

Bacterial Genetics

GMS6038

Final Exam

Fall 2010

Write your station code here. _____

You have 2 1/2 hours to complete the exam.

Please note - this is a closed book, closed note exam. All backpacks and notebooks must be in the cubbies against the wall. All cell phones and personal communication devices must be off and put away.

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 exam is in the form of a Word document. Simply type your answers below the questions. You may write on the paper form of the exam, but you will have to turn it in at the end of the exam. The exam center personnel will instruct you on mechanistic of the computer system.

Shown below is pGULIG-12. Note the following genetic elements:

At the top of the map is the origin of replication of the ColE1 plasmid. Note that the *rna-II* is expressed from the *trp* promoter. Note that the *trp* promoter is not followed by any *trp* protein coding sequences.

Moving clockwise:

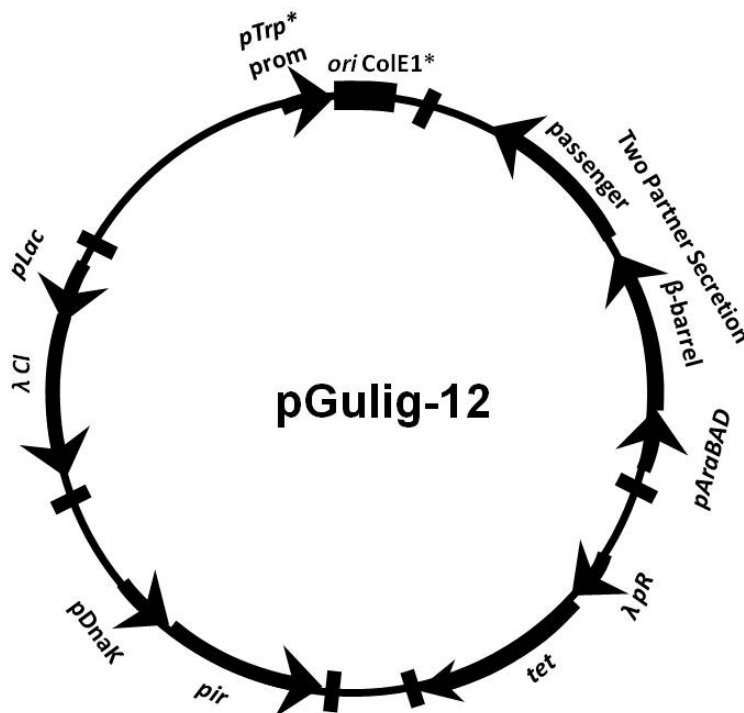
From 1:00-3:00 are the genes for the coding sequences for the two components of a typical two partner secretion system. The β barrel protein and passenger protein genes are indicated. Both of these genes are expressed by the promoter for the *araBAD* genes.

From 4:00-5:00 is the *tet* gene expressed by the λ pR promoter.

From 6:00-8:00 is the *pir* gene expressed from the *dnaK* promoter.

From 8:00-10:00 is the λ CI gene expressed from the *lac* promoter.

There are no other promoters on this plasmid other than those indicated on the map. Assume that all genes have appropriate translation start and stop codons. The short bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements. The host *E. coli* strain **lacks the F plasmid and λ phage**. Any *E. coli* genes that normally regulate the promoters on this plasmid are not encoded on this plasmid unless indicated.



NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. The number of points is the maximum number of facts being looked for, and in some cases one fact is worth 2 points. 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.

The following questions are based on pGulig-12. 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. If you believe that a gene/locus is involved with an answer, but you are not sure of its regulation, be sure to mention this to get partial credit.

1. (6 points) How would you change the copy number of pGULIG-12 by altering growth conditions? Explain for both increasing and decreasing copy number. Be sure to detail how this works at the molecular level.

2. (6 points) If an *E. coli* strain carrying pGULIG-12 is growing in tetracycline, what would happen to the culture under the following conditions? Briefly explain.
 - + IPTG + glucose
 - + IPTG - glucose
 - IPTG +glucose
 - IPTG - glucose

3. (2 points) How might your answer to the first question regarding copy number affect your answer to this question?

4. (2 points) What growth conditions would you use to induce expression of the two partner secretion system? Explain.

5. (4 points) What are the functions of the two proteins in this system?

6. (2 points) Would you expect these proteins to possess typical N terminal signal sequences? Explain.

7. (2 points) Describe the essential components of the DNA sequence between the two protein genes to have this system function.

8. (2 points) What would happen if you subjected the culture to heat shock? Why?

9. (2 points) Is the *trp* promoter on pGULIG-1 under attenuation control? Explain.
10. (4 points) Can pGULIG-12 be packaged into Lambda phage for transduction? Can it be moved by conjugation? Why or why not for both?

The rest of the questions are independent of pGULIG-12.

11. (6 points) Briefly explain how catabolite repression works. In your answer you should specify if the system normally acts as a repressor or activator and how it accomplishes that? Is it possible for the system to act in the opposite manner? Why or why not?
12. (2 points) Your *E. coli* strain has the following alleles listed in its genome. Explain how each one affects the phenotype of the strain: *rpsL*, *rpoB*, *gyrA*
13. (4 points) How would deletion of the *dnaK* gene affect the expression of the other heat shock proteins in otherwise wild-type *E. coli*? Explain.
14. (6 points) Explain the differences in susceptibility to bacitracin and vancomycin between gram-positive and gram-negative and wall-less bacteria. Your answer should include the mechanism of action of these antibiotics.
15. (6 points) Briefly describe how the cell (inner) membrane is involved with energy production, motility, and DNA replication in bacteria.
16. (8 points) What is the difference between specialized transduction and generalized transduction? Can every phage do either? Why or why not.
17. (4 points) What is the difference between a transposon and insertion sequence?
18. (4 points) Why can humans be treated with sulfa drugs and trimethoprim when we use folic acid for single carbon donor reactions just like bacteria? Why are bacteria sensitive to these antibiotics?
19. (4 points) There is a known mechanism for resistance to sulfa drugs and trimethoprim, which we may or may not have covered in class. If you know the mechanism, explain it and its mechanism and genetic basis (hint - resistance to both antibiotics comes in a single package). If you do not know it, then propose two completely different mechanisms for resistance to these drugs and explain their genetic bases.

20. (2 points) If a bacterial culture increased from 10^5 CFU/mL to 10^8 CFU/mL over a 3 hour period, what is the doubling time? You only have to be within 10% of the precise answer, so you can use the rule of thumb as an estimate if you want. You do not need a calculator to do this.
21. (4 points) Describe how looking at a bacterial cell by light and electron microscopy can tell you if it is growing fast or slowly. Explain.
22. (4 points) What nucleic acid sequences are recognized by the CsrA protein? What is the effect of CsrA binding to these sequences?
23. (4 points) How does factor-independent termination work?
24. (6 points) How does the leader sequence of a protein affect its interaction with the Sec export system and the ultimate location of the protein?
25. (4 points) In a pET vector, what is the T7 promoter used for? Where did it come from? If you put a pET plasmid into a normal *E. coli* strain, will the plasmid work for its intended purpose? Why or why not?
26. Extra credit (4 points) What is the physical form of DNA in a cell after the following processes but before any recombination can occur?
- natural transformation
 - generalized transduction
 - electroporation of a plasmid
 - specialized transduction with λ phage