

Bacterial Genetics and Physiology
Final Exam 2000
Due October 13

Attached is a figure depicting the essential and relevant genetic elements of pGulig-2, which is a specific purpose cloning vector. Where a promoter is indicated, it should be assumed to be the sole driving element for the adjacent downstream gene (*lacI*, *N*, RNA-II). If a gene does not have a promoter indicated (*tet*, *araC*), assume it is expressed from its wild-type, normal promoter. Assume that all genes have transcriptional terminators to prevent read through transcription. The loci in the map are definitely not drawn to scale, so don't make any inferences from size. But direction of genes and relative location are important and relevant.

The *N* gene, pL, and tL loci are all derived from lambda phage.

MCS is the multiple cloning site that has the following DNA sequence

NdeI *EcoRI*

pL -> tL -> gctacaggaggatcggtgaactcatt **CATATG** GAATTC ... C-terminus metalloprotease.

The *NdeI* and *EcoRI* sites are in CAPITAL letters, and the **ATG** start codon is **bolded**. The metalloprotease gene is from *Erwinia chrysanthemi* and also contains the amino acid sequence DFVV. In *E. chrysanthemi* (a gram-negative bacterium) the metalloprotease is expressed in the culture supernatant (outside of the cell).

The plasmid contains a tetracycline resistance gene, *tet*.

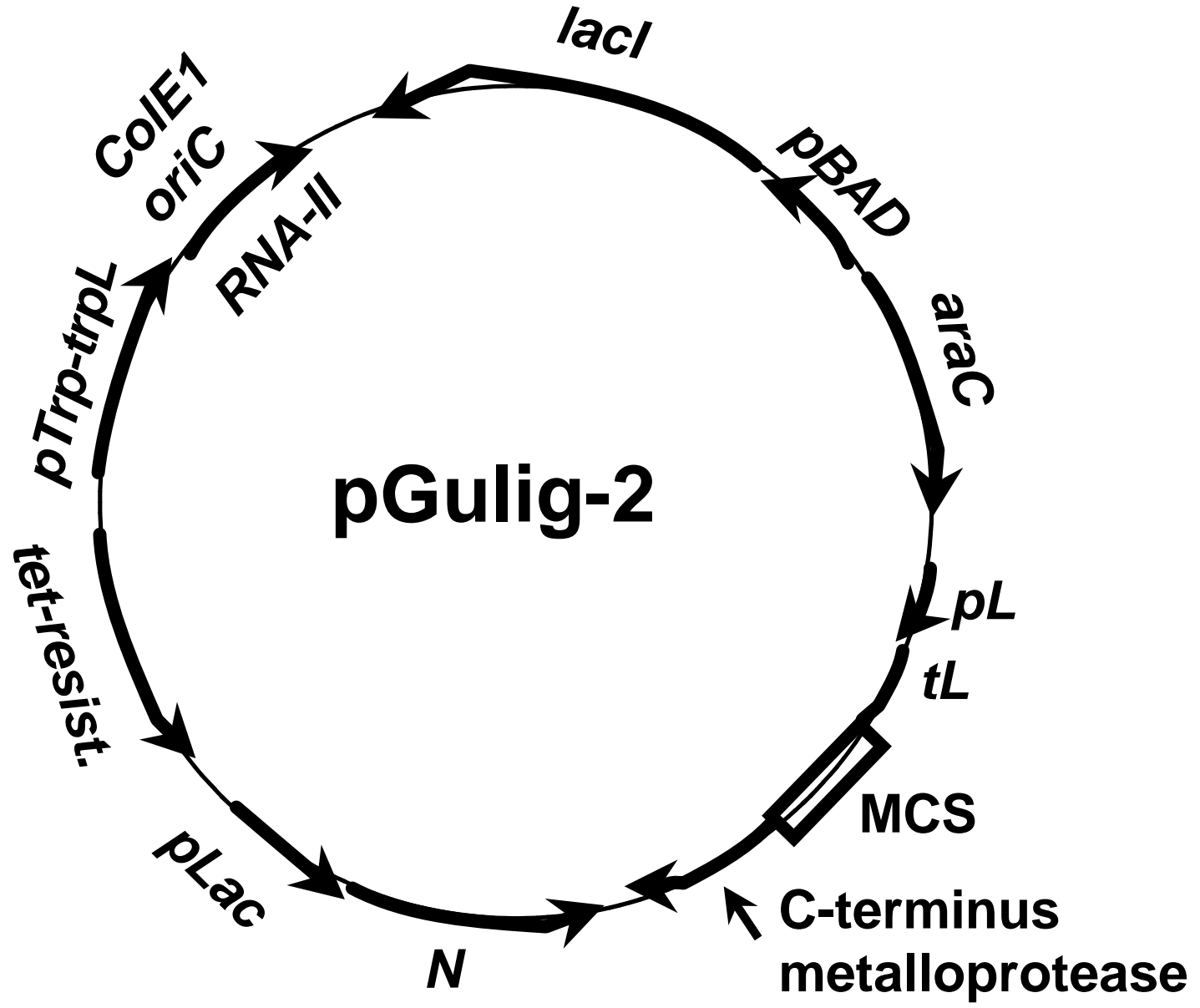
The *lacI* gene is expressed by the pBAD promoter, which is located opposite, but adjacent to, the *araC* gene with its wild-type promoter.

The pTrp promoter and *trpL* gene are in their wild-type configuration and are immediately upstream of and driving the expression of the RNA-II of the ColE1 *oriC* site.

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!



QUESTIONS

1. How would growing the *E. coli* host strain containing pGulig-2 in either L broth (tryptone, yeast extract, NaCl) versus minimal medium (glucose, NaCl, phosphate, ammonium salts) affect the stability of this plasmid at both the single cell and population levels? Explain.
2. What is codon usage or codon bias? How might codon bias by different bacterial species affect the stability of this plasmid at the single cell (not population) level?
3. How would adding fusaric acid to a culture of *E. coli* containing pGulig-2 affect the stability of the plasmid in the culture (population). Explain.
4. So if you wanted to cure a culture of *E. coli* of pGulig-2, what growth conditions and additives to the growth medium would you use to most rapidly bring about the loss of the plasmid from the culture? Explain.
5. Based on the indicated loci, and NOT counting for plasmid copy number, how would you maximize the transcription of your favorite gene cloned into the *NdeI* site of the MCS? Explain.
6. Conversely, how would maximally shut down transcription of your favorite gene, if you were afraid it might be toxic, using the loci provided? Explain.
7. What would happen to expression of your favorite gene if you simultaneously added arabinose and IPTG to the culture? Explain.
8. What would happen to expression of the MCS if a second compatible plasmid constitutively expressing the lambda phage *C_I* gene was placed in the same cell?
9. Remember that pBAD and pLac are wild-type for all cis active regulatory sequences. What would happen to transcription at the MCS if growth medium with glucose versus a lesser carbon source, e.g., galactose, was used? Explain.
10. Look at the DNA sequence of the MCS. Identify the relevant sites for expression of your favorite gene cloned at the *NdeI* site. Could this DNA sequence be improved to increase expression of your favorite gene? If so, how and why?
11. What is the most likely purpose for the C-terminus of the *E. chrysanthemi* metalloprotease? To achieve this purpose, does this sequence need to be in frame with your favorite gene? Why or why not?
12. To achieve this special purpose, does the N-terminus of your favorite protein need to possess a special, yet common amino acid motifs (other than a start codon!)? Explain why or why not.