

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.

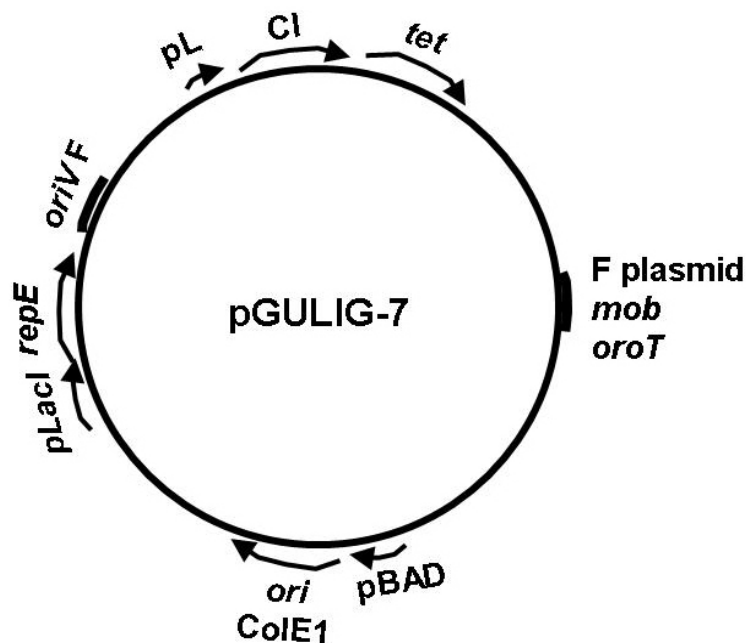
Observe pGULIG-7. Note the following genetic loci:

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.

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.

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

a. + arabinose, + glucose

b. + arabinose, - glucose

c. - arabinose, + glucose

d. - arabinose, - glucose

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

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

ColE1 origin of replication

CI gene of Lambda phage

promoter of the lacI gene

tet tetracycline resistance gene.

repE gene of the F plasmid

mob (oriT) site of the F plasmid

oriV of the F plasmid

pBAD promoter

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

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.

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.

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

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.

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?

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.

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.

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

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.

2.

3.

4.

5.

6.

B. In terms 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?

13. (5 points) If you are trying to express you 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.

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