

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.

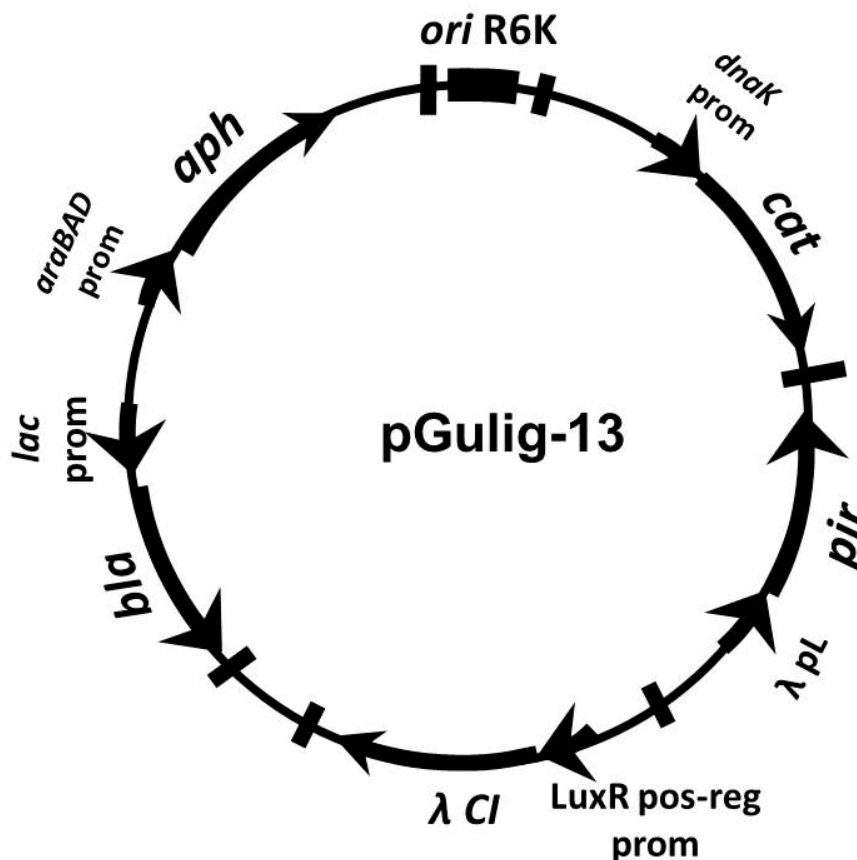
At the bottom is the Lambda CI gene expressed by an unnamed promoter that this positively regulated by the *V. harveyi* LuxR protein.

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.

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.

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.

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?

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

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

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

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.

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.

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

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.

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)?

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).

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.

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.

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

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

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?

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.

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

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

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).

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

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

gram-positive

ampicillin

gram-negative

bacitracin

wall-less

polymyxin

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.

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).

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.

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

B. if the secreted protein utilizes the General Export Pathway

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

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

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

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.

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.