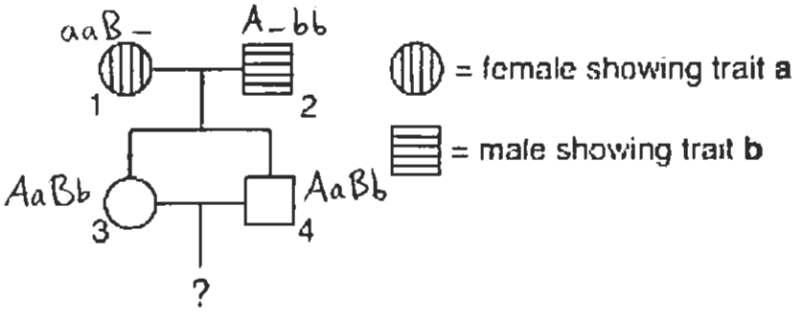
Exam #1 2003 **Name:** KEY

1. The following mouse pedigree shows the segregation of two different autosomal recessive traits. (Assume all phenotypes are completely penetrant).



(**a** 5 pts.) What is the genotype of mouse designated **3**? Use **A** and **a** to designate the dominant and recessive alleles of the gene for trait **a**; and use **B** and **b** to designate the dominant and recessive alleles of the gene for trait **b**.

AaBb

(**b** 10 pts.) If the genes for trait **a** and trait **b** are unlinked, what is the probability that a progeny mouse indicated by **?** will NOT show either recessive trait?

$$p(A\text{-}B\text{-}) = p(A\text{-})\,p(B\text{-}) = (3/4)(3/4) = 9/16$$

(**c** 12 pts.) If the genes for trait **a** and trait **b** are 20 cM apart on the same autosomal chromosome, what is the probability that a progeny mouse indicated by **?** will NOT show either recessive trait?

Parental gametes
Ab = 40%
aB = 40%
Recombinant gametes
ab = 10%
AB = 10%

	(.4) Ab	(.4) aB	(.1) ab	(.1) AB
(.4) Ab	\	.16	\	.04
(.4) aB	.16	\	\	.04
(.1) ab	\	\	\	.01
(.1) AB	.04	.04	.01	.01

Sum = 0.51

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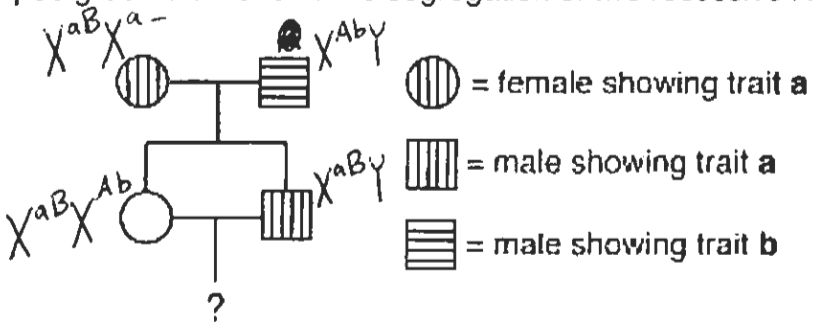
(**d** 8 pts.) The pedigree below shows the segregation of two recessive X-linked traits.



If the genes for the two traits are 20 cM apart on the X chromosome, what is the probability that that a **female** progeny mouse indicated by **?** will NOT show either recessive trait?

receives X^{aB} from father.

must receive X^{A-} from mother.

$$p(X^{A-} \text{ from mother}) = p(X^{AB}) + p(X^{Ab}) = 0.1 + 0.4$$

$$= 0.5$$

2. (**a** 5 points) You have obtained a strain of *Drosophila*, which has cinnabar colored eyes (cn^-) and becomes paralyzed at high temperature because of a shibire mutation ($shi\text{-}1^-$). You mate this strain to a true breeding wild type fly and obtain F1 flies, all of which have the wild type phenotype. F1 females are then mated to males of the starting strain (cn^-, $shi\text{-}1^-$). Among 100 progeny from this cross you observe the following phenotypes:

	Phenotype	Number
parental	wild type (not paralyzed, red eyes)	44
	paralyzed, cinnabar eyes	41
recombinant	not paralyzed, cinnabar eyes	7
	paralyzed, red eyes	8

From this data what is the distance between the **cn** and **shi** genes?

$$\text{distance} = 100 \times \frac{\text{recombinant gametes}}{\text{total gametes}}$$

$$= 100 \times \frac{(7+8)}{100} = 15 \text{ cM}$$

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(b 8 points) You isolate a second allele of the shibire gene designated shi-2$^-$, which also causes paralysis at high temperature. Flies from a true breeding shi-2$^-$ strain are crossed to flies from the true breeding cn$^-$, shi-1$^-$ strain described above. The resulting F1 flies are paralyzed at high temperature and have normal red eyes. F1 females are then mated to males from the true breeding cn$^-$, shi-1$^-$ strain. You collect 10,000 progeny from this cross and note that although almost all the flies are paralyzed at high temperature, there are 10 that are not paralyzed. What is the distance between the shi-1$^-$ and shi-2$^-$ mutations?

Parentals

$\frac{shi2^-\quad +}{+\quad shi1^-}$ (paralyzed)

$\frac{+\quad shi1^-}{+\quad shi1^-}$ (paralyzed)

Recombinants

$\frac{shi2^-\quad shi1^-}{+\quad shi1^-}$ (paralyzed)

$\frac{+\quad +}{+\quad shi1^-}$ (not paralyzed)

We see 10 recombinant, non-paralyzed progeny, but there are an equal number of paralyzed recombinant progeny: $d = 100 \times \frac{10 \times 2}{10{,}000} = 0.2$ cM

(c 12 points) Among the 10 progeny flies that are not paralyzed that result from the cross described in part (b), 8 have cinnabar eyes and 2 have normal red eyes. On the basis of this information as well as the results from parts (a) and (b), draw a genetic map showing the order of the cn$^-$, shi-1$^-$, and shi-2$^-$ mutations and your best estimates of the relevant map distances.

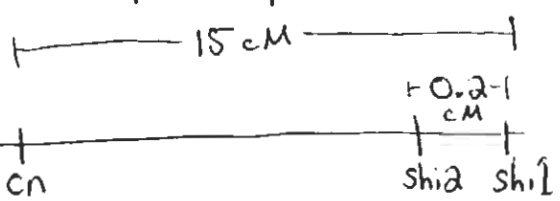
from part (a) $d_{cn-shi1} = 15$ cM

from part (b) $d_{shi1-shi2} = 0.2$ cM

gene order: cn - shi2$^-$ - shi1

Single recombination between shi2 and shi1 gives a non-paralyzed class with cinnabar eyes

double recombination gives a non-paralyzed class with red eyes.



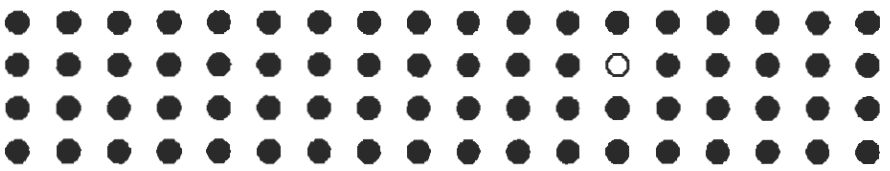
Name: KEY

3. Some yeast mutants with defects in enzymes in the pathway for adenine biosynthesis form red colonies because of the accumulation of an intermediate in the pathway, which is a red pigment.

(**a** 4 points) You have isolated two different red colored mutants in haploid strains of different mating types, which you call *ade1*$^-$ and *ade2*$^-$. When either the *ade1*$^-$ or *ade2*$^-$ mutant is mated to wild type, the resulting diploid forms white colonies like those of wild type yeast. When the *ade1*$^-$ mutant is mated to the *ade2*$^-$ mutant the resulting diploid makes red colonies. From these observations, describe as much as you can about the *ade1*$^-$ and *ade2*$^-$ mutations and the relationship between them.

- ade1$^-$ and ade2$^-$ are recessive to wild-type
- ade1$^-$ and ade2$^-$ fail to complement, and therefore are mutations in the same gene

(**b** 4 points) Next, you sporulate the diploid that was formed by mating the *ade1*$^-$ and *ade2*$^-$ haploid strains. From the 18 tetrads shown below only one spore clone is white, the rest are red.



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

What does this result tell you about the distance between the *ade1*$^-$ and *ade2*$^-$ mutations?

17 PD
1 TT

They are tightly linked.
The distance between them is

$$= 100 \times \left(\frac{TT + 6NPD}{2\Sigma}\right)$$

$$= 100 \times \left(\frac{1 + 6(0)}{2(18)}\right) = 2.78\ cM$$

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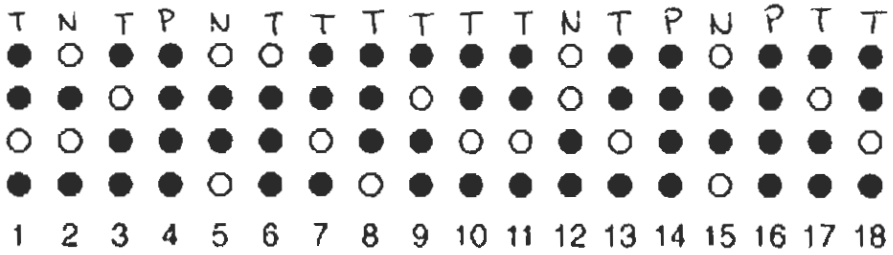
(**c** 4 points) Next, you isolate a new red colored mutant, which you call *ade3*$^-$. When the *ade3*$^-$ mutant is mated to wild type the resulting diploid is red. You mate the *ade3*$^-$ mutant to an *ade1*$^-$ mutant and the resulting diploid is red also. What do these results tell you about the *ade3*$^-$ mutant and the relationship between the *ade3*$^-$ and *ade1*$^-$ mutations?

- ade3$^-$ is dominant to wild-type
- complementation test is inconclusive
  nothing can be concluded about relationship between ade1$^-$ and ade3$^-$

(**d** 6 points) When you sporulate the diploid that was formed by mating the *ade3*$^-$ and *ade1*$^-$ haploid strains you obtain the results shown below:



From the 18 dissected tetrads shown how many tetrads of each type (PD, NPD or T) are there?

3 PD

11 TT

4 NPD

(**e** 4 points) What do the results from this tetrad analysis tell you about the relationship between the *ade3*$^-$ and *ade1*$^-$ mutations? Can these mutations be in the same gene?

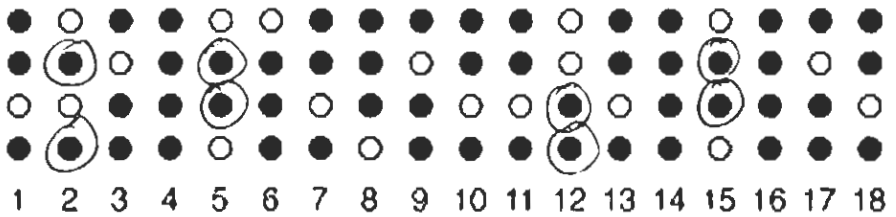
3:11:4 is approximately 1:4:1

ade3$^-$ and ade1$^-$ are unlinked

they cannot be mutations in the same gene

Name: KEY

(f 4 points) Suppose that you wanted to do some experiments with an *ade3*$^-$*ade1*$^-$ double mutant. On the image of the tetrads from the cross between the *ade3*$^-$ and *ade1*$^-$ mutants, circle each spore clone that you can be sure is double mutant, without any further testing.



circle red spores from NPD.

(g 4 pts.) If you crossed an *ade3*$^-$ mutant to an *ade2*$^-$ mutant and dissected 18 tetrads, how many **T**(etratype) tetrads would you expect to see?

Since ade1$^-$ and ade2$^-$ are tightly linked and since ade2$^-$ and ade3$^-$ are unlinked, you would expect a ratio of 1 PD: 4 TT: 1 NPD. You would expect 12 of the 18 tetrads to be tetratypes.

Grading section

Question 1 35 points:_____

Question 2 25 points:_____

Question 3 30 points:_____

Total :_____

7.03 Exam 2

Name: KEY

TA:

Section time:

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

Please write your name on each page.

Only writing on the front sides of each page will be graded

Question 1	**14 points**
Question 2	**24 points**
Question 3	**28 points**
Question 4	**34 points**

7.03 EXAM #2 FALL 2003

Name: KEY

1. (**a** 8 pts.) The sequence of the amber stop codon is $^{5'}UAG^{3'}$ and the sequence of the Trp codon is $^{5'}UGG^{3'}$. Write out the DNA sequence of the anti-codon portion of an amber suppressing allele of $tRNA^{trp}$ (label the 5' and 3' ends of both DNA strands and indicate which strand is used as the *template* during transcription of the tRNA).

mRNA amber stop codon 5' U A G 3'

tRNA anti-codon 3' A U C 5' (equivalent to 5' C U A 3')

DNA for tRNA anti-codon

5' C T A 3'
3' G A T 5' ← template strand

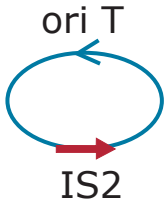
(**b** 6 pts.) You have isolated a mutation in the Lac I gene, which causes constitutive Lac gene expression. DNA sequencing reveals that the mutant gene has an amber mutation in about the middle of the Lac I coding sequence. However, you find that when you introduce the amber suppressing allele of $tRNA^{trp}$ described in part (**a**) into the strain carrying the Lac I mutation, the strain still expresses Lac genes constitutively. Propose two different explanations for why the amber suppressing allele of $tRNA^{trp}$ fails to suppress this particular amber mutation.

1) There is an insufficient amount of wild-type protein to restore regulation.

2) Insertion of tryptophan at that position does not restore normal protein function.

Name: KEY

2. Below is a diagram of the F factor showing the direction of the origin of transfer (ori T) and an **IS2 element** carried on F.



Figurey MIT OCW.

From a wild type F^+ strain you isolate an Hfr strain that transfers $PyrD^+$ early and efficiently, but does *not* transfer the neighboring markers $PyrC^+$, $PurB^+$ and $TrpA^+$ until after 90 minutes of a mating reaction.

(**a** 8 pts.) On the map of a small segment of the *E. coli* chromosome shown below, draw in an **IS2 element** (represented by an arrow to show proper orientation) that could have recombined with the **IS2 element** on F to produce the Hfr.

(**b** 8 pts.) You mate the Hfr strain isolated in part (**a**) to an F^- $PyrC^-$ strain and after a brief (~10 minute) mating you isolate a rare $PyrC^+$ recipient strain. In subsequent matings the newly isolated $PyrC^+$ strain can transfer $PyrC^+$ and $PurB^+$ early and efficiently, but cannot transfer either $PyrD^+$ or $TrpA^+$. On the map below show the position and orientation of a *second* additional chromosomal **IS2 element** (represented by an arrow to show orientation) that would allow the formation of an F' element with these properties.

Name: KEY

2. Below is a diagram of the F factor showing the direction of the origin of transfer (ori T) and an **IS2 element** carried on F.

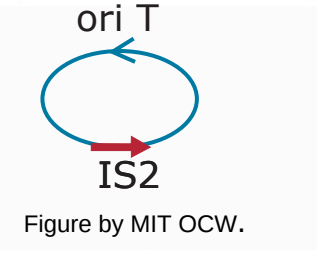


Figure by MIT OCW.

From a wild type F^+ strain you isolate an Hfr strain that transfers $PyrD^+$ early and efficiently, but does *not* transfer the neighboring markers $PyrC^+$, $PurB^+$ and $TrpA^+$ until after 90 minutes of a mating reaction.

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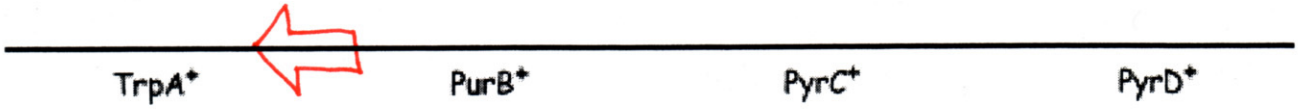


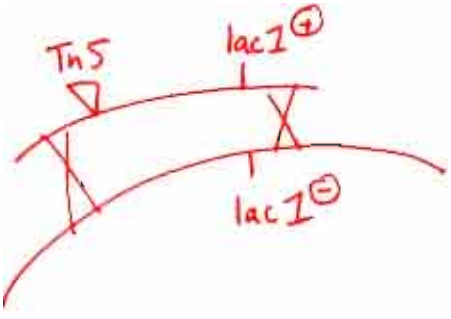
Name: KEY

(c 8 pts.) You have isolated a new PurB$^-$ allele that cannot grow unless purine is added to the medium. However, you find that when the F' isolated in part (**b**) is mated into this strain the resulting recipients bearing the F' remain unable to grow in the absence of purine. Propose an explanation for this finding.

The new PurB$^{\oplus}$ allele is dominant to wild-type.

3. You have isolated a mutant that cannot grow on lactose (Lac$^-$), which you call Lac1$^-$.

(a 8 pts.) You have a wild type (Lac$^+$) strain carrying a Tn5 insertion known to be near Lac genes on the *E. coli* chromosome. You grow P1 phage on this strain and use the resulting phage lysate to infect the Lac1$^-$ strain, selecting for kanamycin resistance (Kan^r). Among 50 Kan^r transductants, you find that 10 are Lac$^-$ and 40 are Lac$^+$. Express the distance between Tn5 and the Lac1$^-$ mutation as a cotransduction frequency.

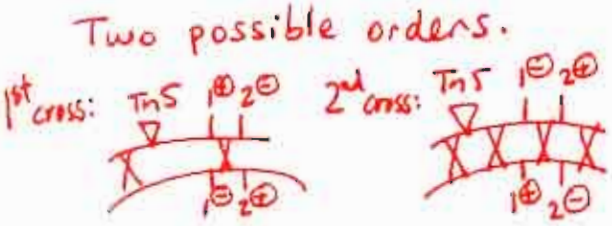


cotransduction: $\left(\frac{40}{50}\right) \times 100\% = 80\%$

Name: KEY

(**b** 12 pts.) You isolate a second Lac⁻ mutation, which you designate Lac2⁻. To map Lac2⁻ relative to Lac1⁻ you set up two reciprocal crosses. In the first cross you grow P1 on a strain that carries the Tn5 insertion described in part (**a**) and the Lac2⁻ mutation. You then use this lysate to infect a Lac1⁻ mutant and select for Kan^r. From 100 Kan^r transductants examined, 96 are Lac⁻ and 4 are Lac⁺. In the second cross you grow P1 on a strain that carries the Tn5 insertion and the Lac1⁻ mutation. You then use this lysate to infect a Lac2⁻ mutant, and select for Kan^r. From 100 Kan^r transductants examined all are Lac⁻. Draw a genetic map showing the relative positions of the Tn5 insertion and the Lac1⁻ and Lac2⁻ mutations.

Two possible orders.



For this order, the second cross should give fewer Lac⁺. This is consistent with data.

For this order, the first cross should give fewer Lac⁺. This is not consistent with data.

Order: 

(**c** 8 pts.) Further analysis of the Lac1⁻ and Lac2⁻ mutations reveals that the Lac1⁻ mutant does not express ß-galactosidase (even in the presence of the inducer IPTG) but expresses permease normally, whereas the Lac2⁻ mutant does not express either ß-galactosidase or permease even in the presence of inducer. What type of mutation best explains the properties of Lac1⁻? What type of mutation best explains the properties of Lac2⁻.

Lac1$^\ominus$ is a mutation in the LacZ gene. → LacZ⁻

Lac2$^\ominus$ is either a mutation in the Lac operon promoter, or it is a mutation that results in a super repressor. → P⁻ or I^s

Name: KEY

4. In studying the regulation of sucrose utilization in a new bacterial species you find that the enzyme sucrase is expressed only when sucrose is added to the growth medium.

(**a** 8 pts.) You mutagenize the bacteria by generating a collection of random insertions of the transposon Tn5 into the bacterial chromosome. By screening for altered expression of sucrase, you find an insertion mutation, designated Suc1$^-$, which gives *constitutive* sucrase expression regardless of whether sucrose is present. Mapping of the Tn5 insertion shows that Suc1$^-$ is not linked to the sucrase gene. Classify the Suc1$^-$ mutation in terms of its likely genetic properties taking into account the type of mutation usually caused by a transposon insertion (explain your reasoning). Finally, propose the type of regulatory function probably encoded by the wild type Suc1 gene.

Suc1$^\ominus$ is
- constitutive (given)
- trans-acting (because unlinked to sucrase enzyme)
- recessive (transposon insertions generally result in loss of function mutations)

Suc1$^\oplus$ is likely a negative regulator

(**b** 12 pts.) You isolate a second Tn5 insertion mutation, designated Suc2$^-$, which also shows *constitutive* sucrase expression. The Tn5 insertion in Suc2$^-$ is *not* linked either to Suc1$^-$ or to the gene for sucrase. Diagram the *two* possible models for linear regulatory pathways for sucrase that account for the behavior of the Suc1 and Suc2 genes. For each model include a role for the inducer sucrose.

Suc2$^\ominus$ is also constitutive, trans-acting, and recessive. It is also likely a negative regulator.

Two possible linear regulatory pathways:

1) sucrose $\dashv^{\ominus}$ Suc1 $\xrightarrow{\oplus}$ Suc2 $\dashv^{\ominus}$ sucrase

2) sucrose $\dashv^{\ominus}$ Suc2 $\xrightarrow{\oplus}$ Suc1 $\dashv^{\ominus}$ sucrase

Name: KEY

(**c** 6 pts.) Explain why the phenotype of a Suc1$^-$ Suc2$^-$ double mutant will not help to distinguish between the models in part (**b**).

In order to carry out an epistasis test, the single mutants must exhibit distinct phenotypes. Here, however, both Suc 1$^\ominus$ and Suc 2$^\ominus$ are both constitutive.

(**d** 8 pts.) Next, you mutagenize the Suc1 gene with a chemical mutagen and find that most Suc1 gene mutations cause *constitutive* sucrase expression similar to the Tn5 insertion mutation. However, you are able to isolate a rare Suc1 allele, which you designate Suc1*, that causes *uninducible* sucrase expression. You construct a Suc1* Suc2$^-$ double mutant and find that it gives *uninducible* sucrase expression. Which of the models in part (**b**) is consistent with this information. Propose a molecular description of the type of effect the Suc1* mutation might have on the function of the Suc1 gene.

Suc 1* = uninducible

Suc 2$^\ominus$ = constitutive

Suc 1* Suc 2$^\ominus$ = uninducible.

Suc 1* is epistatic, and thus, model 2 is consistent.

Suc 1* is a super repressor. It may be mutated such that it is always bound to the operator of sucrase. Alternatively, its own operator may be defective such that it is constitutively expressed.

Question 1 14 points:_____

Question 2 24 points:_____

Question 3 28 points:_____

Question 4 34 points:_____

Total :_____

7.03 EXAM 3
FALL 2003

Name: KEY

1. Trekking in the Himalayas, you discover a "founder generation" of 1000 goats barricaded on all sides by high peaks and massive glaciers. This founder generation consists of 200 **AA** goats, 200 **Aa** goats, and 600 **aa** goats.

(**a** 4 pts.) What are the frequencies of alleles **A** and **a** in the founder generation?

$f(A) = f(AA) + \frac{1}{2} f(Aa) = \frac{200}{1000} + \frac{1}{2}\left(\frac{200}{1000}\right) = 0.3 = p$

$f(a) = 1 - f(A) = 0.7 = q$

(**b** 5 pts.) Is the founder generation in Hardy-Weinberg equilibrium? Show your work.

In H-W equilibrium, $f(aa) = q^2$, $f(Aa) = 2pq$, $f(AA) = p^2$

Here, $q^2 = (0.7)^2 = 0.49$ but $f(aa) = 0.6$

Not in H-W equilibrium

(**c** 3 pts.) What is the frequency of the **A** allele n the second generation (that is, in the generation after the founder generation)? (Mating of the founder generation goats is random, fitness does not differ among the three genotypes, and mutation occurs at a negligible rate.)

$f(A)$ does not change.

$f(A) = 0.3$

(**d** 8 pts.) What are the frequencies of the **AA**, **Aa**, and **aa** genotypes in the second generation?

$f(AA) = p^2 = (0.3)^2 = 0.09$

$f(Aa) = 2pq = 2(0.3)(0.7) = 0.42$

$f(aa) = q^2 = (0.7)^2 = 0.49$

Name: KEY

Let's now return to the purely random-breeding population in which this rare disease (fatal in childhood) has an incidence of four per million births. What rate of mutation (per generation) is required to maintain this incidence if:

(e 5 pts.) The disease exhibits autosomal dominant inheritance.

$\Delta q_{mut} = \Delta q_{sel}$

$\mu = \frac{1}{2}(4 \times 10^{-6}) = 2 \times 10^{-6}$

(f 5 pts.) The disease exhibits autosomal recessive inheritance.

$\Delta q_{mut} = \Delta q_{sel}$

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

Assume now that a new therapy allows many children with the disease to survive, such that affected individuals end up having 80% as many offspring as the population average. After many generations, a new steady-state balance between mutation and selection is achieved. At this new steady state, what would the incidence of the disease be if:

Assume μ is same (from (e) to (g) and from (f) to (h))

(g 5 pts.) The disease exhibits autosomal dominant inheritance.

$\Delta q_{mut} = \Delta q_{sel}$

$\mu = s \cdot \frac{1}{2} \cdot 2p$

$2 \times 10^{-6} = (0.2)p$

$p = 1 \times 10^{-5}$

Incidence $= 2pq \approx 2p = 2 \times 10^{-5}$

(h 5 pts.) The disease exhibits autosomal recessive inheritance.

$\Delta q_{mut} = \Delta q_{sel}$

$\mu = s q^2$

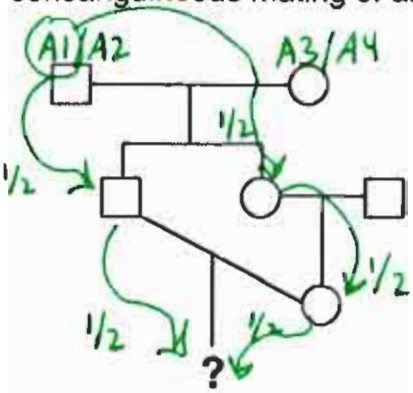
$4 \times 10^{-6} = (0.2)\, q^2$

$q^2 = 2 \times 10^{-5}$

Incidence $= q^2 = 2 \times 10^{-5}$

Name: KEY

2. Diagrammed below is a consanguineous mating of an uncle and niece.



(a 6 pts.) Calculate the inbreeding coefficient for this mating.

$p(A1|A1 \text{ by descent}) = (\frac{1}{2})^5 = (\frac{1}{32})$

$p(\text{homozygous by descent}) = 4(\frac{1}{32}) = \frac{1}{8}$

(b 4 pts.) Calculate the expected number of genes at which the resulting child will be homozygous by descent. (Assume that there are 30,000 genes in the human genome.)

$\frac{30,000}{8} = 3750$

Now consider a rare disease (fatal in childhood) whose incidence in a random-breeding population is four per million births. In parts (c) and (d), calculate the incidence of the disease in the next generation assuming that 1% of all matings are between uncles and nieces (all other matings being random) and:

(c 5 pts.) The disease exhibits autosomal dominant inheritance.

Inbreeding has no effect on incidence of autosomal dominant diseases.

Incidence = 4×10^{-6}

(d 5 pts.) The disease exhibits autosomal recessive inheritance.

$f(a)$, 1st generation (random mating) $= \sqrt{4 \times 10^{-6}} = 0.002 = q$

$f(aa)$, 2nd generation (non-random mating $= (0.99)q^2 + (0.01)(F)(q)$

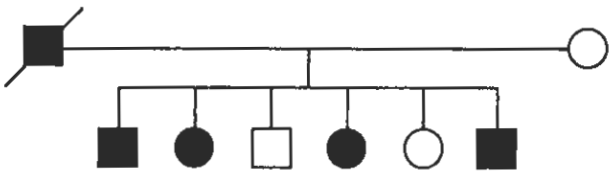
incidence $= 6.46 \times 10^{-6}$

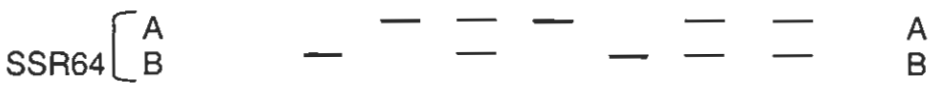
Name: KEY

3. You are genetically mapping webbed toes, a rare trait that shows autosomal dominant inheritance.

Alleles: + (normal) WT (associated with webbed toes)

Here is a family in which some individuals are affected: ↖ called "Θ" in diagrams below



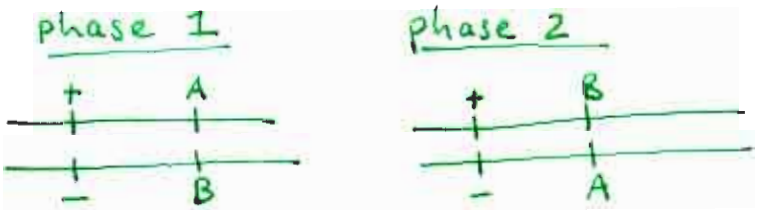


(a 3 pts.) What is the (deceased) father's genotype at SSR64?

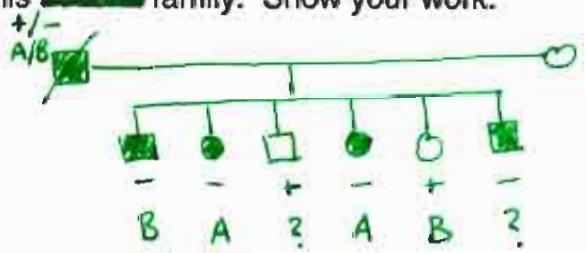
AB

(b 6 pts.) Diagram the phase relationship(s) between the WT alleles and the SSR64 alleles in the (deceased) father.

Both phases possible.



(c 16 pts.) Calculate the LOD score for linkage at θ = 0.2 between the webbed-toes gene and SSR64 in this [redacted] family. **Show your work.**



paternal alleles

Leave uninformative children out of LOD calculation.

$$LOD_{\theta=0.2} = \log_{10} \frac{P(\text{linked at } \theta = 0.2)}{P(\text{unlinked})} = \log_{10} \frac{\frac{1}{2} P(\text{data if phase 1}) + \frac{1}{2} P(\text{data if phase 2})}{P(\text{unlinked})}$$

$$= \log_{10} \frac{\frac{1}{2}\left((0.4)^1 (0.1)^3\right) + \frac{1}{2}\left((0.4)^3 (0.1)^1\right)}{(.25)^4} = -0.06$$

Name: KEY

4. On Problem Set 5, we observed that mice homozygous for a $P_{amylase}$-LacZ transgene insertion displayed a serious heart defect. A reasonable explanation for this observation was that the transgene had randomly inserted into a gene required for heart development or function. This transgene-induced heart defect reminded you of the recessive phenotype associated with *small heart* (***sh***), a previously identified mutation on mouse chromosome 12.

(**a** 9 pts) Like the human genome, the mouse genome is rich in SSR polymorphisms. The transgene insertion exists in mice of Strain A. You also have wild-type mice of Strain B, which differs from Strain A at many SSRs on each of the 20 mouse chromosomes. Propose a genetic linkage mapping experiment to test the hypothesis that your $P_{amylase}$-LacZ transgene has inserted somewhere in chromosome 12 (which has a genetic length of 80 cM).

- Cross Strain A (assumed to be homozygous for transgene insertion) to Strain B
- Resulting progeny are heterozygous for the transgene, and they all have an "A type" and a "B type" SSR at each SSR locus.
- Carry out a brother-sister mating with these progeny.
- Select F2s that display recessive phenotype.
- Examine SSRs on Ch. 12. If the transgene is on Ch. 12, we expect "A type" SSRs to greatly outnumber "B type" SSRs.

(**b** 6 pts.) Propose a breeding experiment to test the hypothesis that the ***sh*** mutation is in the same gene as the transgenic insertion mutation.

Since both the transgene insertion and the sh mutation are recessive, we can carry out a complementation test.

Strain A (assumed homozygous for transgene) × $\frac{sh}{sh}$

↓

If progeny show the "small heart" phenotype, the insertion is in the sh gene. (i.e. non-complementation)

7.03 Final Exam 2003

Name: Answer Key

Section: _____ **TA:** _____

There are 15 pages including this cover page. Verify that you have all 15 pages.

Please write your name on each page.
Only writing on the front side of each page will be graded.

Question 1	**22 points**	_____
Question 2	**16 points**	_____
Question 3	**26 points**	_____
Question 4	**28 points**	_____
Question 5	**26 points**	_____
Question 6	**23 points**	_____
Question 7	**24 points**	_____
Question 8	**10 points**	_____
Question 9	**25 points**	_____
Total	**200 points**	_____

Name: KEY

1. You have isolated two different mutant mouse strains that are true-breeding for the albino trait. When a mouse from each mutant is crossed to a wild type mouse all of the progeny appear normal. both are recessive mutations

(a 5 pts.) If the two albino mutations were **unlinked**, what fraction of the **F1** progeny from a cross between the two strains would be expected to be albino?

aaBB x AAbb

↓

AaBb → no albinos

(b 5 pts.) If the two albino mutations were **unlinked**, what fraction of the **F2** progeny from a cross between the F1 progeny derived in part **a** would be expected to be albino?

AaBb x AaBb

↓

9/16 A_B_
3/16 aaB_
3/16 A_bb
1/16 aabb

} albino → 7/16

(c 6 pts.) If the two albino mutations were **1 cM** apart in the **same** gene, what fraction of the **F1** progeny from a cross between the two strains would be expected to be albino?

$a^1a^1A^2A^2$ x $A^1A^1a^2a^2$

↓

$\frac{a^1 A^2}{A^1 a^2}$ no complementation (no functional copy of gene produced) → all albinos

(d 6 pts.) If the two albino mutations were **1 cM** apart in the **same** gene, what fraction of the **F2** progeny from a cross between the F1 progeny derived in part **c** would be expected to be albino?

parental gametes:
0.495 a^1A^2
0.495 A^1a^2
recombinant gametes:
0.005 a^1a^2
0.005 A^1A^2 (functional gene)

fraction albino = $4(0.495)^2 + 4(0.495)(0.005) + (0.005)^2 = 0.990025 \approx 0.99$

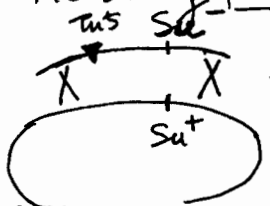
Name: KEY

2. **(a** 7 pts) You have isolated an *E. coli* mutant that carries both an amber mutation in the HisC gene ($HisC^{am}$) and an amber suppressor mutation (Su^+). Therefore this strain is phenotypically His^+ (it can grow without histidine added to the medium). You obtain a strain, which carries the $HisC^{am}$ mutation and has a Tn5 insertion known to be linked to the HisC gene; this strain is phenotypically His^- and is kanamycin resistant (Kan^r). You grow P1 phage on the $HisC^{am}$ Tn5 strain and use the resulting lysate to infect the $HisC^{am}$ Su^+ strain, selecting for Kan^r. Among 100 Kan^r transductants, you find that 20 are His^- and 80 are His^+. What distance are you measuring in this experiment? What is the distance?

$HisC^{am}$, Tn5, Su^- infect $HisC^{am}$, Su^+

Select for Kan^r

Measuring Tn5 to Su distance:

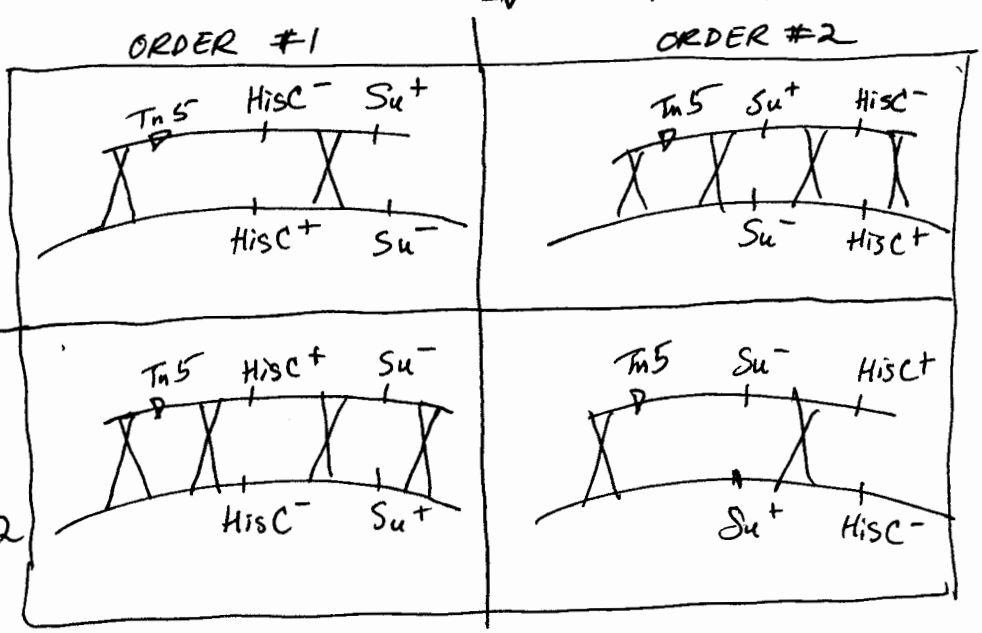


$\Rightarrow His^{\oplus}$

$$\text{distance} = \frac{His^{\oplus}}{\text{total}} = \frac{20}{100} = 20\%$$

(b 9 pts.) Next, you set up two reciprocal crosses. In the first cross you grow P1 on a strain that carries the Tn5 insertion described in part (**a**) as well as the $HisC^{am}$ and Su^+ mutations. You then use this lysate to infect a wild type strain ($HisC^+$ and Su^-) and select for Kan^r. From 100 Kan^r transductants examined, 99 are His^+ and 1 is His^-. In the second cross you grow P1 on a strain that carries the Tn5 insertion and is $HisC^+$ and Su^-. You use this lysate to infect a strain with $HisC^{am}$ and Su^+ mutations selecting for Kan^r. From 100 Kan^r transductants examined, 90 are His^+ and 10 are His^-. Draw a genetic map showing the relative positions of the Tn5 insertion and the $HisC^{am}$ and Su^+ mutations on the chromosome. What, if any, genetic distances can be obtained from these crosses?

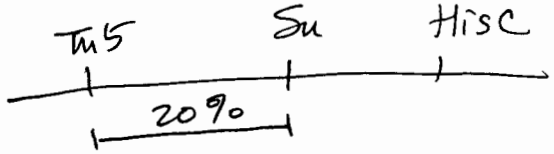
Consider 2 orders: Tn5 HisC Su —OR— Tn5 Su HisC



(note: $HisC^- = HisC^{am}$)

Data shows more $His^{\oplus}$ from Cross 2 than Cross 1.

This is consistent w/ ORDER 2



No other distances can be obtained.

Name: KEY

3. You are studying a yeast strain that will grow on the sugar raffinose. The gene for raffinase enzyme (Raf1) is expressed when raffinose is present, but it is not expressed when raffinose is absent. To study the regulation of Raf1, you construct a fusion of the Raf1 promoter to the *E. coli* LacZ gene and place this gene fusion on an extrachromosomal plasmid designated (Raf1-LacZ). Yeast cells carrying this plasmid express ß-galactosidase only in the presence of raffinose. You identify two new regulatory mutants designated Raf2$^-$ and Raf3$^-$. The effect of these mutants on expression of the Raf1-LacZ reporter is shown below:

	ß-galactosidase activity		
	+ raffinose	– raffinose	
Wild type (Raf1-LacZ)	+	–	
Raf2$^-$ (Raf1-LacZ)	+	+	constitutive, trans
Raf3$^-$ (Raf1-LacZ)	–	–	uninducible, trans

You then construct three diploids strains (each carrying the Raf1-LacZ reporter plasmid) with phenotypes shown below:

	ß-galactosidase activity		
	+ raffinose	– raffinose	
Raf2$^-$ / Raf2$^+$(Raf1-LacZ)	+	–	recessive
Raf3$^-$ / Raf3$^+$(Raf1-LacZ)	+	–	recessive
Raf2$^-$ / Raf3$^-$ (Raf1-LacZ)	+	–	complementation

(a 6 pts.) What do these results tell you about the relationship between the Raf2$^-$ and Raf3$^-$ mutations?

Raf 2$^-$ and Raf 3$^-$ are mutations in different genes, because there is complementation.

Name: KEY

(b 4 pts.) Next, you sporulate the diploid produced by crossing Raf2⁻ and Raf3⁻ strains. Out of a total of 50 tetrads, 35 are Type 1, 8 are Type 2, and 7 are Type 3.

TT : Type 1	NPD: Type 2	PD: Type3
constitutive	uninducible	constitutive
uninducible	uninducible	constitutive
uninducible	regulated	uninducible
regulated	regulated	uninducible

Is the Raf2⁻ Raf3⁻ double mutant regulated, constitutive, or uninducible?

Uninducible. (Phenotype of Raf 3⁻; so Raf 3 is epistatic to Raf 2.)

(c 8 pts.) On the basis of your answer for part **(b)** and from the rest of the information given in this problem, diagram a molecular model of Raf1 regulation. In your model, include Raf1, Raf2, Raf3, and raffinose.

Raf 2 = negative regulator
Raf 3 = positive regulator

Raffinose ⊣ Raf 2 ⊣ Raf 3 → Raf 1

(d 8 pts.) You isolate a dominant allele of the Raf2 gene that gives uninducible expression of Raf1-LacZ expression. The mutation in this allele (designated Raf2ᵘ) lies within the coding sequence of the Raf2 gene. In a cross between Raf2ᵘ and Raf2⁻ mutant strains, what kind(s) of tetrads would you expect to get and in what relative frequencies? (Specify the tetrad types in terms of the spore phenotypes with respect to Raf1-LacZ expression.)

Since they are tightly linked, expect PD >>> TT.

PD	TT
Uninducible	Uninducible
Uninducible	constitutive
constitutive	regulated
constitutive	Unknown (double mutant phenotype)

Name: KEY

4. The human genome spans about 3,000 Mb and contains about 30,000 genes. In the human genome, 1 cM corresponds approximately to 1 Mb. H_{nuc}, the average heterozygosity per nucleotide site, is approximately 0.001 in human populations. Assuming an average gene size of 20 kb (excluding the promoter), and an average mRNA size of 1.5 kb, calculate:

(a 4 pts.) The percentage of the human genome that is transcribed.

$$\frac{(20\,kb/gene)(30{,}000\,genes)}{(3000\,Mb)(1000\,kb/Mb)} = 20\%.$$

(b 4 pts.) The percentage of the human genome that is accounted for by introns.

$$\frac{(20\,kb - 1.5\,kb)(30000\,genes)}{(3000\,Mb)(1000\,kb/Mb)} = 18.5\%.$$

(c 5 pts.) For a randomly selected 20-kb autosomal gene, the expected number of intronic nucleotides at which a person's paternally and maternally inherited alleles differ.

18.5 kb of introns in a 20 kb gene

$H_{nuc} = 0.001$, so 1/1000 bases will be different between the maternal and paternal alleles

$(18.5\,kb)(0.001) = 18.5$ nucleotides

(d 5 pts.) What would your answer to question (**c**) be if the person were a monozygotic twin? A dizygotic twin? Briefly explain your answer.

Question (c) is looking at differences between an individual's parents. Therefore it doesn't matter whether or not the person in question is a twin or not, the answer is still 18.5 nucleotides

Name: KEY

(e 5 pts.**)** What would your answer to question **(c)** be if the person were a product of a brother-sister mating? Briefly explain your answer.

The inbreeding coefficient for a brother-sister mating is 1/4. Therefore the maternal and paternal genomes will have 1/4 of their genomes in common, and only the remaining 3/4 can potentially be different.

$$(3/4)(18.5 \text{ nucleotides}) = 13,875 \text{ nucleotides}$$

(f 5 pts.**)** Consider a randomly selected 20-kb gene in a human sperm. (We'll refer to the man who produced the sperm as the "father.") What is the likelihood that the gene is recombinant, that is, that its 5' end derives from one "grandparent" and its 3' end from the other "grandparent"?

$$1\text{cM} \approx 1\text{Mb}$$

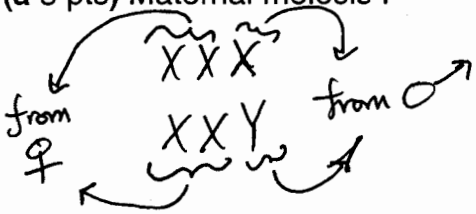
$$\frac{\text{Recombination likelihood}}{1\text{cM}} = \frac{20\text{ kb}}{(1\text{Mb})(1000\text{ kb/Mb})} = 0.02$$

Recombination likelihood = 0.02cM = 0.02%.

Name: KEY

5. Three sex chromosome trisomies are observed in humans: XXX, XXY, and XYY. Which of these sex chromosome trisomies could result from nondisjunction in:

(a 3 pts) Maternal meiosis I



(b 3 pts) Maternal meiosis II

XXX
XXY

(c 3 pts) Paternal meiosis I

XXY

(d 3 pts) Paternal meiosis II

XXX
XYY

(e 3 pts) Post-zygotic mitosis

XXX
XXY
XYY

General Rule:

MI NDJ ⇒ one of each chromosome
mom gives XX
dad gives XY

MII NDJ ⇒ two of the same chromosome
mom gives XX
dad gives XX or YY

Name: KBH

A physician asks your advice in the case of an XXY boy with hemophilia, an X-linked recessive trait. Neither of the boy's parents has hemophilia. Paternity has been confirmed using autosomal SSRs.

(f 7 pts**)** Assuming no new mutations, what sort(s) of meiotic nondisjunction (maternal? paternal? meiosis I? meiosis II?) would readily account for the boy's hemophilia? Sketch the proposed meiotic event(s) in which nondisjunction occurred. As necessary, include the wildtype (+) and mutant (hph) alleles at the hemophilia gene in your sketch.

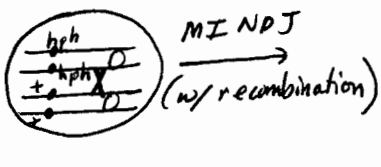
XXY boy w/ hemophilia ⇒ both of his X's have the hph allele:

$X^{hph} X^{hph} Y$

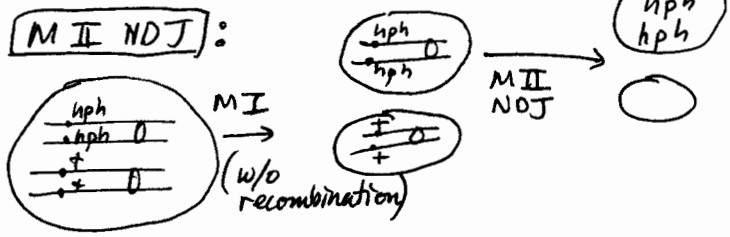
Mother must have been carrier: $X^{hph} X^{+}$

Since we do not know whether hph is centromere-linked, NDJ could have occurred in MI or MII in the mother

MI NDJ:



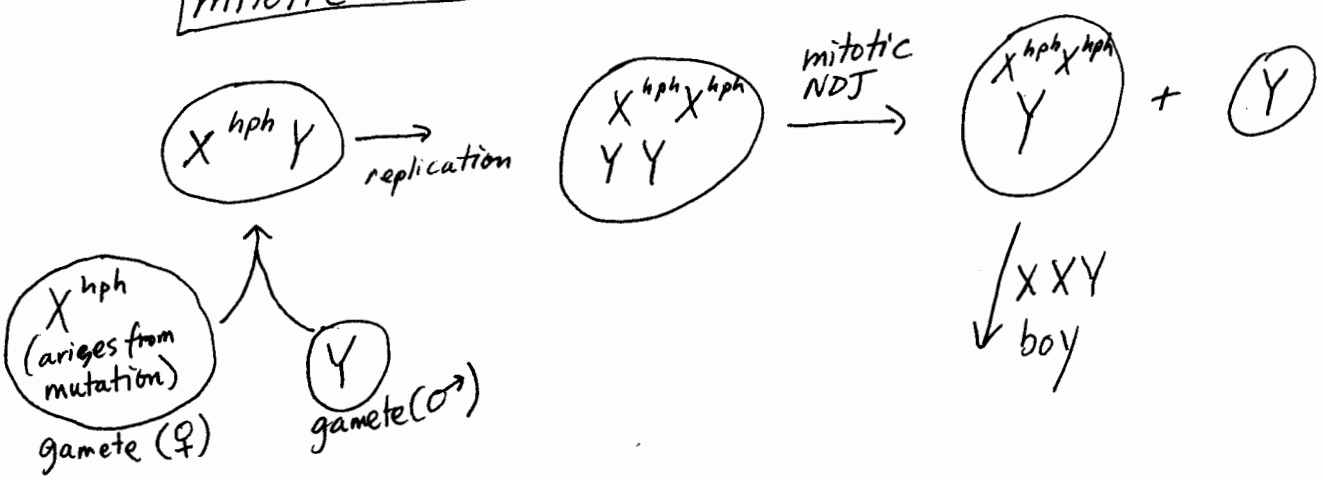
MII NDJ:



(g 4 pts**)** Assuming a new (hph) mutation arises on a single chromatid during meiosis in one parent, what sort of nondisjunction (mitotic? meiotic?) could account for this case? Briefly explain your answer. No sketch needed.

Because there is only one copy of hph, there is no way the boy got the disease through meiotic NDJ (he has to have two copies of the hph allele).

However, he could have two copies because of post-replication mitotic NDJ:

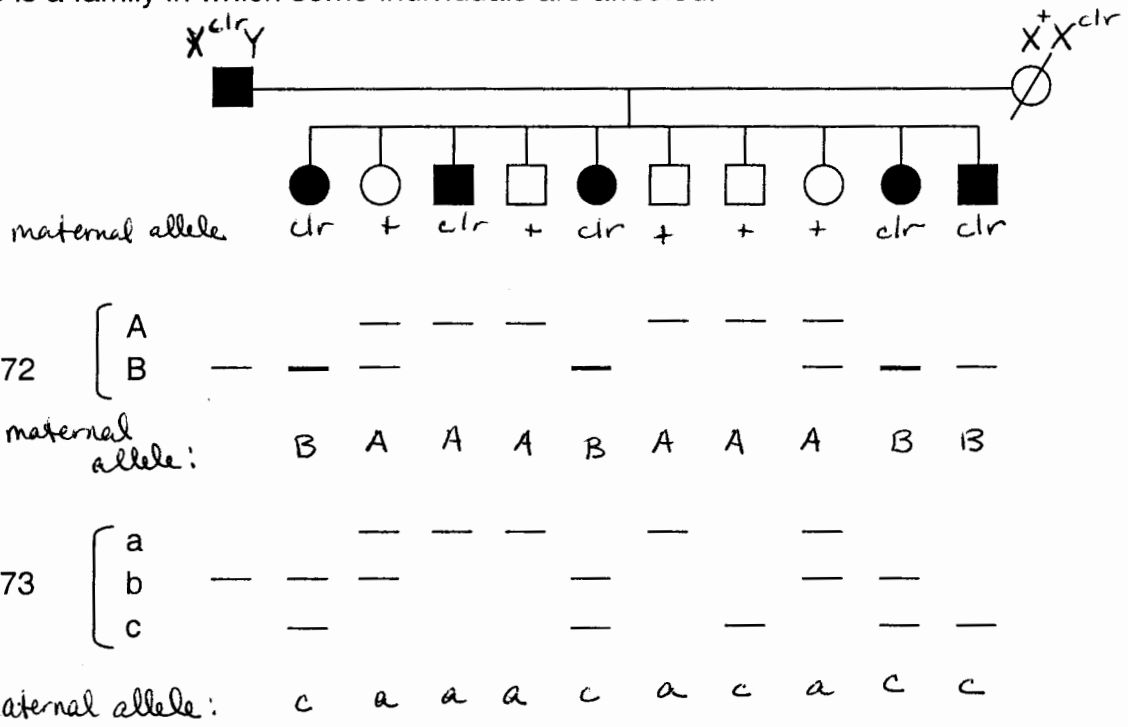


Name: KEY

6. Your UROP project is to genetically map color blindness, an X-linked recessive trait, with respect to SSR markers. Like many X-linked recessive traits, color blindness is usually found in males. However, the mutant allele frequency is sufficiently high that colorblind females do occur.

Alleles: + (normal) clr (associated with color blindness)

Here is a family in which some individuals are affected:



(a 2 pts**)** What is the (deceased) mother's genotype at SSR72?

AB

(b 2 pts**)** What is the (deceased) mother's genotype at SSR73?

ac

(c 2 pts**)** Diagram the two possible phase relationships between the SSR72 and SSR73 alleles in the mother.

Name: KEY

(d 5 pts) Calculate the LOD score for linkage at θ = 0.2 between SSR72 and SSR73 in this family. phase is unknown:

$$LOD_{\theta=0.2} = \log_{10} \frac{\frac{1}{2}(.4)^9(.1)^1 + \frac{1}{2}(.4)^1(.1)^9}{(1/4)^{10}} = \log_{10} 13.7 = \boxed{1.14}$$

(e 2 pts) Diagram the two possible phase relationships between the SSR72 and color blindness alleles in the mother.

(f 5 pts) Calculate a LOD score for linkage at θ = 0.1 between SSR72 and color blindness in this family. phase is unknown:

$$LOD_{\theta=.1} = \log_{10} \frac{\frac{1}{2}(.45)^9(.05)^1 + \frac{1}{2}(.45)^1(.05)^9}{(\frac{1}{4})^{10}} = \log 19.8 = \boxed{1.297}$$

(g 5 pts) If SSR72, SSR73, and the color blindness gene are all located in the same region of the X chromosome, what is their most likely order on the chromosome? Briefly justify your answer.

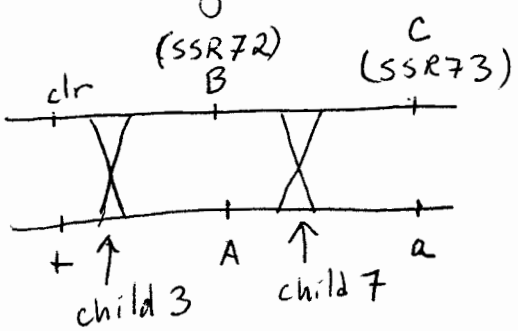
Look at recombinant children.

Child 7 is recombinant between SSR72 and SSR73

Child 3 is recombinant between the disease and SSR72

Both are recombinant between the disease and SSR73

Therefore, because more recombination events are seen between the disease and SSR73, these sites are most likely to be farther away from each other:



Most children are either clr, B, c or +, A, a

Child 3 has recombination between SSR72 and disease, and is clr, A, a

Child 7 is +, A, c

Name: KEY

7. As discussed in 7.03, one in 20,000 human males is an XX male. If an XX male has a twin, what is the likelihood that twin is an XX male assuming:

(a 4 pts.) The twins are dizygotic.

$p(\text{XX male})\,p(\text{male})\left(\frac{1}{20{,}000}\right)\left(\frac{1}{2}\right) = \frac{1}{40{,}000}$

(b 4 pts.) The twins are monozygotic.

100%

As discussed in 7.03, one in 200 individuals in the industrialized world suffers from HNPCC (Hereditary NonPolyposis Colon Cancer), an autosomal dominant trait. If an individual with HNPCC has a twin, what is the likelihood that twin has HNPCC assuming:

(c 4 pts.) The twins are dizygotic.

50%

(d 4 pts.) The twins are monozygotic.

100%

For mothers 30 years of age, the incidence of trisomy 21 is one per 885 births. Consider an individual, born to a 30-year-old mother, who has trisomy 21 due to meiotic nondisjunction in the mother. If the trisomic individual has a twin, what is the likelihood that twin has trisomy 21 assuming:

(e 4 pts.) The twins are dizygotic.

$p(\text{trisomy 21 for child of 30 year old mother}) = 1/885$

(f 4 pts.) The twins are monozygotic.

100%

Name: KEY

8. HNPCC (Hereditary NonPolyposis Colon Cancer) shows autosomal dominant inheritance in humans. As discussed in 7.03, some individuals with HNPCC are heterozygous for a loss-of-function mutation in the mismatch repair gene MSH2. These individuals frequently develop cancer of the colon, ovary, uterus, or other organs before age 50.

(a 5 pts.**)** Which of the following two approaches would yield a better mouse model of HNPCC:

1) random integration of a transgene consisting of a mutant human MSH2 gene (from an HNPCC patient)

OR

(2) knockout of the mouse MSH2 gene?

Briefly justify your answer.

like the HNPCC individuals, we want a mouse heterozygous for a loss-of-function mutation in MSH2, so we want to knock-out one of the mouse's MSH2 gene copies

(b 5 pts.**)** Would you expect mice homozygous for the modification you chose in question (**a**) to develop cancer more quickly, more slowly, or at the same rate as heterozygotes? Briefly justify your answer.

more quickly, homozygote knock-out mice have both MSH2 gene copies already missing whereas heterozygotes have to lose function in their remaining MSH2 gene to reach the same point as the homozygotes because the single copy that is functional still allows wild-type level of function

Name: KEY

9. **(a** 6 pts) When an Hfr *E. coli* strain carrying a wild type Lac operon ($I^+ Z^+$) is mated to a strain in which the entire Lac operon is deleted, there is a transient burst of ß-galactosidase expression even in the absence of the inducer lactose. However, when an Hfr carrying $I^+ Z^+$ is mated to an $I^+ Z^-$ mutant, no ß-galactosidase is synthesized. Explain these results given what you know about the mechanism of Hfr conjugation and Lac gene regulation.

Hfr conjugation only transfers DNA, not proteins. Therefore no repressor protein is transferred to the recipient, so as soon as the recipient receives the lac operon, the Z^+ gene will be transcribed and translated. It will take time for the repressor to be transcribed and translated, so there is a transient burst of LacZ expression before LacI repressor protein is made.

In the second case there is already functional LacI repressor present in the recipient, so LacZ is repressed immediately

(b 7 pts) *E. coli* strains that are lysogens for phage λ carry a phage genome integrated at a specific site in the *E. coli* chromosome. The genes for phage multiplication are kept from being expressed by the phage λ repressor protein. Given the results from the mating experiments in part **a**, what do you expect would happen when an Hfr strain that was lysogenic for phage λ was mated to a F^- strain not carrying a λ lysogen?

There is no λ repressor protein in the recipient. Therefore the genes required for λ replication will be expressed, and the strain will become lytic.

Name: KEY

(c 5 pts**)** The λ repressor protein is the product of the phage cI gene. Mutants of λ that are cI⁻ cannot form lysogens and therefore form clear plaques. Will a λ cI⁻ mutant be able to form plaques on a λ lysogen? Explain your reasoning.

No! The λ repressor from the λ lysogen is already being produced, so it will prevent transcription of genes involved in phage replication of the λ cI⁻ mutant (as well as its own).

(d 7 pts**)** You isolate a new mutant of phage λ that can form plaques on *E. coli* strains that are lysogenic for phage λ. Given what you know about the operon model of gene regulation by a repressor, propose a molecular mechanism to explain the behavior of your new mutant phage.

(A) The new mutant can have a mutant operator so that the λ repressor cannot bind to repress phage replication genes.

(B) The new mutant can have a mutation in a suppressor of the λ repressor (super-repressor of λ repressor).

(C) The new mutant can have a mutant λ repressor that is a dominant negative (although unlikely as λ repressor acts as a monomer).