2005 7.03 Problem Set 1

Due before 5 PM on WEDNESDAY, September 21, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. Wild-type flies are brown in color. You have discovered two genes that control body color in flies -- gene A and gene B, which are on different autosomal chromosomes. You have three true-breeding mutant strains, all of which have black bodies.

Strain One (A*/A*) is homozygous for a dominant mutation in gene A.
Strain Two (A^{-}/A^{-}) is homozygous for a recessive mutation in gene A.
Strain Three (B^{-}/B^{-}) is homozygous for a recessive mutation in gene B.

Group Four are flies that result from mating Strain One to Strain Two.
Group Five are flies that result from mating Strain One to Strain Three.
Group Six are flies that result from mating Strain Two to Strain Three.
Group Seven are flies that result from mating Strain One to wild-type.
Group Eight are flies that result from mating Strain Two to wild-type.
Group Nine are flies that result from mating Strain Three to wild-type.

Predict the phenotypic ratio (the numerical ratio and the phenotype of each phenotypic class) of the offspring resulting from a cross between:

(a) Group Five and Group Nine

(b) Group Six and Group Eight

(c) Group Four and Group Seven

(d) Strain Three and Group Four

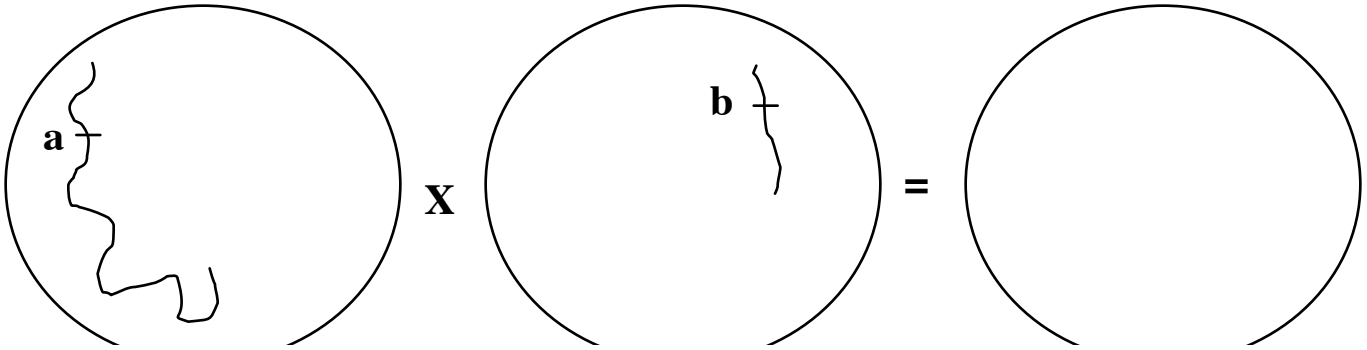
(e) Strain Two and Strain Three

(f) Strain One and Strain Two

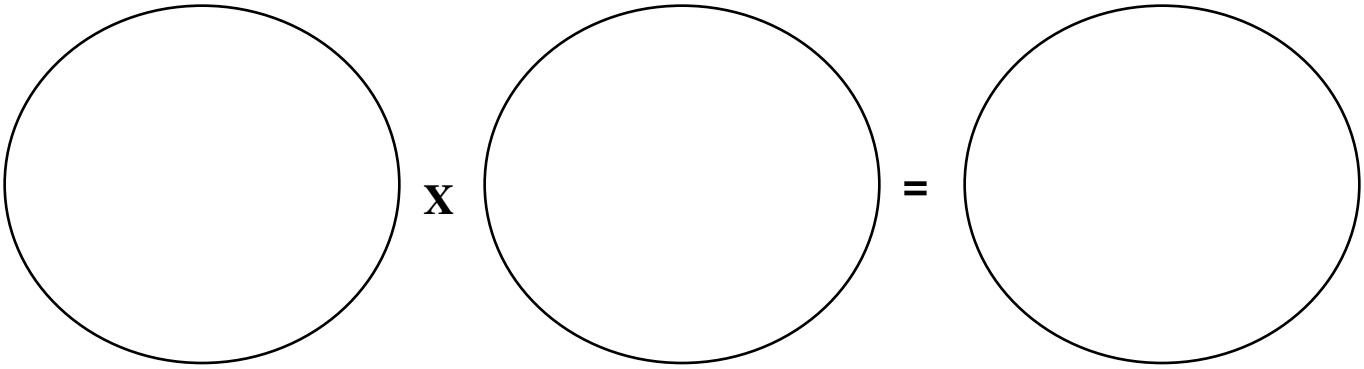
(g) List all of the above six crosses (parts **(a)** – **(f)**) that are proper complementation tests which clearly reveal whether two mutations are in the same gene or different genes.

2. You are studying a type of yeast that has two different chromosomes in its genome. You have isolated three mutations, “a,” “b” and “d,” each of which causes the same phenotype. When you mate a strain containing any one of these three mutations to wild-type, the resulting diploid exhibits the wild-type phenotype. You are in the process of doing complementation tests with these mutants. You discover that “a” and “b” do complement each other, but “a” and “d” do not. The corresponding wild-type alleles are “A,” “B” and “D.” Draw in the correct alleles that exist at each of these loci (A, B, and D) in each of the nine yeast cells drawn below. Make sure to put the alleles in their correct locations, as determined by those already drawn in for you. Also make sure to draw in the chromosomes to any cell whose chromosome(s) is/are missing.

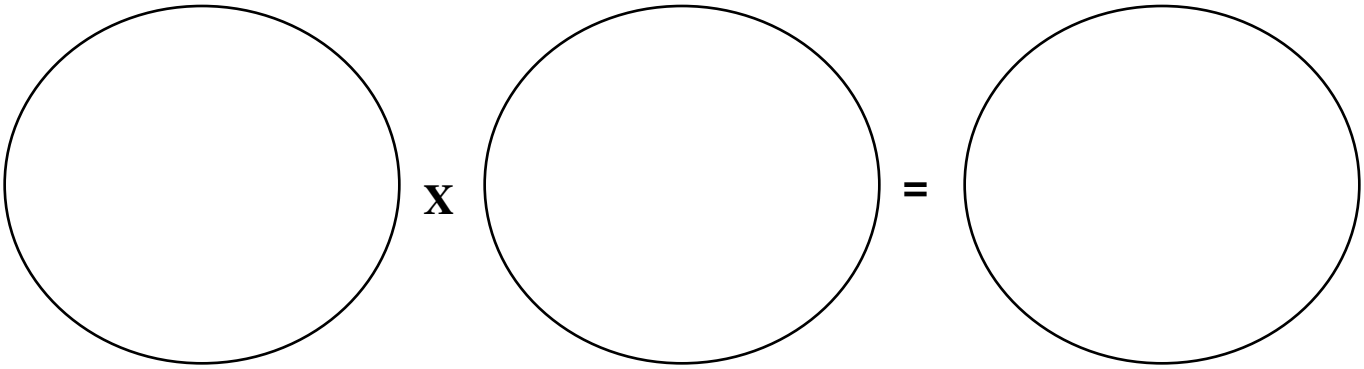
First cross: You mate haploid yeast of genotype “a” to haploid yeast of genotype “b.”



Second cross: You mate haploid yeast of genotype “a” to haploid yeast of genotype “d.”



Third cross: You mate haploid yeast of genotype “b” to haploid yeast of genotype “d.”



3. You are studying the inheritance of feather color in a new species of bird. You cross a true-breeding dark green bird to a true-breeding pale green bird. All of the resulting F1 birds are medium green. You then cross two medium green F1 birds, and analyze the resulting F2 generation. You obtain 50 birds: 13 are dark green, 23 are medium green, and 14 are pale green. We have not covered linkage and sex-linkage yet, so do not take those considerations into account during this problem.

(a) Propose a one-gene genetic model that explains the inheritance of feather color in this bird that is consistent with these results. By “propose a genetic model,” we mean define all possible genotypes and their associated phenotypes. Then give the genotypes of the birds in each generation of each cross described.

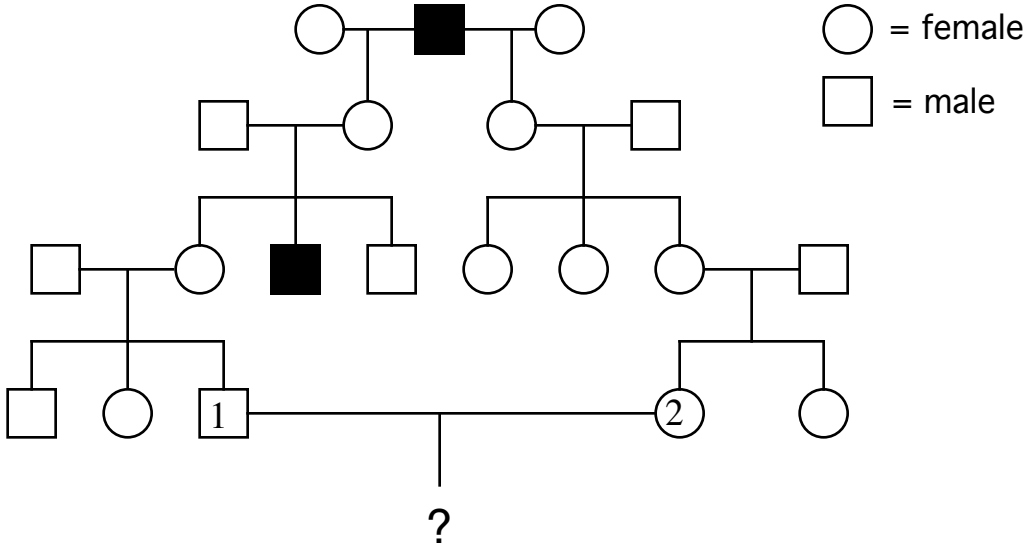
(b) You cross a true-breeding dark green bird to a true-breeding blue bird. All of the resulting F1 birds are blue. You then cross two blue F1 birds, and analyze the resulting F2 generation. You obtain 50 birds: 37 are blue, 4 are dark green, 7 are medium green, and 2 are pale green.
Propose a two-gene genetic model that explains the inheritance of color in this bird that is consistent with all of the data in this problem.

(c) Use chi-square analysis to test whether the numbers of F2 progeny you saw in part **(b)** correlate with the expected numbers based on your genetic model. For the chi square test you do, give the numbers of observed and expected organisms in each phenotypic class, the degrees of freedom, and your calculated value for χ^2. Also state what your conclusion is based on the results of this chi-square test.

p value:	.995	.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
df = 1	.000	.000	.016	.46	2.7	3.8	5.0	6.6	7.9
df = 2	.01	.05	.21	1.4	4.6	6.0	7.4	9.2	10.6
df = 3	.07	.22	.58	2.4	6.3	7.8	9.3	11.3	12.8

(d) Based on your model, how many different results might you expect to get if you crossed one randomly selected light green bird from the F2 to one randomly selected blue bird from the F2? For each possible result, state the phenotypic ratio present in the progeny obtained.

4. This problem deals with the following pedigree, which shows the inheritance of a very rare trait.



(a) Assume that the inherited disorder is expressed with complete penetrance and that there are no new mutations. What mode(s) of inheritance is/are consistent with this pedigree? (Your choices are: X-linked dominant, X-linked recessive, autosomal recessive, autosomal dominant.)

(b) For **each** consistent mode of inheritance, what are the probabilities that Individuals 1 and 2 will have:

… an affected son?

… an affected daughter?

… an unaffected son?

… an unaffected daughter?

(c) Use Bayes' theorem to calculate the probability that the next child of Individuals 1 and 2 will be affected with the disorder, given the new knowledge that the couple already has two healthy sons. Do this calculation for each mode of inheritance consistent with the pedigree.

2005 7.03 Problem Set 1 ANSWER KEY

1. Wild-type flies are brown in color. You have discovered two genes that control body color in flies -- gene A and gene B, which are on different autosomal chromosomes. You have three true-breeding mutant strains, all of which have black bodies.

Strain One (A*/A*) is homozygous for a dominant mutation in gene A. **A*A* B^+B^+ black**
Strain Two (A^-/A^-) is homozygous for a recessive mutation in gene A. **A^-A^- B^+B^+ black**
Strain Three (B^-/B^-) is homozygous for a recessive mutation in gene B. **A^+A^+ B^-B^- black**

Group Four are flies that result from mating Strain One to Strain Two. **A*A^- B^+B^+ black**
Group Five are flies that result from mating Strain One to Strain Three. **A*A^+ B^-B^+ black**
Group Six are flies that result from mating Strain Two to Strain Three. **A^-A^+ B^-B^+ brown**
Group Seven are flies that result from mating Strain One to wild-type. **A*A^+ B^+B^+ black**
Group Eight are flies that result from mating Strain Two to wild-type. **A^-A^+ B^+B^+ brown**
Group Nine are flies that result from mating Strain Three to wild-type. **A^+A^+ B^-B^+ brown**

Predict the phenotypic ratio (the numerical ratio and the phenotype of each phenotypic class) of the offspring resulting from a cross between:

(a) Group Five and Group Nine

---------- 5 BLACK----------: ------- 3 BROWN----------
1 A*A^+ B^-B^- : 1 A*A^+ B^+B^+ : 2 A*A^+ B^-B^+ : 1 A^+A^+ B^-B^- : 2 A^+A^+ B^+B^-: 1 A^+A^+ B^+B^+

(b) Group Six and Group Eight
------- 2 BLACK---------- : ---------- 6 BROWN ----------
1 A^-A^- B^+B^-: 1 A^-A^- B^+B^+ : 2 A^+A^- B^+B^-: 2 A^+A^- B^+B^+ : 1 A^+A^+ B^+B^- : 1 A^+A^+ B^+B^+

(c) Group Four and Group Seven
1 A*A* B^+B^+ : 1 A*A^- B^+B^+ : 1 A^+A*B^+B^+ : 1 A^+A^- B^+B^+
---------- 3 BLACK---------- : 1 BROWN

(d) Strain Three and Group Four
1 $A^*A^+B^+B^-$: 1 $A^+A^-B^+B^-$
1 BLACK : 1 BROWN

(e) Strain Two and Strain Three
all $A^+A^-B^+B^-$
all BROWN

(f) Strain One and Strain Two
all $A^*A^-B^+B^+$
all BLACK

(g) List all of the above six crosses (parts **(a)** – **(f)**) that are proper complementation tests which clearly reveal whether two mutations are in the same gene or different genes.

Part e) is a proper complementation test that clearly shows that wild-type alleles of A and B will rescue the mutant phenotype if the mutations are on different genes but not if they are on the same gene.

Other crosses are not complementation tests because, either, mutations giving dominant phenotypes were used (and these cannot be used in complementation tests), or strains that were not true-breeding were used.
Using strains that are not true-breeding is not the proper way to do a complementation test, because then multiple genotypes and phenotypes are produced instead of just one, which allows room for error in reading results (and a reliance on getting the expected ratios, rather than simply seeing all mutant organisms or all wild-type organisms).

2. You are studying a type of yeast that has two different chromosomes in its genome. You have isolated three mutations, “a,” “b” and “d,” each of which causes the same phenotype. When you mate a strain containing any one of these three mutations to wild-type, the resulting diploid exhibits the wild-type phenotype. You are in the process of doing complementation tests with these mutants. You discover that “a” and “b” do complement each other, but “a” and “d” do not. The corresponding wild-type alleles are “A,” “B” and “D.” Draw in the correct alleles that exist at each of these loci (A, B, and D) in each of the nine yeast cells drawn below. Make sure to put the alleles in their correct locations, as determined by those already drawn in for you. Also make sure to draw in the chromosomes to any cell whose chromosome(s) is/are missing.

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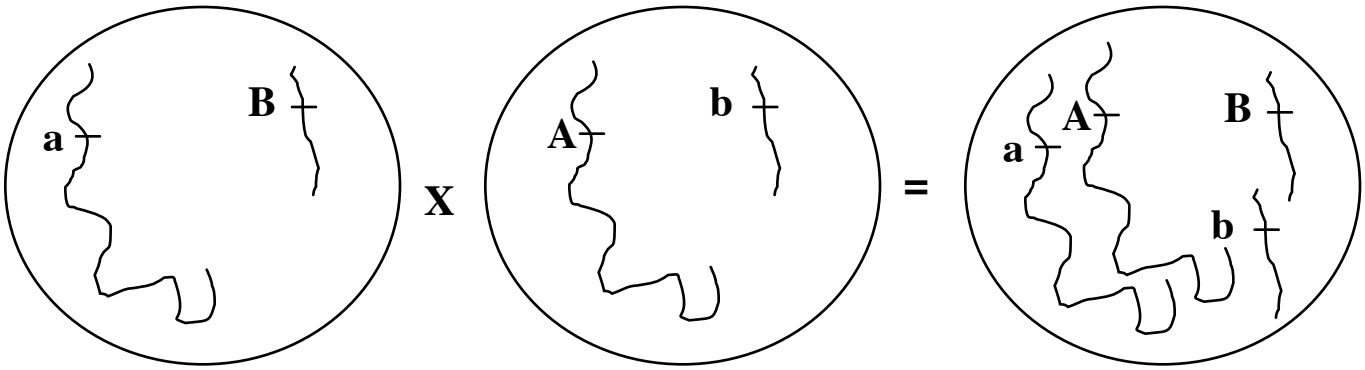
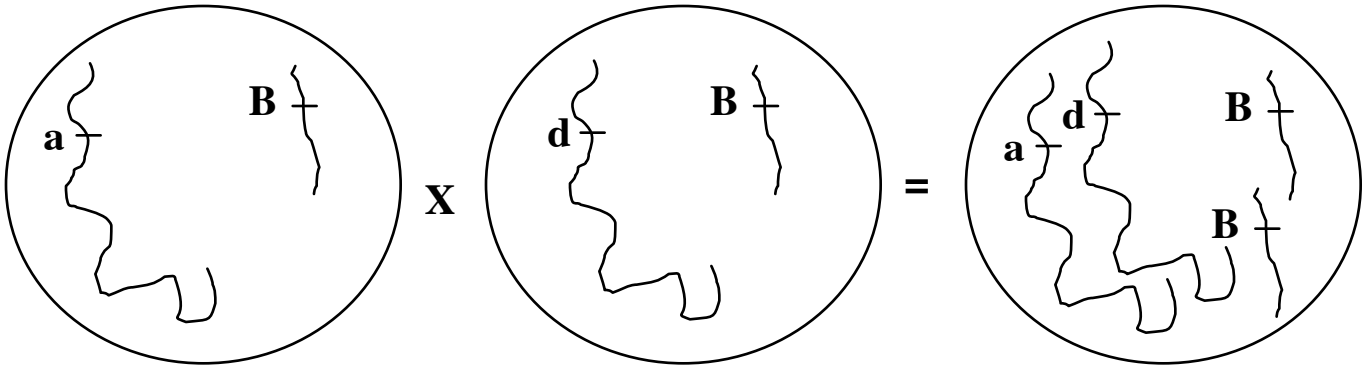
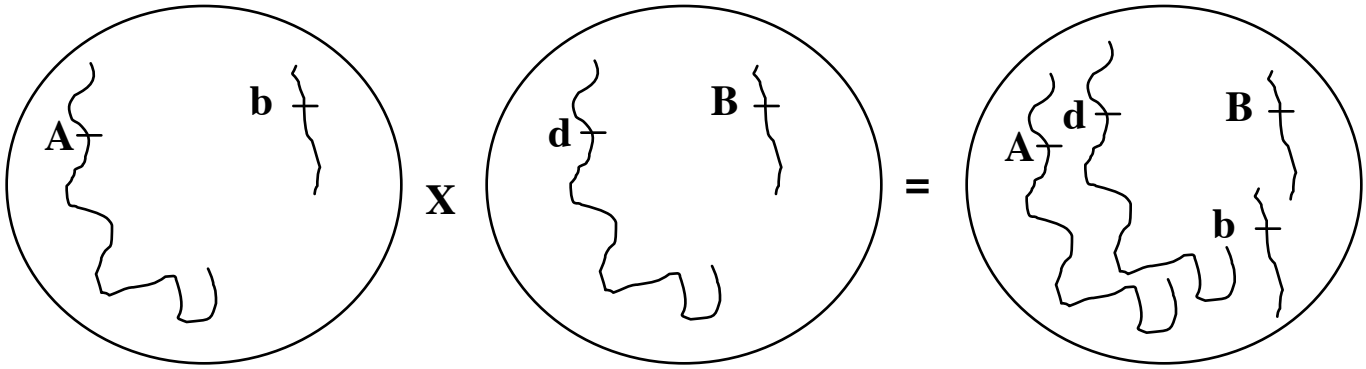


Second cross: You mate haploid yeast of genotype “a” to haploid yeast of genotype “d.”



Third cross: You mate haploid yeast of genotype “b” to haploid yeast of genotype “d.”



3. You are studying the inheritance of feather color in a new species of bird. You cross a true-breeding dark green bird to a true-breeding pale green bird. All of the resulting F1 birds are medium green. You then cross two medium green F1 birds, and analyze the resulting F2 generation. You obtain 50 birds: 13 are dark green, 23 are medium green, and 14 are pale green. We have not covered linkage and sex-linkage yet, so do not take those considerations into account during this problem.

(a) Propose a one-gene genetic model that explains the inheritance of feather color in this bird that is consistent with these results. By "propose a genetic model," we mean define all possible genotypes and their associated phenotypes. Then give the genotypes of the birds in each generation of each cross described.

We see three phenotypes from one gene so we can guess that we have incomplete dominance, giving us the 3rd phenotype as a blending between the other two.
GG= dark green
Gg= medium green
gg= pale green

P: GG x gg = all Gg (F1)
F1: Gg x Gg = 1 GG : 2 Gg : 1 gg
(13: 23: 14 is roughly equal to 1:2:1)

(b) You cross a true-breeding dark green bird to a true-breeding blue bird. All of the resulting F1 birds are blue. You then cross two blue F1 birds, and analyze the resulting F2 generation. You obtain 50 birds: 37 are blue, 4 are dark green, 7 are medium green, and 2 are pale green.
Propose a two-gene genetic model that explains the inheritance of color in this bird that is consistent with all of the data in this problem.

GgBB, or **ggBB,** or **GGBB,** or **GgBb,** or **ggBb,** or **GGBb = blue**
ggbb = pale green
Ggbb = medium green
GGbb = dark green

P: GGbb x ggBB = GgBb (F1) (since all of these birds are blue, we assume that the presence of blue overpowers the ability to be green – these two genes must be acting together to determine color. The B locus determines whether the bird is blue or not. If the bird is not blue, then the G locus is allowed to determine which shade of green the bird is.)

F1: GgBb x GgBb =
12 [GgBB, or **ggBB,** or **GGBB,** or **GgBb,** or **ggBb,** or **GGBb]: 1 GGbb : 2 Ggbb : 1 ggbb**
(since we see our green birds return, we assume that a bird can not express any blue without a B allele)

(c) Use chi-square analysis to test whether the numbers of F2 progeny you saw in part **(b)** correlate with the expected numbers based on your genetic model. For the chi square test you do, give the numbers of observed and expected organisms in each phenotypic class, the degrees of freedom, and your calculated value for χ^2. Also state what your conclusion is based on the results of this chi-square test.

p value:	.995	.975	0.9	0.5	0.1	0.05	0.025	0.01	0.005
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df = 3	.07	.22	.58	2.4	6.3	7.8	9.3	11.3	12.8

Don't reject hypothesis **can reject hypothesis**

Observed = 37 blue, 7 medium green, 4 dark green, 2 pale green
Expected = 37.5 blue, 6.25 medium green, 3.125 dark green, 3.125 pale green

Chi squared value = $\frac{(0.5)^2}{37.5} + \frac{(0.75)^2}{6.25} + \frac{(0.875)^2}{3.125} + \frac{(1.125)^2}{3.125} = 0.747$

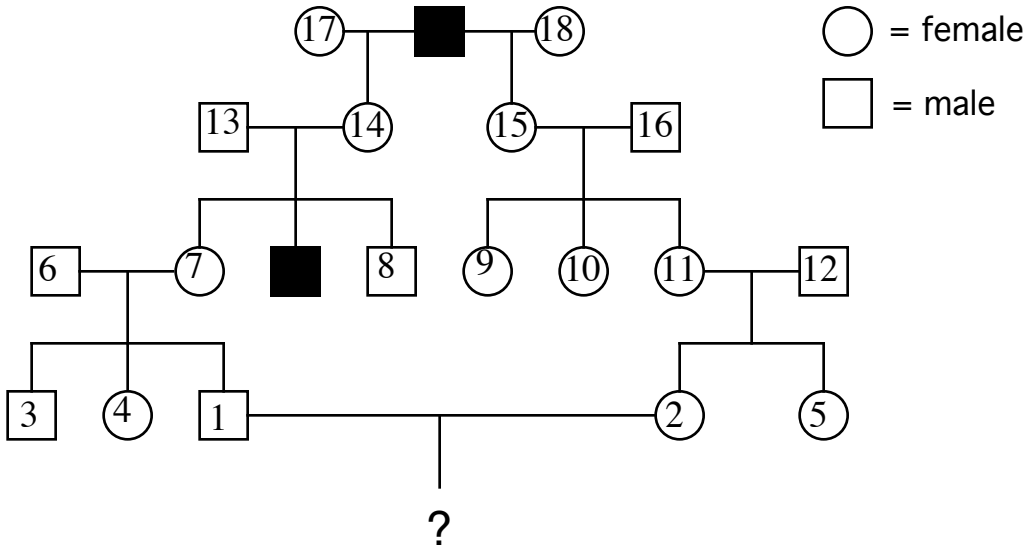
Degrees of freedom= # of classes(which is 4) – 1 = 3
p = 0.747
We can not reject our hypothesis, which is outlined in (b)

(d) Based on your model, how many different results might you expect to get if you crossed one randomly selected light green bird from the F2 to one randomly selected blue bird from the F2? For each possible result, state the phenotypic ratio present in the progeny obtained.

There are four different results, depending on the genotype of the randomly selected blue bird. The randomly selected light green bird will always be ggbb. The four possible outcomes are: all blue, OR half blue and half medium green, OR half blue and half light green, OR half blue and one-quarter medium green and one-quarter light green.

ggBB	**x**	**ggbb**	**=**	**all ggBb (all blue)**
GgBB	**x**	**ggbb**	**=**	**1 GgBb: 1ggBb (all blue)**
GGBB	**x**	**ggbb**	**=**	**all GgBb (all blue)**
ggBb	**x**	**ggbb**	**=**	**1 ggBb: 1ggbb (1blue: 1light green)**
GgBb	**x**	**ggbb**	**=**	**1 GgBb: 1Ggbb: 1ggBb: 1ggbb (2blue: 1 med. gr: 1 lt. gr)**
GGBb	**x**	**ggbb**	**=**	**1 GgBb: 1 Ggbb (1blue: 1 med Green)**

4. This problem deals with the following pedigree, which shows the inheritance of a very rare trait.



(a) Assume that the inherited disorder is expressed with complete penetrance and that there are no new mutations. What mode(s) of inheritance is/are consistent with this pedigree? (Your choices are: X-linked dominant, X-linked recessive, autosomal recessive, autosomal dominant.)

X-linked recessive
The two affected males are X^aY
Individuals #13, 16, 6, 8, 12, 3, and 1 are X^AY
Individuals #14 and 15 are $X^A X^a$
Individuals #17 and 18 are assumed to be $X^A X^A$ (because the trait is very rare)
Individuals #7, 9, 10, 11, 4, 2, and 5 are either $X^A X^a$ or $X^A X^A$

The disease can not be dominant because we have affected children with unaffected parents. The disease is also assumed to NOT be autosomal recessive because the affected man in the third generation would have gotten an "a" allele from Individual #13. However the statement is made that the trait is very rare. With very rare traits, you should assume that people marrying into a pedigree are not carrying the rare allele.

(b) For **each** consistent mode of inheritance, what are the probabilities that Individuals 1 and 2 will have:

… an affected son?

p(Ind #2 is carrier) = p(Ind. #11 is carrier) * p(egg contains "a" allele) = 1/2*1/2 = 1/4

p(affected son) = p(Ind. #2 is carrier) * p(egg contains "a" allele) * p(sperm contains Y)
= (1/4) * (1/2) * (1/2) = 1/16

… an affected daughter?

0%
The father must have an "A" allele since he is unaffected so this couple will never have an affected daughter.

… an unaffected son?

p(unaffected son)
= [p(Ind #2 not carrier) * p(egg contains wt allele) * (sperm contains Y)]
+ [p(Ind #2 carrier) * p(egg contains wt allele) * p(sperm contains Y)]
= [3/4 * 1 * 1/2] + [1/4 * 1/2 * 1/2] = 7/16

… an unaffected daughter?

p(unaffected daughter) = 1 – p(affected son) – p (unaffected son) – p(affected daugter)
= 1 – (1/16) – (7/16) – (0)
= 0.5

(c) Use Bayes' theorem to calculate the probability that the next child of Individuals 1 and 2 will be affected with the disorder, given the new knowledge that the couple already has two healthy sons. Do this calculation for each mode of inheritance consistent with the pedigree.

X = Individual Two is a carrier
notX = Individual Two is not carrier and is therefore $X^A X^A$
Y = the two sons that Individual Two has had are both healthy

P(X) = 1/4 (this is because Individual #11 has a 1/2 chance of being a carrier)
P(notX) = 3/4
P(Y|X) = p(1st egg contains "A" allele) * p(2nd egg contains "A" allele) = 1/2*1/2 = 1/4
P(Y|notX) = p(1st egg contains "A" allele) * p(2nd egg contains "A" allele) = 1*1 = 1

$$P(X/Y) = \frac{p(Y/X) * p(X)}{[p(Y/X) * p(X)] + [p(Y/notX) * p(notX)]}$$

P(X|Y) = 1/13
p(next child is an affected child)
= p(mother is carrier) * p(egg contains "a" allele) * p(sperm contains Y)
= 1/13 * 1/2 * 1/2
= 1/52

2005 7.03 Problem Set 2

Due before 5 PM on FRIDAY, September 30, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. A single gene determines coat color in a fuzzy creature you have discovered. The males of this species are either gray or brown, and the females are either gray, brown, or gray-and-brown striped.

(a) What is the most likely mode of inheritance for coat color in this creature?

(b) A very large litter of pups is produced, and every brown pup in the litter is male. What are all of the possible combinations of parents that produced this litter? (Include both the phenotype and genotype of both the mother and father for each possible mating.)

2. You are studying three X-linked recessive mutations in flies. Two of these mutations are in the same gene, the bl gene. The bl-1 mutation causes the phenotype of black bodies (wild-type flies have brown bodies). The bl-2 mutation also causes the phenotype of black bodies. The cw mutation causes the phenotype of curly wings (wild-type flies have straight wings). You cross true-breeding bl-1 curly-winged females to bl-2 males to obtain an F1 generation. You then cross female F1 flies to wild-type males. You analyze 5000 resulting males, and find the following numbers of flies:

Phenotype		Number of flies
Brown bodies	Straight wings	1
Brown bodies	Curly wings	6
Black bodies	Straight wings	2505
Black bodies	Curly wings	2488

(a) What are the phenotype(s) and genotype(s) of the F1 females?

(b) What are the phenotype(s) and genotype(s) of the F1 males?

(c) Why is it not necessary to cross the F1 females to homozygous recessive males (as it is in other three factor crosses we discussed in class)?

(d) Why do we only see four phenotypic classes in the F2 generation, instead of eight (as we saw in other three factor crosses we discussed in class)?

(e) Draw a map showing each of the possibilities for the relative order of the bl-1, bl-2, and cw loci. Draw any orders that are possible before you analyze the number of F2 flies.

(f) For each of your possible maps above, state the minimum number of crossovers required (during meiosis in the F1 female) to create a brown straight-winged male, and the minimum number of crossovers required to create a brown curly-winged male.

(g) Draw the map that shows the correct relative locations of the bl-1, bl-2, and cw loci.

3. Consider the above problem, in which we discussed how you have two mutations in the bl gene of Drosophila. Each mutation on its own (bl-1 or bl-2) or both mutations together will cause flies to have black bodies instead of brown bodies (like wild-type flies). Say you mate a true-breeding bl-1 female to a bl-2 male to generate the F1 generation.

(a) Draw a cell in an F1 female that is neither going through mitosis or meiosis (but is rather dormant in the G0/G1 stage of the cell cycle). Make sure to draw the X chromosome as being long, so that it would be much longer than the Y chromosome. Also please draw one other chromosome of an intermediate length that is an autosome. Also be sure to mark the alleles present on each chromosome at the bl-1 and bl-2 loci.

You cross an F1 female with a wild-type male, and obtain one brown-bodied male after much searching. Draw one cell from the F1 female gonad going through the various stages of meiosis to generate an egg cell that could have produced this brown-bodied male fly following fertilization. Each time you draw a step, follow the format described in part **(a)**. Draw the following steps only.

(b) The cell after DNA replication, but before the first cell division of meiosis has begun.

(c) The cell in metaphase I with its chromosomes lined up, after any recombination events have occurred, but before the crossing-over has been resolved.

(d) The two cells in metaphase II with their chromosomes lined up.

(e) The four final products of the meiosis. (Please indicate the gamete that led to the creation of the brown-bodied male with a star.)

4. You are studying two different mutations in yeast that both give the same phenotype. Either the Ts-1$^-$ mutation or the Ts-2$^-$ mutation alone causes the phenotype of temperature sensitivity, in the sense that each single mutant yeast is able to grow at 30°C and 33°C as usual, but is not able to grow at 36°C.

You are interested in determining whether the Ts-1 and Ts-2 loci are linked to each other, and whether the Ts-1$^-$ and Ts-2$^-$ mutations are in the same gene or not.

You mate a Ts-1$^-$ haploid mutant strain to a Ts-2$^-$ haploid mutant strain, producing a diploid strain. You can then starve these diploid yeast to induce meiosis and produce tetrads, each of which is a group of four haploid spores bundled together. Theoretically, you could get three different types of tetrads out of this experiment. You know what phenotype to expect from a wild-type spore and a single mutant spore, but not a double mutant spore.

(a) Given what you know, fill out the three tables below. Note that a few lines are already filled in for you. **Remember, the three tetrad types should all be distinct from one another.**

Tetrad Type One
Type of Tetrad (circle one): PD or NPD or TT

	Genotype		**Phenotype**	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A				
Spore B			**no**	**no**
Spore C				
Spore D			**no**	**no**

Tetrad Type Two
Type of Tetrad (circle one): PD or NPD or TT

	Genotype		**Phenotype**	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A				**no**
Spore B				**no**
Spore C				**yes**
Spore D				**no**

Tetrad Type Three

Type of Tetrad (circle one): PD or NPD or TT

	Genotype		**Phenotype**	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A				
Spore B				
Spore C				
Spore D				

State how many PDs, NPDs, and TTs would result (out of a total of 36 tetrads), given that each of the three different scenarios are true:

(b) The Ts-1 and Ts-2 loci lie extremely close to each other in the same gene.

(c) The Ts-1 and Ts-2 loci are unlinked.

(d) The Ts-1 and Ts-2 loci are about 3 cM apart and are in different genes.

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(a) What is the most likely mode of inheritance for coat color in this creature?
X-linked codominant

The fact that the females (who have two alleles of an X-linked gene) can show both phenotypes (gray and brown) at the same time, but males (who have one allele only of an X-linked gene) can only either be gray or brown implies codominance. Note that incomplete dominance would have yielded the results that females could be either gray, brown, or one color spread evenly throughout their fur that is a color that is a blend of gray and brown.

X^GX^G: gray female
X^BX^G: brown-and-gray striped female
X^BY: brown male
X^GY: gray male

(b) A very large litter of pups is produced, and every brown pup in the litter is male. What are all of the possible combinations of parents that produced this litter? (Include both the phenotype and genotype of both the mother and father for each possible mating.)

mother: brown X^BX^B or X^BX^G
father: gray X^GY

The problem states that every brown pup is male, so we know that the mother carries allele X^B. The father does not, as if he did, some brown pups would be female. So we know the father's genotype is X^GY and the mother is $X^BX^?$. However, while we are given that every brown pup is male, this does not mean that every male pup is brown, so therefore some males could be gray. The mother can therefore have the genotype of X^BX^B or X^BX^G.

2. You are studying three X-linked recessive mutations in flies. Two of these mutations are in the same gene, the bl gene. The bl-1 mutation causes the phenotype of black bodies (wild-type flies have brown bodies). The bl-2 mutation also causes the phenotype of black bodies. The cw mutation causes the phenotype of curly wings (wild-type flies have straight wings). You cross true-breeding bl-1 curly-winged females to bl-2 males to obtain an F1 generation. You then cross female F1 flies to wild-type males. You analyze 5000 resulting males, and find the following numbers of flies:

Phenotype		Number of flies
Brown bodies	Straight wings	1
Brown bodies	Curly wings	6
Black bodies	Straight wings	2505
Black bodies	Curly wings	2488

(a) What are the phenotype(s) and genotype(s) of the F1 females?
cw+ = straight wings and cw = curly wings

The original P generation flies were $X^{bl\text{-}1\ cw}\ X^{bl\text{-}1\ cw}$ crossed to $X^{bl\text{-}2\ \ cw+}\ Y$

All female flies resulting from this cross would be:
$X^{bl\text{-}2\ \ cw+}\ X^{bl\text{-}1\ cw}$
black bodies, straight wings

(b) What are the phenotype(s) and genotype(s) of the F1 males?

The original P generation flies were $X^{bl\text{-}1\ cw}\ X^{bl\text{-}1\ cw}$ crossed to $X^{bl\text{-}2\ \ cw+}\ Y$

All male flies resulting from this cross would be:
$X^{bl\text{-}1\ \ cw}\ Y$
black bodies, curly wings

(c) Why is it not necessary to cross the F1 females to homozygous recessive males (as it is in other three factor crosses we discussed in class)?

We usually cross to a homozygous recessive male because we want to see in the F2 every contribution that the heterozygous mother made to the offspring. By crossing to a homozygous recessive male, any allele that the mother gives to her offspring automatically determines the offspring's phenotype. However, when the trait is X-linked, there is another way to ensure that the mother entirely controls the phenotype of her offspring, and this is to analyze only males in the F2 (which is what the introduction to this

question states that we do). If we are only looking at F2 males, and all of the loci we are examining are X-linked, then the mother automatically is the sole influence on the phenotype of her (male) offspring. Thus we can cross the F1 heterozygous mother to any male, as long as we only examine males in the F2.

(d) Why do we only see four phenotypic classes in the F2 generation, instead of eight (as we saw in other three factor crosses we discussed in class)?

Two of the "factors" give the same phenotype. In other three factor crosses we did in class, each of the three "factors" controlled different aspects of phenotype, and there were two possible phenotypes (wild-type or mutant) resulting from each factor. Thus there were 2 X 2 X 2 = 8 possible phenotypic classes in the F2. Here there are only 2 X 2 = 4 phenotypic classes (because the flies can be either black or brown, and either straight or curly).

(e) Draw a map showing each of the possibilities for the relative order of the bl-1, bl-2, and cw loci. Draw any orders that are possible before you analyze the number of F2 flies.

____bl-1 _bl-2 _____cw___ OR ____bl-2_bl-1_____cw___

The other putative order (cw in the middle) is not possible, knowing that bl-1 and bl-2 are alleles of the same gene. However, for typical three factor crosses in which all three mutations are alleles of distinct genes, all three putative orders could be true before you analyze the F2 progeny.

(f) For each of your possible maps above, state the minimum number of crossovers required (during meiosis in the F1 female) to create a brown straight-winged male, and the minimum number of crossovers required to create a brown curly-winged male.

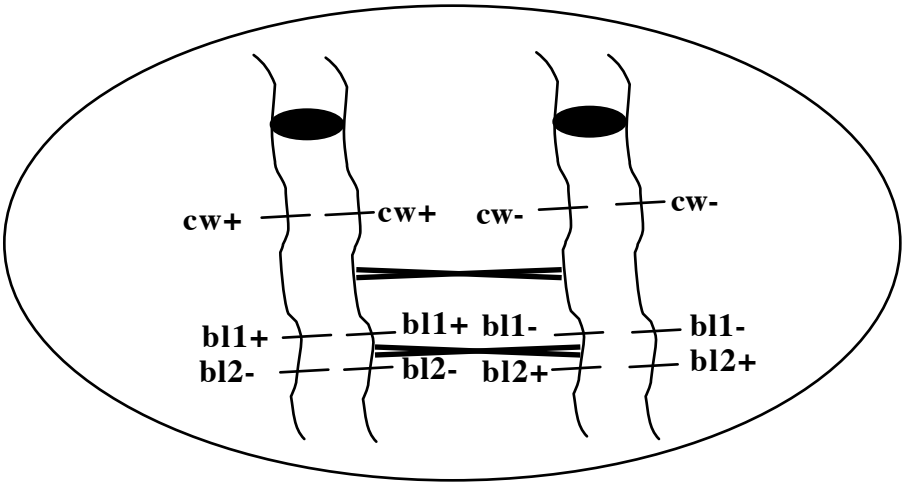
If bl-1 is in the middle:
2 crossovers for brown, curly-winged male
1 crossover for brown, straight-winged male

If bl-2 is in the middle:
1 crossover for brown, curly-winged male
2 crossovers for brown, straight-winged male

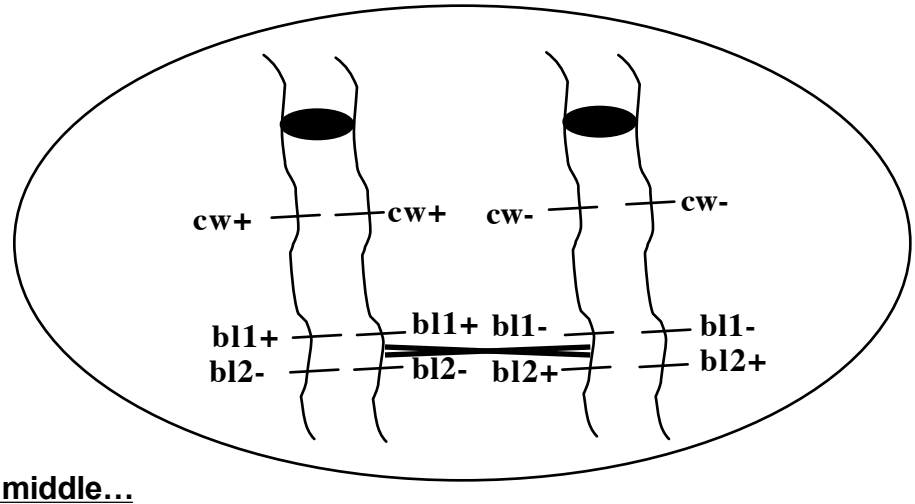
Consider the homologous chromosomes in the F1 female recombining during meiosis:

<u>If bl-1 is in the middle…</u>

To make a chromosome during meiosis that would generate a brown curly male, the following recombination events would have had to occur during meiosis I:

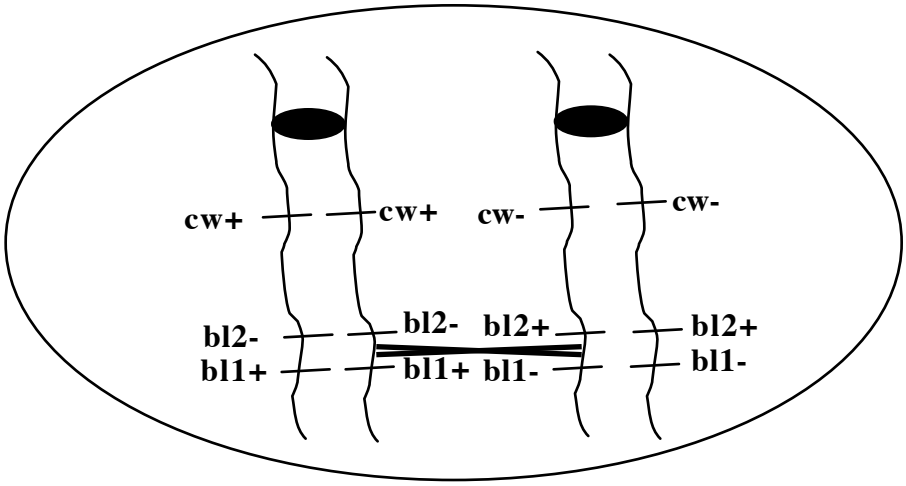


To make a chromosome during meiosis that would generate a brown straight male, the following recombination events would have had to occur during meiosis I:

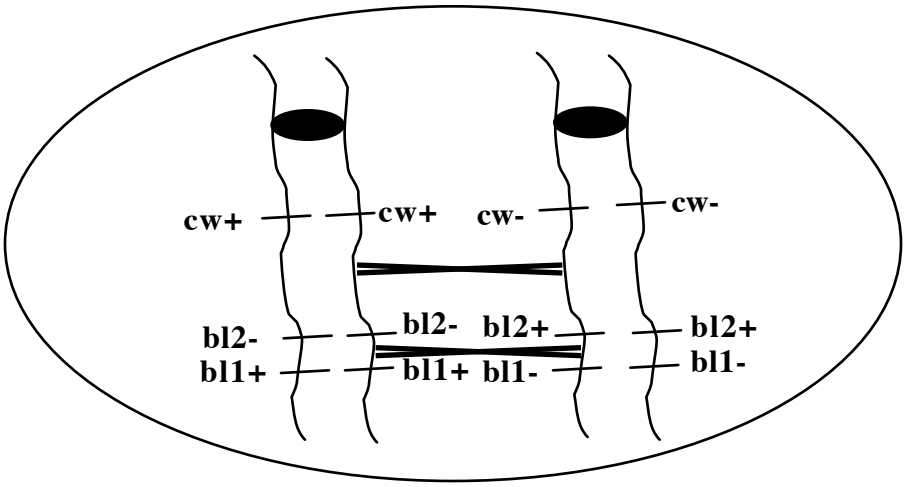


<u>If bl-2 is in the middle…</u>

To make a chromosome during meiosis that would generate a brown curly male, the following recombination events would have had to occur during meiosis I:



To make a chromosome during meiosis that would generate a brown straight male, the following recombination events would have had to occur during meiosis I:



(g) Draw the map that shows the correct relative locations of the bl-1, bl-2, and cw loci.

Now, when you look at the data, you see that it was more frequent to get a brown curly male than a brown straight male. This data from the F2 is consistent with the order:

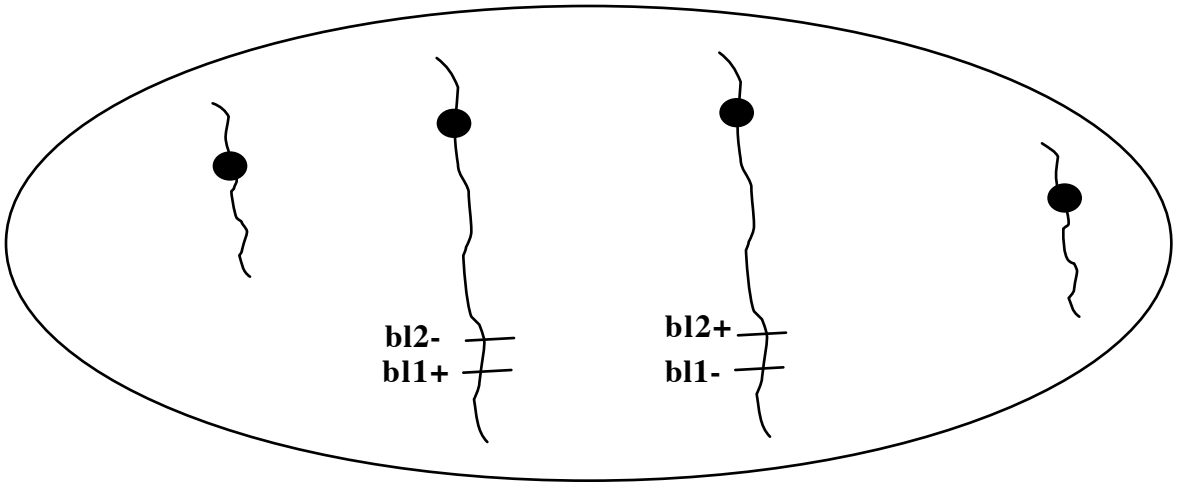
____bl-1 _bl-2 _____cw___

(bl-2 in the middle)

This is because single crossovers are more common than double crossovers. Thus, for the order with bl-2 in the middle, one would predict from part (f) that brown curly males would be more frequent than brown straight males. (Part (f) also tells you that, if the order had been that in which bl-1 is in the middle, you would expect brown straight males to be more frequent than brown curly males.)

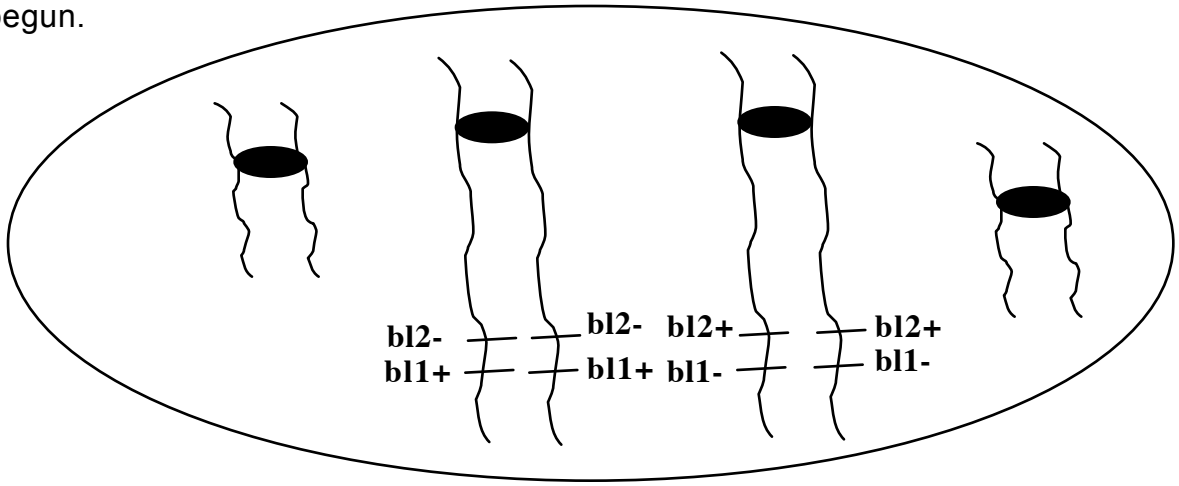
3. Consider the above problem, in which we discussed how you have two mutations in the bl gene of Drosophila. Each mutation on its own (bl-1 or bl-2) or both mutations together will cause flies to have black bodies instead of brown bodies (like wild-type flies). Say you mate a true-breeding bl-1 female to a bl-2 male to generate the F1 generation.

(a) Draw a cell in an F1 female that is neither going through mitosis or meiosis (but is rather dormant in the G0/G1 stage of the cell cycle). Make sure to draw the X chromosome as being long, so that it would be much longer than the Y chromosome. Also please draw one other chromosome of an intermediate length that is an autosome. Also be sure to mark the alleles present on each chromosome at the bl-1 and bl-2 loci.

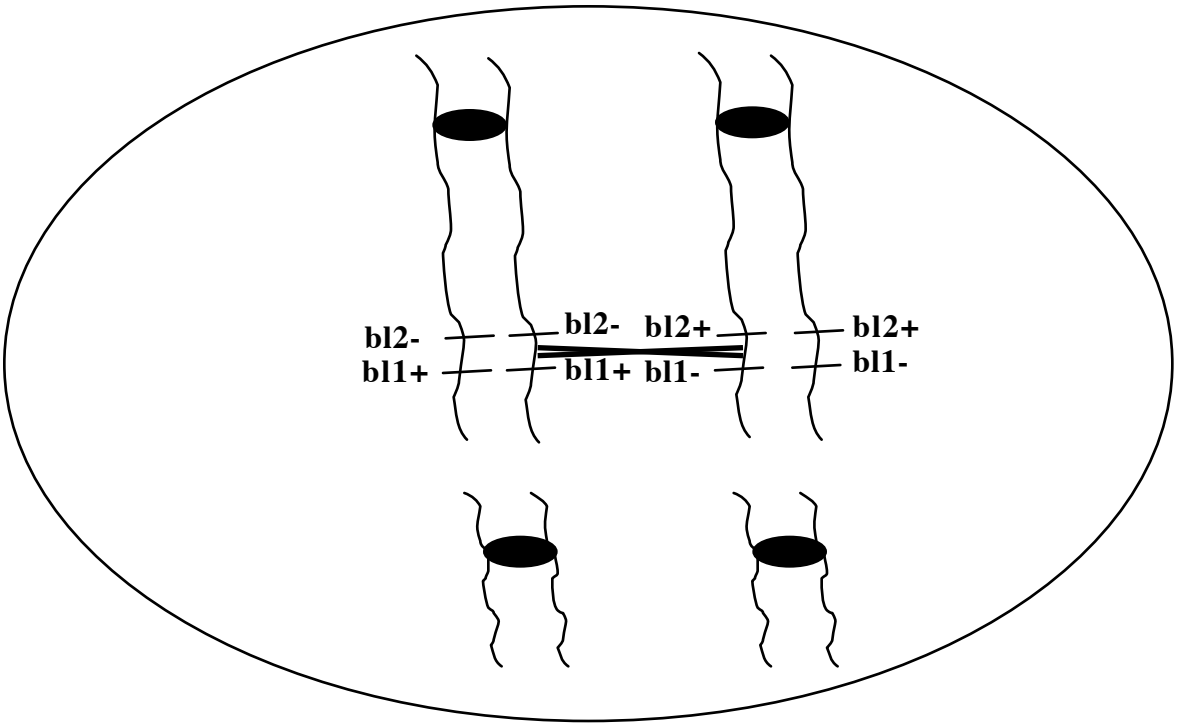


You cross an F1 female with a wild-type male, and obtain one brown-bodied male after much searching. Draw one cell from the F1 female gonad going through the various stages of meiosis to generate an egg cell that could have produced this brown-bodied male fly following fertilization. Each time you draw a step, follow the format described in part **(a)**. Draw the following steps only.

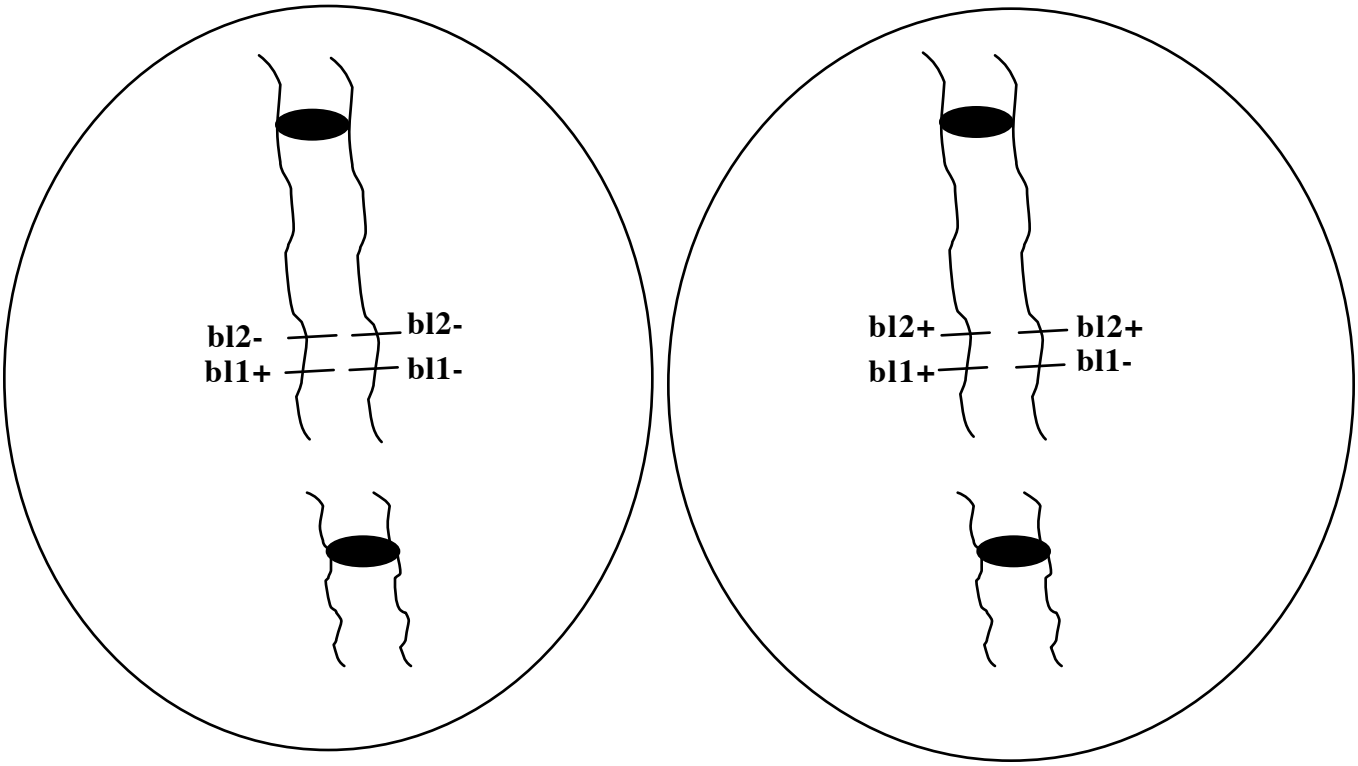
(b) The cell after DNA replication, but before the first cell division of meiosis has begun.



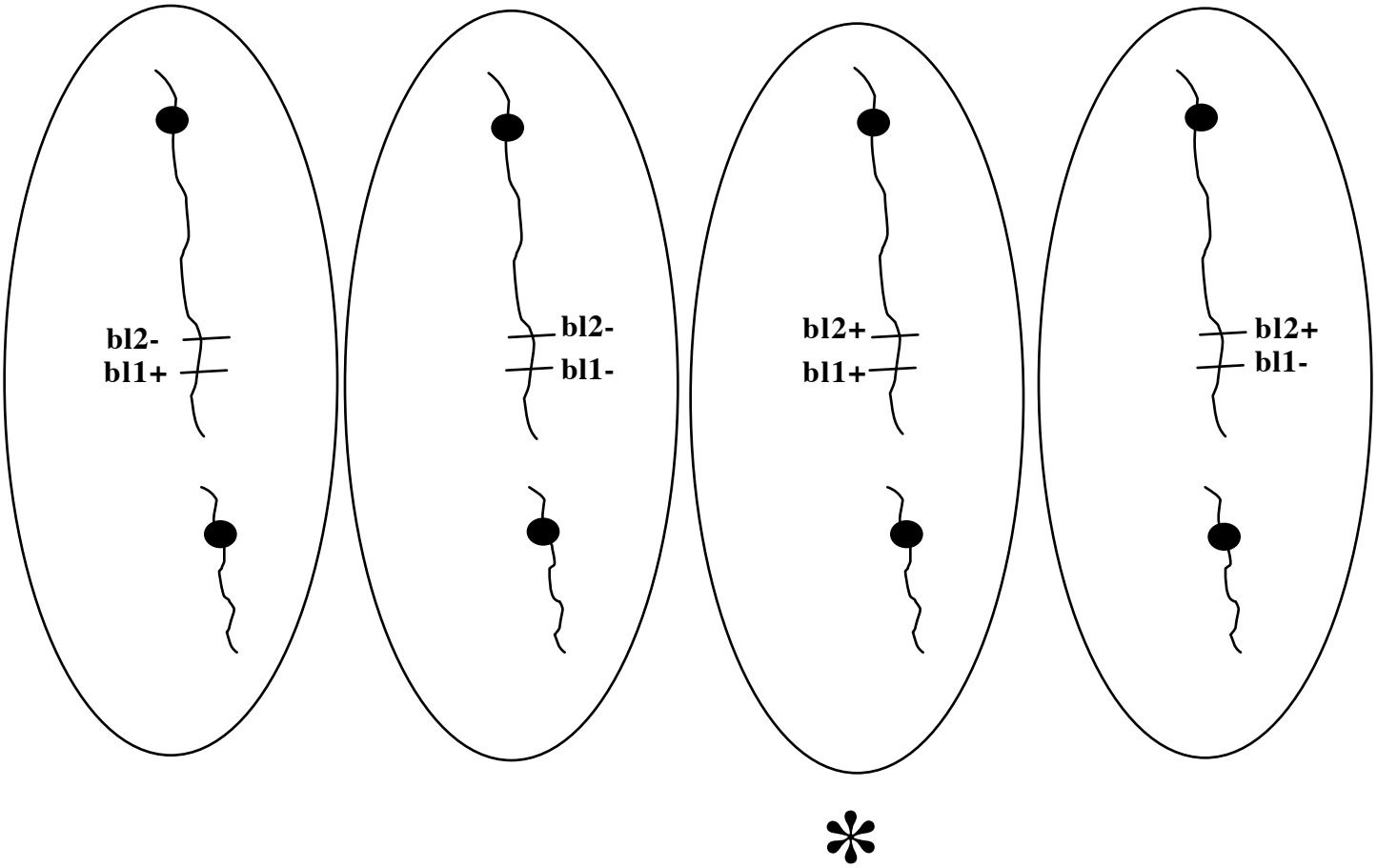
(c) The cell in metaphase I with its chromosomes lined up, after any recombination events have occurred, but before the crossing-over has been resolved.



(d) The two cells in metaphase II with their chromosomes lined up.



(e) The four final products of the meiosis. (Please indicate the gamete that led to the creation of the brown-bodied male with a star.)



4. You are studying two different mutations in yeast that both give the same phenotype. Either the Ts-1$^-$ mutation or the Ts-2$^-$ mutation alone causes the phenotype of temperature sensitivity, in the sense that each single mutant yeast is able to grow at 30°C and 33°C as usual, but is not able to grow at 36°C.

You are interested in determining whether the Ts-1 and Ts-2 loci are linked to each other, and whether the Ts-1$^-$ and Ts-2$^-$ mutations are in the same gene or not.

You mate a Ts-1$^-$ haploid mutant strain to a Ts-2$^-$ haploid mutant strain, producing a diploid strain. You can then starve these diploid yeast to induce meiosis and produce tetrads, each of which is a group of four haploid spores bundled together. Theoretically, you could get three different types of tetrads out of this experiment. You know what phenotype to expect from a wild-type spore and a single mutant spore, but not a double mutant spore.

(a) Given what you know, fill out the three tables below. Note that a few lines are already filled in for you. **Remember, the three tetrad types should all be distinct from one another.**

Tetrad Type One

Type of Tetrad (circle one): PD or **NPD** or TT

Let "+" indicate wild-type and "-" indicate ts-1 or ts-2

	Genotype		**Phenotype**	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A	+	+	Yes	yes
Spore B	-	-	**No**	**no**
Spore C	+	+	Yes	yes
Spore D	-	-	**No**	**no**

Tetrad Type Two

Type of Tetrad (circle one): PD or NPD or **TT**

	Genotype		**Phenotype**	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A	+	-	Yes	**no**
Spore B	-	+	yes	**no**
Spore C	+	+	Yes	**yes**
Spore D	-	-	No	**no**

Tetrad Type Three

Type of Tetrad (circle one): **PD** or NPD or TT

	Genotype		Phenotype	
	at Ts-1 (+ or $^-$)	at Ts-2 (+ or $^-$)	Growth at 33°C (yes or no)	Growth at 36°C (yes or no)
Spore A	+	-	Yes	no
Spore B	+	-	Yes	no
Spore C	-	+	Yes	no
Spore D	-	+	Yes	no

State how many PDs, NPDs, and TTs would result (out of a total of 36 tetrads), given that each of the three different scenarios are true:

(b) The Ts-1 and Ts-2 loci lie extremely close to each other in the same gene.

Almost entirely PDs (36 PDs).

An exact number cannot be calculated without more information. However, when two genes of interest are linked, PD is much greater than NPD. NPDs are products of double crossovers and TTs are products of single crossovers or double crossovers, so both would be rare or nonexistent for small samples sizes (i.e. 36 tetrads) in cases of extreme linkage.

(c) The Ts-1 and Ts-2 loci are unlinked.

6 PD, 24 TT, and 6 NPD

This follows the rule that, when two loci are unlinked, one sees a ratio of 1 PD : 4 TT : 1 NPD due to random segregation of Ts-1 with respect to Ts-2 during meiosis.

Given the following associations of Ts-1 with Ts-2, the resultant tetrad types are on the right. This gives us 1 PD to 4 TT to 1 NPD.

Ts-1	**Ts-1**	**+**	**+**	
Ts-2	Ts-2	+	+	NPD
Ts-2	+	Ts-2	+	TT
Ts-2	+	+	Ts-2	TT
+	Ts-2	+	Ts-2	TT
+	Ts-2	Ts-2	+	TT
+	+	Ts-2	Ts-2	PD

(d) The Ts-1 and Ts-2 loci are about 3 cM apart and are in different genes.

TT = 2 (or 3)
NPD = 0
PD = 34 (or 33)

Use the formula

$$\text{map distance} = \frac{6\,\text{NPD} + \text{TT}}{2 \times (\#\ \text{tetrads})} \times 100$$

you can see that, even if you got 1 NPD and no TTs, the distance you would calculate from your data would be much greater than 3cM. Thus you must have gotten no NPDs. Substituting 0 = # of NPDs, you then calculate:

$$\frac{\text{TT}}{72} \times 100 = 3$$

Thus TT = 2.16

This makes sense, because 3cM translates to a 3% chance of getting recombination between Ts-1 and Ts-2. This means that these two loci are quite close to each other, making the probability that you will see double crossovers very low, especially if you only look at 36 tetrads.

2005 7.03 Problem Set 3

Due before 5 PM on WEDNESDAY, October 19, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. The following sequence is a wild-type gene called *lyeT* that encodes a short protein that is required for a certain bioluminescent species of bacteria to produce light. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

```
5′–ACTTCGATATGCCTAATATATCGATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA–3′
3′–TGAAGCTATACGGATTATATAGCTAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT–5′
```

(a) Which strand (the upper or the lower) is used as the template in transcription?

(b) Write out the entire sequence of the mRNA made from this wild-type gene.

5'-

(c) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

The following sequence is a mutant version of the above gene (*lyeT*$^{-}$) that is present in a bacterial strain that does not produce light. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

```
5′–ACTTCGATATGCCTAATATATAGATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA–3′
3′–TGAAGCTATACGGATTATATATCTAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT–5′
```

(d) Which strand (the upper or the lower) is used as the template in transcription?

(e) Write out the amino acid sequence of any protein that is encoded by this mutated gene.

The following sequence is a wild-type gene that encodes a tRNA-ser molecule that recognizes the codon 5'-UCG-3' on all mRNAs in the bacterial cell. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

```
5′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCATCGCTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGTAGCGAACGAGTACGACTATGCGCGTTGCCA–5′
```

(f) Which strand (the upper or the lower) is used as the template in transcription? (Remember that tRNAs are DIRECTLY transcribed from tRNA-encoding genes. There is no mRNA intermediate in the production of a tRNA molecule from a tRNA gene!)

(g) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

(h) Put a box around the double-stranded DNA portion of the wild-type tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing at the top of the page.)

The following sequence is a mutant gene that encodes a nonsense-suppressing version of the tRNA-ser gene. This mutation suppresses the effects of *lyeT*$^-$. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

```
5′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCATAGCTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGTATCGAACGAGTACGACTATGCGCGTTGCCA–5′
```

(i) Which strand (the upper or the lower) is used as the template in transcription?

(j) Put a box around the double-stranded DNA portion of the mutated tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing in the middle of the page.)

(k) Would a strain produce light if it contains the wild-type version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

(l) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

(m) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the mutant version of the *tRNA-ser* gene?

2. Wild-type bacteria are capable of a type of movement called "swarming," in which many bacterial cells bundle together to form rafts that can swim through solid media of a low agar concentration. Someone has given you a mutant strain of bacteria that has the mutant phenotype of being unable to "swarm." This person tells you that the mutation (*swrM*⁻) which causes this phenotype is either an ochre mutation or an amber mutation. You have another bacterial strain containing a mutant version of a tRNA gene (Su+) that encodes an ochre suppressor tRNA . This tRNA gene is 60% linked (by cotransduction frequency) to a Tn5 KanR transposon in this strain. This transposon is not linked to *swrM*.

(a) You decide to perform a cotransduction experiment to determine whether the *swrM*⁻ mutation is an amber mutation or an ochre mutation. Fill in the blanks in the following paragraph to show what experiment you decide do:

You grow P1 phage on bacteria of the genotype _____.

You use the resulting phage lysate to infect bacteria of the genotype _____.

You select for transductants that can grow on plates containing _____.

(b) Describe the two possible results you could get from this experiment if you analyzed 1000 transductants. (Include in each answer the predicted number of transductants of each phenotypic class, and the genotypes of the transductants in each class.)

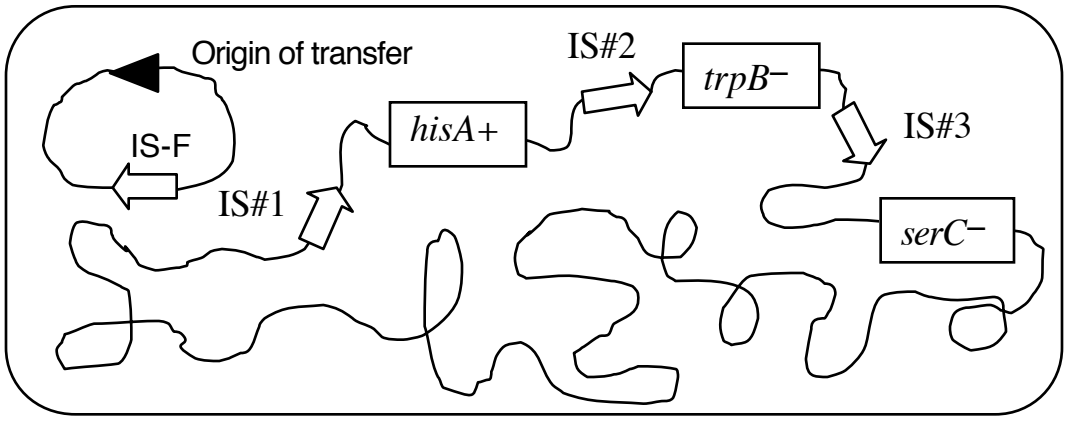
If the *swrM*⁻ mutation is an ochre mutation:

If the *swrM*⁻ mutation is an amber mutation:

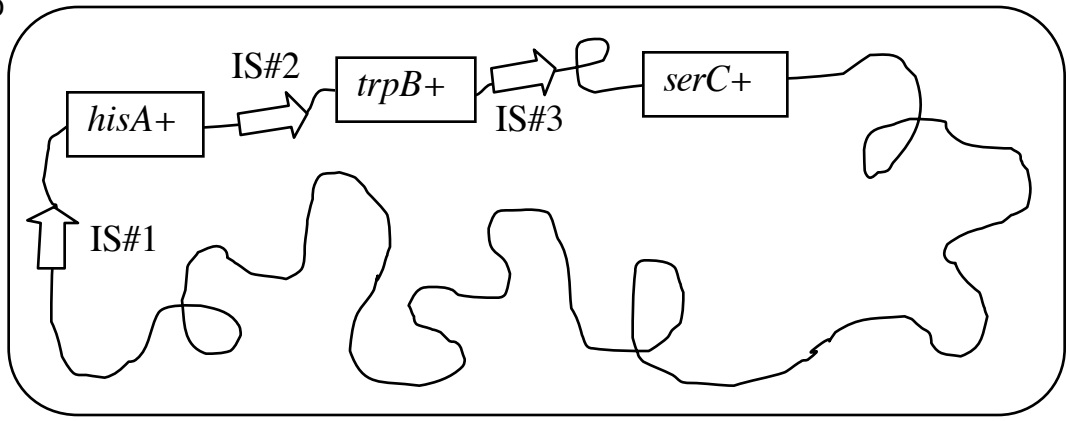
(c) You find a new gene (*swrA*) in which a specific ochre mutation causes the phenotype of being unable to swarm. This gene is linked to the transposon you used in part **(a)** with a cotransduction frequency of 30%. Draw all of the possibilities for a map of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the whole chromosome, and the positions and relative order of the Tn insertion, the tRNA locus, the *swrA* locus, and the *swrM* locus.

3. You are studying two strains of *E. coli*. Below are diagrams of the two different strains, showing their chromosomes and the F plasmid (in Strain One, which contains it).

Strain One



Strain Two



(a) Using the notation from above, draw the new strain that would result if Strains One and Two mated together. (Call the resulting strain Strain Three.)

(b) Using the notation from above, draw the new strain that would result if IS-F and IS#3 recombined together in a cell from Strain Three. (Call the resulting strain Strain Four.)

(c) Using the notation from above, draw the events that would occur in the recipient cell that had been mated into, if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating only for a short time, and you selected for exconjugants on minimal medium that lacks supplemental **serine**. (Call the resulting strain Strain Five.)

(d) Using the notation from above, draw the new strain that would result if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating for only a short time, and you selected for exconjugants on minimal medium that lacks supplemental **tryptophan**. (Call the resulting strain Strain Six.)

(e) Using the notation from above, draw the new strain that would result if IS#1 recombined with the IS sequence that is farthest away from IS#1 in a cell from Strain Four.

(f) State whether each of the following strains is an F**−** strain, an F**'** strain, an F**+** strain, or an Hfr strain:

Strain One --

Strain Two --

Strain Three --

Strain Four --

Strain Five –

Strain Six --

2005 7.03 Problem Set 3 ANSWER KEY

Due before 5 PM on WEDNESDAY, October 19, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. The following sequence is a wild-type gene called *lyeT* that encodes a short protein that is required for a certain bioluminescent species of bacteria to produce light. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5′–ACTTCGATATGCCTAATATATCGATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA–3′
3′–TGAAGCTATACGGATTATATAGCTAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT–5′

(a) Which strand (the upper or the lower) is used as the template in transcription?

lower, because it is the only one of the two that would encode an mRNA with a start codon

(b) Write out the entire sequence of the mRNA made from this wild-type gene.

5'- ACUUCGAUAUGCCUAAUAUAUCGAUCGAUCUGUGGGGCCUAGCUAGCUAA CCAGAGACGCUACCGA-3' because, if the lower strand is used as the template, then the mRNA will look like the upper strand.

(c) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

Met Pro Asn Ile Ser Ile Asp Leu Trp Gly Leu Ala Ser (or MPNISIDLWGLAS)

The following sequence is a mutant version of the above gene (*lyeT*$^{-}$) that is present in a bacterial strain that does not produce light. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5′–ACTTCGATATGCCTAATATA**TAG**ATCGATCTGTGGGGCCTAGCTAGCTAACCAGAGACGCTACCGA–3′
3′–TGAAGCTATACGGATTATAT**ATC**TAGCTAGACACCCCGGATCGATCGATTGGTCTCTGCGATGGCT–5′

(d) Which strand (the upper or the lower) is used as the template in transcription?

lower

(e) Write out the amino acid sequence of any protein that is encoded by this mutated gene.

Met Pro Asn Ile (or MPNI) because of the nonsense mutation bolded above

The following sequence is a wild-type gene that encodes a tRNA-ser molecule that recognizes the codon 5'-UCG-3' on all mRNAs in the bacterial cell. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCA**TCG**CTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGT**AGC**GAACGAGTACGACTATGCGCGTTGCCA–5′

(f) Which strand (the upper or the lower) is used as the template in transcription? (Remember that tRNAs are DIRECTLY transcribed from tRNA-encoding genes. There is no mRNA intermediate in the production of a tRNA molecule from a tRNA gene!)

upper, since it is the only one of the two that would encode a tRNA transcript that would include the proper anticodon 5'-CGA-3' (which can base pair with 5'-UCG-3')

(g) Write out the amino acid sequence of any protein that is encoded by this wild-type gene.

None, this gene does not code for any protein. This gene encodes a tRNA.

(h) Put a box around the double-stranded DNA portion of the wild-type tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing at the top of the page.)

The following sequence is a mutant gene that encodes a nonsense-suppressing version of the tRNA-ser gene. This mutation suppresses the effects of *lyeT*$^-$. The sequence given is from the point where transcription starts (called "+1") to the point where transcription ends (called the "terminator").

5′–CCCGTTGCTCAGATCTGGATATCCATCCTGCATGCA**TAG**CTTGCTCATGCTGATACGCGCAACGGT–3′
3′–GGGCAACGAGTCTAGACCTATAGGTAGGACGTACGT**ATC**GAACGAGTACGACTATGCGCGTTGCCA–5′

(i) Which strand (the upper or the lower) is used as the template in transcription?

Upper. Note that this tRNA now has the anticodon 5'-CUA-3', which pairs with the codon in an mRNA of 5'-UAG-3' (which is normally a stop codon but would be read by this mutant tRNA and translated as a serine)

(j) Put a box around the double-stranded DNA portion of the mutated tRNA gene that encodes the anticodon portion of the tRNA. (Do this in the drawing in the middle of the page.)

(k) Would a strain produce light if it contains the wild-type version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

yes, because wild-type cells of this species produce light

(l) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the wild-type version of the *tRNA-ser* gene?

no, because a single mutant lyeT– strain has the phenotype of inability to make light

(m) Would a strain produce light if it contains the mutant version of the *lyeT* gene and the mutant version of the *tRNA-ser* gene?

yes, the mutant *tRNA-ser* would suppress the effects of the amber mutation in the *lyeT* gene. The 5'-UAG-3' in the lyeT mRNA would be read as a serine, not as a stop. Thus the double mutant cell (harboring the original lyeT mutation and the suppressor mutation) would produce functional LyeT protein, and thus would make light.

2. Wild-type bacteria are capable of a type of movement called "swarming," in which many bacterial cells bundle together to form rafts that can swim through solid media of a low agar concentration. Someone has given you a mutant strain of bacteria that has the mutant phenotype of being unable to "swarm." This person tells you that the mutation (*swrM–*) which causes this phenotype is either an ochre mutation or an amber mutation. You have another bacterial strain containing a mutant version of a tRNA gene (Su+) that encodes an ochre suppressor tRNA . This tRNA gene is 60% linked (by cotransduction frequency) to a Tn5 KanR transposon in this strain. This transposon is not linked to *swrM*.

(a) You decide to perform a cotransduction experiment to determine whether the *swrM–* mutation is an amber mutation or an ochre mutation. Fill in the blanks in the following paragraph to show what experiment you decide do: **Move Su+ into a swrM- strain.**

You grow P1 phage on bacteria of the genotype **Tn5 KanR Su+**.

You use the resulting phage lysate to infect bacteria of the genotype **Su- swrM-**.

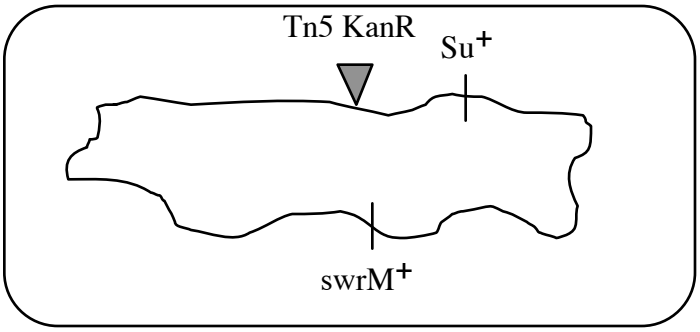
You select for transductants that can grow on plates containing **Kanamycin**.

(b) Describe the two possible results you could get from this experiment if you analyzed 1000 transductants. (Include in each answer the predicted number of transductants of each phenotypic class, and the genotypes of the transductants in each class.)

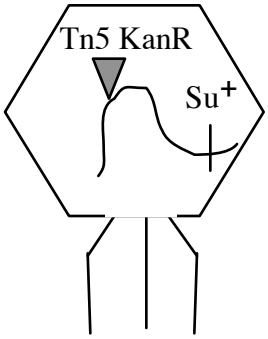
If the *swrM–* mutation is an ochre mutation:
600 can swarm (Tn5 KanR Su+ swrM-)
400 cannot swarm (Tn5 KanR Su- swrM-)

60% cotransduction means that 60% of the time (600/1000), Su+ will be transduced along with the selectable Tn5 transposon. 40% of the time, the Tn5 transposon will enter the cell without Su+ coming in as well.

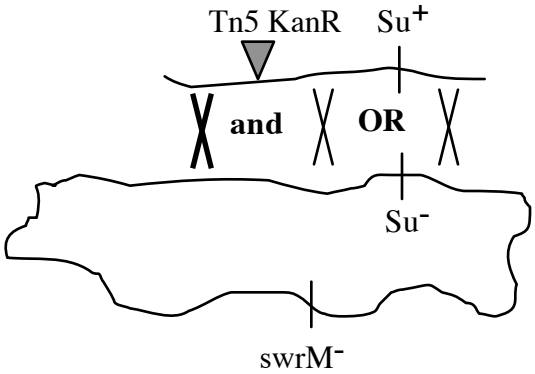
The original cell on which the phage was grown looked like:



When phage infect this cell, replicate, and burst the cell (releasing progeny phage), a small percentage of phage contain bacterial DNA instead of phage DNA. We select for the very small portion of phage that carry the part of the bacterial chromosome carrying Tn5, because we select for transductants on medium containing kanamycin. Thus the transductants we see have been infected with phage carrying a specific piece of the host chromosome:



When these phage infect our recipent strain, this DNA enters the recipient and aligns with homologous sequences in the recipient chromsome. This DNA can integrate into the recipient chromosome by an even number of crossover events. (An odd number of crossovers creates a linear product, and bacteria cannot maintain linear DNA.) One of the two crossovers must be to the left of KanR, because we are selecting for kanamycin resistance, so the KanR gene must have integrated into the recipient genome. 60% of the time, the second crossover will bring Su+ in with KanR. 40% of the time, the second crossover will be to the left of Su+, thus only bringing in KanR.

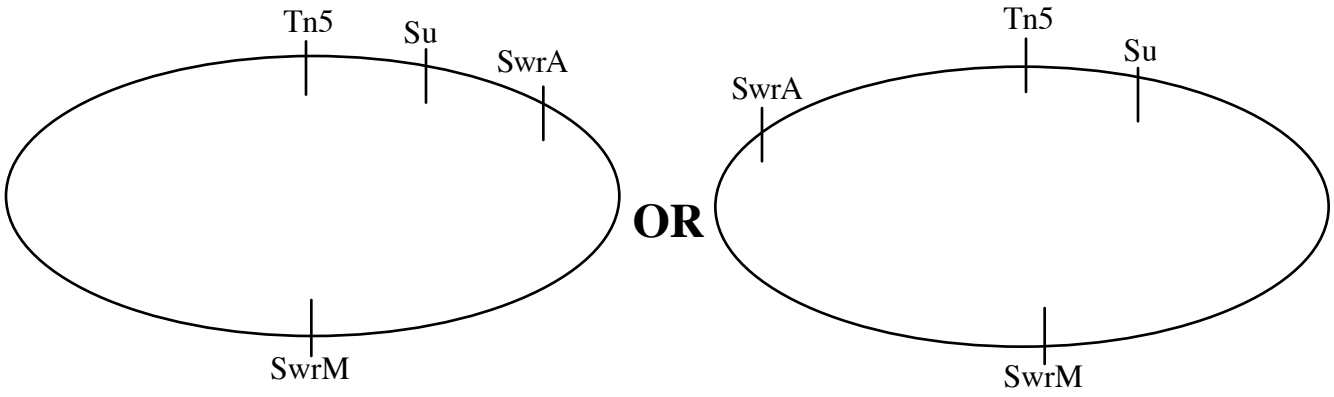


If the *swrM*– mutation is an amber mutation:

1000 cannot swarm. We will still see 600 [Tn5 KanR Su+ swrM-] transductants and the 400 [Tn5 KanR Su- swrM-] transductants, but, in this case, the ochre suppressor cannot suppress the swrM mutation so none will swarm. The pictures of the DNA being transduced in would look the same as the above pictures, however.

(c) You find a new gene (*swrA*) in which a specific ochre mutation causes the phenotype of being unable to swarm. This gene is linked to the transposon you used in part (a) with a cotransduction frequency of 30%. Draw all of the possibilities for a map of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the whole chromosome, and the positions and relative order of the Tn insertion, the tRNA locus, the *swrA* locus, and the *swrM* locus.

There are two possible orders:



A higher cotransduction frequency corresponds to tighter genetic linkage, because higher cotransduction frequencies mean that the two markers come together into a new cell more often. Thus they are closer together physically on the chromosome.

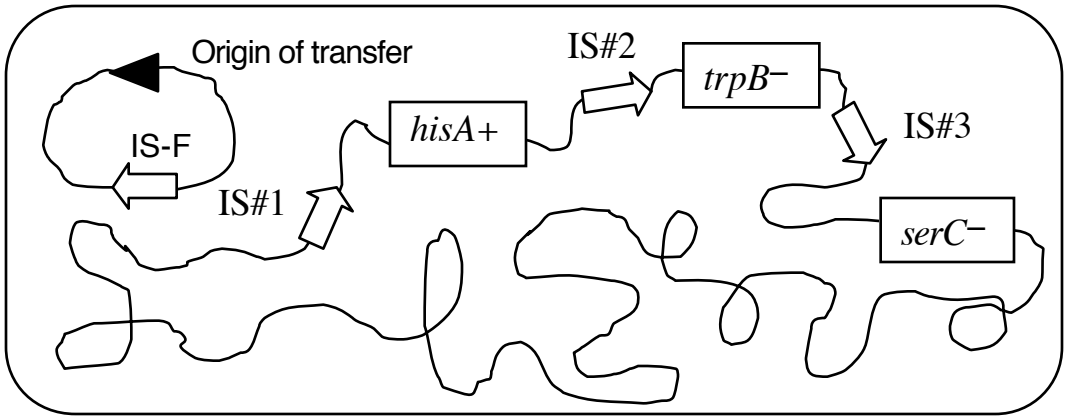
Remember that a bacterial cell of most species contains one circular chromosome.

The information that you have with which to make the map is:
1. The tRNA gene is 60% linked (by cotransduction frequency) to a Tn5 KanR transposon in this strain.
2. The transposon is not linked to *swrM*.
3. The swrA gene is linked to the transposon with a cotransduction frequency of 30%.

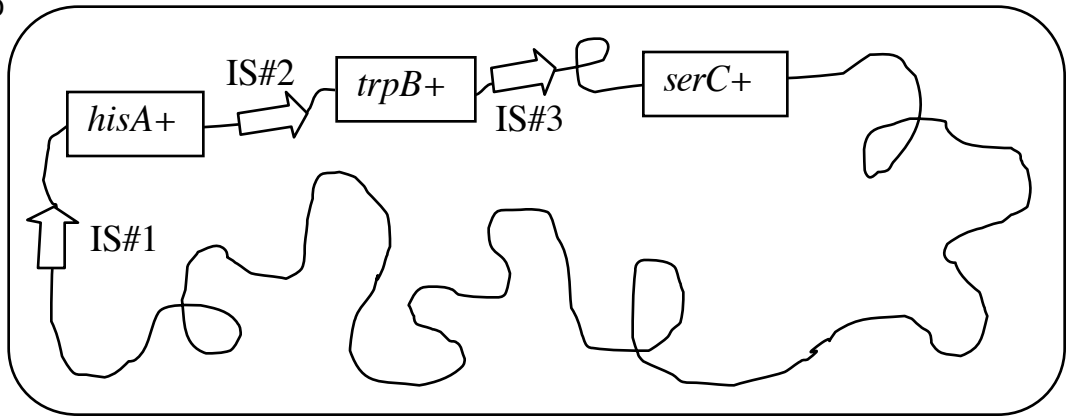
Thus swrA must be farther from the transposon than the Su locus. However, these three loci must all be within the same chromosomal region because they are all linked at a cotransduction distance of >0%. SwrM must be very far away, because it is not linked to the transposon at all.

3. You are studying two strains of *E. coli*. Below are diagrams of the two different strains, showing their chromosomes and the F plasmid (in Strain One, which contains it).

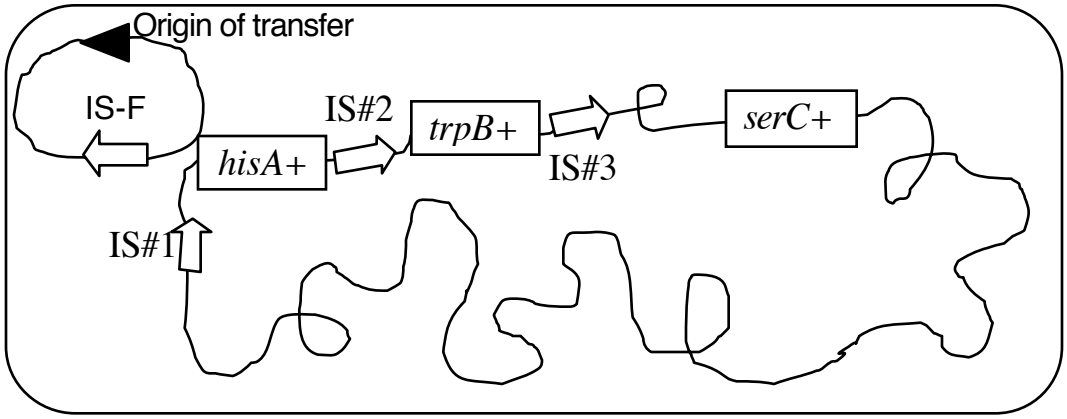
Strain One



Strain Two

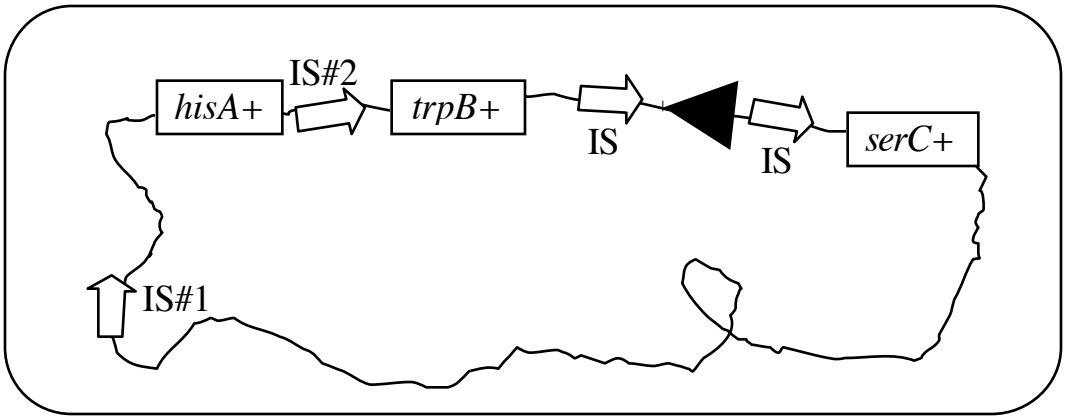


(a) Using the notation from above, draw the new strain that would result if Strains One and Two mated together. (Call the resulting strain Strain Three.)

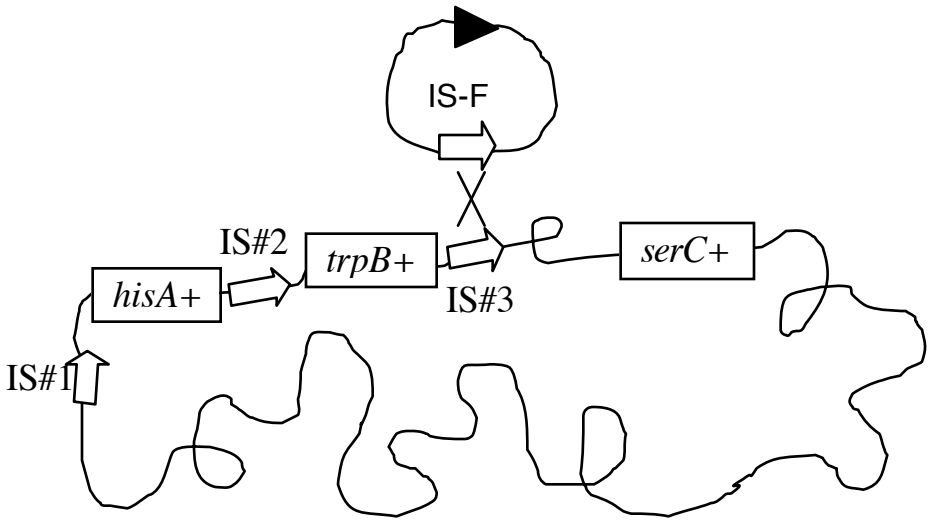


When an F+ cell mates to an F- cell, the F+ cell simply transfers the F plasmid to the F- cell.

(b) Using the notation from above, draw the new strain that would result if IS-F and IS#3 recombined together in a cell from Strain Three. (Call the resulting strain Strain Four.)

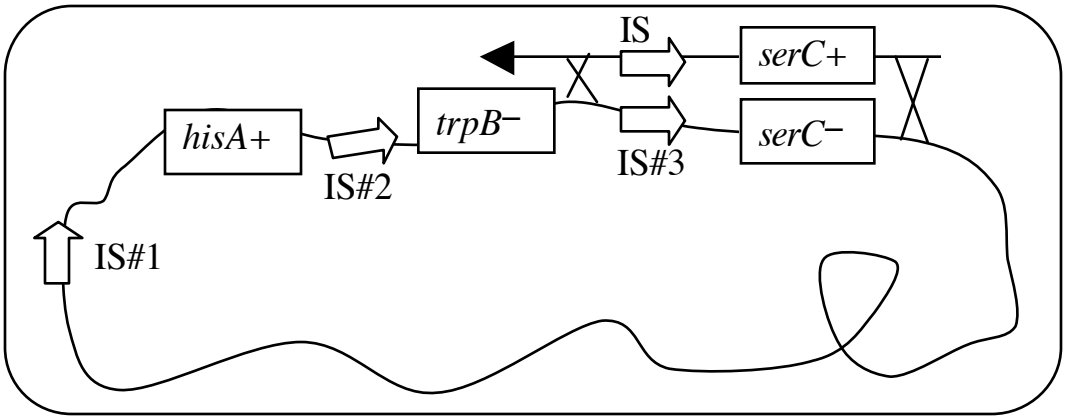


When one recombination event occurs between homologous sequences on two circular pieces of DNA, the result is one large circular piece of DNA. No genetic information is lost or gained in this process. The way to determine the order of the genes on this resulting circular piece of DNA is to draw the two initial DNAs aligned, such that their homologous sequences are lined up with each other. Then, draw a recombination event between them, and trace the resulting product in the shape of a "figure-8." The aligned pieces of DNA will look like this:



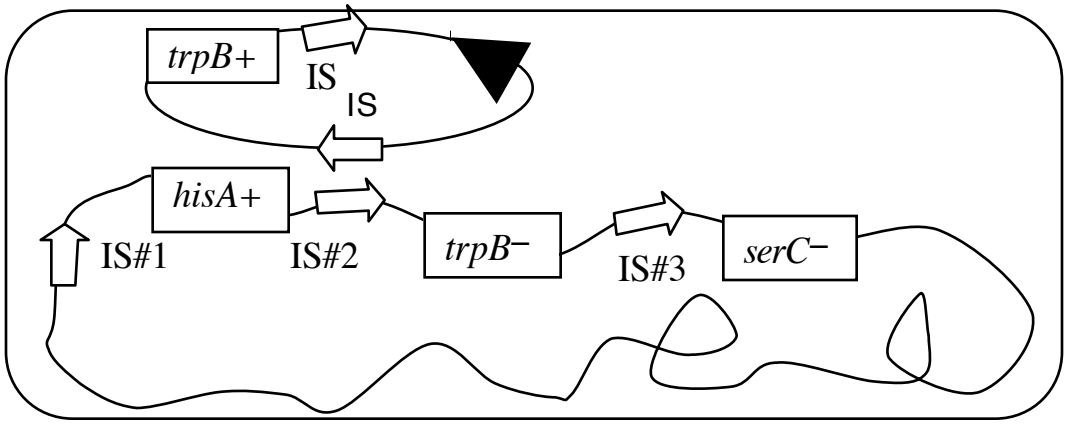
If you trace this structure through the recombination event in the shape of a "figure-8," the resulting large circle of DNA will be as it is drawn in the cell above.

(c) Using the notation from above, draw the events that would occur in the recipient cell that had been mated into, if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating only for a short time, and you selected for exconjugants on minimal medium that lacks supplemental **serine**. (Call the resulting strain Strain Five.)



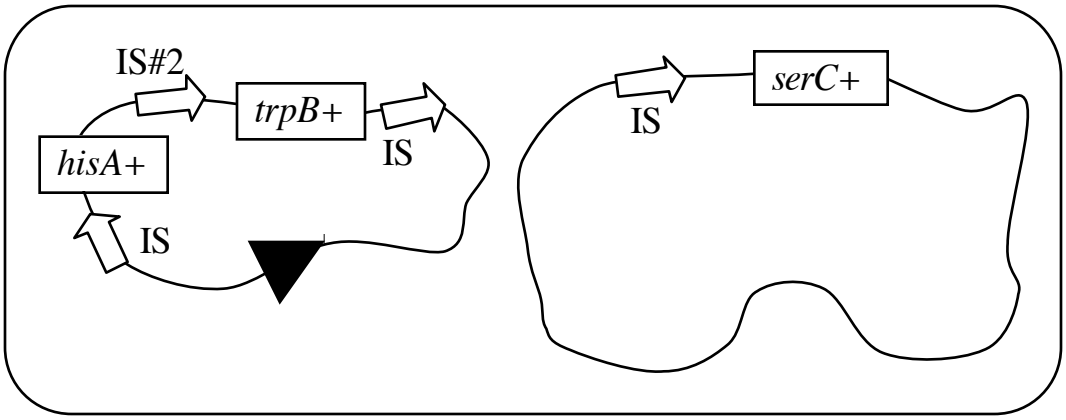
When an Hfr cell mates to an F- cell, the Hfr cell begins to transfer its chromosome into the F- cell, beginning with the origin of transfer, and followed by the first gene behind the blunt end of the origin of transfer symbol (◀). If a short time is allowed for the mating, only the "early markers" will be transferred into the recipient cell. These markers may integrate into the recipient chromosome through a double recombination event between the recipient chromosome and the transferred host chromosome. There must be an even number of crossover events in order for genes from a linear piece of DNA to integrate into a bacterial chromosome, which is circular. If you are selecting for exconjugants on medium that lacks serine, then you are selecting for exconjugants who have acquired the serC+ gene into their chromosomes. This occurs only after transfer of the genome from the Hfr, and an even number of crossover events flanking the serC gene.

(d) Using the notation from above, draw the new strain that would result if Strain Four was mated to a variant of Strain One that had lost its F plasmid. The conditions of the mating are that you allowed mating for only a short time, and you selected for exconjugants on minimal medium that lacks supplemental **tryptophan**. (Call the resulting strain Strain Six.)



The way you can select for F' formation is by mating an Hfr strain to an F- strain, and selecting for a late marker being transferred early. TrpB would normally be the last marker to be transferred by the Hfr (Strain Four). If you end a mating early, then trpB should not be transferred in the way described above in the answer to part (c). The only way trpB can be transferred early is if a recombination event occurred in the donor cell such that two IS sequences in the Hfr chromosome recombined together, forming an F' plasmid. Then that F' would be transferred to Strain 1. F' plasmids are quite small compared to the chromosome, and an entire F' plasmid can be transferred quite quickly to a recipient cell. More about F' plasmids are described in the answer to part (e).

(e) Using the notation from above, draw the new strain that would result if IS#1 recombined with the IS sequence that is farthest away from IS#1 in a cell from Strain Four.



If two IS sequences that flank the origin of transfer in an Hfr chromosome recombine together, an F' plasmid is formed. The region of the Hfr chromosome in between the two IS sequences becomes the F' plasmid, and the remaining parts of the chromosome remain in the circular chromosome.

Note that the chromosome (on the right, ~5,000 kb) is much larger than the F' plasmid (on the left, ~100 kb); this drawing is not to scale.

(f) State whether each of the following strains is an F**−** strain, an F**'** strain, an F**+** strain, or an Hfr strain:

Strain One – **F+ because any strain harboring the F plasmid with no extra bacterial genes inside the plasmid is an F+ strain**

Strain Two – **F- because any strain harboring no origin of transfer anywhere in the cell is an F- strain**

Strain Three – **F+**

Strain Four – **Hfr because any strain with an origin of transfer in the chromosome is an Hfr that can transfer its own chromosome to other cells**

Strain Five – **F- because the homologous recombination events do not bring the origin of transfer into the chromosome. Thus the strain that is formed has exchanged chromosomal markers (serC+ for serC-) but does not have an origin of transfer anywhere in the cell and is thus an F- cell**

Strain Six – **F' because any strain harboring a form of the F plasmid that contains bacterial genes that are normally located on the chromosome is an F' strain**

2005 7.03 Problem Set 4

Due before 5 PM on FRIDAY, October 28, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. For each of the following merodiploid strains, predict the number of units of enzyme activity that will be displayed by a strain of the given genotype, grown under the given conditions. Assume that, when no repressor is bound to DNA, 100 units of ß-galactosidase activity are produced from each functional copy of the **LacZ** gene. Assume that, when repressor is fully bound to DNA, only 1 unit of enzyme is produced for each functional copy of **LacZ**. The presence of **Lac I^{-d}** protein will fully prevent any other forms of the repressor in the same cell from binding to DNA. The strain genotypes are written in the following format:

on the chromosome/ on the F' factor

****Note** that whenever strains are denoted this way anything not listed on the chromosome is wild-type, and anything not listed on the F' factor is absent from that plasmid.**

	ß-galactosidase activity –IPTG	ß-galactosidase activity +IPTG
Lac O^{-} P^{-} Z^{+} / F' Lac I^{-d} O^{+} P^{+} Z^{+}	_____	_____
Lac Y^{-} / F' Lac I^{-} O^{+} P^{+} Z^{+}	_____	_____
Lac I^{-d} / F' Lac I^{+} O^{+} P^{+} Z^{+}	_____	_____
Lac I^{-} / F' Lac I^{S}	_____	_____
Lac O^{-} Z^{+} / F' Lac I^{-} O^{+} P^{+} Z^{+}	_____	_____
Lac P^{-} Z^{-} / F' O^{+} P^{+} Z^{+}	_____	_____

2. You are studying the regulation of a bacterial gene that encodes a toxin that is necessary for the successful infection of mice by the bacteria. This gene is turned on by a signal that is produced by the bacteria only when they are at a high enough cell density to successfully infect and colonize a mouse. You name the toxin-encoding gene *virR*.

(a) Why does it make sense for the bacterium to keep expression of the VirR protein off when the bacteria are growing at low cell density? (Think in terms of why it might be that genes are regulated at all, as opposed to having all genes be constitutively expressed.)

You isolate four strains of this bacterium, each of which harbors a single mutation: *virW*$^{-}$, *virX*$^{-}$, *virY*$^{-}$, or *virZ*$^{-}$. The *virX*$^{-}$ and *virY*$^{-}$ mutations cause *virR* to be constitutively expressed (regardless of whether the bacteria are at high cell density or low cell density). The *virW*$^{-}$ and *virZ*$^{-}$ mutations prevent all *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You make the following strains and note their phenotype:

Strain #	Genotype	Expression of VirR:	
		At low cell density	At high cell density
1.	Z^{-} / F' Z+	off	on
2.	Y^{-} R^{-} / F' R+	on	on
3.	Y^{-} Z^{-}	off	off
4.	X^{-} / F' X+ R^{-}	off	on
5.	W^{-} Z+ / F' W+ Z^{-}	off	on
6.	W^{-} / F' W+	off	on
7.	X^{-} W^{-}	on	on
8.	Z^{-} / F' Z+ R^{-}	off	on
9.	X^{-} / F' X+	off	on
10.	X^{-} Z^{-}	on	on

You find that the *virW* and *virY* loci are very tightly linked to each other by cotransduction mapping, and then you do DNA sequencing and find that these two mutations are two alleles of the same gene.

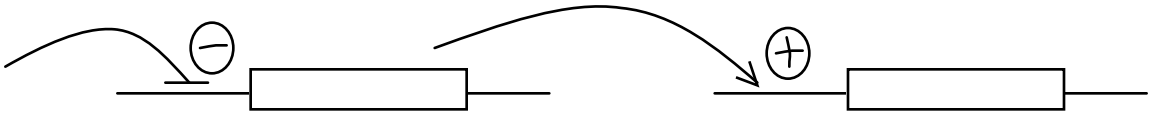
(b) Classify the *virX*$^{-}$ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

(c) Classify the *virY*$^{-}$ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

(d) Classify the *virZ*$^{-}$ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

(e) Which strains will you use to order *virX*, *virY*, and *virZ* in a pathway for *virR* regulation? List all of the strains you will use by strain number.

(f) Given all of the information in this question, draw a linear genetic pathway that shows the pathway by which the *virR* gene is regulated. Be sure to include the wild-type functions of *virR*, *virW*, *virX*, *virY*, and *virZ*. Also include the signal of high cell density. For each model, be sure to use the proper notation of:



to indicate negative regulation by a trans-acting factor

to indicate positive regulation by a trans-acting factor

(g) Which of the proteins (VirX, VirY, and/or VirZ) might potentially bind to DNA sequences in the *virR* gene that lie upstream of the *virR* coding region?

(h) In a sentence, describe the specific function that might be performed by the protein(s) listed in part **(g)** when they are bound to DNA sequences in the *virR* gene.

3. In the previous problem, you found two different mutant alleles (*virW*$^-$ and *virY*$^-$) of one gene that regulates expression of the *virR* gene. The *virY*$^-$ mutation causes *virR* to be constitutively expressed, and the *virW*$^-$mutation prevents *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You want to isolate a Tn5 Kan^r transposon insertion that is linked to the *virW/Y* gene.

(a) Why might you want to isolate such a transposon insertion? What would an insertion like that help you do that you can't do currently?

You have a collection of 2,000 bacterial strains, each of which harbors a single Tn5 insertion somewhere in the genome. This collection is called a transposon library. If you grow P1 phage on this collection of bacteria, you can collect a group of phage, each of which contains a different piece of DNA from the transposon library. You then infect *virW*$^-$ bacteria with this group of phage, and then select for infected bacteria that are now Kan^r.

(b) You screen through your new collection of bacteria, and find that one colony of bacteria now properly regulates the expression of *virR*. This colony harbors a Tn5 insertion near to the *virW/Y* gene. How would you measure the cotransduction distance between the Tn5 insertion and the *virY* locus? Fill in the blanks in the following paragraph to show what experiment you would do:

You grow P1 phage on bacteria of the genotype _____.

You use the resulting phage lysate to infect bacteria of the genotype _____.

You select for transductants that can grow on plates containing _____.

You then screen the transductants that grow on those plates for their ability to properly regulate *virR* expression. You screen 100 colonies, and find that 30 have the phenotype of _____, so you conclude that the cotransduction distance between Tn5 and *virY* is 30%.

You now do two different crosses to determine the order of the transposon insertion you have isolated, the *virW* locus, and the *virY* locus.

The first cross: You grow P1 phage on *virY*$^-$ bacteria containing the Tn5 insertion. You use the resulting phage lysate to infect *virW*$^-$ bacteria. You select for KanR transductants and find that 200 transductants grow. 62 of those express *virR* constitutively and 138 do not ever express *virR*.

The second cross: You grow P1 phage on *virW*$^-$ bacteria containing the Tn5 insertion you isolated. You use the resulting phage lysate to infect *virY*$^-$ bacteria. You select for KanR transductants and find that 200 transductants grow. 131 of those express *virR* constitutively, 65 do not ever express *virR*, and 4 properly regulate *virR* expression.

(c) Draw a map showing the relative order of the *virY* and *virW* loci and the site of the Tn5 insertion.

(d) Draw the recombination events that occurred between the transduced DNA and the bacterial chromosome to create a regulated transductant from the second cross. Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.

(e) What is the genotype of a transductant created by a quadruple crossover event between the transduced DNA and the bacterial chromosome in the second cross? Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.

2005 7.03 Problem Set 4 **KEY**

Due before 5 PM on FRIDAY, October 28, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. For each of the following merodiploid strains, predict the number of units of enzyme activity that will be displayed by a strain of the given genotype, grown under the given conditions. Assume that, when no repressor is bound to DNA, 100 units of ß-galactosidase activity are produced from each functional copy of the **LacZ** gene. Assume that, when repressor is fully bound to DNA, only 1 unit of enzyme is produced for each functional copy of **LacZ**. The presence of **Lac I^{-d}** protein will fully prevent any other forms of the repressor in the same cell from binding to DNA. The strain genotypes are written in the following format:

on the chromosome/ on the F' factor

Note that whenever strains are denoted this way anything not listed on the chromosome is wild-type, and anything not listed on the F' factor is absent from that plasmid.

	ß-galactosidase activity –IPTG	ß-galactosidase activity +IPTG
Lac O^- P^- Z^+ / F' Lac I^{-d} O^+ P^+ Z^+	**100**	**100**

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will never be expressed because it has no functional promoter. There is also another LacZ reporter that is fully wild-type (O+ P+ Z+).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and dominant negative repressor floating around. The dominant negative repressor will "soak up" and inactivate any wild-type repressor, so there is actually no functional repressor in the cell.

Together, this means that there is one functional reporter gene, and no functional repressor protein. Thus the one functional reporter will always express b-gal.

Lac Y^- / F' Lac I^- O^+ P^+ Z^+	**2**	**200**

First consider what forms of the LacZ reporter are in the cell. In this cell, there are two LacZ reporters that are both fully wild-type (O+ P+ Z+).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and non-functional repressor floating around. The wild-type repressor can float around and bind to any LacZ reporter that has a functional operator sequence.

Together, this means that there are two functional reporter genes, and functional repressor protein. Thus the two functional reporters will both show normal inducible expression of b-gal.

Lac I^{-d} / F' Lac I^{+} O^{+} P^{+} Z^{+} **200** **200**

First consider what forms of the LacZ reporter are in the cell. In this cell, there are two LacZ reporters that are both fully wild-type (O+ P+ Z+).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and dominant negative repressor floating around. The dominant negative repressor will "soak up" and inactivate any wild-type repressor, so there is actually no functional repressor in the cell.

Together, this means that there are two functional reporter genes, and no functional repressor protein. Thus the two functional reporters will both always express b-gal.

Lac I^{-} / F' Lac I^{S} **1** **1**

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that is fully wild-type (O+ P+ Z+) on the chromosome.

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is non-functional repressor and super-repressor floating around. The super-repressor will always bind to all reporters in the cell, and constantly repress them.

Together, this means that there is one functional reporter gene, and super-repressor protein. Thus the one functional reporter will always be repressed.

Lac O^{-} Z^{+} / F' Lac I^{-} O^{+} P^{+} Z^{+} **101** **200**

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will always be expressed because it has no functional operator. There is also another LacZ reporter that is fully wild-type (O+ P+ Z+).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor and non-functional repressor floating around. The wild-type repressor can float around and bind to any LacZ reporter that has a functional operator sequence.

Together, this means that there is one functional reporter gene and one constitutive reporter gene, and functional repressor protein. Thus the one functional reporter will show normal inducible regulation, and the other reporter will be constitutive.

Lac P^- Z^- / F' O^+ P^+ Z^+ **1** **100**

First consider what forms of the LacZ reporter are in the cell. In this cell, there is one LacZ reporter that will never be expressed because it has no functional promoter. There is also another LacZ reporter that is fully wild-type (O+ P+ Z+).

Now consider what forms of trans-acting regulators are diffusing throughout the cell. In this cell, there is wild-type repressor being expressed from the chromosome.

Together, this means that there is one functional reporter gene, and functional repressor protein. Thus the one functional reporter will show normal inducible expression of b-gal.

2. You are studying the regulation of a bacterial gene that encodes a toxin that is necessary for the successful infection of mice by the bacteria. This gene is turned on by a signal that is produced by the bacteria only when they are at a high enough cell density to successfully infect and colonize a mouse. You name the toxin-encoding gene *virR*.

(a) Why does it make sense for the bacterium to keep expression of the VirR protein off when the bacteria are growing at low cell density? (Think in terms of why it might be that genes are regulated at all, as opposed to having all genes be constitutively expressed.)

It takes energy and resources to produce an mRNA and a protein from a gene, so an organism may want to express a gene only when it can be used, and may want to repress expression of that gene when it is useless.

In this case, when the bacteria are at a low cell density, the production of the toxin (VirR) is unnecessary because the bacteria aren't be able to successfully colonize the mouse at low cell density. This means the VirR product would be wasted if it was produced.

You isolate four strains of this bacterium, each of which harbors a single mutation: *virW*$^-$, *virX*$^-$, *virY*$^-$, or *virZ*$^-$. The *virX*$^-$ and *virY*$^-$ mutations cause *virR* to be constitutively expressed (regardless of whether the bacteria are at high cell density or low cell density). The *virW*$^-$ and *virZ*$^-$ mutations prevent all *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You make the following strains and note their phenotype:

Strain #	Genotype	Expression of VirR:	
		At low cell density	At high cell density
1.	Z^- / F' Z+	off	on
2.	$Y^- R^-$ / F' R+	on	on
3.	$Y^- Z^-$	off	off
4.	X^- / F' X+ R^-	off	on
5.	W^- Z+ / F' W+ Z^-	off	on
6.	W^- / F' W+	off	on
7.	$X^- W^-$	on	on
8.	Z^- / F' Z+ R^-	off	on
9.	X^- / F' X+	off	on
10.	$X^- Z^-$	on	on

You find that the *virW* and *virY* loci are very tightly linked to each other by cotransduction mapping, and then you do DNA sequencing and find that these two mutations are two alleles of the same gene.

(b) Classify the *virX*$^-$ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

Strain 9 shows that *virX*$^-$ is recessive & Strain 4 shows that *virX*$^-$ is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 9 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virX*$^-$ is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 4 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virX* passes the trans test.

(c) Classify the *virY*$^-$ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

Strain 6 shows that *virW*$^-$ is recessive & Strain 5 shows that *virW*$^-$ is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 6 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virW*⁻ is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 5 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virW* passes the trans test.

virY and virW are alleles of the same gene. This means that, if virW is trans, then Y is also trans. Once you deduce that *virY*⁻ is dominant, Strain 2 confims that *virY*⁻ is trans, but this is not necessary.

The virW⁻ mutation gives a recessive phenotype of uninducible. This means that the wild-type function of virW is that it is an activator protein. The fact that virW⁻ is recessive tells us that virW⁻ is a loss-of-function mutation, and we can deduce the wild-type function of a gene from loss-of-function mutations. Since virY⁻ is an allele of a gene that encodes an activator protein, and *virY*⁻ gives a constitutive phenotype, *virY*⁻ must be a superactivator allele. Superactivator alleles are gain-of-function alleles that show dominant phenotypes.

(d) Classify the *virZ*⁻ mutation based on its genetic properties (cis vs. trans, dominant vs. recessive). For each conclusion, list the piece of information (eg. the strain number) on which you based your conclusion.

Strain 1 shows that *virZ*⁻ is recessive & Strain 8 shows that *virZ*⁻ is trans.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene should be used as a dominant/recessive test. Strain 1 is such a strain. This strain shows a wild-type phenotype, so the conclusion is that *virZ*⁻ is recessive.

A (mero-)diploid strain that has one copy of the wild-type gene and one copy of the mutant gene AND one functional reporter that is on a different piece of DNA than the dominant allele of the regulatory gene should be used as a trans test. Strain 8 is such a strain. This strain shows the dominant phenotype, so the conclusion is that *virZ* passes the trans test.

(e) Which strains will you use to order *virX*, *virY*, and *virZ* in a pathway for *virR* regulation? List all of the strains you will use by strain number.

Strains 3, 7, and 10. We use double mutant strains to order genes into pathways – strains that lack function in two different genes are used for epistasis tests, and epistasis tests are how we order genes into pathways.

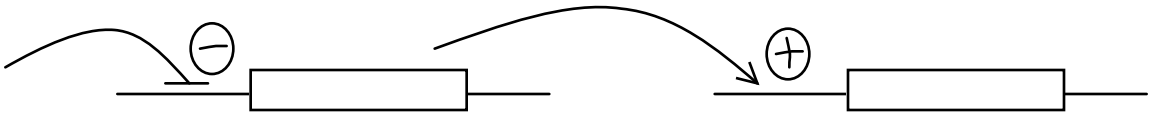
To get the order of the genes in the pathway:

Strain 7 and strain 10 both show the virX$^-$ phenotype so we know that virX is downstream of both virW/Y and virZ.

Strain 3 has the virZ$^-$ phenotype, telling you that virZ is downstream of virY/W.

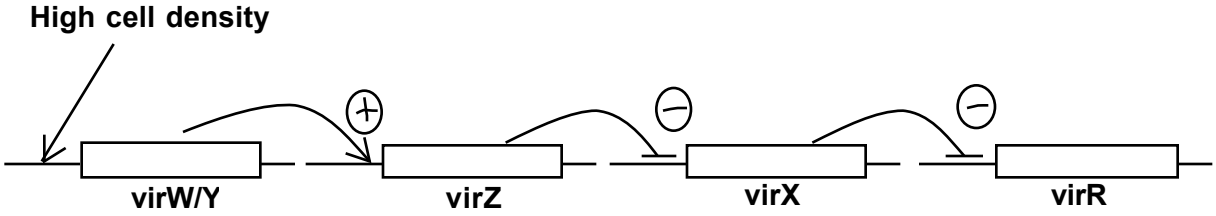
VirW/Y must be the most upstream gene to act in the pathway.

(f) Given all of the information in this question, draw a linear genetic pathway that shows the pathway by which the *virR* gene is regulated. Be sure to include the wild-type functions of *virR*, *virW*, *virX*, *virY*, and *virZ*. Also include the signal of high cell density. For each model, be sure to use the proper notation of:



to indicate negative regulation by a trans-acting factor

to indicate positive regulation by a trans-acting factor



To assign positive or negative signs to the arrows, we need to consider the net function (positive or negative) of each wild-type gene on virR. Do this by working from right to left, so that we start with the gene closest to virR.

VirX is a net repressor because X is trans and, when you lose function in X, you get a constitutive phenotype.
VirZ is a net activator because Z is trans and, when you lose function in Z, you get an uninducible phenotype. Since we know that virX acts after virZ, then to get positive regulation of virR, virZ must act negatively on virX.

VirW is a net activator because W is trans and, when you lose function in W, you get an uninducible phenotype. This means that virWY must act positively on virZ to get a final net output of positive regulation of virR.

Finally, high cell density acts positively on virR expression, so high cell density must act positively on virYW.

(g) Which of the proteins (VirX, VirY, and/or VirZ) might potentially bind to DNA sequences in the *virR* gene that lie upstream of the *virR* coding region?

VirX

Since VirX acts last in the pathway, it is the only one with the potential to bind the DNA sequences that are upstream of the virR region. All other genes in the pathway operate indirectly on virR.

(h) In a sentence, describe the specific function that might be performed by the protein(s) listed in part **(g)** when they are bound to DNA sequences in the *virR* gene.

VirX acts negatively on VirR, so it is a repressor of VirR (as long as there are no further unknown regulatory components in the virR regulation pathway). Repressor proteins prevent RNA polymerase from binding to the promoter and thereby allowing transcription of RNA from a gene.

3. In the previous problem, you found two different mutant alleles (*virW*$^-$ and *virY*$^-$) of one gene that regulates expression of the *virR* gene. The *virY*$^-$ mutation causes *virR* to be constitutively expressed, and the *virW*$^-$mutation prevents *virR* expression (even when the bacteria have colonized a host mouse at very high cell density). You want to isolate a Tn5 Kan^r transposon insertion that is linked to the *virW/Y* gene.

(a) Why might you want to isolate such a transposon insertion? What would an insertion like that help you do that you can't do currently?

It would us to move the virW and virY loci from one strain to another. We can't select for virW and virY, so it is extremely hard to move the virWY gene around between cells. We need to move this gene around in order to map the virY and virW loci, and make strains with different genotypes at virW and virY. If we isolate a transposon that is linked to virWY, then we can move the transposon around by selecting for it, and since the transposon is linked to virWY, then virWY will come along with the transposon sometimes.

You have a collection of 2,000 bacterial strains, each of which harbors a single Tn5 insertion somewhere in the genome. This collection is called a transposon library. If you grow P1 phage on this collection of bacteria, you can collect a group of phage, each of which contains a different piece of DNA from the transposon library. You then infect *virW*– bacteria with this group of phage, and then select for infected bacteria that are now Kan^r.

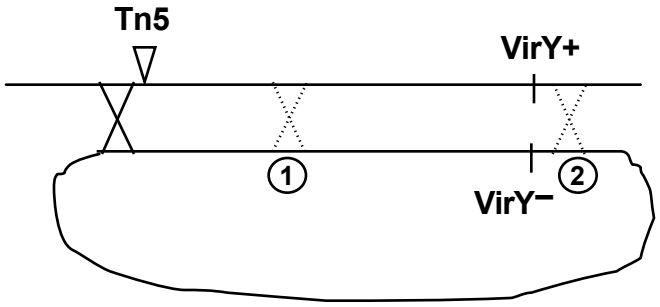
(b) You screen through your new collection of bacteria, and find that one colony of bacteria now properly regulates the expression of *virR*. This colony harbors a Tn5 insertion near to the *virW/Y* gene. How would you measure the cotransduction distance between the Tn5 insertion and the *virY* locus? Fill in the blanks in the following paragraph to show what experiment you would do:

You grow P1 phage on bacteria of the genotype _____**Tn5 (Kan^r) VirW/Y+**_____.

You use the resulting phage lysate to infect bacteria of the genotype _____**VirY-**_____.

You select for transductants that can grow on plates containing _____**kanamycin**_____.

You then screen the transductants that grow on those plates for their ability to properly regulate *virR* expression. You screen 100 colonies, and find that 30 have the phenotype of _____**regulated virR expression**_____, so you conclude that the cotransduction distance between Tn5 and *virY* is 30%.



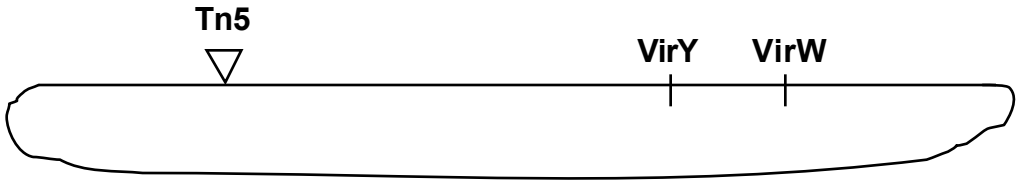
If the second Xover is at position 1, then VirY+ will not be cotransduced with the Tn, and you will get constitutive expression of virR. If the second Xover is at position 2, then VirY+ will be cotransduced with the Tn, and you will get regulated expression of virR.

You now do two different crosses to determine the order of the transposon insertion you have isolated, the *virW* locus, and the *virY* locus.

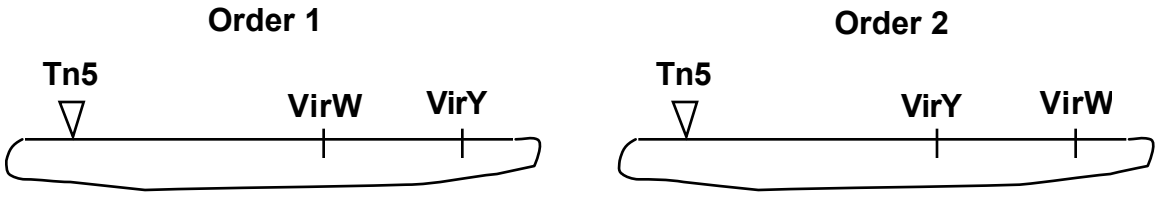
The first cross: You grow P1 phage on *virY*– bacteria containing the Tn5 insertion. You use the resulting phage lysate to infect *virW*– bacteria. You select for KanR transductants and find that 200 transductants grow. 62 of those express *virR* constitutively and 138 do not ever express *virR*.

The second cross: You grow P1 phage on *virW*– bacteria containing the Tn5 insertion you isolated. You use the resulting phage lysate to infect *virY*– bacteria. You select for KanR transductants and find that 200 transductants grow. 131 of those express *virR* constitutively, 65 do not ever express *virR*, and 4 properly regulate *virR* expression.

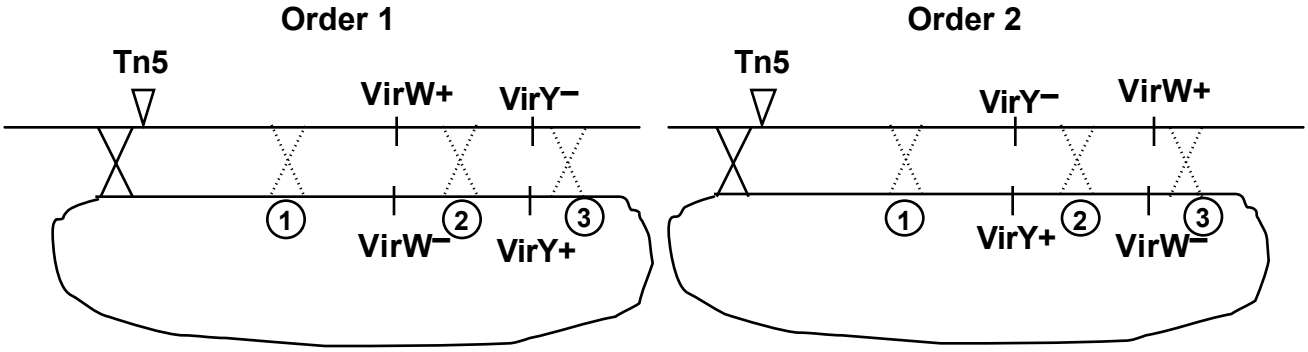
(c) Draw a map showing the relative order of the *virY* and *virW* loci and the site of the Tn5 insertion.



Since VirW and VirY are in the same gene, the transposon can't be between them. If the transposon were between them, then it would disrupt the function of the virWY gene. This is not possible because we isolated a colony in part (b) that contained the Tn but showed regulated expression of VirR. This means there are two possible remaining map orders:



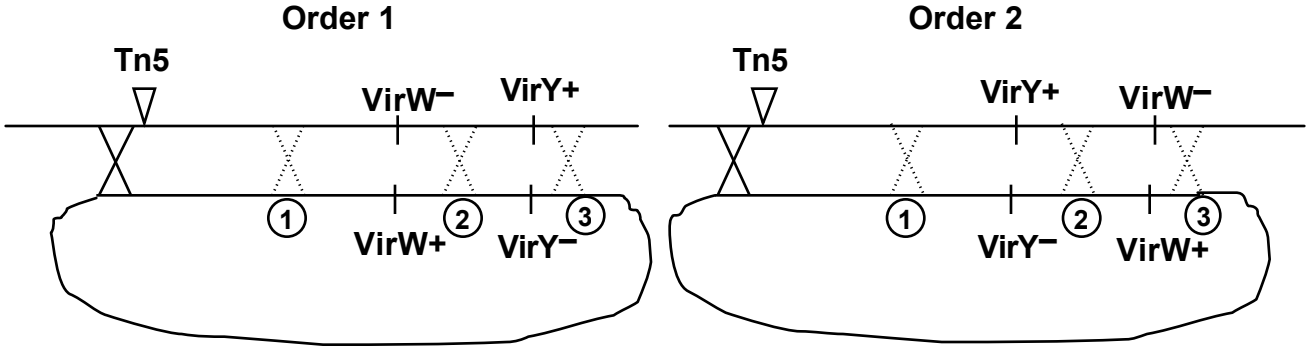
In each of the crosses, one of the crossovers must occur to the left of Tn5 because it is what we selected for. There are three possible locations for the other cross over events.
Cross One:



Possible Genotypes from Cross 1:

The 2nd crossover is at	For Order 1	For Order 2
1	Tn5, virW−, virY+	Tn5, virW−, virY+
2	Tn5, virW+, virY+	Tn5, virW−, virY−
3	Tn5, virW+, virY−	Tn5, virW+, virY−
Quadruple Xover	Tn5, virW−, virY−	Tn5, virW+, virY+

Cross Two:

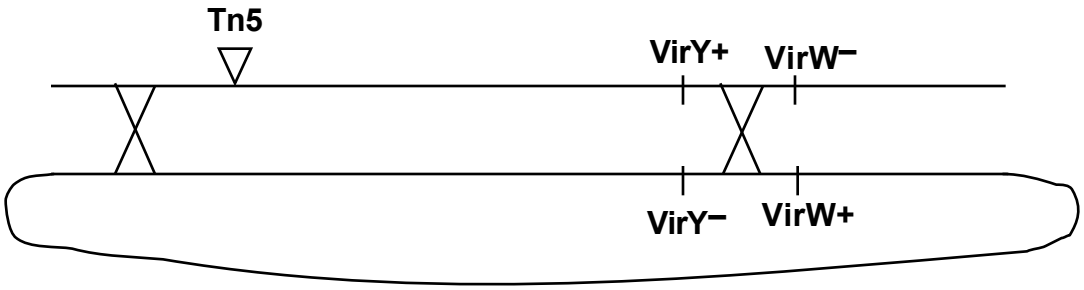


Possible Genotypes from Cross 2:

The 2nd crossover is at	For Order 1	For Order 2
1	Tn5, virW+, virY−	Tn5, virW+, virY−
2	Tn5, virW−, virY−	Tn5, virW+, virY+
3	Tn5, virW−, virY+	Tn5, virW−, virY+
Quadruple Xover	Tn5, virW+, virY+	Tn5, virW−, virY−

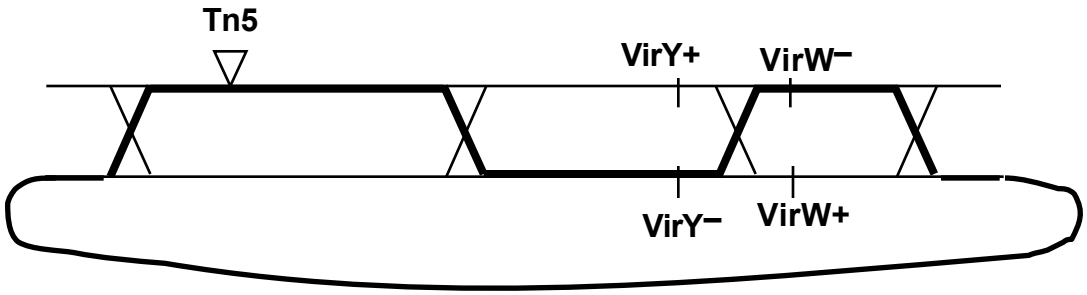
If the Order One is correct, then in there should be more wild-type transductants in cross 1 than in cross 2. If Order Two is correct, then there should be more wild-type transductants in cross 2 than in cross 1. This is because a quadruple crossover event is much less likely to occur than a double crossover event. Since the actual data tell us that cross 2 has more wild type transductants than cross 1, Order Two must be correct.

(d) Draw the recombination events that occurred between the transduced DNA and the bacterial chromosome to create a regulated transductant from the second cross. Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.



(e) What is the genotype of a transductant created by a quadruple crossover event between the transduced DNA and the bacterial chromosome in the second cross? Be sure to show the proper order of the loci as you drew them in part **(c)** and mark the alleles present at those loci.

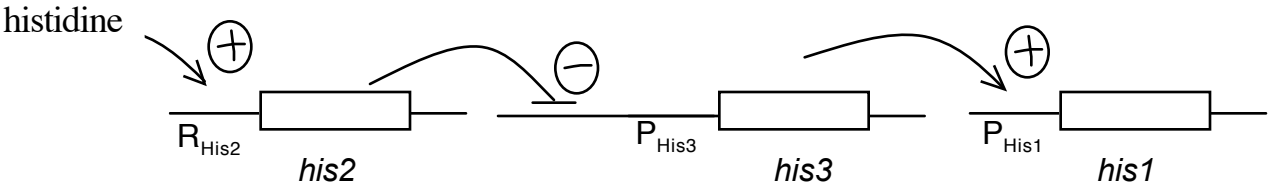
Tn5, VirY−, VirW−



2005 7.03 Problem Set 5

Due before 5 PM on WEDNESDAY, November 16, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. You are studying the regulation of a yeast gene (His1), which is necessary for synthesis of the amino acid histidine. To begin your analysis of the regulation of His1, you fuse the cis regulatory region ("P_{His1}") that lies upstream of the His1 open reading frame to the LacZ coding sequence. You then place this hybrid gene on a yeast plasmid. This reporter gene construct behaves the way you expected based on the pathway for the regulation of His1, which is as follows:



Keep in mind that this model is a genetic pathway that should not be interpreted as a molecular model.

You monitor the expression of the *his1* gene using your reporter gene construct in order to perform a genetic screen looking for mutants that do not properly regulate expression of *his1*. In your screen, you isolate a series of haploid mutant strains that either show constitutive or uninducible expression of *his1*. You identify the genes that are mutated in the mutants you find, and discover that you have identified new alleles of two genes, *his2* (which lies on chromosome #1), and *his3* (which lies on chromosome #5).

In your screen, you isolate five strains, each of which contains one of the following single mutations:

his2a, which is in the **coding region** of *his2*. This mutation gives a recessive phenotype.
his2b, which is in the **coding region** of *his2*. This mutation gives a constitutive phenotype.
his3c, which is in the **coding region** of *his3*. This mutation gives a constitutive phenotype.
his3d, which is in the **coding region** of *his3*. This mutation gives a recessive phenotype.
R_{His2}^{-}, which is a deletion in the **cis regulatory region** in front of *his2.*

(a) Is *his2a* cis-acting or trans-acting with respect to *his1*?

(b) Is R_{His2} cis-acting or trans-acting with respect to *his1*?

(c) What is the phenotype of a *his2a his3d* double mutant with respect to expression of *his1*?

(d) Would the *his3c* mutation give a dominant or recessive phenotype with respect to expression of *his1*?

(e) What type(s) of mutation might *his2b* be with respect to *his1*? (Your choices are: repressor⁻, activator⁻, promoter⁻, UAS⁻, URS⁻, dominant negative repressor, dominant negative activator, super-repressor, super-activator.)

(f) You cross a *his3d* haploid mutant strain to a *his2a* haploid mutant strain. What is the phenotype of the resulting diploid with respect to expression of *his1*?

(g) You induce sporulation of the diploid from part **(f)**. You analyze 90 tetrads. Three distinct tetrad types are obtained. Below, fill in each blank with the phenotype of each of the spores that is not provided to you.

Type 1:	**Type 2:**	**Type 3:**
regulated (wt)	_____	constitutive
_____	_____	_____
_____	_____	_____
constitutive	_____	_____

(h) How many “Type 3” tetrads would you have most likely observed?

2. You are studying the metabolism of a sugar called struliose by yeast cells. (Note that yeast will use struliose even when glucose is present.) You have already isolated one gene that is necessary for the use of struliose as a carbon source. This gene is induced whenever struliose is present. You want to do a genetic procedure (i.e. a screen or selection) to look for more genes involved in struliose metabolism, and you have two reagents that could help you do this. One reagent is a reporter gene that you have created by attaching the promoter region of the known struliose-utilization gene to the open reading frame for *E.coli lacZ*. The other reagent is a form of struliose (called toxo-struliose) that can be metabolized in the same way as struliose, but when it is metabolized, it creates a byproduct that is toxic to yeast cells. You have a collection of thousands of haploid yeast, and each yeast is mutant in a different gene. However, you don't know which of these yeast are mutant in "struliose metabolism" genes (versus which yeast are mutant in any of the other genes in the yeast genome that have nothing to do with struliose metabolism).

(a) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use the reporter gene (but not toxo-struliose). To outline your procedure, include: **i)** the type(s) of growth medium you would plate your yeast mutants on (i.e. what would have to be added to a basic growth medium that contains everything necessary for yeast to grow except a carbon source), **ii)** how you would identify the yeast mutants you are looking for (i.e. what would mutants and non-mutants look like on each type of growth medium), and **iii)** whether this method is a screen or a selection.

i)

ii)

iii)

(b) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use toxo-struliose (but not the reporter gene).

i)

ii)

iii)

3. You have a true-breeding mouse that displays the phenotype of big feet. This phenotype is caused by a specific allele of the "FT1" gene called FT1*. You isolate the FT1 gene from this mutant mouse, and inject it into a fertilized egg produced by the mating of two wild-type mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born has big feet.

(a) What specific conclusion can you draw regarding FT1* from this experiment?

(b) Which breeding experiment could you have done to reach the same conclusion that you reached from part **(a)**?

You make a transgenic mouse that is transgenic for a gene that is involved in determining petal color in petunias. This mouse has no detectable mutant phenotype. You then mate two transgenic mice together to generate a mouse that has two copies of the same transgene. These TG+/TG+ mice now have a phenotype of slow movement. You hypothesize that this slow movement is caused either:
-- by the presence of two copies of the petunia transgene (for unknown reasons)
-- because each of the transgenes disrupted one copy of the "Dext" gene, a gene that is important for mouse motor skills

The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For each mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting techniques
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg **or** ES cells
iv) what is the **genotype** of the fertilized egg or the ES cells you would start with
v) **where** in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera **or** not
vii) which **additional breeding** steps you would do to make the mouse you wanted
viii) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusions you would make for each result

Create a genetically modified mouse to distinguish between your two hypotheses if:

(c) You can use the TG+ DNA, but not the “Dext” gene.
i)

ii)

iii)

iv)

v)

vi)

vii)

viii)

(d) You can use the “Dext” gene, but not the TG+ DNA.
i)

ii)

iii)

iv)

v)

vi)

vii)

viii)

2005 7.03 Problem Set 5

Due before 5 PM on WEDNESDAY, November 16, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

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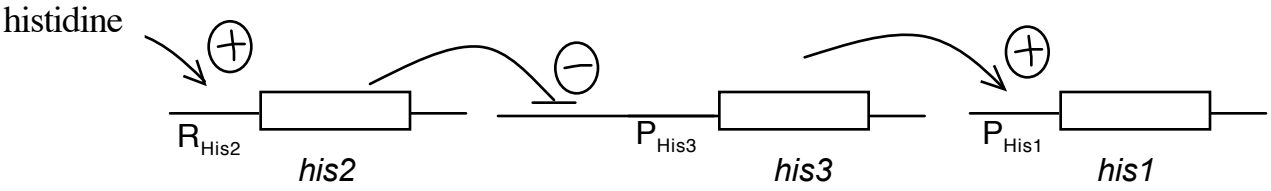

Keep in mind that this model is a genetic pathway that should not be interpreted as a molecular model.

You monitor the expression of the *his1* gene using your reporter gene construct in order to perform a genetic screen looking for mutants that do not properly regulate expression of *his1*. In your screen, you isolate a series of haploid mutant strains that either show constitutive or uninducible expression of *his1*. You identify the genes that are mutated in the mutants you find, and discover that you have identified new alleles of two genes, *his2* (which lies on chromosome #1), and *his3* (which lies on chromosome #5).

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his3d, which is in the **coding region** of *his3*. This mutation gives a recessive phenotype.
R_{His2}^{-}, which is a deletion in the **cis regulatory region** in front of *his2.*

(a) Is *his2a* cis-acting or trans-acting with respect to *his1*?

Trans. His1 and his2 are 2 different coding regions that exist in separate parts of the genome. Thus His2 acts in trans on His1. You can also see from the diagram that His2 acts in trans on His1, because His2 is drawn as a protein-encoding gene (indicated by an open box). If his2 were in cis to His1, His2 would have been drawn as a non-coding DNA sequence that was directly upstream of the His1 open reading frame.

(b) Is R_{His2} cis-acting or trans-acting with respect to *his1*?

Trans. Although R_{His2} acts in cis to his2, his2 then acts in trans with his1, so R_{His2} is also trans-acting with respect to his1.

(c) What is the phenotype of a *his2a his3d* double mutant with respect to expression of *his1*?

Uninducible. This is an epistasis test. The phenotype you see is the phenotype of the single mutation in the gene that acts closer to the reporter in the regulation pathway. Therefore you will see the his3d phenotype. Since you know that his3d is recessive, you know that it is a loss of function mutation in an activator gene, which gives you an uninducible phenotype.

(d) Would the *his3c* mutation give a dominant or recessive phenotype with respect to expression of *his1*?

Dominant. Because this mutation is in the coding region of an activator and gives a constitutive phenotype, it must be a gain of function mutation and therefore will be dominant. Also, if His3c is always activating His1, even if you add a regulated form of the His3 gene, it cannot overpower the constant activation of his1 by his3c. Note that His3c is a superactivator allele of the His3 gene.

(e) What type(s) of mutation might *his2b* be with respect to *his1*? (Your choices are: repressor$^-$, activator$^-$, promoter$^-$, UAS$^-$, URS$^-$, dominant negative repressor, dominant negative activator, super-repressor, super-activator.)

Dominant negative repressor or repressor$^-$. We know that his2 is a net repressor, so all alleles of the his2 gene must be alleles of a gene that encodes a repressor. The only options listed that are alleles of a repressor gene are the two correct answers and superrepressor. The two repressor mutations that give constitutive phenotypes are dominant negative repressor and repressor$^-$. (Superrepressor alleles cause uninducible phenotypes.) Since we don't know whether the his2b mutation is dominant

or recessive, we cannot determine whether it is a dominant negative repressor or repressor⁻ allele.

(f) You cross a *his3d* haploid mutant strain to a *his2a* haploid mutant strain. What is the phenotype of the resulting diploid with respect to expression of *his1*?

Inducible. Since his3d and his2a are both recessive mutations and are on separate genes, there will be complementation and you will get a wild-type phenotype. The cross you did is his3d his2+ X his3+ his2a. The result from crossing two haploid yeast strains to each other is a diploid yeast, which in this case has the genotype: his3d his2+ / his3+ his2a. Note that there are wild-type alleles of both his3 and his2 in this diploid.

(g) You induce sporulation of the diploid from part **(f)**. You analyze 90 tetrads. Three distinct tetrad types are obtained. Below, fill in each blank with the phenotype of each of the spores that is not provided to you.

Type 1: TT	**Type 2: NPD**	**Type 3: PD**
regulated (wt)	**_regulated_____**	constitutive
uninducible_____	**_regulated_____**	**_constitutive_____**
uninducible_____	**_uninducible_____**	**_uninducible_____**
constitutive	**_ uninducible_____**	**_uninducible_____**

If you induce sporulation of a diploid yeast that has the genotype his3d his2+ / his3+ his2a, the three types of tetrads you can get out are:

Type 1: TT	**Type 2: NPD**	**Type 3: PD**
3+ 2+	**3+ 2+**	**3+ 2a**
3d 2+	**3+ 2+**	**3+ 2a**
3d 2a	**3d 2a**	**3d 2+**
3+ 2a	**3d 2a**	**3d 2+**

Your parental spores will be his2+3d (which gives an uninducible phenotype) and his2a3+ (which gives a constitutive phenotype because it is a loss of function mutation in a repressor). Therefore the PD will have 2 constitutive and 2 uninducible spores.

The non-parental spores will be his2+3+ (which will be regulated because it is a wild-type spore) and his2a3d (which is uninducible (see part c)). Therefore the NPDs will have 2 regulated and 2 uninducible spores.

The tetratypes will have 2 parental and 2 non-parental spores so there will be a regulated spore, 2 uninducible spores and 1 constitutive spore. Since this is the only type that has both regulated and constitutive spores, it must be Type 1, so we know that the two blanks are uninducible.

Type 3 must then be the PD because NPD does not have any constitutive spores. We can then fill in that there must be one more constitutive spore and two uninducible spores.

By process of elimination, Type 2 must be the NPD and have two regulated and two uninducible spores.

(h) How many "Type 3" tetrads would you have most likely observed?

15. Type 3 tetrads are the parental ditypes. The ratio of PD:TT:NPD is 1:4:1 when two genes are unlinked, and you know that his2 and his3 are unlinked because they lie on different chromosomes (see the introduction to this question). Therefore the PDs will be 1/6 of the total or 15/90.

2. You are studying the metabolism of a sugar called struliose by yeast cells. (Note that yeast will use struliose even when glucose is present.) You have already isolated one gene that is necessary for the use of struliose as a carbon source. This gene is induced whenever struliose is present. You want to do a genetic procedure (i.e. a screen or selection) to look for more genes involved in struliose metabolism, and you have two reagents that could help you do this. One reagent is a reporter gene that you have created by attaching the promoter region of the known struliose-utilization gene to the open reading frame for *E.coli lacZ*. The other reagent is a form of struliose (called toxo-struliose) that can be metabolized in the same way as struliose, but when it is metabolized, it creates a byproduct that is toxic to yeast cells. You have a collection of thousands of haploid yeast, and each yeast is mutant in a different gene. However, you don't know which of these yeast are mutant in "struliose metabolism" genes (versus which yeast are mutant in any of the other genes in the yeast genome that have nothing to do with struliose metabolism).

(a) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use the reporter gene (but not toxo-struliose). To outline your procedure, include: **i)** the type(s) of growth medium you would plate your yeast mutants on (i.e. what would have to be added to a basic growth medium that contains

everything necessary for yeast to grow except a carbon source), **ii)** how you would identify the yeast mutants you are looking for (i.e. what would mutants and non-mutants look like on each type of growth medium), and **iii)** whether this method is a screen or a selection.

i) Plate cells on minimal medium + struliose + glucose + Xgal and also on plates with just minimal medium + glucose + Xgal. The X-gal will allow you to see whether or not the lacZ gene that is regulated by the struliose promoter is being expressed because the colonies will be blue when LacZ is expressed and white when it is not.

ii) Nonmutant colonies will be blue on struliose glucose Xgal plates and white on glucose Xgal plates. Mutants will be either blue on both plates (constitutive expression) or white on both plates (uninducible expression). You need to plate the cells on both struliose glucose Xgal plates and glucose Xgal plates in order to isolate both constitutive and uninducible mutants. You need glucose in both plates because you need to feed the cells with a carbon source so that all cells (even mutants that cannot metabolize struliose) will be able to grow.

iii) Screen – You have to look through all of the colonies to find the mutations you are looking for. Both mutants and non-mutants grow in a genetic screen.

(b) Outline a genetic procedure that you would do to find more genes involved in struliose metabolism. In your procedure, use toxo-struliose (but not the reporter gene).

i) Plate the cells on minimal medium containing toxo-struilose and glucose. (The glucose is necessary because, without glucose, the mutants you want will have no sugar to eat, because they cannot eat struliose.)

ii) Any cells that can grow must have a mutation that prevents them from metabolizing struliose. (All other cells cannot grow, because they WILL be able to metabolize toxo-struliose, and will thus produce the toxic byproduct that will kill them.)

iii) Selection – only the mutants that you are looking for will be able to grow on the toxo struilose plate. Everything you don't want will be killed. Only mutants grow in a genetic selection.

3. You have a true-breeding mouse that displays the phenotype of big feet. This phenotype is caused by a specific allele of the "FT1" gene called FT1*. You isolate the FT1 gene from this mutant mouse, and inject it into a fertilized egg produced by the mating of two wild-type mice. You then transfer this injected fertilized egg into a pseudopregnant mouse. The mouse that is born has big feet.

(a) What specific conclusion can you draw regarding FT1* from this experiment?

FT1* gene is dominant. There are two wildtype copies of the FT1+ allele in the egg in which FT1* gets inserted into the genome, but the egg that contains both FT1+ and FT1* shows the phenotype (big feet) that is associated with FT1*. This means that the big feet phenotype is dominant.

(b) Which breeding experiment could you have done to reach the same conclusion that you reached from part **(a)**?

Mate true-breeding mutants to true-breeding wild type. All of the offspring would be heterozygous for the FT1* mutation and you could therefore determine that it was dominant because all the children would have big feet.

You make a transgenic mouse that is transgenic for a gene that is involved in determining petal color in petunias. This mouse has no detectable mutant phenotype. You then mate two transgenic mice together to generate a mouse that has two copies of the same transgene. These TG+/TG+ mice now have a phenotype of slow movement. You hypothesize that this slow movement is caused either:
-- by the presence of two copies of the petunia transgene (for unknown reasons)
-- because each of the transgenes disrupted one copy of the "Dext" gene, a gene that is important for mouse motor skills

The scenario in this question asks a biological question that can be addressed by creating genetically engineered mice. When creating engineered mice, the following 8 steps need to be considered. **For each mouse you make**, please state:

i) whether you are using pronuclear injection **or** gene targeting techniques
ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
iii) whether you would put the DNA into a fertilized egg **or** ES cells
iv) what is the **genotype** of the fertilized egg or the ES cells you would start with
v) **where** in the mouse genome the DNA you introduced would integrate
vi) whether creating the mouse should involve the generation of a chimera **or** not

vii) which **additional breeding** steps you would do to make the mouse you wanted

viii) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusions you would make for each result

Create a genetically modified mouse to distinguish between your two hypotheses if:

(c) You can use the TG+ DNA, but not the "Dext" gene.

i) **pronuclear injection – you want to add the TG+ gene in. Therefore you need to use pronuclear injection, because pronuclear injection is the easiest way to add DNA into the genome of a mouse.**

ii) **DNA includes a promoter region and the TG + gene**. **You need to the TG+ gene to be expressed in order to observe the phenotype, so you must attach it to a promoter region that will allow it to be expressed.**

iii**) fertilized eggs. For pronuclear injection, the DNA is injected into the paternal pronucleus before it fuses with the maternal pronucleus**

iv) **you would start with a wildtype egg – you want to see the effects that the TG+ gene has when inserted into the genome and whether or not it causes the mouse to move slowly, so you want all the DNA to start out being normal. Note that the mouse does NOT have endogenous copies of TG, because TG is a petal color gene from petunias, so a mouse would not have its own endogenous TG.**

v) **it would insert randomly. In pronuclear injection, the inserted DNA incorporates itself into the genome at a random location**

vi) **the mice would not be chimeric – you insert the DNA into a fertilized egg, which, once it begins dividing, creates all of the cells in that organism. Thus any DNA that inserts itself into the genome of a fertilized egg will be propagated into every cell of the mouse that grows up from that fertilized egg.**

vii) **You would then have to mate two of these TG+ mice together to get a homozygous TG+/TG+ mouse – you are trying to determine the effects of having two copies of TG+, but pronuclear injection only gives you heterozygotes because only one copy of the DNA sequence inserts at a time. Thus, to get a homozygote, you need to mate 2 hets and take the 1/4 of their offspring that have 2 copies of the TG+ to be studied.**

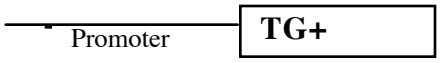
viii) **Your homozygous mice could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype of your original TG+/TG+ mouse was a result of the Dext gene being disrupted.**

*****Note that this method relies upon the fact that the second time you do this experiment, the TG insertion will not occur in the same place as the TG insertion did the first time you did this experiment (in the introduction to this question). TG insertion is random, so the chances of TG inserting into the same place in two different mouse eggs is essentially zero.**

ALTERNATIVE ANSWER TO PART C:

i) ***pronuclear injection***

ii) ***DNA includes a promoter region and the TG + gene***



iii) ***fertilized eggs***

iv) ***TG+ egg that has only one copy of the transgene (from a mating of two mice that each had a single copy of the transgene that you made in the introduction to this question)***

v) ***it would insert randomly***

vi) ***the mice would not be chimeric***

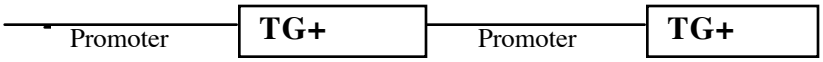
vii) ***no additional mating***

viii) ***your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene. Note that this method relies upon the fact that the second TG insertion will not occur in the same place as the first TG insertion did, because TG insertion is random, so the chances of two TGs inserting in the same place is essentially zero.***

ANOTHER ALTERNATIVE ANSWER TO PART C:

i) ***pronuclear injection***

ii) ***DNA includes a promoter region and two copies of the TG + gene***



iii) ***fertilized eggs***

iv) ***wild-type***

v) ***it would insert randomly***

vi) ***the mice would not be chimeric***

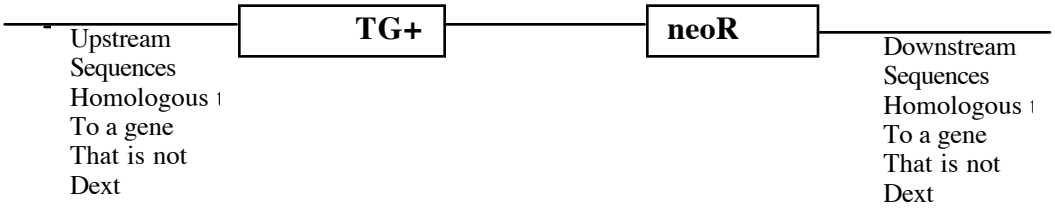
vii) ***no additional mating***

viii) ***your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.***

YET ANOTHER ALTERNATIVE ANSWER TO PART C:

i) ***gene targeting***

ii) ***DNA includes a TG gene and a neoR***



iii) ***ES cells***

iv) ***wild-type***

v) ***it would insert at the locus you chose***

vi) ***the mice would be chimeric***

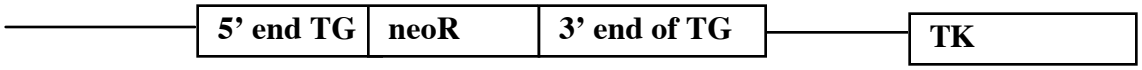
vii) ***mate a chimera to wt to get non-chimeric heterozygotes and mate to hets together to get a homozygote***

viii) ***your mice with two copies of the TG could either move slowly, in which case you would know that the TG+ gene causes this phenotype, or they could move normally, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.***

STILL ANOTHER ALTERNATIVE ANSWER TO PART C:

i) ***gene targeting***

ii) ***DNA includes a TG gene disrupted by neoR***



iii) ***ES cells***

iv) ***TG+/TG+***

v) ***it would insert at the locus where the TG is***

vi) ***the mice would be chimeric***

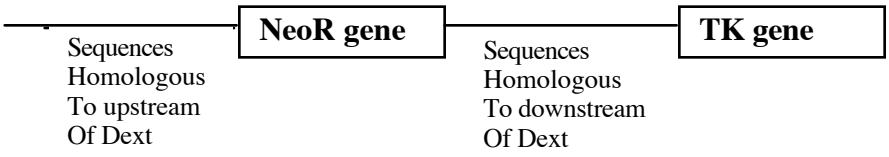
vii) ***mate a chimera to wt to get non-chimeric heterozygotes (TG-KO) and mate those hets to TG+/TG+ homozygotes to get heterozygotes (TG+/TG-KO)***

viii) ***your mouse could either move fast, in which case you would know that the TG+ gene causes this phenotype, or they could move slowly, in which case you could conclude that the phenotype you saw before was a result of the disrupted Dext gene.***

(d) You can use the "Dext" gene, but not the TG+ DNA.

i) **gene targeting. You think that the slow phenotype could be caused by two inactive versions of the Dext gene. To test this, you need to knock out this gene, which can ONLY be done using gene targeting.**

ii) **The DNA would include sequences homologous to the DNA surrounding the Dext gene and then a drug resistance gene in between. The construct needs homologous sequences on either end so that it can recombine into the genome and replace the dext gene, therefore knocking it out. The construct also requires having a TK gene outside of the region of homology to dext. This is so that we can select against TK, thereby selecting against integration of the construct by nonhomologous recombination.**



iii) **you would put the DNA into ES cells – for gene targeting, you use embryonic stem cells which the DNA can incorporate in to**

iv) **you would start with wildtype cells or TG+/TG+ cells**

v) **the DNA would integrate into the place previously held by the Dext gene because the homologous sequences on either side of your construct would recombine with the endogenous Dext locus and integrate it in.**

vi) **the mice would be chimeric**. Y**ou put the manipulated stem cells back together with other wild-type cells that already exist in the wild-type blastocyst into which you inject the manipulated cells. Thus, when the embryo grows up, parts of the embryo will have developed from your manipulated cells, but other parts of the embryo will have developed from the wild-type cells that were present in the blastocyst you began with.**

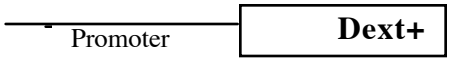
vii) **You would have to mate the chimeric mice to wildtype mice in order to obtain non-chimeric heterozygous mice, and then you would have to mate two carriers so that 1/4 of their offspring will be homozygous knockout mice**

viii) **If the knockout mice move slowly, you know that the phenotype was caused by the disruption of the Dext gene. If they move normally, you can conclude that the phenotype you saw in your original TG+/TG+ mice was a result of the two copies of the TG + gene.**

ALTERNATIVE ANSWER TO PART D:

i) ***pronuclear injection***

ii) ***DNA includes a promoter region and the Dext + gene***



iii) ***fertilized eggs***

iv) ***you would start with a TG+/TG+ egg***

v) ***it would insert randomly***

vi) ***the mice would not be chimeric***

vii) ***no additional mating***

viii) ***your transgenic mice could either move slowly, in which case you would know that the TG+ gene causes this phenotype, OR they could move normally, in which case you could conclude that the phenotype was a result of the disrupted Dext gene (because adding in a wt copy of Dext rescued the mutant phentoype)***

2005 7.03 Problem Set 6

Due before 5 PM on WEDNESDAY, November 23, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. Two populations (Population One and Population Two) send a randomly selected group of their inhabitants to settle on a new and previously uninhabited island. Before sending anyone off to the island, both populations are at Hardy-Weinberg Equilibrium for an autosomal recessive trait displayed by individuals with the genotype bb. In Population One, 1 out of 2,000 people express the trait. In Population Two, the frequency of the b allele is 0.1.

(a) What is the allele frequency of the b allele in Population One?

The two populations send off their settlers to the island, where the 100,000 settlers randomly mate. Each couple has two children, and thereby creates a new generation of 100,000 people (500 of whom express the trait).

(b) What is the allele frequency of the b allele in the new merged population on the island?

Answer the next parts assuming that the two populations of settlers have merged on the island and have reached Hardy Weinberg equilibrium.

(c) If you selected a female at random, what would the probability be that she was a carrier?

(d) What proportion of all b alleles are present in carriers?

(e) What fraction of all matings are between parents who are the same genotype as one another?

2. A rare X-linked recessive trait affects 1/5000 males in a certain population.

(a) What is the allele frequency of the allele for the recessive trait?

(b) How many affected men are there for each affected woman?

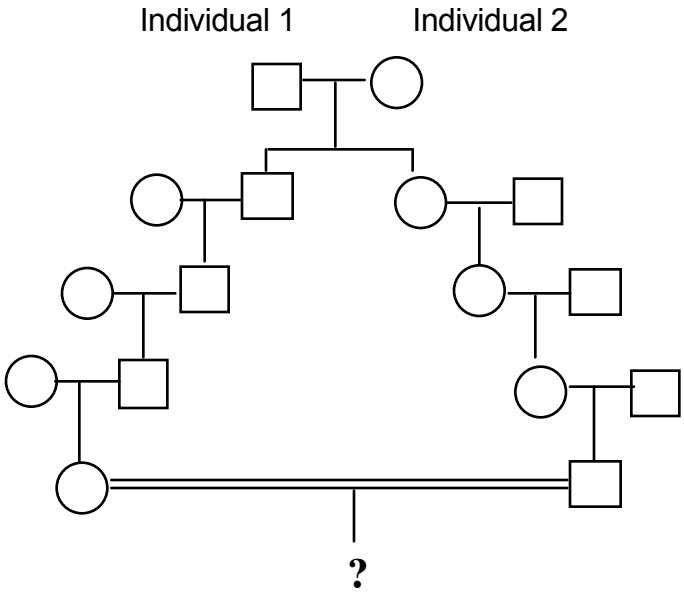
(c) In what proportion of matings would this trait affect half of the children of each sex?

(d) In what proportion of matings would the children have no chance of having the trait?

(e) Now assume that individuals showing the trait have a fitness of 70% as compared to individuals that do not show the trait. There are no other factors affecting the frequency of the trait. By how much would the allele frequency change between the current generation and the next generation?

(f) Now assume that there are new mutations being introduced into the population. The mutation rate at the locus associated with this trait is 10^{-4}. What would the allele frequency of the allele associated with the trait equal when a steady state between mutation and selection is reached?

3. Consider the following pedigree, which illustrates a family in a very large population. The two relatives shown are considering having a child, and if they do, they will be the first example of inbreeding in the entire population.



Assume no new mutations occur, that penetrance is complete, and that selection is negligible. Also assume that all traits are rare.

(a) What is the inbreeding coefficient for this pedigree?

(b) For the child indicated by a question-mark, how many genes on average will he/she be homozygous by descent (i.e. the child has inherited two alleles of a gene, both of which came from the same DNA in one of the great great great grandparents)? (There are about 20,000 genes in the human genome.)

(c) Assume that you do not know the genotypes of the great great great grandparents, but that you do know that neither exhibits an **autosomal recessive** trait with an incidence in the population of 10^{-6}. What is the chance that Individual 2 is a carrier of the allele for the trait, **and** Individual 1 is not a carrier?

(d) For the same trait described in part **(c)**, if Individual 2 is a carrier of the allele for the trait, and Individual 1 is not a carrier (and does not show the trait), what is the chance that the unborn child denoted in the pedigree by a question-mark will have the trait?

(e) Assume that an **autosomal recessive** trait in this population has an incidence of 10^{-6}. Imagine that, in the next generation of the population, every person decides to inbreed with a person of the same degree of relatedness as the two relatives who are mating in the pedigree shown above. What will the frequency of the trait be in the next generation?

(f) Assume that an **autosomal dominant** trait in this population has an incidence of 10^{-6}. Now say that you know that Individual 2 is heterozygous for the allele for the trait, and Individual 1 does not have the trait. What is the chance that the unborn child denoted in the pedigree by a question-mark will have the trait?

2005 7.03 Problem Set 6 KEY

Due before 5 PM on WEDNESDAY, November 23, 2005.
Turn answers in to the box outside of 68-120.
PLEASE WRITE YOUR ANSWERS ON THIS PRINTOUT.

1. Two populations (Population One and Population Two) send a randomly selected group of their inhabitants to settle on a new and previously uninhabited island. Before sending anyone off to the island, both populations are at Hardy-Weinberg Equilibrium for an autosomal recessive trait displayed by individuals with the genotype bb. In Population One, 1 out of 2,000 people express the trait. In Population Two, the frequency of the b allele is 0.1.

(a) What is the allele frequency of the b allele in Population One?

0.022

The allele "b" is recessive, so the frequency of "b" is equal to "q" in the Hardy-Weinberg equation. We know that 1 out of 2,000 people in Population 1 express the trait, which means that 1 / 2,000 people are b/b homozygotes. 1 / 2,000 = 0.0005 = q^2. The frequency of b, therefore, is the square root of q^2, which is 0.022.

The two populations send off their settlers to the island, where the 100,000 settlers randomly mate. Each couple has two children, and thereby creates a new generation of 100,000 people (500 of whom express the trait).

(b) What is the allele frequency of the b allele in the new merged population on the island?

0.071

500 / 100,000 people are b/b, so q^2 = 500 / 100,000 = 0.005. The value of q is 0.071.

Answer the next parts assuming that the two populations of settlers have merged on the island and have reached Hardy Weinberg equilibrium.

(c) If you selected a female at random, what would the probability be that she was a carrier?

0.132

The frequency of b ("q") on the island is 0.071. The frequency of B ("p"), therefore, must be 1 – q = 0.929.
p (carrier) = 2pq = 0.132

(d) What proportion of all b alleles are present in carriers?

93%

The total number of b alleles in the population = q * the total number of alleles in the population (which is 200,000 because there are 100,000 people and each person has 2 alleles at this autosomal locus). Thus the total number of b alleles in the population is 200,000 * 0.071 = 14200.
The total number of heterozygotes in the population = 2pq * the number of individuals in the population = 0.132 * 100,000 = 13200. Each heterozygote possesses one b allele.

Thus the fraction of b alleles that are present in heterozygotes = 13200/14200 = 93%.

Note that 93% is actually the value of p.

(e) What fraction of all matings are between parents who are the same genotype as each other?

76.2%

Since the island's population is in Hardy-Weinberg equilibrium, we know that mating is random:
p (B/B mating with B/B) = $p^2 * p^2 = 0.929^4 = 0.745$
p (B/b mating with B/b) = $2pq * 2pq = 0.13^2 = 0.017$
p (b/b mating with b/b) = $q^2 * q^2 = 0.071^4 \approx 0$
=> p (mating between parents with same genotype) = 0.745 + 0.017 + ~0 = 0.762

2. A rare X-linked recessive trait affects 1/5000 males in a certain population.

(a) What is the allele frequency of the allele associated with the recessive trait?

1/5000

Since this is an X-linked trait, males are haploid at the locus for this gene (males only have one X chromosome). In this case, the frequency of affected males is equal to the frequency of males with the recessive allele: 1 / 5000 = q = 0.0002.

(b) How many affected men are there for each affected woman?

5000

q = 0.0002 = proportion of men affected
q^2 = 0.00000004 = proportion of women affected
Ratio of affected men : affected women = 0.0002 / 0.00000004 = 5000 : 1

(c) In what proportion of matings would this trait affect half of the children of each sex?

0.00000008

Half of all offspring will show the trait only if the mother is a heterozygous carrier, and the father has the trait. The mother would pass on the recessive allele 1/2 of the time, and the father would pass on the recessive allele every time.

p = 1 – q = 0.9998
With random mating,
p (mother is a carrier) = 2pq = 2 * 0.0002 * 0.9998 = 0.0004
p (father is affected) = q = 0.0002
=> p (half of offspring affected) = 2pq * q = 0.0004 * 0.0002 = 0.00000008

(d) In what proportion of matings would no children have the trait?

99.96%

In order for no children to be affected, there are two possible matings: (1) a wild-type mother and a wild-type father, and (2) a wild-type mother and an affected father.

With random mating,
p (1) = p^2 * p = 0.9998^3 = 0.9994
p (2) = p^2 * q = 0.9998^2 * 0.0002 = 0.0002
=> p (no offspring affected) = p(1) + p(2) = 0.9994 + 0.0002 = 99.96%

(e) Now assume that individuals showing the trait have a fitness of 70% as compared to individuals that do not show the trait. There are no other factors affecting the frequency of the trait. By how much would the allele frequency change between the current generation and the next generation?

It would decrease by 0.00002.

The current frequency of the allele is q = 0.0002. The change in frequency in the next generation is equal to Δq_{sel} = –(1/3) Sq, where S is the selective disadvantage. The fitness is 0.7 in this case, so S = 1 – 0.7 = 0.3. Since the overwhelming majority of individuals with the trait will be male, only affected males will be subjected to decreased fitness. The proportion of males subject to selection is the proportion of males that have the trait, which is q. Thus the effect of selection acting on males is –Sq. However you must then

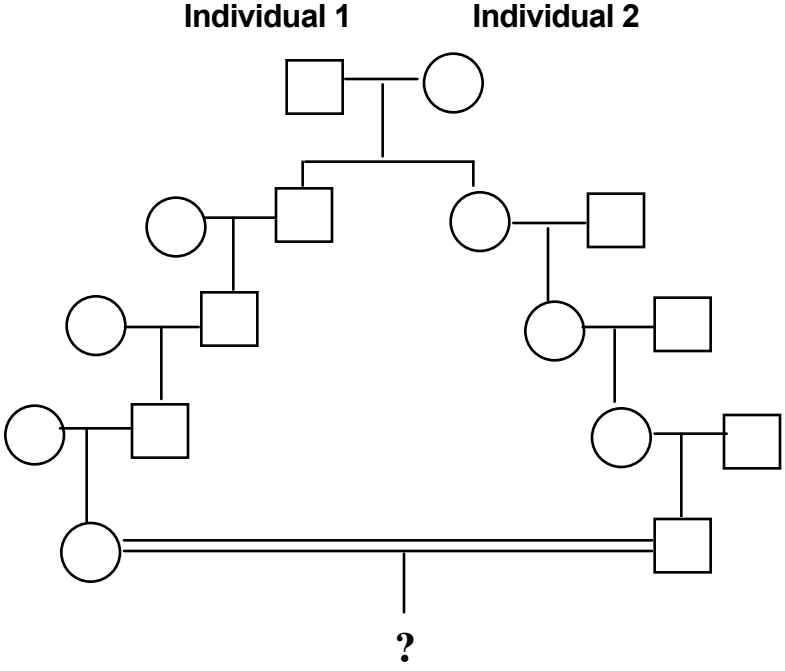
multiply –Sq by 1/3, because 1/3 of all X chromosomes in the population will be in males. This means that only 1/3 of the total recessive alleles are actually acted on by selection. The other 2/3 of X chromosomes with the recessive allele will be in females, who will not be subjected to decreased fitness. Therefore,
$\Delta q_{sel} = – (1/3)\ 0.0002 * 0.3 = – 0.00002.$

(f) Now assume that there are new mutations being introduced into the population. The mutation rate at the locus associated with this trait is 10^{-4}. What would the allele frequency of the allele associated with the trait equal when a steady state between mutation and selection is reached?

The new q would be 0.001

At the steady state, the $\Delta q_{sel} + \Delta q_{mut} = 0$. Δq_{mut} is equal to the mutation rate μ. Δq_{sel} is equal to –Sq/3, and so at steady state, $–Sq/3 + \mu = 0$. The mutation rate μ is 10^{-4} and S = 0.3. Solving for q, we get a new q at steady state = 0.001.

3. Consider the following pedigree, which illustrates a family in a very large population. The two relatives shown are considering having a child, and if they do, they will be the first example of inbreeding in the entire population.



Assume no new mutations occur, that penetrance is complete, and that selection is negligible. Also assume that all traits are rare.

(a) What is the inbreeding coefficient for this pedigree?

F = 1/256

The great great great grandparents of the child have a total of four alleles at any autosomal locus in the genome. We can label the great great grandfather as being A1/A2 and the great great grandmother as being A3/A4. We use these non-specific allele notations because we have no idea which alleles these grandparents possess. Now we can consider the probability that the child in the pedigree will be homozygous by descent at that locus. The child could become homozygous by descent for any of the four alleles possessed by the great great grandparents.

p (A1/A1) = 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/4 = 1/1024
p (A2/A2) = 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/4 = 1/1024
p (A3/A3) = 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/4 = 1/1024
p (A4/A4) = 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/2 * 1/4 = 1/1024

p (homozygous at A locus by descent) = 4 * 1/1024 = 1/256 = F

(b) For the child indicated by a question-mark, how many genes on average will he/she be homozygous by descent (i.e. the child has inherited two alleles of a gene, both of which came from the same DNA in one of the great great great grandparents)? (There are about 20,000 genes in the human genome)

about 78

There is a 1/256 chance that each gene will be homozygous by descent, so the number of homozygous loci would be about 1/256 * 20,000, which is equal to about 78 loci in the genome.

(c) Assume that you do not know the genotypes of the great great great grandparents, but that you do know that neither exhibits an **autosomal recessive** trait with an incidence in the population of 10^{-6}. What is the chance that Individual 2 is a carrier of the allele for the trait, **and** Individual 1 is not a carrier?

0.002

$q^2 = 10^{-6}$
therefore
q = 0.001, p = 0.999
p (Individual 2 is a carrier) = 2pq = 2 * 0.001 * 0.999 = 0.002
p (Individual 1 is not a carrier) = p^2 = 0.999 * 0.999 = 0.998
=> p (2 carrier and 1 not carrier) = 0.002 * 0.998 = 0.002

Note that these are approximations. In fact, since we know that Individual 2 and Individual 1 are not affected, the most precise way to calculate their chances of being the respective genotypes are:

p (Individual 2 is a carrier) = [2pq / (2pq + p^2)] ≈ 2 * 0.001 * 0.999 ≈ 0.002
p (Individual 1 is not a carrier) = [p^2 / (2pq + p^2)] ≈ 0.999 * 0.999 ≈ 0.998

This is an example of a simple conditional probability – e.g. what is the probability that Individual 1 is AA given that Individual 1 is NOT aa?

(d) For the same trait described in part **(c)**, if Individual 2 is a carrier of the allele for the trait, and Individual 1 is not a carrier (and does not show the trait), what is the chance that the unborn child denoted in the pedigree by a question-mark will have the trait?

1/1024

This question is asking for the chance that the child will be homozygous aa. Individual 2 is Aa, and Individual 1 is AA, so the answer to this question is really 1/4 F. This is because F is the chance that the child is homozygous by descent for any of the four alleles possessed by the great great grandparents, while we are asking here what is the chance that the child will be homozygous for 1/4 of the 4 alleles possessed by the great great grandparents (i.e. only for the "a" allele possessed by the great great grandmother).
p(child is aa) = p("A4"/"A4") = 1/1024

(e) Assume that an **autosomal recessive** trait in this population has an incidence of 10^{-6}. Imagine that, in the next generation of the population, every person decides to inbreed with a person of the same degree of relatedness as the two relatives who are mating in the pedigree shown above. What will the frequency of the trait be in the next generation?

The new incidence would be 4 * 10^{-6}

$q^2 = 10^{-6}$
q = 0.001
Mating is no longer random; instead, now the probability of homozygosity by descent in each individual of the next generation is F = 1/256. The frequency of the allele in the previous generation was q, so the frequency of affected individuals in the population will be p (affected by trait) = Fq. This is because q is the chance that any child will receive a "a" allele, and F is the chance that any child will be homozygous. Thus the chance that any child will have "a" AND be homozygous = p(child is aa) = F * q.
q^2 does not enter into this calcuation, because q^2 would be the frequency of affected individuals as a result of random mating, and no random mating is occurring any more in this population.
=> p (affected by trait in next generation) = Fq = (1/256) * 0.001 = 4 * 10^{-6}

(f) Assume that an **autosomal dominant** trait in this population has an incidence of 10^{-6}. Now say that you know that Individual 2 is heterozygous for the allele for the trait, and Individual 1 does not have the trait. What is the chance that the unborn child denoted in the pedigree by a question-mark will have the trait?

6.15%

Designating the dominant trait allele as "A", and the wild-type allele as "a," there are 3 combinations of the parental genotypes that could produce a child with the trait:

Mom Dad
(mating 1) = aa x Aa
(mating 2) = Aa x aa
(mating 3) = Aa x Aa

The probability of one of the parents of "child ?" having the "A" allele passed down from individual 2 is 1/16. Thus the probability that the mom of "child ?" is Aa = 1/16. The probability that the dad of "child ?" is Aa = 1/16.

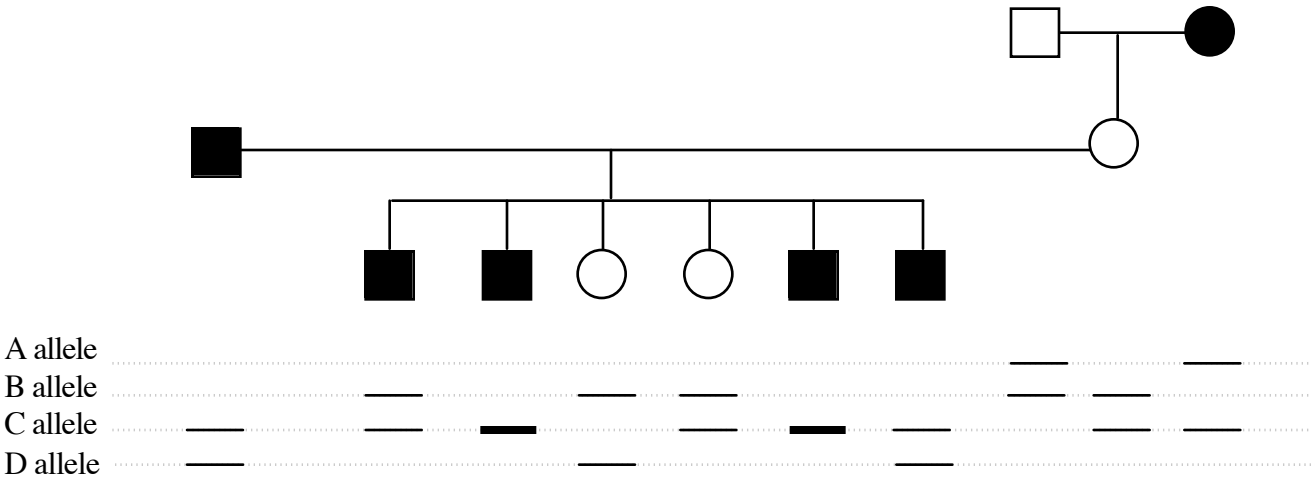
Neither the mom or the dad of "child ?" can be AA (realistically speaking, because this would require that someone mating into the family has the A allele, and the chance of that is 1/1,000,000). Thus the probability of the mom of "child ?" being aa is 15/16 and the probability that the dad of "child ?" is aa is 15/16.
Therefore:
p (mating 1) = (15/16) * (1/16)
p (mating 2) = (1/16) * (15/16)
p (mating 3) = (1/16) * (1/16)

The probability of having a child who displays the dominant trait is equal to 1/2 in (mating 1) and (mating 2), and is equal to 3/4 in (mating 3). The final probability of having an affected "child ?" is:
p(affected child) =
(15/16) * (1/16) * (1/2)
(1/16) * (15/16) * (1/2) +
(1/16) * (1/16) * (3/4)
= 0.0615
Note that the chance of a person mating into the family randomly contributing the "A" allele is much much lower than the chance that the A allele is passed on to "child ?" from the first generation of the pedigree. This is why we can ignore the possibility that rare alleles can be contributed from people marrying into a pedigree for all rare traits.

2005 7.03 Problem Set 7

NO DUE DATE. This problem set is to provide practice on concepts from lectures 30-36.

1. The following pedigree shows the inheritance of an autosomal recessive trait in a specific family. This trait is caused by a specific allele "g" at the G/g locus. You have some reason to suspect that the G/g locus is linked to an SSR on chromosome 6 called SSR41. You obtain blood samples from each member of the family, and perform a PCR reaction on the DNA of each individual that allows for the genotyping of SSR41. The results of the PCR reactions are shown below each family member in the pedigree, in a schematic of an agarose gel in which you have loaded the PCR reactions from each family member into a separate well in the gel.



paternally inherited allele at SSR41						
maternally inherited allele at SSR41						

paternally inherited allele at G/g locus						
maternally inherited allele at G/g locus						

(a) Fill in the tables above to indicate which alleles have been passed on to each child from their mother and father.

(b) Whose alleles (the mother's or the father's or both) should you follow to calculate the LOD score for the linkage of the SSR to the G/g locus?

(c) Draw all possible phases for the parent(s) you listed in part **(b)**.

(d) For each phase you drew you drew in part **(c)**, state how many children are recombinants and how many children are parentals given that phase.

(e) Calculate the LOD score for this family at theta = 0.04 for the linkage of the SSR to the G/g locus.

(f) At what theta value would you achieve the maximal LOD score for this family, knowing everything you know about them?

(g) What is the LOD score value for the theta value you listed in part **(f)**?

(h) If you had never seen the genotyping results for this family, and only had their pedigree available, what would have been the theoretical maximum LOD score value that you could have ever calculated for this family? (**Hint:** Start by thinking about which theta value could give you the maximum possible LOD score.)

(i) If you had never seen the genotyping results for this family, and only had their pedigree available, what is the minimum number of kids that the family would have had to have contained in order to reach a theoretical maximum LOD score that is > 3?

2. A tumor results when a cell in the body loses control over cell growth and division such that the cell divides many times, forming a ball of cells. Cancer can be extremely harmful to the organism when these balls of cells either physically interfere with function of an essential organ, or begin to steal the nutrients away from cells of essential organs. Cells become capable of growing and dividing inappropriately when they have accumulated multiple mutations in genes (such as oncogenes and tumor suppressor genes) whose normal functions are to control cell growth and division (i.e. to control the cell cycle).

(a) Why is the notion of there being "a cure for cancer" unreasonable?

(b) What is the wild-type function of an oncogene?

(c) What phenotype may result if an oncogene gets mutated so that it becomes over-active?

(d) Would an over-active allele of an oncogene cause a dominant or a recessive phenotype?

(e) Would an over-active allele of an oncogene be the result of a loss-of-function mutation or a gain-of-function mutation?

(f) Could a mutation in an oncogene (that caused the gene to become over-active) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of an oncogene could lead to an over-active mutant allele of an oncogene.

(g) What is the wild-type function of a tumor-suppressor gene?

(h) What phenotype may result if a tumor-suppressor gene is mutated so that it no longer functions?

(i) Would the inactivation of a tumor suppressor gene cause a dominant or a recessive phenotype?

(j) Would the inactivation of a tumor suppressor gene be the result of a loss-of-function mutation or a gain-of-function mutation?

(k) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the coding region of the gene? If so, give an example of how a change in the coding region of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

(l) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

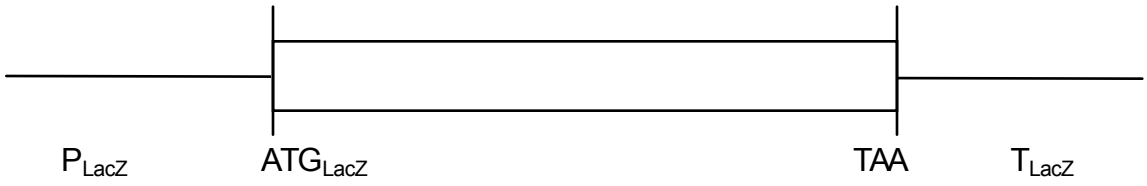
3. You are studying a yeast gene (Act1) which encodes a transcriptional activator protein; Act1 activates the Yst1 gene, which encodes a yeast enzyme that helps yeast cells deal with high salt conditions. Act1 is normally transcribed only when yeast cells are grown in high salt concentrations. You create two different DNA constructs that will allow you to visualize when and/or where Act1 is expressed in cells. Each DNA construct is a fusion of part of the Act1 gene to part of the *E. coli* LacZ gene. You make these two fusion constructs because you want to visualize when and/or where Act1 is expressed in yeast cells, but you don't have a good assay for measuring the presence or activity of Act1 protein. You do, however, have a good assay for measuring the presence and activity of *E. coli* beta-galactosidase, because you know that this enzyme cleaves X-gal and releases a blue-colored compound.

The first DNA construct you make is called an "Act1-LacZ transcriptional fusion." To make this construct, you fuse the cis regulatory region ("P_{Act1}") that lies upstream of the Act1 open reading frame to the LacZ coding sequence and terminator. You then place this hybrid gene on a yeast plasmid.

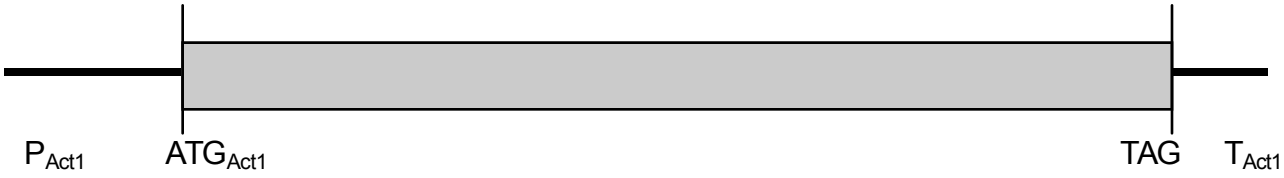
The second DNA construct you make is called an "Act1-LacZ translational fusion." To make this construct, you fuse almost the entire Act1 gene (beginning with its promoter and ending right before its stop codon) directly upstream of a portion of the LacZ gene (from the start codon through the terminator). You then place this hybrid gene on a yeast plasmid.

The beta-galactosidase enzyme (which is encoded by the lacZ gene) is found in the cytoplasm of *E. coli* bacterial cells. When beta-galactosidase is expressed in yeast cells, it is also found in the cytoplasm. The transcriptional activator protein Act1 is found in the nucleus of yeast cells.

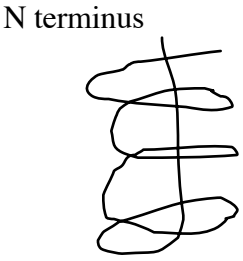
The gene for beta-galactosidase (which is *lacZ*) looks like this: (T = transcription terminator)



The gene for Act1 looks like this:



The gene for beta-galactosidase produces a protein that looks like this:



The gene for Act1 produces a protein that looks like this:

(a) Why does it make sense that the transcriptional activator protein Act1 is found in the nucleus of yeast cells?

(b) What might be the functions of the two different protein domains possessed by the Act1 protein?

(c) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the transcriptional Act1-LacZ fusion.

(d) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the translational Act1-LacZ fusion.

(e) Under what cellular conditions is Act1 normally transcribed and translated?

(f) Under what cellular conditions is beta-galactosidase normally transcribed and translated?

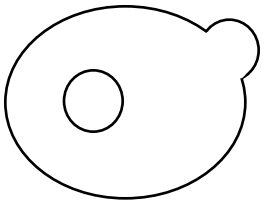
(g) Under what conditions would the protein be made that is produced from the transcriptional Act1-LacZ fusion?

(h) Under what conditions would the protein be made that is produced from the translational Act1-LacZ fusion?

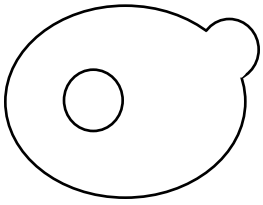
(i) Based on the diagrams above, draw the protein that would be produced from the transcriptional Act1-LacZ fusion.

(j) Based on the diagrams above, draw the protein that would be produced from the translational Act1-LacZ fusion.

(k) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the transcriptional Act1-LacZ fusion. (To answer parts **(k)** and **(l)**, you must know that most signals used by the cell to direct intracellular protein localization are found at the very N terminus of the protein.)



(l) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the translational Act1-LacZ fusion.



(m) Say that you had a haploid yeast strain that had the endogenous chromosomal copy of Act1 deleted. If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

(n) If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?

(o) If you transformed the act1^{-} haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

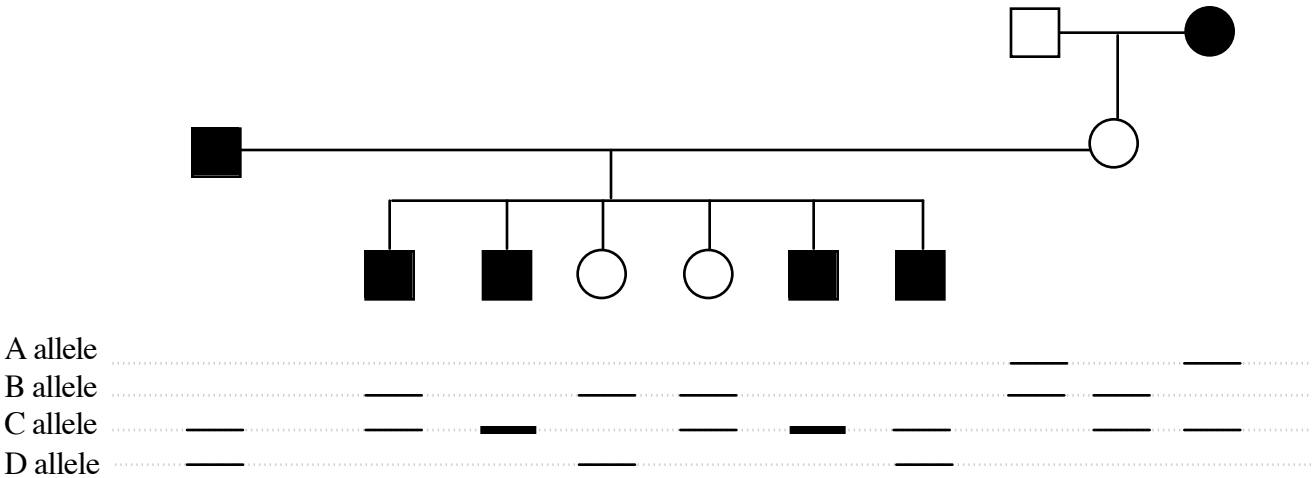
(p) If you transformed the act1^{-} haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?

KEY

2005 7.03 Problem Set 7 KEY

NO DUE DATE. This problem set is to provide practice on concepts from lectures 30-36.

1. The following pedigree shows the inheritance of an autosomal recessive trait in a specific family. This trait is caused by a specific allele "g" at the G/g locus. You have some reason to suspect that the G/g locus is linked to an SSR on chromosome 6 called SSR41. You obtain blood samples from each member of the family, and perform a PCR reaction on the DNA of each individual that allows for the genotyping of SSR41. The results of the PCR reactions are shown below each family member in the pedigree, in a schematic of an agarose gel in which you have loaded the PCR reactions from each family member into a separate well in the gel.



paternally inherited allele at SSR41	**C**	**C**	**D**	**C**	**C**	**D**
maternally inherited allele at SSR41	**B**	**C**	**B**	**B**	**C**	**C**

paternally inherited allele at G/g locus	**g**	**g**	**g**	**g**	**g**	**g**
maternally inherited allele at G/g locus	**g**	**g**	**G**	**G**	**g**	**g**

(a) Fill in the tables above to indicate which alleles have been passed on to each child from their mother and father.

KEY

(b) Whose alleles (the mother's or the father's or both) should you follow to calculate the LOD score for the linkage of the SSR to the G/g locus?

You want to follow the alleles of the parent who is heterozygous at both the disease locus and the SSR locus (so you can tell which of the children are parental and which are recombinant). In this case that parent is the mother.

(c) Draw all possible phases for the parent(s) you listed in part **(b)**.

G B inherited from grandpa
g C inherited from grandma

Since you know the genotypes of the mother's parents, there is only one possible phase for the mother.

(d) For each phase you drew you drew in part **(c)**, state how many children are recombinants and how many children are parentals given that phase.

GB from mother = parental
gC from mother = parental
GC from mother = recombinant
gB from mother = recombinant

Therefore, child 1 is recombinant, the other 5 are all parental.

(e) Calculate the LOD score for this family at theta = 0.04 for the linkage of the SSR to the G/g locus.

For the numerator of the odds ratio, you assume that the SSR and G/g locus are linked. Since theta =0.04, this means you'd expect 4% recombinants and 96% parentals. There are two types of recombinants, GC and gB. Each type of recombinant will occur at equal frequencies, so there is a 2% chance of any child getting GC from the mother and a 2% chance of any child getting gB from the mother. Each type of parental will occur at equal frequencies, so there is a 48% chance of any child getting GB from the mother and a 48% chance of any child getting gC from the mother.
Chances of the mom creating each type of gamete:
GB-48%
gC-48%
GC-2%
gB-2%

KEY

Child one got Bg. The chance of this is 2%.
Child one got Cg. The chance of this is 48%.
Child one got BG. The chance of this is 48%.
Child one got BG. The chance of this is 48%.
Child one got Cg. The chance of this is 48%.
Child one got Cg. The chance of this is 48%.
The chance that you saw these six kids would be 2% * 48% * 48% * 48% * 48% * 48% .

For the denominator of the odds ratio, you assume that the SSR and G/g locus are NOT linked. UN-linkage corresponds to theta = 0.5, because UN-linkage gives 50% parentals and 50% recombinants.

Since theta =0.5, this means you'd expect 50% recombinants and 50% parentals.
There are two types of recombinants, GC and gB. Each type of recombinant will occur at equal frequencies, so there is a 25% chance of any child getting GC from the mother and a 25% chance of any child getting gB from the mother. Each type of parental will occur at equal frequencies, so there is a 25% chance of any child getting GB from the mother and a 25% chance of any child getting gC from the mother.
Chances of the mom creating each type of gamete:
GB-25%
gC-25%
GC-25%
gB-25%
Child one got Bg. The chance of this is 25%.
Child one got Cg. The chance of this is 25%.
Child one got BG. The chance of this is 25%.
Child one got BG. The chance of this is 25%.
Child one got Cg. The chance of this is 25%.
Child one got Cg. The chance of this is 25%.
The chance that you saw these six kids would be 25% * 25% * 25% * 25% * 25% * 25% .

LOD = log of the odds ratio

$$\log_{10}\frac{(0.48)^5(0.02)^1}{(0.25)^6} = 0.32$$

KEY

(f) At what theta value would you achieve the maximal LOD score for this family, knowing everything you know about them?

You'd expect to achieve the maximal LOD score when theta = the fraction of recombinant children that you actually have in your family. In this case 1 in 6 children were recombinant so you'd expect the maximum LOD score when theta = 1/6 = 0.17.

(g) What is the LOD score value for the theta value you listed in part **(f)**?

If theta = 0.17, you'd expect 83% total parentals and 17% total recombinants.

$$\log_{10}\frac{(0.42)^5(0.08)^1}{(0.25)^6} = 0.63$$

(h) If you had never seen the genotyping results for this family, and only had their pedigree available, what would have been the theoretical maximum LOD score value that you could have ever calculated for this family? (**Hint:** Start by thinking about which theta value could give you the maximum possible LOD score.)

The theoretical maximum LOD score for an SSR and a trait locus from a 6-child family in which there is one relevant parent whose phase you do indeed know occurs if all children are parentals. If all children are parentals, then the optimal LOD score value would occur at a theta = 0. Thus the theoretical maximum is:

$$\log_{10}\frac{(0.5)^6}{(0.25)^6} = 1.8$$

(i) If you had never seen the genotyping results for this family, and only had their pedigree available, what is the minimum number of kids that the family would have had to have contained in order to reach a theoretical maximum LOD score that is > 3?

$$\log_{10}\frac{(0.5)^x}{(0.25)^x} \geq 3$$ **Solving for x, x = at least 10 children.**

KEY

2. A tumor results when a cell in the body loses control over cell growth and division such that the cell divides many times, forming a ball of cells. Cancer can be extremely harmful to the organism when these balls of cells either physically interfere with function of an essential organ, or begin to steal the nutrients away from cells of essential organs. Cells become capable of growing and dividing inappropriately when they have accumulated multiple mutations in genes (such as oncogenes and tumor suppressor genes) whose normal functions are to control cell growth and division (i.e. to control the cell cycle).

(a) Why is the notion of there being "a cure for cancer" unreasonable?

As we discussed in class, we use the word "cancer" to describe a variety of diseases with a variety of causes. Each different specific type of cancer is caused by an accumulation of roughly 6 different mutations in a collection of oncogenes and tumor suppressor genes. No two types of cancer are caused by the same collection of 6 mutations. Therefore the notion that there will be one cure that will be effective for all types of cancers is unreasonable.

(b) What is the wild-type function of an oncogene?

The wild-type function of an oncogene is to promote cell growth and cell division when it is an appropriate (and in the appropriate cell types).

(c) What phenotype may result if an oncogene gets mutated so that it becomes over-active?

If an oncogene gets mutated so that it becomes over-active, you'd expect the phenotype of uncontrolled growth. A wild-type oncogene stimulates the cell cycle whever appropriate, so an overactive mutant version of an oncogene would stimulate the cell cycle even when not appropriate. Cancer is the result of uncontrolled cell proliferation, so stimulating the cell cycle at inappropriate times leads to cancer.

(d) Would an over-active allele of an oncogene cause a dominant or a recessive phenotype?

An over-active allele of an oncogene would cause a dominant phenotype. If you have a cell that has one overactive mutant allele and one wild-type allele that is properly regulated, the sum total activity that you would see would be overactivity. Adding back a wild-type version of an oncogene does not help a cell whose problem is that it has a mutant overactive version of an oncogene.

(e) Would an over-active allele of an oncogene be the result of a loss-of-function mutation or a gain-of-function mutation?

KEY

An over-active allele of an oncogene would be the result of a gain of function mutation. The mutated oncogene has gained the ability to stimulate the cell cycle all of the time (when really it is supposed to only stimulate the cell cycle when appropriate, i.e. when the cell receives growth signals from the environment).

(f) Could a mutation in an oncogene (that caused the gene to become over-active) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of an oncogene could lead to an over-active mutant allele of an oncogene.

Yes. One example would be if the cis-acting region of an oncogene was no longer able to bind to a transcriptional repressor. In this case, the repressor would no longer regulate the oncogene, so the oncogene would be transcribed constitutively.

(g) What is the wild-type function of a tumor-suppressor gene?

The wild-type function of a tumor-suppressor gene is to inhibit cell growth and division whenever it is not appropriate for cells to be going through the cell cycle. (Some tumor suppressor genes' wild-type roles are to promote apoptosis, or programmed cell death.)

(h) What phenotype may result if a tumor-suppressor gene is mutated so that it no longer functions?

If a tumor suppressor gene gets mutated so that it becomes inactive, you'd expect the phenotype of uncontrolled growth. A wild-type tumor suppressor gene inhibits the cell cycle whever appropriate, so an inactive mutant version of a tumor suppressor gene would allow cells to go through the cell cycle even when not appropriate. Cancer is the result of uncontrolled cell proliferation, so allowing the cell cycle to progress at inappropriate times leads to cancer.

(ALTERNATELY, FOR OTHER KINDS OF TUMOR SUPPRESSOR GENES: If a tumor suppressor gene gets mutated so that it becomes inactive, you'd expect the phenotype of uncontrolled growth. A wild-type tumor suppressor gene promotes apoptosis whever appropriate, so an inactive mutant version of a tumor suppressor gene would prevent cells from dying even when they should be dying. Cancer is the result of uncontrolled cell proliferation, so allowing cells to survive and proliferate even when they are supposed to die leads to cancer.)

(i) Would the inactivation of a tumor suppressor gene cause a dominant or a recessive phenotype?

KEY

The inactivation of a tumor suppressor gene would cause a recessive phenotype. If you have a cell that has one inactive mutant allele and one wild-type allele that is properly regulated, the sum total activity that you would see would be normal activity. Adding back a wild-type version of a tumor suppressor gene does help a cell whose problem is that it has a mutant inactive version of a tumor suppressor gene.

(j) Would the inactivation of a tumor suppressor gene be the result of a loss-of-function mutation or a gain-of-function mutation?

The inactivation of a tumor suppressor gene would be the result of a loss-of-function mutation. The mutated tumor suppressor gene has lost the ability to inhibit the cell cycle.

(k) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the coding region of the gene? If so, give an example of how a change in the coding region of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

Yes, a premature stop codon (nonsense mutation) could lead to a non-functional and therefore inactive tumor suppressor gene. A missense mutation that led to a change in an amino acid necessary for the tumor suppressor protein to perform its function would also lead to an inactive tumor suppressor gene. Finally, a frameshift mutation could cause the reading frame to shift, thus changing all codons and potentially exposing a premature stop codon that is normally not read.

(l) Could a mutation in a tumor suppressor gene (that caused the gene to become inactive) have occurred in the cis regulatory regions of the gene? If so, give an example of how a change in the cis regulatory regions of a tumor suppressor gene could lead to an inactive mutant allele of a tumor suppressor gene.

Yes, one example would be if the promoter of a tumor suppressor gene was no longer able to bind to a transcriptional activator or to RNA polymerase. In this case, the activator would no longer be able to recruit RNA polymerase, or RNA polymerase would not be able to bind, leading to a lack of transcription of the tumor suppressor gene's mRNA.

KEY

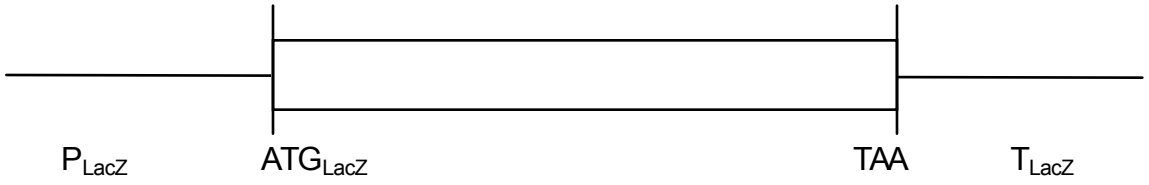
3. You are studying a yeast gene (Act1) which encodes a transcriptional activator protein; Act1 activates the Yst1 gene, which encodes a yeast enzyme that helps yeast cells deal with high salt conditions. Act1 is normally transcribed only when yeast cells are grown in high salt concentrations. You create two different DNA constructs that will allow you to visualize when and/or where Act1 is expressed in cells. Each DNA construct is a fusion of part of the Act1 gene to part of the *E. coli* LacZ gene. You make these two fusion constructs because you want to visualize when and/or where Act1 is expressed in yeast cells, but you don't have a good assay for measuring the presence or activity of Act1 protein. You do, however, have a good assay for measuring the presence and activity of *E. coli* beta-galactosidase, because you know that this enzyme cleaves X-gal and releases a blue-colored compound.

The first DNA construct you make is called an "Act1-LacZ transcriptional fusion." To make this construct, you fuse the cis regulatory region ("P_{Act1}") that lies upstream of the Act1 open reading frame to the LacZ coding sequence and terminator. You then place this hybrid gene on a yeast plasmid.

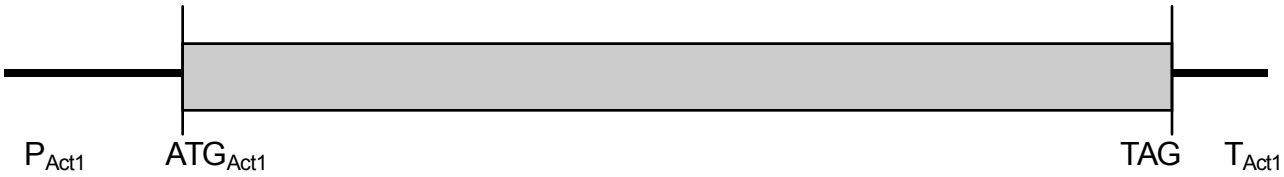
The second DNA construct you make is called an "Act1-LacZ translational fusion." To make this construct, you fuse almost the entire Act1 gene (beginning with its promoter and ending right before its stop codon) directly upstream of a portion of the LacZ gene (from the start codon through the terminator). You then place this hybrid gene on a yeast plasmid.

The beta-galactosidase enzyme (which is encoded by the lacZ gene) is found in the cytoplasm of *E. coli* bacterial cells. When beta-galactosidase is expressed in yeast cells, it is also found in the cytoplasm. The transcriptional activator protein Act1 is found in the nucleus of yeast cells.

The gene for beta-galactosidase (which is *lacZ*) looks like this: (T = transcription terminator)

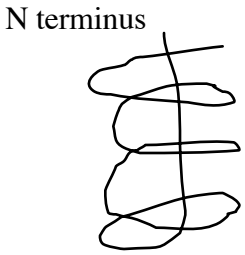


The gene for Act1 looks like this:



KEY

The gene for beta-galactosidase produces a protein that looks like this:



The gene for Act1 produces a protein that looks like this:

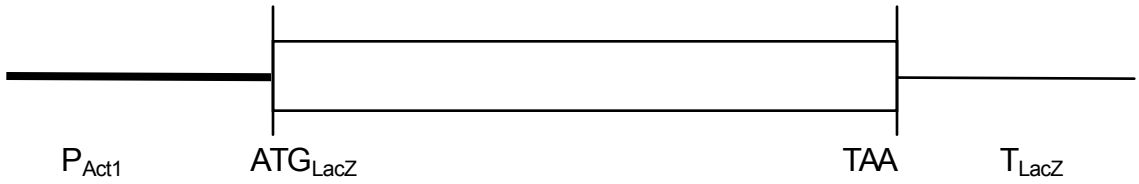
(a) Why does it make sense that the transcriptional activator protein Act1 is found in the nucleus of yeast cells?

In eukaryotes, transcription happens in the nucleus, therefore it makes sense that a transcriptional activator would also be found in the nucleus. The job of a transcriptional activator protein is to bind to DNA and activate transcription. DNA lives in the nucleus of eukaryotic cells, so that is where activators are found in the cell.

(b) What might be the functions of the two different protein domains possessed by the Act1 protein?

The job of a transcriptional activator protein is to bind to DNA and activate transcription. The way that activator proteins activate transcription is by recruiting the transcriptional machinery (RNA polymerase and its associated factors). Thus activator proteins have two different domains: 1) the DNA binding domain (the transcriptional activator must bind to DNA in order to activate transcription) and 2) the activation domain that recruits the transcriptional machinery.

(c) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the transcriptional Act1-LacZ fusion.

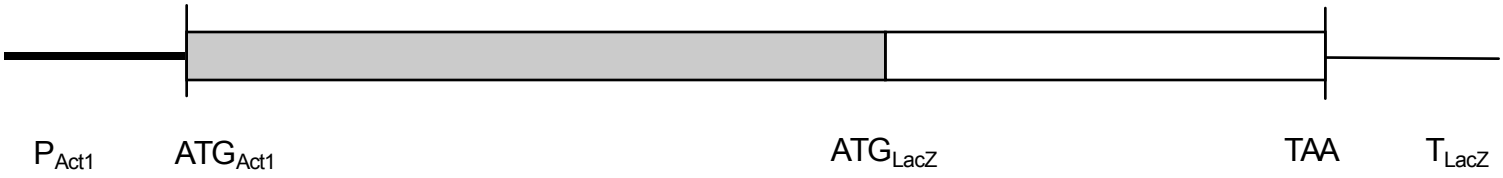


KEY

FROM THE INTRODUCTION TO THE QUESTION: To make this construct, you fuse the cis regulatory region ("P_{Act1}") that lies upstream of the Act1 open reading frame to the LacZ coding sequence and terminator.

(d) Based on the diagrams above, draw a schematic of the DNA construct that would result when you made the translational Act1-LacZ fusion.

(The scale of this drawing is 70% so that it fits on the page.)



FROM THE INTRODUCTION TO THE QUESTION: To make this construct, you fuse almost the entire Act1 gene (beginning with its promoter and ending right before its stop codon) directly upstream of a portion of the LacZ gene (from the start codon through the terminator).

(e) Under what cellular conditions is Act1 normally transcribed and translated?

Act1 is expressed under the condition of high salt in yeast cells. This makes sense, because the job of Act1 is to activate genes that are necessary for yeast cells to deal with high salt conditions (osmotic stress).

(f) Under what cellular conditions is beta-galactosidase normally transcribed and translated?

LacZ is expressed when lactose is present AND when glucose is NOT present in *E. coli* cells. This makes sense, because the job of the LacZ gene product is to metabolize lactose for energy.

(g) Under what conditions would the protein be made that is produced from the transcriptional Act1-LacZ fusion?

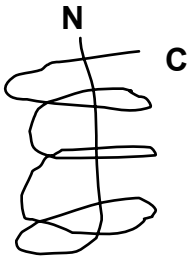
Since the expression of this construct is controlled by the Act1 promoter, you'd expect the product of this gene to be made under the condition of high salt.

KEY

(h) Under what conditions would the protein be made that is produced from the translational Act1-LacZ fusion?

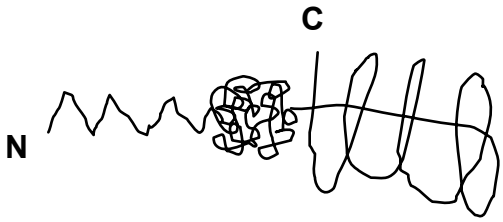
Since the expression of this construct is controlled by the Act1 promoter, you'd expect the product of this gene to be made under the condition of high salt.

(i) Based on the diagrams above, draw the protein that would be produced from the transcriptional Act1-LacZ fusion.

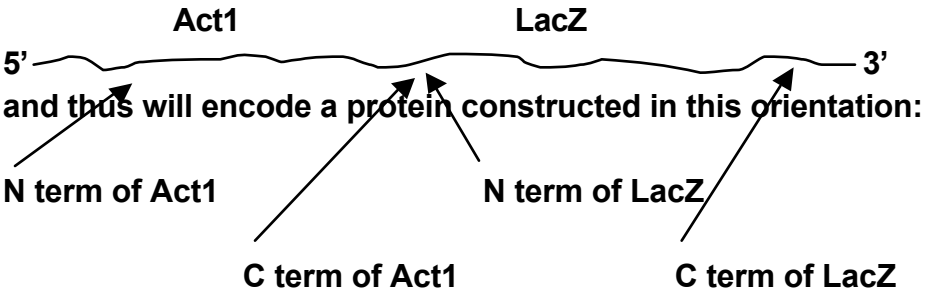


The open reading frame in this reporter gene construct is LacZ. Thus the protein produced by this construct will be identical to LacZ.

(j) Based on the diagrams above, draw the protein that would be produced from the translational Act1-LacZ fusion.



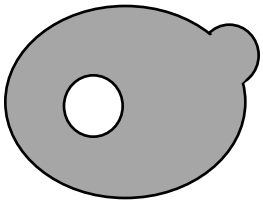
The open reading frame in this reporter gene construct is a fusion of Act1 and LacZ. Thus the protein produced by this construct will be the Act1 protein fused (i.e. covalently linked) to the LacZ protein.
PLEASE NOTE that the directionality with which you draw this fusion protein is very important. ***The 5' end of an mRNA encodes the N terminus of a protein. *
The mRNA produced from this fusion will look like:



KEY

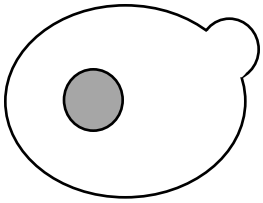
(k) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the transcriptional Act1-LacZ fusion. (To answer parts **(k)** and **(l)**, you must know that most signals used by the cell to direct intracellular protein localization are found at the very N terminus of the protein.)

LacZ will be expressed in the cytoplasm only, since beta-gal is normally used in the cytoplasm and the signal for localizing the beta-gal would still be intact and at the N terminus of the protein produced from the transcriptional fusion. (Most signals used by the cell to direct intracellular protein localization are found at the very N terminus of the protein.)



(l) Below is drawn a budding yeast cell with a nucleus inside the cell. Shade in where the beta-galactosidase enzyme would be found in a yeast cell expressing the translational Act1-LacZ fusion.

In the nucleus only, since Act1 is normally used in the nucleus and the signal for localizing Act1 is at the very N terminus of the protein produced from this translational fusion. (Most signals used by the cell to direct intracellular protein localization are found at the very N terminus of the protein.)



(m) Say that you had a haploid yeast strain that had the endogenous chromosomal copy of Act1 deleted. If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

Yes. Under conditions when Act1 would normally be produced (high salt), now beta-gal would be produced and would be able to cleave X-gal.

(n) If you transformed the act1^{-} haploid yeast strain with the transcriptional Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?

KEY

No. There is no Act1 protein present in these yeast cells, because the chromosomal copy of Act1 has been deleted, and no Act1 protein is produced from the transcriptional fusion. If there is no Act1 protein produced, then there is nothing to activate the Yst1 gene.

(o) If you transformed the act1$^-$ haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to cleave the compound X-gal?

It is possible, yes. LacZ is being produced in these cells, even though the LacZ being produced is fused to Act1. For LacZ to function in the cell, it must be true that having the Act1 protein fused to the LacZ protein doesn't affect the functionality of LacZ. Experimentally, we know that LacZ can function just fine EVEN when it are covalently tethered to other proteins. This may seem counterintuitive, but we know it to be true imperically. NOTE that there is an additional issue involved in answering this question, which is that LacZ protein will now be in the nucleus of the yeast cells. It is also only true that X-gal can be cleaved if X-gal is capable of gaining access to the nucleus. (X-gal normally only needs to gain access to the cytoplasm in normal cells that are expressing LacZ where it should reside, which is in the cytoplasm.)

(p) If you transformed the act1$^-$ haploid yeast strain with the translational Act1-LacZ fusion plasmid, would the strain be able to induce Yst1 gene expression?

It is possible, yes. Act1 is being produced in these cells, even though the Act1 being produced is fused to LacZ. For Act1 to function in the cell, it must be true that having the beta-gal protein fused to the Act-1 protein doesn't affect the functionality of Act1. Experimentally, we know that many proteins can function just fine EVEN when they are covalently tethered to other proteins. This may seem counterintuitive, but we know it to be true imperically.