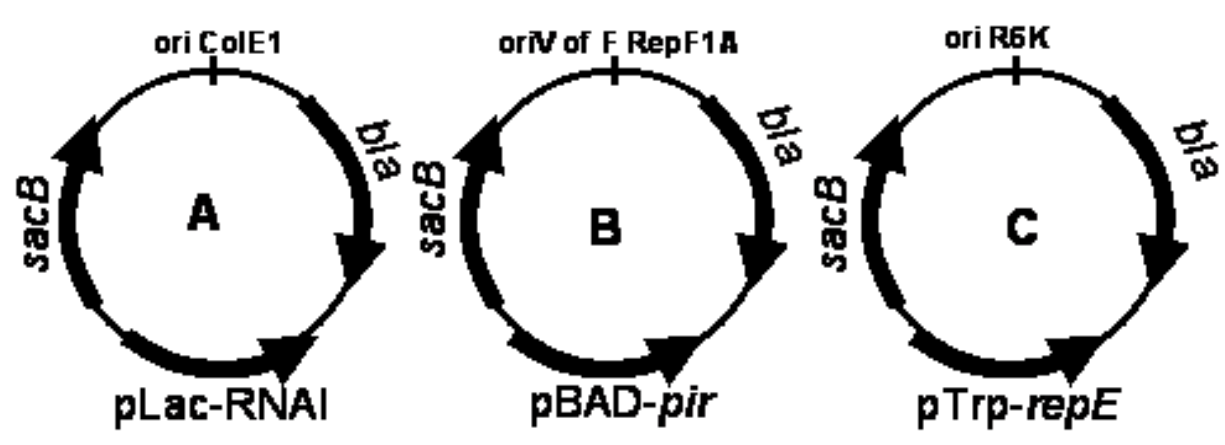
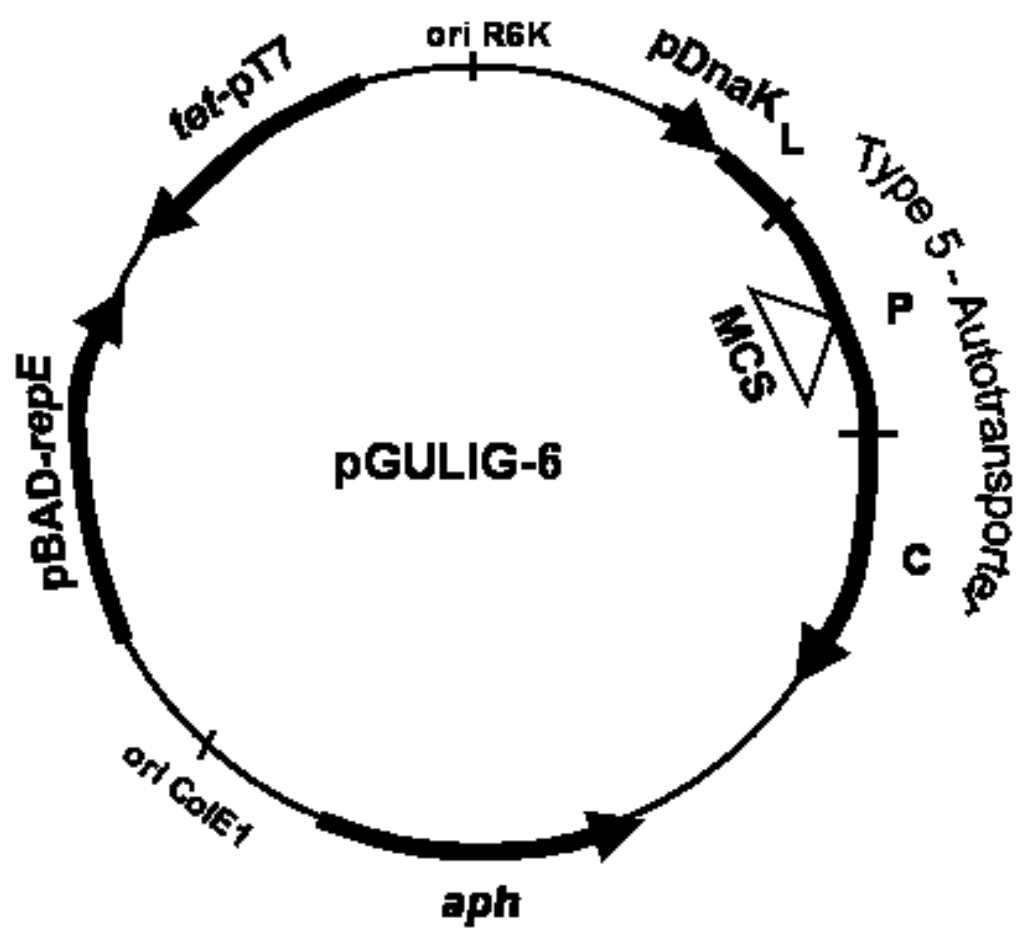

code

**GMS 6038
Bacterial Genetics and Physiology
Final Exam - 2004**

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, 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 2 hours. When you finish, leave your exam on the podium.



Take a look at the attached figure of pGulig-6 and helper plasmids A, B, and C. Note the following to help you understand. The arrows around the circles show the direction of transcription of the indicated genes and their promoters. The genetic elements are not drawn to scale. Assume that every gene is immediately followed by a Rho-independent terminator.

Legend (starting at the top and going clockwise):

pGULIG-6

- oriR6K
- Next is the promoter for *dnaK* driving expression of a Type 5 Autotransporter protein. The three portions of the protein are labeled as: L - leader/signal sequence, P - passenger domain, C - carrier domain. There is a multiple cloning site inserted into the passenger domain.
- At the bottom is the aminoglycoside phosphotransferase gene (*aph*) driven by its own constitutive promoter (not shown)
- Next is the ColE1 plasmid origin of replication (*ori* ColE1). Note that this is only the cis-active origin with no transcripts included.
- At 9:00 is the pBAD promoter driving expression of the F plasmid *repE* gene.
- Finally is the *tet* gene (resistance to tetracycline) driven by the phage T7 promoter (pT7).

The three helper plasmids have the following elements in common:

- On the right is the *bla* gene expressed by its own constitutive promoter (not shown)
- On the left is the *sacB* gene from *Bacillus* driven by its own constitutive promoter (now shown)

At the top of each helper plasmid is the cis-active origin (not encoding any transcripts) of three different plasmids: A - ColE1, B - RepF1A of the F plasmid, C - R6K.

At the bottom of each helper plasmid is a gene expressed from the shown promoter:

A - wild-type Lac promoter driving ColE1 RNA1, B - wild-type pBAD promoter driving the *pir* gene, C - Wild-type Trp promoter driving the F plasmid *repE* gene.

Assume that these plasmids are to be placed into a **wild-type *E. coli* strain** lacking the F plasmid and Lambda phage.

Questions

1. (14 points) Your goal is to make pGULIG-6 be stably maintained in a wild-type *E. coli* strain using ONE of the helper plasmids A, B, or C. You may add antibiotics, add sugars or amino acids, use rich or minimal media, or change the growth conditions of the culture. However, you may NOT introduce any other genetics elements including plasmids or phages. Please note - if you choose the wrong helper plasmid, as long as your answers below are consistent with your choice, you will get at least partial credit.

Which helper plasmid will you choose? Explain how the helper plasmid will enable pGULIG-6 to be stably maintained and how the helper plasmid will function as a plasmid, itself. Explain the specifics of growth conditions to enable the plasmids to function.

Plasmid B - It provides the Pir protein to drive replication of pGULIG-6 at the ori-R6K. pGULIG-6 will provide the RepE protein needed for the ori-F-RepF1A. The ori-ColE1 requires RNA-II. RNA-II might be able to be provided in trans (I doubt it), but in any case, there is no plasmid encoding RNA-II. The RNA-I from plasmid A would act as a repressor or replication from ori-ColE1. Plasmid C will not work because, as I indicated for you during the exam, F and R6K don't interact. This means they can't help each other and they don't interfere with each other (i.e., they are not incompatible).

Growth conditions: Since *pir* and *repE* are driven by the wild-type pBAD promoter using the AraC protein from the wild-type *E. coli* chromosome, you need to add arabinose and NO GLUCOSE (since the system is under catabolite repression). You could add ampicillin to select for plasmid B and kanamycin to select for pGULIG-6. You cannot use tetracycline (see #5) and NO SUCROSE should be added (see #3).

2. (9 points) How would the following mutations in the host *E. coli* genome affect the conditions or ability to maintain your plasmid pair? Explain.

A. A *crp** mutation (constitutive active form of the CRP (CAP) protein).

Many of you did not read this question carefully. Constitutive active means constitutively expressed, but more important - does not require cAMP to be in its active state to be able to relieve catabolite expression. I detailed this in class Wednesday. Since CRP becomes cAMP insensitive, adding glucose to the culture will have no effect.

B. A deletion of the *cya* gene.

Without *cya*, adenylate cyclase, there will be no cAMP, even under low energy conditions. Without any cAMP, CRP will never be active and you cannot express effectively from the pBAD promoter, even with the addition of arabinose. This mutation would effectively not let these plasmids function.

C. A combination of these two mutations.

Since the *Crp** mutation no longer requires cAMP, the loss of adenylate cyclase will have no effect. The answer is the same as A - the system becomes glucose-insensitive and you can add glucose to the culture.

3. (10 points) If you plate 10^9 cells of the culture with your construct on sucrose and kanamycin (in addition to the conditions outlined in answer 1), you see only five colonies.

A. Why did you not see 10^9 colonies? Explain.

Sucrose selects against the *sacB* gene on the helper plasmid. Therefore, any cells with the helper plasmid will be killed because the sucrose will be polymerized and “choke” the cells. Some of you pointed out that the selection is not that strong - that’s OK.

B. What is the most likely explanation for these five colonies (they are not contaminants from other cultures)?

They stopped producing functional SacB protein by spontaneous mutation. The easiest mutation is a nonsense or missense mutation in the gene, itself. A promoter mutation could occur, but the target is much smaller, hence less likely. You could propose a different form of suppressor mutation - as long as it cause SacB not to be made.

You cannot propose loss of the helper plasmid, since it is required for the replication of pGULIG-6, and pGULIG-6 encodes the kanamycin resistance.

4. (12 points) The multiple cloning site (MCS) in the passenger domain of the autotransporter protein is designed for you to be able to clone all or part of your favorite gene to get all or part of your favorite protein expressed so you can immunize animals for antisera.

A. What growth conditions are required to get expression of the autotransporter? Explain.

Heat shock. Heat shock enables the heat shock sigma factor (RpoH or sigma-32) to be functional (post-translationally), and the *dnaK* gene is expressed by RpoH. DnaK usually degrades RpoH, but when there is heat shock, it becomes occupied with other misfolded proteins, and RpoH is freed to act.

B. Should you include a ribosome binding site in the multiple cloning site? Explain.

No, you should NOT put a RBS in the MCS. Your favorite reading frame MUST be in frame with the autotransporter, or the system will not work. The system works as a single peptide with the three domains indicated. Some of mentioned that a RBS was required upstream of the start codon for the autotransporter, and that is true (but it wasn’t the question).

C. As best as you can, explain the roles of the leader sequence, passenger domain, and carrier domain in the autotransporter.

The leader directs the protein to the general secretion pathway (Sec-mediated) to get the protein into the periplasm. The passenger domain is the part that ultimately gets secreted. The carrier domain forms a beta-barrel in the outer membrane and enables the passenger domain to get outside of the cell.

D. Where would you expect to find your favorite protein sequences upon expression? Why?

The most likely place is the supernatant or extracellular fluid. Some autotransporters remain associated with the outer membrane

5. (5 points) Based on the limitations provided above as to how you can manipulate the culture, how could you get the culture to grow in the presence of tetracycline?

There is no way, if you follow the rules. You need the T7 phage RNA polymerase, which is not present in wild-type *E. coli*. Some of you said to clone the T7 RNA polymerase into the MCS. Although that would be cheating, as long as you made it clear you understood the problem, you got credit.

The following questions are independent of pGULIG-6

6. (10 points) How would deletion of the *dnaK* gene affect the expression of the other heat shock proteins in otherwise wild-type *E. coli*? Explain.

Since DnaK normally prevents RpoH from expressing heat shock genes, if you delete *dnaK*, RpoH will be freed to function, and the heat shock genes will be more highly expressed, possibly even constitutive.

7. (10 points) Explain why you get satellite colonies when using *bla* as a selectable marker, but you don't get satellite colonies with *aph* or *cat*.

bla encodes beta-lactamase which inactivates beta-lactam antibiotics. Since these act on peptidoglycan in the periplasm (or outside of gram-positive bacteria), the beta-lactamase must be in the periplasm or secreted. In the periplasm, the beta-lactamase can leak out and inactivate the antibiotic in the surrounding medium, thereby enabling sensitive cells to grow in the protected zone. *aph* and *cat* encode modifying enzymes for kanamycin and chloramphenicol, which act on ribosomes in the cytosol. Although Aph and Cat could be present in the periplasm, they are not - they are located in the cytosol. Therefore, they cannot leak out of the cell to inactivate the antibiotic in the surrounding medium.

8. (10 points) A. Why is the λ phage so good at specialized transduction but so poor at generalized transduction?

Specialized transduction is when a phage integrates into the host genome during lysogeny and accidentally picks up an adjacent piece of host genome for the lytic phase. Lambda integrates, so it can do this.

Generalized transduction is when a phage accidentally picks up a somewhat random piece of host genome during lytic phase. However, Lambda packages phage genome using a very specific *cos* site, which is not present in the *E. coli* genome. Therefore, random pieces of host genome cannot be packaged by Lambda during lytic infection.

B. Would you expect M13 phage to be good at either form of transduction? Why or why not?

M13 exists as a plasmid during its "lytic" phase. Actually, M13's relationship with *E. coli* is almost a hybrid between lytic and lysogeny (see previous year's test question). regardless, M13's genome does NOT integrate into the *E. coli* genome, so there is no possibility for specialized transduction. M13's linear circular genome is packaged in a cis-active manner, that is, the coat protein first binds to a specific site and then processively coats the genome. So first, the *E. coli* genome most likely does not contain this coating initiation site, but equally importantly, there are not going to be single stranded circular pieces of *E. coli* genome during the infection. So M13 would not be able to package and transduce pieces of *E. coli* genome during a normal infective process.

9. (10 points) 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.

This question was a gift - a repeat from a previous exam. I hope everyone got it right. The idea was so important, I thought I would repeat it.

You should not have proposed that plasmids or other bacteria somehow got into your culture. These numbers reflect the relative ease with which spontaneous mutations could produce resistant mutants. Ampicillin is 0 (most difficult) because there are several penicillin binding proteins, and any one being inactivated by ampicillin is enough to kill the cell. Therefore, there would have to be several simultaneous mutations, and the frequency of multiple mutations becomes multiplicatively lower (e.g., $10^{-7} \times 10^{-7} = 10^{-14}$). The second issue is how many possible ways there are to mutate the target of the antibiotic so that it is not affected by the antibiotic but still retains its function. The target of Nal (gyrase alpha subunit) is apparently more forgiving in this than are the ribosomal protein affected by streptomycin or the RNA polymerase beta subunit affected by rifampin.

(continued)

10. (10 points) What are the optimal growth conditions for inducing gene expression from an *E. coli* strain carrying a plasmid with a gene expressed by the Trc promoter? Include growth temperature, complexity of growth medium, and supplements, growth vessel, etc. and briefly explain your answer.

This question was also a gift - a repeat from a previous exam. I hope everyone got it right. The idea was so important, I thought I would repeat it.

The optimal growth conditions are AEROBIC and EXPONENTIAL (LOG) PHASE. Under aerobic conditions, energy is produced very efficiently via aerobic respiration, hence more of the substrates are available for biosynthesis. At exponential phase, the bacteria are at their peak activity for the given medium/environment. Shake the culture in a flask that is shallow with broth. A complex medium such as L broth is better than a minimal or simple medium because the bacteria can repress biosynthetic operons and save energy for making your favorite protein (we didn't discuss this last part in class, so it would count as extra credit). You should avoid anaerobic conditions (e.g., filling a centrifuge tube with broth and having the lid tight) since energy production and growth will be reduced. You should avoid stationary phase since the bacteria have depleted the nutrients and energy sources, hence upon induction with IPTG, they cannot produce your favorite protein. Don't shake the culture overnight and then drop in IPTG.