GMS 6038
Bacterial Genetics and Physiology
Final Exam - 2003

This is an open note/open book exam. However, you may not share materials with other students, nor may you talk with other students. Violating these rules will result in a 0 being recorded for this exam, which will cause failure of the course. You may use the restroom and get drinks of water ONE STUDENT AT A TIME. You may not use cell phones during this exam in or out of the room. Please note that I unfortunately had to deal with a violation of these rules recently in a different course last year, so I am serious.

Please use a code rather than your name on your exam and record your code on the form provided.

You have until 5:00. When you finish, bring your exam to my office.

pGULIG-5

pL → repE

F plasmid

Ptrc →

λ CI

Pcat →

cat

oriV

F plasmid

MCS

PT7 → AGGAGGT... ATG-TISS Leader

PlacI → lacI

Take a look at the attached figure of pGulig-5. Note the following to help you understand. The arrows around the circle show the direction of transcription of the indicated genes and their promoters. The genetic elements are not drawn to scale. All of the promoters are indicated.

Legend (starting at the top and going clockwise):

- F plasmid *repE* gene driven by the Lambda phage left promoter (P_L)
- Next is the Lambda phage *CI* gene driven by the TRC promoter (P_{TRC})
- Next is the chloramphenicol acetyl transferase gene (*cat*) driven by its own constitutive promoter (P_{CAT})
- At the bottom is the F plasmid origin of replication (*oriV*)
- The lower left is a site for you to insert a portion of your favorite gene into a multiple cloning site (MCS). Upstream of the MCS is a phage T7 promoter (P_{T7}) in front of the sequence AGGAGGT followed seven bases later by the ATG codon for a type three secretion system (TTSS) leader. The MCS is fused to the leader sequence.
- Finally is the *lacI* gene driven by its own promoter (P_{LacI}).

Assume that this plasmid is placed into a wild-type *E. coli* strain lacking the F plasmid and Lambda phage.

Questions

1. Describe what would happen to this plasmid (copy number, replication) in wild-type *E. coli* if you grew the culture in L broth with the following additions. Explain your answers.
   A. - glucose, - IPTG
   B. - glucose, + IPTG
   C. + glucose, + IPTG
   D. + glucose, - IPTG

2. If you added chloramphenicol to the conditions in question 1, what would happen to the *E. coli* culture at the molecular (physiological) and cellular levels? Explain.

3. You clone only the open reading frame (coding sequence) of your favorite gene into the MCS so that it is fused in frame with the TTSS leader peptide and put the plasmid in a normal *E. coli*. Will your gene be expressed from this plasmid? Explain. If there are any problems with expression, explain how they could be alleviated or corrected.

4. Will the TTSS leader function this *E. coli* strain? Explain why or why not. If there is a problem with the functioning of the TTSS leader, how could it be alleviated or corrected?

5. If your gene gets transcribed, translated, and the TTSS system works (based on your answer to question 4), what is the potential usefulness of the TTSS system in this plasmid (i.e., what is its function?)?

6. What problems might there be related to expression assuming you get the T7 promoter to work (question 3) and TTSS to function (questions 4 and 5), depending on the identity of your favorite gene? That is, other than getting the promoter and secretion systems to work, what other concerns would you have about your favorite protein? Explain.

7. What is the significance of the AGGAGGT upstream of the ATG codon? How does it work? If this sequence was deleted, how would <u>transcription</u> AND <u>translation</u> of your favorite gene be affected?

The following questions are independent of pGULIG-5

8. Explain why adding lactose induces *lacZ* but adding tryptophan represses the *trp* operon. Do not simply state the molecular interactions of the regulators, but explain the biology.

9. If you are going to induce gene expression from a *lac* promoter in a cloning vector using IPTG, generally what are the optimal growth conditions to maximize expression of your favorite gene product? What growth conditions would you avoid when using IPTG induction? Explain.

10. What are the major benefits of using phage M13 cloning vectors?

How does resistance to chloramphenicol by the *cat* gene fit with the general pattern of genetics of antibiotic resistance (nature of resistance mechanism versus location of resistance gene)?

11. 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 moelcular leve, why would your strain be resistant?

chloramphenicol, ampicillin, nalidixic acid

12. There is a central, overriding theme of how bacteria regulate each of the numerous functions that are required for growth (DNA replication, transcription, translation). What is this theme and provide at least one example where this theme is not followed (hint - we discussed at least two exceptions in class).