Bacterial Genetics
GMS6038
Final Exam
Fall 2008

Make up a code name for yourself and put it here. ____________________________

Write down the code with your name on a sheet for the class so I can grade the exam in a blinded manner.

You have 2 hours to complete the exam.

Please note - this is a closed book, closed note exam. All backpacks and notebooks must be against the wall, not at your table.

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.

Shown below is pGULIG-10. Note the following genetic elements:

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

Moving clockwise:

From 1:00-4:00 the *araBAD* promoter drives the *pir* gene followed immediately by the *lacI* gene. The *araC* gene is placed in its natural context relative to the *araBAD* promoter.

At the bottom is the phage T7 promoter driving the expression of the *bla* gene. The *bla* gene has a multiple cloning site (MCS) inserted.

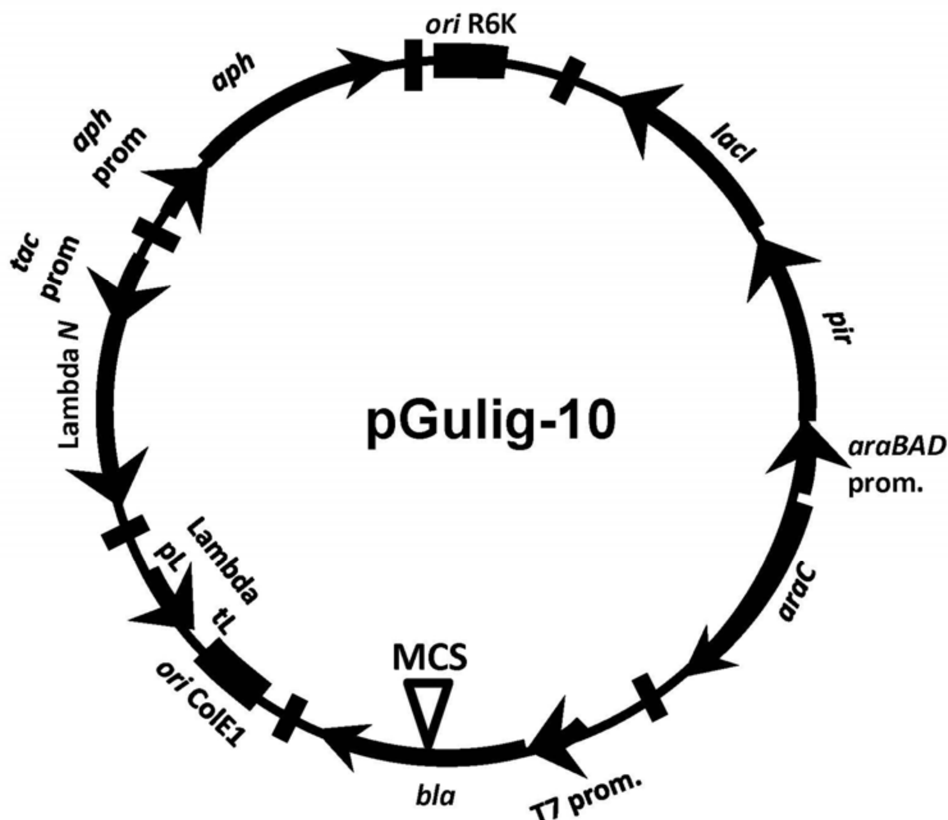
At about 7:00 is the origin of replication of the ColE1 plasmid WITHOUT its Rna-I and Rna-II loci. Instead immediately upstream is the Lambda phage pL promoter followed by the tL terminator.

At about 9:00 is the Lambda phage N gene expressed from the tac promoter.

Finally from 10:00-11:00 is the *aph* gene driven by its own promoter, which is constitutive.

There are no other promoters on this plasmid other than the those indicated on the map. Assume that all genes have appropriate translation initiation and termination sequences. The thin 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 its *lacI* gene deleted**, **no F plasmid**, and **no Lambda phage** or any other phages.



NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. 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.

Write your answers legibly on separate lined paper with your code on each page.

Questions 1 through xx are based on pGulig-10. Here is a clue to help work these questions. Start at the genetic element that is being asked about and then work your way through the rest of the plasmid looking for genes that are related to those. Don't try to take in the whole plasmid at once. Don't forget to consider genes contributed by the *E. coli* host (or not).

1. (3 points) Which of the sites on pGulig10 are necessarily cis active? Explain.

2. (2 points) Is pGulig10 conjugative, mobilizable, or not? Explain.

3. (5 points) If you want to maintain the plasmid based on the origin of replication from the R6K plasmid, what conditions would you use. Explain.

4. (5 points) If you want to maintain the plasmid expressed from the ColE1 origin, what conditions would you use. Explain.

5. There is a multiple cloning site in the middle of the *bla* gene.

a. (2 points) If this plasmid is placed in the *E. coli* strain described, what will be required to get the *bla* gene expressed? Explain.

b. (4 points) What advantage or special use is there to having the MCS in the *bla* gene? To get the benefit of cloning your favorite sequences into the *bla* gene, what must you ensure about the placement of the MCS and the sequences cloned within it?

6. a. (2 points) What is the function of the *aph* gene?

b. (1 point) What is meant by "constitutive"?

The rest of the questions are independent of pGULIG-10.

7. (6 points) How do the Lambda phage *cII* and *cro* genes interact to regulate Lambda gene expression? Include in your answer what gene(s) is being regulated and the effects of this regulation.

8. (2 points) Why is the trp operon not under catabolite repression?

9. (4 points) If the *trpR* gene was mutated so that it no longer bound tryptophan, how would this affect the regulation of the trp promoter? What would ultimately happen to the levels of tryptophan in the cells? Explain.

10. (4 points) Would M13 make a good specialized transducing phage? Why or why not? Would M13 make a good generalized transducing phage? Why or why not?

11. (4 points) How would a mutation that knocked the *polA* gene encoding DNA polymerase 1 affect chromosomal replication of *E. coli*. Do you believe that such a mutant would be viable? Explain.

12. (4 points) In *E. coli*, what determines if a protein is directed to the general export pathway by SecB or SRP? Your answer should include a description of the structure of the protein and what the consequences to the protein are if it is handled by SecB or SRP.

13. (6 points) Compare and contrast the type 2 and type 3 secretion systems in terms of ultimate location of secreted protein and dependence on general export pathway.

14. (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

15. (10 points) Sketch the gram-positive and gram-negative cell membrane/wall. Label the unique components of each.

16. (6 points) If you wanted to make an *E. coli* strain that was resistant to an antibiotic to use in the lab, but you only had the non-resistant parent strain, L agar media, and the antibiotics themselves, which of the following antibiotics would you use to develop your resistant strain? Explain how you would do this and explain your choice of antibiotic. At the molecular level, why would your strain be resistant?
tetracycline, ampicillin, rifampin

17. (10 points) Diagram the essential elements for expression of a bacterial gene that encodes a protein. Include in your diagram the role of each element, that is, what

factors/functions act at each site. Do not put the DNA sequences (ACGT), just the name and function.

18. (4 points) You have a culture of E. coli at a density of $1x10^6$ CFU/mL. You incubate it at room temperature in L broth for 3 hours, and the final density is $6.4x10^7$ CFU/mL. What was the doubling time of the culture? What was the growth rate? You do not need a calculator to do this.

19. (6 points) Why is the response to heat shock a stimulon but the response to arabinose a regulon? Do not simply define stimulon and regulon, but also biologically explain why heat shock needs to be a stimulon and arabinose a regulon.

20. (4 points) What is a suicide plasmid and what is it used for? What are the critical elements of a suicide plasmid?

21. Extra credit – (4 points) How does the GroEL chaperonin function?