

**Bacterial Genetics and Physiology**  
**Final Exam 2000**  
**Due October 13**  
**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.

Growing *E. coli* with pGULIG-2 in L broth would cause the plasmid to become unstable at the single cell level and at the population level. This is because the *trp* promoter drives RNA-II, which forms the RNA primer for initiation of DNA replication for pGULIG-2, and the *trp* promoter is repressed by TrpR in the presence of tryptophan. Furthermore, the presence of Trp-tRNA will cause attenuation to inhibit transcription of RNA-II.

In contrast, in minimal medium with no tryptophan, there will be no repression or attenuation, so RNA-II will be transcribed, and the plasmid copy number will increase.

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? Codon usage or bias is the preferential use of different redundant codons for amino acids by different bacteria. Hence, if a gene encoding a protein uses codons that are rare for the host bacterium, expression of the protein may go down. Since pGULIG-2 is regulated by RNA, as opposed to protein, codon bias will be of less concern.

One way that codon bias could affect pGULIG-2 would be if the Trp codons for attenuation were different, this form of regulation could become ineffective.

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.

Fusaric acid selects against tetracycline resistance. So adding fusaric acid would select against bacteria with tetracycline resistance-conferring pGULIG-2 and cause the plasmid to become unstable at the population level. (At the cellular level, there would be no effect on plasmid replication.)

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.

To cure a culture of the plasmid, I would grow the bacteria in L broth, possibly with tryptophan being added, but with fusaric acid (and no tetracycline).

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.

To maximize expression of the MCS, I would grow the bacteria in the absence of glucose (to inhibit catabolite repression of the N gene), with IPTG (to stimulate transcription of the N gene from the lac promoter). The pL promoter driving transcription of the MCS is naturally strong (as long as there is no CI protein present, and that would be true here). However, the tL terminator will prevent transcription from getting to the MCS unless the N protein is made to act as an anti-terminator. I would leave out arabinose to prevent the production of LacI, which could inhibit the lac promoter and production of N protein.

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.

To shut down production from the MCS, do not provide IPTG or lactose (fail to induce N from the lac promoter), but do provide arabinose to activate expression of the LacI repressor, and do not provide glucose (to prevent catabolite repression of the arabinose regulation of lacI). Arabinose will bind to the AraC protein to induce the expression of lacI from the pBAD promoter.

7. What would happen to expression of your favorite gene if you simultaneously added arabinose and IPTG to the culture? Explain.

If both arabinose and IPTG were added, the gene would be expressed. Arabinose would cause production of the LacI repressor, but the IPTG would act as an inducer to allow expression of N and thereby cause anti-termination of the MCS.

8. What would happen to expression of the MCS if a second compatible plasmid constitutively expressing the lambda phage *CI* gene was placed in the same cell?

If the CI gene were placed into the cell from a second plasmid, expression from the pL promoter would decrease and expression from the MCS would decrease. CI is the natural repressor of the pL promoter.

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.

Adding glucose would inhibit expression from pBAD as well as pLac. Therefore, there would be less N and transcription of MCS would decrease.

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?

The ribosome binding site (aggagg) is too far away from the ATG start codon and needs to be moved to within 5 to 10 bases of the start codon.

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?

The C-terminus of the metalloprotease is there to cause your favorite protein to be exported out of the bacterial cells by the type 1 secretion system. It must be part of the translocated protein, so it must be in frame.

12. To achieve this special purpose, does the N-terminus of your favorite protein need to possess any special, yet common amino acid motifs (other than a start codon!)? Explain why or why not.

The N-terminus of your favorite protein would normally contain a leader peptide sequence to direct it to the general secretion pathway (GSP) for eventual export. However, the type 1 secretion system is independent of the GSP, hence no leader peptide is needed.