Problem Sets

Fall 1996

7.03 Problem Set 1

due before 5 PM on Thursday, September 19
Hand in answers in recitation section or in the box outside the class

1. You have just isolated twelve new Met⁻ yeast strains. These mutants will only grow on minimal medium when methionine is included. Six of the mutants were isolated in a strain of mating type α (MATα) and six were isolated in a strain of mating type **a** (MAT**a**). You are interested in finding out how many different genes are represented by your mutants, so you cross each of the MATα strains to each of the MAT**a** strains and test the diploids for the ability to grow on medium without methionine. Being a good geneticist you also include wild-type controls. In the table below, a (+) indicates diploids that <u>can</u> grow on medium without methionine and a (−) indicates diploids that <u>can not</u> grow without methionine.

strains of mating type α

	1	2	3	4	5	6	wild type
7	+	+	+	+	+	+	+
8	+	+	+	+	+	−	+
9	+	+	+	+	+	+	+
10	+	−	+	−	+	+	+
11	+	+	+	+	+	+	+
12	+	+	+	+	+	+	+
wild type	+	+	+	+	+	+	+

strains of mating type **a**

(a) Which mutations do you know to be in the same gene?

(b) What is the minimum number of genes required for methionine synthesis represented by your twelve mutants?

(c) What is the maximum number of genes required for methionine synthesis represented by your twelve mutants?

A colleague of yours isolates twelve more Met⁻ mutants in his own lab. He performs complementation tests among his mutants just as you did. The results of the tests on his mutants are given in the table below.

strains of mating type α

	13	14	15	16	17	18	wild type
19	−	−	−	+	−	−	+
20	−	−	−	+	−	−	+
21	−	−	−	+	−	−	+
22	−	−	−	+	−	−	+
23	+	+	+	+	−	+	+
24	−	−	−	+	−	−	+
wild type	+	+	+	+	−	+	+

strains of mating type **a** (rows 19–24 and wild type)

(d) How do you explain the behavior of mutant 17?

**(e)** Which of his mutations do you know to be in the same gene?

(f) What is the minimum number of genes required for methionine synthesis represented by your colleagues twelve mutants?

(g) What is the maximum number of genes required for methionine synthesis represented by your colleagues twelve mutants?

2. **(a)** You are given a male and a female mouse that have chocolate brown fur. When you cross these mice all of the F_1 progeny have chocolate brown fur. Similarly, crosses among the F_1 mice give F_2 mice that all have chocolate brown fur. What can you say about the alleles governing coat color in the two mice that you were given?

(b) Wild mice have gray fur. When you cross one of the chocolate brown mice to a wild mouse all of the F_1 progeny look like wild mice. What can you say about the relationship between the coat-color alleles in the chocolate brown mice relative to wild mice?

(c) You produce a large number of F_1 progeny by crossing chocolate brown mice to wild mice. Crosses among these F_1 mice give 500 F_2 mice. About half of the F_2 mice look like wild mice, many have coats with intermediate properties, and 5⚬ look just like the chocolate brown grandparents. What is your best guess for the number of coat-color genes that differ between the chocolate brown mice and wild mice.

(d) Evaluate your answer in part **c** by performing three different Chi-square tests. For the first test, use your answer for **c** to derive the expected number of chocolate brown F_2 mice. For the other tests, use one more and one less than your answer for **c** to derive the expected number of chocolate brown mice. Give the p values for each of the tests.

3.   Each of the families below exhibits a different very rare genetic disorder where individuals expressing the disorder are shown by solid symbols. Assume complete penetrance and also assume that no new mutations have arisen in these families. Give the most likely mode of inheritance for each pedigree (autosomal recessive, X-linked recessive, or autosomal dominant). Also indicate the predicted genotypes of each individual in the pedigree using **A** for dominant alleles and **a** for recessive alleles (in ambiguous cases give all possible genotypes). Finally, calculate the probability that the next child indicated by a (?) will be affected.
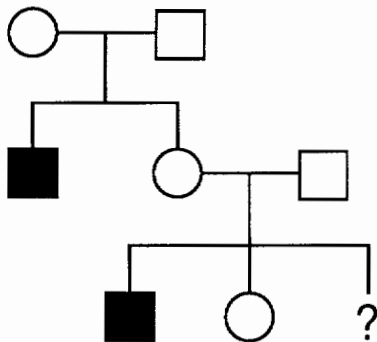
(a)
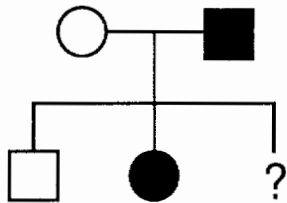
(b)
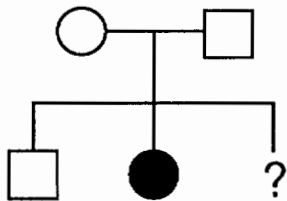
(c)

Problem Set #1 Answers
Biology 7.03

1.   In this problem we are assuming that each mutant has a single gene mutated .

     (a) Mutants 2, 4 and 10 are in the same gene and mutants 6 and 8 are in the same gene because they fail to complement which is the hallmark of two mutations being in the same gene.

     (b) The minimum number of genes is 4. Mutants 2, 4 and 10 represent one gene. Mutants 6 and 8 represent one gene. Mutants 7, 9, 11 and 12 could be in the same gene and mutants 1, 3, and 5 could be in the same gene.

     (c) The maximum number of genes is 9. Mutants 2, 4 and 10 represent one gene and mutants 6 and 8 represent one gene by their failure to complement. However, mutants 7, 9, 11 and 12 although they have the same complementation pattern in the complementation grid may all be in different genes as may mutants 1, 3 and 5. To confirm that they were mutations in the same gene we would need to show that they all fail to complement the same mutation. Only failure to complement is taken as proof of the mutations being in the same gene, not an identical pattern of complementation on the complementation grid.

     (d) Mutant 17 is a dominant mutation because it does not complement the wild type strain.

     (e) Mutants 13, 14, 15, 18, 19, 20, 21, 22 and 24 are in the same gene because they fail to complement each other.

     (f) The minimum number of genes represented by these 12 mutants is 3. We know mutants 13, 14, 15, 18, 19, 20, 21, 22 and 24 to be in the same gene and we also have mutants 16 and 23 which are in different genes. Mutant 17 is a dominant mutation so we cannot tell what gene it is in, so it could be a mutation in one of those 3 genes.

     (g) The maximum number of genes represented by these 12 mutants is 4 if mutant 17 where to be in a different gene then all the other mutants.

2. (a) Since a different coat color does not appear in the progeny, the line is likely to be true breeding, that is homozygous for the chocolate brown fur allele.

(b) The grey coat color allele (wild type) is dominant to chocolate brown, (i.e., brown is recessive to grey).

(c) In the F2 generation we have 5 out of 500 mice that express the recessive trait, that is 1/100. If the number of coat color genes involved is 1, we would expect 1/4 of the F2 generation to be chocolate brown; if there are two genes, one would expect 1/16; if three genes are involved: 1/64; and if there are four, 1/256. 1/100 is closest to 1/64; therefore, the best guess for the number genes involved is three.

(d) Since we do not know the exact number of wild type mice and mice with intermediate properties, assume two phenotypic classes: chocolate brown and non-brown.

Assuming that *three genes* are involved, the expected number of chocolate brown mice is $500 \times 1/64 = 8$. Hence,

	observed	expected
non-brown	495	492
brown	5	8

$\chi^2 = (O - E)^2 / E = (495 - 492)^2 / 492 + (5 - 8)^2 / 8 = 1.14$
degrees of freedom = # classes -1 = 2 - 1 = 1
Using the χ^2 table, we get: $0.1<p<0.5$. That is, the three genes model is well within the limits.

Similarly, for *two genes:*

	observed	expected
non-brown	495	469
brown	5	$31 = 500 \times 1/16$

$\chi^2 = (O - E)^2 / E = (495 - 469)^2 / 469 + (5 - 31)^2 /31 = 23$
df=1
The p value is much lower than 0.005; hence the two gene hypothesis is rejected.

Finally, for *four genes:*

	observed	expected
non-brown	495	498
brown	5	$2 = 500 \times 1/256$

$\chi^2 = (O - E)^2 / E = (495 - 498)^2 / 498 + (5 - 2)^2 /2 = 4.52$
df=1
The p value is less than 0.05 $(0.025<p<0.05)$; hence the four gene hypothesis is also rejected.

3.	(a) The most likely mode of inheritance is autosomal dominant. The mother has a genotype aa, the father Aa, the male child aa and the female child Aa. The probability of the next child being affected is .5, the probability that he/she will get the A allele from his/her father.

(b) The most likely mode of inheritance is autosomal recessive. The mother has a genotype Aa, the father Aa, the male child Aa or AA and the female child aa. The probability of the next child being affected is .25, the probability that he/she will get the a allele from both his/her parents.

(c) The most likely mode of inheritance is X-linked recessive. The mother has a genotype Aa, the father AY the male child aY and the female child Aa. This female child married a male who was AY and had a male child who was aY and a female child who was AA or Aa. The probability of the next child being affected is .25. The probability of the child being affected if female is 0 because the father has a wild type A allele. If the child is male then the probability is .5. We multiple that probability by the probability that the child is male (.5) and get (.5)(.5)=.25.

7.03 Problem Set 2

1. Imagine three closely-linked autosomal recessive *Drosophilia* mutations in genes **a**, **b**, and **c** (for simplicity, we will use these letters to designate the recessive alleles and **+** to designate the corresponding wild-type allele of each gene). The genes are in order **a–b–c** and by two-factor crosses the distance between **a** and **b** is 10 cM and the distance between **b** and **c** is 10 cm. A male from a true-breeding strain that is **a + c** is crossed to a female from a true-breeding strain that is **+ b +**. Female F_1 flies are then test-crossed to males from a true breeding strain of genotype **a b c** and the number of progeny of each phenotype are counted (also for simplicity we will use the letters **a**, **b** and **c** to designate the recessive traits and **+** to designate each of the corresponding wild-type traits). State whether each of the statements below is true or false and give your reason why.

(a) **a + c** and **+ b +** will be the most common phenotypes among the progeny.

(b) **a** and **b** will be recombined in 10% of the progeny.

(c) **a** and **c** will be recombined in 20% of the progeny.

(d) 1% of the progeny will have phenotype **a b c**.

(d) Given that there are no crossovers in meiosis in male *Drosophilia*, if the above experiment were carried out with male F_1 flies, genes **a** and **c** would be completely linked (distance of 0 cM).

2. Wild-type yeast are sensitive to the antifungal compound nystatin and are therefore phenotypically nys^s. To study how nystatin kills yeast cells, you isolate a set of mutants that are resistant to nystatin (nys^r). Six of the mutants were isolated in a strain of mating type α (MATα) and four were isolated in a strain of mating type **a** (MAT**a**). You cross each of the mutants to a wild-type strain of the opposite mating type and you find that all of the resulting diploids are nys^r.

(a) What does this tell you about your nystatin-resistant mutants? Why can't you now perform complementation tests to figure out how many genes are represented by your mutants?

(b) Another way to figure out whether two mutations are in the same gene is by a linkage test. You cross each of the MATα nys^r mutants to each of the MATa nys^r mutants. You sporulate each of the resulting diploids, dissect ten tetrads, and test the resulting spore clones for resistance to nystatin. In the table below a (+) indicates crosses that produced all nys^r spores and a (−) indicates crosses that produced both nys^r spores and nys^s spores. How many genes are represented by the mutations and which mutations appear to be in the same genes.

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

strains of mating type **a**

(c) For the cross of mutant 3 by mutant 9 no nys^s spores were found in ten tetrads. However, when you dissect 90 more tetrads from this cross, you find four tetrads that have three nys^r spores and one nys^s spore. What is the distance between these mutations in cM? Is it possible that these mutations are in the same gene?

3. You have just developed a new method for visualizing in a microscope yeast chromosomes during meiosis. In your investigation of a new yeast species you determine that the diploid yeast has four pairs of homologous chromosomes. In each cell undergoing meiotic division, you see an average of two chiasma per pair of homologous chromosomes.

(a) Assuming that each chiasma leads to a crossover between two of the four chromatids, estimate the total genetic length of the genome of this new yeast in cM.

(b) From physical measurements of the DNA from this new yeast you estimate that the length of the haploid genome is 16×10^6 base pairs. If you isolate two mutations in the same gene that are 1×10^3 base pairs apart, on average how many tetrads would you need to look at before you identified a single tetratype tetrad in a cross between the two mutations?

1. a----b----c
    10cM 10cM

        male <u>a+c</u> X female <u>+b+</u>
             a+c   +b+
                   |
                   \/

         F1: female <u>a+c</u> X male <u>abc</u>
                    +b+   abc
                          |
                          \/
                          F2

a)TRUE. a+c and +b+ are the phenotypes associated with no crossovers, so they will be the most common.
b)TRUE. 10cM = 10%recombinant progeny
c)FALSE.

        a-\/-b----c   10% chance of recombination between
        a-/\-b----c   a and b

        a----b-\/-c   10% chance of recombination between
        a----b-/\-c   b and c

However...

        a-\/-b-\/-c   1% chance of having a double crossover
        a-/\-b-/\-c   (10%chance between a-b)x(10% chance between b-c)

Thus, the chance that a and c will be recombined is:
    10%+10%-1%=19%

d)FALSE. Again, the chance of a double crossover will be 1%. This means that 0.5% of the progeny will be abc and 0.5% will be +++. *TRUE.*
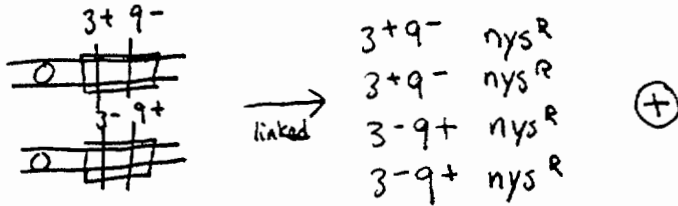e)~~FALSE.~~ ~~██████████████████████████.~~ Using the formula, you will calculate a distance of 0cM since there will be no recombinants (no crossovers). However, you cannot measure the *physical* distance between genes using recombinant mapping in organisms with no crossovers.

2.a) Since all <u>nys^r</u> have nysr phenotype, nys^r is dominant.
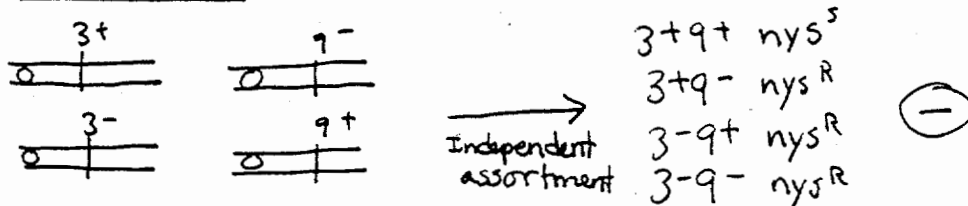   nys^s
   ->complementation test cannot be used for dominant alleles

b) + = all nys^r spores = same gene
  - = some nys^r, some nys^s spores = different genes

10

Same Gene:

$3^+ 9^-$

$3^- 9^+$

$\xrightarrow{\text{linked}}$

$3^+ 9^-$ nys^R
$3^+ 9^-$ nys^R
$3^- 9^+$ nys^R
$3^- 9^+$ nys^R

$\oplus$

Different Gene:

3^+ 9^-

3^- 9^+

$\xrightarrow[\text{assortment}]{\text{Independent}}$

$3^+ 9^+$ nys^S
$3^+ 9^-$ nys^R
$3^- 9^+$ nys^R
$3^- 9^-$ nys^R

$\ominus$

Therefore, there are 2 genes:(2,4,7,8,10) and (1,3,5,6,9)

10

c) 3nys^r:1nys^s -> Tetratype

Map Distance = $\dfrac{T + 6NPD}{2(\text{tot. tetrads})}$ X 100 = $\dfrac{4 + 0}{200}$ X 100 = 2cM $\big)$ 8

RF for yeast = 293.3cM/10^{6}bp

so... 2cM/(293.3cM/10^{6}bp) = 6,800bp ->probably not on the same $\big)$ 2
                                                        gene

3.a) # chiasma = # crossovers = 8 crossovers = x/50cM
   (since 1 crossover = 50cM)

$$\text{--> } x = 400\text{cM}$$

(handwritten: 10)

(handwritten: $=0-1$: 7 points)

(handwritten: 3 points)

b) $\dfrac{y}{1 \times 10^6 \text{bp}} = \dfrac{400\text{cM}}{16 \times 10^6 \text{bp}}$

(handwritten: 20)

$$\text{--> } y = 0.0250\text{cM apart}$$

(handwritten: 6p – R.F.)

(handwritten: 6 pt — 1000 bp)

Plug into mapping function:

$$cM = 100 \times \frac{T + 6NPD}{2(\text{tot.tetrads})}$$

Because it is a small distance, we will ignore double crossovers.(ie:T>>NPD)

$$cM = 100 \times \frac{T}{2(\text{tot.tetrads})}$$

$$0.0250 = 100 \times \frac{T}{2(\text{tot.tetrads})}$$

$$5 \times 10^{-4} = \frac{T}{\text{tot.tetrads}}$$

(handwritten: 8)

to get T = 1, tot. tetrads = **2,000 tetrads.**

7.03 Problem Set 3

Note: Question #1 was
purposely omitted.

2. As with higher organisms, three-factor crosses can be used to determine the order of closely linked mutations in phage. A cl⁻ mutant of phage λ makes clear plaques. You have isolated two different mi⁻ mutants that make small plaques. To map these mutants, you first do two-factor crosses between pairs of mutants to get a general idea of the arrangement of the mutant sites. First, you infect a host with one of the mi⁻ mutants together with the cl⁻ mutant and then plate the resulting phage and examine the morphology of 100 of the plaques that are produced. In the table below the numbers of each type of plaque are given for each of the crosses.

mi-1 x cl⁻

Plaque Type	Number
small, turbid	45
large, clear	41
small, clear	6
large, turbid	8

mi-2 x cl⁻

Plaque Type	Number
small, turbid	50
large, clear	38
small, clear	4
large, turbid	8

(a) Give the distances between mi-1 and cl and between mi-2 and cl. Is the difference between these distances statistically significant?

(b) When the mi-1 mutant is crossed to the mi-2 mutant, 100 plaques are examined and one large plaque (the mi⁺ phenotype) is found. What is the approximate distance between mi-1 and mi-2 in map units?

(c) For a three-factor cross, a cl⁻ mi-1 phage is crossed to a cl⁺ mi-2 phage. Of 1000 plaques examined, there are 14 that are large. Although about half of the total plaques are clear, all 14 of the large plaques are turbid. What is the order of cl, mi-1, and mi-2?

3.   In a transduction experiment, phage P1 is grown on a bacterial host of genotype $A^+ B^+ C^+$ and the resulting lysate is used to infect a recipient strain of genotype $A^- B^- C^-$. The markers are in order $A - B - C$ and the distance between A and B is greater than the distance between B and C. Transductants that are A^+ are selected. State whether each of the following statements is true or false and why.

(a)   If none of the A^+ transductants were also C^+ then the distance between A and C would be greater than about 50 kbp.

(b)   The distance between A and B can be obtained from the fraction of A^+ transductants that are $A^+ B^+ C^+$ and $A^+ B^+ C^-$.

(c)   The number of A^+ transductants that are C^+ will be greater than the number of A^+ transductants that are B^+.

(d)   The number of A^+ transductants that are B^+ and C^- will be much greater than the number of A^+ transductants that are B^- and C^+.

7.03 Problem Set 3 Solutions

2.(a) genetic distance = (recombinant plaques / total plaques) X 100 m.u.

The two recombinant plaque phenotypes are small, clear and large, turbid, so the distance for **mi-1** is (6+8)/100 X 100 m.u. = **14 m.u.**and for **mi-2** (4+8)/100 X 100 m.u. = **12 m.u.**

Given that each distance is determined using only a sample size of 100 plaques, the difference between 12 and 14 m.u. is **not significant**. One might claim this simply by inspection, or one could perform a more rigorous statistical test such as a Chi-square test.

A Chi-square test could be set up in a number of ways. If you are asking the question, "is 14 m.u. really different from 12 m.u.?", you could see if the observed values for mi-1 (14 m.u.) fit the expected ratio of 12 recombinants out of 100 plaques.

For the mi-1 X cl⁻ cross:

class	observed	expected	$(O-E)^2/E$
recombinant	14	12	0.333
parental	86	88	0.045

$X^2 = 0.333 + 0.045 = 0.378.$ For df = 2-1 = 1, then $0.5 < p < 0.9$.

We cannot reject the model, thus the data for mi-1 fits a model of 12 m.u. just as well as a model of 14 m.u.

The X^2 test could also have been performed using expected ratios for 14 m.u. and observed values for mi-2 (12 m.u.) with similar results. Finally, the test can also be performed using 4 classes (small & turbid, large & clear, small & clear, small & turbid) instead of 2 classes (recombinant vs. parental). In all of these cases, the p-value is much greater than the required 5%:

Expected	Observed	# classes	df	X^2
14 m.u.	12 m.u.	2	1	0.332
12 m.u.	14 m.u.	4	3	0.894
14 m.u.	12 m.u.	4	3	3.149

2.(b) the large plaque is one of the two possible recombinant phenotypes form a recombination event between mi-1 and mi-2. Thus the actual number of recombinant plaques is 2 X the number of large (mi$^+$) plaques.

genetic distance (mi-1 to mi-2) = (2 X 1)/100 X 100 m.u. = **2 m.u.**

2.(c) **THE GENE ORDER IS cl -- mi-1 -- mi-2.** Of the 14 large recombinants (i.e.- recombinants between mi-1 and mi-2), all are turbid. If the gene order was cl--mi-2--mi-1, then the large turbid recombinants require a double crossover. If the gene order was cl--mi-1--mi-2, then only a single crossover is required to produce large turbid recombinants. The second case is more likely.

Note: cl- is clear, and cl+ is turbid.

Note: among the large recombinants, none are clear. Thus large and clear is the RAREST (double crossover) class.

3.(a) **FALSE.** Zero percent cotransduction would indicate a distance of greater than about 100 kbp as this is the maximum length of DNA that can be packaged into the P1 phage.

3.(b) **TRUE.** These two classes represent all the A+B+ cotransductants.

3.(c) **FALSE.** Since the order of markers is A-B-C, A$^+$ B$^+$ cotransduction is more likely than A$^+$ C$^+$ cotransduction. Gene distances and cotransduction frequencies are inversely related.

3.(d) **TRUE.** A$^+$ B$^+$ C$^-$ transductants result from a double crossover, one before A, and the other crossover between B and C. This is more likely than A$^+$ B$^-$ C$^+$ transductants, which require a quadruple crossover (one crossover in each of the 4 intervals).

7.03 Problem Set 4

1. An Hfr donor whose genotype is $A^+ B^+ C^+$ is mated to a recipient whose genotype is $A^- B^- C^- Str^s$. the order of transfer is A — B — C. None of these genes is transferred early and they are not near the Str^r marker. The distance between A and B is greater than the distance between B and C. Recombinants are selected by plating on media that contains streptomycin and that lacks particular nutrients. State whether each of the following statements is true or false and why.

(a) After mating is carried out for a long time the number of $A^+ Str^r$ recombinants will be greatr than the number of $B^+ Str^r$ recombinants.

(b) All of the $C^+ Str^r$ recombinants will also be B^+.

(c) The number of $A^+ B^- C^+ Str^r$ recombinants will be less than the number of $A^+ B^+ C^+ Str^r$ recombinants.

(d) The number of $A^+ B^+ C^- Str^r$ recombinants will be less than the number of $A^+ B^- C^- Str^r$ recombinants.

(e) Most of the $A^+ B^+ C^- Str^r$ recombinants will themselves be Hfrs.

2. (a) Consider a portion of the *E. coli* chromosome that contains three genetic markers in the following order: HisB — LeuC — TrpA. Starting with an F^+, $HisB^+$, $LeuC^+$, $TrpA^+ Str^s$ strain, you isolate an Hfr that transferrs $TrpA^+$ very efficiently but transfers the $HisB^+$ and $LeuC^+$ markers inefficiently. Draw a map of the relevant portion of the *E. coli* chromosome in the Hfr showing the integrated F (indicate both the site of integration and the orientation of the origin of transfer).

(b) You want to isolate an F' from the Hfr described in part **(a)**. To do this you mate the Hfr to a to an F^-, $HisB^-$, $LeuC^-$, $TrpA^- Str^r$ strain and select for $HisB^+$ and Str^r after a brief period of mating. Why was it better to select for $HisB^+$ than $TrpA^+$? Would you expect the F's that are isolated to be able to transfer $TrpA^+$ (explain your answer)? What is the purpose of including the selection for Str^r?

(c) The F' strains isolated in part **(b)** all transfer both HisB⁺ and LeuC⁺ efficiently. If the F's had been isolated by selecting for LeuC⁺ (rather than HisB⁺) would you expect all of them to effeciently transfer HisB⁺? Explain why.

(d) By mating, one of the F' strains isolated in part **(b)** is introduced into an F⁻, HisB⁻, LeuC⁻, TrpA⁺ strain. From this new F' strain an Hfr is isolated that can transfer TrpA⁺ early. Draw out the three different recombination events that could give such an Hfr. For each of the three types of recombination, indicate whether HisB⁺ would be transferred early or late and whether LeuC⁺ would be transferred early or late, by the resulting Hfr.

(e) You have isolated a new HisB⁻ mutation in an F⁻, LeuC⁻ strain. You introduce into this strain an F' that carries HisB⁺ LeuC⁺ by mating to one of the F' strains isolated in part **(b)**. Strains that have recieved the F' are selected by selection for LeuC⁺. To your suprise you notice that these strains that carry both the new HisB⁻ mutation and the F' are not HisB⁺. Give a simple genetic explanation for this observation.

3. The rhmJ (or J) gene is one of the structural genes involved in metabolism of rhammnose (rhm) in *Shigella problematica*. The closely linked genes L and N encode regulators of the J gene. You have a collection of mutants that produce abnormal levels of the J protein. You assay the levels of the J gene product in these mutants to obtain the following data:

<u>levels of J⁺</u>

	no rhm added	rhm added
J+ L+ N+	5	50
J+ L+ N-	50	50
F' L+ N+ / J+ L+ N-	5	50
J+ L1- N+	<0.1	<0.1
F' L+ N+ / J+ L1- N+	5	50
J+ L2- N+	50	50
F' L+ N+ / J+ L2- N+	50	50

Note: L1 and L2 are two different mutations in the L gene.

(a) What is the role of the N gene in controlling expression of J?

(b) What is the role of L? Explain the behavior of both the L1 and the L2 mutations.

(c) What phenotype do you expect of a null mutation in the L locus?

(d) You isolate a new mutant (K-) that is very closely linked to J. When you assay for levels of J+, you obtain the following results:

<div align="center">

levels of J$^+$

</div>

	no rhm added	rhm added
K- J+	<0.1	<0.1
F' K+ J+ / K- J-	5	50
F' K-J+ / K+ J-	<0.1	<0.1

Explain the behavior of the K mutation.

7.03 Problem Set 4
Solutions

1. (a) **True.** Because of the stochastic breaking of the bridge between the donor and the recipient cell, the final number of exconjugants will always be bigger for a marker transferred earlier.

(b) **False.** A quadruple crossover could result in B-C+ recombinant.

(c) **True.** To get an A+B-C+ recombinant, you need four crossovers, but to get an A+B+C+ recombinant two crossovers are sufficient. Four crossovers are less likely than two.

(d) **True.** Starting from the origin of transfer, an A+B+ recombinant need a larger piece of donor DNA transferred into the recipient cell, which will happen less frequently.

(e) **False.** Fertility is very rarely transferred from an Hfr strain. For that to happen, the entire bacterial chromosome would need to be transferred.

2. (a)
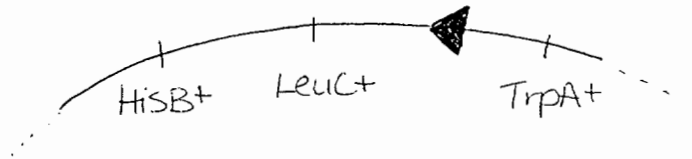
(b) It is better to select for HisB+ than for TrpA+ because if you were to select for TrpA+ you could get a simple transfer of TrpA+ from the Hfr instead of making an F'.

Since you did not select for TrpA+, you would not expect the F's you isolate to carry TrpA+ because usually chromosomal sequences from only one side of the incorporated F factor are looped out to make the F'.

The selection for Str^r is to kill the donor strain.

(c) If the F's had been isolated by selecting for LeuC+ you would not expect all of them to transfer HisB+, because if the recombination event to generate the F' occurred between HisB and LeuC, the F' would include LeuC+, but not HisB+.

(d)
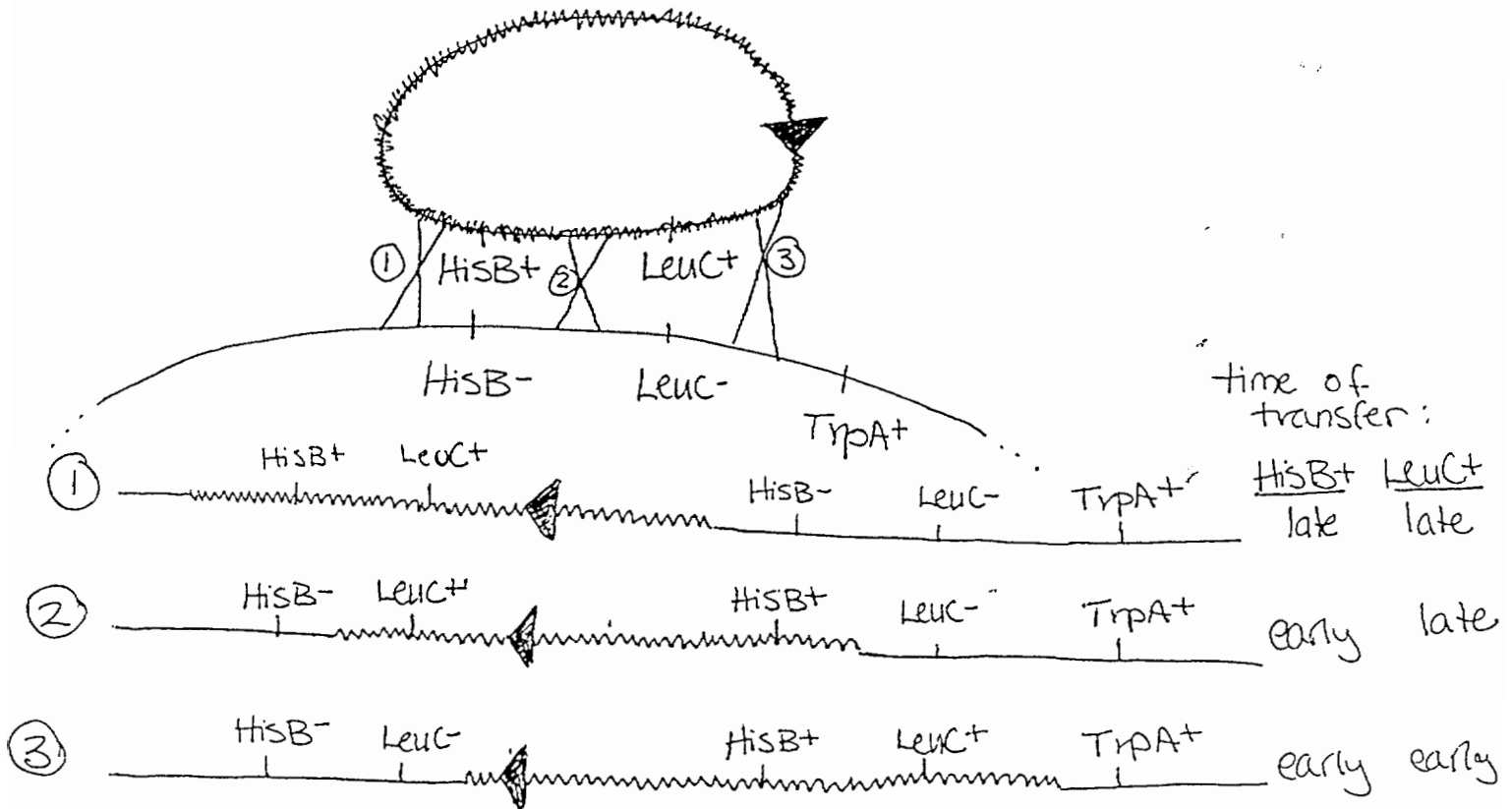
(e) The new HisB- mutation is dominant to the wild type HisB+.

3. (a). A recessive mutation in N causes constitutive expression of J; hence the N gene encodes a **repressor** of J expression.

   (b) L is an **activator** of J expression. The L1 mutation is recessive and likely to be loss of function. This mutation causes uninducible expression. The L2 mutation is dominant, most likely a gain of function mutation, and the phenotype is constitutive expression of J.
       For your information:
       L could activate J expression directly (model 1), or could be a repressor of repressor N (model 2). To distinguish between these two possibilities, one would need to perform an epistasis analysis. If the phenotype of an L1 N-double mutant is constitutive J expression, then N is epistatic to L, and the more likely model is that L is a repressor of repressor. If the phenotype of the double mutant is uninducible, then L is epistatic, and is more likely to activate J expression directly.

model 1:
```
               L------------>
                             J expression
               N------------/
```

or: N-----------/ L---------> J expression

model 2:   L-----------/ N--------/ J expression

(c) Null mutations are loss of function. Hence the mutation would be like the L1 mutation: recessive and uninducible.

(d) K- is a cis-acting mutation that makes J expression uninducible. The mutation is either in the promoter or in the DNA site to which an activator protein binds.
The mutation could also be a polar mutation in the coding sequence upstream of J in the same operon. However, this topic has not been covered in lecture, so you are not responsible for this information.

7.03 Problem Set 5

Question 1.

For this question, you must show your work to obtain full credit. Express your answers as fractions (rather than as decimals or percents).

Consider a gene regulated by negative control by a repressor R. R^{-d} (dominant negative) mutants are obtained and introduced into an F^- R^+ strain on an F'.

1.a. What is the fraction of functional repressor in the partial diploid assuming that it is a dimer and the heterodimer is non-functional. What if the heterodimer is functional?

1.b. Carry out the same analysis as in part 1.a. assuming the repressor acts as a tetramer (4 molecules of R are required to make a repressor molecule).

1.c. A second copy of the R^{-d} is introduced into the partial diploid strain on a specialized transducing phage. Carry out the same analysis as in parts 1.a. and 1.b. for this strain.

Note: For questions 2 and 3, use genetic terms in describing your models and refrain from using molecular descriptions when possible. In describing regulatory mutants, indicate whether they are dominant or recessive, cis or trans acting, and whether they result in constitutive or uninducible phenotypes. From these properties you should be able to deduce if the regulatory mutations are in proteins or DNA elements and whether they are in positive or negative regulators of transcription.

Note: Question #2 was purposely omitted.

Question 3.

You are studying a yeast fatty acid desaturase (FADS) gene which is regulated as shown in the table below. When cells are grown at 15°C, the gene is induced 100-fold by the addition of saturated fatty acids to the media. When grown at 30°, addition of saturated fatty acids only induces the gene 2-fold.

3.a. A mutation, a-, is isolated with shows no induction of FADS at either temperature. A diploid strain A+/a- shows normal induction at both temperatures, as shown in the table. Propose a role for the A gene in the induction of FADS.

3.b. A second mutation, c-, also shows poor induction. Diploid strain heterozygous for C+/c- and FADS+/fads- were also assayed; the results are shown in the table. Propose a role for C in the regulation of FADS. Predict a map position for C as part of your answer.

3.c. Another mutation, b-, allows equally robust induction at 30°C as at 15°C. The B+/b- diploid behaves like wild type in all respects. Propose a role for B in the regulation of FADS.

3.d. Yet another mutation, e-, supports only poor induction at both temperatures. The diploid E+/e- behaves like wild type. Propose a role for E in the regulation of FADS. Propose two models for the relationship between B, E, and temperature in this regulation.

3.e. Propose a simple genetic experiment to distinguish between your two models from part d, and explain what result would be consistent with each model.

level of FADS RNA

Strain	Temp (°C)	- sat. fatty acids	+ sat. fatty acids
WT	15	10	1000
	30	10	20
a-	15	10	10
	30	10	10
A+/a-	15	10	1000
	30	10	20
c-	15	10	50
	30	10	10
C+fads-/c-FADS+	15	10	50
	30	10	10
C+FADS+/c-fads-	15	10	1000
	30	10	20
b-	15	10	1000
	30	10	1000
B+/b-	15	10	1000
	30	10	20
e-	15	10	20
	30	10	20
E+/e-	15	10	1000
	30	10	20

Problem Set 5 - Solutions

1a.) The probability of having the dominant allele or the WT allele as part of a dimer is 1/2. Therefore the probability of having two dominant alleles or two W.T. alleles in a dimer is 1/2 x 1/2 = 1/4. The probability of having the heterodimer is 2(1/2 x 1/2)=1/2. Therefore if the heterodimer is non-functional then the fraction of functional repressor is 1/4. If the heterodimer is functional then the fraction of functional repressor is 3/4.

1b.) As in a.) p(WT allele or dominant allele as part of a tetramer) = 1/2. Therefore p(tetramer is 4 dominant alleles or 4 WT alleles)=1/2x1/2x1/2x1/2=1/16. p(heterotetramer with 3 WT or 3 dominant alleles and 1 of the other allele)=4(1/2x1/2x1/2x1/2)=1/4. p(heterotetramer with 2 WT alleles and 2 dominant alleles)=6(1/2x1/2x1/2x1/2)=3/8. Therefore if none of the heterotetramers is functional, the fraction of functional repressor is 1/16. If one assumes that all of the heterotetramers are functional, the fraction of functional repressor is 1/16+1/4+1/4+3/8=15/16. Assuming that at least 3 WT alleles must be present to make a functional repressor gives 1/16 + 1/4= 5/16 functional repressor. Assuming that at least 2 WT alleles must be present in a functional heterotetramer gives 3/8 + 1/4 + 1/16= 11/16 functional repressor.

1c.) p(WT allele as part of tetramer) = 1/3. p(dominant allele as part of tetramer) = 2/3.
     As in part a): p(dimer all WT)=1/3x1/3=1/9. p(heterodimer)=2x1/3x2/3=4/9. p(dimer all Dom.)=2/3x2/3=4/9. Therefore if the heterodimer is non-functional then the fraction of functional repressor=1/9. If the heterodimer is functional then the fraction of functional repressor = 5/9.
     As in b): p(Tetramer is composed of all WT alleles) = 1/3x1/3x1/3x1/3= 1/81. p(Tetramer is composed of all dominant alleles) = 2/3x2/3x2/3x2/3= 16/81. p(3 Dom:1 WT)= 4(2/3x2/3x2/3x1/3)= 32/81. p(3WT:1Dom.)= 4(1/3x1/3x1/3x2/3)= 8/81. p(2WT:2Dom.) = 6(1/3x1/3x2/3x2/3)= 24/81. Therefore if the heterotetramers are non-functional the fraction of functional repressor is 1/81. If one assumes that all the heterotetramers are functional then then 32/81+24/81+8/81+1/81= 65/81 functional repressor. Assuming 3 WT alleles needed for functional heterotetramer gives 8/81+1/81= 9/81 functional repressor. Assuming 2 WT alleles needed for functional repressor gives 24/81+8/81+1/81 = 33/81 functional repressor.

3a.) A is a positive regulator of the FADS gene in the presence of saturated fatty acids. The mutant fails to show induction and is recessive. A could be a trans-acting protein or a cis-acting DNA element. We have no cis-trans complementation data to distinguish.

3b.) C is a binding site for a transcriptional activator which could be, but does not have to be, A. The mutant is severely impaired in fatty acid induction, and the mutation is cis-dominant. Thus C could be in the promoter region or in an upstream activating sequence (UAS) of the FADS gene.

3c.) B must be involved in negative regulation of FADS transcription at 30°C, because the mutant is induced to levels only seen at 15°C. The mutation is recessive, so we are dealing with a trans-acting factor (because this is negative regulation). Thus B encodes a repressor of FADS expression at 30°C. Note: This does not mean necessarily that B is a direct repressor, but it is, genetically speaking, a repressor.

3d.) E is another recessive mutation mutation. In the mutant transcription is induced only to the levels seen normally at 30°C. Therefore E is responsible for the additional induction seen at 15°C and, genetically speaking, can be considered a positive regulator. The data is consistent with E being cis or trans acting (because this is positive regulation one cannot distinguish).

Two models for the relationship of E, B, and temperature are:

i)

at 15°C

E--------| B-----------| FADS

ii)

at 30°C

B-------| E-----------> FADS

3e.) To differentiate between these models, you want to look for <u>epistasis</u>. Construct an e-b- double mutant and analyze FADS RNA levels upon fatty acid induction at 15°C & 30°C. If they are both 1000, as in b- then B is epistatic and model i is correct. If they are both 20, as in e-, then E is epistatic and model ii is correct.

Note: Problem Set #6 was
purposely omitted.

Note: Questions #1 + #2 were
purposely omitted.

3. a) Imagine a large human population with a recessive trait that greatly increases the ability of the body to store fat. Knowing that 1 in 90,000 individuals within the population have the trait and that mating within the population is random, give the allele frequency for the trait and the frequencies of the three genotypes.

b) Assume that the fitness of the individuals that have the recessive trait is 0.1. What mutation frequency would be needed to give the observed allele frequency in the population?

c) Now suppose that a period of famine ensues so that the fat-storing trait becomes advantageous in the <u>heterozygous</u> state. If the fitness of the heterozygote increases by 3%, what will the new allele frequency for the trait eventually become when the selective disadvantage for the homozygote is balanced by the selective advantage of the heterozygote?

d) Now suppose that a new high fat food source is developed which everyone in the population begins to eat. The fitness of homozygotes with the fat storing trait becomes 0 because of premature heart attacks and the fitness of heterozygotes is the same as for homozygous normal individuals (i.e. there is no longer a heterozygote advantage). In the first generation after initiation of the high fat diet what will be the fractional reduction in the allele frequency? About how many years will it take for the allele frequency to fall by a factor of two given a generation time of 25 years?

3a. Assuming Hardy-Weinberg equilibrium is in effect:

1/90,000 people are homozygous recessive (let q be the frequency for the mutant allele

$q^2 = 1.1 \times 10^{-5}$ $q = 3 \times 10^{-3}$

Since q is so small we can assume that p (the frequency of the wild type allele is about 1.

Therefore the frequency of homozygous wild type individuals is also about 1 (.994 for the math fans)

And the heterozygous individuals are f(aA) = 2pq = 2q = .006

3b. if fitness of the homozygous individuals is .1 then S= .9

$q = \sqrt{\frac{\mu}{S}}$ $3 \times 10^{-3} = \sqrt{\frac{\mu}{.9}}$

so $\mu = 8.1 \times 10^{-6}$

3c. in the case of a balanced polymorphism q=h/S so q= $\frac{.03}{.9}$ = .033

3d. After selection the loss of q due to selection is $-S q^2$ which here is just $q^2 = .0011$

So in one generation q = .032

$2 q = .031$ 18 .019

3   .03   19 .0185

4   .029   20 .0182

5   .028   21 .0189

6. .027   22 .0178

5   .026   23 .0173

6   .025   24 .0170

7   .0245   25 .0167

8   .0240   26 .0164

9   .0235   27 .0161

10 .023   phew! -

11 .0225   27 generation (50 yes apop

12 .022   = 1,350 years

13 .0215

14 .0210

15 .0205

16 .02

17 .0195