

**Bacterial Genetics**  
**GMS6038**  
**Final Exam**  
**Fall 2009**

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 on the next page is pGULIG-11. Note the following genetic elements:**

At the top of the map is the origin of replication of the F plasmid. Since I used both *oriS* and *oriV* in class, I have provided both names here, but they are considered to be synonymous.

Moving clockwise:

From 1:00-2:00 is the *lacI* gene expressed by its own promoter.

From 2:00-4:00 the *rom/rop* (synonymous names) gene is expressed from the wild-type pBAD promoter which is in its natural configuration relative to the *araC* gene, also expressed by its own natural promoter.

At 5:00 is the *oriT* locus of the F plasmid.

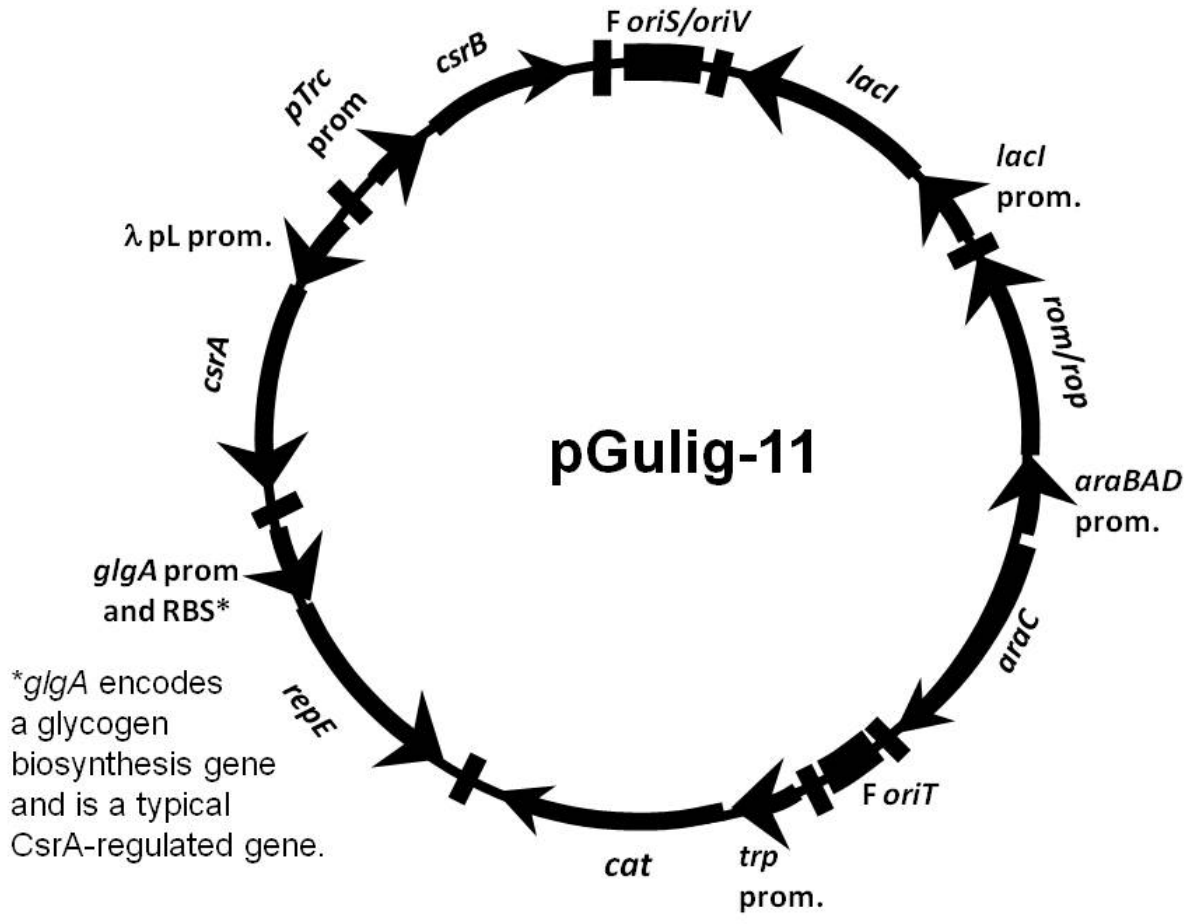
At the bottom is the *trp* (*trpL*, not *trpR*) promoter driving the expression of the *cat* gene.

At 7:00-8:00 is the *repE* gene expressed from the *glgA* gene promoter, including the *glgA* ribosome binding site (RBS). We did not mention the *glgA* gene by name in class. However, all you need to know about *glgA* is that it is a typical CsrA-regulated gene, meaning that it follows the most common relationship between the CsrA protein and a CsrA-regulated gene.

At 9:00 is the *csrA* gene expressed from phage  $\lambda$  pL promoter.

Finally from 10:00-11:00 is the *csrB* gene driven by the pTrc 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 short bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements. Every gene that is present on pGulig-11 has been deleted from the *E. coli* chromosome in the strain that houses pGulig-11. The host *E. coli* strain also **lacks the F plasmid and  $\lambda$  phage**.



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.

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

Questions 1 through 6 are based on pGulig-11. 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. (5 points) If you wanted to express the *rom/rop* gene, what growth conditions would you use? Explain. What would be the effect of expression *rom/rop* relative to replication of pGulig-11? Explain.
  
2. a. (2 points) What conditions are necessary for expression of *csrA*? Explain.  
b. (3 points) What conditions are necessary for expression of *csrB*? Explain.  
c. (4 points) How does CsrA affect the expression of the *repE* gene (not just increase or decrease, but what does CsrA do at the molecular level)?
  
3. (2 points) What are the growth conditions that are necessary for pGulig-11 to be replicated in the host *E. coli* strain described above? Explain. You do not have to repeat any detail already provided in answers above – just mention the relationships between the conditions and the relevant genetic loci.
  
4. (3 points) Is pGulig-11 conjugative, mobilizable, or neither in this *E. coli* host? Explain.
  
5. (5 points) What antibiotic resistance does this plasmid confer? What growth conditions are essential for expression of the antibiotic resistance? Explain. How does this resistance mechanism work? How does the antibiotic work (i.e., what is its target?).
  
6. a. (3 points) Could pGulig-11 be used as a suicide plasmid? Your answer should make it clear that you know what a suicide plasmid is. Why or why not?  
  
b. (3 points) What would you add to pGulig-11 to either make it a suicide plasmid or make it a better suicide plasmid, depending on your answer above. How would your addition improve pGulig-11 as a suicide plasmid?

**The rest of the questions are independent of pGULIG-11.**

7. (3 points) If a *Staphylococcus aureus* strain that was previously sensitive to methicillin became resistant to methicillin (i.e., became a MRSA – methicillin resistant *S. aureus*) in a patient (not just by itself in the lab), which of the following most likely was responsible for this change: spontaneous point mutation, transduction, or acquisition of a *bla* gene on a plasmid? Explain.
8. (5 points) For which of the 6 secretion systems in gram-negative bacteria would the secreted protein (target protein) lack a typical signal (leader) sequence? Explain.
9. (3 points) If someone did an experiment that mapped transcription initiation sites, and they told you that the initiation site was 3 nucleotides upstream of the ATG start codon of a particular gene, would be surprised by this result? Explain.
10. (3 points) Why is it important for the third amino acid in the peptidoglycan building block to have an amino group-containing side chain?
11. (4 points) What is the difference between smooth and rough lipopolysaccharide? Which type of change would be easiest to accomplish by a single point mutation – smooth to rough or rough to smooth? Explain.
12. (3 points) If you want *E. coli* to produce your favorite protein from a cloned gene, irrespective of the regulation of the promoter driving the recombinant gene's expression, what growth conditions would you use to get a maximal yield? Explain.
13. (6 points) List 2 antibiotics that have different spectra (cell types that are affected). Explain the reason for the limited spectrum.
14. (5 points) You have cloned your favorite gene into a pET plasmid that uses a phage T7 promoter to express your gene. How does using this type of plasmid affect the host *E. coli* strain that you should use? Explain. How does using this plasmid affect your use of the cloned gene, i.e., what is this type of expression most often used for? Why?
15. (4 points) In general terms, what is phage display? Why is it useful?

16. (6 points) What be the effects of mutating each of the following  $\lambda$  phage genes be on  $\lambda$  infection of an *E. coli* cell? Explain.
- CI
  - CII
  - Cro
17. (3 points) Your strain of *E. coli* has a doubling time of 30 minutes. If you start with a cell density of  $10^3$  CFU/mL and stationary phase is at  $10^9$  CFU/mL, how many hours will it take for the culture to reach stationary phase, assuming that the bacteria start in exponential phase, with no lag phase? Your answer should be within 1 hour and be rounded to the hour. You do not need a calculator to do this, even if you use the mathematical formula taught in class. If you use the "rule of thumb" estimate regarding estimating numbers of generations, your answer will be correct.
18. (4 points) Explain how DnaK is involved in its own expression.
19. (2 points) Why does DNA polymerase I have 5'-3' exonuclease activity, but DNA polymerase III lacks this activity?
20. (4 points) What two ways does the replication rate of bacteria affect the composition/size of the cell? Explain.
21. (4 points) Are the F plasmid and ColE1 plasmid compatible or incompatible? Explain?
22. (4 points) SRP and SecB are both involved with export of proteins. What is different between the proteins that interact with SRP and SecB in terms of their:  
a) signal sequence and b) ultimate location in the *E. coli* cell.
23. (6 points) Briefly explain how a factor-independent (Rho-independent) terminator works in *E. coli*. You should include in your answer the essential elements of the terminator sequence.
24. (4 points) How would each of the following mutations affect the sensitivity of the maltose operon to the presence of glucose in the medium? Explain.
- cya* gene
  - crp* (*cap*) gene

Extra credit – 5 points – Briefly explain how a marker plasmid works to enable the determination if differential yields of two bacterial strains from an animal infection are due to differential growth of the bacteria or differential killing by the host during infection. You don't have to explain how to make a suicide plasmid, just how it would be used.