

# **Bacterial Genetics**

## **GMS6038**

### **Final Exam**

### **Fall 2010**

Write your station code here. \_\_\_\_\_

You have 2 1/2 hours to complete the exam.

Please note - this is a closed book, closed note exam. All backpacks and notebooks must be in the cubbies against the wall. All cell phones and personal communication devices must be off and put away.

You may use the rest room one person at a time.

Any cheating will result in a 0 for the exam and failure of the course.

This exam is in the form of a Word document. Simply type your answers below the questions. You may write on the paper form of the exam, but you will have to turn it in at the end of the exam. The exam center personnel will instruct you on mechanistic of the computer system.

**Shown below is pGULIG-12. Note the following genetic elements:**

At the top of the map is the origin of replication of the ColE1 plasmid. Note that the *rna-II* is expressed from the *trp* promoter. Note that the *trp* promoter is not followed by any *trp* protein coding sequences.

Moving clockwise:

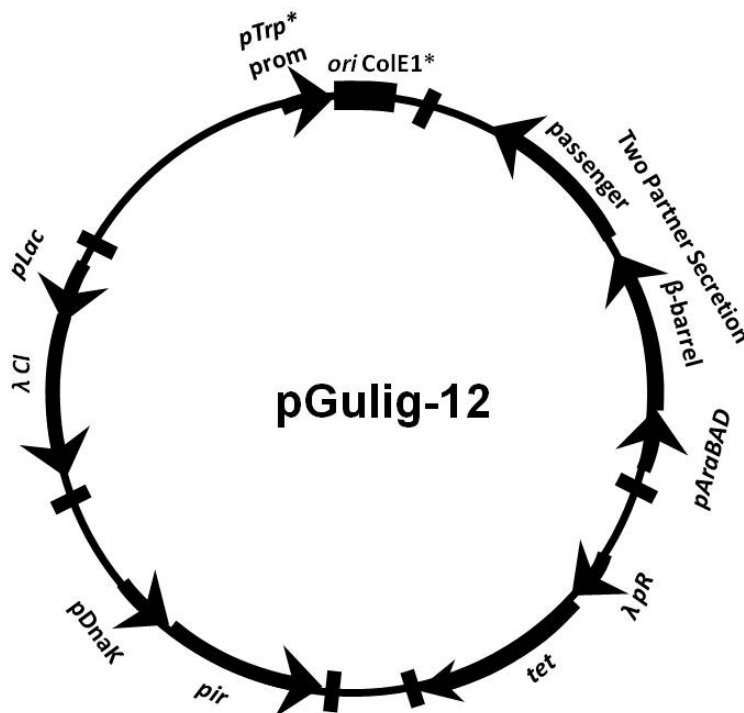
From 1:00-3:00 are the genes for the coding sequences for the two components of a typical two partner secretion system. The  $\beta$  barrel protein and passenger protein genes are indicated. Both of these genes are expressed by the promoter for the *araBAD* genes.

From 4:00-5:00 is the *tet* gene expressed by the  $\lambda$  pR promoter.

From 6:00-8:00 is the *pir* gene expressed from the *dnaK* promoter.

From 8:00-10:00 is the  $\lambda$  CI gene expressed from the *lac* promoter.

There are no other promoters on this plasmid other than those indicated on the map. Assume that all genes have appropriate translation start and stop codons. The short bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements. The host *E. coli* strain **lacks the F plasmid and  $\lambda$  phage**. Any *E. coli* genes that normally regulate the promoters on this plasmid are not encoded on this plasmid unless indicated.



NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. The number of points is the maximum number of facts being looked for, and in some cases one fact is worth 2 points. Keep to what is being asked. Adding extra material hoping to include the right answer somewhere in the middle could count against you if your answer reveals a lack of understanding or includes incorrect information, even if unrelated to the original question.

The following questions are based on pGulig-12.

1. (6 points) How would you change the copy number of pGULIG-12 by altering growth conditions? Explain for both increasing and decreasing copy number. Be sure to detail how this works at the molecular level.

There is only one origin of replication on the plasmid, the ori of the ColE1 plasmid. It is an RNA-based origin, so the promoter of the initiating RNA, RNA-2, determines the replication and copy number. The promoter is the trp promoter without any coding sequences, i.e., trpL, the leader protein that encodes the attenuation system. So this promoter is regulated strictly by the trpR regulation. To increase expression of the trp promoter, you make the cells deficient for tryptophan since trp is the corepressor that acts in conjunction with the TrpR repressor protein. So growing the culture in a minimal medium or any medium without trp will do the job to increase copy number. To decrease the copy number, add trp to the medium or use a complex medium like L broth that is loaded with amino acids.

2. (6 points) If an *E. coli* strain carrying pGULIG-12 is growing in tetracycline, what would happen to the culture under the following conditions? Briefly explain.

OK, so we must get the tet gene expressed. It is expressed from the Lambda pR promoter, which is normally expressed - unless it is repressed by the Lambda CI protein. The Lambda CI gene is on the plasmid and expressed from the wild-type lac promoter. So, the question revolves around if the CI gene is expressed or not. If it is not expressed, the tet gene will be expressed, and the cells will grow. If CI is expressed, the pR will be repressed and the cells will not grow in tet.

+ IPTG + glucose

IPTG will prevent LacI from working (induction), but glucose will not enable cAMP to be made and bind to CRP/CAP to relieve catabolite repression, so CI will not be made. The cells will grow.

+ IPTG - glucose

Induction + relieving catabolite repression = CI expression = repression of pR and the tet gene. The cells will not grow.

- IPTG +glucose

No induction, no relieving of catabolite repression, no CI, tet is expressed, the cells will grow

- IPTG - glucose

No induction, but relieving of catabolite repression, no CI, no pR repression, tet is expressed = growth

3. (2 points) How might your answer to the first question regarding copy number affect your answer to this question?

OK, this was a little deep. Remember that I said that there are only 4 copies of the LacI repressor protein in an E. coli cell, and that if you had a multicopy plasmid with a lac promoter you usually encoded lacI on the plasmid itself to make sure there was enough available to repress the lac promoter on the plasmid. So, if the copy number of pGULIG-12 goes up, there will no longer be enough LacI to repress CI and IPTG becomes irrelevant. However, you still cannot have glucose in the culture or else you will not relieve catabolite repression.

4. (2 points) What growth conditions would you use to induce expression of the two partner secretion system? Explain.

Expressed from the pBAD promoter of the ara system. So, you should add arabinose to the culture (induction) and not add glucose (relieve catabolite repression). Ara acts with the AraC activator protein to induce pBAD expression.

5. (4 points) What are the functions of the two proteins in this system?

The beta barrel forms a channel in the outer member to enable the passenger protein to get through and be secreted to the outside. The passenger protein is the effector protein that might be a toxin, for example.

6. (2 points) Would you expect these proteins to possess typical N terminal signal sequences? Explain.

Yes. Both proteins rely on the Sec (general export) system to get into the periplasm.

7. (2 points) Describe the essential components of the DNA sequence between the two protein genes to have this system function.

There must be a ribosome binding site to enable translation of the passenger protein.

8. (2 points) What would happen if you subjected the culture to heat shock? Why?

Trick question! Heat shock would induce expression from the dnaK promoter, which drives the pir gene. The Pir protein is essential for replication at the R6K plasmid origin, which is irrelevant for this plasmid. So heat shock (at least at the first level of analysis) would have no effect on this plasmid.

9. (2 points) Is the *trp* promoter on pGULIG-1 under attenuation control? Explain.

As noted in question 1, there is no attenuation since it relies on the double trp codons of the leader protein in the trpL operon. (You didn't have to go into detail on the mechanism of attenuation for this answer)

10. (4 points) Can pGULIG-12 be packaged into Lambda phage for transduction? Can it be moved by conjugation? Why or why not for both?

No transduction because it cannot get packaged into Lambda particles because it lacks the cos site. No for conjugation because 1) it does not encode the tra functions and 2) it lacks a mob/oriT site.

**The rest of the questions are independent of pGULIG-12.**

11. (6 points) Briefly explain how catabolite repression works. In your answer you should specify if the system normally acts as a repressor or activator and how it accomplishes that? Is it possible for the system to act in the opposite manner? Why or why not?

It is actually activation. When energy is low (no glucose), cAMP is made by the adenylate cyclase enzyme (*cya* gene). cAMP binds to the CRP/CAP protein and binds to CRP binding sites in DNA in the promoter region of specific genes. This usually causes increased transcription because it has affinity for the RNA polymerase. Yes, it can repress, most easily if the CRP binding sequence is too close to the -35 -10 region and interferes with the ability of the RNA polymerase to bind.

12. (2 points) Your *E. coli* strain has the following alleles listed in its genome. Explain how each one affects the phenotype of the strain: *rpsL*, *rpoB*, *gyrA*

*rpsL* - mutation in ribosomal protein that is the target for streptomycin. This will be Str resistant.

*rpoB* - RNA polymerase beta subunit - rifampin resistant

*gyrA* - gyrase A subunit - nalidixic acid resistant.

13. (4 points) How would deletion of the *dnaK* gene affect the expression of the other heat shock proteins in otherwise wild-type *E. coli*? Explain.

DnaK has a post-translational negative effect on the RpoH heat shock sigma subunit (it causes its degradation). So, if *dnaK* is deleted, RpoH protein would be increased and the other heat shock genes would be induced.

14. (6 points) Explain the differences in susceptibility to bacitracin and vancomycin between gram-positive and gram-negative and wall-less bacteria. Your answer should include the mechanism of action of these antibiotics.

bacitracin - inhibits peptidoglycan synthesis by interfering with the lipid transporter that moves the building blocks from the cytoplasm to outside where polymerization occurs.

vancomycin - binds to D-Ala-D-Ala and interferes with PBPs binding there (inhibits transpeptidation and transport as well (you only had to say transpeptidation).

G+ are sensitive because these antibiotics can access their targets.

G- are resistant because these antibiotics are too large to get through the porins of the outer membrane.

Wall-less are resistance because they don't have peptidoglycan.

15. (6 points) Briefly describe how the cell (inner) membrane is involved with energy production, motility, and DNA replication in bacteria.

energy production - electron transfer and H<sup>+</sup> ATPase

motility - the motor that spins the flagella is in the cell membrane driven by proton motive force

DNA replication - the chromosomes are attached to the membrane and get dragged into the progeny cells during division

16. (8 points) What is the difference between specialized transduction and generalized transduction? Can every phage do either? Why or why not.

specialized transduction - temperate phage integrates into the host genome, during prophage genomic excision an adjacent host gene is included with the phage genome, during lysogeny of recipient cell the adjacent gene is inserted, too, no recombination necessary

generalized transduction - during packaging of phage DNA during lytic infection, a host DNA fragment gets packaged by accident - there is no phage DNA in the transducing particle, the phage particle injects the DNA into a recipient cell where it must recombine

Only temperate phages that insert their DNA into the host genome can do specialized transduction.

Only phages that have sloppy packaging can do generalized transduction.

17. (4 points) What is the difference between a transposon and insertion sequence?

An insertion sequence is inverted repeats that flank a transposase gene that recognizes those repeats to move the IS within a cell. That's it - no other genes.

A transposon is a complex IS, 2 ISs that flank a gene or genes (most importantly antibiotic resistance genes) so that when the IS transposases moves the whole set, the resistance gene (or some other observable genes) is moved.

18. (4 points) Why can humans be treated with sulfa drugs and trimethoprim when we use folic acid for single carbon donor reactions just like bacteria? Why are bacteria sensitive to these antibiotics?

We lack the target of sulfa drugs - dihydropteroate synthase, whereas bacteria must have this enzyme to create folic acid. This is because folic acid is an essential vitamin for us (i.e., we can't make it).

We have the target for trimethoprim, dihydrofolate reductase, to recycle folic acid, but ours is different enough from the bacterial form that it can be targeted.

19. (4 points) There is a known mechanism for resistance to sulfa drugs and trimethoprim, which we may or may not have covered in class. If you know the mechanism, explain it and its mechanism and genetic basis (hint - resistance to both antibiotics comes in a single package). If you do not know it, then propose two completely different mechanisms for resistance to these drugs and explain their genetic bases.

The known mechanism is a plasmid-encoded set of genes encoding an alternative folic acid pathway that is resistant to these antibiotics.

One hypothetical mechanism would be enzymes that break down the antibiotics. Another would be pumps. These would be on a plasmid since a single point mutation would not enable these.

You should not have proposed a single point mutation affecting resistance to both antibiotics, since their targets are different.

20. (2 points) If a bacterial culture increased from  $10^5$  CFU/mL to  $10^8$  CFU/mL over a 3 hour period, what is the doubling time? You only have to be within 10% of the precise answer, so you can use the rule of thumb as an estimate if you want. You do not need a calculator to do this.

$10^8/10^5 = 10^3$ -fold difference. For every 10-fold increase there are about 3 generations.  $3 \times 3 = 9$  generations over a 3 hour period = 9 gen/3h = 3 gen/hr. That translates into 20 min/gen.

21. (4 points) Describe how looking at a bacterial cell by light and electron microscopy can tell you if it is growing fast or slowly. Explain.

light microscopy - If the cell is large, it is growing fast. Fast growing cells require multiple copies of the chromosome being made at the same time to keep up with division.

EM - Look for more dense concentration of ribosomes for fast growing cells. Fast growing cells need more ribosomes to keep up with protein synthesis.

22. (4 points) What nucleic acid sequences are recognized by the CsrA protein? What is the effect of CsrA binding to these sequences?

It resembles the ribosome binding site (you didn't have to put the sequence!) on mRNA (not DNA). When CsrA binds to this site on an mRNA it either prevents translation or increases decay of the mRNA. In either case, there will be less protein made.

23. (4 points) How does factor-independent termination work?

There is a GC-rich stem with a loop followed by U's in the mRNA. When the step-loop forms, it causes the weak U-A RNA-DNA binding in the transcription bubble to be dislodged, and the RNA polymerase falls off the DNA, stopping transcription.

24. (6 points) How does the leader sequence of a protein affect its interaction with the Sec export system and the ultimate location of the protein?

If the leader sequence is short, it will tend to interact with SecB, which will stabilize the protein and guide it to the SecYEG system for post-translational export into the periplasm for G<sup>-</sup> or outside of the cell for G<sup>+</sup>.

If the leader sequence is longer, it will interact with the SRP, which will pause translation until it docks with the Sec system for co-translational export. The leader sequence becomes the first transmembrane domain of an inner/cell membrane protein.

25. (4 points) In a pET vector, what is the T7 promoter used for? Where did it come from? If you put a pET plasmid into a normal *E. coli* strain, will the plasmid work for its intended purpose? Why or why not?

The T7 promoter drives the expression of the cloned gene. It is from the T7 bacteriophage. In a regular *E. coli* strain, the T7 promoter will not work because it requires the T7 RNA polymerase to function. (In commercially available strains, this is BL21(DE3).)

26. Extra credit (4 points) What is the physical form of DNA in a cell after the following processes but before any recombination can occur?

natural transformation - linear single stranded

generalized transduction - linear double stranded

electroporation of a plasmid - circular double stranded (supercoiled, too)

specialized transduction with  $\lambda$  phage - linear double stranded, just like the Lambda phage DNA exists in the phage particle. However, it will circularize before lysogeny.