

Name: _____KEY_____

7.03 Final Exam -- 2005 KEY

Name: _____KEY_____

The exam starts at 9 am and ends at 12 pm.

There are 18 pages including this cover page.

Please write your name on each page.

Only writing on the front of every page will be graded.

Question 1 **24 pts**_____

Question 2 **26 pts**_____

Question 3 **20 pts**_____

Question 4 **24 pts**_____

Question 5 **24 pts**_____

Question 6 **22 pts**_____

Question 7 **34 pts**_____

Question 8 **26 pts**_____

TOTAL **out of 200**_____

1. (24 pts) You are studying three autosomal mutations in flies. Each of these three mutations lies in a different gene. All three genes lie on the same autosome. The $wn1^-$ mutation is recessive and causes the phenotype of short wings (wild-type flies have long wings). The $wn2^-$ mutation is recessive and also causes the phenotype of short wings. The ey^- mutation is dominant and causes the phenotype of small eyes (wild-type flies have big eyes). You cross true-breeding $wn1^- wn2^-$ short-winged females to true-breeding ey^- males to obtain an F1 generation. You then cross female F1 flies to true-breeding $wn1^- wn2^-$ big-eyed males. You analyze the resulting progeny, and find that there are flies in the progeny from all four phenotypic classes:

- Short wings Small eyes
- Long wings Small eyes
- Short wings Large eyes
- Long wings Large eyes

For parts (a) – (c), write out complete genotype(s) and phenotype(s) of the flies we ask for. By complete genotype, we mean the genotype at all loci discussed in the problem. By complete phenotype, we mean the phenotype at all traits discussed in the problem. If there are multiple answers, write ALL POSSIBLE answers. Use “+” to indicate wild-type alleles.

(a, 6pts) Write out complete genotype(s) and phenotype(s) of both parents.

	Phenotype	Genotype
P generation mother	Short wings, big eyes	<u>$wn1^- wn2^- ey^+$</u> $wn1^- wn2^- ey^+$
P generation father	Long wings, small eyes	<u>$wn1^+ wn2^+ ey^-$</u> $wn1^+ wn2^+ ey^-$

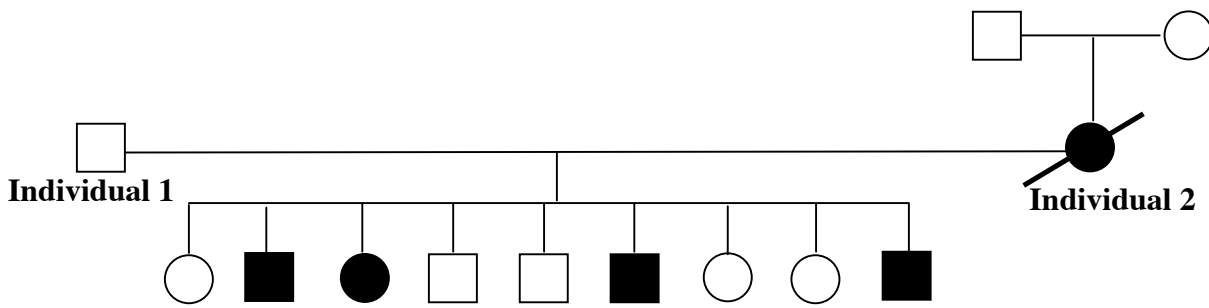
(b, 6pts) Write out complete genotype(s) and phenotype(s) of F1 flies.

	Phenotype	Genotype
F1 generation (mother of F2)	Long wings, small eyes	<u>$wn1^+ wn2^+ ey^-$</u> $wn1^- wn2^- ey^+$
Father to whom you cross the F1 mother	Short wings, big eyes	<u>$wn1^- wn2^- ey^+$</u> $wn1^- wn2^- ey^+$

2. (26 pts) You are studying a new species of primate that is diploid, and has four pairs of autosomes. You have found a rare autosomal recessive disease that is lethal in old age, and is prevalent in a primate family living in the wild. The mother (Individual 2) has already died from this disease. You want to find the genetic locus responsible for this disease, and decide to use SSR mapping to do so. Your first step is to determine which chromosome the locus responsible for the disease is located on. You have access to blood samples of all living members of the family, and you use these blood samples to genotype each living member of the family at four SSRs:

- SSR12, on chromosome 1
- SSR13, on chromosome 2
- SSR14, on chromosome 3
- SSR17, on chromosome 4

The pedigree of the primate family, and the SSRs possessed by each family member, are shown in the chart below. Assume complete penetrance and no new mutations.



<u>SSR</u>	1	2	3	4	5	6	7	8	9	10	11	12	13
12	AB	AA	AA	AB	BB	AB	AA	BB	AA	BB	AB	AB	AB
13	BC	AC	BB	BB	AC	AC	BB	AC	BC	BB	AA	AB	BB
14	AC	AB	BC	BC	AB	AB	AB	BC	BC	AB	BC	B?	AB
17	AB	AB	AB	AB	AB	BB	BB	AB	BB	BB	AB	B?	BC

(a, 4pts) Fill in the empty column of the chart, which indicates the deceased mother's genotypes at each of the four SSRs. Indicate any ambiguous alleles with a question-mark (?).

(b, 4pts) If you want to determine the LOD score for this family for the locus responsible for the disease and one SSR, which parent(s) would be relevant (Individual One, Individual Two, or both)?

Individual One, because he is heterozygous at all loci involved.

(c, 4pts) Do you know the phase of the parent(s) you listed in part **(b)**?

No, because you do not know his parents.

For parts **(d)** and **(e)**, calculate the LOD score at $\theta = 0.1$ for this family for the locus responsible for the disease and each of the following SSRs. For each LOD score, clearly write the expression you used to calculate the LOD score.

(d, 7pts) the locus responsible for the disease and SSR12.

$$\log \frac{(1/2) (0.45)^3 (0.05)^4 + (1/2) (0.05)^3 (0.45)^4}{(0.25)^7} = -1.33$$

Since you do not know the genotypes of the parents of Individual One, there are two possible phases for Individual One. We will call + the allele that does not confer the disease, and - the allele that does confer the disease.

Phase One	Phase Two
-B from father = parental	-B from father = recombinant
+A from father = parental	+A from father = recombinant
-A from father = recombinant	-A from father = parental
+B from father = recombinant	+B from father = parental

- The first child received +A
- The second child received -A
- The third child received - and ???
- The fourth child received +B
- The fifth child received + and ???
- The sixth child received -A
- The seventh child received +B
- The eighth child received +A
- The ninth child received -B

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****You cannot tell whether children three and five are recombinants or parentals, because you cannot tell which allele was given to these children by Individual One. Thus these meioses are not informative, and cannot be included in the analysis.****

**For phase one, there are three parental children and four recombinant children.
For phase two, there are four parental children and three recombinant children.**

**Since $\theta = 0.1$, this means you'd expect 10% recombinants and 90% parentals.
The chances of Individual One creating each type of gamete are:**

Phase One	Phase Two
-B from father = 45%	-B from father = 5%
+A from father = 45%	+A from father = 5%
-A from father = 5%	-A from father = 45%
+B from father = 5%	+B from father = 45%

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

The LOD score is the log of the ratio of the odds (linkage over un linkage):

$$\log \frac{(1/2) (0.45)^3 (0.05)^4 + (1/2) (0.05)^3 (0.45)^4}{(0.25)^7}$$

(e, 7pts) the locus responsible for the disease and SSR13.

$$\log \frac{(1/2) (0.45)^9 (0.05)^0 + (1/2) (0.05)^9 (0.45)^0}{(0.25)^9}$$

Since you do not know the genotypes of the parents of Individual One, there are two possible phases for Individual One. We will call + the allele that does not confer the disease, and - the allele that does confer the disease.

Phase One	Phase Two
-B from father = parental	-B from father = recombinant
+C from father = parental	+C from father = recombinant
-C from father = recombinant	-C from father = parental
+B from father = recombinant	+B from father = parental

Name: _____KEY_____

The first child received +C
The second child received -B
The third child received -B
The fourth child received +C
The fifth child received +C
The sixth child received -B
The seventh child received +C
The eighth child received +C
The ninth child received -B

For phase one, there are nine parental children.
For phase two, there are nine recombinant children.

Since $\theta = 0.1$, this means you'd expect 10% recombinants and 90% parentals.
The chances of Individual One creating each type of gamete are:

Phase One	Phase Two
-B from father = 45%	-B from father = 5%
+C from father = 45%	+C from father = 5%
-C from father = 5%	-C from father = 45%
+B from father = 5%	+B from father = 45%

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

The LOD score is the log of the ratio of the odds (linkage over un linkage):

$$\log \frac{(1/2) (0.45)^9 (0.05)^0 + (1/2) (0.05)^9 (0.45)^0}{(0.25)^9}$$

3. (20 pts) Hemophilia is an inherited bleeding disorder. People with hemophilia lack the ability to clot because of an absence in their blood of clotting factors, which are proteins necessary for clotting. There are several types of hemophilia; one common form is hemophilia A. Hemophilia A is an X-linked recessive disorder possessed by people who lack any functional clotting factor VIII. You want to create a genetically engineered mouse model for hemophilia A. Mice have a homolog of the human gene encoding clotting factor VIII; the mouse homolog also lies on the X chromosome. You are interested to see whether a mouse lacking functional clotting factor VIII will show the same phenotype as a human lacking functional clotting factor VIII.

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(a, 8pts) You want to create a genetically engineered mouse model for hemophilia A. **For the mouse you make**, please state:

- i) whether you are using pronuclear injection **or** gene targeting
- ii) what **DNA** you would introduce into the mouse cells (also draw the DNA)
- iii) what is the **genotype** of the fertilized egg or the ES cells you would start with
- iv) which **additional breeding** steps you would do to make the mouse you wanted
- v) **two possible** phenotypic results you could get from the newly made mice, **and** the corresponding conclusion you would make for each result

i) gene targeting, because you are trying to remove information from the genome

ii) the gene that encodes clotting factor VIII, but with a drug resistance gene inserted in the middle of it

iii) wild-type ES cells

iv) The mouse that would be born would be a heterozygous chimera. You should take this mouse and breed it to wild-type mice to generate some heterozygous females that are not chimeras. You can then mate this heterozygous female to a wild-type male, and half of her sons would be the mice that you want.

v) If half of the sons of a heterozygous female are hemophiliacs, then lacking clotting factor VIII has the same phenotype in mice as it does in humans. If all of the sons of a heterozygous female are able to clot, then lacking clotting factor VIII plays a different role in mice than it does in humans.

(b, 4pts) You find that you are successful in creating a mouse model of hemophilia A. However, you are unable to keep any of the mice with hemophilia A alive, because even the smallest movement gives them injuries that are lethal. How are you going to maintain your genetically engineered strain of mice, given that mice with the disease have an S equal to 100%?

You can maintain this strain because females are diploid for the X chromosome, so you can maintain heterozygous females (who will not have hemophilia), but any time you want to generate hemophiliacs, you can simply mate the heterozygous female to a wild-type male, and half of her sons will have hemophilia.

(c, 4pts) Which aspect of the creation of your strain of genetically engineered mice would demonstrate that the gene for clotting factor VIII is “haplosufficient” (i.e. NOT haploinsufficient)? Explain your answer in one sentence.

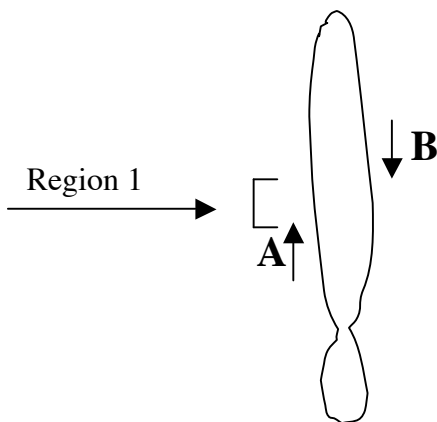
The fact that heterozygous females are not hemophiliacs means that this gene is haplosufficient. HaploINsufficiency refers to genes that are necessary in two functional copies in order to give a wild-type phenotype. Most genes are haplosufficient, so a deletion in one copy of the gene will not cause a phenotype.

(d, 4pts) You want to test whether clotting factor VIII, which is mutated in people with hemophilia A, physically interacts with clotting factor IX, which is mutated in people with hemophilia B. Which technique that we have discussed would you use to test this?

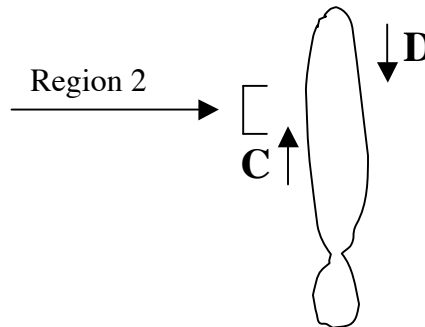
The two-hybrid assay. This technique allows you to test whether any two proteins of interest physically interact with one another.

4. (24 pts) You are a human geneticist studying cancer. You have four cell types that have been derived from four different tumors (each from a different patient with a different type of cancer). You have designed a PCR-based assay to detect large chromosomal abnormalities such as deletions, duplications, inversions, and translocations. It turns out that each of your cell types has a different one of these abnormalities affecting either one or both of the following chromosomal regions (Regions 1 & 2). In each cell type, this chromosomal abnormality contributes to the development of the cancer in these cells. In the diagram below, the small arrows indicate PCR primers you will be using in your assay. Note that Regions 1 and 2 are not the same size (i.e. they are not drawn to scale in the drawing).

Schematic of Chromosome 3



Schematic of Chromosome 7



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You do PCR using four different pairs of primers (in four separate reactions) on each of the four cell lines, and wild-type cells. The primers used are listed at the top of each lane in the gel.

A&B A&D B&C C&D A&B A&D B&C C&D A&B A&D B&C C&D A&B A&D B&C C&D



Wild-type Cells
or Cell Type T
(both look the same)

(a, 10pts) State which type of chromosomal abnormality is present in each cell type, and whether you think it is present in a heterozygous or homozygous state. If you cannot conclude, write “*inconclusive*.”

	Type of rearrangement	Heterozygous or homozygous
Cell Type Q	Translocation	Heterozygous
Cell Type R	Deletion	Homozygous
Cell Type S	Duplication	Heterozygous
Cell Type T	Inversion	inconclusive

Q is a translocation because both chromosomes (3 and 7) are affected. It is heterozygous because one band for chromosome 3 and one band for chromosome 7 show the wild-type patterns.

R is a deletion because one of the PCR products becomes smaller than it is supposed to be. It is homozygous because the organism’s two homologous chromosomes both give the smaller sized fragment using primers A&B.

Name: _____KEY_____

S is a duplication because one of the PCR products becomes larger than it is supposed to be. It is heterozygous because one band in the last lane of the gel still shows the wild-type pattern, even though the other band in the last lane of the gel stays the correct size.

T is an inversion because an inversion within the boundaries set by the PCR primers will not affect the size of the PCR product. One cannot tell whether it is homozygous or heterozygous because the original chromosome and the inverted chromosome look the same in this PCR-based assay.

(b, 3pts) Do you think that Cell Type R's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

A tumor suppressor gene, because deletions remove information from the chromosome, so they are typically loss of function alleles. Loss of function alleles of tumor suppressor genes lead to cancer, but loss of function alleles of oncogenes do not lead to cancer.

(c, 3pts) Do you think that Cell Type S's abnormality was more likely to affect an oncogene or a tumor suppressor gene?

An oncogene, because duplications add information to the genome, so they are typically gain of function or increased function alleles. Gain of function alleles of oncogenes lead to cancer, but gain of function alleles of tumor suppressor genes do not lead to cancer.

(d, 8pts) The general type of chromosomal abnormality found in Cell Type Q can cause cancer by either affecting an oncogene or a tumor suppressor gene. Give an example of how the general type of chromosomal abnormality found in Cell Type Q could affect each type of gene, and thereby lead to cancer.

Oncogene:

A translocation can lead to the placement of a strong promoter (normally in front of a different gene) in front of an oncogene. This increases expression of an oncogene, which leads to cancer.

Tumor Suppressor Gene:

If the translocation breakpoint is within a tumor suppressor gene, that gene would be torn apart, and thus inactivated. If the translocation were homozygous, then both copies of the tumor suppressor gene would be inactivated, and this would lead to cancer.

5. (24 pts) There are 10 people living on an island. You take their blood samples and genotype them for a specific autosomal SSR that is in a non-coding region of the genome, and that has no functional effect. During your studies, assume that no mutations occur at this SSR locus, and that no people move to or leave the island. Below is a gel indicating the results of your genotypic analysis.

Individuals from Generation One:

1 2 3 4 5 6 7 8 9 10



(a, 6pts) What are the frequencies of each possible genotype in Generation One?

$f(AA) = 2/10$

$f(AB) = 1/10$

$f(AC) = 1/10$

$f(BC) = 0/10$

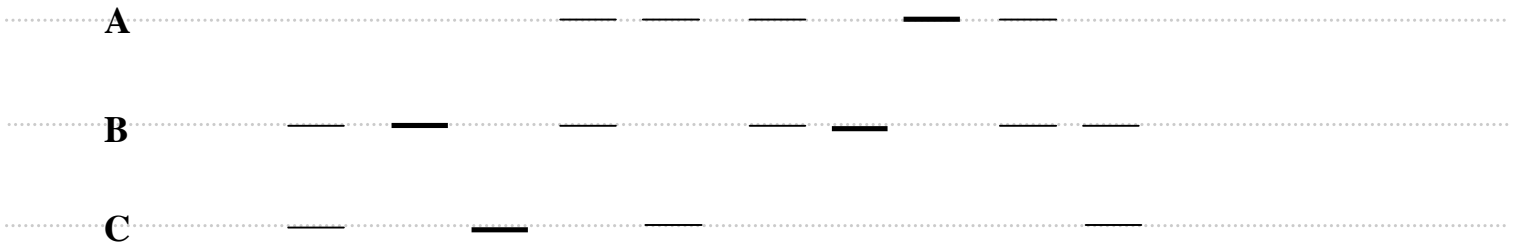
$f(BB) = 4/10$

$f(CC) = 2/10$

The ten individuals then split into 5 couples randomly, and each have two children to create a second generation of ten people. Below is a gel indicating the results of your genotypic analysis on this next generation.

Individuals from Generation Two:

1 2 3 4 5 6 7 8 9 10



(b, 6pts) What are the frequencies of each allele in Generation Two?

$$f(A) = 6/20$$

$$f(B) = 9/20$$

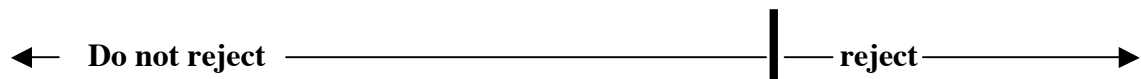
$$f(C) = 5/20$$

(c, 4pts) What fraction of all **B** alleles are found in heterozygotes in Generation Two?

5/9. There are 9 total B alleles in the population of 20 alleles, and 5 of those are found in heterozygotes.

(d, 8pts) Does Generation Two have genotypic frequencies that are consistent with the island population being at Hardy-Weinberg equilibrium? Use chi-square analysis to support your answer. For the chi square test you do, give the observed and expected **number** of individuals, the degrees of freedom, and your calculated value for χ^2 . Finally, give your conclusion given the test results.

<i>p</i> 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



Generation One and Two both have the same allele frequencies:

$$f(A) = 6/20 = p = 0.3$$

$$f(B) = 9/20 = q = 0.45$$

$$f(C) = 5/20 = r = 0.25$$

For Generation Two to be at Hardy-Weinberg equilibrium, it would have to have the observed numbers of individuals of each genotype that match the expected number of individuals of each genotype that are predicted by the equations:

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

$$f(AB) = 2pq = 2 * 0.3 * 0.45 = 0.27$$

$$f(AC) = 2pr = 2 * 0.3 * 0.25 = 0.15$$

$$f(BC) = 2qr = 2 * 0.45 * 0.25 = 0.225$$

$$f(BB) = q^2 = 0.45 * 0.45 = 0.2025$$

$$f(CC) = r^2 = 0.25 * 0.25 = 0.0625$$

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Since there are 10 total individuals in Generation Two, you can multiply the frequency of each expected genotype by 10 to get the number of individuals of each expected genotype.

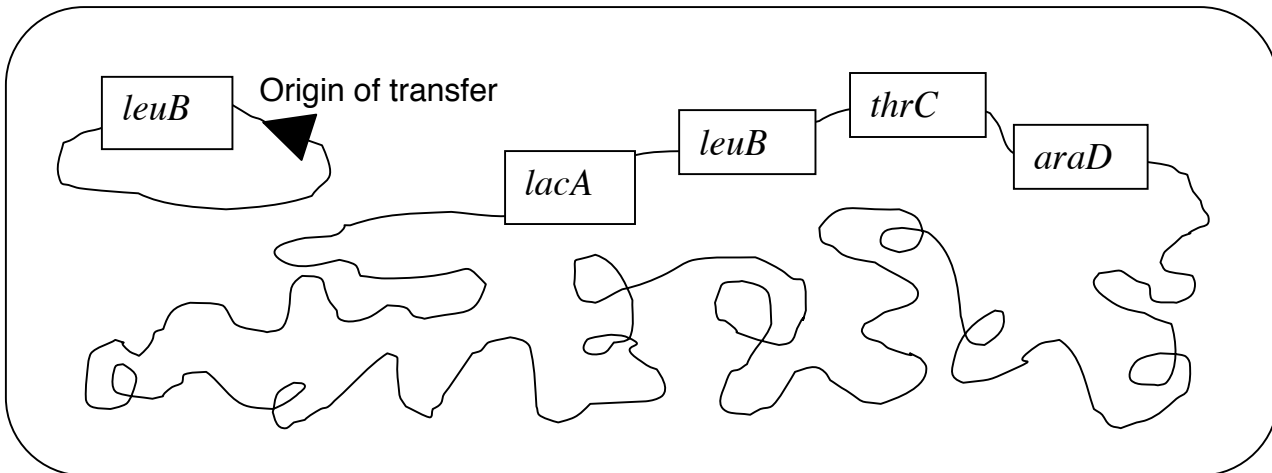
Genotype	Expected number	Observed Number	$\frac{(O - E)^2}{E}$
AA	0.9	1	0.0111
AB	2.7	3	0.0333
AC	1.5	1	0.1666
BC	2.25	2	0.0277
BB	2.025	2	0.0003
CC	0.625	1	0.225

To get the chi-squared value, you sum the numbers in the last column to get = 0.464.

The degrees of freedom = # classes - 1 = 5.

The chi-squared value you get is so small that, even if you had one df, you would still not be able to reject your hypothesis that Generation Two is at Hardy Weinberg equilibrium.

6. (22 pts) You are studying a merodiploid strain of *E. coli* that contains a form of the F factor. Below is a diagram of all of the genetic material in the strain. The size of the chromosome is about 50 times larger than that of the extrachromosomal element.



(a, 2pts) Is the strain drawn above an F⁺ strain, an Hfr strain, an F'⁺ strain, or an F⁻ strain?

An F'⁺ strain, because it contains a form of the F plasmid that harbors an extra gene that is normally found on the bacterial chromosome.

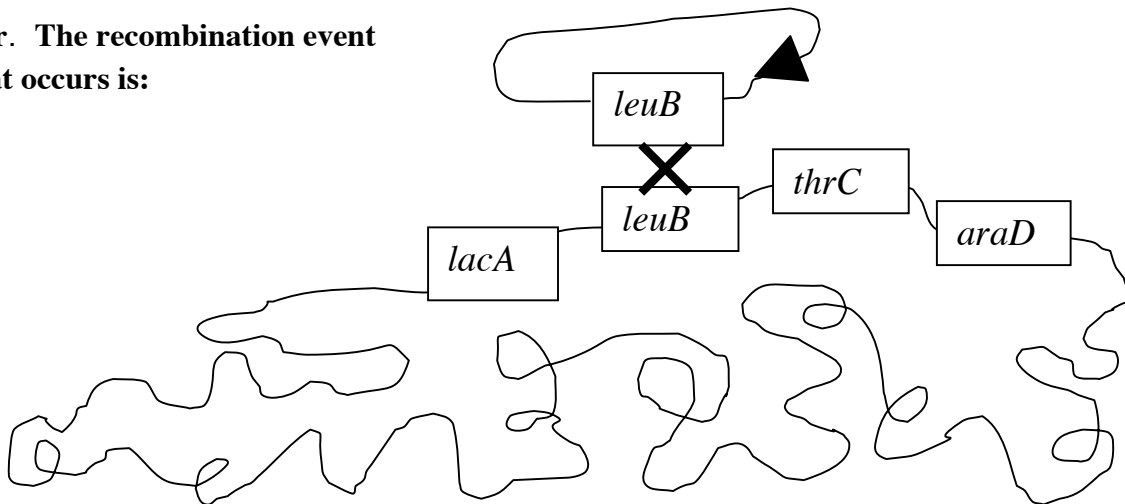
Name: _____KEY_____

(b, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain drawn above.

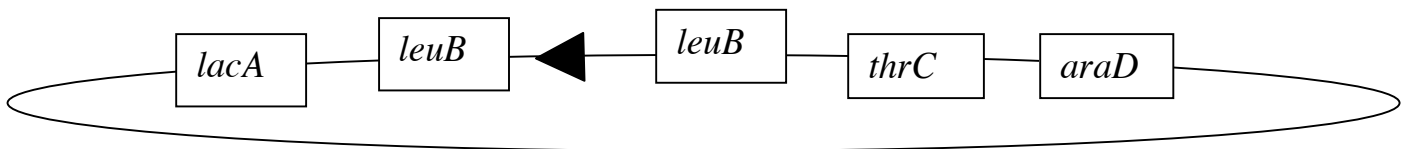
Gene Name	How transferred?
<i>lacA</i>	Never (because the only copy of <i>lacA</i> is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)
<i>leuB</i>	Early (because a copy of <i>leuB</i> is on the very small F' plasmid that can be entirely transferred to a new cell very quickly and efficiently)
<i>thrC</i>	Never (because the only copy of <i>thrC</i> is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)
<i>araD</i>	Never (because the only copy of <i>araD</i> is on the chromosome, and the chromosome has no origin of transfer on it, so that chromosome can never be transferred)

(c, 2pts) Now assume that a single homologous recombination event occurs between the extrachromosomal element and the chromosome. Is the resulting strain an F+ strain, an Hfr strain, an F' strain, or an F- strain?

Hfr. The recombination event that occurs is:



And the product is:



This is an Hfr because the origin of transfer is now on the bacterial chromosome itself.

Name: _____KEY_____

(d, 4pts) For each gene listed below, state whether it is transferred early/efficiently, late/inefficiently, **OR** never by the strain discussed in part (c).

Gene Name	How transferred?
<i>lacA</i>	Late, because, in the Hfr that is formed, <i>lacA</i> is the second to last gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.
<i>leuB</i>	Early, because, in the Hfr that is formed, <i>leuB</i> is the first gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.
<i>thrC</i>	Early, because, in the Hfr that is formed, <i>thrC</i> is the second gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.
<i>araD</i>	Early, because, in the Hfr that is formed, <i>araD</i> is the third gene out of all of the genes on the chromosome that lies behind the blunt end of the origin of transfer.

(e, 10pts) Circle all of the following methods that could be used to make a merodiploid bacterial strain if you started with a strain that is an F⁻ strain. The possibilities are:

mating it with an Hfr strain

mating it with an F⁺ strain

mating it with an F' strain

transducing it with wild-type P1 phage

infecting it with wild-type phage lambda

transforming it with a wild-type R factor

Mating it with an Hfr strain gives an F⁻ strain.

Mating it with an F⁺ strain gives an F⁺ strain.

Mating it with an F' strain gives an F' strain, which is a merodiploid strain because the bacterial chromosome has one copy of every bacterial gene, and the F' plasmid has one copy of a few bacterial genes on it too. Thus the bacterial strain is diploid for any bacterial genes carried on the F' plasmid.

Transducing it with wild-type P1 phage leads to lysis.

Infecting it with wild-type phage lambda leads to lysis.

Transforming it with a wild-type R factor leads to the strain acquiring antibiotic resistance, but wild-type R factors do not contain any genes that are found on the unmodified bacterial chromosome, so the resulting strain is not a merodiploid.

7. (34 pts) Below is a segment of the messenger RNA produced from the *C. elegans* wild-type **lin-14** gene. This segment (shown below) is perfectly complementary to a segment of the microRNA produced from the wild-type *lin-4* gene.

wild-type **lin-14** mRNA = 5'—...CUCAGGGAAC...—3'

(a, 2pts) Write out as much of the sequence of the *lin-4* RNA as you can predict, using the format of the drawing above.

***lin-4* RNA is a microRNA that is perfectly complementary to this region of the lin-14 mRNA. Thus the *lin-4* RNA sequence would be 5'- GUUCCCUGAG -3'.**

(b, 4pts) Write out as much of the sequence of the double-stranded *lin-4* gene as you can predict, using the format of the drawing above. Clearly label the strand that is used as a template during transcription.

The *lin-4* RNA sequence is 5'- GUUCCCUGAG -3' so the *lin-4* gene would be:

5' —...GTTCCCTGAG...— 3'

3' —...CAAGGGACTC...—5' and the bottom is the strand used as a template

You isolate a point mutation in **lin-14** that causes increased function of **lin-14**. The phenotype of a **lin-14** (gf) worm is that the L1 stage is repeated over and over again during development. The sequence of the gain-of-function mutant **lin-14** RNA is:

mutant **lin-14** (gf) mRNA = 5'—...CUCAAGGAAC...—3'

You do a genetic screen in order to isolate suppressor mutations that return development back to wild-type in **lin-14** (gf) worms. You isolate a single strain (which is homozygous for both **lin-14** (gf) and a suppressor mutation “sup*”) in your screen. This strain contains an extragenic suppressor mutation that is a point mutation.

(c, 4pts) Write out exactly which sequence change has been acquired by your suppressed strain, and state where the sequence change is located in the genome.

When the sequence of lin-14 changes in the region that is complementary to *lin-4*, lin-14 becomes impervious to regulation by *lin-4*. This means that the levels of lin-14 protein will no longer drop when the levels of the *lin-4* microRNA begin to rise. A way to suppress this would be to mutate *lin-4* so that it regains perfect complementarity to lin-14 and thus regains the ability to regulate lin-14. Thus the change would be in the *lin-4* gene, such that it now has the sequence:

Name: _____KEY_____

5' -...GTTCCCTGAG...-3'

3' -...CAAGGAACTC...-5'

This gene will produce a *lin-4* microRNA that can bind to and regulate the mutated *lin-14* that already exists in your original single mutant strain.

(d, 8pts) You make the following strains of *C. elegans*. Which developmental phenotype (mutant or wild-type) would these strains show? If mutant, list the exact mutant phenotype you would see. (In this chart, "wt" = wild-type.)

Strain Genotype	Developmental Phenotype
lin-14 (gf) / lin-14 (gf) sup* / wt	Wild-type, because <i>lin-14</i> is mutated so that it cannot be regulated by wild-type <i>lin-4</i>, but there is mutant <i>lin-4</i> present that has the ability to regulate the mutant <i>lin-14</i>.
lin-14+ / lin-14+ sup* / sup*	L1 stage repeated over and over, because <i>lin-14</i> is wild-type so it can only be regulated by wild-type <i>lin-4</i>, but there is no wild-type <i>lin-4</i> present. Thus <i>lin-14</i> has increased function, and the phenotype of worms with too much <i>lin-14</i> function is that the L1 larval stage is repeated over and over.
lin-14+ / lin-14+ sup* / wt	Wild-type, because <i>lin-14</i> is wild-type so it can be regulated by wild-type <i>lin-4</i>, and there is wild-type <i>lin-4</i> present.

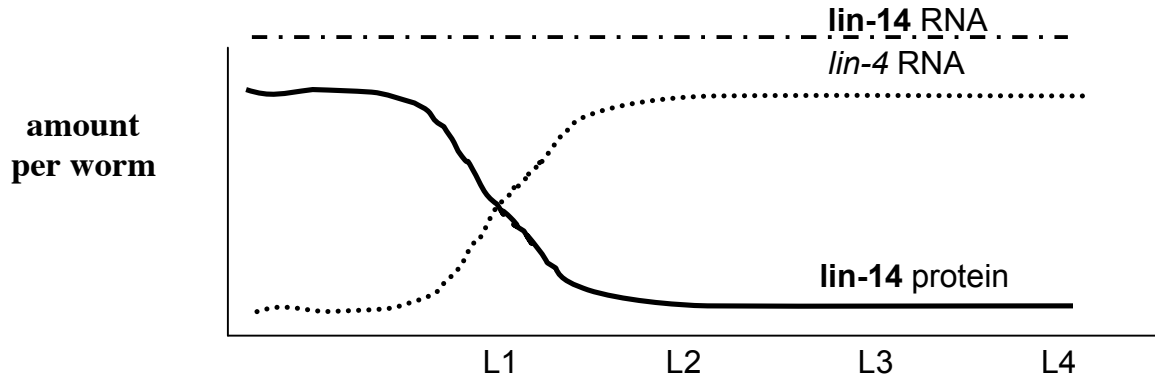
(e, 3pts) You do an experiment to test the levels of the *lin-14* RNA throughout the L1, L2, L3, and L4 larval stages. Which technique would you use to do this experiment?

Northern blot. You would isolate RNA from the four different larval stages, run them out on a denaturing agarose gel, transfer the RNA in the gel to a membrane, and probe the membrane with a probe that is complementary to the *lin-14* RNA.

(f, 2pts) Write the sequence of the probe you would use in the experiment from (e).

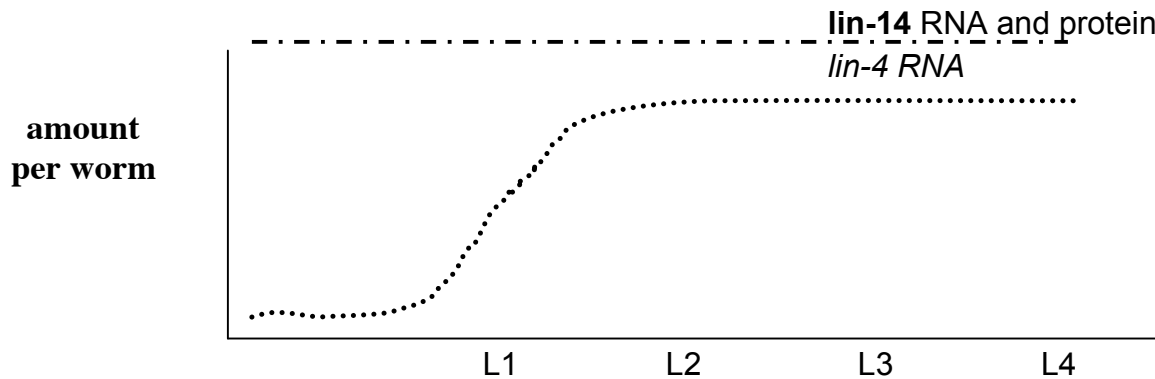
5'- GUUCCCUGAG -3' or 5'- GTTCCCTGAG -3'. One could use a DNA or an RNA probe in a Northern. Your probe should be perfectly complementary to the *lin-14* mRNA whose levels you are measuring.

(g, 3pts) Your experiment tells you that **lin-14** expression is **not** regulated at the transcriptional level. In class, the following slide was shown that denotes levels of the *lin-4* RNA and the **lin-14** protein during the four developmental stages of the worm. Add one line to the diagram that shows **lin-14** RNA levels during the development of a wild-type worm.



The *lin-14* gene is not regulated at the level of transcription, which means that a constant amount of RNA is being produced from this gene at all times.

(h, 8pts) Below, draw in the *lin-4* RNA levels, **lin-14** protein levels, and **lin-14** RNA levels that would exist in your original single mutant worm with the **lin-14** (*gf*) mutation in it.



The *lin-14* gene is not regulated at the level of transcription, which means that a constant amount of RNA is being produced from this gene at all times. The production of *lin-14* protein from *lin-14* RNA is normally inhibited whenever *lin-4* RNA is being made. However the original single mutant strain has a mutation in *lin-14* such that it becomes insensitive to regulation by *lin-4*. Thus the *lin-14* protein will be unaffected by the absence or presence of *lin-4* RNA in this strain.

8. (26 pts) You have discovered a new restriction-modification gene pair in a bacterial species. One gene of the pair, *rstR*, encodes a restriction enzyme, and the other gene (which is adjacent to *rstR* in the genome), *rstM*, encodes a modification enzyme. You have two mutant strains of bacteria. Strain One is an *rstR*⁻ strain that contains an ochre mutation early in the *rstR* coding sequence. Strain Two is an *rstM*⁻ strain that contains an amber mutation early in the *rstM* coding sequence. Strain Two also contains: 1) a nonsense-suppressing allele of a tRNA gene that is unlinked to the *rstM* locus, **and** 2) a Tn5 KanR transposon linked to the *rstM* locus with a cotransduction frequency of 80%.

(a, 6pts) Strain Two actually **must** contain a nonsense-suppressing allele of a tRNA gene. Why do you think that is?

Strain Two has a genotype of *rstM*⁻ *rstR*⁺. This strain produces functional restriction enzyme from the *rstR*⁺ allele, but non-functional methylase modifying enzyme from the *rstM*⁻ allele. This strain's restriction enzyme would chew up its own DNA, thereby destroying itself, unless it produced functional methylase to methylate the DNA sites usually recognized by the restriction enzyme to prevent cutting. Thus the nonsense-suppressing allele of the tRNA gene must exist to suppress the inability of an *rstM*⁻ *rstR*⁺ strain to produce functional methylase.

(b, 3pts) Give your best estimate of the distance between the transposon insertion and *rstR* (expressed as a cotransduction frequency).

80%. *rstR* and *rstM* are right next to each other in the genome, so the distance between the transposon and *rstM* should be the same as (or very close to) the distance between the transposon and *rstR*.

(c, 3pts) Give your best estimate of the distance between *rstM* and *rstR* (expressed as a cotransduction frequency).

1000%. *rstR* and *rstM* are right next to each other in the genome, so they should essentially always move together into a new cell by cotransduction.

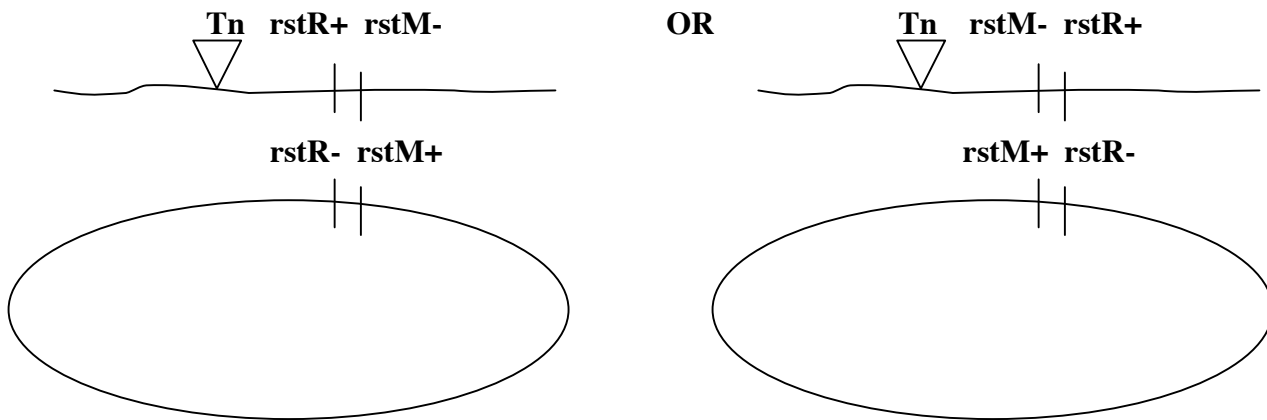
(d, 3pts) Do you think that *rstM* is cis-acting or trans-acting?

Trans-acting. *rstM* encodes a protein that can float around the cell and work on its substrate (DNA), which it methylates. Cis-acting sequences are DNA elements that do not encode free-floating gene products.

(e, 5pts) You want to determine the relative order of the transposon insertion, the *rstM* locus, and the *rstR* locus. You grow P1 phage on Strain Two and use the resulting phage lysate to infect Strain One. You select for transductants using kanamycin, and obtain 20 transductants that can grow. Estimate the number of Kan^R transductants that would be of each of the following genotypic classes.

Genotype	Number
<i>rstM</i> ⁺ <i>rstR</i> ⁺	0
<i>rstM</i> ⁻ <i>rstR</i> ⁻	0
<i>rstM</i> ⁻ <i>rstR</i> ⁺	0
<i>rstM</i> ⁺ <i>rstR</i> ⁻	20

The transduction experiment that you are doing is pictured below:



Either way, *rstR* and *rstM* are so close together that it is unlikely to get a crossover in between them. The transposon and the *rst* genes will be cotransduced 80% of the time, and the other 20% of the time, the transposon will come in without the *rst* genes. Thus, for either experiment, 80% of the cotransductants will be *rstR*⁺ *rstM*⁻, and 20% will be *rstR*⁻ and *rstM*⁺. However, *rstR*⁺ *rstM*⁻ cells cannot live without a nonsense-suppressing allele of a tRNA gene. You are growing phage on the strain that has this nonsense-suppressing allele, but the tRNA gene is unlinked to the *rst* genes and thus will never come along into the new strain. Thus the *rstR*⁺ *rstM*⁻ transductants will die (the restriction enzymes in these cells will chew up the genome of these cells), and you will be left with only *rstR*⁻ *rstM*⁺ cells.

Name: _____KEY_____

(f, 6pts) 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 *rstM* locus, the tRNA gene, and the *rstR* locus.

You do not know the order of *rstR* and *rstM*, but you do know that they are right next to each other. You know that the transposon is linked to them at a distance of 80% cotransduction, and that the tRNA gene is unlinked to them. Thus there are two possible orders:

