Problem Sets

Fall 1995

7.03 Problem Set 1

due in TA's box outside the class
by 12 noon, Friday, September 22

1. You wrote to a colleague at Berkeley to request ten yeast strains with genotypes listed below. MATα means that the strain is of mating type α and MAT**a** means that the strain is of mating type **a**. Remember that only α and **a** strains can mate to form a diploid. HIS2 and HIS4 are genes for two different enzymes required for histidine synthesis. Strains that lack the function of either of these enzymes grow only when histidine is provided in the medium. $his2^-$ and $his4^-$ are recessive mutations and $his4^{-d}$ is a dominant mutation that blocks the activity of the HIS4 enzyme.

MATα His^+
MATα $his2^-$
MATα $his4^-$
MATα $his2^-$ $his4^-$
MATα $his4^{-d}$
MAT**a** His^+
MAT**a** $his2^-$
MAT**a** $his4^-$
MAT**a** $his2^-$ $his4^-$
MAT**a** $his4^{-d}$

Your colleague sent the strains you requested numbered 1-10 (not necessarily in the same order as the list above). You know that strains 1-5 are mating type α and that strains 6-10 are mating type **a.** When you were cleaning your desk you accidentally threw out the letter that came with the strains giving the genotype of each strain. Because you are too embarrassed to admit your mistake you decide to try to figure out which strain is which by complementation testing. You mate each α strains to each of the **a** strains and test the resulting diploid for the ability to grow on medium that does not contain histidine. In the table below, when a diploid can not grow on medium without histidine a (–) is indicated at the intersection of the two parental strains. When the resulting diploid can grow without histidine a (+) is indicated.

strains of mating type **a**	strains of mating type α				
	1	2	3	4	5
6	−	+	+	+	+
7	−	−	−	−	−
8	−	−	+	+	−
9	−	−	+	−	+
10	−	−	+	−	−

From these complementation tests, determine the genotype of each of the ten strains.

2. You are studying a plant that normally has light blue flowers and you have isolated two different true breeding mutant lines. One has dark blue flowers and the other has white flowers. When either mutant line is crossed to wild type, all of the F1 plants have light blue flowers.

(a) What can you say about the alleles that cause dark blue and white flowers?

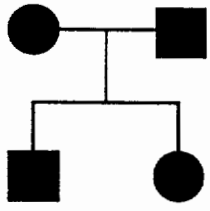
(b) When the dark blue line is crossed to the white line all of the F1 plants have light blue flowers. Now what can you say about the alleles that cause dark blue and white flowers? Why?

(c) The F1 plants from part (b) are crossed to produce 160 F2 plants. Of these 93 are light blue, 29 are dark blue and 38 are white. You examine the different flowers under the microscope and find that in white flowers none of the cells are pigmented, in dark blue flowers all of the cells are pigmented, and in light blue flowers there are alternating stripes of pigmented and unpigmented cells. Guessing that the phenotypic ratios for the F2 are 9 : 3 : 4 (light blue : dark blue : white), propose a model for the role of the different mutant alleles in color determination that fits all of the data.

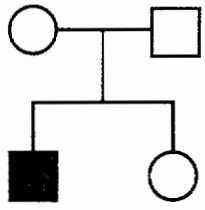
(d) While you are gloating over what you consider to be a very good model, a colleague in your lab points out that the numbers of F2 plants are also consistent with a phenotypic ratio of 10 : 3 : 3. First, check your colleagues assertion that the data is consistent with 10 : 3 : 3 by calculating the p value for this ratio with a Chi-square test. Propose a new model for the role of the different alleles in determining flower color that would give an F2 phenotypic ratio of 10 : 3 : 3.

3. Consider the following simple pedigrees where individuals expressing a trait are shown by solid symbols. For each pedigree, state whether the pattern of inheritance for the trait is either consistent or inconsistent with the trait being recessive, dominant, or X-linked recessive. For those cases where the inheritance pattern is inconsistent with the trait being either dominant or X-linked recessive, state whether the pattern would be consistent if it were known that the trait was incompletely penetrant. Similarly, for these cases where the inheritance pattern is inconsistent with the trait being either dominant or X-linked recessive, state whether it would be consistent if a new mutation had arisen in the family shown and state in which individual (mother, father daughter or son) the mutation would have occurred.

(a)



(b)



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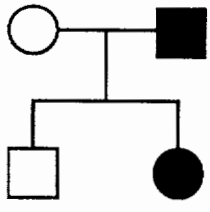
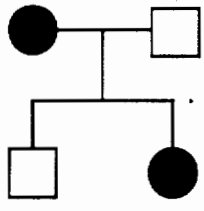
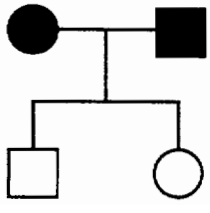


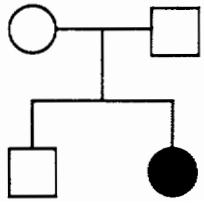
(d)



(e)



(f)



9/22/95

7.03 Problem Set #1 Solutions

1)

	1	2	3	4	5
6	-	+	+	+	+
7	-	-	-	-	-
8	-	-	+	+	-
9	-	-	+	-	+
10	-	-	+	-	-

STRAIN	GENOTYPE
1	MAT α his 4^{-d}
2	MAT α his 2- his 4-
3	MAT α His^{+}
4	MAT α his 4-
5	MAT α his 2-
6	MAT **a** His^{+}
7	MAT **a** his 4^{-d}
8	MAT **a** his 2-
9	MAT **a** his 4-
10	MAT **a** his 2- his 4-

Strains 1 and 7 must carry the dominant his 4^{-d} allele because they do not complement any of the other strains, including the wild-type.

Strains 2 and 10 must be the double mutants, his 2- his 4-, because they are only complemented by the wild-type strain.

Strains 3 and 6 must be wild-type strains because they complement all the other strains except the dominant mutant.

Strains 4 and 9 constitute a complementation group, as do strains 5 and 8, since they fail to complement one another. This data is not sufficient to determine which group represents the his 2^{-} gene and which represents the his 4- gene. However, you can conclude strains 4 and 9 carry mutations in the same gene, and likewise strains 5 and 8

carry mutations in the same gene. Assigning either His mutation to the groups would be an acceptable answer.

2)

a) Cross I: white flower x wild-type Cross II: dark blue flower x wild type

F1 All wild-type F1 All wild-type

The above crosses demonstrate that both mutant alleles are complemented by the corresponding wild-type alleles. Therefore you can state both mutant alleles are *recessive* to the wild-type.

b) Cross III: dark blue flower x white flower

F1 all wild-type

Cross III indicates that the two mutant strains complement becasue all their progeny exhibit a wild-type phenotype. We can conclude from this data that the two lay in different genes. If we label the gene which leads to the white phenotype the A gene and the gene which leads to the dark blue phenotype the B gene we can write the genotypes of Cross III as:

(dark blue) +/+ b/b x (white) a/a +/+

F1 (wild-type) a/+ b/+

c) Cross IV: F1 wild-type a/+ b/+ x F1 wild-type a/+ b/+

F2: 160 total plants

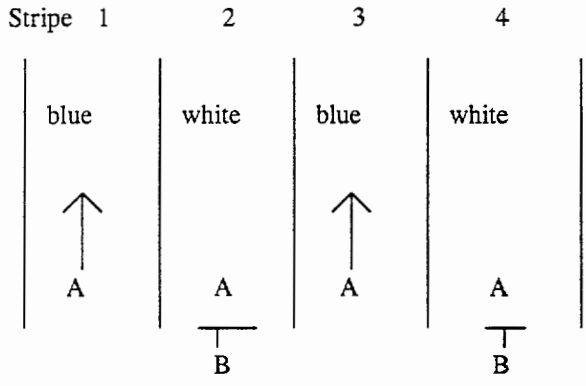
# of plants	phenotype
93	light blue (wild-type)
29	dark blue
38	white

We know a dihybrid cross produces the following expected genotypic ratios:

ratio	genotype	phenotype
9	+/- +/-	wild-type
3	+/- b/b	dark blue
3	a/a +/-	white
1	a/a b/b	white (assuming 9:3:4 observed ratio)

Before generating a model to explain the data we must recognize the new genotypic class generated by Cross IV. The new double homozygous mutant class exhibits the white flowered phenotype, therefore in the proposed model, the A gene must function

prior to the B gene, i.e the A gene must be *epistatic* to the B gene. One such model is shown below:



In this model the A gene product is necessary for the production of a blue pigment and is found in every flower cell. A plant with an a/a genotype therefore has the white flower phenotype. The B gene product is a protein which inhibits the function of the A gene product. In a wild type plant, the B gene product is only expressed in every other stripe & thus produces the wild type pigmentation pattern of alternating stripes. In a b/b mutant plant the A gene product is active in every stripe of the flower, and the dark blue phenotype results. A plant which is a double homozygous mutant, a/a b/b, cannot make any pigment without a functional A gene product and therefore exhibits the white flower phenotype.

d)

F1 a/+ b/+ x F1 a/+ b/+

F2: 93 wild-type
29 dark blue
38 white

If the data indicates a 10:3:3 phenotypic ratio then a new model is required.

Chi Square Test

Hypothesis: The data indicates a phenotypic ratio of 10:3:3 (wild-type:dark:white)

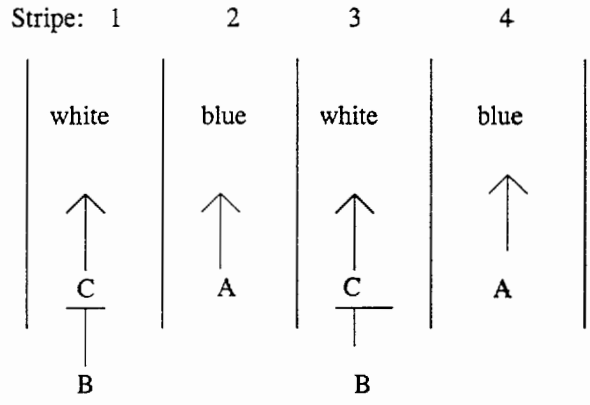
Degrees of freedom: df=(3-1)=2

phenotype	expected	observed	$(O-E)^2/E$
wild type	100	93	0.49
dark blue	30	29	0.033
white	30	38	2.133

$\chi^2 = 0.49+0.033+2.133= 2.656$ with df=2 $0.5>p>0.1$

Since $p>>0.05$ accept the hypothesis.

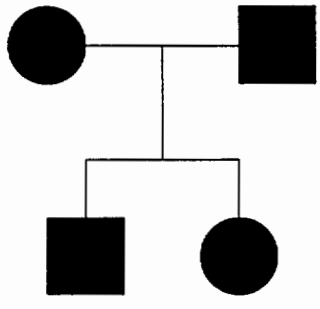
This reexamination of the data indicates the fourth genotypic class, a/a b/b, has a wild type phenotype. A model for this data must allow the double mutant to exhibit a wild type phenotype. One such model is shown below:



In this model there are two pathways which can produce blue pigment. In a wild-type flower blue stripes are produced via the action of the A gene product. The A gene is only expressed in every other stripe. In the white stripes of a wild-type flower the B gene product inhibits the function of a third gene product, the C gene. The C gene, when functioning, can also lead to blue pigment production, however it is only active in stripes where the A gene is not expressed. A flower with an a/a genotype produces no blue pigment because neither pathway can function. A b/b flower has both pathways active and therefore exhibits the dark blue phenotype. The double mutant flower, a/a b/b, has an obseverable wild-type color, however the stripes are reversed relative to a wild-type flower.

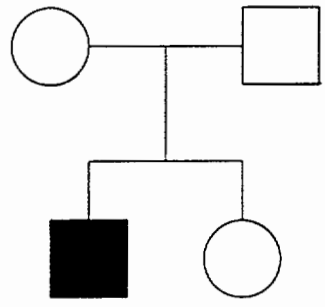
3) For the pedigrees the following labels will be used to identify the individuals: 1=mother, 2=father, 3=son, 4=daughter.

a)



Recessive	Dominant	X-linked recessive
1- a/a	1- A/?	1- X^aX^a
2- a/a	2- A/?	2- X^aY
3- a/a	3- A/?	3- X^aY
4- a/a	4- A/?	4- X^aX^a
Consistent	Consistent	Consistent

b)



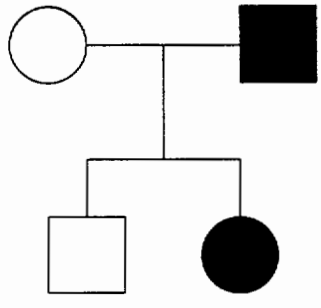
Recessive	Dominant	X-linked recessive
1- A/a	Not Consistent	1- X^AX^a
2- A/a		2- X^AY
3- a/a		3- X^aY
4- A/?		4- $X^AX^?$
Consistent		Consistent

The pedigree is inconsistent with a dominat allele because unaffected parents cannot have an affected child if the allele is dominat.

If the trait was incompletely penetrant the pedigree would be consistent with a dominant allele. One of the parents could carry the dominat allele and not exhibit the affetced phenotype.

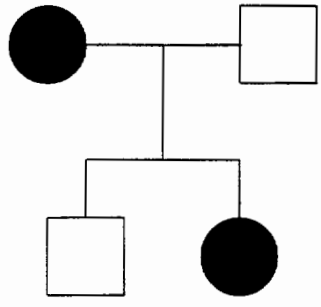
The pedigree would also be consistent if a new dominant mutant allele had arisen in the germ line of either parent, or in the soma of individual 3.

c)



Recessive	Dominant	X-linked recessive
1- A/a	1- a/a	1- $X^A X^a$
2- a/a	2- A/a	2- $X^a Y$
3- A/a	3- a/a	3- $X^A Y$
4- a/a	4- A/a	4- $X^a X^a$
Consistent if mom is a carrier	Consistent	Consistent if mom is a carrier

d)

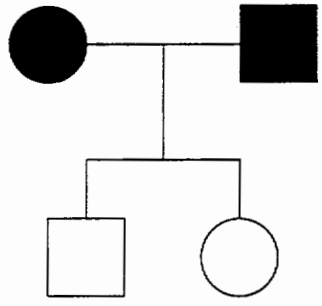


Recessive	Dominant	X-linked recessive
1- a/a	1- A/a	Not consistent because an affected mothe cannot have an unaffected son. & an unaffected father cannot produce an affected daughter.
2- A/a	2- a/a	
3- A/a	3- a/a	
4- a/a	4- A/a	
Consistent if dad is a carrier	Consistent	

If the trait is incompletely penetrant the phenotype may be caused by an X-linked recessive allele. In this scenerio both father and son would carry the recessive mutant allele on their X-chromosome but be unaffected by the trait.

If new mutations occured this pedigree may be consistent with an X-linked recessive allele, however it would be highly unlikely. For an unaffected father and an affected mother to produce an unaffected son, a reversion mutation must occur in the mother's germ line. And for the same parents to produce an affected daughter a new mutation would have to occur in the father's germ line. Since the frequency of mutation in humans is very low, these two independent events both occuring in a pedigree this small is highly unlikely.

e)

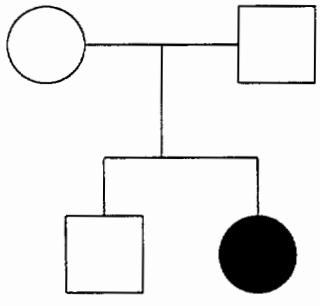


Recessive	Dominant	X-linked recessive
Inconsistent, two affected parents must have 100% of their progeny affected	1- A/a 2- A/a 3- a/a 4- a/a Consistent	Not consistent because if both parents are affected then 100% of their progeny would be affected.

If the trait was incompletely penetrant then the pedigree could be consistent with an X-linked recessive allele. In this case the daughter would be homozygous recessive but not exhibit the trait, and the son would have an $X^a Y$ genotype, but also appear unaffected.

It is possible, but highly unlikely that this trait is inherited in an X-linked recessive fashion, even if new mutations occured. In this pedigree both children would have to receive a revertant dominant "normal" allele from one of their parents. This new mutation could occur in the germ line of either parent, however for both children to inherit an allele produced in the usually extremely rare manner is very improbable.

f)



Recessive	Dominant	X-linked recessive
1- A/a 2- A/a 3- A/? 4- a/a Consistent	Not Consistent Domiant alleles cannot skip generations	Inconsistent, an unaffected father cannot have an affected daughter if a trait is caused by an X-linked recessive allele.

If the trait is incompletely penetrant the pedigree could be consistent with a dominant or an X-linked recessive allele. If the allele was dominant one of the parents would have to carry the dominant allele but not exhibit the trait.. If it was X-linked recessive then the father must carry the recessive allele but not exhibit the trait and the mother must be (at least) a heterozygote to produce an affetced daughter.

The pedigree may be caused by a dominant allele if a new dominat mutation arose in the germ line of either parent or in the soma of the daughter. If the trait was X-linked recessive there are several possible scenerios, the most likely of which is that the mother was a carrier and a new mutation arose in the father's germ line.

7.03 Problem Set 2

due in TA's box outside the class by 12 noon, Friday, September 29

1. Consider two hypothetical human genes that are 20 cM apart. The first gene, has two alleles S and s. Individuals that are S/S or S/s express a particular blood antigen whereas s/s individuals do not. The second gene has two alleles D and d. Individuals that are d/d are deaf whereas individuals that are D/d or D/D have normal hearing. Assume that both traits are completely penetrant and that no new mutations arise in the family under consideration. A woman who has normal hearing and expresses the antigen marries a man who is deaf and does not express the antigen. Their first child is deaf and does not express the antigen.

(a) What is the probability that their next child will be deaf? What is the probability that their next child will express the antigen?

(b) If you knew that one of the woman's parents was deaf and didn't express the antigen, what probability would you give that the couple's second child would be deaf and not express the antigen?

2. A diploid yeast cell is sporulated and 25 tetrads are dissected. Four tetrads have three His^- and one His^+ spore while the rest of the tetrads have two His^+ and two His^- spores.

(a) Was either of the haploid parents of the diploid His^+?

(b) What is the distance in cM between the two His genes in this cross?

Problem Set # 2 Solutions 9/29/95
7.03

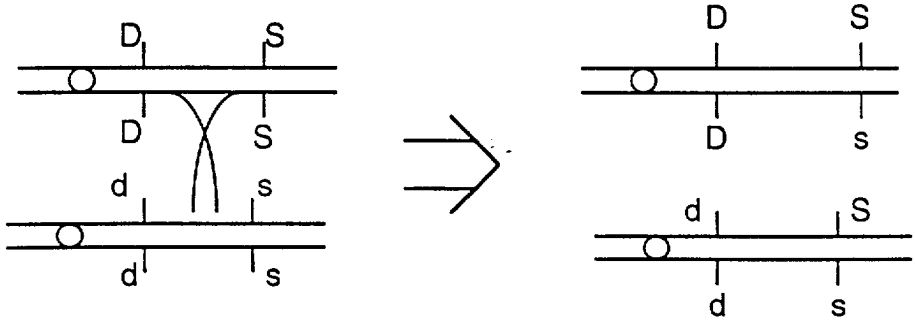
1. (a) The father must be d/d s/s becuase he is deaf and does not express the blood antigen.
The mother must be D/d S/s because: (i) she is not deaf and expresses the blood antigen and must therefore carry at least one copy of the dominant allele for each gene, and (ii) she must carry a copy of the recessive allele for each gene in order to pass them on to her deaf son who does not express the antigen.
The probability of the next child being deaf (d/d) is:

p(d/d) = p(receives d allele from father) * p(receives d allele from mother)
= 1 * 1/2
= 1/2

The probability of the next child not expressing the antigen (s/s) is:

p(s/s) = p(receives s allele from father) * p(receives s allele from mother)
= 1 * 1/2
= 1/2

(b) We know the two genes are linked. If one of the mother's parents was deaf and did not express the blood antigen she must have had the genotype (ds/ds) and therefore the mother's genotype is (ds/DS). The two major (parental) classes of gametes she will produce are therefore (ds) and (DS). However, the 20 cM distance between the two genes implies that 20% of the total gametes will be recombinant: (dS) and (Ds).



The frequency of the different gametes she produces will be:
DS = 40 %
ds = 40 %
Ds = 10 %
dS = 10 %
because the 20 % of the gametes which are recombinant will be evenly divided between the two types of recombinant gametes.

Therefore, the probability of having a deaf child who does not express the blood antigen is:

p(ds/ds) = p(receives (ds) gamete from mother) * p(receives (ds) gamete from father)
= 2/5 * 1
= 2/5

2. (a) If one gene were involved, and one parent were His- and one His+, all the tetrads would be 2 His- : 2 His+. Therefore we must assume 2 genes are involved. Let us label the genes His1 and His2, with the two mutant alleles His1- and His2- giving us His- phenotypes. The diploid must be heterozygous for both genes in order to produce spores which are both His+ and His- phenotypically. The haploid parents are thus either:

1: His1- His2- and His1+ His2+
2: His1- His2+ and His1+ His2-

Only case 1 has a haploid parent which is phenotypically His+.
Since the vast majority of tetrads are of one type, the two genes are linked,with the most common tetrad being a parental ditype. Case 1 produces a parental ditype tetrad which is phenotypically 2 His+ : 2 His-:

genotype	phenotype
His 1- His 2-	His-
His1- His2-	His-
His1+ His2+	His+
His1+ His2+	His+

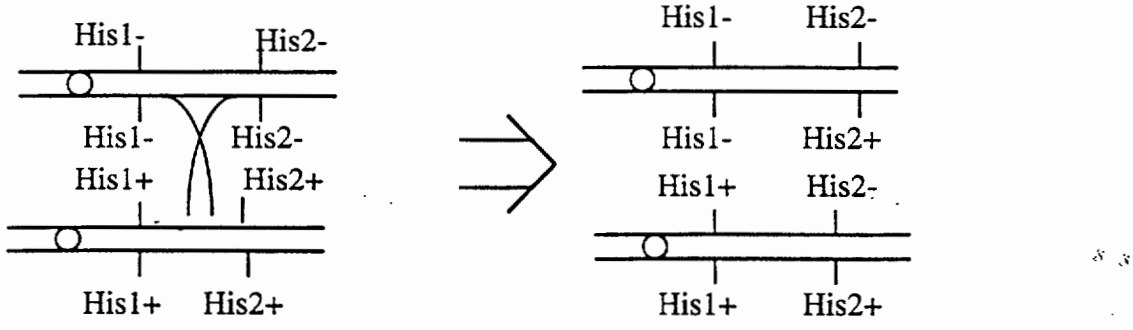
Case 2 produces a PD tetrad which is phenotypically 4 His-: 0 His+:

genotype	phenotype
His1- His2+	His-
His1- His2+	His-
His1+ His2-	His-
His1+ His2-	His-

Therefore Case1 is correct, and one of the haploid parents is His+.

(b) As discussed above, the two haploid parents are (His1- His2-) and (His1+ His2+). The 21 tetrads which are 2His- : 2His+ are parental ditype, also shown above. A single crossover event between the two genes will give the other 4 tetrads observed, which are 3His- : 1 His+ and are tetratype tetrads:

genotype	phenotype
His1- His2-	His-
His1- His2+	His-
His1+ His2-	His-
His1+ His2+	His+



Using the formula: genetic distance = $\frac{T + 6\,NPD}{2 * \text{total gametes}} * 100$

$= 4(100)/2(25) = 8cM$

Note:

Problem Set 3 was purposely omitted.

7.03 Problem Set 4

due in TA's box outside the class by 12 noon, Friday, October 20

1. Below is a map of an **Hfr** *E. coli* strain showing the region of the chromosome that carries the integrated **F** plasmid.

This **Hfr** is now going to be used to study mutations in the **Lac** operon. A **LacO^c** mutation is introduced into the **Lac** operon as follows. Phage **P1** is grown on a strain that is **ProA$^+$ LacO^c**. The resulting phage lysate is used to transduce the **Hfr**, selecting for **ProA$^+$**. The **Pro$^+$** transductants are then screened for constitutive **LacZ** expression indicating acquisition of the **LacO^c** allele.

(a) How would you isolate an **F'** that carries both the **Lac** operon and the **ProA** gene from the **Hfr**? Specify the genotype of the strains used and how you would select for the ~~Hfr~~ F'?

(b) The **F'** carrying **LacO^c** that was constructed in part (**a**) is mated to an **F$^-$ Lac I^s** strain. What will the genotype of this strain be? What will the behavior of **Lac** expression in this strain be? (regulated, uninducible, or constitutive).

(c) Derivatives of the strain in part (**b**) where the **F'** has integrated back into the chromosome are isolated by screening for strains that can transfer **PurE$^+$**. There will be three different types of **Hfr** produced by recombination between **F'** and the chromosome. Draw a map for each of the **Hfr**s showing the region of the chromosome that carries the integrated **F** plasmid. Be sure to explicitly indicate the position of the different **Lac** alleles. Also for each of the three **Hfr**s indicate the behavior of **Lac** expression that will be transferred <u>early</u> when the **Hfr** is mated. (Assume that the **F$^-$** recipient strain is deleted for the **Lac** operon.)

2. Consider a hypothetical amylase enzyme in *E. coli* that is produced only when starch is available. Several mutations have been isolated that affect the regulation of amylase. The first mutation, Mut1$^-$ is uninducible. Various merodiploids are constructed for analysis of Mut1. In this analysis, mutations in the structural gene for amylase are designated Am$^-$.

Genotype	amylase regulation
Mut1$^-$ / F' Mut1$^+$	regulated
Mut1$^-$ Am$^-$ / F' Mut1$^+$ Am$^+$	regulated
Mut1$^-$ Am$^+$ / F' Mut1$^+$ Am$^-$	regulated

(a) Propose a function for the Mut1 gene product and an explanation for the behavior of the Mut1$^-$ mutation.

(b) Mut2$^-$ is a mutation in the same gene as Mut1$^-$ but the phenotype of a Mut2$^-$ mutation is uninducible amylase. On the basis of the genetic tests below, propose an explanation for the behavior of the Mut2$^-$ mutation.

Genotype	amylase regulation
Mut2$^-$ / F' Mut2$^+$	uninducible
Mut2$^-$ Am$^-$ / F' Mut2$^+$ Am$^+$	uninducible
Mut2$^-$ Am$^+$ / F' Mut2$^+$ Am$^-$	uninducible

(c) Mut3$^-$ is a mutation in the same gene as Mut1$^-$ and Mut2$^-$ but the phenotype of a Mut3$^-$ mutation is constitutive amylase. On the basis of the genetic tests below, propose an explanation for the behavior of the Mut3$^-$ mutation.

Genotype	amylase regulation
Mut3$^-$ / F' Mut3$^+$	constitutive
Mut3$^-$ Am$^-$ / F' Mut3$^+$ Am$^+$	constitutive
Mut3$^-$ Am$^+$ / F' Mut3$^+$ Am$^-$	constitutive

3. The principles of prokaryotic regulatory circuits can also be applied to multicellular organisms. In sex determination the regulatory input signal can be thought of as the presence of a particular sex chromosome and the output is the expression of either male or female traits. Consider a hypothetical organism with X and Y sex chromosomes. The presence of the Y chromosome dictates expression of male specific genes. Without the Y chromosome, the default state is expression of female specific genes. Thus XY individuals are male and XX individuals are female. Recessive mutations in three genes have been isolated in this organism that alter sex determination. The mutations are designated gene1$^-$, gene2$^-$, and gene3$^-$. The effects of the different mutations are shown below.

Genotype	Phenotype	Genotype	Phenotype
XY	male	2$^-$ / 2$^-$ XY	male
XX	female	2$^-$ / 2$^-$ XX	male
1$^-$ / 1$^-$ XY	female	3$^-$ / 3$^-$ XY	male
1$^-$ / 1$^-$ XX	female	3$^-$ / 3$^-$ XX	male

With respect to expression of male specific genes, gene1 mutations can be thought of as uninducible whereas gene2 and gene3 mutations can be thought of as constitutive.

To organize the genes into a regulatory hierarchy, the following double-mutant epistasis tests are performed .

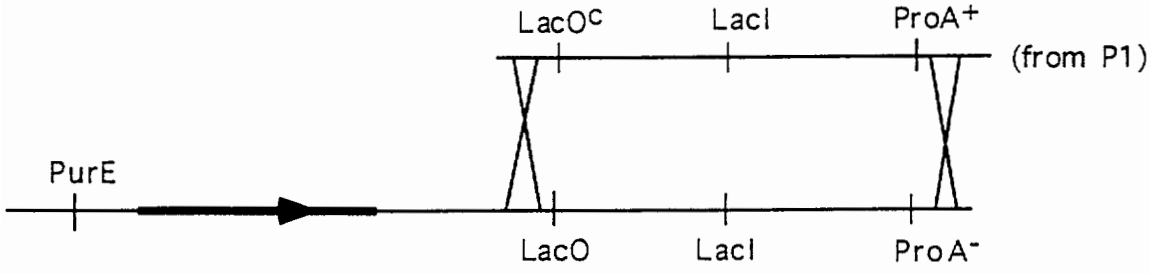
Genotype	Phenotype
1$^-$ / 1$^-$, 2$^-$ / 2$^-$ XY	male
1$^-$ / 1$^-$, 2$^-$ / 2$^-$ XY	male
1$^-$ / 1$^-$, 3$^-$ / 3$^-$ XY	female
1$^-$ / 1$^-$, 3$^-$ / 3$^-$ XY	female

(a) Assuming that all three genes express either transcriptional repressors or activators, make as simple a model as you can to explain the regulation of sex determination in this organism. You may give your answer in the form of a diagram like the ones developed in lecture that show either positive or negative influence of one gene product on expression of another gene.

(b) If there is a single gene on the Y chromosome that determines maleness and that gene encodes a repressor protein, what gene is the repressor encoded on the Y chromosome likely to regulate?

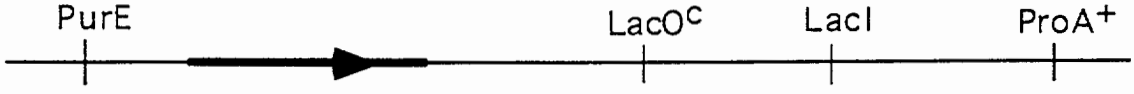
7.03 Problem Set 4 Solutions

1a) The problem states that lysate from phage P1 grown on a strain that is ProA$^+$ LacO^c is used to transduce the Hfr as shown below:



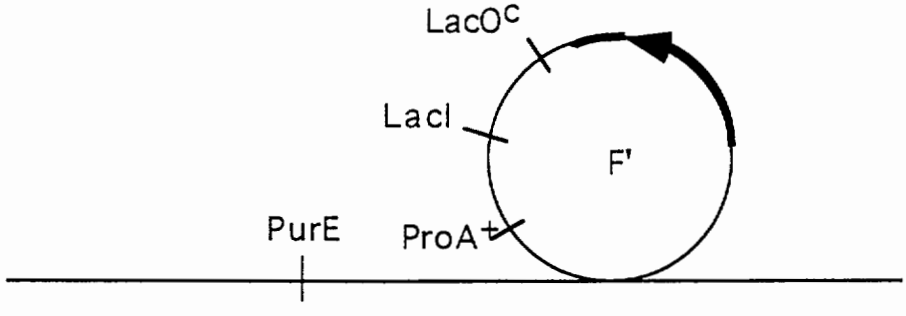
Selected ProA$^+$ transductants constituative for LacZ expression have the following map:

STRAIN A:



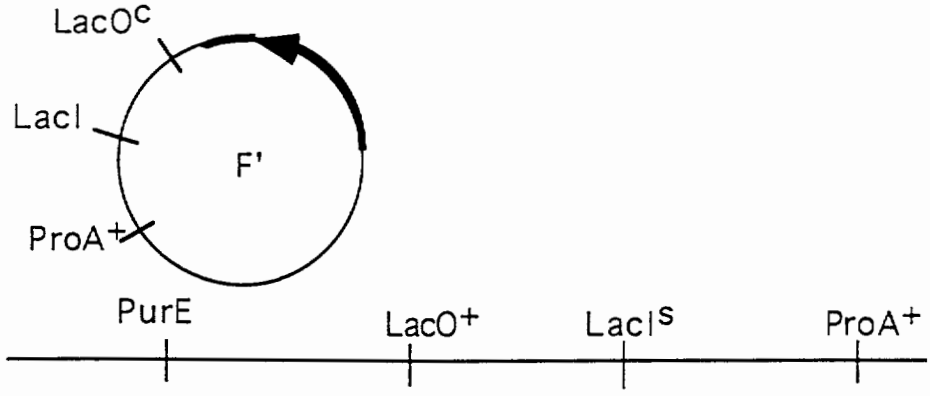
This Hfr (Strain A) transfers ProA+ and the Lac operon late. To isolate an F' which carries both the LacO^c operon and the ProA$^+$ gene from the Hfr, Strain A should be mated to an F$^-$ strain that is LacO$^+$ ProA$^-$ PurE$^-$ and str^r (assuming Strain A is str^s). ProA$^+$ str^r strains can then be selected and screened for constituative LacZ expression. Derivative strains that now transfer fertility, ProA$^+$ and constituative LacZ expression early, but never transfer PurE$^+$,will have undergone the event diagrammed below to produce the F':

STRAIN B:



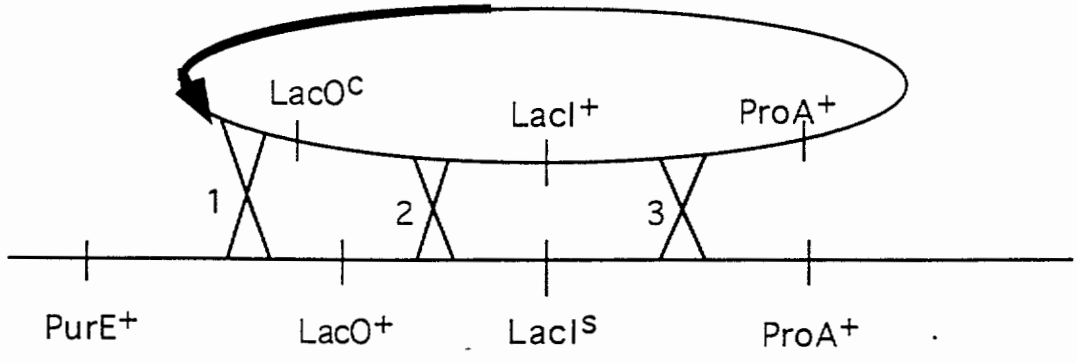
1b) The genotype of a strain produced by mating Stain B to an F⁻ LacI^s strain will be:

$I^sO^+Z^+/F'\ I^+O^cZ^+$



Since the super repressor from the recipient strain cannot bind the constituative LacO^c operator introduced into the cell on the F', the behavior of this strain will be constituative LacZ expression.

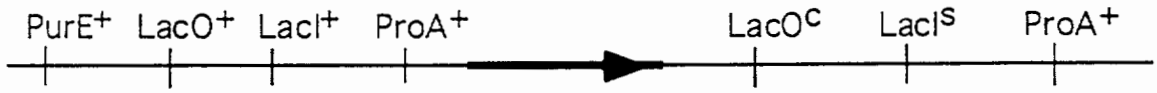
1c) Recombination between the F' and the chromosome results in 3 different types of Hfrs:



Crossover 1:

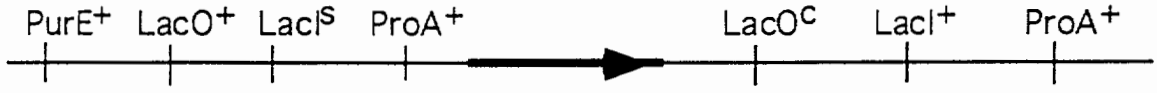
Constituative LacZ expression will be transferred early when this Hfr is mated to an F⁻ strained deleted for the Lac operon.

Crossover 2:



Regulated LacZ expression will be tranferred early.

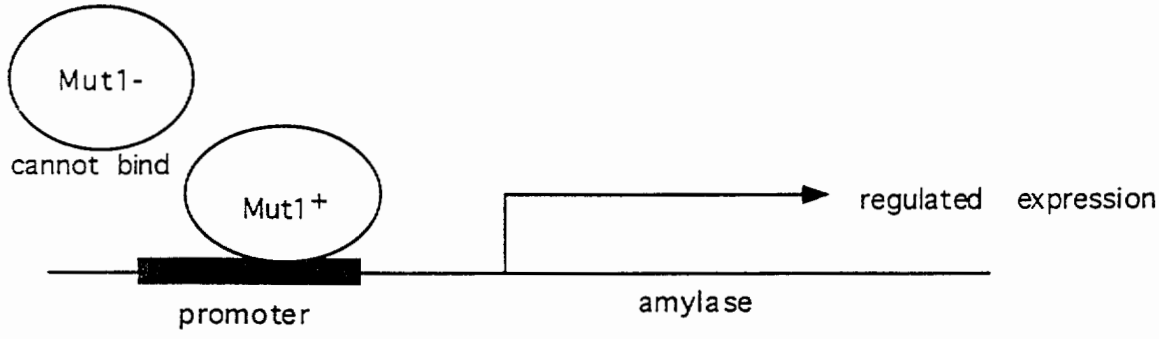
Crossover 3:



Uninducible LacZ expression will be transferred early.

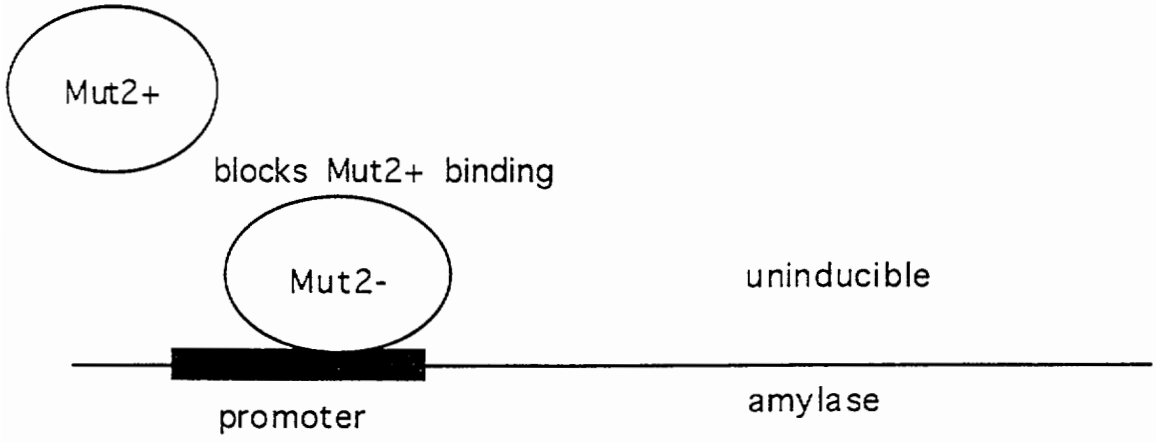
2a) The data from analysis of Mut1⁻/F'Mut1⁺ indicates that the Mut1⁻ mutation is recessive. The cis-trans test shows that the Mut1 gene product is trans-acting. The Mut1 gene product may be a diffusible activator of the amylaze enzyme.

A possible mode¹· Mut1⁻ causes the activator to be unable to bind the promoter upstream of the amylase enzyme resulting in uninducible amylase expression. However, in a merodiploid, wild type Mut1 is diffusible and can bind the promoter to activate amylase expression.

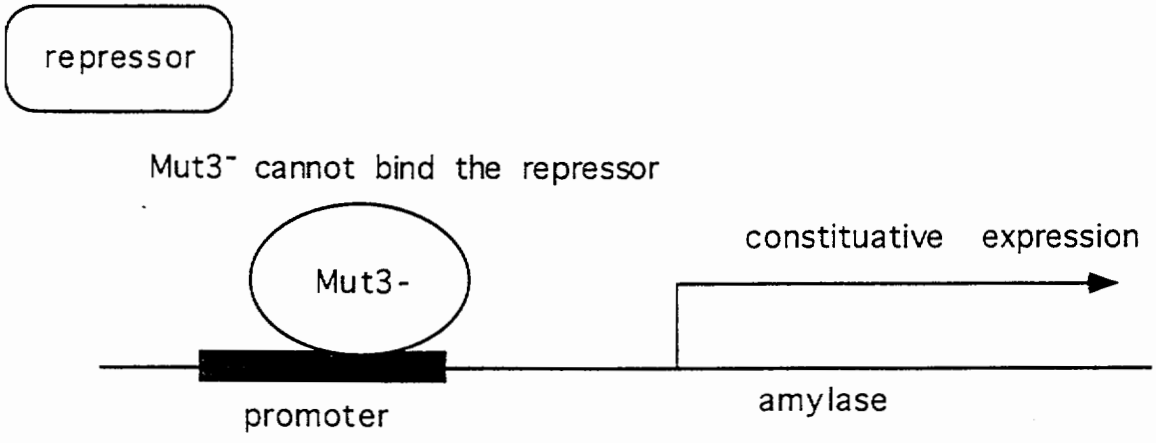


2b) The data from analysis of Mut2⁻/F'Mut2⁺ indicates that the Mut2⁻ mutation is dominant to wild type. Like the LacI⁻ᵈ mutation, the Mut2⁻ mutation is dominant in cis or trans and is ttherefore trans-acting.

A possible model: Since both Mut1⁻ and Mut2⁻ mutations are in the same gene, the Mut2⁻ mutation may lie in the activation domain of the protein. The gene product of a Mut2⁻ mutation can bind the promoter sequence but cannot activate transcription of the amylase gene. Mut2⁻ would thus appear as a dominant mutation since the mutant gene product is diffusible and can bind the promoter sequence and block wild type Mut2 from binding the promoter to active transcription.



2c) The data from analysis of Mut3$^-$/F'Mut3$^+$ indicates that the Mut3- mutation is dominant. Since amylase expression is constituative in the absence of starch, the data implies that this third mutation in the activator protein causes the Mut3$^-$ gene product to be irrepressible. In other words, the mutation may disrupt the activator's binding site for the repressor protein. In a merodiploid, the wild type Mut3 that binds amylase promoter sequences will block transcription in the absence of starch, but the Mut3$^-$ gene product in the same cell will activate transcription constituatively since it cannot bind the repressor.



3a) The problem states that the presence of the Y chromosome dictates expression of male specific genes. Without the Y chromosome, male specific genes (located on other chromosomes besides the Y chromosome) will remain unexpressed.

The first part of the problem deals with single mutants. An XY organism homozygous for gene 1$^-$ is female. Since the problem tells us that with respect to expression of male specific genes, gene 1 mutations can be thought of as uninducible. This implies that Gene 1 normally promotes the expression of male specific genes or blocks a repressor of male specific genes (2 negatives= positive in regulatory pathways).

An XX organism homozygous for gene 2$^-$ is male. The problem states that this mutation may be considered constituative with respect to male specific genes. The wild type fuction of Gene 2 may therefore be to repress the expression of male specific genes.

Similar to the gene 2$^-$ mutation, the phenotype of a gene 3- mutation in an XX organism is constituative expression of male specific genes. Therefore Gene 3 is also a likely negative regulator of male specific genes.

The information in the second part of the problem deals with epistatis. The phenotype (male) of a homozygous gene 1$^-$ gene2$^-$ double mutant in an XX organism is the same as the phenotype of a gene 2$^-$ single mutant. Therefore, Gene 2 is epistatic to Gene 1 (i.e. Gene 2 acts downstream from Gene 1 in the regulatory pathway).

A gene 1$^-$ gene 3$^-$ homozygous XY organism is phenotypically female like the single mutant homozygous gene 1$^-$ XY organism. Therefore Gene 1 is epistatic to Gene 3.

A regulatory pathway that fits the above data is as follows:

Gene 3 ⊣ Gene 1 ⊣ Gene 2 ⊣ Male Specific Genes

3b) If a single gene on the Y chromosome determines maleness and encodes a repressor protein, that repressor is likely to negatively regulate Gene 3. A repressor which blocks Gene 3 allows Gene 1 to now block the Gene 2, which ultimately results in the expression of male specific genes.

Y repressor protein ⊣ Gene 3 ⊣ Gene 1 ⊣ Gene 2 ⊣ Male Specific Genes

Genetics 7.03 Problem Set V

Due Nov. 3, 1995 by 11:55AM

1. Some strains of clover produce high levels of cyanide, while others produce only low levels. The beef industry funds you as a UROP student to investigate cyanide production.

You initiate your work by crossing a true-breeding, high-cyanide producing strain by a true-breeding, low-cyanide producing strain.

High X Low

↓

F1 All High

↓

F2 297 High producing plants
101 Low producing plants

A. What does this experiment tell you about the low cyanide strain? Designate the genotypes of the parental and F2 plants, and define your symbols.

Next you are given a different true-breeding strain that produces low levels of cyanide. You cross this to the first low cyanide strain. .

Low 1 X Low 2

↓

F1 All High

↓

F2 269 High producing plants
208 Low producing plants

B. Explain how all of the F1 plants can be high cyanide producers. Explain the ratios of high and low producing plants observed in the F2 generation.

You observe that the Low 1 strain accumulates a compound P that is an intermediate in cyanide synthesis, and strain Low 2 accumulates compound T. You decide to look for these intermediates in low cyanide producing plants of the F2 generation of part B. Of the 208 plants you find that 89 accumulate compound P and 119 accumulate compound T.

Next you take the 89 Low plants that accumulate P and cross them to both the Low 1 and to Low 2 strains.

89 Low cyanide, P accumulators X Low 1	89 X Low 2
↓	↓
F1 All Low cyanide producers	2/3 High 1/3 Low

You also take the 119 Low plants that accumulate T and cross them to both Low 1 and Low 2 strains. You find they fall into two classes.

90 Low cyanide, T accumlators X Low 1	90 X Low 2
↓	↓
F1 2/3 High cyanide producers 1/3 Low cyanide producers	All Low

However, the remaining 29 have the following properties:

29 Low cyanide, T accumulators X Low 1	29 X Low 2
↓	↓
F1 All Low cyanide producers	All Low

C. Give the genotypes of the 89 Low cyanide, P accumulators, the 90 Low cyanide, T accumulators, and the 29 Low cyanide, T accumulators.
Propose a model for cyanide production in clover. Explain the phenotypes in the Low 1 and Low 2 strains and account for intermediates P and T.

Note: Questions #2 + #3 were purposely omitted.

7.03 Problem Set 5 Solutions
November 3, 1995

1. In this problem, we are dealing with the production levels of cyanide in different strains of the same species of clover.

1A. In part A, the question asks for you to provide the genotypes of the parental and F2 plants from the results of the experimental crosses given to you. By crossing the true breeding strain high-cyanide strain to the true breeding low-cyanide strain, you find that all of the F1's are High. This tell you that the Low strain is recessive to the High strain. Then from the F2 results, from which you quickly recognize as a 3:1 ratio, you are able to determine that it is simple example of one gene, with a dominant and recessive allele.

Let High = A and Low = a

High (AA) x Low (aa)

↓

F1= all high (Aa)

↓

F2= 1 (AA, high) : 2 (Aa, high) : 1 (aa, low)

1B. In the next part, you are given another true breeding strain that produces low levels of cyanide. So when you cross this to the first strain and get all high phenotypes, this is an example of a complementation cross. Because they are all high, this shows that you are dealing with two different genes, which are both recessive to there respective dominant alleles. To produce high levels of cyanide, a plant must have at least one copy of the dominant allele for both genes. So...

let Low 1 = A and a for their respective alleles
let Low 2 = B and b

Low 1 (aaBB) x Low 2 (AAbb)

↓

F1 = all high (AaBb)

↓

F2 = 3/4 A__ x 3/4 B__ : 9/16 A__B__

1/4 bb : 3/16 A__bb

1/4 aa	x	3/4 B__	: 3/16 aaB__
		1/4 bb	: 1/16 aabb

Thus, the observed phenotypical ratio of High : Low is equal to 9 : 3 + 3 + 1 or 9 : 7.

1C. Now, you analyze the different types of low cyanide producing strains produced from the F2 in part B. Of the 208 low producing strains, you find that they have the following characteristics:

89 accumulate compound P
119 accumulate compound T

When you cross the 89 Low P's to the two different true breeding low strains, you get the following results:

89Low aaB_ x Low1 aaBB → F1: all low aaB_

and

89Low aaB_ x Low2 AAbb → F1: 2/3 high and 1/3 low

these numbers come from remembering that in the 89Low, the possible genotypes are 1/3 aaBB and 2/3 aaBb, and when you cross these with the Low2 strain (which is 1/1AAbb), you set up
1/3aaBB x 1AAbb = 1/3 AaBb and
2/3aaBb x 1 AAbb = 1/3 AaBb , 1/3 Aabb

Now when you take the 119 Low plants that accumulate T and cross them to the different strains, you find that you have two different types. The first type, 90LowT follows in the same pattern as the above LowT's:

90Low A_bb x Low2 AAbb → F1: all low A_bb

and

90Low A_bb x Low1 aaBB → F1: 2/3 high and 1/3 low (same reason as above)

But in the remaining 29LowT's, when you cross them to either the Low1 or Low2 strains, you still get only low production of cyanide in all plants. This represents your double negative genotypes from the original F2 cross, in which case it is impossible to get a copy of any dominant alleles when you cross with it, which is what is necessary when crossing with either Low1 or Low2, both of which we know is missing a dominant copy of either gene A or B.

Only one gene needs to be homozygous recessive for the plant to be a low cyanide producer. This would indicate the following pathway from the results:

$$T \xrightarrow{B} P \xrightarrow{A} \text{cyanide}$$

A mutation in the B or A causes low cyanide production since B may be an enzyme that

converts the intermediate T into P. A is then an enzyme that converts P into cyanide. In a Low1 strain, A is mutated not allowing the pathway to pass the P step, which leads to the accumulation of P. If B is mutated as in Low2 strain, T builds up. If both are homozygous recessive at their locus, as in the double recessive aabb, T accumulates as is seen in the 29LowT's, which show the same phenotype as the other LowT's. Using this, we are able to deduce that B is epistatic to A, remembering that in a biochemical pathway, it is the upstream gene that is epistatic to the downstream gene.

Note: Problem Set #6 was purposely omitted.

Genetics 7.03 Problem Set VII

Due Friday, Dec. 8, 1995 by 11:55AM

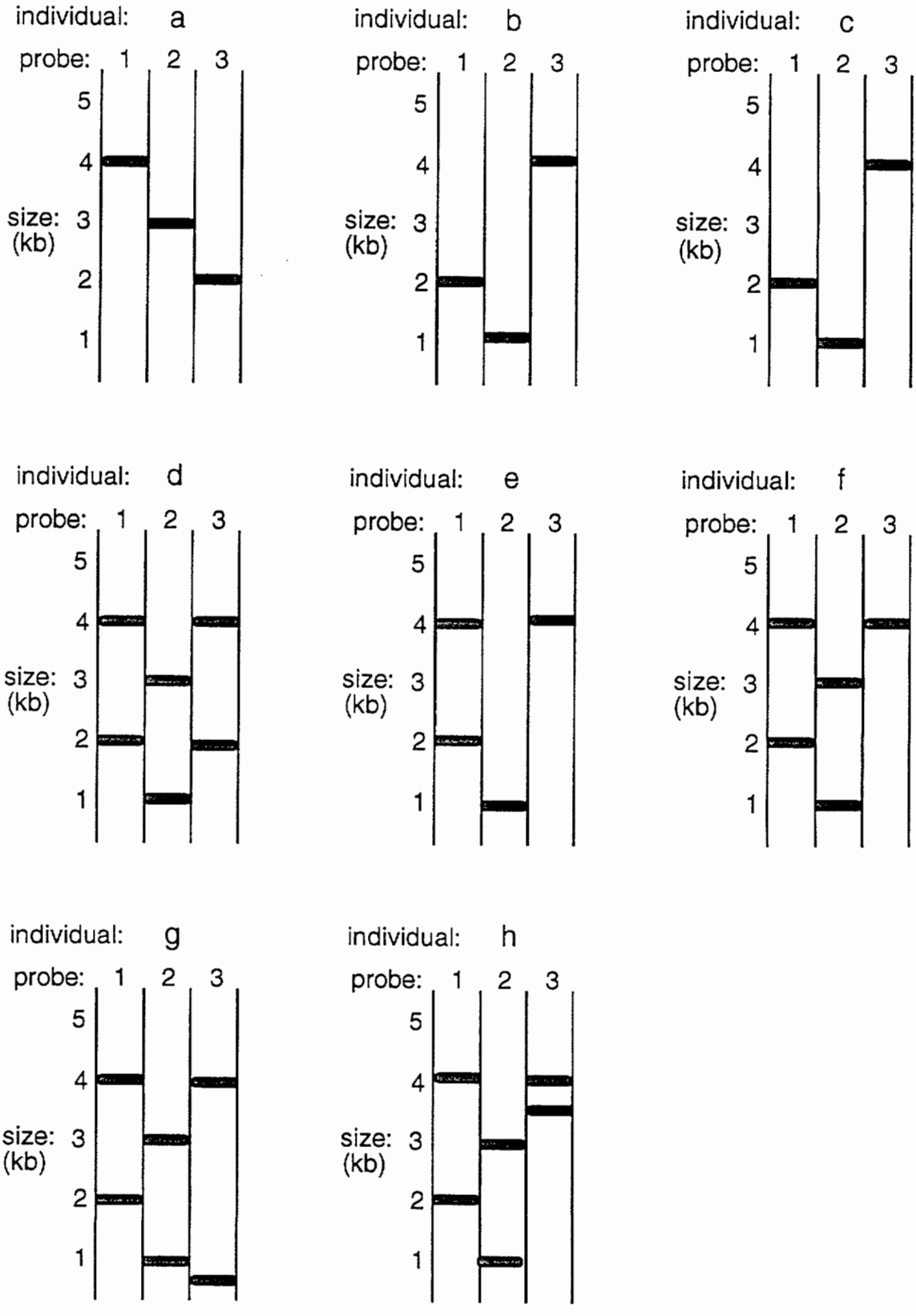
Note: Question #1 was purposely omitted.

2. Wilms' tumor of the kidney segregates as an autosomal dominant single-gene trait. Although the disease is inherited as a dominant trait, the actual cause of the disease is loss of function of a particular gene; this loss of function is recessive. Thus, an individual inherits a single mutant copy of the gene affected in Wilms' tumor from a parent, and then a second event must occur to eliminate expression of the wild-type gene that was inherited from the other parent.

You are interested in determining what types of events lead to expression of the recessive phenotype, so you perform RFLP analysis of members of the following pedigree:

Individuals affected with Wilms' tumor are indicated with solid symbols.

Results of RFLP analysis:



a. Which RFLP marker is likely to be closest to the locus affected in Wilms' tumor ?

b. For individuals c, d, e, f, g, and h, describe the most likely event that led to the tumor.

c. Explain why a child who is heterozygous for the affected locus almost always develops the disease.

Problem Set VII Solutions

2. a) The Wilm's tumor (WT) trait is dominant: an individual who is heterozygous for the responsible mutation will have the disease (i.e. develop tumors in the kidney). However, the mutation is a recessive loss of function in the gene; therefore, for a single kidney cell in a heterozygous individual to become a tumor, an event must occur within that cell to disrupt the function of the wild type copy of the gene. All other cells in the kidney and the rest of the individual remain unaffected; only the descendants of cells in which the remaining normal gene function is lost will become cancerous.

Individual (a) is homozygous for each marker; thus we see only one band with each probe. Individual (b) may be homozygous for each marker as well, although he must be heterozygous for the WT mutation. However, since the RFLP analysis is done on DNA from a kidney tumor, the individual may also be heterozygous for the markers and have lost the chromosome carrying the unaffected gene in only the tumor's cells.

Each affected child has received the chromosome from parent (b) carrying the WT mutation and the markers seen in gel (b). Each has also received an chromosome carrying an unaffected gene from parent (a). To make a tumor, the unaffected gene copy has to lose its function. In each affected child except for (d), the 2kb fragment recognized by probe #3 [on the chromosome containing the normal WT gene] is either lost or altered. The other two markers on this chromosome are unchanged in 4 or 5 of the 6 children. **Therefore, the RFLP marker recognized by probe #3 is most closely linked to the WT locus.**

(b) c. We see none of the markers from parent (a), which is best explained by loss of the entire chromosome carrying the normal WT locus. This is caused by **mitotic nondisjunction.**

d. All of the parental markers are unchanged, so the WT locus in this individual underwent a **small rearrangement or point mutation** which eliminated gene function but did not grossly affect the chromosome's structure.

e. Both markers 2 and 3 from the chromosome inherited from (a) are missing. This can be explained by a **mitotic recombination** event between the centromere and marker 2 or a **large deletion** encompassing both markers.

f. Marker 3 from parental chromosome (a) is missing. Again, **mitotic recombination** between marker 2 and marker 3 or a **large deletion** encompassing all of marker 3 could be responsible.

g The fragment recognized by probe #3 on parental chromosome (a) is now 1kb instead of 2kb in size. This is the result of a **deletion** removing only part of the region covered by probe#3 (as well as affecting the WT locus).

h The fragment recognized by probe #3 on parental chromosome (a) is now 3.5kb rather than 2kb. This could be the result of an **insertion** event within the region of probe #3 or a **deletion of the restriction site** at one of the two ends of RFLP fragment #3.

(c) In a heterozygous individual, only one wild type copy of the WT locus must be affected to generate the disease. As we have seen, a variety of events can occur during mitotic division which disrupt the activity of the wild type copy of the WT locus. The probability of these events, although small, is high enough that it is likely that at least one of the many cells produced during kidney development will undergo one of these disruptions, thus losing all wild type function from the WT locus and developing into a tumor. These events occur at the same frequency in all cells within the organism and are not induced by the WT mutation.