Exams

Fall 1999

7.03 Exam 1

Name: _____

Section: _____ **TA:** _____

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

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

Please...

- 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	**30 points**
Question 2	**40 points**
Question 3	**30 points**

Name: _____

1. A true breeding mouse strain exhibits two different rare traits. When a male from the true breeding strain is crossed to a wild-type female, all of the female F_1 progeny exhibit both traits whereas all of the male F_1 progeny look wild type.

(a 10 pts.**)** What is the mode of inheritance of the two traits?

(b 10 pts.**)** The male and female F_1 mice described above are crossed to one another to produce F_2 progeny. Of the male F_2 progeny, 40% have both traits (the rest of the F_2 males either appear wild type or have only one trait or the other). What fraction of the female F_2 progeny would you expect to have both traits?

(c 10 pts.**)** What is the map distance (in cM) between the genes for the two traits?

Name: _____

2. **(a** 6 pts.) You have isolated a yeast mutant that makes small colonies. When you mate your haploid mutant to a haploid wild-type strain, the resulting diploids look like wild type. What does this observation tell us about your mutant?

(b 8 pts.) When the diploids from part **(a)** are sporulated, all of the tetrads appear to be PDs in the sense that they each have two small colonies and two normal sized colonies. What does this observation tell us about your mutant?

(c 6 pts.) You isolate a second mutant that also makes small colonies. When a haploid of one small mutant is mated to a haploid of the other small mutant, the resulting diploids appear normal. What is the relationship between the two small mutant strains?

Name: _____

(d 10 pts.**)** When the diploids from part **(c)** are sporulated, three types of tetrads are found.

Type I have 4 small colonies

Type II have 1 normal and 3 small colonies

Type III have 2 normal and 2 small colonies

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

What is the map distance between the two mutations?

(e 10 pts.**)** Give your best estimate for the number of tetrads (out of 50 total) described in part **(d)** that result from two crossovers in the interval between the two mutations.

Name: _____

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

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

(a 10 pts.) What is the distance between the **r1⁻** and the **cI⁻** mutations in map units?

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

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

(b 10 pts.) What is the distance between the **r1⁻** and **r2⁻** mutations in map units?

Name: _____

(c 10 pts.**)** Draw a genetic map showing the relative order of the **cI**$^{-}$, **r1**$^{-}$, and **r2**$^{-}$ mutations as well as the distances that you have determined.

7.03 Exam 1

Name: SOLUTIONS

Section: _____ TA: _____

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

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Question 1	**30 points**
Question 2	**40 points**
Question 3	**30 points**

Name: Solutions

1. A true breeding mouse strain exhibits two different rare traits. When a male from the true breeding strain is crossed to a wild-type female, all of the female F_1 progeny exhibit both traits whereas all of the male F_1 progeny look wild type.

(a 10 pts.) What is the mode of inheritance of the two traits?

X-linked Dominant

A, B are dominant:

$X^{AB}Y \times X^{++}X^{++}$

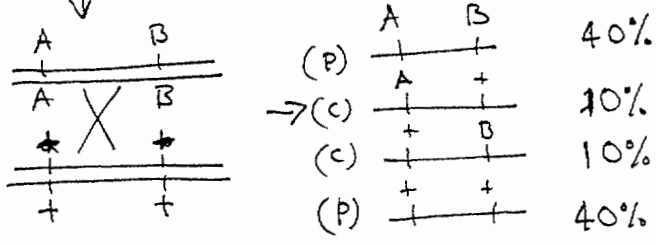
$\downarrow$

F_1 $\underbrace{X^{AB}X^{++}}_{\text{Exhibits both traits}}$ and $\underbrace{X^{++}Y}_{\text{wild-type}}$

(b 10 pts.) The male and female F_1 mice described above are crossed to one another to produce F_2 progeny. Of the male F_2 progeny, 40% have both traits (the rest of the F_2 males either appear wild type or have only one trait or the other). What fraction of the female F_2 progeny would you expect to have both traits?

From part (a): $X^{AB}X^{++} \times X^{++}Y$

$\downarrow$



Since A, B are dominant: $X^{AB}X^{++}$ show both traits.

$\therefore$ 40%

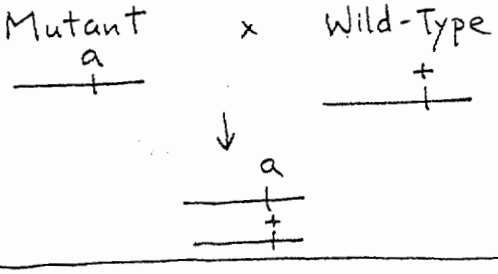
(c 10 pts.) What is the map distance (in cM) between the genes for the two traits?

$$\text{Distance} = \frac{\#\text{ crossover gametes}}{\text{total }\#\text{ gametes}} \times 100$$

$$= \frac{10+10}{100} \times 100 = \boxed{20\text{ cM}}$$

Name: Solutions

2. **(a** 6 pts.) You have isolated a yeast mutant that makes small colonies. When you mate your haploid mutant to a haploid wild-type strain, the resulting diploids look like wild type. What does this observation tell us about your mutant?



Since diploid a/+ is wild-type, mutation is recessive.

(b 8 pts.) When the diploids from part **(a)** are sporulated, all of the tetrads appear to be PDs in the sense that they each have two small colonies and two normal sized colonies. What does this observation tell us about your mutant?

Since all are PDs:

① Only ONE gene involved (more likely)

or

② 2 mutations very, very close together (less likely)

(c 6 pts.) You isolate a second mutant that also makes small colonies. When a haploid of one small mutant is mated to a haploid of the other small mutant, the resulting diploids appear normal. What is the relationship between the two small mutant strains?

Because the mutants complement each other, there are 2 recessive mutations on DIFFERENT genes.

Name: Solutions

(d 10 pts.) When the diploids from part **(c)** are sporulated, three types of tetrads are found.

		☆
Type I have 4 small colonies	PD	24
Type II have 1 normal and 3 small colonies	T	24
Type III have 2 normal and 2 small colonies	NPD	2

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

What is the map distance between the two mutations?

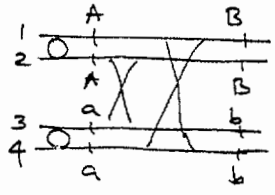
$$\text{Distance} = \frac{T + 6NPD}{2\Sigma} \times 100 = \frac{24 + 6(2)}{2(50)} \times 100 = \boxed{36cM}$$

(e 10 pts.) Give your best estimate for the number of tetrads (out of 50 total) described in part **(d)** that result from two crossovers in the interval between the two mutations.

2 Possible Answers:

① MORE LIKELY:

BETTER ANSWER



1st crossover	2nd crossover	Type
2, 3	2, 3	PD
2, 3	1, 4	NPD
2, 3	1, 3	T
2, 3	2, 4	T

∴ For every NPD, there are 3 more "hidden" double crossovers, so total # of double crossovers is equal to $4(NPD) = 4(2) = 8$.

OR

② $\#\text{d.c.o.} = p(\text{two crossovers}) * (\text{total \# of tetrads})$

$= (.36)(.36) * (50) = \sim 6.5$

↑ probability of ONE crossover.

Name: Solutions

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

$$\frac{r1^- \quad cI^+}{r1^+ \quad cI^-} \times \downarrow$$

Plaque Phenotype	Number	
rough, turbid	44	$r1^- cI^+$ (P)
rough, clear	4	$r1^- cI^-$ (C)
smooth, turbid	6	$r1^+ cI^+$ (C)
smooth, clear	46	$r1^+ cI^-$ (P)

(a 10 pts.) What is the distance between the **r1⁻** and the **cI⁻** mutations in map units?

$$\text{Distance} = \frac{4+6}{100} \times 100 = \boxed{10 \text{ m.u.}}$$

don't need to multiply by 2 b/c no "hidden" crossover phenotypes

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

Plaque Phenotype	Number	
rough, turbid	491	
rough, clear	499	
smooth, turbid	9	] crossovers
smooth, clear	1	] crossovers

(b 10 pts.) What is the distance between the **r1⁻** and **r2⁻** mutations in map units?

There are 10 crossovers, but there are reciprocal pairs with phenotypes that are "hidden" (i.e. double $r1^- r2^-$ mutants that appear as "rough"). So we need to multiply by 2:

$$\therefore \text{Distance} = \frac{2(10)}{100} \times 100 = \boxed{2 \text{ m.u.}}$$

Name: Solutions

(c 10 pts.) Draw a genetic map showing the relative order of the **cI⁻**, **r1⁻**, and **r2⁻** mutations as well as the distances that you have determined.

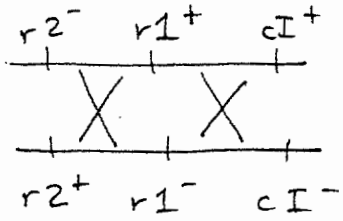
From part (b), the RAREST class is smooth, clear

or $r1^+$ $r2^+$ cI^-

The mutants we crossed were:

$r2^-$ $r1^+$ cI^+ × $r2^+$ $r1^-$ cI^-

↓



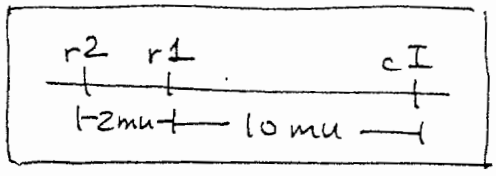
d.c.o. →

$r2^-$ $r1^-$ cI^+

$r2^+$ $r1^+$ cI^-

↑ smooth clear (rarest class)

∴ The gene map must be:



7.03 Exam 2

Name: _____

Section: _____ **TA:** _____

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

There are eight pages including this cover page
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Question 1	**25 points**
Question 2	**35 points**
Question 3	**40 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. **(a** 7 pts.**)** Hydroxylamine causes C•G to T•A mutations. After treatment of λ phage with hydroxylamine you isolate a mutant that forms clear plaques on a wild-type *E. coli* host, but will form turbid plaques on a host that carries an amber (UAG) suppressor. List the codons in wild-type λ that could have been mutated to produce the mutant phage.

(b 8 pts.**)** Would you expect mutagenesis of the λ mutant described above by treatment of the phage with hydroxylamine to generate revertants that can make turbid plaques on wild-type *E. coli*? Why or why not?

Name: _____

(c 10 pts.) The following sequence (and encoded amino acids) lies within the coding sequence of a wild-type *E. coli* gene:

```
CTC TCT TTC ATG ACT AGG CTG TTG AAG
leu ser ser met thr arg leu leu lys
```

A mutant is isolated that has an additional A residue giving the sequence:

```
CTC TCT TTC ATG ACAT AGG CTG TTG AAG
```

Describe a possible suppressor mutation that might revert the defect of the mutation shown above (do not simply describe the back mutation). For your answer, (i) State whether this is an intragenic or extragenic suppressor, (ii) show the exact sequence change that gives the suppressor mutation, (iii) give the amino acid sequence of the mutant gene sequence with the suppressor, and (iv) describe any properties that this part of the protein sequence must have in order for the suppressor to restore function to the mutated gene.

Name: _____

2. The **ProB** gene lies close to the **Lac** operon, and **Lac** mutations and **ProB** mutations are cotransduced at a frequency of about 80%. You have isolated a new **Tn5** insertion near **ProB** and **Lac**. In transduction experiments with this insertion, **Lac** mutants are cotransduced 45% with Kan^r, and **ProB** mutants are cotransduced 35% with Kan^r.

(a 8 pts.) Draw a map showing the relative order of **ProB**, **Lac**, and **Tn5**. Also indicate as many of the map distances as you can.

(b 6 pts.) From an otherwise wild-type strain carrying the **Tn5** insertion described above, you isolate an **Hfr** that transfers **ProB** early and efficiently, but transfers **Lac** genes late and inefficiently. Draw a diagram showing the structure of the **Hfr** and indicate whether Kan^r will be transferred early or late.

(c 6 pts.) You want to use the **Hfr** described in part **(b)** to isolate an **F'** that carries the **Lac** genes. Describe briefly how you would select for the desired **F'**. Be sure to indicate the genotypes of all strains that you use.

Name: _____

Next, you want to map the **Tn5** insertion described in part **(a)** relative to mutations within the **Lac** operon. To do this you perform two reciprocal crosses. In cross 1 you grow P1 phage on a host that has the **Tn5** insertion and a **LacO^{c}** mutation (this strain gives constitutive expression of **LacZ**). The resulting phage are then used to infect a **LacI^{s}** strain (this strain gives uninducible expression of **LacZ**). Among the Kan^{r} transductants, 50% are constitutive, 48% are uninducible, and 2% are regulated by IPTG. For cross 2, you grow P1 phage on a host that has the **Tn5** insertion and a **LacI^{s}** mutation. The resulting phage are then used to infect a **LacO^{c}** strain, and among the Kan^{r} transductants 50% are constitutive and 50% are uninducible.

(d 10 pts.**)** Draw a genetic map showing the relative order of **Tn5**, **LacO**, and **LacI**. Also give any relevant distances expressed as cotransduction frequencies.

(e 5 pts.**)** For each of the crosses there is a transductant class that you know is the result of a quadruple crossover. Give the phenotype of the quadruple crossover class from cross 1.

Name: _____

3. You are studying the regulation of methanol utilization in bacteria. Methanol oxidase, encoded by the **Mox** gene, is the key enzyme in the methanol utilization pathway. Methanol oxidase is expressed at high levels when methanol is present in the growth medium, but methanol oxidase is not expressed when methanol is absent. You find a mutation designated $\mathbf{A^-}$, which gives constitutive **Mox** expression and is closely linked to **Mox** gene mutations. You have $\mathbf{Mox^-}$ and $\mathbf{A^-}$ mutations as well as an **F'** that carries the **Mox** gene along with neighboring genes and regulatory sites, you carry out the following genetic tests:

	Methanol oxidase activity	
	– methanol	+ methanol
$A^+ Mox^+$	–	+
$A^- Mox^+$	+	+
$A^- Mox^+ / F' A^+ Mox^+$	–	+
$A^- Mox^+ / F' A^+ Mox^-$	–	+
$A^- Mox^- / F' A^+ Mox^+$	–	+

(a 10 pts.) Give as complete a description as you can of the properties of the $\mathbf{A^-}$ mutation, and propose a molecular function for the regulatory component that is affected by the $\mathbf{A^-}$ mutation.

Next, you isolate two regulatory mutations that are not linked to **Mox** but that are very closely linked to each other. You call these mutations $\mathbf{B1^-}$ and $\mathbf{B2^-}$. An **F'** is isolated that carries the region of the chromosome where the **B** mutations lie. Genetic tests reveal the following properties:

	Methanol oxidase activity	
	– methanol	+ methanol
$B1^- Mox^+$	+	+
$B2^- Mox^+$	–	–
$B1^- Mox^+ / F' B^+$	–	+
$B2^- Mox^+ / F' B^+$	–	–

Name: _____

(b 5 pts.) Why can't you use a complementation test to determine whether the **B1⁻** and **B2⁻** mutations lie in the same gene?

(c 10 pts.) Assuming that the **B1⁻** and **B2⁻** mutations are in fact in the same gene, propose a molecular function for the regulatory component encoded by the **B** gene. Also describe the molecular defects caused by the **B1⁻** and **B2⁻** mutations (be as specific as possible).

(d 12 pts.) Draw two different models showing the possible relationships between the two different regulatory factors encoded by **A** and **B**. For your answer be sure to include the **Mox** gene and to indicate where and how the inducer methanol is acting.

Name: _____

(e 5 pts.) To distinguish the two models, you construct an **A^- $B2^-$** double mutant. Why is it better to choose the **$B2^-$** rather than the **$B1^-$** allele for this double mutant epistasis test?

(f 8 pts.) You find that the **A^- $B2^-$** double mutant has the following behavior:

	Methanol oxidase activity	
	– methanol	+ methanol
A^- $B2^-$ Mox^+	–	–

Draw a final model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the **Mox** gene and to indicate where and how methanol acts.

7.03 Exam 2

Name: Solutions

Section: _____ TA: _____

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	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: Solutions

1. (**a** 7 pts.) Hydroxylamine causes C•G to T•A mutations. After treatment of λ phage with hydroxylamine you isolate a mutant that forms clear plaques on a wild-type *E. coli* host, but will form turbid plaques on a host that carries an amber (UAG) suppressor. List the codons in wild-type λ that could have been mutated to produce the mutant phage.

Mutated λ: mRNA: 5′ U A G 3′
DNA: 5′ T A G 3′

Original λ: DNA: ① CAG ② TGG

mRNA: CAG → Gln; UGG → Trp

③ The G in TAG was not the result of a mutation because hydroxylamine can cause G→A mutations BUT NOT A→G mutations

(**b** 8 pts.) Would you expect mutagenesis of the λ mutant described above by treatment of the phage with hydroxylamine to generate revertants that can make turbid plaques on wild-type *E. coli*? Why or why not?

No. Hydroxylamine can only make the base pair changes: C→T and G→A, so the mutated λ DNA 5′ T A G 3′ could only be further mutated to 5′ T A A 3′. TAA gives us UAA in the mRNA, which codes for a STOP codon. Therefore, hydroxylamine cannot cause back mutations.

Name: Solutions

(c 10 pts.) The following sequence (and encoded amino acids) lies within the coding sequence of a wild-type *E. coli* gene:

```
CTC TCT TTC ATG ACT AGG CTG TTG AAG
leu ser ser met thr arg leu leu lys
```

A mutant is isolated that has an additional A residue giving the sequence:

```
CTC TCT TTC ATG ACAT AGG CTG TTG AAG
```

Describe a possible suppressor mutation that might revert the defect of the mutation shown above (do not simply describe the back mutation). For your answer, (i) State whether this is an intragenic or extragenic suppressor, (ii) show the exact sequence change that gives the suppressor mutation, (iii) give the amino acid sequence of the mutant gene sequence with the suppressor, and (iv) describe any properties that this part of the protein sequence must have in order for the suppressor to restore function to the mutated gene.

i) An intragenic suppressor: ① single bp deletion (-1 frameshift) OR ② two bp insertion (+2 frameshift)

* Note: Either suppressing mutation must occur before the "G" nucleotide following the original insertion. Otherwise, translation will be terminated by a premature stop codon.

ii) Single bp deletion (the "C" right before the original insertion):
Mutant gene seq with suppressor:

CTC TCT TTC ATG AAT AGG CTG TTG AAG

iii) leu ser phe met asn arg leu leu lys
(asn: changed a.a.)

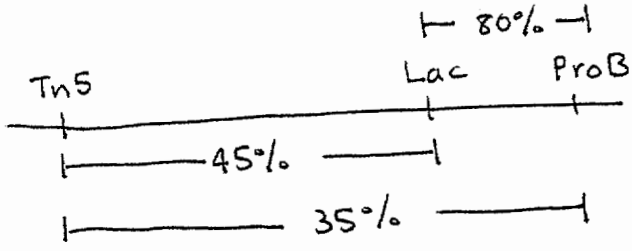
iv) In order for the suppressor to restore function to the mutated gene:

- the Thr → Asn mutation must NOT lie in a part of the protein that is essential for catalysis and/or proper folding.

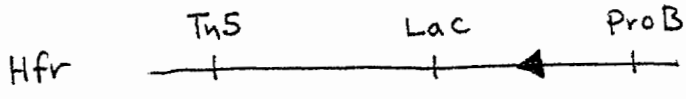
Name: Solutions

2. The **ProB** gene lies close to the **Lac** operon, and **Lac** mutations and **ProB** mutations are cotransduced at a frequency of about 80%. You have isolated a new **Tn5** insertion near **ProB** and **Lac**. In transduction experiments with this insertion, **Lac** mutants are cotransduced 45% with Kan^r, and **ProB** mutants are cotransduced 35% with Kan^r.

(**a** 8 pts.) Draw a map showing the relative order of **ProB**, **Lac**, and **Tn5**. Also indicate as many of the map distances as you can.



(**b** 6 pts.) From an otherwise wild-type strain carrying the **Tn5** insertion described above, you isolate an **Hfr** that transfers **ProB** early and efficiently, but transfers **Lac** genes late and inefficiently. Draw a diagram showing the structure of the **Hfr** and indicate whether Kan^r will be transferred early or late.



Transfers Kan^r late

(**c** 6 pts.) You want to use the **Hfr** described in part **(b)** to isolate an **F'** that carries the **Lac** genes. Describe briefly how you would select for the desired **F'**. Be sure to indicate the genotypes of all strains that you use.

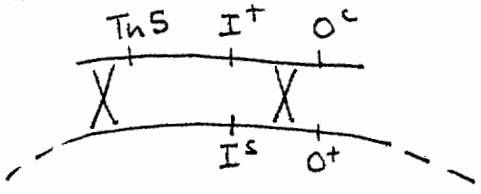
- Mate to a strain that is Kan^s, Lac$^-$
- Select for Kan^r and early transfer of Lac$^+$
- To select against donor (Kan^r, Lac$^+$, ProB$^+$), you'd also select for ProB$^-$.

Name: Solutions

Next, you want to map the **Tn5** insertion described in part **(a)** relative to mutations within the **Lac** operon. To do this you perform two reciprocal crosses. In cross 1 you grow P1 phage on a host that has the **Tn5** insertion and a **LacO^c** mutation (this strain gives constitutive expression of **LacZ**). The resulting phage are then used to infect a **LacI^s** strain (this strain gives uninducible expression of **LacZ**). Among the Kan^r transductants, 50% are constitutive, 48% are uninducible, and 2% are regulated by IPTG. For cross 2, you grow P1 phage on a host that has the **Tn5** insertion and a **LacI^s** mutation. The resulting phage are then used to infect a **LacO^c** strain, and among the Kan^r transductants 50% are constitutive and 50% are uninducible.

(d 10 pts.) Draw a genetic map showing the relative order of **Tn5**, **LacO**, and **LacI**. Also give any relevant distances expressed as cotransduction frequencies.

Cross I:

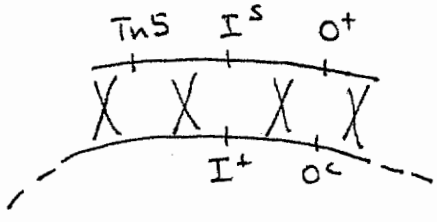


50% constitutive = Kan^r O^c I$^+$
Kan^r O^c I^s (rare)

48% uninducible = Kan^r O$^+$ I^s

2% inducible = Kan^r O$^+$ I$^+$

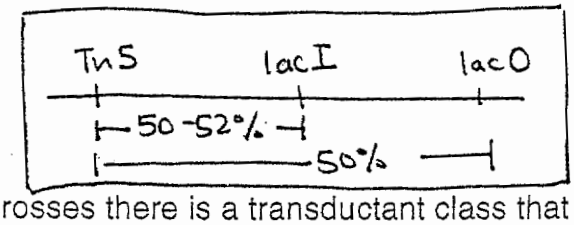
Cross II:



50% constitutive = Kan^r O^c I^s
Kan^r O^c I$^+$

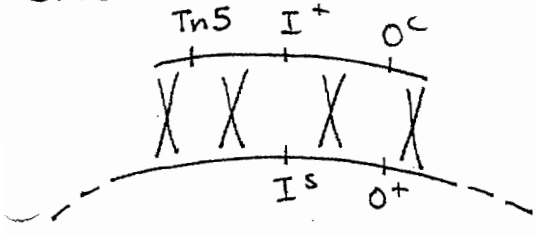
50% uninducible = Kan^r O$^+$ I^s

0% inducible = Kan^r O$^+$ I$^+$



(e 5 pts.) For each of the crosses there is a transductant class that you know is the result of a quadruple crossover. Give the phenotype of the quadruple crossover class from cross 1.

Cross I:



= Tn5 I^s O^c = constitutive

Name: Solutions

3. You are studying the regulation of methanol utilization in bacteria. Methanol oxidase, encoded by the **Mox** gene, is the key enzyme in the methanol utilization pathway. Methanol oxidase is expressed at high levels when methanol is present in the growth medium, but methanol oxidase is not expressed when methanol is absent. You find a mutation designated $\mathbf{A^-}$, which gives constitutive **Mox** expression and is closely linked to **Mox** gene mutations. You have $\mathbf{Mox^-}$ and $\mathbf{A^-}$ mutations as well as an **F'** that carries the **Mox** gene along with neighboring genes and regulatory sites, you carry out the following genetic tests:

		Methanol oxidase activity: − methanol	+ methanol	
w.t.	$A^+ Mox^+$	−	+	inducible
	$A^- Mox^+$	+	+	constitutive
dom/rec?	$A^- Mox^+ / F' A^+ Mox^+$	−	+	recessive
cis/trans?	$A^- Mox^+ / F' A^+ Mox^-$	−	+	} trans-acting
	$A^- Mox^- / F' A^+ Mox^+$	−	+	

(a 10 pts.) Give as complete a description as you can of the properties of the $\mathbf{A^-}$ mutation, and propose a molecular function for the regulatory component that is affected by the $\mathbf{A^-}$ mutation.

Recessive, trans-acting, constitutive:

REPRESSOR

Next, you isolate two regulatory mutations that are not linked to **Mox** but that are very closely linked to each other. You call these mutations $\mathbf{B1^-}$ and $\mathbf{B2^-}$. An **F'** is isolated that carries the region of the chromosome where the **B** mutations lie. Genetic tests reveal the following properties:

		Methanol oxidase activity: − methanol	+ methanol	
	$B1^- Mox^+$	+	+	constitutive
	$B2^- Mox^+$	−	−	uninducible
dom/rec?	$B1^- Mox^+ / F' B^+$	−	+	recessive
	$B2^- Mox^+ / F' B^+$	−	−	dominant

Name: Solutions

(**b** 5 pts.) Why can't you use a complementation test to determine whether the **B1**$^-$ and **B2**$^-$ mutations lie in the same gene?

B2$^-$ is a dominant mutation

$\Rightarrow$ cannot perform complementation test.

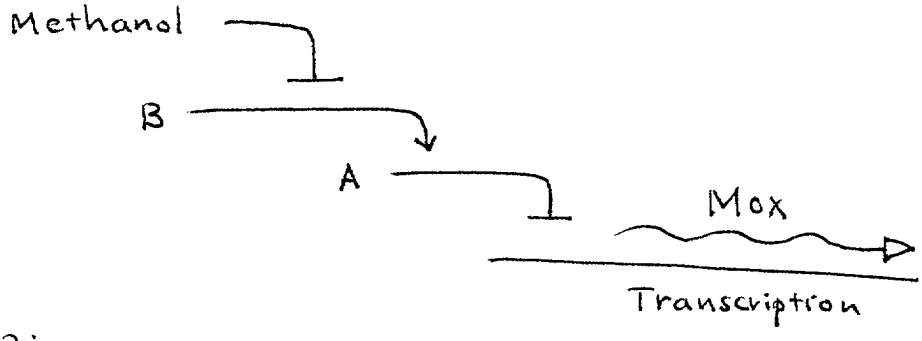
(**c** 10 pts.) Assuming that the **B1**$^-$ and **B2**$^-$ mutations are in fact in the same gene, propose a molecular function for the regulatory component encoded by the **B** gene. Also describe the molecular defects caused by the **B1**$^-$ and **B2**$^-$ mutations (be as specific as possible).

B could also be a repressor:

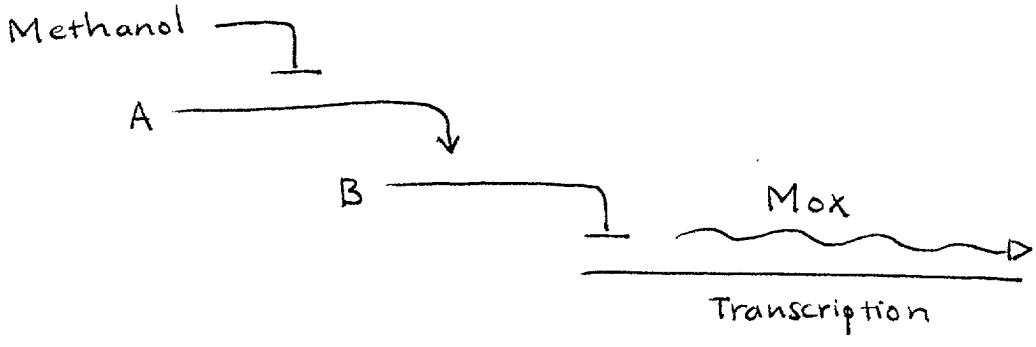
- B1$^-$ could be a mutation which prevents the repressor from binding to the operator (just like loss of function mutation in lacI$^-$)
- B2$^-$ could be a mutation which prevents the inducer from binding to the repressor, which normally inactivates the repressor (just like the "super repressor" mutation in lacI^s)

(**d** 12 pts.) Draw two different models showing the possible relationships between the two different regulatory factors encoded by **A** and **B**. For your answer be sure to include the **Mox** gene and to indicate where and how the inducer methanol is acting.

Model 1:



Model 2:



Name: Solutions

(e 5 pts.) To distinguish the two models, you construct an **A⁻ B2⁻** double mutant. Why is it better to choose the **B2⁻** rather than the **B1⁻** allele for this double mutant epistasis test?

B1⁻ constitutive
B2⁻ uninducible
A⁻ constitutive

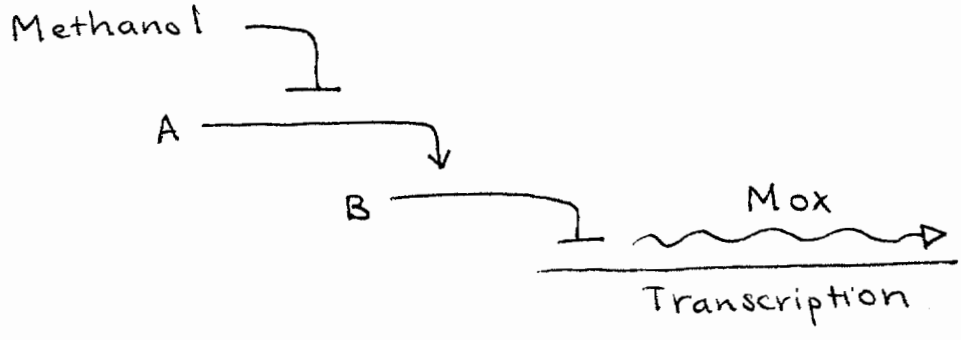
- In order to do an epistasis test, you need to have mutations with 2 DIFFERENT phenotypes to be able to distinguish between them.

(f 8 pts.) You find that the **A⁻ B2⁻** double mutant has the following behavior:

	Methanol oxidase activity		
	– methanol	+ methanol	
$A^- B2^- Mox^+$	–	–	uninducible

Draw a final model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the **Mox** gene and to indicate where and how methanol acts.

B is epistatic to A, so Model 2 is the correct one:



7.03 EXAM III
Review Session Problems

1. Consider an eukaryotic gene regulatory pathway where a small molecule **X** activates the expression of **YFG** (your favorite gene). You have isolated a recessive mutation in J which gives constitutive expression of **YFG** and a recessive mutation in K which gives uninducible expression of **YFG**. Genes **J** and **K** are not linked to each other and neither gene is linked to **YFG**.

a) Draw out two models showing the relationships between J, K, YFG, and small molecule X.

J^- constitutive → neg regulator

K^- uninducible → pos regulator

X activates exp → overall pos regulation

Model 1: X → K ⊣ J ⊣ YFG

Model 2: X ⊣ J ⊣ K → YFG

b) How would you go about determining which model is the correct one? Explain how you would interpret your results.

Do an epistasis test on double mutant J^-K^-. If double mutant is constitutive, J is epi to K, and Model 1 is correct. If K is epi to J (double mutant is uninducible), then Model 2 is correct.

Assume that you are studying this regulatory pathway in yeast. Based on your logic for part (b), you cross a MATa **J-K+** with a MATα **J+K-** and get the following types of tetrads:

Type I	**Type II**	**Type III**
2 uninducible J^+K^-	1 regulated J^+K^+	2 regulated J^+K^+
2 constitutive J^-K^+	1 constitutive J^-K^+	2 uninducible J^-K^-
PD	2 uninducible J^+K^-, J^-K^-	NPD

double mutant! uninducible

c) What do these results tell you?

J^-K^- is uninducible, which means K is epistatic to J, which means Model 2 is correct!

d) Now assume that you are studying this regulatory pathway in *Drosophila*. If you cross a J-/J- K+/K+ male to a J+/J+ K-/K- female. Based on what you know from part (c), give the expected phenotype of the F_1 flies from this cross. Now you cross the F_1 flies among themselves to produce F_2 flies. Give the expected ratio of phenotypes among the F_2 flies.

♂ J^-/J^- K^+/K^+ × ♀ J^+/J^+ K^-/K^-

↓

F_1 J^-/J^+ K^-/K^+ regulated (assuming mutations are recessive)

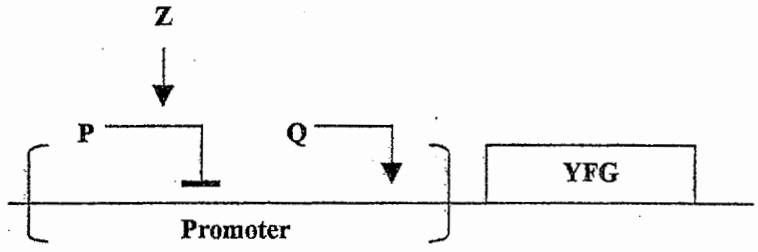
↓

F_2		
$J^+\_$ $K^+\_$	9 reg	9 : 3 : 4
$J^-J^-K^+\_$	3 const	R : C : U
$J^+\_$ K^-K^-	3 unind	
$J^-J^-K^-K^-$	1 unind	

2. Based on the Gal Operon, fill in the following table. (DB = DNA-binding domain, AD = activation domain, UAS = upstream activation sequence, URS = upstream repression sequence, Δ = deletion).

Gal 1 Expression				
	- gal	**+ gal**	**+ gal, + glu**	**Why?**
Gal1+	—	+	—	"wild-type", normal regulation
Gal1-	—	—	—	mutation prevents proper Gal1 exp
Gal4(ΔBD)	—	—	—	Gal4 can't bind to UAS
Gal4(ΔAD)	—	—	—	Gal4 can't interact w/ RNAP
Gal80-	+	+	—	Gal80 can't bind to Gal4, leaving Gal4's AD free to interact w/ RNAP
Gal81-	+	+	—	mutation in Gal4's AD that prevents Gal80 fr. binding
ΔUAS	—	—	—	No place for Gal4 to bind
ΔTATA	—	—	—	No place for TBP+RNAP to bind
ΔURS	—	+	+	No place for repressor protein complex to bind, so no way to turn off Gal operon

4. Given the following model of regulation, you want to figure out where **P** and **Q** bind on the promoter, so you perform a promoter bashing experiment:



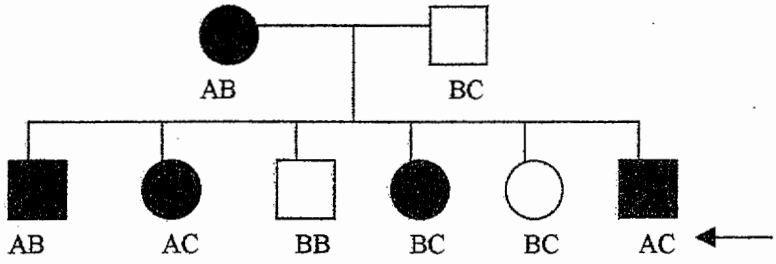
	-300 -250 -200 -150 -100 -50 +1	-Z	+Z
1.	----------	+	--
2.	------- ----------	--	--
3.	---------- ----------	--	--
4.	---------- ----------	+	--
5.	---------- ----------	+	+
6.	---------- ----------	+	--
7.	---------- ----	--	--

a) Identify the regions where P and Q are likely to bind and any other important regions on the promoter.

P⁻ constitutive
Q⁻ uninducible

Therefore:
P binds to region -100 to -150
Q binds to region -200 to -300
TATA box is in region ~+1 to -50

8. For the following autosomal dominant disease, calculate the LOD score for θ = 0.16. (Just show your set-up).



These represent the alleles genotyped for a marker thought to be linked fo the disease.

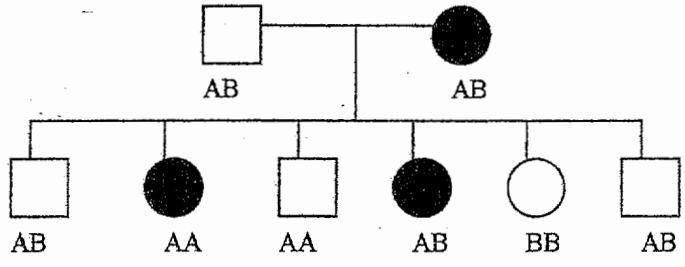
Phase unknown

Phase 1 $\frac{A\ D}{B\ +}$ ⇒ AD: 42, B+: 42, A+: 8, BD: 8

Phase 2 $\frac{A\ +}{B\ D}$ ⇒ A+: 42, BD: 42, AD: 8, B+: 8

$$\log_{10} \frac{\frac{1}{2}(.42^5 \times .08) + \frac{1}{2}(.08^5 \times .42)}{.25^6}$$

9. For the folloing autosomal dominant disease, calculat the LOD score for θ = 0.30. (Just show your set-up).

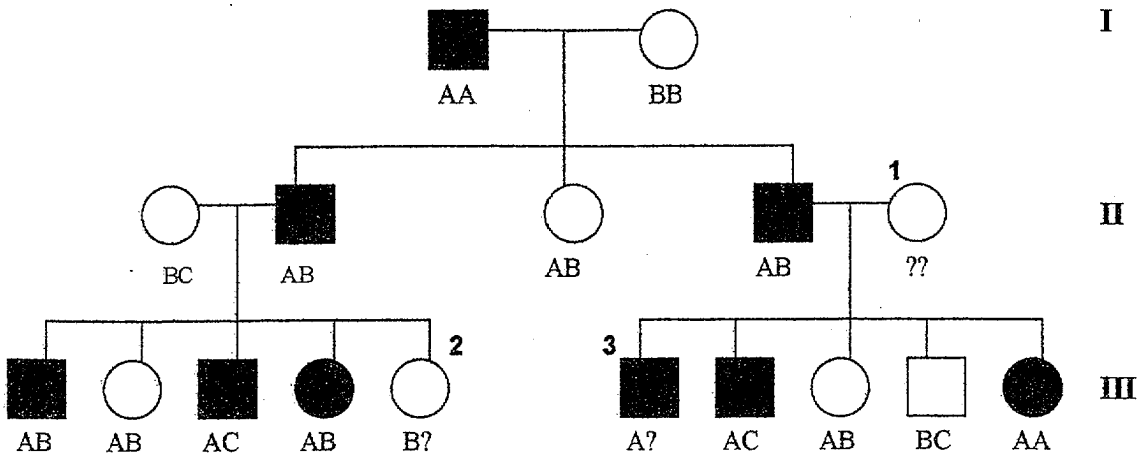


Only 2nd, 3rd, and 5th ind. informative

Phase unknown

$$\log_{10} \frac{\frac{1}{2}(.35^2 \times .15) + \frac{1}{2}(.15^2 \times .35)}{(.25)^3}$$

10.



a) Given that the disease is autosomal dominant and that the LOD score for $\theta = 0.1$ is negative, fill in the missing pedigree genotypes for individuals **1**, **2**, and **3**.

① AC
② AB
③ AB

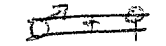
b) Fill in the pedigree so as to maximize the LOD score for $\theta = 0.1$.

① AC
② BB or BC
③ AA or AC

c) From part (b), which individual(s), if he/she developed the disease, would raise the LOD score? (From generation II only.) Which would decrease the LOD score? (From generation III only.)

III

Increase : daughter 2nd to left

Decrease : unaffected  in family on right
brother + sister

also ind ②

7.03 Exam 3

Name: _____

Section: _____ TA: _____

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

There are 7 pages including this cover page
Please write your name on each page.

Question 1	34 points
Question 2	26 points
Question 3	40 points

Name: _____

1. You are studying how yeast cells grow on maltose and you find that both maltose and glucose regulate expression of the principal enzyme for maltose utilization, called maltase. In cells grown without maltose, maltase is not expressed, but maltase is induced when maltose is added to the growth medium. In cells grown in medium that contains both maltose and glucose, maltase is not expressed. You have isolated mutations in three different genes that alter maltase regulation, called **A⁻**, **B⁻** and **C⁻**. All three mutations are recessive and none of the mutations are linked either to maltase or to each other. The maltase expression of wild type and each of the three mutants are shown below.

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

(a 6 pts.) For each of the three genes, state whether it affects regulation by maltose or glucose and whether it is a positive regulator or a negative regulator.

A

B

C

Name: _____

Next you cross an **A⁻** mutant to a **B⁻** mutant. After tetrads are dissected and evaluated for maltase expression in either the presence or absence of maltose, the following tetrad types are observed.

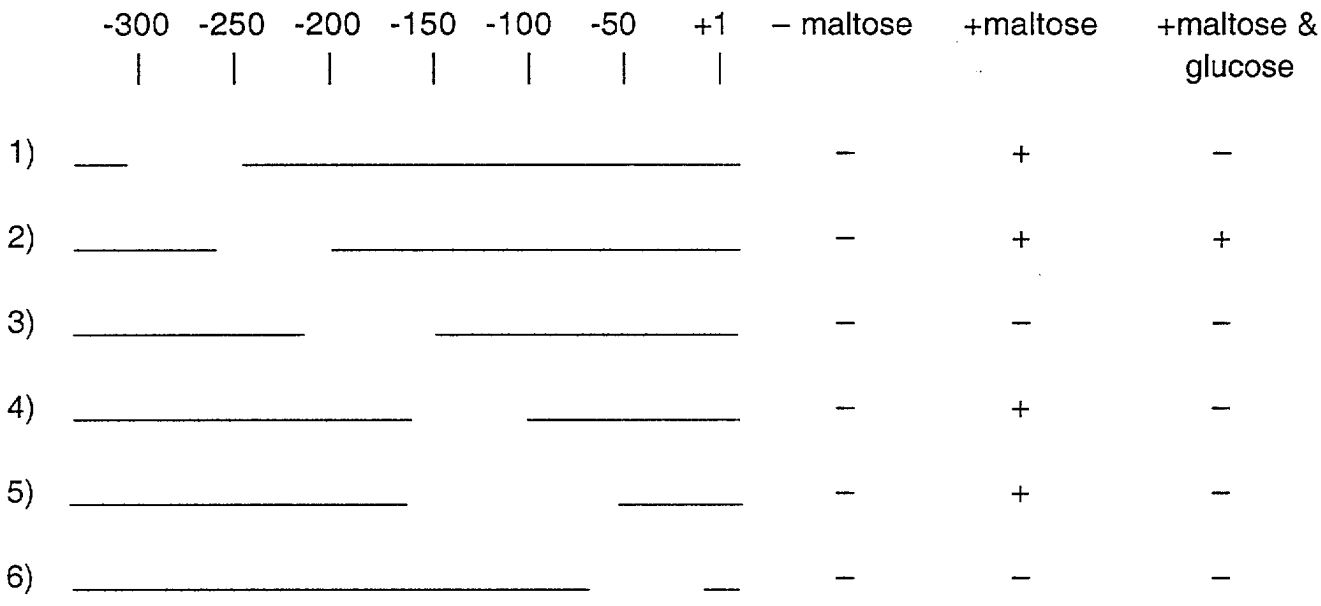
Type 1	Type 2	Type3
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible

(b 8 pts.) What is the phenotype of the **A⁻ B⁻** double mutant? Explain how you arrived at your answer.

(c 10 pts.) Draw a model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the maltase gene and to indicate where and how maltose acts.

Name: _____

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



(d 5 pts.) The DNA sequence of gene **C** reveals that this gene is likely to encode a DNA-binding protein. Assuming that the product of gene **C** binds to the promoter region of the maltase gene, where is it most likely to bind?

(e 5 pts.) In general, upstream activation sequences function normally regardless of their distance from the TATA sequence. Which of the deletion mutants shown above demonstrate that the distance between upstream activation and TATA sequences is of little or no consequence?

Name: _____

2. Suppose that body color in cockroaches is controlled by an autosomal gene G. GG and Gg cockroaches are black, and gg cockroaches are white. Let us consider a population of cockroaches that lives in your apartment and that mates at random.

(a 5 pts.) You count a week's worth of newborn cockroaches in your apartment and find that they include 99,990 black cockroaches and 10 white cockroaches. Estimate the frequency of the g allele in this population.

(b 7 pts.) Assume that the cockroach population in your apartment has held steady for more than a year. Throughout this period, you have disliked cockroaches and have smashed them whenever you spotted them. White cockroaches are easy to spot on the black floor of your apartment, and thus white cockroaches (gg) have suffered a selective disadvantage. White cockroaches are only 20% as likely as black cockroaches to survive to reproductive age. What is the mutation rate (G → g) per generation in this population?

(c 7 pts.) You now start graduate school and have less time to rid your apartment of these pests. This environmental change results in white cockroaches being 60% as likely as black cockroaches to survive to reproductive age. What would be the new frequency of the g allele at steady state?

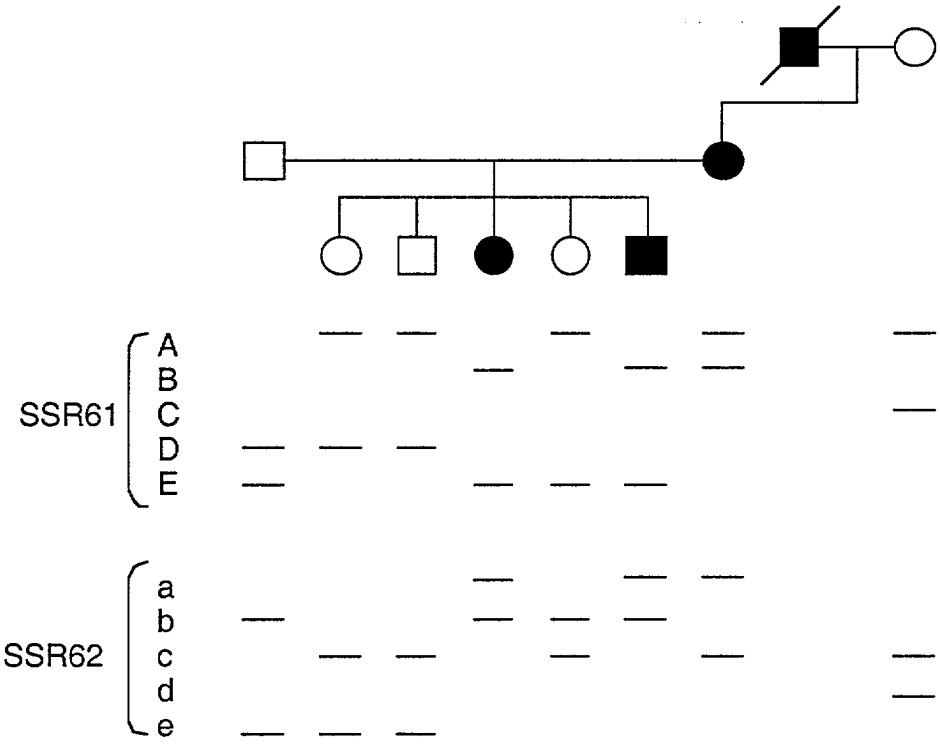
(d 7 pts.) After many years, you clear the toxic wastes from your apartment, and the G → g mutation rate falls to zero. Simultaneously you apply a pesticide that kills many of the cockroaches. Unfortunately, the g allele confers partial resistance to this pesticide so that, in the presence of the pesticide, Gg heterozygotes have 20% more offspring than do GG cockroaches. White cockroaches continue to be 60% as likely as black cockroaches to avoid smashing prior to reproductive age. What would be the new frequency of the g allele at steady state?

Name: _____

3. You are genetically mapping a rare form of osteoporosis (weakened, brittle bones) that shows autosomal dominant inheritance.

Alleles: + (normal) OS (associated with osteoporosis)

Here is a family in which some individuals are affected:



(a 3 pts.) What allele at SSR61 did the affected mother inherit from her father (deceased)?

(b 3 pts.) What allele at SSR62 did the affected mother inherit from her father (deceased)?

(c 5 pts.) Diagram the phase relationship between the osteoporosis and SSR61 alleles in the affected mother.

Name: _____

(d 9 pts.) Calculate the LOD score for linkage at $\theta = 0$ between osteoporosis and SSR61 in this family.

(e 5 pts.) Diagram the phase relationship between the SSR61 and SSR62 alleles in the affected mother.

(f 5 pts.) Diagram the two possible phase relationships between the SSR61 and SSR62 alleles in the unaffected father.

(g 10 pts.) Calculate the LOD score for linkage at $\theta = 0$ between SSR61 and SSR62 in this family.

1997

7.03 Exam III

Name: Solutions

1. You are studying how yeast cells grow on maltose and you find that both maltose and glucose regulate expression of the principal enzyme for maltose utilization, called maltase. In cells grown without maltose, maltase is not expressed, but maltase is induced when maltose is added to the growth medium. In cells grown in medium that contains both maltose and glucose, maltase is not expressed. You have isolated mutations in three different genes that alter maltase regulation, called **A⁻**, **B⁻** and **C⁻**. All three mutations are recessive and none of the mutations are linked either to maltase or to each other. The maltase expression of wild type and each of the three mutants are shown below.

	Maltase activity			
	– maltose	+maltose	+maltose & glucose	
Wild type	–	+	–	
A⁻	+	+	–	const
B⁻	–	–	–	unind
C⁻	–	+	+	const

(a 6 pts.) For each of the three genes, state whether it affects regulation by maltose or glucose and whether it is a positive regulator or a negative regulator.

A Maltose - negative regulator

B Maltose - positive regulator

C Glucose - negative regulator

Name: Solutions

Next you cross an **A⁻** mutant to a **B⁻** mutant. After tetrads are dissected and evaluated for maltase expression in either the presence or absence of maltose, the following tetrad types are observed.

A^-B^+ const × A^+B^- unind

Type 1	Type 2	Type3
constitutive A^-B^+	constitutive A^-B^-	constitutive A^-B^+
constitutive A^-B^-	constitutive A^-B^-	constitutive A^-B^+
regulated A^+B^+	regulated A^+B^+	uninducible A^+B^-
uninducible A^+B^-	regulated A^+B^+	uninducible A^+B^-
T	NPD	PD

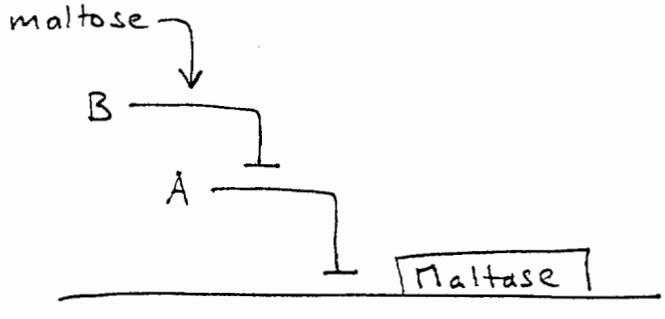
(b 8 pts.) What is the phenotype of the **A⁻ B⁻** double mutant? Explain how you arrived at your answer.

constitutive → because in NPD (Type 2), the double mutant A^-B^- is constitutive

(c 10 pts.) Draw a model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the maltase gene and to indicate where and how maltose acts.

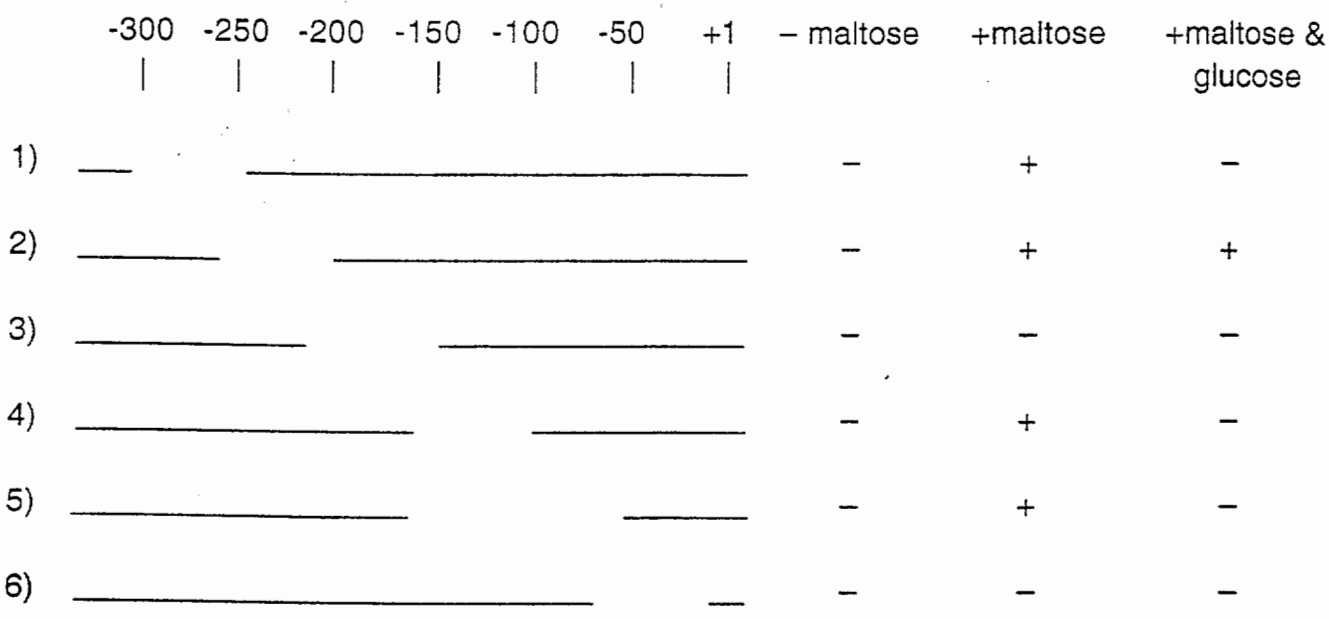
From part (b), A is epistatic to B :

∴



Name: Solutions

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



(d 5 pts.) The DNA sequence of gene **C** reveals that this gene is likely to encode a DNA-binding protein. Assuming that the product of gene **C** binds to the promoter region of the maltase gene, where is it most likely to bind?

Deletion #2 shows same phenotype as C⁻ mutant.

∴ C binds between -200 and -250

(e 5 pts.) In general, upstream activation sequences function normally regardless of their distance from the TATA sequence. Which of the deletion mutants shown above demonstrate that the distance between upstream activation and TATA sequences is of little or no consequence?

Deletion mutants 4 & 5

(Deletion mutant 6 is a deletion of TATA sequence)

Name: Solutions

2. Suppose that body color in cockroaches is controlled by an autosomal gene G. GG and Gg cockroaches are black, and gg cockroaches are white. Let us consider a population of cockroaches that lives in your apartment and that mates at random.

(a 5 pts.) You count a week's worth of newborn cockroaches in your apartment and find that they include 99,990 black cockroaches and 10 white cockroaches. Estimate the frequency of the g allele in this population.

$$f(g/g) = \frac{10}{100{,}000} = \frac{1}{10{,}000} = q^2 \;;\; \boxed{q = \sqrt{\frac{1}{10{,}000}} = 0.01}$$

(b 7 pts.) Assume that the cockroach population in your apartment has held steady for more than a year. Throughout this period, you have disliked cockroaches and have smashed them whenever you spotted them. White cockroaches are easy to spot on the black floor of your apartment, and thus white cockroaches (gg) have suffered a selective disadvantage. White cockroaches are only 20% as likely as black cockroaches to survive to reproductive age. What is the mutation rate (G → g) per generation in this population?

survival rate = $1-S = 0.2$

$S = 0.8$

Autosomal Recessive

so: $\hat{q} = \sqrt{\frac{\mu}{S}} \rightarrow \mu = Sq^2 = (0.8)(0.01)^2 = \boxed{0.00008}$

(c 7 pts.) You now start graduate school and have less time to rid your apartment of these pests. This environmental change results in white cockroaches being 60% as likely as black cockroaches to survive to reproductive age. What would be the new frequency of the g allele at steady state?

survival rate = $1-S = 0.6$

$S = 0.4$

$$\hat{q} = \sqrt{\frac{\mu}{S}} = \sqrt{\frac{0.00008}{0.4}} = \boxed{0.014}$$

(d 7 pts.) After many years, you clear the toxic wastes from your apartment, and the G → g mutation rate falls to zero. Simultaneously you apply a pesticide that kills many of the cockroaches. Unfortunately, the g allele confers partial resistance to this pesticide so that, in the presence of the pesticide, Gg heterozygotes have 20% more offspring than do GG cockroaches. White cockroaches continue to be 60% as likely as black cockroaches to avoid smashing prior to reproductive age. What would be the new frequency of the g allele at steady state?

$H = 0.2$

$S = 0.4$

At steady-state for balanced polymorphism:

$$\Delta q = 0 \rightarrow \underbrace{Sq^2}_{f(a/a)} = \frac{1}{2} \cdot \underbrace{2pq}_{f(A/a)} \cdot H \quad \text{(Note: } p \neq 1 \text{ in this case)}$$

$$Sq = pH$$

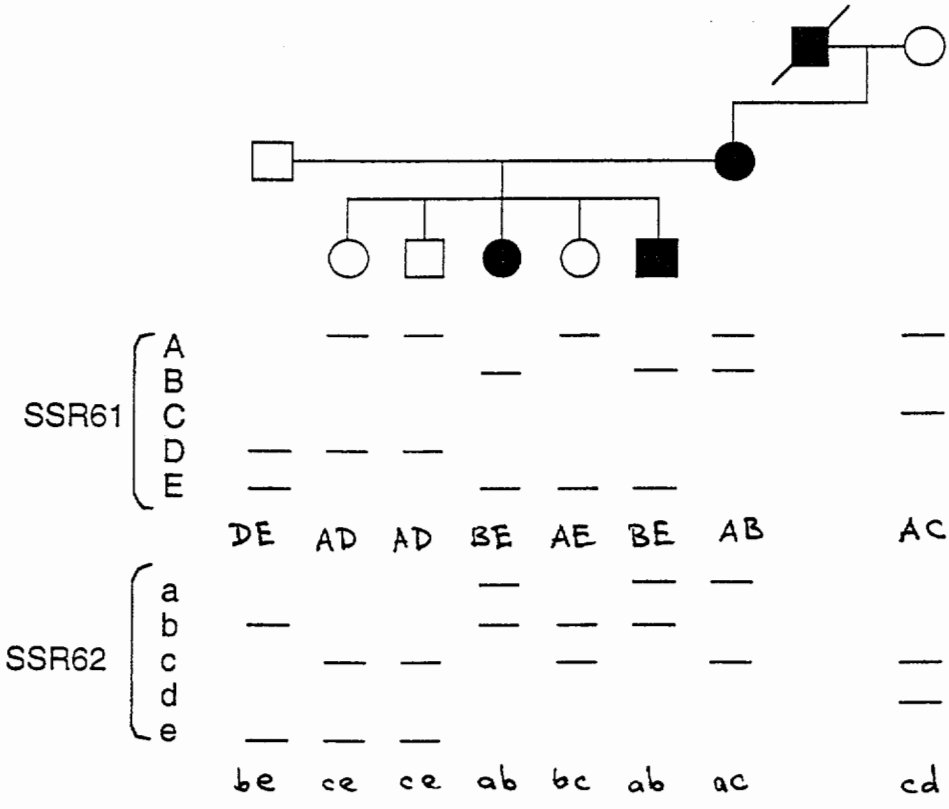
since $p = 1-q$: $Sq = (1-q)H \rightarrow \hat{q} = \frac{H}{S+H} = \frac{0.2}{0.4+0.2} = \boxed{\frac{1}{3}}$

Name: Solutions

3. You are genetically mapping a rare form of osteoporosis (weakened, brittle bones) that shows autosomal dominant inheritance.

Alleles: + (normal) OS (associated with osteoporosis)

Here is a family in which some individuals are affected:



(a 3 pts.) What allele at SSR61 did the affected mother inherit from her father (deceased)?

B allele

(b 3 pts.) What allele at SSR62 did the affected mother inherit from her father (deceased)?

a allele

(c 5 pts.) Diagram the phase relationship between the osteoporosis and SSR61 alleles in the affected mother.

B OS

A +

(we know the phase!)

Name: Solutions

(d 9 pts.) Calculate the LOD score for linkage at θ = 0 between osteoporosis and SSR61 in this family.

$$LOD_{\theta=0} = \log_{10}\left(\frac{(0.5)^5}{(0.25)^5}\right) = \boxed{1.51}$$

* Don't need a $\frac{1}{2}$ in the numerator b/c we know the phase.

(e 5 pts.) Diagram the phase relationship between the SSR61 and SSR62 alleles in the affected mother.

$$\frac{B \quad a}{A \quad c}$$

(again, we know the phase!)

(f 5 pts.) Diagram the two possible phase relationships between the SSR61 and SSR62 alleles in the unaffected father.

Phase 1: $\frac{D \quad b}{E \quad e}$ Phase 2: $\frac{D \quad e}{E \quad b}$

(g 10 pts.) Calculate the LOD score for linkage at θ = 0 between SSR61 and SSR62 in this family.

Mom:

$$LOD_{\theta=0} = \log_{10}\left(\frac{(0.5)^5}{(0.25)^5}\right) = 1.51$$

Dad:

$$LOD_{\theta=0} = \log_{10}\left(\frac{\frac{1}{2}(0^5) + \frac{1}{2}(0.5)^5}{(0.25)^5}\right) = 1.20$$

Total LOD = 2.71

7.03 Final Exam

Name: Solutions

Section: _____ TA: _____

There are 17 pages including this cover page

Please write your name on each page.

Question 1	30 points
Question 2	25 points
Question 3	15 points
Question 4	30 points
Question 5	18 points
Question 6	33 points
Question 7	25 points
Question 8	24 points

Name: _____

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

(a 5 pts.) A male from a true-breeding **w** strain is crossed to a female from a true-breeding **ap** strain. All of the female F_1 progeny from this cross have pale apricot colored eyes. What colored eyes should the male F_1 progeny have?

Apricot

♂ $X^{w}Y \times X^{ap}X^{ap}$

♂ $Y\,X^{ap}$ ♀ $X^{w}X^{ap}$ = pale apricot

from father, from mother

(b 5 pts.) Are the **w** and **ap** mutations alleles of the same gene or alleles of different genes? Explain your reasoning.

Same gene. Both w and ap are recessive to wild-type. ~~the females are pale apricot~~

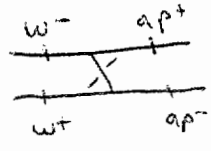
In the cross in (a), this is like a complementation test (for the ♀'s). Since they do not complement (pale-apricot ♀ vs. red) the mutations are in the same gene.

(c 10 pts.) A female F_1 fly (with pale apricot eyes) is crossed to a wild-type male and 1000 male progeny from this cross are examined. Among the male progeny there are 496 flies with white eyes, 499 flies with apricot eyes, and 5 flies with normal red eyes. What is the distance between **w** and **ap** in cM? (Be sure to state any assumptions that you make.)

white and apricot are parental classes
the red flies are a recombinant class
and are $w^+ ap^+$

~~there is a "hidden"~~

The $w^- ap^-$ are "hidden" and must also be counted. (btw, they are white)



$w^- ap^- \Rightarrow ?$

$w^+ ap^+ \Rightarrow$ red

$$\frac{5 \cdot 2}{499+496+5} \times 100 = \boxed{1\,cM}$$

(5 · 2: for the hidden class)

i.e. $w^- ap^- \neq w^- ap^+ / w^+ ap^-$

Note: a genotype of $X^{w^- ap^-}Y \neq X^{w^- ap^+}X^{w^+ ap^-}$

Name: _____

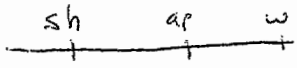
(d 10 pts.) A mutation that gives short bristles (known as **sh**) is linked to the **w** and **ap** mutations. A fly from a true breeding **sh w** strain (short bristles, white eyes) is crossed to a fly from a true breeding **ap** strain (long bristles, apricot eyes). The female F_1 progeny are then crossed to wild type males and only males from this cross are examined. The following phenotypic classes and numbers are seen:

Bristles	Eye color	Number
Short	white	398
Long	apricot	405
Short	apricot	90
Long	white	102
Short	red	5

Draw a genetic map showing the relative order of the **sh**, **w**, and **ap** mutations. (Hint: note the absence of a phenotypic class with long bristles and red eyes).

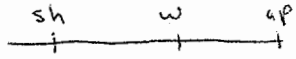
Double clo class is : Long red = 0
the other dclo is hidden (short otw = white)

order 1 :

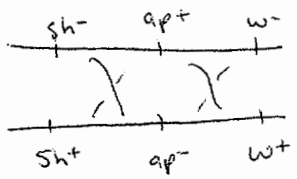


vs

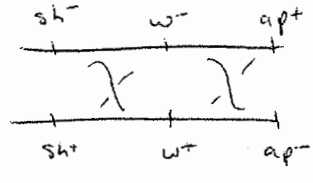
order 2



the cross :



vs.



double clo class:

$sh^- ap^- w^-$ = ?

$sh^+ ap^+ w^+$ = Long red

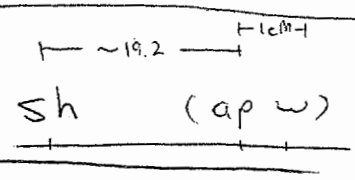
vs.

$sh^- w^+ ap^+$ = short red

$sh^+ w^- ap^-$ = ?

So, this is correct

Order is



distances were not necessary

Name: _____

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

(a 10 pts.) You grow P1 phage on a host that carries the **Tn5-LacZ** insertion and then use the resulting lysate to infect a **mut1 lacZ⁻** strain selecting for kanamycin resistance. Among the kan^r transductants, 20% give constitutive expression of ß-galactosidase, whereas 80% only express ß-galactosidase when melibiose is present. Is **mut1** linked to the melibiase structural gene, and if so what is the distance from the **Tn5-LacZ** insertion in terms of cotransduction frequency?

Yes, mut1 is linked to the melibiase structural gene.

The distance from mut1 to Tn5-Lac Z is 80% cotransduction

(b 5 pts.) You obtain an **F'** that carries the melibiase structural gene (this **F'** includes chromosomal sequences that span >100 kbp on either side of the melibiase gene). You select a kan^r transductant from part (**a**) that gives constitutive ß-galactosidase expression and mate the **F'** into this strain. The resulting merodiploid still gives constitutive expression of ß-galactosidase. What does this observation tell you about the nature of **mut1**?

The results of this merodiploid analysis is that mut1 is a dominant mutation.

Name: _____

(c 10 pts.) You further examine the F' strain constructed in part (b), and find that melibiase expression is regulated normally despite the fact that ß-galactosidase expression is constitutive. State what this observation tells you about the nature of mut1 and provide as detailed a model as possible for the molecular defect caused by the mut1 mutation.

This analysis is a Cis-Trans test. This cis-Trans test indicates that mut I is a cis acting mutation. We already know that mut I is a dominant mutation. A cis-acting dominant mutation linked to the structural genes is most likely in the operator. This operator-constitutive mutation most likely ~~either~~ prevents some repressor ~~binding~~ binding of.

Name: _____

3.

1st position (5′ end)	2nd position U	2nd position C	2nd position A	2nd position 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

(a 7 pts.) Write out the DNA base sequence of the segment of the tRNA^{trp} gene that codes for the anticodon sequence (tRNA^{trp} = tryptophan tRNA). For your answer, show both strands of the DNA and indicate the 5′ and 3′ ends of each strand. Also indicate which strand is used as the template for transcription of the tRNA molecule.

mRNA 5′-U G G-3′
tRNA 3′-A C C-5′ → 5′-C C A-3′ / 3′-G G T-5′

(b 8 pts.) After mutagenesis, two different nonsense suppressing alleles of tRNA^{trp} can be isolated. Use the same format as above to write out the DNA base sequence of both nonsense suppressing alleles and indicate which nonsense codon(s) can be suppressed by each allele.

STOPS: mRNA UAA UAG UGA — tRNA UUA CUA UCA

DNA 5′-C C A-3′

mRNA 5′-C C A-3′ — UCA CUA

DNA 5′-T C A-3′ / 3′-A G T-5′ — suppresses stop UGA codon

5′-C T A-3′ / 3′-G A T-5′ — suppresses: UAG stop codon

Name: _____

4. You are studying the yeast genes needed to metabolize organic phosphates. The key regulated enzyme is phosphastase, which is needed to release inorganic phosphate from organic phosphate compounds in the medium. Phosphatase is not expressed in medium that contains inorganic phosphate, but is induced to high levels in medium with no inorganic phosphate. You have isolated a recessive mutation that shows uninducible phosphatase regulation, which you call **pho4⁻**.

	Phosphatase activity	
	+ phosphate	–phosphate
Wild type	–	+
pho4⁻	–	–
pho4⁻ / pho4⁺	–	+

Starting with an uninducible **pho4⁻** strain, you isolate three different revertants (called revertant 1, 2, and 3) that restore phosphatase expression on medium without phosphate.

(a 10 pts.) Revertant 1 shows regulated expression of phosphatase. A cross of revertant 1 to wild type gives the following tetrad types. (Type 1 is the most abundant class).

Type 1	Type 2	Type3
regulated	regulated	regulated
regulated	regulated	regulated
regulated	regulated	uninducible
uninducible	regulated	uninducible
T	PD	NPD

What kind of mutation could produce the behavior of revertant 1. Be as explicit as possible and explain your reasoning.

Extragenic Suppressor because PD ≠ T

so therefore, unlinked.

Name: _____

(b 10 pts.) Revertant 2 also shows regulated expression of phosphatase. In a cross of revertant 2 to wild type only one tetrad type is observed.

Type 1
regulated
regulated
regulated
regulated

PD

What kind of mutation could produce the behavior of revertant 2. Be as explicit as possible and explain your reasoning.

back mutation
or
tightly-linked intragenic suppressor
because only PDs.

(c 10 pts.) Revertant 3 shows constitutive expression of phosphatase. A cross of revertant 3 to wild type gives the following tetrad types. (Type 1 is the most abundant class).

Type 1	Type 2	Type3
constitutive	constitutive	constitutive
constitutive	constitutive	constitutive
regulated	regulated	uninducible
uninducible	regulated	uninducible
T	PD	NPD

Give an explanation for the type of mutation that could produce the behavior of revertant 3.

Rev 3 epi to Pho4
diff gene

Name: _____

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

(a 4 pts.) In this population, what is the frequency of the recessive allele responsible for albinism? (Assume Hardy-Weinberg equilibrium.)

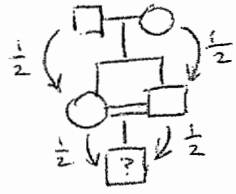
$f(a/a) = \frac{1}{5500} = q^2 \rightarrow q = \sqrt{\frac{1}{5500}} = \boxed{0.013}$

(b 4 pts.) Inbreeding can occur not only in humans but also in other animals, including hamsters. What are the inbreeding coefficients for the following matings?

brother-sister: $\frac{1}{4}$

1st cousins: $\frac{1}{16}$

Example: bro-sis



$\left(\frac{1}{2}\right)^4 = \frac{1}{16} \times 4 = \frac{1}{4}$

(c 5 pts.) In this population of hamsters, what is the probability that an animal resulting from a 1st cousin mating will be albino?

$f(a/a) = F \times q = \frac{1}{16} \times 0.013 = \boxed{0.0008}$

(c 5 pts.) In this population of hamsters, 1 in every 800 matings is between 1st cousins. (Assume that all other matings are random.) In this population, what fraction of all albino offspring will come from 1st cousin matings?

probability of albino offspring resulting fr 1st cousin matings = $\left(\frac{1}{800}\right)(F \times q)$

probability of albino offspring resulting fr random matings = q^2

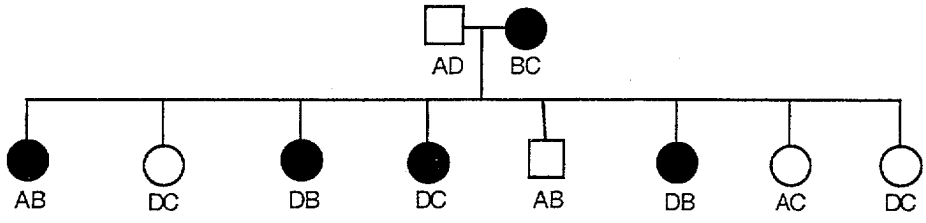
Fraction of all albino offspring coming from 1st cousin matings:

$$\frac{\left(\frac{1}{800}\right)(F \times q)}{\left(\frac{1}{800}\right)(F \times q) + q^2} = \frac{\left(\frac{1}{800}\right)(0.0008)}{\left(\frac{1}{800}\right)(0.0008) + 0.00018} = \boxed{0.0055}$$

1st cousin matings → Total Albino offspring

Name: _____

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



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

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

(a 10 pts.) Calculate the LOD score for linkage between breast cancer and SSR126 at $\theta = 0.1$.

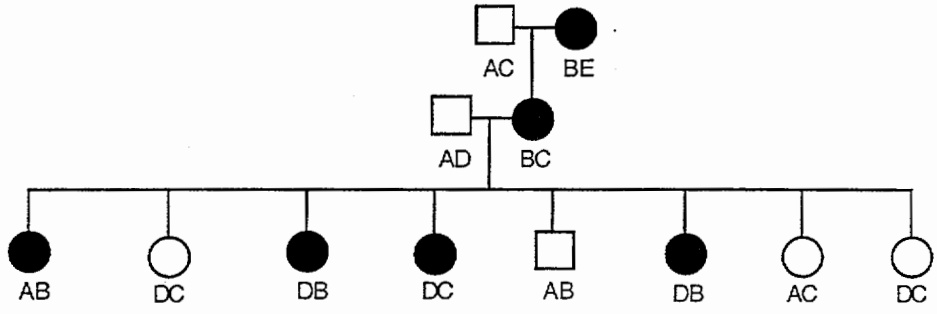
male is uninformative. phase is unknown

$$LOD_{\theta=.1} = \log_{10}\left(\frac{\frac{1}{2}(.45)^6(.05)^1 + \frac{1}{2}(.05)^6(.45)^1}{(.25)^7}\right)$$

$$= .53$$

Name: _____

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



(b 7 pts.) Recalculate this family's LOD score for linkage between breast cancer and SSR126 at $\theta = 0.1$.

male still uninformative. phase is now known.

$$LOD_{\theta=.1} = \log_{10}\left(\frac{(.45)^6(.05)^1}{(.25)^7}\right)$$

$$= .83$$

(c 3 pts.) No woman in this family developed breast cancer before the age of 37, despite the presence of a predisposing mutation. Why does it take so long for the predisposing mutation to manifest itself? (Focus on the cellular level in your answer. A ONE SENTENCE answer is sufficient.)

You need multiple mutations in multiple genes to develop cancer. It takes many years to develop all the mutations.

Name: _____

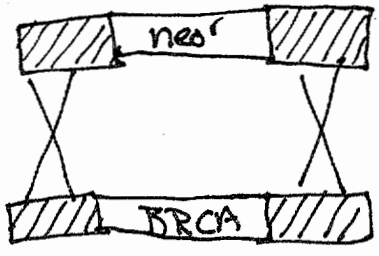
You subsequently pinpoint, at the molecular level, the gene that predisposes to breast cancer in this family. You 1) name the gene BRCA, 2) demonstrate that affected women in this family are heterozygous for a loss-of-function mutation in BRCA, and 3) demonstrate that BRCA is a "tumor suppressor" gene. You identify a mouse homolog of the human BRCA gene and decide that you want to generate a mouse model of breast cancer.

(d 3 pts.) What type of modification to the mouse genome would you make to study BRCA's role in breast cancer? Explain your choice.

Since BRCA is a tumor suppressor gene, loss of function of the BRCA gene is one of the mutations leading to cancer. To model this you would make a knockout mouse missing a copy of the BRCA gene like in humans.

(e 7 pts.) Draw the DNA construct you would use to modify the mouse genome, and explain how your construct would integrate into the mouse genome.

DNA construct (targeting vector):



/// = ~~homologous~~ identical region of homology outside BRCA.

endogenous mouse BRCA gene

The DNA would insert into the BRCA locus by homologous recombination.

(f 3 pts.) You create mice that are heterozygous for your construct. You cross these BRCA +/- mice with each other and obtain 185 progeny, 125 of which are BRCA +/-, and 60 of which are +/+. What might explain these breeding results?

You would expect 1 +/+ : 2 +/- : 1 -/-.
No -/- mice are ~~born~~ born indicating that the deletion of the gene is lethal to the embryo.
BRCA must be an essential gene.

Name: _____

7. The incidence of insulin-dependent diabetes in the general population is about 0.4%. The incidence of insulin-dependent diabetes in relatives of affected individuals is as follows:

	% affected
Siblings	9%
Parents	8%
Aunts and Uncles	1.6%

(a 3 pts.) Is this data consistent with simple autosomal dominant inheritance (complete penetrance; no environmental influence) of insulin-dependent diabetes? Justify your answer.

No, % affected for siblings would be 50% and at least one parent of an affected child would be affected.

(b 3 pts.) Is this data consistent with simple autosomal recessive inheritance (complete penetrance; no environmental influence) of insulin-dependent diabetes? Justify your answer.

No, % affected for siblings would be 25%.

(c 3 pts.) What additional data would allow you to draw conclusions about the role, if any, of environment in causing insulin-dependent diabetes?

Monozygotic twin studies. Since they share 100% of their genes, if they are not 100% concordant then there are environmental influences

In 1994, human geneticists interested in insulin-dependent diabetes typed 96 affected (concordant) sib-pairs (and their parents) for more than 200 SSRs scattered throughout the human genome. The investigators expected to find random allele sharing at most SSRs, but sought to identify one or more SSRs at which the hypothesis of random allele sharing could be ruled out. Some of the SSR findings in this study are shown below. (Realize that, at any autosomal locus, sib-pairs can share 0, 1, or 2 alleles by descent. In this study of 96 sib-pairs, there are, at each locus, a total of 96 x 2 = 192 alleles to be examined for identity or non-identity by descent.)

SSR	Chromosome	# of Alleles Identical by Descent	# of Alleles NOT Identical by Descent	Total # of Alleles
SSR1	5	101	91	192
SSR2	5	86	106	192
SSR3	6	137	55	192
SSR4	6	99	93	192
SSR5	11	90	102	192
SSR6	11	118	74	192

Name: _____

(d 2 pt.) What fraction of all alleles at all loci are siblings (in this or any other study) expected to share by descent?

$\frac{1}{2}$

(e 1 pt.) In this study, with 192 alleles examined (per locus), what number of alleles (per locus) is expected to be identical by descent, assuming random allele sharing?

96

(f 7 pts.) Based on the affected sib-pair results shown on the previous page, can the hypothesis of random allele sharing be ruled out at one or more SSRs? Base your conclusions on Chi-squared analysis.

$\chi^2 = \sum \frac{(O-E)^2}{E}$ $\quad df = \#\text{classes} - 1 = 1$

SSR2 - E 96 96, O 86 106 $\quad \chi^2 = \frac{100}{96} + \frac{100}{96} = 2.08$ $p \approx 0.2$ can not rule out

SSR3 E 96 96, O 137 55 $\quad \chi^2 = \frac{41^2}{96} + \frac{41^2}{96} = 35$ $p << .05$ rule out random hypothesis

SSR6 E 96 96, O 118 74 $\quad \chi^2 = \frac{22^2}{96} + \frac{22^2}{96} = 10.08$ $p << .05$ rule out random allele sharing

Table 5-3. Critical Values of the χ^2 Distribution

df \ p	0.995	0.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005	df
1	.000	.000	0.016	0.455	2.706	3.841	5.024	6.635	7.879	1
2	0.010	0.051	0.211	1.386	4.605	5.991	7.378	9.210	10.597	2
3	0.072	0.216	0.584	2.366	6.251	7.815	9.348	11.345	12.838	3
4	0.207	0.484	1.064	3.357	7.779	9.488	11.143	13.277	14.860	4
5	0.412	0.831	1.610	4.351	9.236	11.070	12.832	15.086	16.750	5
6	0.676	1.237	2.204	5.348	10.645	12.592	14.449	16.812	18.548	6
7	0.989	1.690	2.833	6.346	12.017	14.067	16.013	18.475	20.278	7
8	1.344	2.180	3.490	7.344	13.362	15.507	17.535	20.090	21.955	8
9	1.735	2.700	4.168	8.343	14.684	16.919	19.023	21.666	23.589	9
10	2.156	3.247	4.865	9.342	15.987	18.307	20.483	23.209	25.188	10
11	2.603	3.816	5.578	10.341	17.275	19.675	21.920	24.725	26.757	11
12	3.074	4.404	6.304	11.340	18.549	21.026	23.337	26.217	28.300	12
13	3.565	5.009	7.042	12.340	19.812	22.362	24.736	27.688	29.819	13
14	4.075	5.629	7.790	13.339	21.064	23.685	26.119	29.141	31.319	14
15	4.601	6.262	8.547	14.339	22.307	24.996	27.488	30.578	32.801	15

Name: _____

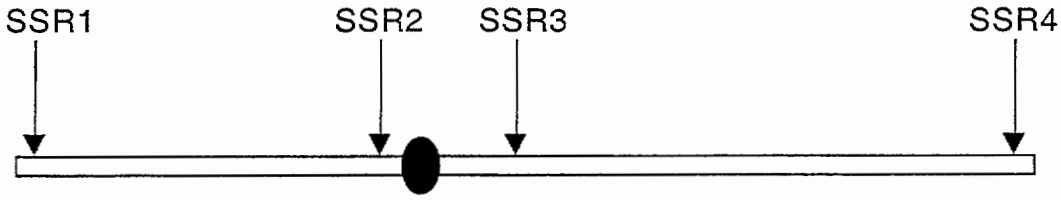
(g 6 pts.) Based on this affected sib-pair analysis, how many genes contribute to insulin-dependent diabetes? Justify your answer.

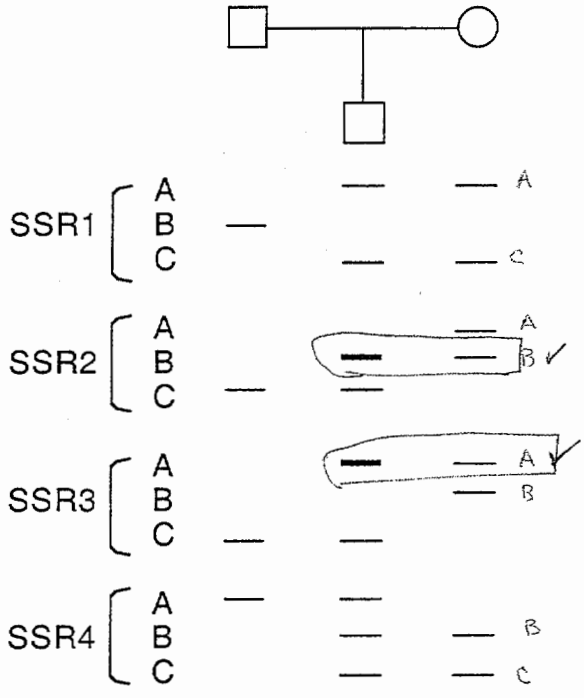
at least 2 From the X^2 test we can rule out random allele sharing for 2 SSR's. Thus, these SSRs seem to contribute to insulin dependent diabetes. There could be more genes involved since the SSR's did not span the entire genome.

Name: _____

8. While home on winter vacation, you are called by your family physician to provide an expert genetic opinion on an unusual patient: a 47,XXX boy.

You prepare DNA samples from the boy and from his parents. You confirm that the stated father is in fact the biological father by testing the family for a large number of autosomal SSRs. You also test the family for a series of SSRs distributed along the X chromosome:





(a 2 pts.) In which parent did nondisjunction occur?

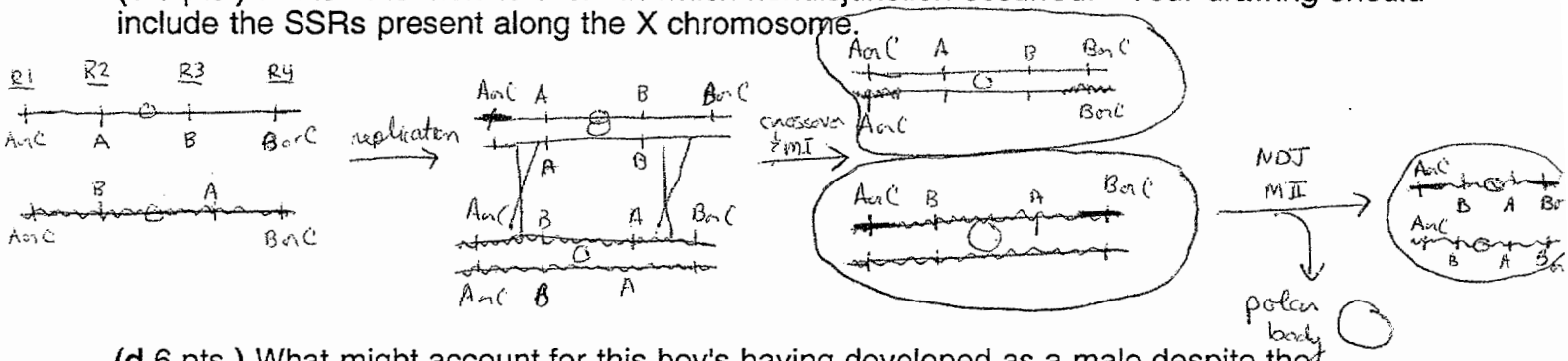
Mom

(b 3 pts.) In which division of meiosis did nondisjunction occur?

Meiosis II

Name: _____

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



(d 6 pts.) What might account for this boy's having developed as a male despite the presence of three X chromosomes? Explain how you would test your hypothesis.

~~Increased~~ recombination between X & Y in the father such that SRY ended up on the X chromosome

test: - look for Y chromosome SSRs on X

- PCR SRY

(e 5 pts.) How would you account for the absence in the XXX boy of a paternal allele for SSR1?

paternal X's SSR1 was lost during recombination w/ Y chromosome as described above

(f 3 pts.) Write an equation to estimate the frequency of such XXX males in human populations. (No calculation needed.)

= p(nondisjunction in mother) x p(X-Y recombination in father)