Name: _____

7.03 Exam Two -- 2005

Name: _____

Exam starts at 11:05 am and ends at 11:55 am.

There are 7 pages including this cover page.

Please write your name on each page.

Only writing on the **FRONT** of every page will be graded.
(You may use the backs, but only as scratch paper.)

Question 1	**31 pts_____**
Question 2	**31 pts_____**
Question 3	**38 pts_____**
TOTAL	**out of 100_____**

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1. (31 pts) You have isolated three bacterial mutants that cannot grow without supplemental serine being included in the growth medium. These three mutations lie in two genes, SerC and SerB. The SerC$^-$ mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1$^-$ mutation is a nonsense mutation that produces a protein product that is 30 kDa. The SerB2$^-$ mutation is a frameshift mutation that produces a protein product that is 12 kDa.

The first cross: You grow P1 phage on SerC$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ bacteria. (ProA$^-$ bacteria have a disruption in the ProA gene, which is required for the bacteria to synthesize their own proline.) You select for KanR transductants. All 200 of the transductants you analyze can grow on plates containing kanamycin and serine and proline, but cannot grow on plates containing kanamycin and serine (but not proline).

(a, 6pts) What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

The second cross: You grow P1 phage on SerB2$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ SerB1$^-$ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on plates lacking serine. The other 67 can only grow on plates containing serine.

(b, 5pts) Are ProA and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

(c, 5pts) Are SerC and SerB **definitely**, **maybe**, or **definitely not** linked by cotransduction?

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The third cross: You grow P1 phage on SerB1$^-$ bacteria. You use the resulting phage lysate to infect ProA$^-$ SerB2$^-$ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 400 transductants you analyze, 3 can also grow on plates lacking serine. The other 397 can only grow on plates containing serine.

(d, 9pts) In the table below, fill in the genotypes (at the ProA, SerB1, and SerB2 loci) of the different phenotypic classes of transductants obtained from this third cross. Be sure to list **all possible genotypes** in each category.

GENOTYPE: **Phenotype:**	**at the ProA locus (+ or $^-$)**	**at the SerB locus (be sure to include the genotype at SerB1 and SerB2) (+ or $^-$)**
Don't require supplemental serine		
Require supplemental serine		

(e, 6pts) Draw all of the possibilities for a map of the region of the bacterial chromosome that is consistent with all of the data in this problem. Your map should show the positions and relative order of the ProA, SerB1, and SerB2 loci.

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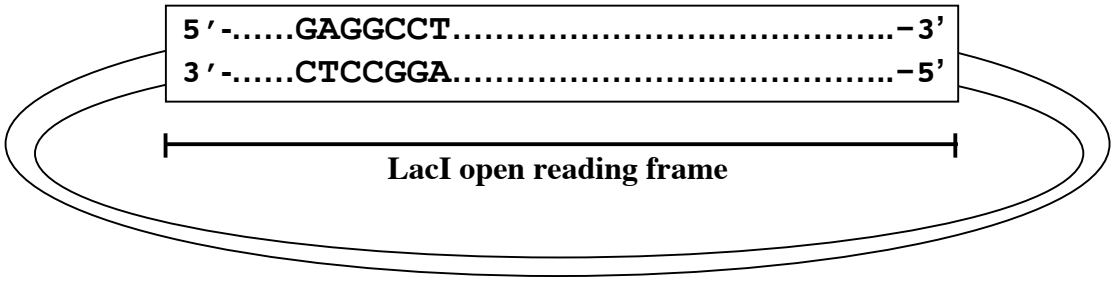
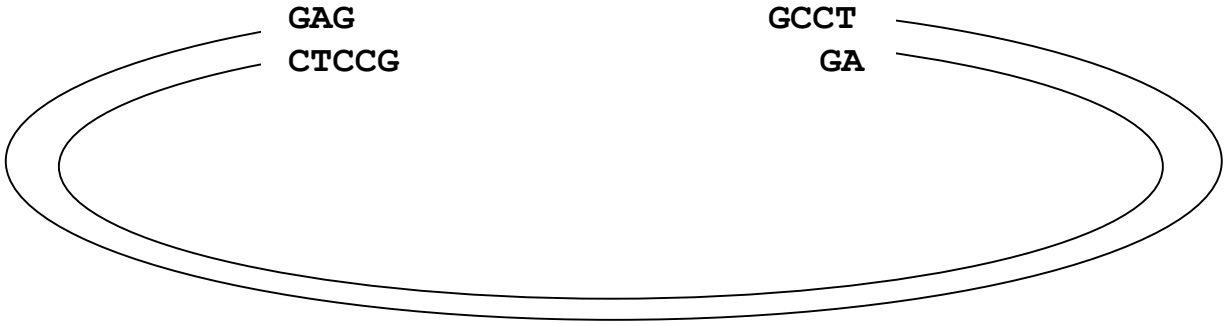
2. (31 pts) You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, the strain carrying the plasmid shows normal inducible expression of beta-galactosidase. A diagram of your plasmid is shown below. For this problem we are going to focus on a highlighted region of DNA sequence present early in the LacI open reading frame.



(a, 5pts) Write out the sequence that would result from transcription of the LacI gene by RNA polymerase, if the lower strand was used as a template. Be sure to give the sequence corresponding to the short segement that is highlighted, and label any 5’ and 3’ ends in your drawing.

(b, 5pts) Label the correct reading frame of this gene, given that a tRNA with the anticodon 5’-GCC-3' is supposed to base-pair with the region of the transcript that you drew in part **(a)**. Label the reading frame in the original plasmid drawing by circling a set of nucleotides that should be read as one codon.

(c, 6pts) The drawing below shows the original plasmid after being cut by a restriction enzyme that recognized the highlighted sequence.



Draw what would result if this cut plasmid were incubated with DNA polymerase in the presence of all four normal nucleotides. DO NOT do the drawing over – simply modify the drawing we gave you. Label any 5’ and 3’ ends in your drawing.

(d, 4pts) You next add DNA ligase to the product you drew in part **(c)**. DNA ligase will reseal the free DNA ends of that product so that a circular molecule reforms. You now transform a *lacI*Δ *E. coli* strain with the new plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase (uninducible, constitutive, or inducible)?

(e, 5pts) In one sentence, explain how the specific molecular change to the LacI gene made in the new plasmid led to the phenotype you predicted above.

(f, 6pts) You now transform a *lacI*Δ *E. coli* strain with the new plasmid that you made in part **(d) <u>and</u>** the original plasmid. What phenotype do you think that the transformed strain will display with respect to expression of beta-galactosidase? Explain your answer in one sentence.

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3. (38 pts) You are studying the regulation of a bacterial gene (TolU) that encodes an enzyme that is necessary for the bacterium to degrade toluene for use as a carbon source. The tolU gene is only transcribed when simple sugars are not available as a carbon source. You isolate three mutant strains of this bacterium, each of which harbors a single mutation: tolA$^-$, tolB$^-$, or tolC$^-$. TolA, TolB, and TolC are all regulatory components involved in TolU regulation. Below are the phenotypes of different strains that you have constructed.

	Activity of TolU when:	
Genotype	Simple sugars absent	Simple sugars present
A+ B+ C+ U+	+	–
A$^-$	–	–
C$^-$ / **F'** A+ B+ C+ U+	+	+
A+ U$^-$ / **F'** A$^-$ B+ C+ U+	–	–
C$^-$ U+ / **F'** A+ B+ C+ U$^-$	+	+
A$^-$ B+ / **F'** A+ B$^-$ C+ U+	+	–
B$^-$	+	+
C$^-$	+	+
C$^-$ U$^-$ / **F'** A+ B+ C+ U+	+	–
A$^-$ U+ / **F'** A+ B+ C+ U+	+	–

(a, 7pts) Classify the tolA$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(b, 7pts) Classify the tolB$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(c, 7pts) Classify the tolC$^-$ mutation as cis or trans, constitutive or uninducible, and dominant or recessive.

(d, 8pts) Given your answers to parts **(a)** – **(c)**, draw a genetic pathway that shows the way by which the tolU gene is regulated. Be sure to indicate the wild-type functions of tolU, tolA, tolB, and tolC. Also include a role for simple sugars.

NOTE: Answer all of the remaining parts of this problem based on the model you drew in part **(d)**.

(e, 4pts) What would you predict to be the double mutant phenotype of a tolA$^-$ tolC$^-$ double mutant with respect to tolU expression? (Your choices are: uninducible, constitutive, or regulated.)

(f, 5pts) You isolate an allele at the TolB locus that gives an uninducible phenotype. What kind(s) of mutation could this new allele be with respect to TolU? (Your choices are: repressor$^-$, activator$^-$, promoter$^-$, operator$^-$, super repressor, super activator, dominant negative repressor, dominant negative activator.)