

# **Bacterial Genetics**

## **GMS6038**

### **Final Exam**

### **Fall 2011**

You should be able to complete the exam in 2 hours, but there is extra time allowed if necessary.

This is a closed book, closed note exam. All backpacks and notebooks must be against the wall, not at your workstation. Follow the rules of the exam center.

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 IS NOT AN ESSAY EXAM. Thoughtful answers will be short. The points allotted for each part of each question mainly correspond to the number of facts/concepts are being looked for in the answer. For example, a 1 or 2 point question should be able to be answered in a single (compound) sentence.

To complete the exam, simply type your text beneath each question. You may use the printed exam form to organize your thoughts. It will be turned in when you are done.

Shown below is pGULIG-13. Note the following genetic elements:

At the top of the map is the origin of replication of the R6K plasmid.

Moving clockwise:

From 1:00-3:00 the *cat* gene expressed from the *dnaK* promoter.

From 3:00-5:00 is the *pir* gene expressed by the Lambda phage pL promoter.

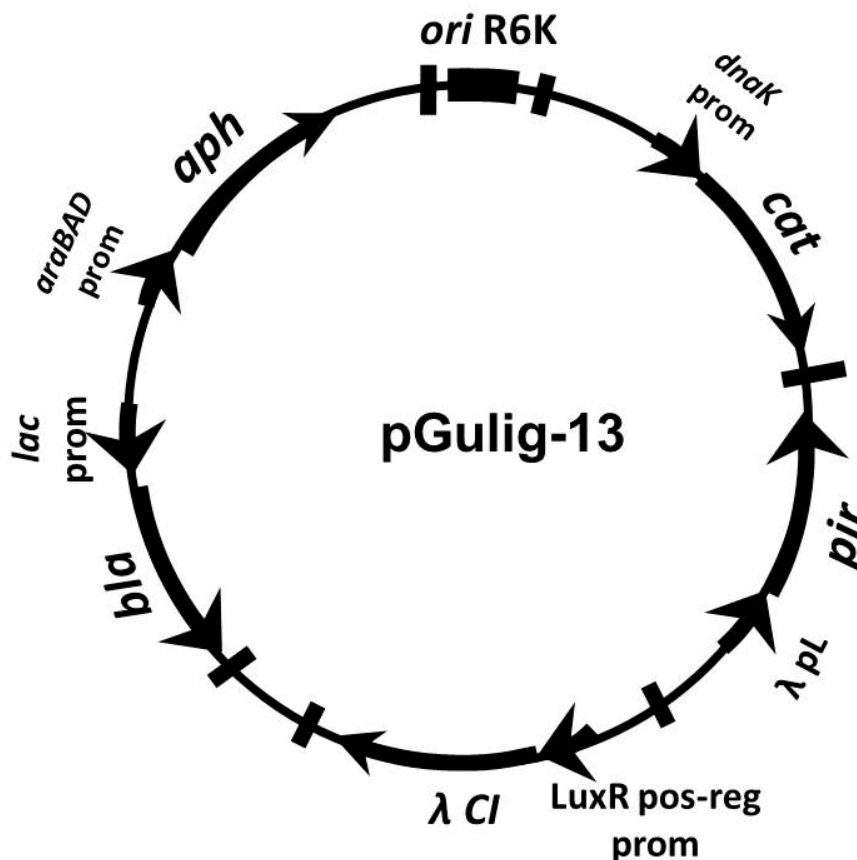
At the bottom is the Lambda CI gene expressed by an unnamed promoter that this positively regulated by the *V. harveyi* LuxR protein.

From 7:00-9:00 is the *bla* gene expressed from the wild-type *lac* promoter.

From 10:00-11:00 is the *aph* gene driven by the wild-type *araBAD* promoter.

There are no other promoters on this plasmid other than those indicated on the map. Assume that all genes have appropriate translation initiation and termination sequences. The bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements.

The host *E. coli* K12 strain has the normal chromosomal genes except that it has **no F plasmid** and **no Lambda phage**. For purposes of this exam, this *E. coli* has the complete set of genes for the *Vibrio harveyi* quorum sensing system in the chromosome, including autoinducer production, sensing, and signal transduction.



The following questions pertain to pGULIG-13.

1. A. (4 points) If the *E. coli* culture is growing at a moderately low cell density, explain what is governing the plasmid copy number.

The copy number is regulated by the OriR6K origin of replication. This requires the Pir protein to be expressed from the *pir* gene. The *pir* gene is expressed by the Lambda left promoter (pL), which will be expressed unless it is repressed by the Lambda CI protein. The CI gene is expressed from a LuxR positively regulated promoter. Therefore, the question is if the LuxR protein will be expressed for activation of CI expression. LuxR is the quorum sensing master regulator, so it will only be expressed at high cell density. At high cell density it will be expressed and will activate CI, which will repress *pir*, which will prevent plasmid replication (lower copy number). However, the question is about low cell density, so LuxR will not be expressed, CI will not be expressed, and *pir* will be expressed, and copy number will be higher. Note that you didn't have to go into so much detail in your answer - I wrote this out to best explain the answer.

B. (6 points) What happens to copy number of pGULIG-13 when the culture reaches very high cell density? Explain the whole chain of events starting from what happens in the culture at high cell density. Here's a clue - this question is focused on quorum sensing, not stationary phase physiology.

As noted above, at high cell density, LuxR will be expressed, CI will be expressed, the pL will be repressed, Pir will not be expressed, and copy number will go down.

The whole chain of events is this: Autoinducer molecules will accumulate in the culture. They will be sensed by membrane two component regulatory systems that will initiate a chain of dephosphorylation events. First LuxU then LuxO. Dephosphorylated LuxO will prevent the production of small regulatory RNAs (sRNAs) that would normally repress expression of LuxR by destabilizing its mRNA.

C. (2 points) Would this change in copy number be harmful or beneficial to the cells if pGULIG-13 was essential for viability, e.g., one of the relevant selective pressures to which this plasmid encodes resistance, was present in the culture medium? Explain.

This is a very complex question and answer. First, if the cells were in stationary phase, the antibiotics would not have a great effect on the cells. Second, if the cells are in stationary phase, they will not be replicating, and the plasmid would not be lost (cells in the culture "lose" the plasmid when there is not a copy for them to receive at division). If any cells arose from replication that did not maintain the plasmid, this would be detrimental since expression of the antibiotic resistance genes encoded on the plasmid would cease. If you think about it even one step further, as cell density decreases when cells die off or get diluted away, the remaining cells with the plasmid will not be under quorum sensing conditions, so they will replicate the plasmid again.

2. A. (5 points) Which of the two genes would you rather use as positive selection for this plasmid, especially if the culture was going to be a broth, as opposed to plate: *aph* or *bla*? Explain your answer, and be sure to say what these genes do. What is the explanation for the differences in the nature of the gene products that makes one better than the other for purposes of selecting for this plasmid using one of these genes?

*aph* - because the aminoglycoside phosphotransferase protein is localized to the cytoplasm and will not leak out of the cells. The beta-lactamase is in the periplasm and leaks out of cells and breaks down beta-lactams in the culture. Therefore, plasmid-free cells can still grow. *Aph* is protecting the ribosomes from kanamycin so it is best localized to the cytoplasm. Because *Bla* protects penicillin-binding proteins involved with peptidoglycan synthesis in the periplasm, that is where this protein is localized.

B. (3 points) What would the relevant composition of the growth medium be to get *aph* and *bla* expressed individually? Explain.

*aph* - pBAD - + arabinose, - glucose: pBAD is the arabinose operon which is positively activated by arabinose. No glucose to prevent catabolite repression. Arabinose binds to AraC to activate pBAD

*bla* - pLac - + lactose, - glucose: pLac is the lactose operon which is positively activated by lactose. No glucose to prevent catabolite repression. Lactose binds to the LacI repressor to prevent its repression of pLac.

3. A. (2 points) What conditions would you use to get expression of the *cat* gene? Explain.

*cat* is expressed from the *dnaK* promoter. *dnaK* is a heat shock-regulated promoter, so increasing the temperature to 42C would cause its transient expression mediated by RpoH.

B. (2 points) What does the *cat* gene product do?

chloramphenicol acetyl transferase - chloramphenicol resistance

C. (2 points) Relative to the regulation for *aph* and *bla* collectively, is the way that *cat* is regulated more beneficial or detrimental to the health of the cells if the selective pressure for this gene was present? Explain. Note that this question does not relate to the enzyme activities of any of these genes, but the way that they are regulated and how this affects their overall ability to achieve their purpose for the health of the cell under selective pressure.

Detrimental - because the heat shock response is transitory and the cells will shut off *cat* expression. There is only a brief increase in expression of heat shock genes after a heat shock, even though the temperature remains high. Once the cells adjust to the elevated temperature, they shut off heat shock proteins. On the other hand, the *lac* and *ara* operons will remain on as long as the inducers are present.

4. A. (4 points) A thought question with more than one possible correct answer - If you were going to insert a multiple cloning site into the middle of one of the genes on pGULIG-13 so that you could get regulated expression of your favorite protein whose gene is cloned into the multiple cloning site, but the health of the cells couldn't be compromised, which genes would it be and why? Note that you will need to have at least one method of selecting for the plasmid in your culture.

You could use *bla* or *aph* and then regulate expression with the appropriate inducer. You could use *cat* if you wanted transitory expression from a heat shock. You could use *CI* if you want a quorum sensing stimulus (probably wouldn't be good since cells are tired and getting quiescent by the time they are in quorum sensing mode).

B. (2 points) Which gene would be the worst for cloning into? Why?

Main thing is that it can't be *pir*, or the plasmid will not replicate.

These questions are independent of pGULIG-13.

5. In this course, we discussed two proteins that are involved with helping RNAs do their jobs, Rop (Rom) and Hfq.

A. (4 points) Describe the functions of these proteins in interacting with the relevant RNAs. Be sure to mention the specific RNA(s) that are affected and the molecular function of the protein (i.e., what does it do to/with the RNA?). Read the next question before answering this part to avoid redundancy.

Rom - stabilizes the binding of the RNA-1 and RNA-2 of the ColE1 plasmid.

Hfq binds to the quorum sensing sRNAs and helps them bind to the LuxR mRNA.

B. (4 points) What is the end result of each protein interacting with the RNA (be sure and say what the RNA in question does)?

Rom - by stabilizing RNA-1 and RNA-2, RNA-2 cannot act as a primer for ColE1 replication, so replication is inhibited.

By guiding sRNA-luxR mRNA binding, the luxR mRNA is destabilized and LuxR protein will not be expressed. The quorum sensing positively regulated genes will not be expressed.

C. (2 points) Conversely, what would be the effect on the relevant phenotype if the *rop* and *hfq* genes were individually deleted (i.e., I'm not looking for any relationships between these different proteins and their RNAs - this is two independent questions).

*rop/rom* - ColE1 plasmid copy number would increase

*hfq* - quorum sensing regulated genes would be expressed more

6. The pET plasmids employ the phage T7 expression system to get your favorite gene expressed.
- A. (4 points) Explain the critical parts of the expression aspect of the pET plasmids, especially which components are from the T7 phage and how they work. Your answer should include if there are any special requirements about the host *E. coli* strain that you use.

The promoter that drives expression of your favorite gene is a T7 phage promoter. It is expressed using the T7 RNA polymerase, which is cloned elsewhere in the host *E. coli* strain, driven by the *lac* promoter. The T7 RNA polymerase acts as a single subunit very strongly active RNA polymerase. You could throw in the T7 lysozyme, which represses the activity of the T7 RNA pol to make expression even more tightly regulated.

- B. (2 points) Using the pET system, what do you do to turn the system on? Explain at the level of molecules working together what happens when you make your change. Note that you do not have to repeat information from part A here. You can just link the two answers.

Add IPTG to bind *LacI* and turn on the T7 RNA pol which will turn on the T7 promoter driving expression of your favorite gene.

- C. (4 points) What are the benefits and problems with using the T7 expression systems?

Benefits - very high expression of your protein. Problems - this can make the cells sick and unable to perform other functions, such as virulence. They might even die. They might make inclusion bodies which make isolation of your protein difficult. However, you can also purify inclusion bodies pretty easily, and if you can get the protein back into solution and refolded correctly, you can get pretty clean protein.

7. (3 points) Do all *E. coli* promoters have similar sequences at the -10 and -35 regions? Why or why not?

No. First, the -10 and -35 sequences are specific for the different sigma subunits of the RNA polymerase, and there are several different sigmas for different purposes (heat shock, sporulation, stationary phase). Second, even for the standard housekeeping sigma factor, genes that rely on regulatory proteins for differential expression often have non-canonical -10 and/or -35.

8. A. (5 points) How are proteins that are destined for the inner membrane handled differently than proteins destined for the periplasm or complete secretion out of the cell?

inner membrane - longer signal sequence that causes them to be recognized by the SRP particle. They are exported through the membrane by the Sec system. The leader sequence becomes the first transmembrane domain (not cleaved by Lep usually). Transport is co-translational, energy is from translation.

periplasm/secretion - shorter signal sequence bound by SecB and SecA that uses the Sec system for transport. The leader sequence is cleaved off by Lep. Energy is primarily from ATP.

B. (4 points) Briefly describe the two major/essential characteristics you would expect to see in a protein that is exported via the sortase system. Explain what these things do.

Leader sequence to get the protein through the Sec secretion system  
Sortase recognition sequence that gets the protein attached to the peptidoglycan.

C. (1 point) What type of bacteria have the sortase system?

Gram-positive

9. A. (2 points) What is the function of a ribosome binding site?

Translation initiation. Gets the ribosome to bind to the mRNA near the ATG start codon.

B. (1 point) What is the significance of this sequence, i.e., why is this not a random sequence (not the same answer as part A).

complementary to the 16S rRNA.

10. (3 points) In catabolite repression, is cAMP an inducer or corepressor? Explain.

inducer - its binding to Crp protein enables it to bind to promoter sequences and generally induce gene expression (there are some examples of repression, though, in which case it would be technically a co-repressor, but you didn't have to say that.)

11. (6 points) Match the cell type with the antibiotic to which it is inherently resistant. Briefly explain why it is resistant.

gram-positive - polymyxin - binds LPS of gram-negative bacteria  
gram-negative - bacitracin - too big to fit through the outer membrane porins  
wall-less - ampicillin - no peptidoglycan, polymyxin - no LPS

12. A. (3 points) If I showed you a light micrograph (1000X) of an *E. coli* cell, how could you tell me if it was rapidly growing or in stationary phase? Explain. Do not tell me that you can see the cell dividing. I am going to show you a single cell not in the process of dividing, right after cell division.

If it is extra long, it is dividing because there would be multiple copies of the chromosome to keep up with cell division.

B. (2 points) If I showed you an electron micrograph of an *E. coli* cell, how could you tell me if it was rapidly growing or in stationary phase? Explain. (This answer must be different from part A).

Numerous ribosomes - rapid growth - need to increase ribosome number to make proteins fast enough for rapid cell division.

13. (4 points) How do trimethoprim and sulfonamide antibiotics typify the two general principles that lead to usable antibiotics in terms of therapeutic index? Your answer should show that you know what therapeutic index is.

sulfa - we lack the target (dihydropteroate synthase), so there is a high TI (max dose tolerated by us divided by lowest effective dose against the bacteria)

trimethoprim - we have the same target (dihydrofolate reductase), but ours is different enough from the bacteria that we can target it with an antibiotic, so there is a lower (but acceptable) TI

14. (8 points total) Compare and contrast (a table or list is adequate) the type 2 and type 3 terminal secretion pathways in terms of:

A. final location of the secreted protein

T2 - outside of cell in extracellular space

T3 - injected into host cell

B. if the secreted protein utilizes the General Export Pathway

T2 - yes      T3 - no

C. the cellular component that most resembles or depends on each pathway

T2 - type 4 pili

T3 - flagella

D. if the common laboratory *E. coli* strain K12 has the terminal pathway

T2 YES (I taught no, so that was accepted, but it turns out there are cryptic genes), T3 - no

15. (4 points) What is the difference between specialized transduction and lysogenic conversion?

Specialized transduction - a temperate phage that integrates into the host genome accidentally packages a host gene next to the integration site upon excision. The host gene is transmitted upon the next lysogenic event.

Lysogenic conversion - the gene of interest is an inherent part of the phage genome and is transmitted every time the phage lysogenizes a host cell.

16. (2 points) If a bacterial culture grows from 1,000 CFU to 1,000,000 CFU over a period of 3 hours, what is the doubling time? You do not need a calculator to do this. You may use the "rule of thumb" for estimation.

$10^6/10^3 = 10^3$ -fold increase. For each 10-fold increase it takes about 3 generations, so a total of 9 generations. 3 h/9 gen or 0.33 h/gen or 20 min/gen.

Extra credit question for thought (5 points) What are some advantages and disadvantages of bacteria growing in biofilms? Make sure that your answer shows that you know something about biofilm biology.

Disadvantage - they are crowded and must slow down or stop growing.

Advantage - since they are not replicating rapidly, they are resistant to antibiotics and other insults. Biofilms help bacteria stick to surfaces and colonize them. In animal infections, biofilms are very difficult to fight with the immune system.