

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.

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.

Note - These are the answers that I had in mind when I wrote the questions. I'm sure that some of you came up with some answers that are acceptable that I will give either full or part credit for when I read them.

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

OK, first off, here's what's happening. The promoter being affected by IPTG is the Trc promoter driving the *CI* gene. As discussed in class, Trc, unlike the wild-type Lac promoter, is NOT under catabolite repression, so the presence or absence of glucose has no effect. For there to be repression of the Trc promoter, there must be *LacI*, which is encoded on the plasmid itself, in addition to being present on the *E. coli* chromosome. If you mentioned that it was better since *LacI* was encoded on the plasmid for copy number effect, I would give you some extra credit for that. So *CI* is usually repressed by the *LacI*, and when you add IPTG it becomes induced. What will *CI* do? It will repress the Lambda left promoter, P_L , which is driving the expression of the *repE* gene. It is important to note that P_L is normally expressed by itself. *RepE* is the positive regulator of plasmid replication at the F plasmid *oriV* locus. So without IPTG, *CI* is not expressed and therefore *repE* is expressed, and the plasmid replicates. The copy number might be high. That covers situations A and D - no IPTG.

When you add IPTG, *CI* is expressed, *CI* represses *repE*, and the plasmid stops replicating because *RepE* is essential for plasmid replication. Therefore, under conditions B and C, the plasmid will not replicate, the copy number will go down, and the plasmid will be lost from the population of *E. coli* as it grows. Of course, there will always be a few cells that contain the plasmid since it is neither degraded nor expelled from the cells.

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.

Under the conditions above, when no IPTG is added and the plasmid replicates, the bacteria will survive because the plasmid encodes chloramphenicol resistance from the *cat* gene. The *Cat* gene product will acetylate chloramphenicol and inactivate it.

When you add IPTG, the plasmid will not replicate, hence new bacterial cells will not contain the plasmid and will become chloramphenicol-sensitive. As a result, their protein synthesis will cease, and growth will stop. I believe that chloramphenicol is bacteriostatic, so the bacterial will not die - but then again - you had the textbook in front of you! There will be a remnant of cells that will survive since they will have the copies of pGULIG-5 present when IPTG was added.

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.

The promoter driving the TTSS and MCS is the phage T7 promoter. This requires the T7 RNA polymerase, which is NOT present in normal *E. coli*. Therefore, your gene will not be expressed.

This can be alleviated by providing a copy of the T7 RNA polymerase gene somewhere in the *E. coli* in a way that enables regulation of its expression. This is what happens with *E. coli* BL21 (DE3) used with the pET vectors that we discussed in class.

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?

This was a pretty hard question that we discussed in class. Normal *E. coli* does NOT encode TTSSs. The TTSS is a complex set of proteins that make a secretion apparatus with a needle and syringe-like organelle to inject proteins into host cells. Although the TTSS leader sequence is present in your clone, the secretion apparatus is not present, so your fusion protein will NOT be secreted. To alleviate this problem, you need to clone and express the TTSS apparatus somewhere in your *E. coli* strain. You might have been able to say that the flagella secretion system is very similar to a TTSS and you might be able to get secretion from there. If you had such insight, I'll give you credit.

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)?

As noted above, the TTSSs inject proteins into host cells - some are involved with plants, others animals. So you would only use this system if you wanted your protein injected into host cells.

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.

As we discussed in class, you cannot simply tell *E. coli* to secrete proteins wherever you want them to go. Remember, we discussed fusing LacZ to an outer membrane protein and how that was lethal. So if you fuse a large globular protein to the TTSS leader, the fusion protein might not get secreted and therefore inhibit membrane function to the point of being lethal.

A second consideration which you should have mentioned is that, similar to pET vectors, the T7 promoter is very strong, and the *E. coli* often kill themselves or at least inhibit their growth by producing too much of even a benign protein. That is why cloning/expression vectors have a variety of promoters of different strengths and abilities to be regulated.

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

This is the ribosome binding site (RBS), otherwise known as the Shine-Delgarno sequence. This sequence on the mRNA is complementary to the 16s rRNA of the small subunit of the ribosome. Therefore, it causes the small subunit to bind to the mRNA upstream of the ATG start codon and initiate translation. It would have NO effect on transcription.

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.

This has to do with regulating catabolism (use of nutrients) versus biosynthesis. Lactose is a nutrient. There is no point in making the utilization pathway unless there is lactose present to use. Therefore, it is normally repressed, and the nutrient acts as an inducer to enable expression of the utilization genes. Of course, catabolite repression fits in here, too, since the bacteria want to use the best carbon source, glucose, first, but that was not part of the question. Since I implied I wanted molecular description, I will add that lactose binds to the repressor LacI and prevents the binding of LacI to the Lac operator. Therefore, in the absence of LacI binding to the operator, the *lacZYA* operon will be transcribed because the RNA polymerase can reach the promoter.

For *trp*, this is biosynthesis. If the amino acid is being provided in the environment/medium, there is no point wasting energy making it for yourself. Therefore, if there is Trp available, it will repress the biosynthetic genes. This occurs via TrpR acting as a repressor and Trp acting as the co-repressor. When there is no Trp, TrpR does not bind to the promoter for repression. When there is Trp available, Trp binds to TrpR and enables it to bind, thereby inhibiting RNA polymerase from binding to the promoter. There is also the whole thing about attenuation, but that was not really relevant to this question.

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.

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.

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

M13 has two major benefits. First, if you isolate the phage after they have leaked out of the *E. coli*, the DNA is single stranded, and single stranded DNA has many useful purposes (better DNA sequencing, used for hybridization probes).

Second, since M13 is not a typical head and tail phage, there is no size restriction for the amount of DNA that can be cloned into the phage vectors.

You might come up with other good answers.

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)?

The general rule is that if the target of the antibiotic is altered so that the antibiotic no longer binds and inhibits the target, this is usually accomplished by a point mutation in the gene encoding the target and will therefore be in the chromosome.

If the antibiotic is inactivated by acetylation, phosphorylation, or being pumped out of the cell, a whole new enzyme or protein is required. This cannot be accomplished by a simple point mutation, hence the new gene will have been acquired via a plasmid. Antibiotic-modifying resistances are usually plasmid-encoded.

Cat fits into the latter category, hence would be expected to be plasmid-encoded. Now that I think about it, it was probably unclear that this question was related to the plasmid (I had originally moved this question to that part but then moved it back). You may not have been aware that *cat* is almost always found on plasmids. If it becomes clear that this was NOT clear to you, I will make appropriate considerations for you.

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 molecular level, why would your strain be resistant?

chloramphenicol, ampicillin, nalidixic acid

You simply plate a large number of the parent *E. coli* strain on an agar plate containing the appropriate antibiotic. Pre-existing spontaneous mutants which are resistant to the antibiotic will then be selected on the plate. Chloramphenicol and ampicillin resistances are of the type in which the antibiotic is altered or degraded, hence they are plasmid encoded (related to the previous question). Since you have no source of plasmid, you will not obtain spontaneous mutants that are resistant to these antibiotics.

On the other hand, mutations in the *gyrA* gene which make the gyrase target protein resistant to nalidixic acid occur with reasonable frequency. So you can select for and identify spontaneous nalidixic acid-resistant mutants of *E. coli* very easily.

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).

This may have been confusing. The central theme is that bacteria regulate these functions by regulating the INITIATION of the event, not the RATE of processivity (e.g., the rate of translation or DNA polymerization). So DNA replication is regulated by the initiation of replication at the origin (for both chromosomes and plasmids), RNA synthesis is regulated by the initiation of transcription (promoter strength and repression/induction), and protein synthesis is regulated by the strength of the initiation (the RBS). The exception to the transcription rule is attenuation, where, although initiation is regulated typically, attenuation can terminate transcription if there is sufficient Trp available. In a way, the post-translational regulation of the heat shock response can be used as an example. Rather than regulating the expression of RpoH at the transcriptional level, it is post-translationally regulated by having DnaK directing the degradation of the RpoH protein, unless DnaK becomes occupied by other misfolded proteins. There may be other examples that you could come up with.