

Protein Purification For the Study of Protein-Protein Interaction

Over the last several years, enormous attention is now increasingly being drawn towards the study of the functions of proteins in the context of cellular molecular machines and functional networks, and the study of protein-protein interactions is becoming a key step towards the understanding of the functions of proteins and cellular pathways. The biochemical approach – a combination of purification of interacting protein and MS protein identification, has emerged as an important method for this study. In a biochemical approach, either a bait protein immobilized in an affinity column is used to directly fish out its interaction proteins, or antibody against the bait protein is used to directly precipitate the whole protein complex. The most challenging problem is how to purify a protein complex. Unfortunately, since each protein and each protein complex are different, it often takes some hardworking to develop an optimized purification procedure. Here we want offer some suggestions to our customers about some common issues in developing a procedure to purify interacting proteins and protein complex.

1. “Quick and dirty” Approach. The attempt to use some quick and dirty approach to get results fast is often difficult to resist. Although a quick and dirty experiment may work in some other area, it rarely work in protein purification based on our experience. In some cases a researcher tried to use an antibody to immuno-precipitate a protein complex directly from whole cell extract, and identified proteins are often heat shock proteins, glycolytic enzymes or very abundant structural proteins. Considering the time and all the related cost involved, we strongly against using similar “quick and dirty” approach for protein purifications.
2. Fold of purification: We suggest everyone to do some estimation about the fold of purification required to purify the protein complex of your interest before you start a purification process. Then multiply all the fold of purification you may gain in each purification step and compare these two numbers. For example, it often requires more than 10 to 6th fold to purify a mammalian protein complex, and a good affinity column (high affinity antibody or some a tag) may yield 10 to 3rd or 10 to 4th fold of purification. So if two independent good affinity purification steps are used, it is likely to reach to the desired purification level. If only one affinity step is used, it might be necessary to include some other steps in the purification procedure, such a fractionation and other chromatography steps.
3. Starting material: If the localization of a protein complex is known, it is always better to start with the fractionated starting material instead of whole cell extract. For example, to purify a protein complex from nuclei of mammalian cells, starting with nuclear extract alone may yield 10 to 100 fold of purification compared to starting from whole cell extract.
4. Incubation time: Since formation of a protein complex is a kinetics controlled process, it is often necessary to incubate bait protein in the presence of its interacting partners for some time period, ranging from a few hours to overnight. Incubation temperature is often at 4C, although elevated temperature may lead to faster protein complex formation some times. Protease inhibitor is often added to prevent protein degradation during a prolonged incubation.
5. Use of detergent: Including some low concentration in wash buffer may help to reduce non-specific protein binding. However, some loosely associated components may be washed away by such solution. Use of the detergent should be determined experimentally.
6. Control: one of advantages of this biochemical approach is that it is possible to include multiple controls in a purification process. These controls may provide indicator about the quality of the purified proteins before subjected to MS protein identifications. For example, using an identical affinity column except the bait protein as a control may reveal the non-specific bound proteins. Use of two different antibodies against two domains or two proteins in a complex may point to

some common protein bands, which could be more likely to be real interacting proteins.

Please keep in mind that protein purification is still an art. It still takes a lot of time, effort and dedication to master it. However, purification determines the success of all and every step in the study: we can only identify the proteins that you have isolated.

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