

Name: _____

7.03 Final Exam -- 2005

Name: _____

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** _____

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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		
P generation father		

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

	Phenotype	Genotype
F1 generation (mother of F2)		
Father to whom you cross the F1 mother		

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(c, 8pts) Write out complete genotype(s) of the different F2 flies.

Phenotype	Genotype
Short wings Small eyes	
Long wings Small eyes	
Short wings Large eyes	
Long wings Large eyes	

(d, 4pts) Remember from class that, oddly enough, male flies do not undergo recombination during meiosis. You cross true-breeding $wn1^- wn2^-$ short-winged females to true-breeding ey^- males to obtain an F1 generation. You then cross male F1 flies to true-breeding $wn1^- wn2^-$ big-eyed females. If you analyze 2000 resulting progeny, predict the number of the following kinds of flies that you will get:

Phenotype

Number of flies

Short wings Small eyes

Long wings Small eyes

Short wings Large eyes

Long wings Large eyes

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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:

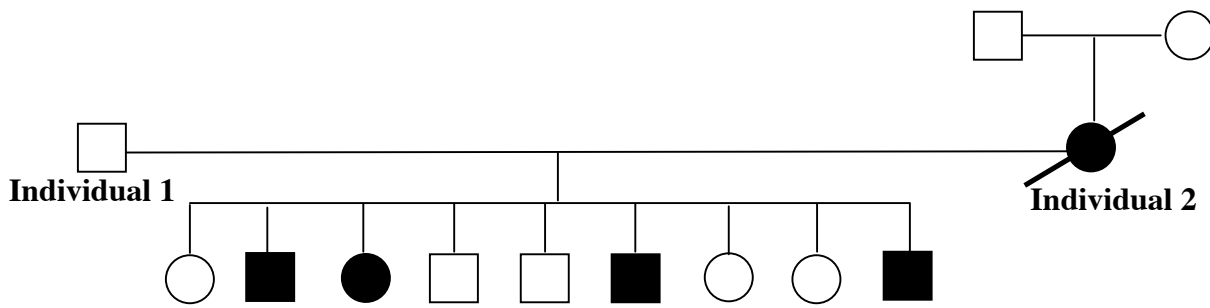
SSR12, on chromosome 1

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.



SSR	1	2	3	4	5	6	7	8	9	10	11	12
12	AB	AA	AA	AB	BB	AB	AA	BB	AA	BB	AB	AB
13	BC	AC	BB	BB	AC	AC	BB	AC	BC	BB	AA	BB
14	AC	AB	BC	BC	AB	AB	AB	BC	BC	AB	BC	AB
17	AB	AB	AB	AB	AB	BB	BB	AB	BB	BB	AB	BC

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(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)?

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

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.

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

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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.

(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)
- ii)
- iii)
- iv)
- v)

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(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%?

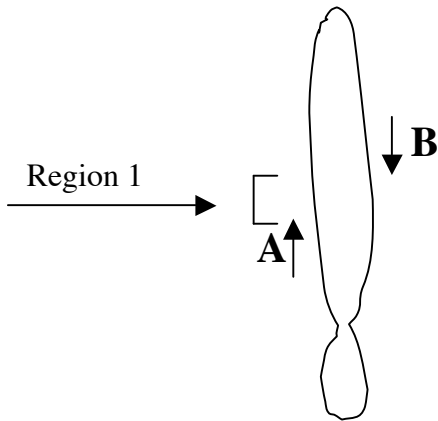
(c, 4pts) Which aspect of the creation of your strain of genetically engineered mice would demonstrate that the gene for clotting factor VIII is “haplosufficient” (i.e. NOT haploinsufficient)? Explain your answer in one sentence.

(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?

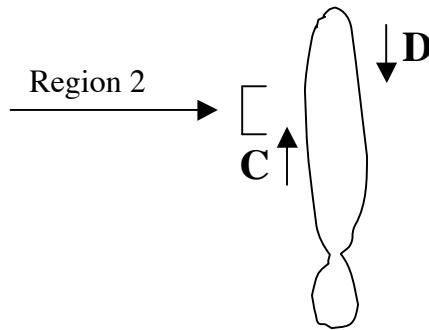
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4. (24 pts) You are a human geneticist studying cancer. You have four cell types that have been derived from four different tumors (each from a different patient with a different type of cancer). You have designed a PCR-based assay to detect large 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



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)

Cell Type Q Cell Type R Cell Type S

Name: _____

(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		
Cell Type R		
Cell Type S		
Cell Type T		

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

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

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(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:

Tumor Suppressor Gene:

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



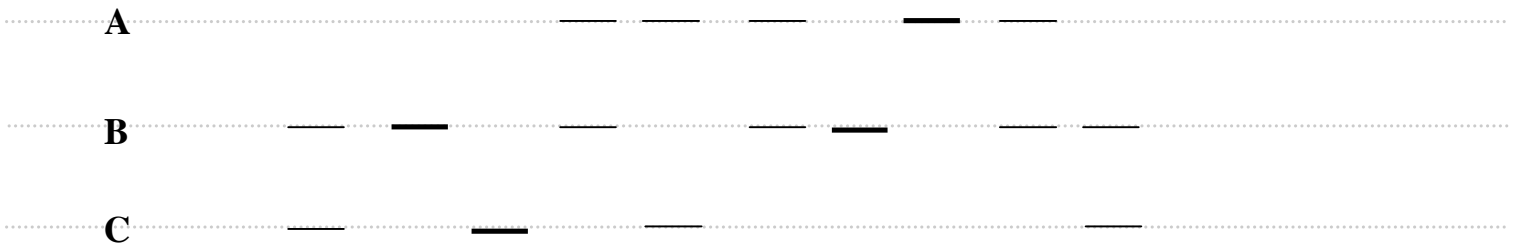
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(a, 6pts) What are the frequencies of each possible genotype in Generation One?

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?

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

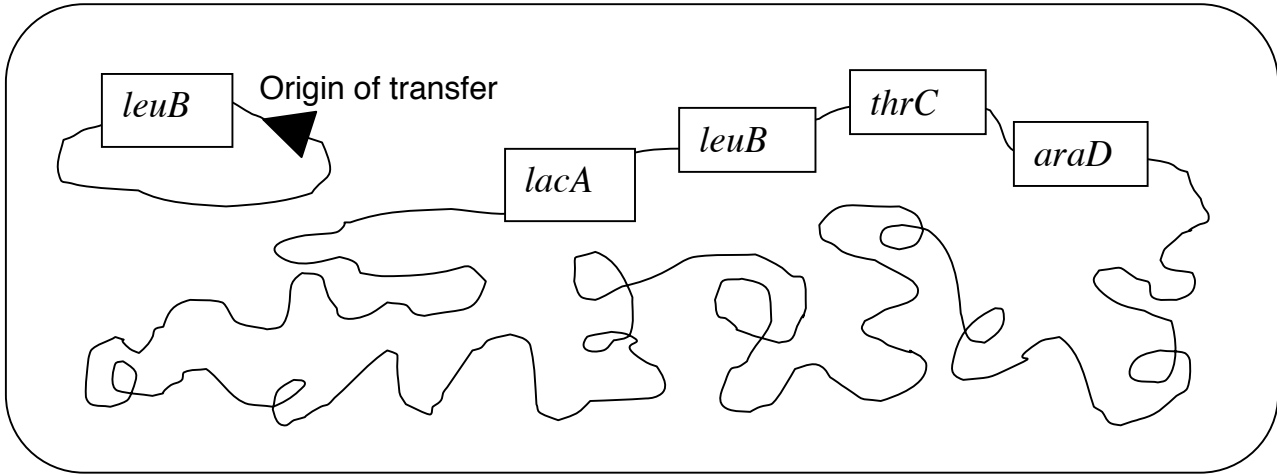
Name: _____

(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

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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?

(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>	
<i>leuB</i>	
<i>thrC</i>	
<i>araD</i>	

(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?

Name: _____

(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>	
<i>leuB</i>	
<i>thrC</i>	
<i>araD</i>	

(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

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.

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(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.

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.

(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.)

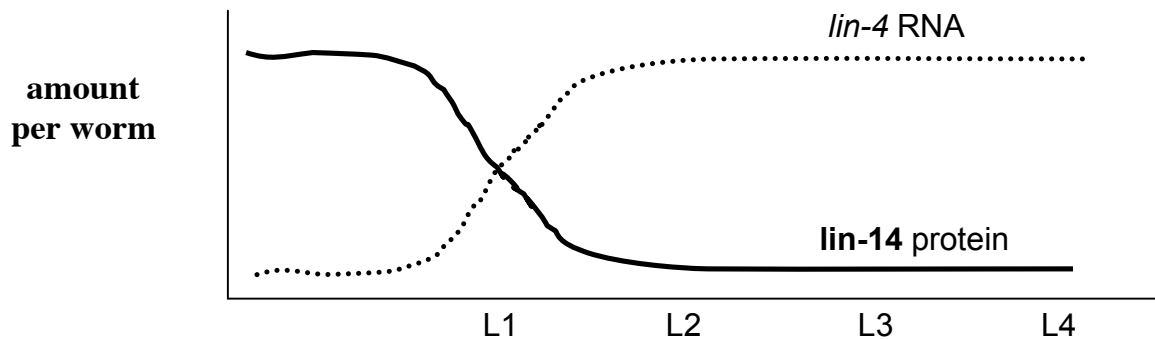
Strain Genotype	Developmental Phenotype
lin-14 (gf) / lin-14 (gf) sup [*] / wt	
lin-14 ⁺ / lin-14 ⁺ sup [*] / sup [*]	
lin-14 ⁺ / lin-14 ⁺ sup [*] / wt	

Name: _____

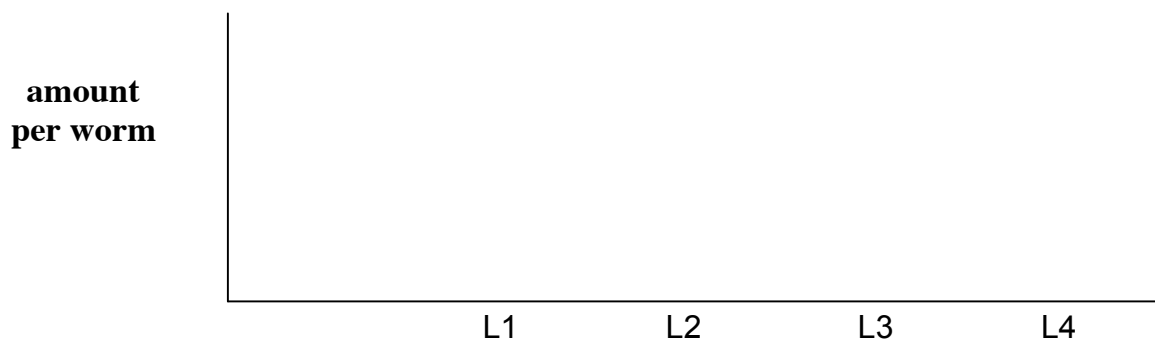
(e, 3pts) You do an experiment to test the levels of the **lin-14** RNA throughout the L1, L2, L3, and L4 larval stages. Which technique would you use to do this experiment?

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

(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.



(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.



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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 Kan^R 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?

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

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

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

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(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> ⁺	
<i>rstM</i> ⁻ <i>rstR</i> ⁻	
<i>rstM</i> ⁻ <i>rstR</i> ⁺	
<i>rstM</i> ⁺ <i>rstR</i> ⁻	

(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.