**Tips For Affinity-Purification ('Pull-Down') Proteomics**

The most common application of proteomics to cell biology and related research involves a 'pull-down' assay to determine the interaction partners for a protein of interest. Pull-down may be achieved by various methods (immunoprecipitation, TAP tags, *in vivo* biotinylation, *etc*.). The following tips may help optimize your experiment.

- Choose an appropriate negative (background) control. Remember that some proteins will stick to beads, antibodies and/or plastic surfaces in a non-specific way. If available, a cell line with knock-down or deletion of the protein of interest makes an excellent negative control.
- Optimize your stringency. Experiment with different salt concentrations to find an optimum balance between reducing background and maintaining *bona fide* low-affinity interactions.
- Use a thin polyacrylamide gel. Unless you need to accommodate large amounts of protein, extra gel only contributes extra background.
- Keep your sample free of irrelevant environmental proteins. Keratin (the major protein of skin and hair) can enter your reagents at any stage. Keep everything clean, from the stock reagents onwards.
- If applicable, isolate only the subcellular region where your complex of interest resides (*e.g.* nucleus).
- If using immunoprecipitation, cross-link your reagents to reduce the amount of antibody in the final sample. If possible, affinity-purify your antibody before use. Selective elution (*e.g.* competitor peptide) can potentially increase the specificity of your pull-down.
- Try pre-clearing your sample (*e.g.* for an immunoprecipitation, pre-incubate with beads but without antibody). If using a centrifugation-based pull-down, pre-clear your sample of non-specific precipitators with a centrifugation step prior to performing the pull-down.
- Try different bead types (*e.g.* magnetic, sepharose, agarose) to see what gives the lowest background for your application.
- Do not over-centrifuge beads or subject them to physical damage.
- Pre-wash sample tubes with ethanol, methanol or other organic solvent to ensure they are protein-free.