

GMS 6038
Bacterial Genetics and Physiology
Final Exam - 2002

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. There will be a restroom/water pass on the board. You must have the pass if you are outside of the exam room. 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 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.

Please look over the exam in its entirety quickly, because I will be leaving for some time at 2:30. Other faculty may stop by, but they may not be able to give as good of help as me.

You have until 5:00. When you finish, leave your exam on the computer console.

Take a look at the attached figure of pGulig-4. 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):

- ColE1 *ori* - with RNA-I and RNA-II shown. Note that the promoter for RNA-II has been replaced by the indicated pBAD-AraC fragment, which encodes the wild-type and complete DNA sequences for the arabinose operon in the indicated orientation. RNA-I has its wild-type promoter. The *ori* is shown by the X on the plasmid
- Next is the *lacI* gene driven by the wild-type *lacI* promoter
- Next is the Lambda Phage *CI* gene driven by the *trc* promoter
- At the bottom is the ColE1 *rom/rop* gene driven by the Lambda Phage P_L (left promoter)
- Next is a site for you to insert an appropriate antibiotic resistance gene (see question 3).
- Finally is a site for you to insert an appropriate expression system to get your favorite gene secreted out of an *E. coli* cell (see question 4). There is a multiple cloning site (MCS) and a place for you to insert a promoter to drive expression of the MCS.

Assume that this plasmid is placed into a wild-type *E. coli* strain. For our purposes that means phage Lambda-negative and F plasmid-negative.

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, - arabinose
- B. - glucose, + arabinose
- C. + glucose, + arabinose
- D. + glucose, - arabinose

2. Under each of the conditions above, explain what would happen to the copy number of pGulig-4 if you added IPTG to the culture.

3. You are given your choice for placing an antibiotic resistance gene into pGulig-4 to enable selection in *E. coli*.

- A. Which antibiotic resistance gene would you choose? Explain your answer.
- B. Which antibiotic resistance gene would you NOT choose? Why not?

4. The purpose of this plasmid is to get your favorite gene secreted into the culture medium by the *E. coli* cells. You should therefore insert into the plasmid the essential genetic element(s) that would accomplish this in wild-type *E. coli* containing this plasmid.

A. Of the six secretion systems that we discussed in class, which one would you incorporate into this plasmid or use, keeping in mind the necessity of keeping this plasmid a reasonable size (less than 10 kb [don't get hung up on the 10 kb])? Explain your answer.

B. Explain briefly how your secretion system will work with the gene that you clone into the multiple cloning site.

C. Do you think your secretion/expression will enable the secretion of ANY gene that you clone in? Why or why not?

D. Given what is shown for pGulig-4, which promoter would you use to drive expression of the MCS? Briefly explain.

5. Can this plasmid be moved by conjugation from a donor *E. coli* strain to a recipient *E. coli* strain? Why or why not?

6. Are any of the genes shown on pGulig-4 constitutive? If so, which? What does constitutive mean?

Other questions not related to pGulig-4:

7. How is regulation of the level of the alternative sigma factor, RpoH (F^{32}), different from the typical regulation of bacterial gene products?

8. You plate 10^9 cells of wild-type *E. coli* onto four different plates that contain either nalidixic acid, rifampin, streptomycin, or ampicillin. You observe the following numbers of colonies on each of the plates after overnight growth: nalidixic acid - 100, rifampin - 10, streptomycin - 1, ampicillin - 0. Explain these results.

9. Define the following terms: cistron, operon, regulon, stimulon

10. Explain the reason for the differences in the sites at which activators and repressors bind relative to the transcriptional start sites.