

Bacterial Genetics
GMS6038
Final Exam
Fall 2007

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-9. Note the following genetic elements:

At the top of the map is the Lambda phage CI gene. It is driven by the *dnaK* promoter.

The rectangle is the *incC/copA* locus from the F plasmid (different names for same site).

The *sacB* gene is driven by the arabinose *araBAD* promoter.

The F plasmid *repE* gene is expressed from the Lambda phage pL promoter.

oriT is from the F plasmid.

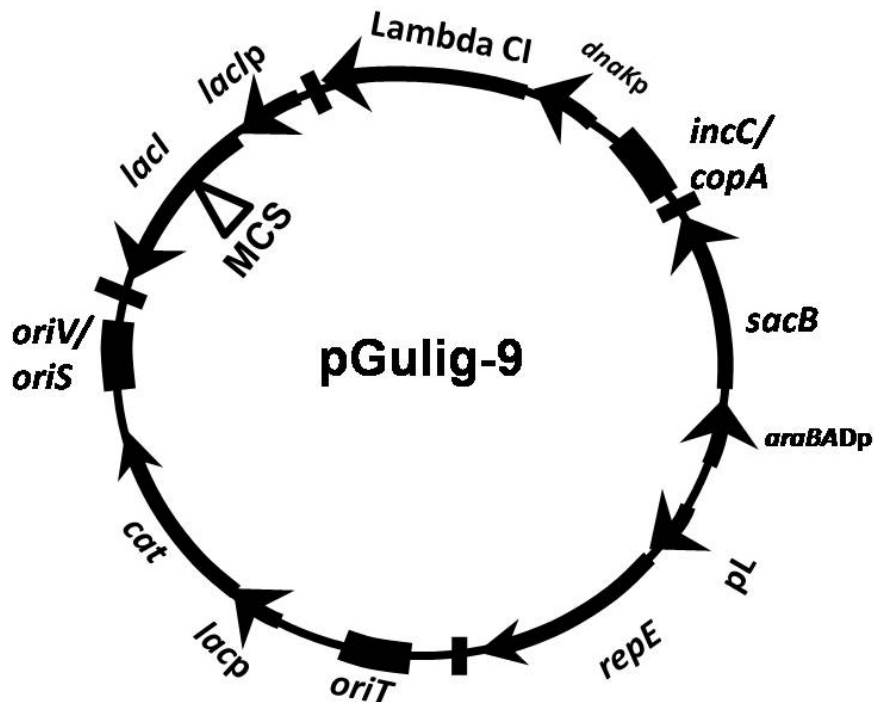
The *cat* gene is expressed from the *lacZYA* promoter.

The rectangle is the *oriV/oriS* locus from the F plasmid (different names for same site).

The *lacI* gene is driven by its own promoter. In the middle of the *lacI* gene is a multiple cloning site (MCS). Just like the MCSs in many favorite cloning vectors, this MCS does not disrupt the functional reading frame of the LacI protein.

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 5 are based on pGulig-9. 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. This plasmid is supposed to act as a suicide plasmid.
 - A. 2 points. In a general sense (i.e., not limited to this plasmid), explain how a suicide plasmid works purely in terms of its replication.
 - B. 6 points. Under what conditions will this plasmid act as a suicide plasmid? Be sure to explain how every relevant element of pGulig-9 is involved with its being a suicide plasmid, as related to plasmid replication.

2. Another aspect of suicide plasmids can be the presence of a counter-selectable marker (one that can be selected against).
 - A. 2 points. What is the counter-selectable marker on pGulig-9?
 - B. 2 points. How does it work as a counter-selectable marker?
 - C. 6 points. Under what conditions would the counter-selectable marker be functional, that is, how would you grow the bacteria with pGulig-9 to select against the plasmid (note that this is actually pretty complex, so be sure and think it through, and don't forget the relevant chromosomally encoded genes)?

3.
 - A. 4 points. What is the function of the *cat* gene? Provide the full name of the gene product and its mechanism of action.
 - B. 6 points. How is *cat* expressed and regulated on this plasmid, that is, under what growth conditions would the gene be expressed? Explain.
 - C. 2 points. How could you use the complex mechanism of regulation coupled with the multiple cloning site to select for successful insertion of cloned sequences into the MCS?

4.
 - A. 4 points. On the F plasmid what is the function of the *incC/copA*? Give a general function and then a molecular explanation.
 - B. 2 points. How might the *incC/copA* affect pGulig-9, especially in light of your answer to question 1?

5. 4 points. Can this plasmid be moved from the host *E. coli* strain described above into a recipient *E. coli*? Why or why not? Explain.

The following questions are independent of pGULIG-9

6. You have a globular protein that you would like to have expressed and exported into the periplasm of *E. coli*. So you cloned the gene into a vector that has the beta-lactamase signal sequence followed by a multiple cloning site. Beta-lactamase is exported through the SecYGE system.

A. 2 points. Describe the essential nature of the DNA sequence between your gene and the beta-lactamase signal sequence to enable export of your protein.

B. 6 points. Briefly explain how the beta-lactamase signal sequence works at the molecular level, including other proteins and *E. coli* factors that it will interact with after it has been translated.

C. 2 points. Unfortunately, you find that your protein is not exported. Is this unexpected or not? Explain.

D. 2 points. Describe another export system that might be useful for getting your protein exported through the inner membrane. Why might this other system be useful?

7. A. 4 points. Why are trimethoprim and sulfamethoxazole frequently mixed together to treat bacterial infections? Explain in terms of their mechanisms of action.

B. 5 points. How do these two antibiotics exemplify the two different reasons behind selective toxicity for antibiotics relative to humans? Based on this answer, which would you expect to have the highest therapeutic index?

C. 5 points. Your favorite *E. coli* strain's genotype has *rpsL* in it. What does this mean? Explain how it works.

8. 10 points. Explain how RNA polymerase, CAP-cAMP (CRP-cAMP), AraC, LacI interact for the *ara* and *lac* systems, respectively, to affect initiation of transcription. Your answer should include the relative locations (not the DNA sequences!) of the DNA sites that these factors bind to. Your answer should focus on what happens at the promoter region, not all of the other things going on before and after initiation of transcription.

9. 4 points. How does the fate of proteins secreted by the sortase system of gram-positive bacteria differ from that of proteins secreted by gram-negative bacteria?

10. A. 4 points. How does a suppressor mutation for a nonsense mutation work?

B. 2 points. What would the consequences be if suppressor mutations were 100% effective?

11. Bacterial cells that are growing rapidly in rich broth are significantly larger than cells growing very slowly or not at all. There are two major reasons that this is true (hint – you would be able to see this with an electron microscope).

A. 4 points. What are the two things that make the cells larger?

B. 4 points. What is the explanation for this phenomenon?

12. 6 points. Explain the differences in sensitivities to ampicillin and vancomycin between gram-positives, gram-negatives, and wall-less bacteria (note there are 6 possible interactions here).