7.03 Exam Two -- 2005

Name: _____KEY_____

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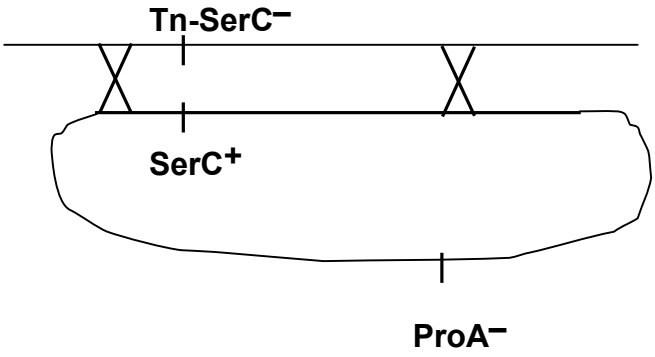
1. (31 pts) You have isolated three bacterial mutants that cannot grow without supplemental serine being included in the growth medium. These three mutations lie in two genes, SerC and SerB. The SerC$^-$ mutation is a Tn5 KanR insertion in the middle of the SerC coding region. The SerB1$^-$ mutation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

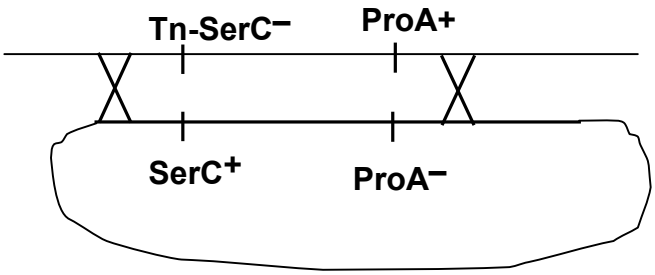
(a, 6pts) What is the genetic distance between the SerC and the ProA loci, expressed as a cotransduction frequency?

0%
There are two possibilities – either A and C are linked, or they are not.
The first cross:

If A and C are unlinked



If A and C are linked



You will never get ProA+ KanR from this.

You can get ProA+ KanR from this.

Given that you never see ProA+ KanR, the two are unlinked.
This problem asked you to express a distance between ProA and SerC as a cotransduction frequency. The cotransduction frequency between ProA and SerC is 0%.

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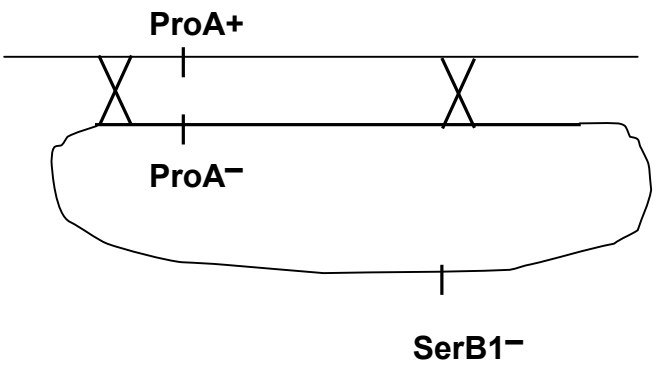
The second cross: You grow P1 phage on SerB2⁻ bacteria. You use the resulting phage lysate to infect ProA⁻ SerB1⁻ bacteria. You select for transductants that can grow on plates containing serine (but not proline). Of the 70 transductants you analyze, 3 can also grow on plates lacking serine. The other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

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

Definitely.

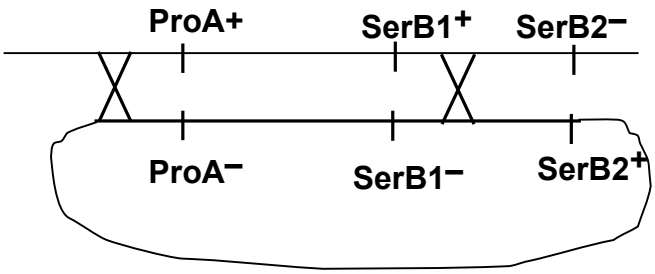
There are two possibilities – either A and B are linked, or they are not.
The second cross:

If A and B are unlinked



You will never get ProA+ Ser+ from this.

If A and B are linked

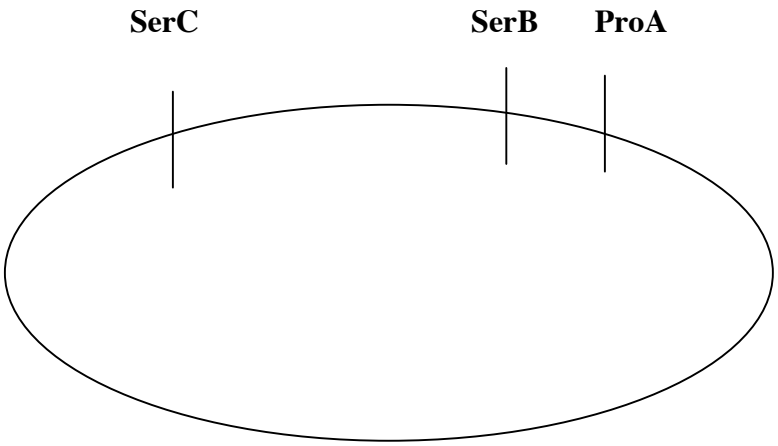


You can get ProA+ SerB+ from this.

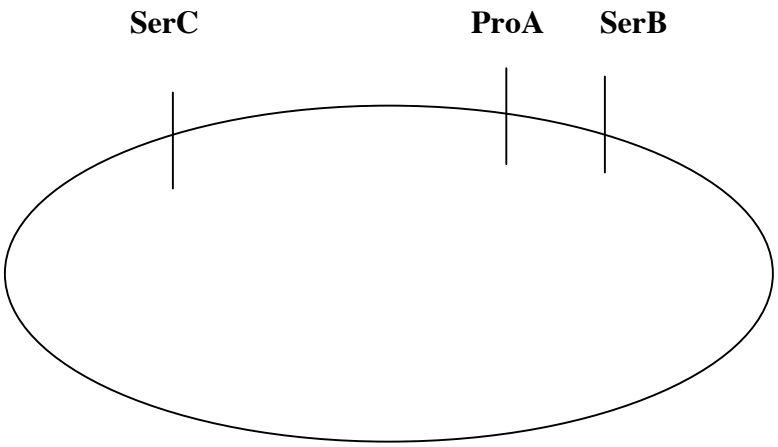
Given that you do see ProA+ Ser+ transductants, A and B must be linked.

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

Maybe. SerC and ProA are unlinked by cotransduction. ProA and SerB are linked by cotransduction. It may be that the map order of these genes is such that SerB is in the middle of SerC and ProA, so SerC and ProA are far enough to be unlinked (more than 10^5 basepairs), but SerC and SerB are close enough to each other to be linked (see diagram below).



However it also may be that the map order of these genes is such that ProA is in the middle of SerC and SerB, so SerC and ProA are far enough to be unlinked (more than 10^5 basepairs), and then SerC and SerB are also unlinked because they are even farther from each other than ProA and SerC (see diagram below).



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.

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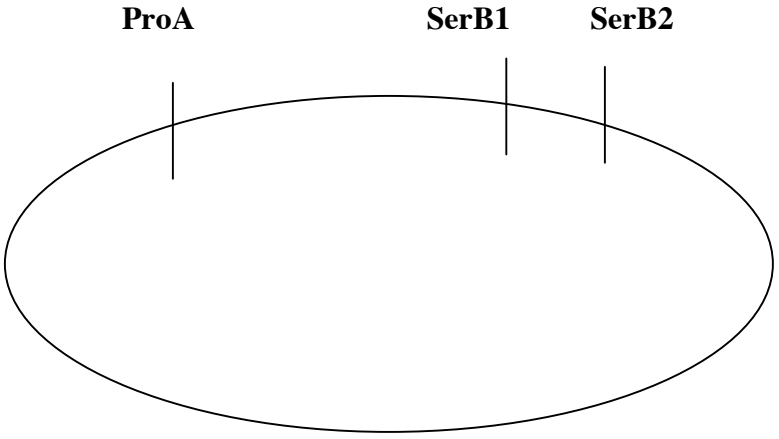
GENOTYPE: **Phenotype:**	**at the ProA locus (+ or ⁻)**	**at the SerB locus (be sure to include the genotype at SerB1 and SerB2) (+ or ⁻)**
Don't require supplemental serine	**+**	**1+ 2+**
Require supplemental serine	**+**	**1+ 2⁻** **1⁻ 2⁻** **1⁻ 2+**

Note that you are selecting for ProA+, so ALL transductants will be ProA+.
Ser+ transductants will only result if both positions in the SerB gene are wild-type.

(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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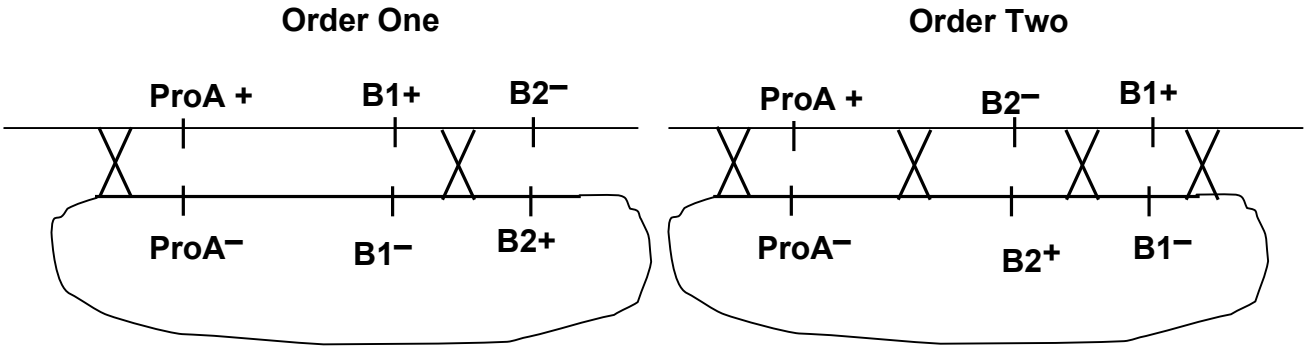
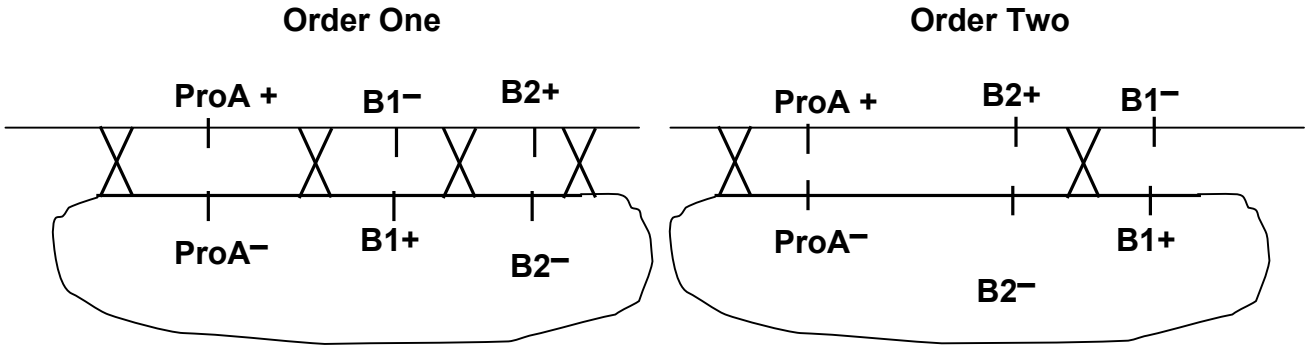
There is only one possible order for 1e. ProA cannot be in the middle, because SerB1 and SerB2 are in the same gene. SerB1 is much more likely to be in the middle because, if B1 is in the middle, you will see a higher frequency of Ser+ transductants in the second cross than in the third cross. (If B2 is in the middle, you would have seen a higher frequency of Ser+ transductants in the third cross than in the second cross). This is because double crossover events are more frequent than quadruple crossover events. Below are drawn the crossovers necessary to create Ser+ transductants. Note that you are selecting for ProA+, so ALL transductants will be ProA+.

Order One – B1 in middle **Order Two - B2 in middle**

The second cross:



The third cross:



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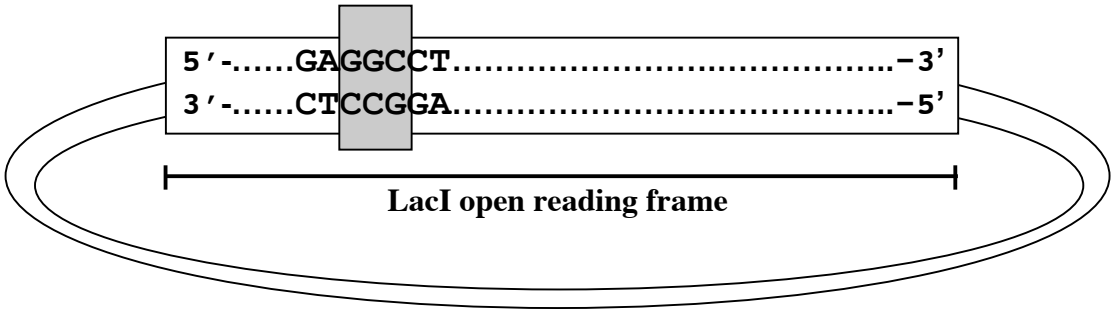
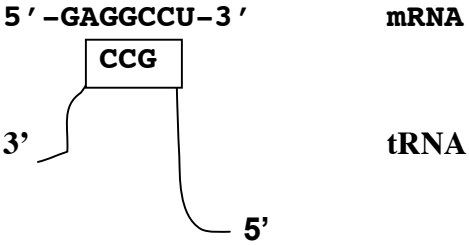
2. (31 pts) You construct a plasmid that has a wild-type copy of the LacI gene from *E. coli*. You transform a *lacIΔ E. coli* strain (that is, a strain with the LacI gene deleted) with this plasmid. You observe that, whereas the original *lacIΔ E. coli* strain shows constitutive expression of beta-galactosidase, 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.

5’-GAGGCCU-3’

(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.



See drawing above. Note that, if you did not label the reading frame in the original plasmid drawing (and instead labeled the reading frame in the mRNA you drew in part a), you did not receive full credit.

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

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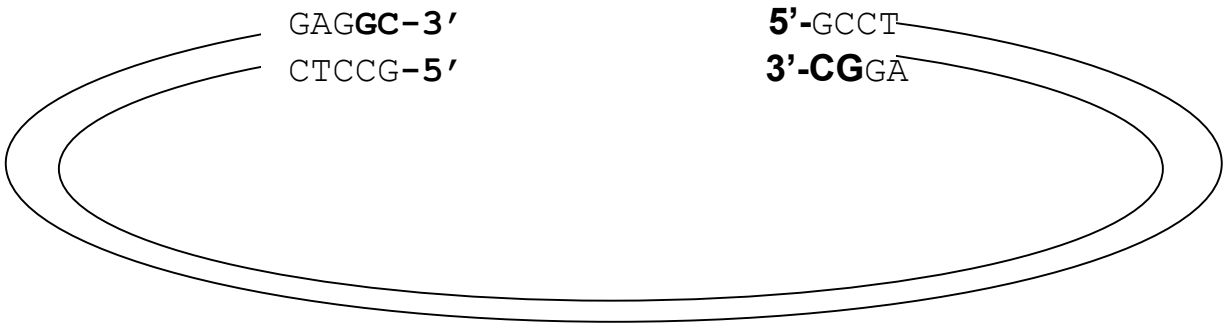


Draw what would result if this cut plasmid were incubated with DNA polymerase in the presence of all four normal nucleotides. DO NOT do the drawing over – simply modify the drawing we gave you. Label any 5’ and 3’ ends in your drawing.

See drawing above. DNA polymerase can do replication, but only of sequences for which there is a template (i.e. you can't fill in other nucleotides in the middle because there is no template there.) Note that you did not receive credit unless you labeled the 5' and 3' ENDS of the DNA molecule. The ends are located at the two opposite sides of the linear product you created in part c).

(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 *lacIΔ 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)?

Constitutive.

(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.

The mutation made in lacI from religating the plasmid is a +2 frameshift mutation (a 2 base pair insertion). This means that the entire frame of LacI (from early on in the gene) is shifted off from the original frame. Thus the protein created from this mutated version of the lacI gene would be non-functional because none of the subsequent codons would be read correctly. LacI is a repressor of LacZ, so losing function in LacI would lead to constitutive expression of LacZ.

(f, 6pts) You now transform a *lacIΔ 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.

Inducible. The mutation made in lacI from religating the plasmid would be recessive, because it is a loss-of-function mutation that destroys the function of LacI. Thus, if you had a cell with wild-type LacI and non-functional LacI, you would see the wild-type phenotype.

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
1. A+ B+ C+ U+	+	–
2. A$^-$	–	–
3. C$^-$ / **F'** A+ B+ C+ U+	+	+
4. A+ U$^-$ / **F'** A$^-$ B+ C+ U+	–	–
5. C$^-$ U+ / **F'** A+ B+ C+ U$^-$	+	+
6. A$^-$ B+ / **F'** A+ B$^-$ C+ U+	+	–
7. B$^-$	+	+
8. C$^-$	+	+
9. C$^-$ U$^-$ / **F'** A+ B+ C+ U+	+	–
10. A$^-$ U+ / **F'** A+ B+ C+ U+	+	–

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

Cis, uninducible, recessive. (This means that A is the promoter sequence in the U gene.)
Uninducible – Strain 2
Recessive – Strain 10
Cis – Strain 4 is the trans test (because A+ is on a different piece of DNA from U+), and A fails the trans test because the trans test strain displays the recessive phenotype (uninducible from A$^-$ is recessive to inducible from A+).

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

Trans, constitutive, recessive. (This means that B is a repressor.)

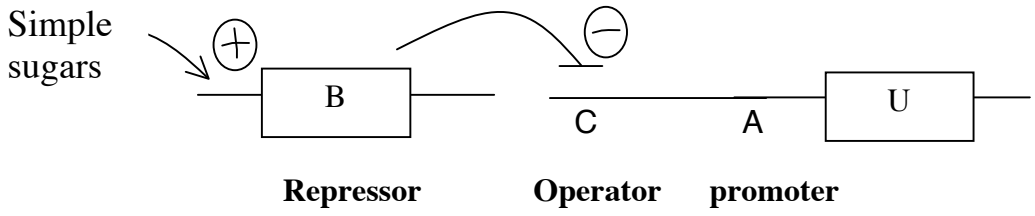
Constitutive – Strain 7
Recessive – Strain 6. Strain 6 is a complementation test for A and B. This strain shows the wild-type phenotype, which can only occur if A and B are both recessive and in different genes.
Trans – Strain 6. Strain 6 is a complementation test for A and B. This strain shows the wild-type phenotype, which can only occur if A and B are both recessive and in different genes. The fact that A is cis to U means that A and U are in the same gene. The fact that B is in a different gene than A means that B is not in the same gene as U. All cis sequences are in the same gene as the reporter, so B must be trans.

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

Cis, constitutive, dominant. (This means that C is the operator sequence in the U gene.)
Constitutive – Strain 8
Dominant – Strain 3
Cis – Strain 9 is the trans test (because C$^-$ is on a different piece of DNA from U+), and C fails the trans test because the trans test strain displays the recessive phenotype (inducible from C+ is recessive to constitutive from C$^-$).

(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.

If you answered parts a – c correctly, then the model is:



Note that operators and promoters are cis-acting sequences. Cis-acting sequences must be linked to the reporter gene (that is, physically linked). Thus you must have drawn A and C physically linked to U to get full credit. Operator and promoter sequences are part of the reporter gene. They are sequences that do not get transcribed or translated, but are instead control sequences that are found before the transcription start site of the reporter gene.

Also, to get full credit:
-- The net effect of B must have been negative, because B is a repressor.
-- The net effect of sugars must have been negative, because sugars inhibit expression of the U gene.
-- The order in your pathway must have been:
Signal (sugars) then B (the trans-acting regulator) then U (the reporter gene).
-- The wild-type functions of the elements in your pathway must have been obvious from your drawing, or clearly labeled and drawn consistently with the label you gave.

PLEASE NOTE that we cannot interpret what you mean if you draw a blocking arrow labeled with a plus sign, and we cannot interpret what you mean if you draw a pointed arrow labeled with a negative sign. Such arrows send us mixed signals.

If you did not answer parts a-c correctly, then the model that you drew had to be consistent with what the predicted wild-type functions of A, B, and C would have been, had your answers to a-c been correct. Thus, if you determined that C was trans, you had to draw C as trans-acting in your model (for example). Note that there are NO cis uninducible dominant mutations, and NO cis constitutive recessive mutations, so if you gave such an answer to parts a, b, or c, then there is nothing that you could have drawn in your model that would have been consistent with the properties that you determined of the mutant.

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.)

If you drew A as the promoter and C as the operator in your model (which is correct), then the correct answer is UNINDUCIBLE, because the U gene would have no promoter and thus could never be expressed.
If you drew A and C as trans-acting regulators (which is incorrect), then the correct answer to part e would be the phenotype of mutating the downstream regulator (whichever one you drew closer to U, either A or C).

(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.)

If you drew B as being a net repressor in your model (which is correct), then the only correct answer is SUPER-REPRESSOR. Drawing B as a repressor in your model tells us that the wild-type function of B is to be a repressor. Therefore, the B gene encodes a

repressor. Therefore any allele of the B gene must be a repressor allele. The only allele of a repressor-encoding gene that gives the uninducible phenotype is the super-repressor allele.

If you drew B as being a net activator in your model (which is incorrect), then the correct answer to part f) is dominant negative activator or activator⁻. This is because there are two kinds of alleles of activator-encoding genes that give uninducible: dominant negative activator or activator⁻.