The Proteomics Resource Center at The Rockefeller University have experience with many different types of protein-protein interaction experiments of which co-immunoprecipitation is one such type [1, 2]. Other examples includes experiments where DNA is use as bait [3] or sets of specific peptides[4]. The PRC also works with APEX [5] and BioID experiments which is a different type of protein-protein study.

Guidelines and thoughts

1) LC-MS is sensitive and we therefore suggest to not further separate a sample. If the sample is so complex that separation is needed it is likely that the protein-protein interaction experiment needs to be further optimized to remove excessive back-ground proteome. Analyzing the protein-protein interaction sample 'in-one-go' decreases technical variability and cost.

2) What can compromise an affinity purification experiment is dealing with a too high dynamic range. Reasons for a too high dynamic range can be if BSA is used to block beads, if antibody or streptavidin has leaked into the sample or if a for example a FLAG peptide is used to competitive elute a FLAG tagged bait.
   The first three examples are often more of an issue.
   BSA – aim to avoid blocking the beads using a protein.
   Streptavadin - use a product where streptavidin is bound tight to the beads. We have seen good results with M280 (https://www.thermofisher.com/order/catalog/product/11205D).
   Antibody – consider if the antibody should be covalent linked to the beads. Options includes BS3 (https://www.thermofisher.com/order/catalog/product/21580) or epoxy beads (M270: https://www.thermofisher.com/order/catalog/product/14301).

3) Most LC-MS is based on reversed phase chromatography and therefore not compatible with detergents. However, you can use non-ionic detergents (e.g. NP-40, Tween and Triton – typically at a maximum conc. of 0.5%) to wash the beads. In general, don't use SDS*. Washing with non-ionic detergent MUST be followed by 3 steps of salt washes WITHOUT detergent. Salt washes removes enough non-ionic detergent to make the sample compatible with in-solution digestion followed by LC-MS. Changing vials between washes can sometimes be needed.
   *) when the interaction proteome is linked either covalent or via biotin, SDS can be used to allow for more stringent washes but must be followed by high urea/non-ionic detergent washes and multiple salt-only washes.

4) Consider if binding capacity is too low to bind all of expressed bait. If not most bait is bound, competition between free and bound bait can results in loss of interactors. Test and compare input and flow through, by WB, during immobilization of bait.

5) Consider how to elute bound bait and interactors. Proteins can be eluted by denaturing (e.g. low pH, high pH, 8M Urea) by competitive elution (e.g. 3x FLAG peptide) or by digestion. The elution is very important for the experiment and efficient elution depends very much on the experiment. Elution can be tested by a combination of WB and staining: Split the protein-protein interaction experiment into multiple fractions to allow to test for different elution conditions. Compare Laemmli buffer elution to for example 8M Urea elution. Split eluents into two to allow for WB (to test elution of bait) and colloidal blue

(https://www.thermofisher.com/order/catalog/product/LC6025) staining (to test for complexity) experiments. Such an experiment series will also allow to gauge the pull-down. For experiment where antibody is conjugated to beads (e.g. protein A/G beads), for experiments where the interaction proteome is linked to beads via biotin and for experiments where bait is linked to a larger volume of agarose beads, often on-bead digestion is best 'elution' option.

6) Protein-protein interaction experiments are somewhat stochastic and so is Data Dependent Acquisition (LC-MS/MS) - the preferred strategy to analyze such samples. We recommend to conduct experiment in biological replicates (n=3 or more). With replicates the results can be filter by: *"protein must be detected in minimum x % of the samples for at least one condition*" and the read-out can be fold differences and a p-values (t-test).

7) For protease inhibitors, best to use non-peptide based (https://www.sigmaaldrich.com/catalog/product/roche/coro?lang=en&region=US). If the inhibitor is peptide based, do let us know.

8) Sample hand-over: if magnetic beads has been used, the samples can be handed-over dry (use magnet to remove the last salt washes and store at -20 °C if needed). Agarose beads/suspension: Keep in a physiological buffer at 4 °C since elution will likely be via on-beads digestion.

9) For most affinity experiments it is preferred that the bait protein is among the more abundant proteins. If the bait protein is 'hidden' by abundant proteins, it can be difficult to find true interactors since they will most likely be of even lower signal. Washing, using LowBind vials and changing vials at least once can help to minimize abundant background. In Figure 1 are plotted signals of measured proteins (replicates) from two different affinity purification experiments. Bait proteins are marked. In the left plot, the bait protein is 'hidden' by much more abundant proteins while the bait protein is one of the most abundant proteins in the right plot.

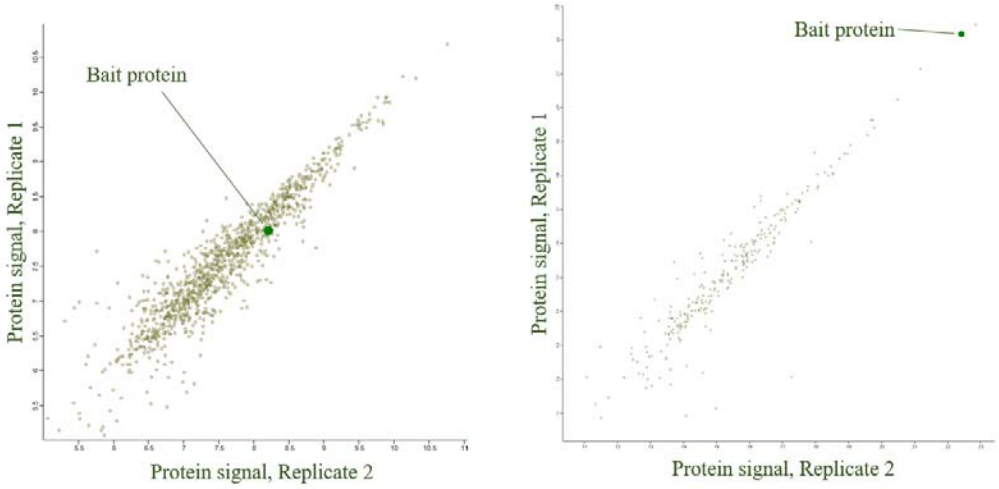

Figure 1. Signal of proteins measured in replicates for two different co-IP experiments. Bait proteins are marked.

References

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