mutations that cause altered regulation of the **Tn5::LacZ** reporter. The **sta1⁻** mutation is unlinked to the **Tn5::LacZ** insertion and has uninducible ß-galactosidase expression. The **sta2⁻** mutation is linked to the **Tn5::LacZ** insertion (50% cotransduction) and expresses ß-galactosidase constitutively. By constructing the appropriate merodiploid you determine that the **sta2⁻** mutation is recessive.

(a 15 pts.) Construct two models for amylase regulation that explains all of the properties of the **sta1⁻** and **sta2⁻** mutations. For your models, represent the relevant gene products as **Sta1** and **Sta2**, include the amylase gene, and be sure to indicate where and how the inducer starch acts.

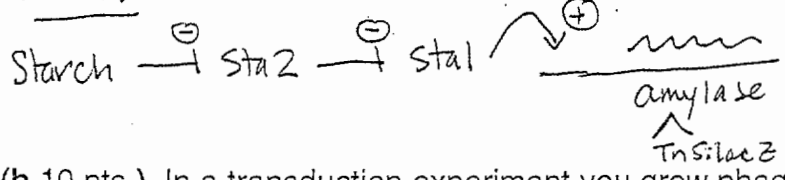
sta1⁻ - trans-acting, uninducible ⇒ activator⁻

sta2⁻ - linked, constitutive ⇒ repressor⁻, not O^c (not dominant)
recessive

Model 1



Model 2



(b 10 pts.) In a transduction experiment you grow phage **P1** on a strain carrying the **Tn5::LacZ** insertion and the **sta2⁻** mutation. You use the resulting phage to infect a **sta1⁻** mutant (which does not carry the **Tn5::LacZ** insertion). About half of the resulting Kan^r transductants constitutively express ß-galactosidase and half have uninducible expression. What does this observation tell you about the phenotype of a **sta1⁻ sta2⁻** double mutant? Which of your models is supported by this observation?

50% cotransduction

recipient - sta1⁻ ⇒ uninducible donor -> constitutive

50% - constitutive ← result of Tn5 + sta2⁻ cotransduction

50% - uninducible ∴ Sta2⁻ is epistatic to Sta1

Model 1 double mutant = constitutive ← Model 1 is correct

Model 2 double mutant = uninducible

7.03 Exam 3

Name: _____

Section: _____ TA: _____

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

There are 6 pages including this cover page
Please write your name on each page.

Question 1	35 points
Question 2	36 points
Question 3	29 points

Name: _____

1. Tay-Sachs disease is an autosomal recessive disorder characterized by degeneration of brain function in infants and death before age 4 years. The disease is more common in Jews of European extraction than in other populations. The incidence of Tay-Sachs disease is about 1 in 250,000 births among non-Jews in North America. Assume random mating in the non-Jewish population in North America.

(a 5 pts.) In non-Jewish North Americans, what is the frequency of the allele (call it allele TS) associated with Tay-Sachs disease?

(b 5 pts.) What is the frequency of heterozygotes among non-Jewish North Americans?

(c 7 pts.) What is the probability that a child born to first cousins (who were non-Jewish North Americans) would have Tay-Sachs disease?

(d 7 pts.) Assuming that the TS allele frequency is at steady state, what is the mutation rate per generation at the Tay-Sachs gene in non-Jewish North Americans?

The incidence of Tay-Sachs disease is much higher in the European Jewish population, where the TS allele frequency is 0.016. Scientists have speculated that the relatively high incidence of Tay-Sachs disease in European Jews might be the result of TS/+ heterozygotes being partially resistant to tuberculosis, an infectious disease that ravaged parts of Europe for centuries.

(e 6 pts.) Calculate the heterozygote advantage h that would (under steady state conditions) account for the frequency of the TS allele in European Jewish populations. Assume random mating in the European Jewish population.

Name: _____

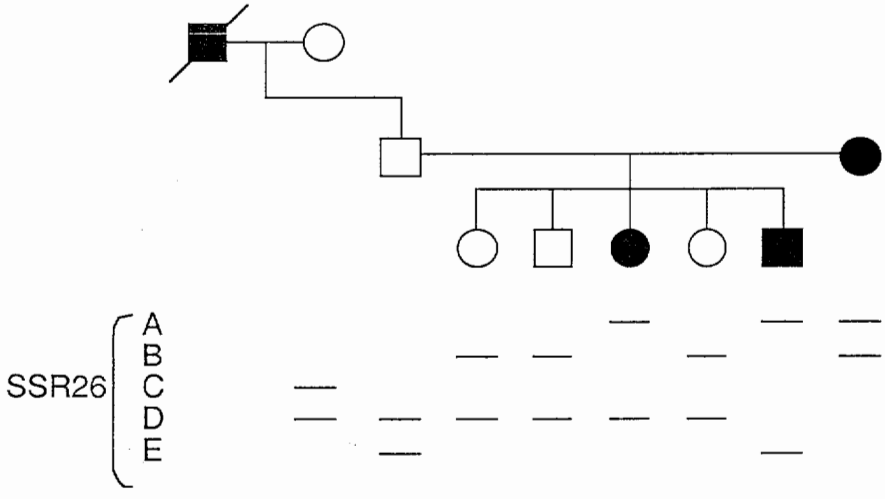
(f 5 pts.) The incidence of tuberculosis in Europe is much lower now than in the past. Assume that, as a result, the heterozygote advantage h falls to zero. By what increment (per generation) would you expect the TS allele frequency to decline in the European Jewish population?

2. On Problem Set 6, we considered the ability to taste PTC (phenylthiocarbamide). The inability to taste PTC is an autosomal recessive trait. The trait is quite common in some populations. You are interested in identifying the critical gene, and you set out to map it through genetic linkage studies.

Alleles: NT (associated with inability to taste PTC)

\+ (associated with ability to taste PTC)

Here is a family in which some individuals (solid circles or squares) could not taste PTC:



(a 3 pts.) What is the genotype of the affected mother (a PTC non-taster) at the PTC gene?

(b 3 pts.) What is the genotype of the father (a PTC taster) at the PTC gene?

(c 5 pts.) What allele at SSR26 did the father inherit from the (deceased) grandfather?

Name: _____

(d 5 pts.) Diagram the phase relationship between the PTC and SSR26 alleles in the father.

(e 5 pts.) Diagram the phase relationship between the PTC and SSR26 alleles in the affected mother.

(f 7 pts.) Calculate the LOD score for linkage at $\theta = 0$ between PTC tasting and SSR26 in this family.

(g 8 pts.) Calculate the LOD score for linkage at $\theta = 0.2$ between PTC tasting and SSR26 in this family.

Name: _____

3. After extensive genetic linkage studies, you map PTC tasting to a 2-centiMorgan (cM) region on human chromosome 7.

(a 5 pts.) Assuming that this 2-cM region is typical of the human genome, how many genes is the region likely to contain?

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

(b 6 pts.) What type of modification to the mouse genome would you make to test the hypothesis that the absence of gene Z results in inability to taste PTC? Explain your choice.

(c 9 pts.) Draw the DNA construct that you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.

(d 4 pts.) Initially you obtain just one mouse that is heterozygous for the genomic modification that you've engineered. How will you obtain homozygotes?

Name: _____

(e 5 pts.) What additional modification to the mouse genome would you make to test the hypothesis that the mouse and human Z genes are functionally interchangeable?

7.03 Exam 3

Name: Key

Section: ∅ TA: _____

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

There are 6 pages including this cover page
Please write your name on each page.

Question 1	35 points
Question 2	36 points
Question 3	29 points

Name: Key

1. Tay-Sachs disease is an autosomal recessive disorder characterized by degeneration of brain function in infants and death before age 4 years. The disease is more common in Jews of European extraction than in other populations. The incidence of Tay-Sachs disease is about 1 in 250,000 births among non-Jews in North America. Assume random mating in the non-Jewish population in North America.

(a 5 pts.) In non-Jewish North Americans, what is the frequency of the allele (call it allele TS) associated with Tay-Sachs disease? $f(TS/TS) = \frac{1}{250,000} \approx q^2$

$$f(TS) = q \approx \sqrt{\frac{1}{250,000}} = 0.002$$

(b 5 pts.) What is the frequency of heterozygotes among non-Jewish North Americans?

$f(TS/+) = 2pq$ $\quad p = 1 - q$, $p = 0.998$

$f(TS/+) = 2(0.998)(0.002) = 0.004$

(c 7 pts.) What is the probability that a child born to first cousins (who were non-Jewish North Americans) would have Tay-Sachs disease?

$p(\text{child born to first cousins has TS}) = Fq = \left(\frac{1}{16}\right)(0.002) = 1.25 \times 10^{-3}$

1st cousins, $F = 4\left(\frac{1}{2}\right)^6 = \frac{1}{16}$

(d 7 pts.) Assuming that the TS allele frequency is at steady state, what is the mutation rate per generation at the Tay-Sachs gene in non-Jewish North Americans?

$\mu = \Delta q_{\text{mutation per generation}}$ $\quad$ at steady state, $0 = \Delta q_{\text{total}} = \Delta q_{\text{mut.}} + \Delta q_{\text{sel.}}$

lethal; $\Delta q_{\text{sel}} = -q^2$ $\quad \hookrightarrow s = 1$

$\mu - q^2 = 0$

$\mu = q^2 = 4 \times 10^{-6}$

The incidence of Tay-Sachs disease is much higher in the European Jewish population, where the TS allele frequency is 0.016. Scientists have speculated that the relatively high incidence of Tay-Sachs disease in European Jews might be the result of TS/+ heterozygotes being partially resistant to tuberculosis, an infectious disease that ravaged parts of Europe for centuries.

(e 6 pts.) Calculate the heterozygote advantage h that would (under steady state conditions) account for the frequency of the TS allele in European Jewish populations. Assume random mating in the European Jewish population. $\mu \approx 0$.

$q = 0.016$ for this population

$q \approx \frac{h}{s}$

$h \approx qs$

$s = 1$

$h \approx 0.016$

or

$\Delta q = 0 = \frac{1}{2}h(2pq) + \mu - q^2$

$\mu \approx 0$

$hpq = q^2 \; (= sq^2)$

$hp = q \; (= sq)$

$p \approx 1$

Name: Key

(f 5 pts.) The incidence of tuberculosis in Europe is much lower now than in the past. Assume that, as a result, the heterozygote advantage h falls to zero. By what increment (per generation) would you expect the TS allele frequency to decline in the European Jewish population?

$h = 0$

$\Delta q_{\text{per gen.}} = \mu - Sq^2$

$\mu \approx 0, \; S = 1$

$\Delta q = -q^2 = -(0.016)^2 = -2.56 \times 10^{-4}$

∴ The TS allele declines by 2.56×10^{-4} per generation

2. On Problem Set 6, we considered the ability to taste PTC (phenylthiocarbamide). The inability to taste PTC is an autosomal recessive trait. The trait is quite common in some populations. You are interested in identifying the critical gene, and you set out to map it through genetic linkage studies.

Alleles: NT (associated with inability to taste PTC)

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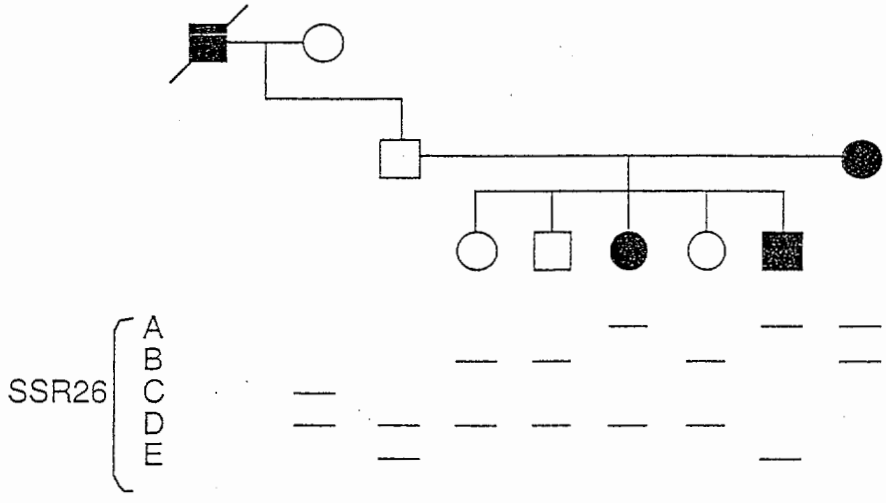
Here is a family in which some individuals (solid circles or squares) could not taste PTC:



(a 3 pts.) What is the genotype of the affected mother (a PTC non-taster) at the PTC gene?

NT/NT

(b 3 pts.) What is the genotype of the father (a PTC taster) at the PTC gene?

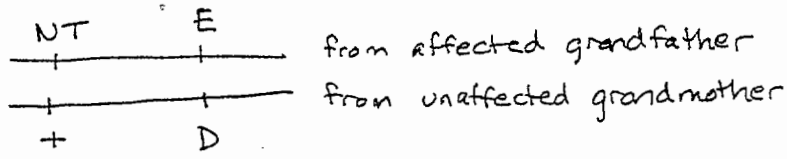
+/NT

(c 5 pts.) What allele at SSR26 did the father inherit from the (deceased) grandfather?

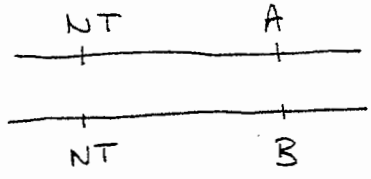
E

Name: Key

(d 5 pts.) Diagram the phase relationship between the PTC and SSR26 alleles in the father.



(e 5 pts.) Diagram the phase relationship between the PTC and SSR26 alleles in the affected mother.



(f 7 pts.) Calculate the LOD score for linkage at θ = 0 between PTC tasting and SSR26 in this family.

$$LOD_\theta = \log_{10} \frac{p(\text{this data if linked at } \theta)}{p(\text{this data if unlinked})}$$

Only consider meioses from heterozygotic father. We know the phase.

$p(\text{if linked at } \theta = 0) = (\frac{1}{2} \cdot 1)^4 (\frac{1}{2} \cdot 0)^1 = 0$

(1 ← child inheriting NT+D (recombination); 4 ← children inheriting E+NT or D++ from father (no recombination))

$p(\text{if unlinked}) = (\frac{1}{2} \cdot \frac{1}{2})^5$

$$LOD_{\theta=0} = \log_{10}(0) = -\infty$$

(g 8 pts.) Calculate the LOD score for linkage at θ = 0.2 between PTC tasting and SSR26 in this family.

$p(\text{if linked at } \theta = 0.2) = (\frac{1}{2} \cdot 0.8)^4 (\frac{1}{2} \cdot 0.2)^1$

$$LOD_{\theta=0.2} = \frac{(0.4)^4(0.1)}{(\frac{1}{4})^5} = 0.418$$

Name: Key

3. After extensive genetic linkage studies, you map PTC tasting to a 2-centiMorgan (cM) region on human chromosome 7.

(a 5 pts.) Assuming that this 2-cM region is typical of the human genome, how many genes is the region likely to contain?

$$\frac{30{,}000 \text{ genes in genome}}{3300 \text{ cM}} \times 2 \text{ cM} \approx 18 \text{ genes}$$

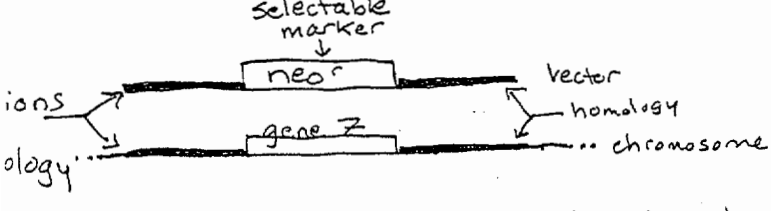
Because of discrepancies in number of genes in genome (30000, 80000, etc), reasonable answers were accepted.

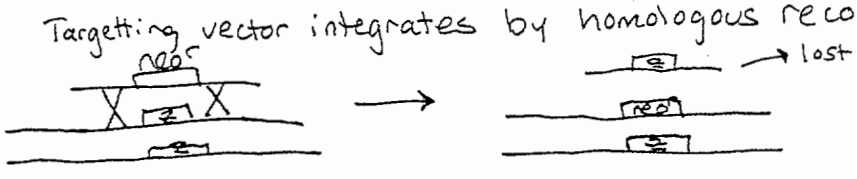
You then discover that some PTC non-tasters are homozygous for a 10-kb deletion within the implicated region. The deletion encompasses gene Z. Your findings suggest but do not prove that the absence of gene Z results in the inability to taste PTC. You decide to test this hypothesis in mice using transgenic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

(b 6 pts.) What type of modification to the mouse genome would you make to test the hypothesis that the absence of gene Z results in inability to taste PTC? Explain your choice.

Make a knock-out of the Z gene, creating a specific deletion of gene Z.

(c 9 pts.) Draw the DNA construct that you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.





(d 4 pts.) Initially you obtain just one mouse that is heterozygous for the genomic modification that you've engineered. How will you obtain homozygotes?

Cross heterozygote to wild type mouse.
Select heterozygotes from the offspring (check by PCR).
½ are heterozygotes, ½ are wild-type.
Breed heterozygotic siblings to each other.
¼ of their offspring will be homozygous.
They can be identified by PCR assay.

Name: Key

(e 5 pts.) What additional modification to the mouse genome would you make to test the hypothesis that the mouse and human Z genes are functionally interchangeable?

Make a transgenic mouse with no copies of the mouse gene and one added copy of the human gene.

You can do this either by adding the human transgene into a knock-out mouse, or by adding the human transgene into a wild-type and breeding the resulting transgenic mouse to a knock-out mouse.

The transgene construct is injected into the male pronucleus of a fertilized egg where it inserts randomly into the genome.

7.03 Final Exam

Name: _____

Images removed due to copyright reasons.

Please identify your TA by circling their name.

Also, please circle the two professors below who taught this course:

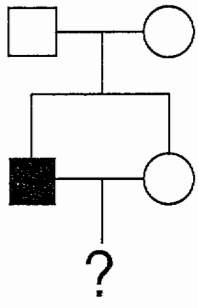
Images removed due to copyright reasons.

There are 13 pages including this cover page.
Please write your name on each page.

Question 1	25 points
Question 2	25 points
Question 3	24 points
Question 4	30 points
Question 5	31 points
Question 6	27 points
Question 7	10 points
Question 8	28 points

Name: _____

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



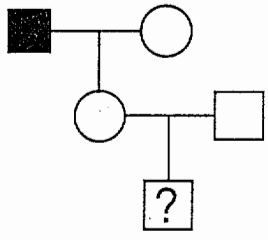
(a 5 pts.) Assuming that the trait is autosomal recessive, calculate the probability that an offspring from the indicated brother-sister mating will exhibit the trait.

(b 5 pts.) Assuming that the trait is X-linked recessive, calculate the probability that a male offspring from the brother-sister mating will exhibit the trait.

(c 5 pts.) Assuming that the trait is X-linked recessive, calculate the probability that a female offspring from the brother-sister mating will exhibit the trait.

Name: _____

(d 10 pts.) Consider the following mouse pedigree in which a male mouse that exhibits two different recessive X-linked traits (indicated by the filled symbol) is crossed to a true breeding wild type female and a female offspring from this cross is mated to a true breeding wild type male.



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

2. Wild type *E. coli* is motile. You have isolated a nonmotile strain that you designate **mot1⁻**. In order to find a transposon linked to **mot1⁻** you start with a large collection of different random **Tn5** insertions in an otherwise wild type *E. coli* strain (these insertion strains are kanamycin resistant (**Kan^r**) and motile (**mot$^+$**)). You grow **P1** phage on a mixture of the entire collection of **Tn5** insertion strains and then infect the **mot1⁻** mutant and select for **Kan^r** transductants. Out of 500 **Kan^r** transductants, 1 is motile (499 are nonmotile). You designate this motile, **Kan^r** transductant **strain 1**. Next, you grow **P1** on **strain 1** and use the resulting phage to infect your original **mot1⁻** strain selecting for **Kan^r** transductants. Out of 100 **Kan^r** transductants 70 are motile and 30 are nonmotile.

(a 8 pts.) What is the distance between the **Tn5** insertion and **mot1⁻** expressed as a cotransduction frequency?

Name: _____

(b 7 pts.) Next, you isolate a second nonmotile mutant, designated **mot2⁻**. You grow **P1** on **strain 1** and use the resulting phage to infect your **mot2⁻** strain. After selection, you isolate 100 **Kan^r** transductants. All of these transductants are nonmotile. Based on this result, what conclusion can you draw about the distance between the **mot2⁻** mutation and the **Tn5** insertion in **strain 1**?

(c 10 pts.) Finally, you isolate a third nonmotile mutation designated **mot3⁻**. In transduction experiments you discover that **mot3⁻** is tightly linked to **mot1⁻**. To determine the relative order of the **mot3⁻** and **mot1⁻** mutations you set up two different transduction experiments. 1) You grow **P1** on **strain 1** that also carries **mot1⁻** and use the resulting phage to infect your **mot3⁻** strain selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants 12 are motile and 488 are nonmotile. 2) You grow **P1** on **strain 1** that also carries **mot3⁻** and use the resulting phage to infect your **mot1⁻** strain selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants all are nonmotile.

Draw a genetic map showing the relative order of **Tn5**, **mot1⁻** and **mot3⁻**. Also give any relevant distances expressed as cotransduction frequencies.

Name: _____

3. (**a** 8 pts.) The **cI** gene of phage lambda encodes a repressor protein that has a molecular weight of 24 kDa. You have isolated a phage mutant with a defective **cI** gene and therefore makes clear plaques rather than the normal turbid plaques. But you find that the mutant phage will produce turbid plaques when plated on an *E. coli* strain that contains an amber suppressing mutation (the amber codon is UAG). You find that the repressor protein is 16 kDa when the mutant phage are grown on wild type *E. coli*, but is 24 kDa when the mutant phage are grown on an amber suppressing strain. What can you deduce about the mutation? Be as specific as possible about the molecular nature of the mutation and where the mutation lies within the **cI** gene.

(**b** 8 pts.) Next you mutagenize the mutant phage described in part **a** to isolate a double mutant. This double mutant still forms clear plaques when plated on wild type *E. coli*, but when you examine the repressor protein produced by the double mutant grown on wild type *E. coli*, you find that it is larger than the protein produced by the original mutant (the protein produced by the original mutant is 16 kDa whereas the protein produced by the double mutant is 17 kDa). Describe what kind of second mutation could give these results, assuming that the second mutation was caused by a single mutational event. Be as specific as possible.

(**c** 8 pts.) The codon for tryptophan is UGG. 1) Write out the DNA base sequence of the segment of the wild type $tRNA^{trp}$ gene that codes for the anticodon sequence ($tRNA^{trp}$ = tryptophan tRNA). For your answer, only show the DNA strand that is used as the template for transcription of the tRNA molecule, indicating the 5' and 3' ends. 2) Using the same format, write out the DNA base sequence of the same segment of an amber suppressing allele of the $tRNA^{trp}$ gene.

Name: _____

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

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

(a 5 pts.) You isolate a recessive mutation, designated **Arg2⁻**, which shows uninducible arginase expression. When you mate an **Arg2⁻** mutant to an **Arg1⁻** mutant, the resulting **Arg2⁻ / Arg1⁻** diploid shows normal arginase expression and regulation. What does this result tell you about the nature of the **Arg2⁻** mutation, and what can you deduce about the normal role in arginase regulation of the cellular function disrupted by the **Arg2⁻** mutation?

(b 10 pts.) Next, you isolate a mutation, designated **Arg3⁻**, which shows constitutive arginase expression. In a cross of an **Arg3⁻** mutant to an **Arg1⁻** mutant gives the following tetrad types. Out of a total of 50 tetrads, 8 are Type 1, 7 are Type 2, and 35 are Type 3.

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

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

Name: _____

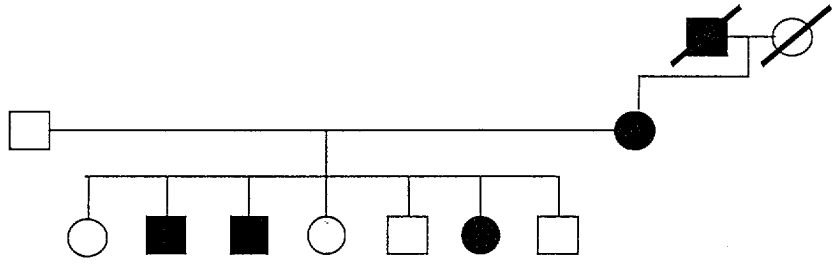
(c 5 pts.) A cross of an **Arg3⁻** mutant to an **Arg2⁻** mutant gives the following tetrad types. Out of a total of 50 tetrads, 35 are Type 1, 8 are Type 2, and 7 are Type 3.

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

Is an **Arg3⁻ Arg2⁻** double mutant regulated, constitutive, or uninducible? Which tetrad type has the most double mutants?

(d 10 pts.) On the basis of your answer for part **c** and from the rest of the information given in this problem, diagram a model to explain the regulation of arginase. For your model, include the **Arg1**, **Arg2** and **Arg3** genes. Also show how arginine itself might act.

5. A physician seeks your advice regarding a family in which several individuals (filled circles or squares below) developed colon cancer in their 30's or 40's. All living, unaffected individuals are in their 60's or older.

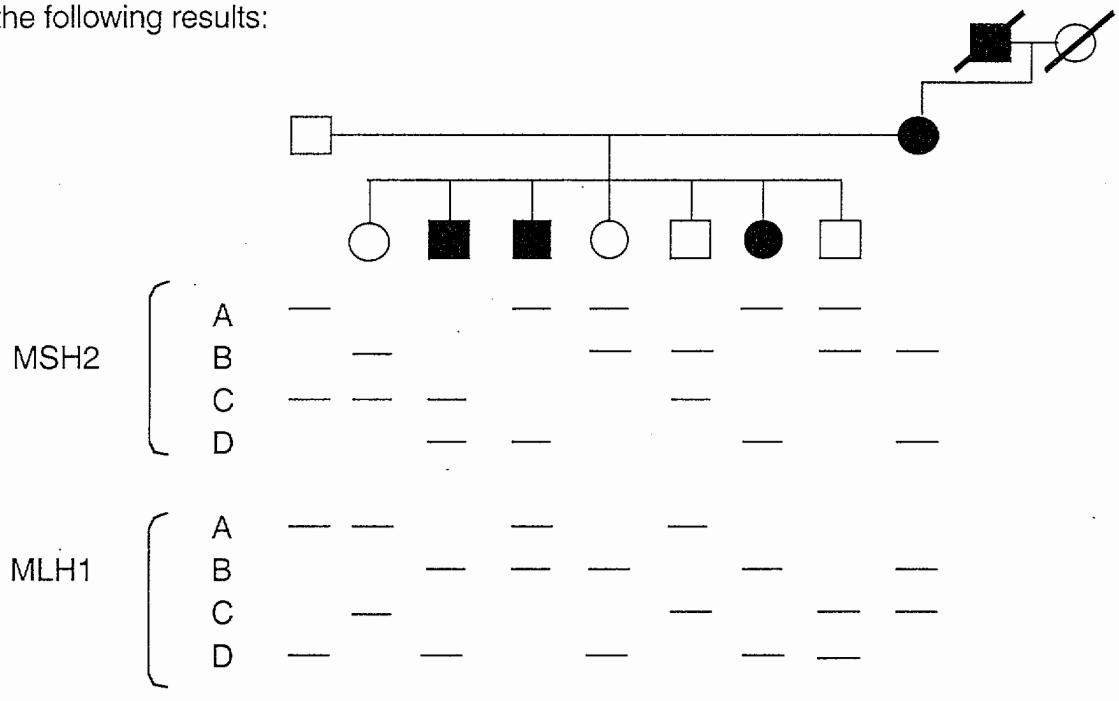


You hypothesize that colon cancer in this family is caused by germline transmission of a mutation in one of two genes involved in mismatch repair: MSH2 or MLH1. To test this hypothesis, you obtain blood DNA samples from all living family members, and you obtain colon tumor DNA samples from the four living, affected individuals.

Name: _____

(a 4 pts.) If your hypothesis is correct, what would you expect to see if you typed the four colon tumor DNA samples for an SSR that is not linked to either MSH2 or MLH1?

You identify an SSR that is located within an intron of the MSH2 gene, and a second SSR that is located within an intron of the MLH1 gene. You type blood DNA samples for these SSRs and obtain the following results:



(b 5 pts.) Calculate a LOD score for linkage at $\theta = 0$ between colon cancer and MSH2 in this family.

(c 5 pts.) Calculate a LOD score for linkage at $\theta = 0.1$ between colon cancer and MLH1 in this family.

Name: _____

(d 3 pts.) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in MSH2? Briefly justify your answer.

(e 3 pts.) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in MLH1? Briefly justify your answer.

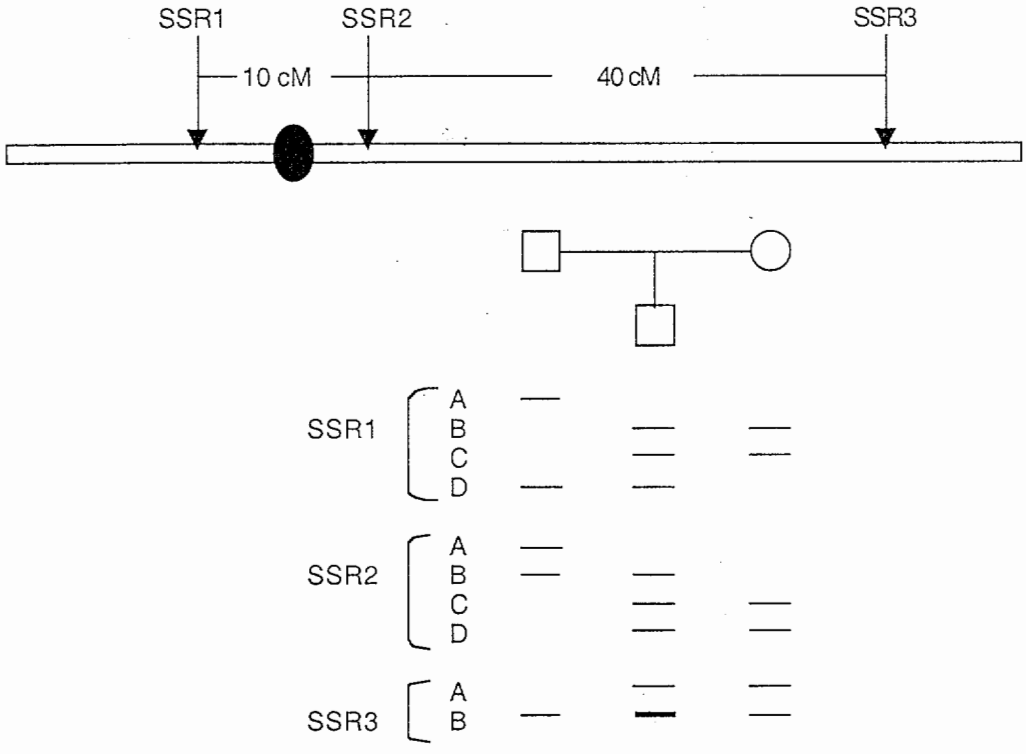
(f 3 pts.) Would your answer to part **e** change if the "MLH1" SSR data shown had been obtained with an SSR located 8 Mb 5' of the MLH1 gene (rather than within an MLH1 intron)? Briefly justify your answer.

(g 3 pts.) You learn that, in this family, the two women who developed colon cancer also developed second cancers: in one case, cancer of the uterus, and in the other case, cancer of the ovary. Would this increase or diminish your suspicion that colon cancer in this family is caused by germline transmission of a mutation in a mismatch repair gene? Briefly justify your answer.

(h 5 pts.) In an unrelated family with colon cancer, you demonstrate that affected individuals are heterozygous for a nonsense mutation in MSH2. A colleague, noting the autosomal dominant inheritance of colon cancer in the second family, suggests that you generate mice to which you've added the mutant human MSH2 gene as a transgene (randomly inserted). Would you expect these transgenic mice to develop cancers more quickly, more slowly, or at the same rate as wild-type mice? Briefly justify your answer.

Name: _____

6. Only a small fraction of human fetuses with trisomy 18 survive to birth, and most of those surviving to birth die in infancy. You prepare DNA samples from umbilical cord blood of a newborn baby with trisomy 18, and from his parents. You then type the baby and his parents for three STSs distributed along chromosome 18:



(a 3 pts.) Did nondisjunction occur before or after fertilization? On what evidence do you base your conclusion?

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

(b 3 pts.) In which parent did nondisjunction occur?

(c 4 pts.) In which division of meiosis did nondisjunction occur?

Name: _____

(**d** 5 pts.) Sketch the meiotic event in which nondisjunction occurred. Your drawing should include the SSRs present along chromosome 18.

(**e** 3 pts.) In humans, how many chromosomes are normally present in the first polar body?

(**f** 3 pts.) In humans, how many chromosomes are normally present in the second polar body?

(**g** 3 pts.) In this case of trisomy 18, how many chromosomes would have been present in the first polar body?

(**h** 3 pts.) In this case of trisomy 18, how many chromosomes would have been present in the second polar body?

Name: _____

7. Birds have ZW sex chromosomes. Males are ZZ, and females are ZW. In birds, it is not known whether sex is determined by the number of Z chromosomes or by the presence or absence of the W chromosome. Being a student of mammalian and fruitfly sex determination, you conclude that the question could be resolved if birds with particular numerical abnormalities of the sex chromosomes could be identified.

(**a** 5 pts.) What numerical abnormalities of the bird sex chromosomes would you seek?

(**b** 5 pts.) Having identified birds with the desired numerical abnormalities, how would you settle the question stated above?

8. Mendel's concept of the gene was first applied to a human trait in Archibald Garrod's landmark1902 paper entitled "The Incidence of Alkaptonuria: A Study in Chemical Individuality." Alkaptonuria is a disease characterized by degenerative arthritis and by urine which turns black upon exposure to air. Because of an enzyme defect, the urine accumulates homogentisic acid, which oxidizes to form a black pigment.

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

(**a** 4 pts.) Is alkaptonuria an autosomal dominant or autosomal recessive disorder? Briefly justify your answer.

Name: _____

(b 5 pts.) In the United Kingdom, what is the frequency of the allele (call it allele AK) associated with alkaptonuria?

(c 3 pts.) In the United Kingdom, what is the frequency of heterozygotes?

(d 4 pts.) What is the expected proportion of all alleles (at all autosomal genes) that first cousins share by descent?

(e 4 pts.) What is the expected proportion of all autosomal genes at which offspring of first cousins are homozygous by descent?

(f 4 pts.) Based on the data given here, estimate the frequency of first-cousin marriages in the United Kingdom.

(g 4 pts.) Now assume allelic heterogeneity. Would you modify your responses to any of the questions above? Briefly justify your answer.

2000

7.03 Final Exam

Name: Key

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Please identify your TA by circling their name.

Also, please circle the two professors below who taught this course:

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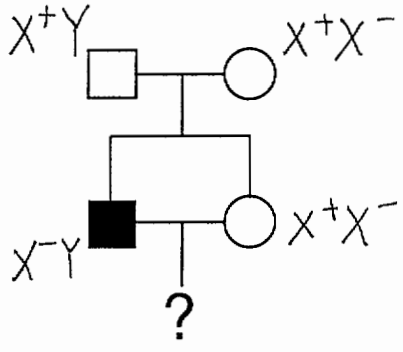
There are 13 pages including this cover page.
Please write your name on each page.

Question 1	25 points
Question 2	25 points
Question 3	24 points
Question 4	30 points
Question 5	31 points
Question 6	27 points
Question 7	10 points
Question 8	28 points

Name: Key

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



+ = WT
− = trait

(a 5 pts.) Assuming that the trait is <u>autosomal recessive</u>, calculate the probability that an offspring from the indicated brother-sister mating will exhibit the trait.

$$P(\text{offspring exhibits trait}) = P(\text{MOM is a heterozygote}) \times 1/2$$
$$= 2/3 \times 1/2$$
$$= 1/3$$

(b 5 pts.) Assuming that the trait is <u>X-linked recessive</u>, calculate the probability that a <u>male</u> offspring from the brother-sister mating will exhibit the trait.

$$P(\text{male offspring exhibits trait}) = P(\text{MOM is } X^+X^-) \times P(\text{getting } X^- \text{ from MOM})$$
$$= 1/2 \times 1/2$$
$$= 1/4$$

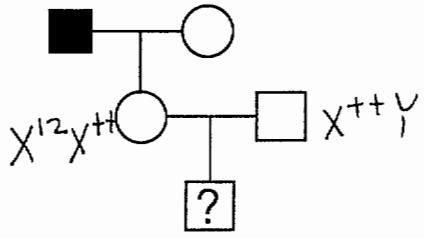
(c 5 pts.) Assuming that the trait is <u>X-linked recessive</u>, calculate the probability that a <u>female</u> offspring from the brother-sister mating will exhibit the trait.

$$P(\text{female offspring exhibits trait}) = P(X^- \text{ from Dad}) \times P(\text{MOM is } X^+X^-) \times P(X^- \text{ from MOM})$$
$$= 1 \times 1/2 \times 1/2$$
$$= 1/4$$

Name: Key

(d 10 pts.) Consider the following mouse pedigree in which a male mouse that exhibits two different recessive X-linked traits (indicated by the filled symbol) is crossed to a true breeding wild type female and a female offspring from this cross is mated to a true breeding wild type male.

1 = recessive trait 1
2 = recessive trait 2
+ = WT.



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

$P(\text{male offspring exhibits both traits}) = P(\text{no crossover in MOM}) \times P(\text{getting } X^{12} \text{ from MOM})$

$= (1 - 0.1) \times 1/2$

$= 0.9 \times 1/2$

$= 0.45$

2. Wild type *E. coli* is motile. You have isolated a nonmotile strain that you designate **mot1⁻**. In order to find a transposon linked to **mot1⁻** you start with a large collection of different random **Tn5** insertions in an otherwise wild type *E. coli* strain (these insertion strains are kanamycin resistant (**Kan^r**) and motile (**mot$^+$**)). You grow **P1** phage on a mixture of the entire collection of **Tn5** insertion strains and then infect the **mot1⁻** mutant and select for **Kan^r** transductants. Out of 500 **Kan^r** transductants, 1 is motile (499 are nonmotile). You designate this motile, **Kan^r** transductant **strain 1**. Next, you grow **P1** on **strain 1** and use the resulting phage to infect your original **mot1⁻** strain selecting for **Kan^r** transductants. Out of 100 **Kan^r** transductants 70 are motile and 30 are nonmotile.

(a 8 pts.) What is the distance between the **Tn5** insertion and **mot1⁻** expressed as a cotransduction frequency?

$$\text{Distance} = \frac{\#\text{ cotransductants of Tn5 \& mot1}}{\text{Total}} \times 100\% = \frac{70}{100} \times 100\% = 70\%$$

Name: Key

(b 7 pts.) Next, you isolate a second nonmotile mutant, designated **mot2⁻**. You grow **P1** on **strain 1** and use the resulting phage to infect your **mot2⁻** strain. After selection, you isolate 100 **Kan^r** transductants. All of these transductants are nonmotile. Based on this result, what conclusion can you draw about the distance between the **mot2⁻** mutation and the **Tn5** insertion in **strain 1**?

No cotransduction of Mot2$^+$ and Tn5. Therefore, distance between Mot2$^-$ and Tn5 insertion is greater than length of DNA which "fits" into one phage head. One phage headful = 10^5 bp. Thus, greater than 10^5 bp.

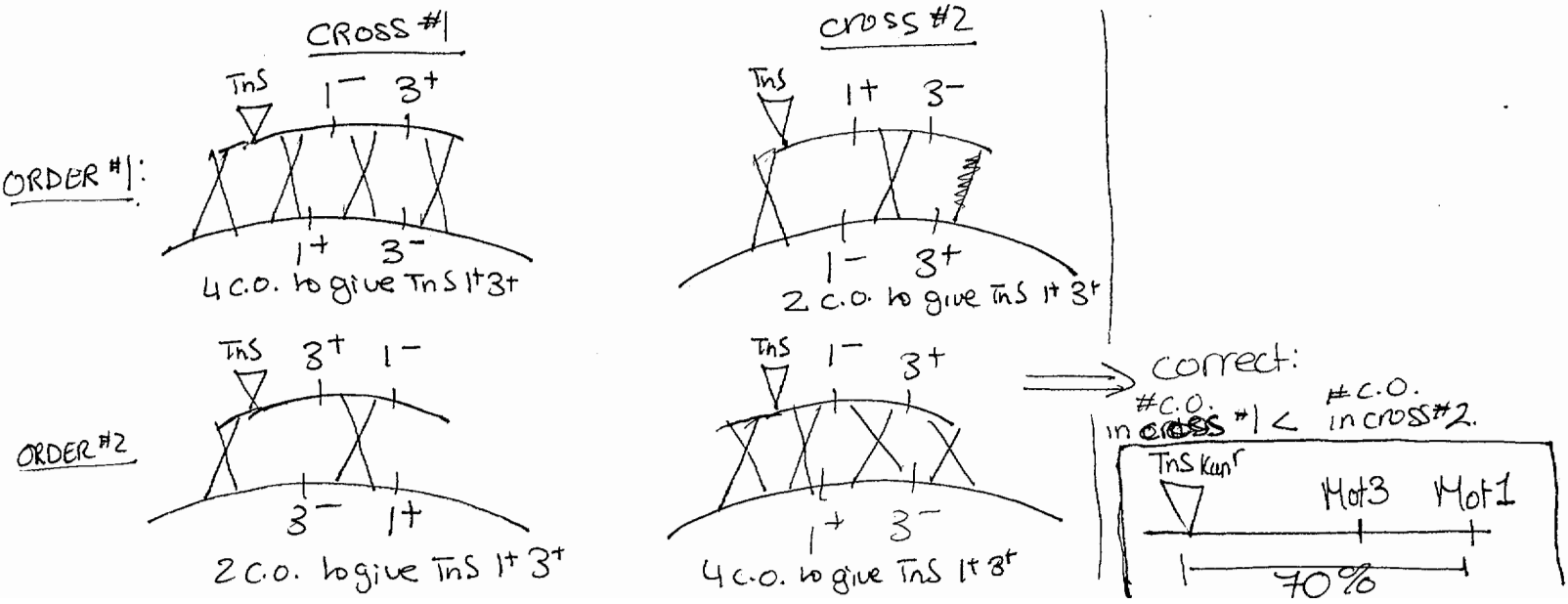
(c 10 pts.) Finally, you isolate a third nonmotile mutation designated **mot3⁻**. In transduction experiments you discover that **mot3⁻** is tightly linked to **mot1⁻**. To determine the relative order of the **mot3⁻** and **mot1⁻** mutations you set up two different transduction experiments. 1) You grow **P1** on **strain 1** that also carries **mot1⁻** and use the resulting phage to infect your **mot3⁻** strain selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants 12 are motile and 488 are nonmotile. 2) You grow **P1** on **strain 1** that also carries **mot3⁻** and use the resulting phage to infect your **mot1⁻** strain selecting for **Kan^r** transductants. Out of 500 **Kan^r** transductants all are nonmotile.

Draw a genetic map showing the relative order of **Tn5**, **mot1⁻** and **mot3⁻**. Also give any relevant distances expressed as cotransduction frequencies.

rare class: Kan^r Mot1$^+$ Mot3$^+$

given: # of rare class from cross #1 > # of rare class from cross #2

∴ # crossovers in cross #1 < # of crossovers in cross #2



Name: Key

3. **(a** 8 pts.) The **cI** gene of phage lambda encodes a repressor protein that has a molecular weight of 24 kDa. You have isolated a phage mutant with a defective **cI** gene and therefore makes clear plaques rather than the normal turbid plaques. But you find that the mutant phage will produce turbid plaques when plated on an *E. coli* strain that contains an amber suppressing mutation (the amber codon is UAG). You find that the repressor protein is 16 kDa when the mutant phage are grown on wild type *E. coli*, but is 24 kDa when the mutant phage are grown on an amber suppressing strain. What can you deduce about the mutation? Be as specific as possible about the molecular nature of the mutation and where the mutation lies within the **cI** gene.

the mutation must be a nonsense mutation that, by definition, makes a stop codon at the codon corresponding to ~ 2/3 of the protein length.

it cannot be a frameshift because then it would not be ~~read~~ suppressed by an amber suppressor

(b 8 pts.) Next you mutagenize the mutant phage described in part **a** to isolate a double mutant. This double mutant still forms clear plaques when plated on wild type *E. coli*, but when you examine the repressor protein produced by the double mutant grown on wild type *E. coli*, you find that it is larger than the protein produced by the original mutant (the protein produced by the original mutant is 16 kDa whereas the protein produced by the double mutant is 17 kDa). Describe what kind of second mutation could give these results, assuming that the second mutation was caused by a single mutational event. Be as specific as possible.

frameshift earlier than the 1st mutation, changing the stop codon @ 16 kDa but creating a new one soon after.

(c 8 pts.) The codon for tryptophan is UGG. 1) Write out the DNA base sequence of the segment of the wild type $tRNA^{trp}$ gene that codes for the anticodon sequence ($tRNA^{trp}$ = tryptophan tRNA). For your answer, only show the DNA strand that is used as the template for transcription of the tRNA molecule, indicating the 5' and 3' ends. 2) Using the same format, write out the DNA base sequence of the same segment of an amber suppressing allele of the $tRNA^{trp}$ gene.

(1)

5' — TGG — 3' DNA template strand

3' (ACC — 5'

$tRNA^{trp}$

(2)

5' — TAG — 3' DNA template strand

3' (AUC — 5'

$tRNA^{trp}$

Name: Key

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

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

(a 5 pts.) You isolate a recessive mutation, designated **Arg2⁻**, which shows uninducible arginase expression. When you mate an **Arg2⁻** mutant to an **Arg1⁻** mutant, the resulting **Arg2⁻ / Arg1⁻** diploid shows normal arginase expression and regulation. What does this result tell you about the nature of the **Arg2⁻** mutation, and what can you deduce about the normal role in arginase regulation of the cellular function disrupted by the **Arg2⁻** mutation?

arg1⁻ & arg2⁻ complement ⇒ diff^ent genes

arg2 is likely to be an activator

(b 10 pts.) Next, you isolate a mutation, designated **Arg3⁻**, which shows constitutive arginase expression. In a cross of an **Arg3⁻** mutant to an **Arg1⁻** mutant gives the following tetrad types. Out of a total of 50 tetrads, 8 are Type 1, 7 are Type 2, and 35 are Type 3.

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

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

Type I = ~~PD~~ TT PD >> NPD ⇒ linked

Type II = NPD

Type III = PD

$$cM = \frac{6NPD + 8T}{2\Sigma} = 50\ cM$$

Name: Key

(c 5 pts.) A cross of an **Arg3⁻** mutant to an **Arg2⁻** mutant gives the following tetrad types. Out of a total of 50 tetrads, 35 are Type 1, 8 are Type 2, and 7 are Type 3.

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

Is an **Arg3⁻ Arg2⁻** double mutant regulated, constitutive, or uninducible? Which tetrad type has the most double mutants?

double mutant is constitutive

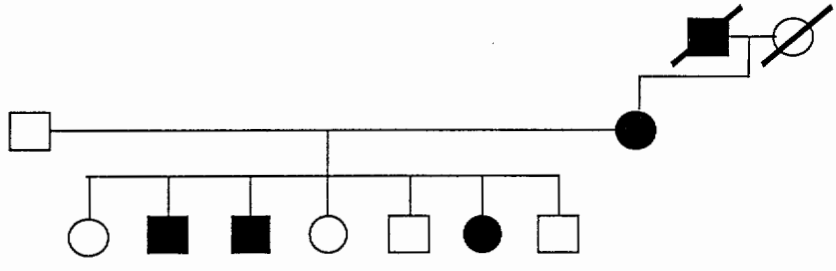
Type 2 = NPD have 2 double mutants per tetrad

(d 10 pts.) On the basis of your answer for part **c** and from the rest of the information given in this problem, diagram a model to explain the regulation of arginase. For your model, include the **Arg1**, **Arg2** and **Arg3** genes. Also show how arginine itself might act.

arg3⁻ is epistatic to arg2⁻ arg1 = arginase

arginine → arg2 ⊣ arg3 ⊣ arg1

5. A physician seeks your advice regarding a family in which several individuals (filled circles or squares below) developed colon cancer in their 30's or 40's. All living, unaffected individuals are in their 60's or older.



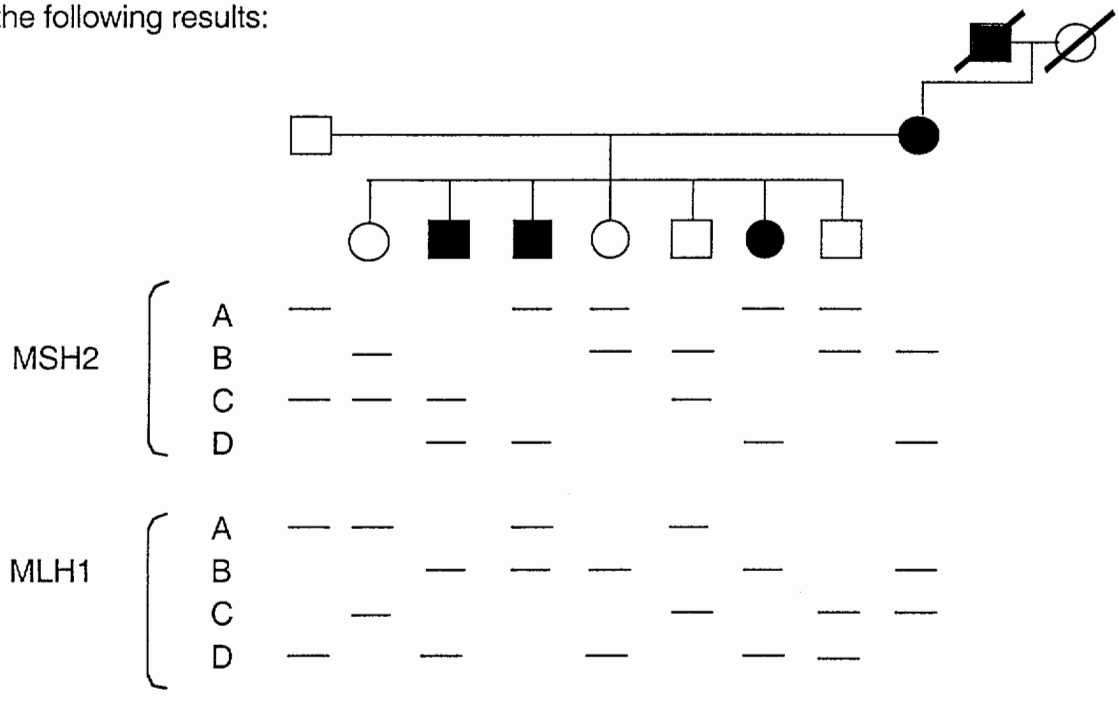
You hypothesize that colon cancer in this family is caused by germline transmission of a mutation in one of two genes involved in mismatch repair: MSH2 or MLH1. To test this hypothesis, you obtain blood DNA samples from all living family members, and you obtain colon tumor DNA samples from the four living, affected individuals.

Name: Key

(a 4 pts.) If your hypothesis is correct, what would you expect to see if you typed the four colon tumor DNA samples for an SSR that is not linked to either MSH2 or MLH1?

The SSR will not show linkage to the disease. More importantly, tumor DNA samples will have multiple new alleles of the SSR (mutator effect) due to loss of function of a mismatch repair gene.

You identify an SSR that is located within an intron of the MSH2 gene, and a second SSR that is located within an intron of the MLH1 gene. You type blood DNA samples for these SSRs and obtain the following results:



(b 5 pts.) Calculate a LOD score for linkage at $\theta = 0$ between colon cancer and MSH2 in this family. Mother is informative because heterozygotic.

$$LOD_{\theta=0} = \log_{10} \frac{P(\text{data if totally linked})}{P(\text{if unlinked})} = \log_{10} \frac{\frac{1}{2}(0)^7 + \frac{1}{2}\left(\frac{1}{2}\right)^7}{\left(\frac{1}{4}\right)^7}$$

$$= 1.8$$

(c 5 pts.) Calculate a LOD score for linkage at $\theta = 0.1$ between colon cancer and MLH1 in this family.

$$LOD_{\theta=0.1} = \log_{10} \frac{\frac{1}{2}(0.05)(0.45)^6 + \frac{1}{2}(0.45)(0.05)^6}{\left(\frac{1}{4}\right)^7} = 0.53$$

Name: _____

(d 3 pts.) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in MSH2? Briefly justify your answer.

Yes. $LOD_{\theta=0} > 0$. The disease shows complete linkage with the SSR in this data. (However, it is not of publishable significance because $LOD < 3$.)

(e 3 pts.) Are these data consistent with the specific hypothesis that colon cancer in this family is caused by germline transmission of a mutation in MLH1? Briefly justify your answer.

No. Because the SSR is in an intron of the gene, the presence of a single recombinant (child 4) is inconsistent with the hypothesis that colon cancer in the family is caused by a mutation in MLH1.

(f 3 pts.) Would your answer to part **e** change if the "MLH1" SSR data shown had been obtained with an SSR located 8 Mb 5' of the MLH1 gene (rather than within an MLH1 intron)? Briefly justify your answer.

Yes. 8Mb ≈ 8cM (because 1Mb ≈ 1cM), so we would expect 8% recombination between this SSR and MLH1 (not 0% as would be expected if the SSR is in an intron). The presence of the single recombinant in the family is not inconsistent with 8cM distance between the disease-causing allele and the SSR. (It does not prove that MLH1 causes disease.)

(g 3 pts.) You learn that, in this family, the two women who developed colon cancer also developed second cancers: in one case, cancer of the uterus, and in the other case, cancer of the ovary. Would this increase or diminish your suspicion that colon cancer in this family is caused by germline transmission of a mutation in a mismatch repair gene? Briefly justify your answer.

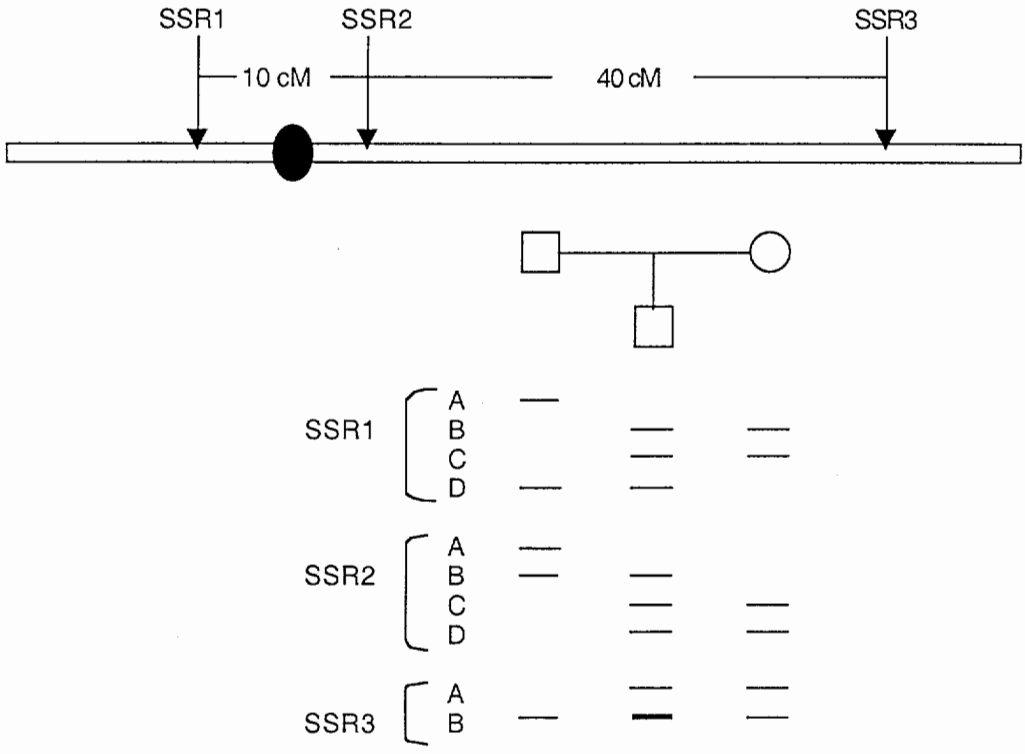
This would increase suspicion, because germline transmission of a mutation in a mismatch repair gene would be expected to cause a higher risk of increased mutation rates (leading to cancers) in all cells in members of the family.

(h 5 pts.) In an unrelated family with colon cancer, you demonstrate that affected individuals are heterozygous for a nonsense mutation in MSH2. A colleague, noting the autosomal dominant inheritance of colon cancer in the second family, suggests that you generate mice to which you've added the mutant human MSH2 gene as a transgene (randomly inserted). Would you expect these transgenic mice to develop cancers more quickly, more slowly, or at the same rate as wild-type mice? Briefly justify your answer.

Cancers should develop at the same rate because the mice still have their own good copies of mismatch repair genes- adding in a mutant gene that doesn't produce a functional product should have no phenotypic effect.

Name: KEY

6..Only a small fraction of human fetuses with trisomy 18 survive to birth, and most of those surviving to birth die in infancy. You prepare DNA samples from umbilical cord blood of a newborn baby with trisomy 18, and from his parents. You then type the baby and his parents for three STSs distributed along chromosome 18:



(**a** 3 pts.) Did nondisjunction occur before or after fertilization? On what evidence do you base your conclusion? BEFORE: - MEIOSIS I IN THE MOTHER OCCURS PRIOR TO FERTILIZATION.

- IF THIS WERE MITOTIC NONDISJUNCTION, YOU COULD NOT HAVE BOTH MATERNAL ALLELES AT SSR1 (WOULD HAVE 2xB OR 2 COPIES OF C)

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

(**b** 3 pts.) In which parent did nondisjunction occur?

THE MOTHER - THE CHILD RECEIVED TWO COPIES OF CHR. 18 FROM HER MOTHER, AS INDICATED BY THE SSRs.

(**c** 4 pts.) In which division of meiosis did nondisjunction occur?

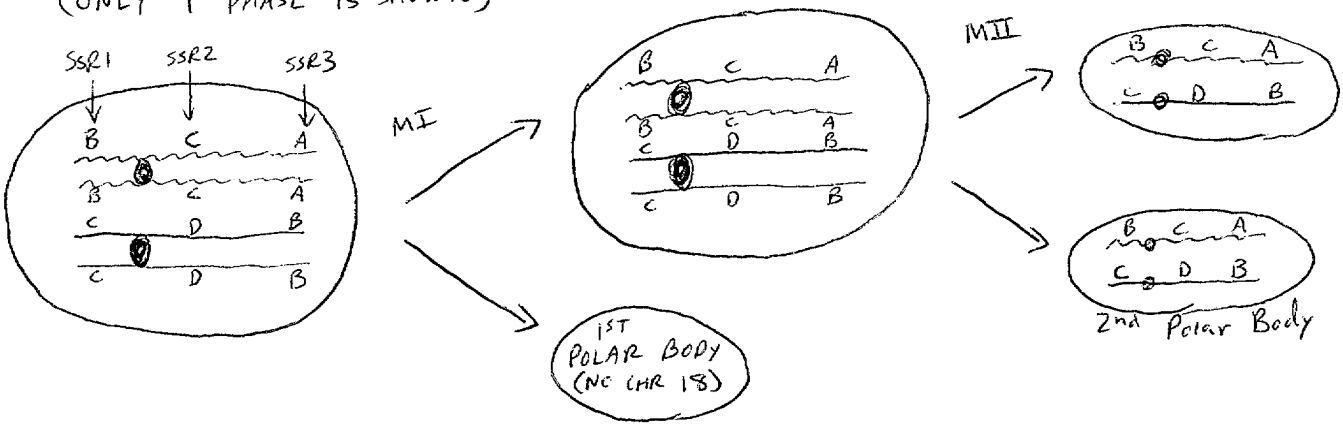
MEIOSIS I - THE TWO CHROMOSOMES THAT THE MOTHER PASSED ON HAD DIFFERENT CENTROMERIC MARKERS (1 COPY OF EACH HOMOLOGUE).

Name: KEY

(d 5 pts.) Sketch the meiotic event in which nondisjunction occurred. Your drawing should include the SSRs present along chromosome 18.

MI NDJ: NO CROSSOVERS (OR TWO BTWN A SET OF MARKERS)

(ONLY 1 PHASE IS SHOWN)



(e 3 pts.) In humans, how many chromosomes are normally present in the first polar body?

23 CHROMOSOMES, CONSISTING OF 46 CHROMATIDS

(f 3 pts.) In humans, how many chromosomes are normally present in the second polar body?

23 CHROMOSOMES (CHROMATIDS HAVE SEP'TED)

(g 3 pts.) In this case of trisomy 18, how many chromosomes would have been present in the first polar body?

22 CHROMOSOMES, CONSISTING OF 44 CHROMATIDS

(h 3 pts.) In this case of trisomy 18, how many chromosomes would have been present in the second polar body?

24 CHROMOSOMES

Name: Key

7. Birds have ZW sex chromosomes. Males are ZZ, and females are ZW. In birds, it is not known whether sex is determined by the number of Z chromosomes or by the presence or absence of the W chromosome. Being a student of mammalian and fruitfly sex determination, you conclude that the question could be resolved if birds with particular numerical abnormalities of the sex chromosomes could be identified.

(a 5 pts.) What numerical abnormalities of the bird sex chromosomes would you seek?

Zϕ , ZZW

(b 5 pts.) Having identified birds with the desired numerical abnormalities, how would you settle the question stated above?

If Zϕ ♀, ZZW♂ then # of Z's determines sex

If Zϕ♂, ZZW♀ then W determines sex

8. Mendel's concept of the gene was first applied to a human trait in Archibald Garrod's landmark1902 paper entitled "The Incidence of Alkaptonuria: A Study in Chemical Individuality." Alkaptonuria is a disease characterized by degenerative arthritis and by urine which turns black upon exposure to air. Because of an enzyme defect, the urine accumulates homogentisic acid, which oxidizes to form a black pigment.

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

(a 4 pts.) Is alkaptonuria an autosomal dominant or autosomal recessive disorder? Briefly justify your answer.

Autosomal recessive.

If the disorder was autosomal dominant, there wouldn't be such higher occurrence of the disorder in offspring of first-cousin matings.

Name: _____

(b 5 pts.) In the United Kingdom, what is the frequency of the allele (call it allele AK) associated with alkaptonuria?

$q^2_{\text{in random}} = \frac{1}{2}\left(\frac{1}{250,000}\right)$; $q^2_{\text{random}} = \frac{1}{500,000}$

$q_{\text{random}} = \sqrt{\frac{1}{500,000}} = \boxed{0.0014}$

(c 3 pts.) In the United Kingdom, what is the frequency of heterozygotes?

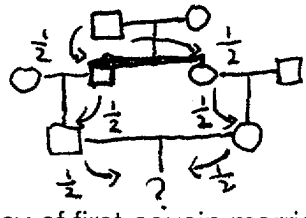
$2pq = 2(1-q)q = 2(0.9986)(0.0014) = \boxed{0.0028}$

(d 4 pts.) What is the expected proportion of all alleles (at all autosomal genes) that first cousins share by descent?

$r = 3$ $\quad \left(\frac{1}{2}\right)^3 = \frac{1}{8}$

(e 4 pts.) What is the expected proportion of all autosomal genes at which offspring of first cousins are homozygous by descent?

$F_{\substack{\text{first}\\\text{cousins}}} = \frac{1}{16}$



$\left(\frac{1}{64}\right)\cdot 4 = \frac{1}{16}$

(f 4 pts.) Based on the data given here, estimate the frequency of first-cousin marriages in the United Kingdom.

$F = \frac{1}{16}$

$F \cdot q \cdot x = \left(\frac{1}{2}\right)\left(\frac{1}{250,000}\right)$

$q = 0.0014$

or

$\boxed{x = 0.022}$ $\quad F \cdot q \cdot x = q^2(1-x)$ $\quad$ or $\quad \frac{F \cdot q \cdot X}{F \cdot q \cdot X + q^2(1-x)} = \frac{1}{2}$

(g 4 pts.) Now assume allelic heterogeneity. Would you modify your responses to any of the questions above? Briefly justify your answer.

No. Because the alleles are in the same gene, it would make no difference.