Troubleshooting Forum

Molecular Biology Techniques Q&A

Protein Methods: Selecting the Best Epitope Tag

This month's questions from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) come 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 they do not represent endorsements by, or the opinions of, *BioTechniques*.

How can I select an expression tag that will not interfere with protein function? (Thread 21630)

Q1 I want to express two proteins in cultured cells and then detect the level of expression. Both proteins are uninvestigated, do not have corresponding antibodies available, and their function is unknown. Can someone suggest a tag that will be least likely to interfere with protein function? And how do I determine if the function has been preserved if I don't know what function to assay?

A1 I have had very good success with the V5 tag and occasionally also with His and Flag tags. The advantage of the His tag is that there are columns available that allow for easy purification of the soluble protein.

A2 His tags are great for purification, but they are notorious for causing aggregation. I recommend the FLAG tag for a small protein.

A3 Try expressing the protein with an N-terminal His tag at 25°C and also as a fusion to something like MBP or SUMO. Do these experiments in parallel and you should get at least some protein to work with fairly quickly.

A4 If you want to express your protein in mammalian cells, I would recommend using an HA tag and making two constructs. The first should have a C-terminal tag and the second should have an N-terminal tag. At least one of those should work.

A5 There are a number of commercial expression vectors available with either N-terminal or C-terminal tags. Which one is best depends on the structure of your protein of interest. You may need to experiment with one or two.

A6 Have you tried homology searches to find putative homologs with functions that you could assay?

A7 If your protein does not share much homology with other proteins at the sequence level, you may want to get a predicted secondary structure of your protein from PSIPRED and see if the arrangement of secondary structure elements matches anything.

Gene arrangements on chromosomes are often conserved too. If you have access to the genome sequence, you may want to check what genes are present on either side of your gene. If the gene arrangement is conserved, you may be able to find a homolog in another organism.

A8 Do you know if the proteins are essential? Try making an RNAi knockdown or a knockout. If you see a phenotype by reducing or eliminating the protein, you can show that your tagged version functions properly if it restores activity.

A9 You could also cleave your tag and repeat your assay to see how the function is affected.

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How can I purify His-tagged proteins that do not bind to Ni-NTA columns? (Thread 21037)

Q2 I am purifying several eukaryotic proteins that are expressed in *E. coli* using Ni-NTA columns. Some of the proteins do not bind the Ni-NTA column even when they are denatured. I am using Tris buffer, pH 7.5 for binding. I also tried MOPS buffer, pH 6.5 for overnight binding, but there was no improvement. Does anyone have any suggestions for how I can purify these problematic proteins?

A1 Are you sure that they still have the His-tag or could it have been cleaved?

A2 Did you confirm that there is actually expression of your protein of interest? You can do this with mini-scale induction. Just spin down 50 µL of induced cells and control cells, boil them in 20 µL of 1× sample buffer, and check for a band showing the induced protein. If the band you see is small, it probably is not real and you will not see a band after the Ni-NTA spin column purification.

A3 What size column are you using? Is your protein indeed overexpressed? If it is poorly expressed, you may be underloading the column so that you will not see any binding. Make sure to verify whether or not you have overexpressed the protein. If there is not an induced band, however, it does not necessarily mean that your target is not expressed. Some recombinant expression can't be detected by that method. If you don't have classical overexpression, you need to load more lysate onto the column to get any appreciable binding.

A4 I can think of a few things that might be occurring:

1) Your protein may not be within the soluble fraction. Did you save the non-soluble fraction? If so, try to further solubilize it. You should save all of your fractions when running the nickel column purification so you can run them on a gel for a Western blot.

2) The protein, especially if it is highly expressed, could be in inclusion bodies and thus needs to be further solubilized. Pierce Biotechnology offers a kit for that.

3) The protein may need to be denatured and re-folded. The His tag may be inaccessible due to incorrect folding.

A5 Once you confirm which fraction holds your protein, check the concentration of the reducing or chelating agents in your buffer. These can interfere a lot with column binding.

A6 If your proteins are N-terminally His-tagged, you can't be sure that the protein translated properly and still carries the tag. You could have a frame-shift or problems in translation that produce an incomplete product. Although some constructs can't properly express C-terminal His tags, with them you at least know that the protein with the tag is likely to be your full-length protein.

A7 If you do not get proper expression, you may wish to correct for codon bias. *E. coli* use tRNAs in different proportions from mammals. We use the Rosetta strain as an expression host to avoid the codon bias in *E. coli*.

Selected by Kristie Nybo, Ph.D.

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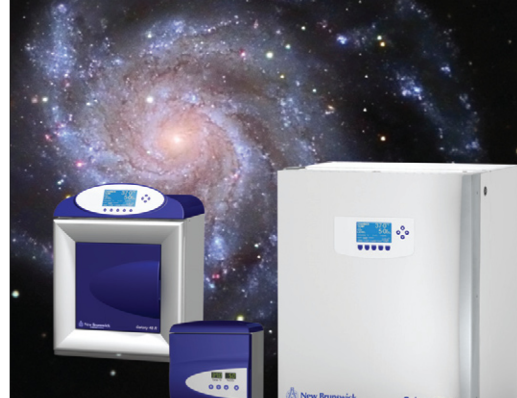

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