

Here is the long-awaited final exam. You may either return your answer to me by email or a hard copy. I think a week is plenty of time, but if want to take a couple of weeks, that is fine with me. Although this is an open book/note exam, you may not confer with anyone else about your answers. Please send me an email indicating that you received the exam and that the text and figure are clear to you. You may wish to copy the exam.gif file and open it with an appropriate graphics program rather than trusting your email browser to do it (it might not fit it onto your screen).

Attached is a file named exam.gif which is a physical and genetic map of a new cloning vector, pGULIG (there will be a hard copy outside of my office on Monday if the electronic version isn't clear). There are several loci and genes indicated on the map, some of which are detailed herein. Please note that *oriT* RK2 = *mob* RK2 and that *oriV* R6K = *ori* R6K, if that helps. Note also that all of the genes/loci in the bottom of the figure labeled "lambda phage" are derived from the lambda phage.

You are to identify the loci/genes and explain what they are in terms of where they came from and why they would be included on pGULIG. Relate their usefulness to their real function.

Very important - if there are any particular characteristics (genes or functions) that need to be present or absent in the host *E. coli* strain, be sure and explain. These characteristics do not necessarily have to be of *E. coli* origin (i.e., they may be phage or plasmid genes).

Look at the DNA sequence of the multiple cloning site (MCS) and explain the critical regions for the site. If there are any problems with the site or necessary/beneficial improvements that could be made, explain. Please note that the MCS is buried within the *lacZ* alpha gene, which in this case is driven by the wild-type *lac* promoter.

T7 RNA promoter → AACTTGCACCATG**CCCATGG** . . . MCS . . . *rrnBT*₁T₂

The ATG start codon for your favorite gene is indicated in bold. It is located within an underlined *Nco*I site, followed by rest of the multiple cloning site restriction sites (MCS), followed by the *rrnBT*₁T₂ sequence.

In addition to explaining all of the indicated genes/loci, answer the following questions about pGULIG (some of these answers are obviously going to be related to your explanations):

1. After you have cloned your favorite gene into the MCS, how would you get this recombinant plasmid into the appropriate *E. coli* strain? Be sure and explain as many relevant methods as appropriate based upon the information given about pGULIG.
2. If something goes wrong with the *asd* gene (be sure and discuss why it's there in the first place, above), what will happen to the host *E. coli* strain? If you had to liken the effects to the administration of an antibiotic to the *E. coli*, which antibiotic would it be? Why?
3. What host proteins would most likely act at *oriV* R6K? Very briefly, what would they do?

4. If you administered rifampin to the host *E. coli* strain carrying your recombinant pGULIG plasmid (and assuming that the host is NOT a spontaneous rifampin-resistant mutant), would there be an immediate and direct effect on the *oriV R6K* of pGULIG? Very briefly explain your answer.

5. What would be the effect of placing this plasmid into an otherwise wild-type *E. coli* strain that expressed a temperature-sensitive allele of the lambda phage CI gene (for example, the CI857ts allele) and raising the temperature to the non-permissive temperature for this CI gene?

Good luck to all of you. If you really get stumped on what I am getting at, you may ask me (not your colleagues) for some guidance at a very slight cost of points (unless I really screwed it up, in which case you may earn yourself bonus points).

