

Bacterial Genetics
GMS6038
Final Exam
Fall 2007

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 below is pGULIG-9. Note the following genetic elements:

At the top of the map is the Lambda phage CI gene. It is driven by the *dnaK* promoter.

The rectangle is the *incC/copA* locus from the F plasmid (different names for same site).

The *sacB* gene is driven by the arabinose *araBAD* promoter.

The F plasmid *repE* gene is expressed from the Lambda phage pL promoter.

oriT is from the F plasmid.

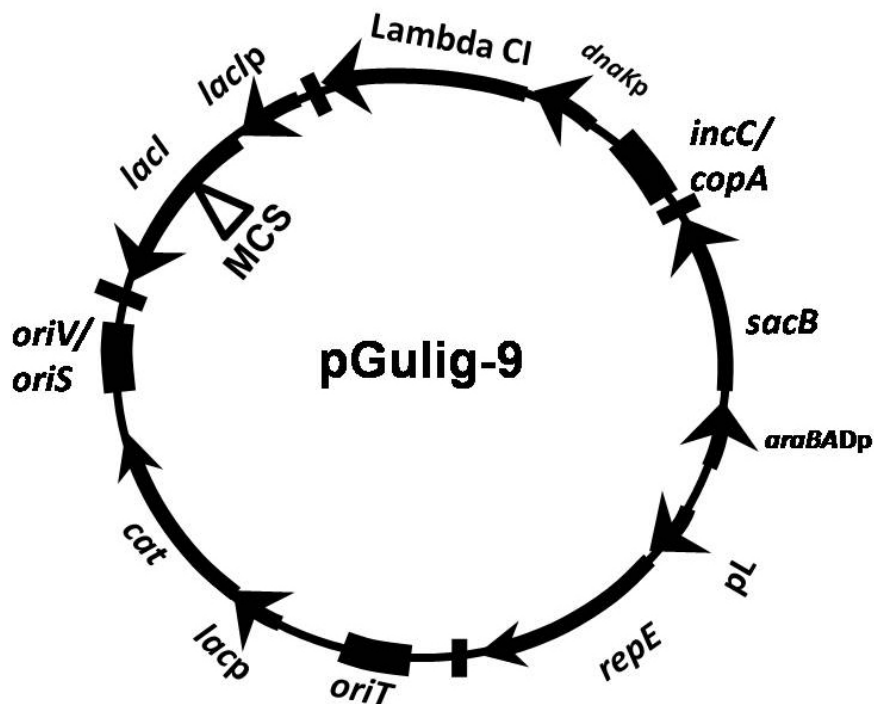
The *cat* gene is expressed from the *lacZYA* promoter.

The rectangle is the *oriV/oriS* locus from the F plasmid (different names for same site).

The *lacI* gene is driven by its own promoter. In the middle of the *lacI* gene is a multiple cloning site (MCS). Just like the MCSs in many favorite cloning vectors, this MCS does not disrupt the functional reading frame of the LacI protein.

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 thin bars crossing the circle represent typical factor-independent terminators that will prevent transcriptional read through between these different elements.

The host *E. coli* K12 strain has the normal chromosomal genes, except that **it has its *lacI* gene deleted, no F plasmid, and no Lambda phage** or any other phages.



NOTE: These questions involve 2 or 3 sentence answers - not paragraphs. 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 separate lined paper with your code on each page.

Questions 1 through 5 are based on pGulig-9. 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. Don't forget to consider genes contributed by the *E. coli* host (or not).

1. This plasmid is supposed to act as a suicide plasmid.

A. 2 points. In a general sense (i.e., not limited to this plasmid), explain how a suicide plasmid works purely in terms of its replication.

A suicide plasmid cannot replicate under certain conditions. This can be the lack of an essential gene (e.g., a necessary replication protein) or condition (e.g., the essential gene is not expressed or is non-functional).

B. 6 points. Under what conditions will this plasmid act as a suicide plasmid? Be sure to explain how every relevant element of pGulig-9 is involved with its being a suicide plasmid, as related to plasmid replication.

The origin of plasmid replication is *oriS/oriV* from the F plasmid. This required the RepE protein. Expression of the *repE* gene will govern replication. *repE* is expressed from the Lambda pL promoter (promoter left), which is normally expressed. However, pGulig-9 also has the Lambda CI gene, and the CI protein represses pL. Therefore, if CI is expressed, pL will be repressed and RepE will not be made. CI is expressed from the *dnaK* promoter. This promoter is activated under heat shock. Therefore, to make this plasmid act as a suicide plasmid, the bacteria would be grown under heat shock (elevated temperature) conditions, CI will be made, pL and *repE* will be repressed, and replication of the plasmid will be inhibited.

2. Another aspect of suicide plasmids can be the presence of a counter-selectable marker (one that can be selected against).

A. 2 points. What is the counter-selectable marker on pGulig-9?

sacB

B. 2 points. How does it work as a counter-selectable marker?

In gram-negative bacteria in the presence of sucrose, a sucrose polymer is formed. This polymer is toxic to gram-negative (but not gram-positive) bacteria.

C. 6 points. Under what conditions would the counter-selectable marker be functional, that is, how would you grow the bacteria with pGulig-9 to select against the plasmid (note that this is actually pretty complex, so be sure and think it through, and don't forget the relevant chromosomally encoded genes)?

First, we need to get the *sacB* gene expressed. It is expressed from the *araBAD* promoter. This promoter required the AraC protein plus arabinose for activation. Although the *araC* gene is not encoded on the plasmid, it is normally present in the *E. coli* chromosome. Therefore, growing the culture with arabinose will provide the first signal for induction. However, the culture cannot have glucose, because the BAD promoter is also under catabolite repression. Finally, unless sucrose is present, the SacB enzyme will not make the toxic product.

Short answer – with arabinose, without glucose, with sucrose

3. A. 4 points. What is the function of the *cat* gene? Provide the full name of the gene product and its mechanism of action.

Chloramphenicol acetyl transferase – it acetylates and inactivates chloramphenicol, so it encodes chloramphenicol resistance

B. 6 points. How is *cat* expressed and regulated on this plasmid, that is, under what growth conditions would the gene be expressed? Explain.

Cat is expressed from the *lacZ_{YA}* promoter, which is normally on. However, it is repressed by the LacI protein. The host *E. coli* has no *lacI* gene, but the pGulig-9 plasmid does. *lacI* is expressed from its own promoter, so it is constitutively expressed. Therefore, unless the repression is relieved, *cat* will not be expressed. To induce the *lacZ_{YA}* promoter, you should provide IPTG or lactose to the culture without glucose, since the *lacZ_{YA}* promoter is under catabolite repression.

C. 2 points. How could you use the complex mechanism of regulation coupled with the multiple cloning site to select for successful insertion of cloned sequences into the MCS?

If you successfully clone a DNA sequence into the MCS in *lacI*, the gene will be inactivated, and there cannot be any repression of the *lacZ_{YA}* promoter for *cat*. Therefore, even if you don't add IPTG when no glucose is present, *cat* will not be repressed, and the bacterial will be chloramphenicol resistant. If you fail to insert DNA into the MCS, without adding IPTG, *cat* will be repressed and the bacteria will be chloramphenicol sensitive. Note, though, that you cannot provide glucose in any case, since here will still be catabolite repression.

I liked this idea so much in writing this question, I might make such a construct for use in my lab. Note that you have to use a *lacI* mutant *E. coli* host strain, but they are easy to find.

4. A. 4 points. On the F plasmid what is the function of the *incC/copA*? Give a general function and then a molecular explanation.

This is involved with reducing copy number of the plasmid. If there is a sufficient amount of RepE protein, it will link (couple or hand-cuff) two copies of the plasmid and inhibit its replication. This required that both plasmids have the *incC* site and that there be sufficient RepE protein.

B. 2 points. How might the *incC/copA* affect pGulig-9, especially in light of your answer to question 1?

OK, this is rather vague (hence only 2 points). If enough RepE is made, it is possible that repression of plasmid replication by coupling by occur. This could keep the plasmid from reaching too high of a copy number, which could be bad if your gene cloned into the MCS is toxic.

5. 4 points. Can this plasmid be moved from the host *E. coli* strain described above into a recipient *E. coli*? Why or why not? Explain.

No. Although there is an origin of transfer from the F plasmid (*oriT*), all of the transfer (*tra*) functions must be provided to make the pilus and DNA transfer functions. Since the host *E. coli* strain lacks the F plasmid, these *tra* functions are not there, and the *oriT* is useless.

The following questions are independent of pGULIG-9

6. You have a globular protein that you would like to have expressed and exported into the periplasm of *E. coli*. So you cloned the gene into a vector that has the beta-lactamase signal sequence followed by a multiple cloning site. Beta-lactamase is exported through the SecYGE system.

A. 2 points. Describe the essential nature of the DNA sequence between your gene and the beta-lactamase signal sequence to enable export of your protein.

Since our favorite gene is relying on the *bla* gene for the export signals (i.e., leader peptide), the two reading frames must be fused in frame. There should not be a ribosome binding site or start codon for our gene. These will be provided by the *bla* gene. Very important – there should not be a stop codon either.

B. 6 points. Briefly explain how the beta-lactamase signal sequence works at the molecular level, including other proteins and *E. coli* factors that it will interact with after it has been translated.

Since Bla is normally exported to the periplasm, its leader sequence will bind SecB, which will guide it to SecA. SecA will help the leader peptide into the SecYGE export machinery. The protein will be exported post-translationally. After export, the leader sequence will be cleaved off by leader peptidase. Note that this is different from inner membrane proteins that are recognized by the SRP and are exported co-translationally.

C. 2 points. Unfortunately, you find that your protein is not exported. Is this unexpected or not? Explain.

Since my protein is globular, I doubt that it will be exported. It likely will fold and not be competent for transport through SecYEG.

D. 2 points. Describe another export system that might be useful for getting your protein exported through the inner membrane. Why might this other system be useful?

The twin arginine translocase (TAT) system is capable of exporting folded proteins. You would have to use a different leader sequence. You might have mentioned some of the terminal secretion systems that bypass the periplasm, but that is where you want your protein to go.

7. A. 4 points. Why are trimethoprim and sulfamethoxazole frequently mixed together to treat bacterial infections? Explain in terms of their mechanisms of action.

Trimethoprim and sulfa drugs inhibit different steps in the folic acid biosynthetic pathway. Sulfa drugs inhibit dihydropteroate synthase and trimethoprim inhibits tetrahydrofolate reductase. Inhibiting the same pathway twice is a good thing.

B. 5 points. How do these two antibiotics exemplify the two different reasons behind selective toxicity for antibiotics relative to humans? Based on this answer, which would you expect to have the highest therapeutic index?

Humans lack dihydropteroate synthase, so there is nothing for the sulfa drugs to act against in our cells. Humans have tetrahydrofolate reductase, but our enzyme is sufficiently different from the bacterial enzyme that trimethoprim can be used against bacteria without harming us.

Therefore, I would predict that sulfa drugs have a higher therapeutic index (the toxic dose for humans divided by the effective dose against bacteria).

C. 5 points. Your favorite *E. coli* strain's genotype has *rpsL* in it. What does this mean? Explain how it works.

rpsL = ribosomal protein, small subunit (protein L). This is a point mutation that renders the ribosomes (and therefore bacteria) resistant to streptomycin (the antibiotic does not bind and inactivate the ribosomes). The *rpsL* gene product is the target of streptomycin. Of course, the ribosomes have retained their function of translation, or the streptomycin-resistant bacteria would be sick.

8. 10 points. Explain how RNA polymerase, CAP-cAMP (CRP-cAMP), AraC, LacI interact for the *ara* and *lac* systems, respectively, to affect initiation of transcription. Your answer should include the relative locations (not the DNA sequences!) of the DNA sites that these factors bind to. Your answer should focus on what happens at the promoter region, not all of the other things going on before and after initiation of transcription.

In both systems, the sigma subunit of RNA polymerase recognizes the promoter sequences, and binds the RNA polymerase holoenzyme to start transcription. The promoter sequences are at -10 and -35 relative to the initiation of transcription.

In both systems CAP-cAMP has affinity for RNA polymerase so its binding site must be close enough help the RNA polymerase bind to the promoter. Usually the CAP binding site is slightly upstream of the promoter.

AraC and LacI are where differences come in. Since AraC is an activator, its job is to bind to the RNA polymerase and help it bind to the promoter. Therefore, the AraC binding site is slightly upstream of the promoter. Conversely, since LacI acts as a repressor, its job it to inhibit RNA polymerase from binding to the promoter. Therefore, the LacI binding site (the operator) is slightly downstream of the promoter sequence.

9. 4 points. How does the fate of proteins secreted by the sortase system of gram-positive bacteria differ from that of proteins secreted by gram-negative bacteria?

The sortase-secreted proteins are bound to the peptidoglycan. There are peptidoglycan-bound proteins in gram-negative bacteria, but they are rare. Usually exported proteins in gram negatives are free in the periplasm or secreted all the way out of the cell. Outer membrane proteins are also considered exported proteins.

10. A. 4 points. How does a suppressor mutation for a nonsense mutation work?

It is a mutation in a tRNA gene that causes the tRNA for a particular amino acid to recognize the stop codon of the nonsense mutation. That amino acid is placed onto the peptide where the stop codon was made enabling the continued translation of the protein.

B. 2 points. What would the consequences be if suppressor mutations were 100% effective?

The cell would be sick because stop codons are an essential part of producing the correct proteins. If all of the stop codons of a particular type (1 or 3) were not read as stops, in theory, 1 of 3 proteins would not be terminated correctly.

11. Bacterial cells that are growing rapidly in rich broth are significantly larger than cells growing very slowly or not at all. There are two major reasons that this is true (hint – you would be able to see this with an electron microscope).

A. 4 points. What are the two things that make the cells larger?

The chromosome is present in multiple copies (as much as 4) and there are more ribosomes.

B. 4 points. What is the explanation for this phenomenon?

It takes 40 minutes to replicate the chromosome. But if the cell is doubling every 20 minutes, there is not time to duplicate the chromosome. Therefore, there are numerous copies of the chromosome being made at any given time in rapidly growing cells. This is enough to make the cells larger.

If the cells are growing rapidly, they are making lots of protein. Ribosomes are the limiting step in making proteins, so you need more ribosomes to grow fast. They will significantly contribute to increased cell size.

You can see the nucleoid (DNA) and ribosomes by EM.

12. 6 points. Explain the differences in sensitivities to ampicillin and vancomycin between gram-positives, gram-negatives, and wall-less bacteria (note there are 6 possible interactions here).

Gram-positive bacteria are sensitive to both antibiotics because they have the target (peptidoglycan biosynthesis and cross-linking) and the targets are available to the antibiotics.

Gram-negatives have both targets of the antibiotics. They are sensitive to ampicillin because it can cross the outer membrane to inhibit the penicillin binding proteins. However, vancomycin is too large to cross the outer membrane, hence gram-negative bacteria are naturally resistant to vancomycin.

Wall-less bacteria have no peptidoglycan, which is the target of these antibiotics, so they are naturally resistant.