7.03 Exam 1

Name: _____

TA (circle one):

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Doug Mills	Rishi Puram	Bethany Redding	Nate Young

Section time: _____

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

There are eight 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	**26 points**
Question 2	**36 points**
Question 3	**38 points**

Name: _____

1. Consider the following autosomal *Drosophila* traits caused by recessive alleles: bent wings (**bn**$^{-}$), short legs (**sh**$^{-}$), and orange eyes (**or**$^{-}$). You cross two true breeding lines to produce F1 flies, all of which have the wild type phenotype (strait wings, long legs, and red eyes). F1 females are then mated to triply homozygous males with bent wings, short legs, and orange eyes. Among 100 progeny from this cross you observe the following phenotypes:

Phenotype	Number
strait wings, long legs, and red eyes	10
bent wings, short legs, and orange eyes	14
strait wings, short legs, and red eyes	26
bent wings, long legs, and orange eyes	30
strait wings, long legs, and orange eyes	8
bent wings, short legs, and red eyes	6
strait wings, short legs, and orange eyes	2
bent wings, long legs, and red eyes	4

(a 6 points**)** What were the genotypes of the two true breeding parental lines that were crossed?

We can determine the genotypes of the parental flies by looking at the two highest classes of progeny in the F2. These classes are the Parental Classes. Based on these classes, we can determine that the original true-breeding strains were

bn-/bn- sh+/sh+ or-/or- and bn+/bn+ sh-/sh- or+/or+

(b 12 points**)** Draw a genetic map showing the order and relevant distances in cM of the **bn**, **sh**, and **or** markers.

There are three possible orders for these genes along the chromosome.

or bn sh or sh bn sh or bn

Since we know what the parental genotypes are, we can draw the three possible F1 chromosome arrangements.

$\frac{or^- \; bn^- \; sh^+}{or^+ \; bn^+ \; sh^-}$ $\frac{or^- \; sh^+ \; bn^-}{or^+ \; sh^- \; bn^+}$ $\frac{sh^+ \; or^- \; bn^-}{sh^- \; or^+ \; bn^+}$

The smallest class of F2 progeny represents the Double-Crossover class. These progeny resulted from a double crossover event during meiosis of the F1 parent.

Double Crossover Class 1 Straight wings, short legs, orange eyes

Double Crossover Class 2 Bent wings, long legs, red eyes

Only the following order can generate these classes via a double crossover event.

$\frac{sh^+ \; or^- \; bn^-}{sh^- \; or^+ \; bn^+}$ $\frac{sh^+ \; or^+ \; bn^-}{sh^- \; or^- \; bn^+}$

To calculate the distance between each of the markers we must add up the total number of recombinant progeny for that interval and divide by the total number of progeny.

The distance between sh and or is:

(10 + 14 + 4 + 2) / 100 X 100 = 30 cM

The distance between or and bn is:

(8 + 6 + 4 + 2) / 100 X 100 = 20 cM

The cumulative map distance between sh and bn is 50 cM

HOWEVER, if you were to calculate the distance between sh and bn by ignoring the "or" locus, you would end up with:

(10 + 14 + 8 + 6) / 100 X100 = 38 cM

The correct map should be the following:

sh____30cM_____or____20cM___bn

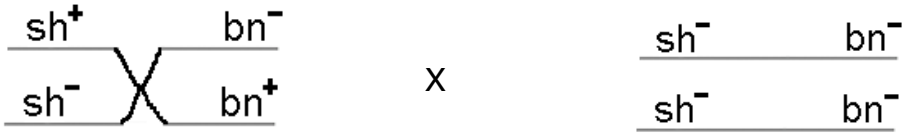
Name: _____

(c 8 points**)** A colleague calls to tell you she plans to carry out the following <u>two-factor</u> cross. A true breeding line with bent wings will be crossed to a true breeding line with short legs (unless specified other traits appear normal). F1 flies will then be crossed to a true breeding strain with bent wings and short legs. Your colleague wants to know what proportion of the progeny from this cross will have bent wings and short legs. What would you tell her?

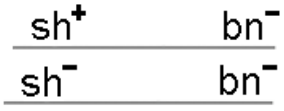
It is very important to realize that this question is dealing with a two-factor cross and not a three-factor cross. Although we have shown the cumulative map distance between "bn" and "sh" is 50 cM via a three-factor cross, the observable map distance in a two-factor cross will only be 38 cM.

This means that 38% of the F2 progeny will be recombinant progeny. One half of these progeny will inherit the sh- bn- chromosome.

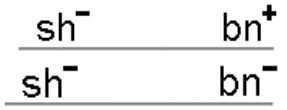
Therefore, 38 / 2 = 19 % of the F2 progeny will have short wings and bent legs.

F1 

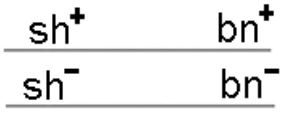
F2



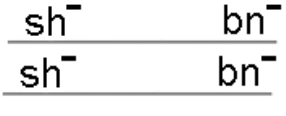
62 / 2 = 31% Bent wings



62 / 2 = 31% Short legs



38 / 2 = 19% Wild-type



38 / 2 = 19% Short legs and Bent wings

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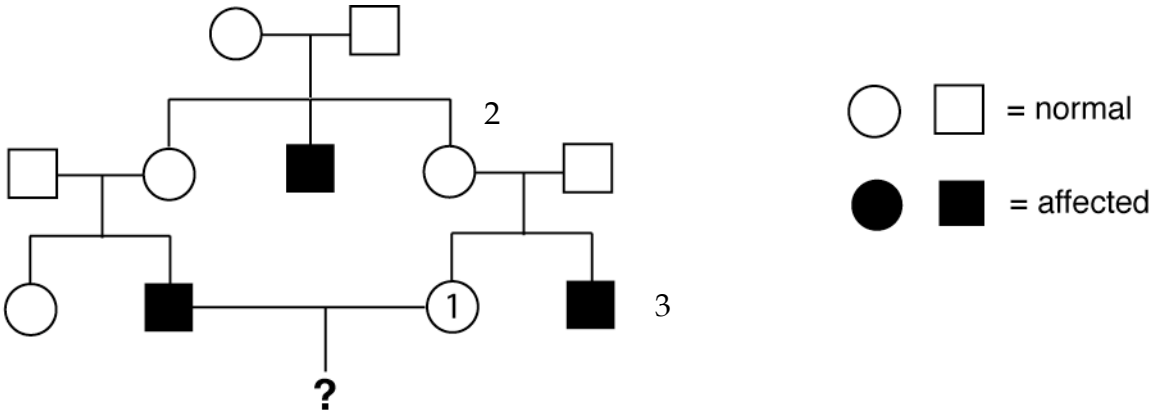
2. The pedigree shows inheritance of an X-linked recessive trait. (Assume the trait is completely penetrant).



(a 4 pts.**)** What is the probability that the female designated **1** is a carrier for the trait?

Given that male #3 is affected, female #2 must be a carrier. As a result, the probability that female #1 is a carrier is $\frac{1}{2}$.

(b 6 pts.**)** If the child indicated by **?** is a boy, what is the probability he will be affected by the trait?

P (boy ? affected) = P (#1 is a carrier) x P (boy ? receives X^{rec} from mom)

$= \frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$

(c 6 pts.**)** If the child indicated by **?** is a girl, what is the probability she will be affected by the trait?

P (girl ? affected) = P (#1 is a carrier) x P (girl ? receives X^{rec} from mom) x P (girl ? receives X^{rec} from dad)

$= \frac{1}{2} \times \frac{1}{2} \times 1 = 1/4$

Name: _____

(d 8 pts.**)** If the child indicated by **?** is affected by the trait, what is the probability that the couple's next child will be affected by the trait?

If the child indicated by ? is affected, then female #1 must be a carrier. Thus, the probability that the next child is affected is $\frac{1}{2}$.

(e 12 pts.**)** If the child indicated by **?** is not affected by the trait, calculate the new probability that the female designated **1** is a carrier for the trait.

Bayes Theorem can be used to compute this conditional probability.

X = #1 is a carrier
Y = the child isn't affected
P (Y/X) = $\frac{1}{2}$
P(X) = $\frac{1}{2}$
P (Y/not X) = 1
P (not X) = 1/2

P (X | Y) = p(Y | X)*p(X) / [p(Y | X)*p(X) + p(Y | not X)*p(not X)]

$$P(X/Y) = \frac{(1/2 \times 1/2)}{[(1/2 \times 1/2) + (1 \times 1/2)]}$$

P (X/Y) = 1/3

After the birth of a child without the trait, the probability that female #1 is a carrier is reduced from 50% to 33.33%.

Name: _____

3. You have isolated two different yeast mutants called *cys1*$^-$ and *cys2*$^-$ that cannot synthesize the amino acid cysteine and therefore require cysteine added to the medium for growth (i.e. they are Cys$^-$).

(a 4 points**)** You mate a *cys1*$^-$ mutant to a *cys2*$^-$ mutant. The resulting diploids don't require cysteine (i.e. they are Cys$^+$). What does this tell you about the *cys1*$^-$ and *cys2*$^-$ mutations?

This result is indicative of complementation between cys1 and cys2. Therefore, the mutations are in <u>different</u> genes.

Next, you sporulate the diploid from part (**a**). Among the 50 tetrads analyzed three different tetrad types are found

Type:	4 Cys$^-$	3 Cys$^-$: 1 Cys$^+$	2 Cys$^-$: 2 Cys$^+$
Number:	39	10	1

(b 6 points**)** Say that you want a *cys1*$^-$*cys2*$^-$ double mutant. What is the easiest way to obtain such a mutant without further analysis?

The double crossover event creating the NPD tetrad resulted in the production of two Cys$^-$ and two Cys$^+$ spores. The two Cys$^-$ spores (*cys1*$^-$*cys2*$^-$) are double mutants.

(c 6 points**)** You choose one of the tetrads for further analysis and the four spores have the following properties: Spore 1 = MAT**a** Cys$^+$, Spore 2 = MAT**a** Cys$^-$, Spore 3 = MATα Cys$^-$, and Spore 4 = MATα Cys$^-$. You carry out the matings that are possible and find that the diploid produced by mating Spore 2 to Spore 3 is Cys$^-$, while the diploid produced by mating Spore 2 to Spore 4 is Cys$^+$. Which spore is the double mutant? Explain your reasoning.

Spore 3 is the double mutant. The cross between spore 2 (Cys$^-$) and spore 4 (Cys$^-$) generated a Cys$^+$ diploid. This is complementation and indicates that spores 2 and 4 are single mutants carrying mutations in different genes. Since we know spore 1 is Cys$^+$, it is not a mutant. This leaves spore 3 as the double mutant.

Name: _____

(d 6 points**)** Given the number of tetrads of each type, what is the distance between the *cys1*$^{-}$ and *cys2*$^{-}$ mutations?

$$\text{Map Distance (cM)} = 100 \times \frac{\text{TT} + 6\,(\text{NPD})}{2 \sum \text{TETRADS}}$$

= 100 x [16 / 100]

= 16 cM

You have isolated a mutation that you call *cysX*$^{-}$ that activates an alternative pathway for cysteine synthesis. A *cysX*$^{-}$ mutation on its own is Cys^{+}, and when a *cysX*$^{-}$ mutation is combined with a *cys1*$^{-}$ mutation, the double mutant is Cys^{+}.

(e 8 points**)** Describe the cross you would perform and the interpretation of the outcome that you would use to determine whether *cysX*$^{-}$ is dominant or recessive?

cysX^{-} *cys1*$^{-}$ (haploid) x *cysX*$^{+}$ *cys1*$^{-}$ (haploid) > *cysX*$^{-}$ *cysX*$^{+}$; *cys1*$^{-}$ *cys1*$^{-}$ (diploid)

If diploid is *cys*$^{+}$, then *cysX*$^{-}$ is dominant to *cysX*$^{+}$

If diploid is *cys*$^{-}$, then *cysX*$^{-}$ is recessive to *cysX*$^{+}$

(f 8 points**)** Say that *cysX*$^{-}$ is 5 cM away from *cys1*$^{-}$. In a cross of a MAT**a** *cysX*$^{-}$ mutant to a MATα *cys1*$^{-}$ mutant what types of tetrads (in terms of the proportion of Cys^{-} : Cys^{+}) would you expect to find and how many of each type would you expect from a total of 50 tetrads?

PD(45)	NPD(0)	TT(5)
cysX^{-} *cys1*$^{+}$	*cysX*$^{-}$ *cys1*$^{-}$	*cysX*$^{-}$ *cys1*$^{-}$
cysX^{-} *cys1*$^{+}$	*cysX*$^{-}$ *cys1*$^{-}$	*cysX*$^{-}$ *cys1*$^{+}$
cysX^{+} *cys1*$^{-}$	*cysX*$^{+}$ *cys1*$^{+}$	*cysX*$^{+}$ *cys1*$^{+}$
cysX^{+} *cys1*$^{-}$	*cysX*$^{+}$ *cys1*$^{+}$	*cysX*$^{+}$ *cys1*$^{-}$
2*cys*$^{+}$: 2*cys*$^{-}$	4*cys*$^{+}$	3*cys*$^{+}$: 1*cys*$^{-}$

Out of 50 tetrads, 0 NPD would be expected. The frequency of a double crossover is .05 x .05 = 0.0025. Of these double crossovers, only $\frac{1}{4}$ represent NPDs. Thus, only one in 1600 tetrads would be expected to be NPD.

$$\text{Map Distance (cM)} = 100 \times \frac{\text{TT} + 6\,(\text{NPD})}{2 \sum \text{TETRADS}}$$

$5 = 100 \times [((TT + (6 \times 0))/(2 \times 50)]$

TT = 5. Thus, the 45 remaining tetrads are PD.

Name: _____

1. You are studying how yeast cells grow on sucrose and you find that both sucrose and glucose regulate expression of the *SUC1* gene, which encodes an enzyme for sucrose breakdown. *SUC1* is not expressed in cells grown without sucrose, but is induced when sucrose is added to the growth medium. In cells grown in medium that contains both sucrose and glucose, *SUC1* is not expressed. You have isolated mutations in three different genes that alter *SUC1* regulation, called **A⁻**, **B⁻** and **C⁻**. All three mutations are recessive and none of the mutations are linked to one another or to *SUC1*. *SUC1* expression in wild type yeast and each of the three mutants are shown below.

	SUC1 expression		
	– sucrose	+ sucrose	+ sucrose & glucose
Wild type	–	+	–
A⁻	+	+	–
B⁻	–	–	–
C⁻	–	+	+

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

Gene **A**

Sucrose – negative regulator

Gene **B**

Sucrose – positive regulator

Gene **C**

Glucose – negative regulator

Name: _____

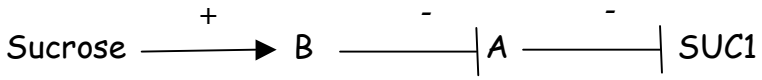
You cross an **A⁻** mutant to a **B⁻** mutant. After tetrads are dissected and evaluated for *SUC1* expression in either the presence or absence of sucrose, (no glucose is present in this experiment) the following tetrad types are observed.

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.

Based on the spore phenotypes, the Type 2 tetrads must be the NPD tetrads. The spores within the NPD tetrad show either a regulated or constitutive phenotype. The regulated phenotype corresponds to an A+ B+ genotype. Thus, the spores showing the constitutive phenotype must have the double mutant genotype (A- B-).

(c 10 pts.**)** Draw a model showing the interactions between the different regulatory factors encoded by **A** and **B**. Be sure to include the *SUC1* gene and to indicate where and how sucrose acts.



Sucrose and gene B are net activators of SUC1 expression, while gene A is a net negative regulator.

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Next, you construct a set of deletions within the promoter region of the *SUC1* gene (+1 indicates the first transcribed nucleotide). The ability of each of these deletions to express *SUC1* in cells grown on different sugars is shown below.

	-300 |	-250 |	-200 |	-150 |	-100 |	-50 |	+1 |	– sucrose	+ sucrose	+ sucrose & glucose
1)	___	_____						–	+	–
2)	_____	_____						–	+	+
3)	_____	_____						–	–	–
4)	_____	_____						–	+	–
5)	_____	_____						–	+	–
6)	_____	__						–	–	–

(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 *SUC1* gene, where is it most likely to bind? Explain your reasoning.

The deletion 2 mutant shows SUC1 expression in the presence of glucose. This suggests that the nucleotide region -250 to -200 is the site where the Gene C regulatory protein binds.

In addition, the deletion 2 mutant and the Gene C mutant exhibit the identical phenotype, which suggests that the deleted -250 to -200 region is the binding site for the Gene C regulatory product.

(e 5 pts.**)** In general, upstream activation sequences function normally regardless of their distance from the start of transcription. Which of the deletion mutants shown above show this to be true for the upstream activation sequence that responds to sucrose activation?

The results above show that the UAS responsive to sucrose lies in the -200 to -150 region. Deletion mutants 4 & 5 show wild-type regulation and reduce the distance between the UAS and promoter and support the hypothesis that a UAS can function normally despite its distance from the transcription start site.

Name: _____

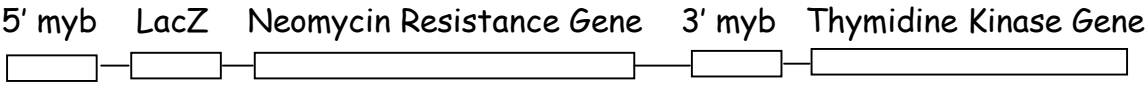
2. **(a** 12 pts.**)** Recently your lab has become interested in the function of a mouse gene called *myb*. To determine when and where *myb* is expressed during development you decide to construct a reporter for *myb* expression that can be examined in developing mice. Describe the basic procedure that you would use to produce a useful reporter for *myb* expression. For your answer include such specifics as cell type(s), a diagram of the DNA construct, the site of integration, and any additional breeding steps needed to obtain mice useful for your study.

A transgenic approach or knock-in approach would be effective.

For the transgenic approach, a gene fusion consisting of the myb promoter ligated to the LacZ or GFP coding region (P_{myb} – LacZ or P_{myb} - GFP) could be introduced into a wild-type fertilized egg by micro-injection. This transgene would insert randomly into the genome. No additional breeding would be required. This transgenic mouse would serve as an effective model to monitor the spatial and temporal pattern of myb gene expression during development.

For the knock-in approach, the endogenous myb gene could be replaced with a reporter gene such as lacZ or GFP under control of the myb promoter.

The DNA construct, which would replace the endogenous myb gene through homologous recombination, would contain the LacZ gene and an anti-biotic resistance gene. DNA homologous to the 5′ and 3′ regions of the myb gene would border these two genes. In addition, a gene encoding thymidine kinase would be placed outside of the homologous 5′ and 3′ regions of the myb gene.



After selecting for homologous recombination of the construct into wild-type embryonic stem cells in culture, the knock-in cells would be introduced into an embryo, which would be injected into the uterus.

The chimeric offspring would be mated with wild-type mice in order to yield heterozygous mice (KI/+). These heterozygous mice would serve as effective models to monitor the spatial and temporal pattern of myb expression during development.

(b 8 pts.**)** From the procedure above you obtain two different lines of mice carrying integrated *myb* reporter constructs. You cross heterozygous mice from Line 1 to one another and score how many of the resulting progeny carry the reporter construct and how many don't. You repeat the procedure for Line 2. The results of these two crosses are shown below.

	Contain Reporter	Do not contain Reporter
Progeny from Line 1 heterozygotes:	74	24
Progeny from Line 2 heterozygotes:	41	23

Provide an explanation for this data, keeping in mind that you did the same number of crosses for each line, but obtained fewer progeny overall from the Line 2 crosses.

The absence of homozygous recessives in line 2 suggests that the reporter gene fusion inserted into an essential gene resulting in a loss of function mutation. The absence of a functional allele of this essential gene in homozygous recessives is lethal.

An alternative explanation is that two copies of the reporter gene fusion in homozygous recessives somehow are lethal due to some sort of toxicity.

Name: _____

(c 10 pts.**)** With Line 1, you design a screen looking for regulators of *myb* expression. After mutagenesis with a chemical mutagen, you find a mutant mouse (M) that has increased expression of your construct. The regulatory mutant M is genetically recessive and after extensive mapping experiments, you narrow down the location of the regulatory mutation to a region that contains two genes, TF1 and Db. Describe how you would construct a useful mouse model to test the idea that Db is a regulator of *myb* expression. For your answer include the cell type(s) you would target, a diagram of the DNA construct, the site of integration, and any additional breeding steps needed to get mice useful for your study.

A knockout or transgenic could be used.

For the knockout approach, the construct illustrated below would homologously recombine into the Db gene of wild-type embryonic stem cells.

After producing chimeric mice, they would be crossed with wild-type mice to generate heterozygous offspring (KO/+). The resulting heterozygous offspring would be mated among themselves to yield homozygous knockout mice (KO/KO). The homozygous knockout could be used to determine if Db is a regulator of myb.

For the transgenic approach, a wild-type Db allele would be injected into a MM fertilized egg through micro-injection. The wild-type Db allele would insert randomly into the MM mutant genome. No additional breeding steps would be required.

The phenotype of the transgenic mouse would enable the role of Db in myb expression to be determined.

(d 8 pts.**)** Using any mice generated in parts **a-c**, describe how you would carry out breeding experiments to test whether the mutation M is an allele of the Db gene. For your answer, include possible results and how you would interpret them.

The following cross could be performed –

KO/KO x M/M

↓

KO/M

and the phenotype of the KO/M mice would be examined.

If no complementation occurs, then M is an allele of the Db gene.

If complementation occurs, then M is an allele of a gene other than Db.

Another cross could be performed –

M/M x Transgenic Mouse

If the progeny show a wild-type phenotype, then M is an allele of the Db gene.

3. **(a** 6 pts.**)** Consider a blood antigen called D. The allele for this antigen is dominant (i.e. individuals who are either D/d or D/D will express the antigen). If 84% of a population in Hardy-Weinberg equilibrium expresses the D antigen, what percent of the population are heterozygous for the D allele?

f(D/D) + f(D/d) = .84; Since f(D/D) + f(D/d) + f(d/d) = 1, f(d/d) = 1 - .84 = .16

f(d/d) = .16, so the frequency of d = .4

D + d = 1, so the frequency of D = 1 - .4 = .6

f(D/d) = 2 (D) (d) = 2 x .6 x .4 = .48

(b 6 pts.**)** The population in part **(a)** mixes in equal numbers with a population in which all of the individuals express the blood antigen. After one generation of random interbreeding between the two populations what percent of the population will express the antigen?

	f(D)	f(d)
Population 1	.6	.4
Population 2	1	~0, so $d^2 = 0$

Mixed Population: $D_{new} = (.5)\, f(D_{population\ 1}) + (.5)\, f(D_{population\ 2})$
$= (.5)\ .6 + (.5)\ 1$
$= .8$

Mixed Population: D + d = 1, so d = .2

f (individuals that express antigen) = f (D/D) + f (D/d)
= .64 + .32 = .96

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(c 9 pts.**)** You find an isolated population in which an unusual allele for the blood antigen (called d*) is quite prevalent. By analyzing the DNA from this population you find that 18% of the population has genotype D/d*, but none of the thousands of individuals examined has genotype d*/d*. Explain this result by assuming that d* is a balanced polymorphism. For your answer calculate parameters h (the heterozygous advantage for d*) and S (the selective disadvantage of d*/d*).

$d^* = \frac{1}{2} f(D/d^*) + f(d^*/d^*)$; Since $f(d^*/d^*) = 0$, $d^* = \frac{1}{2} f(D/d^*)$

$$d^* = \frac{1}{2} f(D/d^*) = 1/2\ (.18) = .09$$

OR $f(D/D) = .82 = D^2$, so D = .905 and d* = 1-.905 = .095

$Sq = h$; S=1, so $q = h = .09$ or .095

(d 9 pts.**)** Consider a population in which 1% of matings are between second cousins, yet all other matings are between unrelated individuals (second cousins have great grandparents in common). If half of the individuals with a recessive trait have parents that are second cousins, what is the (approximate) allele frequency for the trait.

The inbreeding coefficient for second cousins is 1/64 (F = 1/64).

$f(a/a) = .01\ (Fq) + .99\ (q^2)$

$\frac{1}{2} f(a/a) = .01\ (Fq)$

$.02\ (Fq) = .01\ (Fq) + .99\ (q^2)$

$.99\ q = .01\ (1/64)$

$q = 1.58 \times 10^{-4}$