Troubleshooting Forum

Molecular Biology Techniques Q&A

Protein coexpression: sequential induction

This month's question from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) comes from the "Protein Methods" section. Entries have been edited for concision and clarity. Mentions of specific products and manufacturers have been retained from the original posts, but do not represent endorsements by, or the opinions of, *BioTechniques*.

Does anyone know of an inducible vector that responds to something other than IPTG? (Thread 22363)

Q I am planning a co-expression study where I want to induce expression of one protein, wait a few hours, then induce expression of the second protein. To do this, I need a second plasmid with a promoter that is inducible by something other than IPTG. I will be working with *E. coli*.

The pBAD promoter is induced by arabinose and would almost work for me, but it has a *Bam*HI restriction site located in the promoter itself. I don't think I can remove it without changing the activity of the promoter and I need to use *Bam*HI for cloning.

The Tet promoter from pPROtet.E 6xHN might work, but Clontech has apparently discontinued this vector series. It also needs a repressor protein on a separate plasmid, requiring me to use three plasmids per cell or perform additional manipulation to reorganize the vectors.

I'm not worried about compatible origins of replication or selection markers at this point since those are easy concerns to resolve. I just want a simple on/off promoter that doesn't respond to IPTG. Does anyone know of something that could work here?

A I know it would require several extra steps, but you could PCR clone your gene-of-interest from one of your plasmids, re-engineer the *Bam*HI site, then cut the PCR product and ligate it into your target vector.

Q I could do that, but I really don't want to if there is an easier way. I need the *Bam*HI recognition sequence in the multiple cloning site to maintain compatibility with a series of plasmids that I routinely work with and that coordinate with several experimental designs. I'd rather mutate the *Bam*HI site in the promoter. I'm looking through the literature to see if I can find a reference from anyone who has done it before and for any description of the effect on expression.

A The promoter I use is far from the canonical bacterial promoter. It doesn't even have the Shine-Dalgarno sequence completely intact and properly positioned.

As long as you don't disrupt critical signals (ie Shine-Dalgarno) when you remove the *Bam*HI site in the promoter, you should be fine. The fact that there's a *Bam*HI site in there to begin with might indicate that the section surrounding it is not critical. If it were critical, there would be a *Bam*HI site in every organism that used that promoter natively, leaving them highly susceptible to enzymatic attack. So, my guess is that the creators of the plasmid or promoter region actually added the *Bam*HI recognition site in that location because it was convenient and not needed for expression.

I would go ahead and try a minimal point mutation and verify that you can still express that protein independently. Then you should attempt your co-expression experiment and see how it goes.

Q Normally, I would agree with you. But the araBAD promoter might be a little different. First, this region of the promoter, including the *Bam*HI site, is fully conserved in all *E. coli* according to a BLAST search. The AraC activator binds arabinose and subsequently activates the promoter by binding in the region surrounding the *Bam*HI recognition sequence. I think there is a good chance that the mutation might silence the promoter. Therefore, I would like to find a reference indicating that this change is okay before going to the effort.

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A You could use *Bgl*II instead of *Bam*HI in your original multiple cloning site. In that case, you would need to maintain a source vector from which you can prepare your sequence. Any time you cut the section and insert it into another vector, you will have to leave it there since you won't be able to cut it from those same sites again. If you did this, you could clone into any vector's *Bam*HI site, but you wouldn't be able to get it back using either *Bam*HI or *Bgl*II.

A I know of a rhamnose inducible system that might work for you. The promoter name is pRha; it is supposed to offer tighter regulation than pBAD. I also remember seeing a series of tetracycline-based expression systems, but that might actually be the discontinued Tet-on/Tet-off Clontech product that you mentioned earlier.

I have a question for you regarding your experimental design: how critical is the level of expression regulation for your work? All promoters have a basal level of expression, and depending on what you are looking for with your sequential protein expressions, this could influence your results and make them hard to interpret. It might even make data from that experiment meaningless. If that does happen when you try the experiment, you could add glucose to reduce basal expression, but that will require several rounds of optimization for the induction method.

Q I don't need the basal level to be zero in this case; I just need to be able to have some control over when the protein expression gets turned on. I'm conducting a screen, and I read that I would get too many false positives if I turned the genes on at the same time because the second protein stabilizes the first.

I'll look into the rhamnose system. Do you know if it is commercially available? The Tet-on/Tet-off system is also used in mammalian cells, but the only commercial vector for *E. coli* seems to be the Clontech one that I can't order any longer. Of course I could make something, but I'm looking for an easier solution first.

I might be able to switch my vectors and put the bait protein in the pBAD and the screening protein in the T7 system instead. But I would still need to modify the pBAD to get it to work correctly.

It seems that almost every vector out there uses IPTG for induction.

A pASK uses anhydrotetracycline for induction.

A I thought rhamnose systems were commercially available, but I just searched quickly and could not find any.

I did find a reference in my endnote library (Jana, S. and J.K. Deb. 2005. Strategies for efficient production of heterologous proteins in *Escherichia coli*. Appl. Microbiol. Biotechnol. 67:289–298) that might be useful if you need to design something for your experiment. But if you're only interested in commercially available vectors, this might not help.

A I also know of a propionate-inducible system, but I can't find any information about where to buy it.

Q I looked into all the different options and it looks like we have a winner! I just ordered pASK-IBA7C from IBA BioTAGnology.

I will have to replace the multiple cloning site and silence one *Nde*I recognition sequence, but these are not major changes. This will actually be easier than if I had been able to get the Clontech vectors because pASK-IBA7C already has the Tet repressor protein on the expression vector.

A I am working with a pASK vector that I believe is an IBA2 derivative. Something you should keep in mind with these vectors is that they reside in rather low copy numbers in the cells. If you're trying to control relative expression levels between this plasmid and another, you may have some difficulties. The low copy number of the plasmid will also lead to small DNA yields from minipreps. I typically get 50–100 ng/μL from a 3-mL culture volume, depending on the insert. Starting with a 100-mL culture volume and performing Hi Speed Midiprep with 1 mL final volume gives a similar concentration, so I often elute with 500–750 μL instead.

I believe all host strains are good for DNA propagation. I personally use DH5α for the task. For protein expression, I use JM83, which is available in a non-competent form from ATCC, but XL1-Blue may also work.

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