# Bacterial Genetics
# GMS6038
# Final Exam
# Fall 2008

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-10. Note the following genetic elements:**

At the top of the map is the origin of replication of the R6K plasmid.

Moving clockwise:

From 1:00-4:00 the *araBAD* promoter drives the *pir* gene followed immediately by the *lacI* gene. The *araC* gene is placed in its natural context relative to the *araBAD* promoter.

At the bottom is the phage T7 promoter driving the expression of the *bla* gene. The *bla* gene has a multiple cloning site (MCS) inserted.

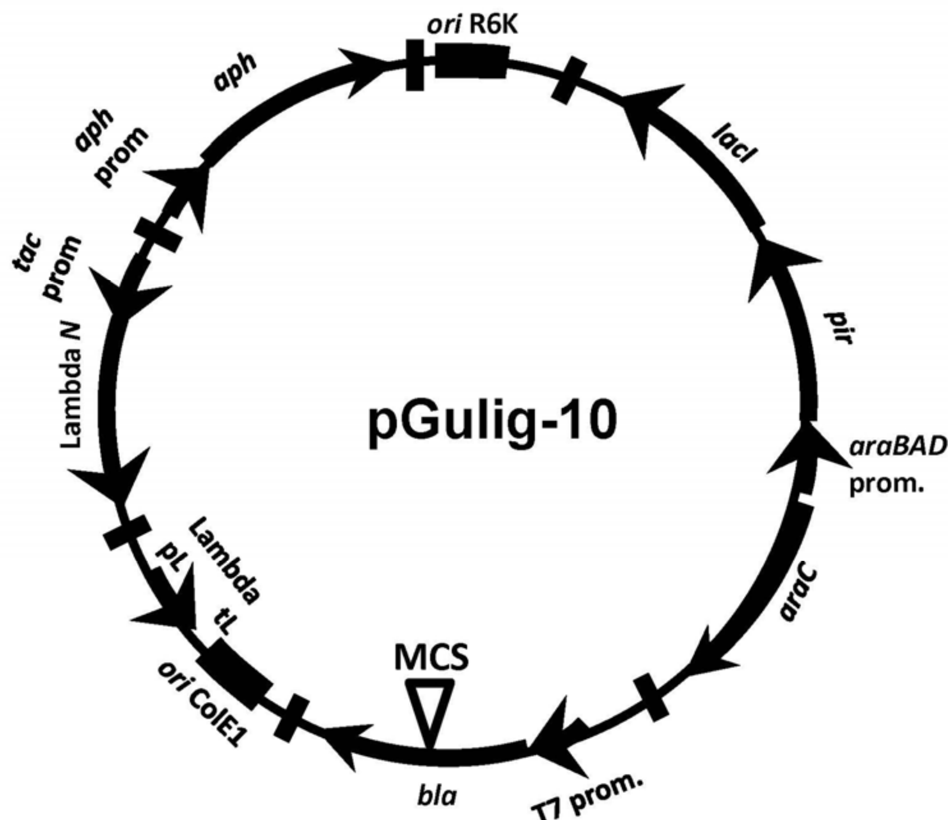
At about 7:00 is the origin of replication of the ColE1 plasmid WITHOUT its Rna-I and Rna-II promoters. Instead immediately upstream is the Lambda phage pL promoter followed by the tL terminator.

At about 9:00 is the Lambda phage N gene expressed from the tac promoter.

Finally from 10:00-11:00 is the *aph* gene driven by its own promoter, which is constitutive.

There are no other promoters on this plasmid other than the 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 xx are based on pGulig-10. 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. (3 points) Which of the sites on pGulig10 are necessarily cis active? Explain.

oriR 6K and ori ColE1 – This is where the DNA synthesis initiates, so they obviously have to be part of the plasmid.

All of the promoters and terminators. Promoters can't initiate transcription unless they are upstream of the gene, and terminators can't stop transcription unless they are downstream of the gene.

2. (2 points) Is pGulig10 conjugative, mobilizable, or not? Explain.

It is neither conjugative nor mobilizable. There are no tra genes (about 30 kb) to encode for the pilus and there is not a mob/oriT site to enable trans active conjugative functions to move the plasmid.

3. (5 points) If you want to maintain the plasmid based on the origin of replication from the R6K plasmid, what conditions would you use. Explain.

ori R6K requires the Pir protein encoded by the pir gene. pir is expressed by the araBAD promoter. So you need to add arabinose (the inducer for AraC) and not have glucose (to relieve catabolite repression).

4. (5 points) If you want to maintain the plasmid expressed from the ColE1 origin, what conditions would you use. Explain.

You need transcription through the ColE1 origin, and since the Rna-II promoter is not there you need to use the only other promoter nearby – pL from Lambda. Since there is no CI around, pL will be constitutive. However, the tL terminator will stop transcription before the origin. So you need the N protein – anti-terminator – to be produced. It is expressed from the tac promoter, which is a hybrid trp and lac promoter. If there is no LacI expressed (remember that the E. coli host has a lacI deletion), the tac promoter will be very strong and constitutive (remember that tac is not under catabolite repression). So, unless lacI is expressed downstream of pir, N will always be made. Unless you add arabinose without glucose, N will always be made, and you don't have to do anything(!). However, if you add arabinose without glucose, LacI will be made, and you need to further add IPTG as the inducer for the tac promoter.

Now, when I wrote the question, I didn't plan ahead and notice that LacI would not be around unless you induced the arabinose system. I should have added a question that asked if the culture was grown only in L broth, which ori would function, and you should have been able to say - ori ColE1. The neat thing about my system is that if you induce pir for the ori R6K, you simultaneously repress N and thereby ori ColE1 (I did have that in mind.). Plasmids don't like being replicated from two origins.

5. There is a multiple cloning site in the middle of the *bla* gene.

a. (2 points) If this plasmid is placed in the *E. coli* strain described, what will be required to get the *bla* gene expressed? Explain.

bla is expressed by a T7 promoter. This requires T7 RNA polymerase, which normal E. coli does not make. So, in the host indicated in the question, you can't get expression – period. However, if you add the DE3 element (or something like it) that encodes T7 RNA polymerase, you can get expression. You cannot infect with T7, because that will kill your culture.

b. (4 points) What advantage or special use is there to having the MCS in the *bla* gene? To get the benefit of cloning your favorite sequences into the *bla* gene, what must you ensure about the placement of the MCS and the sequences cloned within it?

Bla (beta lactamase) is a periplasmic protein. So this construct would be good for getting a peptide sequence fused with Bla into the periplasm. Cloning something into the MCS would also disrupt bla and inhibit ampicillin resistance – providing a screen for successful insertion – although this would be a cumbersome screen. For the MCS to work, it must be placed downstream of the leader/signal sequence and your favorite peptide must be in frame with bla open reading frame (just like for PhoA fusions). Without the in frame fusion (i.e., a gene or peptide fusion), the Bla signal sequence will not get your peptide into the periplasm.

6. a. (2 points) What is the function of the *aph* gene?

kanamycin resistance – this is the selectable marker for this plasmid (bla will not work for the reasons above).

b. (1 point) What is meant by "constitutive"?

Expressed at a constant level. Constitutive does not mean expressed at a high level.

**The rest of the questions are independent of pGULIG-10.**

7. (6 points) How do the Lambda phage *cII* and *cro* genes interact to regulate Lambda gene expression? Include in your answer what gene(s) is being regulated and the effects of this regulation.

CII activates transcription of the CI gene, and Cro represses transcription of CI. CI represses the pL and pR promoters. Without transcription from pL and pR, there can be no lytic phase. CI sets up and maintains lysogeny.

8. (2 points) Why is the trp operon not under catabolite repression?

Catabolite repression helps bacteria utilize the optimal growth substrate (glucose) and hold off on using lesser substrates (lactose, arabinose) until the good stuff gets used up first (catabolism). The trp operon is a biosynthetic operon to make tryptophan, so the energy charge of the cell is mostly irrelevant.

9. (4 points) If the *trpR* gene was mutated so that it no longer bound tryptophan, how would this affect the regulation of the trp promoter? What would ultimately happen to the levels of tryptophan in the cells? Explain.

TrpR is a repressor that uses trp as a corepressor. If TrpR cannot bind trp, it will not repress the trp promoter, and the promoter will become constitutive.

However, attenuation would still work since it is independent of TrpR. So if trp levels rise too high because of the constitutive trp promoter, the sufficient levels of trp-tRNA will cause attenuation in the trpL gene and termination of transcription.

10. (4 points) Would M13 make a good specialized transducing phage? Why or why not? Would M13 make a good generalized transducing phage? Why or why not?

Specialized transducing phages must integrate into the host genome to accidentally drag along an adjacent host gene. M13 does not integrate into the E. coli genome, so it cannot do specialized transduction.

Generalized transducing phages have to be sloppy in packaging phage DNA during phage assembly. First, the M13 genome is ssDNA, and the E. coli will not have ss genomic DNA. Second, M13 requires its origin of replication to begin the packaging process. The phage coat proteins are not going to spontaneously bind to and package host DNA. Third, M13 is not lytic, so it will not break up the host DNA into pieces small enough to make phage particles. On a deeper level, it is doubtful that the rest of the M13 structural proteins would attach to non-M13 DNA to make an infective particle. I don't expect you to get all of this, but at least a couple of these reasons.

11. (4 points) How would a mutation that knocked the *polA* gene encoding DNA polymerase 1 affect chromosomal replication of *E. coli*. Do you believe that such a mutant would be viable? Explain.

PolA is required to synthesize the Okazaki fragments (repair the RNA-DNA hybrids). So without PolA, DNA replication cannot proceed and the cells cannot grow. They would not be viable.

12. (4 points) In *E. coli*, what determines if a protein is directed to the general export pathway by SecB or SRP? Your answer should include a description of the structure of the protein and what the consequences to the protein are if it is handled by SecB or SRP.

The nature of the signal/leader sequence determines if SecB or SRP work. The SRP sequences tend to be longer and more hydrophobic. SecB is for proteins destined for the periplasm or beyond. SRP is for inner membrane (cytoplasmic membrane) proteins.

13. (6 points) Compare and contrast the type 2 and type 3 secretion systems in terms of ultimate location of secreted protein and dependence on general export pathway.

Type 2 – outside of the cell into the growth medium.
Type 3 – injected into a host cell.

Both systems depend on the GEP for their export machinery. Type 2 also uses the GEP to get the exported protein into the periplasm. Type 3 does not use the GEP for the exported protein. It is injected from the cytoplasm of the bacterium into the cytoplasm of the host cell.

14. (6 points) Match the cell type with the antibiotic to which it is inherently resistant. Briefly explain why it is resistant.

| | | | |
|---|---|---|---|
| gram-positive | polymyxin | Polymyxin binds LPS. G+ don't have LPS. | |
| gram-negative | bacitracin | bacitracin is too big to get through the outer membrane | |
| wall-less | ampicillin | bacitracin | both of these act on peptidoglycan, which wall-less do not have. |
| | polymyxin | Polymyxin binds LPS. Wall-less don't have LPS. | |

15. (10 points) Sketch the gram-positive and gram-negative cell membrane/wall. Label the unique components of each.

| | G+ | G- | This is what I'm looking for in your figure. |
|---|---|---|---|
| cell membrane | + | + (inner membrane) | |
| periplasm | - | + | |
| outer membrane | - | + | |
| LPS | - | + | |
| porins | - | + | |
| peptidoglycan | thick | 1 layer | |
| teichoic acid | + | - | |

16. (6 points) 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?
tetracycline, ampicillin, rifampin

The only thing you can do is isolate a spontaneous resistant mutant. Of these antibiotics, the only one that is known to have resistance by point mutation is rifampin. Tet resistance is a membrane pump and amp resistance is by beta lactamase, neither of which will happen by point mutation. You simply grow up the culture and plate more bacteria on the antibiotic plate than the frequency of point mutations to resistance (usually $10^9$ CFU will do). Your Rif-resistant mutation will show up as a colony. There should be a point mutation in the rpoB gene encoding the beta subunit of the RNA polymerase. The RNA polymerase will still function, but it will no longer bind rifampin.

17. (10 points) Diagram the essential elements for expression of a bacterial gene that encodes a protein. Include in your diagram the role of each element, that is, what factors/functions act at each site. Do not put the DNA sequences (ACGT), just the name and function.

promoter: -35 and -10 This is where RNA polymerase binds (sigma subunit is the main recongition factor)

Transcription start site

RBS at -5 to -10 from ATG start. Complementary to the 16s rRNA to get the small ribosomal subunit to start translation.

Start codon (ATG) – Initiation of translation, initiation factors, f-Met-tRNA, ribosome

Stop codon to stop translation. There are stop factors, but you didn't have to mention them.

Terminator (OK, you could leave this off since it is not essential for expression) – to stop transcription. There are no factors here.

18. (4 points) You have a culture of E. coli at a density of $1 \times 10^6$ CFU/mL. You incubate it at room temperature in L broth for 3 hours, and the final density is $6.4 \times 10^7$ CFU/mL. What was the doubling time of the culture? What was the growth rate? You do not need a calculator to do this.

This was supposed to be easy. You didn't need to even know a formula. How many doublings occurred in the 3 hours? To go from 1 to 2, 4, 8, 16, 32, 64 is 6 doublings or generations. So 6 generations happened in 3 hours. So 3 hours/6 generations = a doubling time of 0.5 hours or 30 minutes. The growth rate is the inverse of the doubling time: 2/hour or 0.03/minute (the hours are obviously an easier calculation).

19. (6 points) Why is the response to heat shock a stimulon but the response to arabinose a regulon? Do not simply define stimulon and regulon, but also biologically explain why heat shock needs to be a stimulon and arabinose a regulon.

A stimulon is all of the genes that get expressed in response to an environmental stimulus. It is composed of numerous regulons and operons. Heat shock is a serious stimulus/challenge that requires massive changes in gene expression for survival. Part of that involves alternative sigma factors (RpoH) that will act on numerous regulons and operons.

A regulon is all of the operons regulated by a single regulatory protein. Therefore, it is a much smaller set of operons – likely even just 1. Inducing the utilization of arabinose involves only a single operon, so only a single regulatory protein is needed.

20.  (4 points)  What is a suicide plasmid and what is it used for?  What are the critical elements of a suicide plasmid?

Suicide plasmids are plasmids that do not function as plasmids (replicons) under specific conditions or in specific host cells.  Essentially, their replication is conditional.  That's it.  Suicide plasmids don't necessarily kill the host cell.

The critical element is an origin with conditional function.  The best ones are protein-requiring origins (R6K requires Pir – see pGulig-10).  You could also use an RNA-regulated origin, too (ColE1 as in pGulig-10).

Suicide plasmids are used for a couple of purposes, but they both revolve around putting DNA into a cell in an unstable manner.  We discussed allelic exchange to force a mutated allele of a gene to recombine into the host genome.  You can also use suicide plasmids to deliver transposons into cells, but we didn't talk about that.

For a really good suicide plasmid, there should be a counterselectable marker such as sacB, so that when the 2 step allelic exchange is complete, you can select for loss of the suicide plasmid.

21.  Extra credit – (4 points)  How does the GroEL chaperonin function?

GroEL either refolds or degrades misfolded proteins.  It forms a cylinder that captures misfolded proteins (hydrophobic residues are facing outward rather than being buried inside).  The inside of the cylinder starts off hydrophobic, but a conformational change occurs and the inside becomes hydrophilic.  This makes the misfolded protein "uncomfortable".  It either refolds to become comfortable, or it gets degraded.