

GMS 6038
Bacterial Genetics and Physiology
Final Exam - 2001

Take a look at the attached figure of pGulig-3. Note the following to help you understand. The arrows inside the circle show the direction of transcription of the indicated genes. The dashes between promoters and/or genes indicate that they are fused. The (...) indicate that the genes/elements are closely linked, but not fused. When relevant, details of the sequence between these elements are provided below. Assume that there is no read-through transcription except through (–) and (...). The genetic elements are not drawn to scale.

Legend (starting at the top and going clockwise):

- (F plasmid) *oriV* - note that this is ONLY *oriV* - no other RepF1A sequences are included at this site
- *pgal-repE* - the galactose promoter for *galETK* - including all associated *cis*-active sequences - driving the expression of the F plasmid *repE* gene
- *bla...aphT* - beta-lactamase gene with its own promoter followed by *aphT*, which encodes kanamycin resistance. Here is the DNA sequence at the end of the *bla* open reading frame (TAA stop codon in bold) and the beginning of the *aphT* open reading frame (ATG underlined):
... CAG **TAA** GCTTACGTTCCG ATG GGG ...
- M13 *ori* - phage M13 origin of replication
- *pdnaK* - *Cl* (Lambda) - promoter for the *dnaK* gene driving the expression of the Lambda *Cl* gene
- *ptac*-RBS-ATG-MCS-pilin leader - the *tac* promoter followed by a ribosome binding site, followed by an ATG start codon, followed by the pilin leader peptide sequence in frame with a multiple cloning site. You can assume there is a stop codon after the MCS.
- *ptrp-N* (Lambda) - the *trp* promoter driving the expression of the Lambda *N* gene
- (Lambda) *pR-lacI* - the Lambda *R* promoter driving the expression of the *lacI* gene

Assume that this plasmid is placed into a wild-type *E. coli* strain. For our purposes that means phage Lambda-negative and F plasmid-negative, but pili-positive.

Everything relevant to answering these questions has been covered in class or in reading assignments. You may submit the numbered answers to the questions by email or hard copy.

Although this exam is open note, open book, it is not to be discussed with any person other than me until after all exams have been turned in. Failure to adhere to this policy will result in failure of the course.

If you really get stuck or confused, come by or drop me an email. There usually is some clarification needed on these things!

The answers are due Wednesday October 10. The sooner everyone completes the exam, the sooner I will be able to compute the grades.

Questions

1. Describe what would happen to this plasmid in wild-type *E. coli* if you grew the culture in L broth + glucose. Explain.
2. What would happen to the culture if you grew it in L broth + glucose + ampicillin? Explain.
3. What growth medium would be optimal for maintaining this plasmid in a culture of *E. coli*? Explain.
4. Can this plasmid be moved by conjugation from a donor *E. coli* strain to a recipient *E. coli* strain? Why or why not?
5. Assuming that the plasmid is being maintained in the culture, which antibiotic resistance gene would be most effective for selection? Explain your answer.
6. What is a possible role of the M13 *ori* locus on this vector? Would there be any additional genetic requirements of the host *E. coli* strain or manipulations of the culture to make the M13 *ori* perform its function? Explain.
7. What will happen with the plasmid if the culture is grown with added tryptophan? What will happen if the culture is grown with no added tryptophan? Explain your answers.

The following questions are related to the expression of the MCS.

8. What is the most straight-forward way of inducing transcription at the MCS? What medium components would need to be added or not included? Explain.
9. Using pGulig-3 without any mutations, devise a method of inducing expression at the MCS by changing the growth conditions, but without adding anything to or changing the chemical composition of the growth medium (L broth without glucose)? Explain.
10. You cloned your favorite protein in frame into the multiple cloning site, induced expression of the MCS, and the culture stopped growing, possibly even died (it did not go into stationary phase). Provide at least two unrelated reasons as to why this could happen.

