

You must answer the following 5 questions worth 30 points about pGULIG-7 for your written exam. You must answer at least 35 points of the remaining 70 points for your written exam. Your grade will be proportional to the amount of questions you answer (if you answer 65 points total, your grade is based on the percentage of 65 points correct, if you answer 100 points total, your grade is based on the percentage of 100 points correct. Note that the point value is proportional to the length of the answer expected.

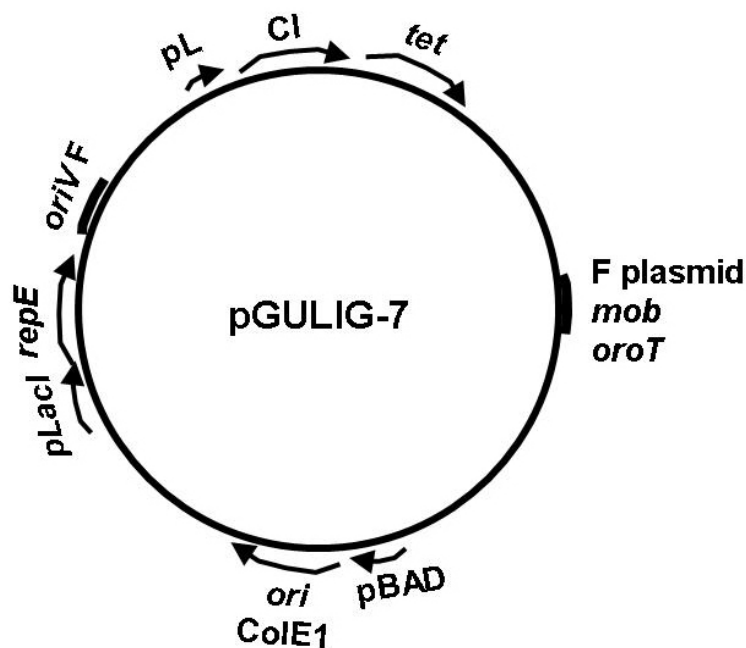
Observe pGULIG-7. Note the following genetic loci:

at 12:00 - pL promoter from Lambda phage driving expression of CI gene of Lambda phage followed by the tet tetracycline resistance gene.

at 3:00 - the *mob* (*oriT*) site of the F plasmid

at 6:00 - the pBAD promoter replacing the RNAlI promoter for the ColE1 origin of replication

at 9:00 - the promoter of the *lacI* gene (not *lacZYA*) driving the *repE* gene of the F plasmid followed by the *oriV* of the F plasmid.



Assume that this plasmid is placed into a completely wild-type (normal) *E. coli* K-12 that does NOT have the Lambda phage or F plasmid. Answer the following questions for 30 points:

1. (4 points) Based solely on the regulation of the tet gene, do you believe a cell with this plasmid will have a high or low level of resistance to tetracycline? Explain your answer.

First, you have to understand how tet is regulated/expressed. The upstream CI gene is not important at first glance, but the pL promoter is very important. What do you know about the pL promoter? It is repressed by CI, which on this plasmid is driven by pL, itself. Therefore, CI will repress its own expression, hence repress the expression of tet. Therefore, the level of tetracycline resistance will be low.

2. (4 points) What is the function of the F plasmid mob/oriT site at 3:00? Will this site be functional in this cell? Explain.

mob/oriT is the origin of transfer. If the pilus generation genes and the genes encoding plasmid DNA transfer are provided in this cell (they are not on this plasmid), the plasmid can be transferred by conjugation into a recipient. Since the host E. coli does not have the F plasmid, these functions are not available, and the mob/oriT will be nonfunctional.

3. (4 points) Explain what happens at the origin of ColE1 replication under the following conditions:

Remember that this is a wild-type E. coli that encodes araC, the activator of the pBAD promoter, which is a trans active protein.

a. + arabinose, + glucose

Adding arabinose will enable AraC to activate the pBAD promoter, but the ara system is also under catabolite repression. With glucose in the medium the promoter will not be induced. Since the ori of ColE1 needs transcription through it to initiate DNA replication (just like RNA-II), the origin will not work.

b. + arabinose, - glucose

Inducer is present and catabolite repression is relieved, now pBAD will be induced and plasmid replication will occur at the ori.

c. - arabinose, + glucose

Catabolite repression will not be relieved, and AraC will not induce the promoter. No plasmid replication.

d. - arabinose, - glucose

Catabolite repression will be relieved, but AraC will not induce the promoter. No plasmid replication.

4. (4 points) Explain what happens at the oriV of the F plasmid under the following conditions:

OK, this was a trick question. lacI is constitutive - low level, but not changed by glucose or lactose. The low level of transcription from pLacI will drive low level expression of repE, which will produce the RepE protein, which will enable low level initiation of plasmid replication at oriV.

Therefore, none of the conditions below will change replication at oriV.

a. + lactose, + glucose

b. + lactose, - glucose

c. - lactose, + glucose

d. - lactose, - glucose

5. (9 points) For every genetic element or locus on pGULIG-7, which are cis-active and which are trans-active?

pL promoter from Lambda phage
cis active (all promoters are cis active)

CI gene of Lambda phage
trans - the protein can float around in the cell to repress pL

tet tetracycline resistance gene - trans

mob (oriT) site of the F plasmid - cis (all origins of replication and transfer are cis)

pBAD promoter cis active (all promoters are cis active)

ColE1 origin of replication - cis (all origins of replication and transfer are cis)

promoter of the lacI gene - cis active (all promoters are cis active)

repE gene of the F plasmid - trans

oriV of the F plasmid - cis (all origins of replication and transfer are cis)

6. (5 points) Between the stop codon of CI and the start codon of the tet gene, which sequences/elements MUST be present for expression of tet?

ribosome binding site (Shine Delgarno sequence) for ribosomes to initiate translation

7. (10 points) There are two models for how the iteron sequences regulate the copy number of plasmids such as the F plasmid. What are the models, and which model is currently accepted. Briefly describe the two models - which cis and trans active sequences are involved and how they work according to the two models. Briefly describe the experimental data that supports the current model and weighs against the previous model.

Titration model - the "useless" iteron sequences at copA/incC bind RepE and prevent it from acting at oriV to initiate plasmid replication, If this were true, then simply overexpressing repE would increase the copy number of F, but that doesn't work as well as expected.

Coupling (handcuffing) model. Since RepE binds to the iterons, it could physically link two different plasmids with iteron sequences and thereby prevent their replication. Adding plasmids with iterons in the presence of RepE inhibits their replication, as well as F.

8. (10 points) What would the phenotype of a mutation in TrpR be if it no longer bound tryptophan under the two different conditions of the bacteria being grown in the presence or absence of tryptophan in the culture medium? Your answer should address the following specific issues: the initiation of transcription at the *trp* promoter and the transcription at the downstream genes in the operon. Explain your answer.

No trp in growth media. There would be no trp to bind to TrpR anyway, so the mutation would be silent. The *trp* promoter would be expressed, as would the downstream genes (there would

be no attenuation since there would not be excess trp for attenuation).

trp added - trp normally binds to TrpR and represses the trp promoter (it is a corepressor). However, if trp doesn't bind to TrpR, the trp promoter will not be repressed. However, because there will be plenty of trp in the cells, attenuation will occur and the downstream genes from trpL will not be transcribed.

9. (5 points) In catabolite repression, is cAMP an inducer or corepressor? Explain.

cAMP is normally an inducer. Inducers bind to the regulator (Cap) and result in activation of a promoter. This is how Cap + cAMP normally works. This was somewhat of a trick question since catabolite repression is not really repression.

10. (20 points) A. Explain why satellite colonies appear with use of ampicillin but not chloramphenicol. Your answer should include the mechanism of action of the antibiotic and resistance.

I can't remember if we covered this in class for 2006, so it may not be a relevant question. bla encodes beta-lactamase which is present in the periplasm and degrades ampicillin. Sometimes the enzyme leaks out of the periplasm into the culture medium and degrades ampicillin surrounding the colony. Any ampicillin-sensitive cells that didn't die before the amp was broken down would be able to grow. Note that if you restreaked these satellite colonies on a fresh amp plate, they would not grow. Oh yes, ampicillin binds to penicillin-binding proteins and inhibits transpeptidation of peptidoglycan.

Chloramphenicol resistance is by cat - chloramphenicol acetyl transferase. This enzyme is in the cytosol, hence does not leak out of the cells into the culture medium. Therefore, it cannot help adjacent cells like beta-lactamase can. Chloramphenicol binds to ribosomes and inhibits protein synthesis.

B. Your answer to part A should include the most common mechanism of resistance to chloramphenicol. How is this mechanism encoded, i.e., where would you find the gene(s) involved? How does this fit or not fit the general relationship between mechanism of action of antibiotic resistance and genetics of that mechanism?

cat is encoded on a plasmid. Usually, when a new enzyme or pathway is required for resistance, the gene or genes are acquired on a plasmid. New enzymes and pathways do not occur by a single point mutation in the target gene or another gene.

C. Considering your answer to part B, propose an alternative mechanism of resistance that would be encoded in a completely different manner. Explain the relationship between your alternative mechanism of resistance and the way that it is encoded.

OK, this was rather unclear. You could propose a point mutation in the ribosomal protein or ribosomal RNA targeted by chloramphenicol (I don't even know which is true!), so that the ribosome retains function but is not affected by chloramphenicol. Point mutations such as this occur on the chromosome where the target function is encoded.

D. Which form of resistance is likely to enable resistance to the greatest level of chloramphenicol - the most commonly known mechanism from part A or your hypothetical mechanism in part C? Explain your answer.

Again, I can't remember if we talked about this in 2006 or not. The point mutation would confer the highest level of resistance. If the antibiotic does not recognize the altered target, you can add all you want, and it won't have any effect. However, with resistance mechanisms that are enzymes that degrade or pump the antibiotic, you can overcome the enzyme by simply adding more antibiotic.

11. (5 points) Explain the differences in susceptibility to bacitracin and vancomycin between gram-positive and gram-negative and wall-less bacteria.

G+ are sensitive because the peptidoglycan is polymerized outside of the cell membrane where these large antibiotics can reach their target.

G- are resistant because peptidoglycan synthesis occurs in the periplasm, and these large antibiotics cannot get to their target because they cannot make it through the porins of the outer membrane.

Wall-less bacteria lack the targets of these antibiotics.

12. (10 points) For some of the terminal secretion pathways the secreted proteins possess typical Sec-dependent leader (signal) sequences while others do not.

A. For secretion mechanisms 1-6, list which use typical Sec-dependent leaders for the secreted proteins. Be sure and use the name (if any) for each secretion system in case you get the numbers mixed up.

- | | |
|--------------------------|--|
| 1. ABC transporter - NO | 2. General Secretion Pathway - Yes |
| 3. Type 3 secretion - NO | 4. Type 4 - NO |
| 5. Autotransporter - YES | 6. Twin Arg Translocase - NO (might not have said this in class) |

B. In terms of the general mechanisms by which these proteins are secreted, explain how some can dispense with the Sec system for their secretion. What is common among those that use the Sec system and common for those that do not use it?

Those that dispense with the Sec pathway secrete the proteins directly from the cytoplasm to the outside of the cell. This requires inner membrane/periplasm/outer membrane spanning protein complexes. Often, these complexes get to their destinations using the Sec system.

Those that use the Sec system have a typical leader sequence, and those that do not use the Sec system lack this leader. However, the Sec-independent secreted proteins could have other recognition sequences for their secretion apparatus.

13. (5 points) If you are trying to express your favorite protein encoded on a plasmid in E. coli that is expressed from the Trc promoter, what are the optimal growth conditions for maximizing expression? Explain.

log phase is always best because of active metabolism; rich medium enables high yield of bacteria; aeration enables high yield because of aerobic respiration; add IPTG - of course to induce the trc promoter; you can add glucose if you want since the trc promoter is not under catabolite repression

14. (5 points) Would M13 phage make a good specialized transducing phage? Why or why not?

No - its genome does not integrate into the host genome.