

**BMS 6038**  
**Bacterial Genetics and Physiology**  
**Final Exam - 2001 -Key**

(Note - I haven't finished grading your tests, so I may find some other acceptable answers other than these.)

**Questions**

1. Describe what would happen to this plasmid in wild-type *E. coli* if you grew the culture in L broth + glucose. Explain.

The plasmid would not replicate in the *E. coli* cells. This is because the *repE* gene, which is essential for maintenance of the plasmid by acting as a positive factor at *oriV*, will not be expressed if these bacteria are grown in L broth + glucose for two reasons. First, *repE* is driven by the *gal* promoter, which requires galactose, and none is provided. Second, glucose will cause catabolite repression, even if galactose was added.

2. What would happen to the culture if you grew it in L broth + glucose + ampicillin? Explain.

Essentially all of the cells will die by lysis. Since the plasmid will not be replicated (see #1), there will be mainly plasmid-cured cells that will not be ampicillin resistant. Now, if you were really on the ball you would have indicated that the few cells that had the plasmid in the first place would continue to survive since they would not degrade or expel the plasmid.

3. What growth medium would be optimal for maintaining this plasmid in a culture of *E. coli*? Explain

L broth with galactose, no glucose, + ampicillin. Galactose to induce the *gal* promoter driving expression of *repE* to produce RepE protein to stimulate replication from *oriV*. No glucose to prevent catabolite repression. The ampicillin would select for the plasmids and select against any cells that lost the plasmid by chance.

4. Can this plasmid be moved by conjugation from a donor *E. coli* strain to a recipient *E. coli* strain? Why or why not?

No. The *tra* functions are not present, so this plasmid cannot move itself, and there is no *oriT* to act as a cis-active site for mobilization by another conjugative plasmid.

5. Assuming that the plasmid is being maintained in the culture, which antibiotic resistance gene would be most effective for selection? Explain your answer.

OK, this was a trick question! The knee jerk response would have been kanamycin, since I told you that it is not leaky (cytoplasmic resistance protein) compared with ampicillin resistance (periplasmic resistance protein). However, close examination of the intergenic region between *bla* and *aphT* reveals no ribosome binding site in front of the *aphT* gene. It would not be translated. Hence the only functional antibiotic resistance gene on this plasmid is *bla*, and the answer is ampicillin.

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.

The M13 *ori* is there to enable you to produce single stranded DNA of one of the strands of this plasmid by having it packaged as a phage particle. This technically is then a phagemid. However, for the packaging to occur, you must provide M13 genes II and IV, along with the other phage genes required for packaging, etc. These are usually provided by infecting the plasmid-containing culture with a helper phage.

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.

OK, this was another trick question. The answer is nothing. Adding tryptophan will repress the *trp* promoter driving the Lambda phage *N* gene. The *N* protein is an anti-terminator of the Lambda *R* and *L* transcriptional units. However, the terminators for these transcripts are NOT present on this plasmid, so there is no place for *N* to act. Looking at the map of Lambda, the *R* and *L* terminators are far away from the promoters, so the fact that I have pR driving *lacI* does not imply that the *R* terminator is present.

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.

The MCS is expressed by the *tac* promoter, which is a *lac* promoter that is no longer under catabolite expression, but still requires induction by IPTG or lactose. The *tac* promoter is repressed by *Lacl*, which on this plasmid is expressed by the Lambda *R* promoter. In this cell, the *R* promoter would be constitutive (just like the *L* promoter would be). These are strong promoters in *E. coli*.

The most straight-forward way to induce the MCS would be to add IPTG to the culture. You can leave glucose in or not, since catabolite repression is not a factor.

9. Can you devise a method of inducing expression at the MCS by changing the growth conditions, but without adding anything to the growth medium? Explain.

OK, I screwed up here a little. When I wrote this question I concentrated on the Lambda *R* promoter expressing the plasmid-encoded *lacI* gene, and I forgot that I told you this was a wild-type *E. coli*, which would encode the wild-type *lacI* gene on its chromosome.

The intentional correct answer was as follows, but I'll judge each answer on its merits: The Lambda *CI* gene, which presses the Lambda *R* promoter, is expressed by the *dnaK* promoter. So if the *dnaK* promoter was activated, *CI* would prevent (plasmid-encoded) *LacI* from being expressed, and, in theory, the *tac* promoter at the MCS would become constitutive. How do you induce the *dnaK* promoter? Heat shock. So if you heat shock the culture, *lacI* will be repressed and the MCS will be expressed. The problem is that the *LacI* from the chromosome would still repress the MCS.

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. Provide at least two unrelated reasons as to why this could happen.

I'm sure there are many answers, but here is what I had in mind:

A. Your favorite protein is a globular cytoplasmic protein. Since you are attempting to get it secreted by the bacteria, it could interfere with normal membrane function and kill the cells (recall the story about early microbiologists trying to get *LacZ* secreted to the outer membrane).

B. Simple over expression of your favorite protein from the MCS could divert too much energy from the normal requirements of the host cell.