

Bacterial Genetics
GMS6038
Final Exam
Fall 2006

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 from when you arrived 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-8. Starting at the top, note the following genetic elements:

At the top is the origin of replication of the ColE1 plasmid. The promoter for RNA-II has been replaced by the lac promoter (for lacZ_YA).

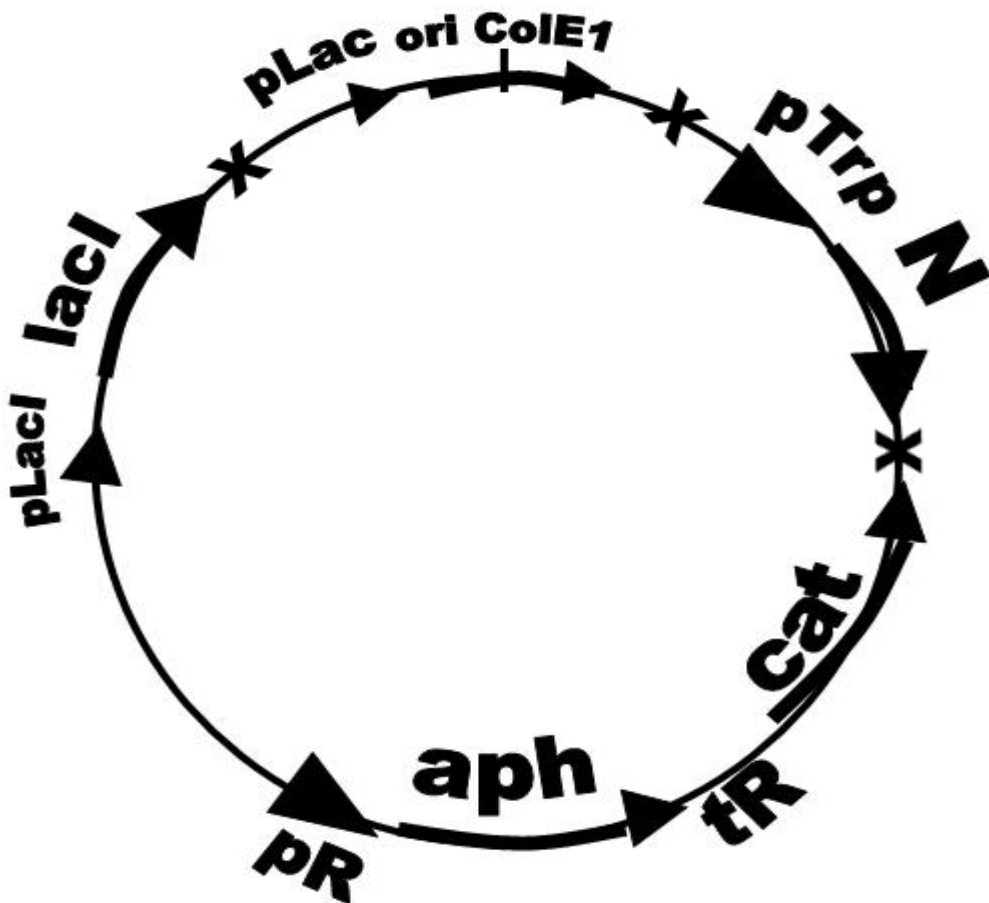
In the upper right is the Lambda phage N gene expressed from the trp promoter (does not include trpL).

In the bottom right is the Lambda phage pR promoter driving expression of the aph gene followed by the Lambda phage tR terminator followed by the cat gene. Note that the cat gene does not have its own promoter.

At the left is the lacI gene driven by its own promoter.

Assume that all genes have appropriate translation sequences. The x's on the map represent typical factor-independent terminators that will prevent transcriptional read through between these different elements.

Don't forget that the host E. coli K12 strain has the normal chromosomal genes, 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 4 are based on pGULIG-8. 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.

1. What would you include or exclude from the growth medium to:
 - A. (5 points) cause the highest copy number of this plasmid? Explain.
 - B. (5 points) cause the lowest copy number of this plasmid (maybe even prevent its replication altogether)? Explain.
 - C. (2 points) If the lac promoter was exchanged with a trc or tac promoter, how would this affect your answers above? Explain.
2. (4 points) What functions are encoded by the aph and cat genes?
3. A. (6 points) How would adding or omitting tryptophan in the growth medium of E. coli possessing this plasmid affect antibiotic resistance? Explain.

B. (2 points) If the trp sequences upstream of the N gene included the trpL gene, how would this affect your answer to part A? Explain.
4. (4 points) If this plasmid was placed into an E. coli strain that was lysogenized by Lambda phage, how might this affect antibiotic resistance conferred by this plasmid? Explain.

The following questions are independent of pGULIG-8

5. A. (2 points) What is the definition of a facultative anaerobe?

B. (6 points) E. coli is a facultative anaerobe. How does this fact relate to the optimal growth conditions for enabling this bacterium to produce your favorite cloned gene? Explain. You only need to discuss optimal growth conditions related to being a facultative anaerobe, but if you discuss all aspects of optimal growth, you can get extra credit.
6. (6 points) Name two unrelated (in different families) antibiotics that affect DNA synthesis. Very briefly explain how each interferes with DNA synthesis.

7. A. (6 points) Let's say that you want to convert your favorite cloning vector into one that can be moved by conjugation. What DNA sequences would you have to add to your vector and what DNA sequences/genetic elements would you need to add to your host donor E. coli strain? Briefly explain how this system will work for conjugation.

B. (6 points) To conjugate your plasmid from the donor to the recipient, you need a selectable genetic marker in the recipient strain so that you can plate the conjugation mixtures of the donor and recipient strains on this antibiotic to kill or inhibit the donor E. coli strain. So you need to create an antibiotic-resistant derivative of your recipient strain. Without using any plasmids or other genetic exchange, briefly explain how you will make your recipient strain antibiotic resistant. Explain at the genetic and functional level why your bacteria are now resistant.

8. (6 points) Why is Lambda phage very good at specialized transduction but terrible at generalized transduction?

9. A. (2 points) What is the mechanism of action of beta-lactamase?

B. (4 points) Where (on what genetic element) would you normally expect the bla gene to be encoded? Why?

C. (6 points) Beta-lactamase enzyme is normally found in the periplasm of E. coli. How does E. coli target beta-lactamase to the periplasm, both in terms of the critical elements of the protein itself and cellular components/functions that get the protein to the periplasm.

D. (2 points) If you specifically removed the portion of the bla gene that encodes the critical elements of the protein required for localization of the protein to the periplasm, do you believe that the protein would still enable resistance to beta-lactam antibiotics? Why or why not?

10. (4 points) Why do bacteria have ribosome binding sites but eukaryotes do not?

11. (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

12. (8 points) Using only a few words or possibly a diagram, describe the **single** most important/characteristic feature of each of the four bacterial cell wall types.

13. You clone your favorite gene into a common expression vector and electroporate it into *E. coli*. You choose a single colony grown on a suitable antibiotic to select for the plasmid and then grow a broth culture overnight. However, you failed to include the antibiotic in the overnight broth. The next day the culture is turbid because it grew to stationary phase. Realizing your mistake, you dilute the culture and plate it on plates with and without the antibiotic. You find that only 10% of the bacteria were capable of growing on the antibiotic-containing plates.

A. (4 points) Assuming that the antibiotic-sensitive bacteria are not contamination, what happened to allow these bacteria to appear?

B. (2 points) How might the gene that you cloned into the vector affect the appearance of the sensitive bacteria?

14. Extra credit (up to 10 points) *rpoH* encodes the heat shock alternative sigma factor. When *E. coli* cells are subjected to a heat shock, the level of transcription of *rpoH* does not increase very much, but the levels of the RpoH sigma factor protein increase a lot. Explain why this happens. This answer could be longer than a couple of sentences!