The Proteomics Resource Center at The Rockefeller University have experience with different types of biotin/streptavidin based enrichment experiments. Examples includes APEX [1] and BioID experiments as well as enrichment of newly generated N-termini [2]. Particular the report by Hung *et al.* should be very useful. Biotin/streptavidin has an ultra-strong affinity and is one of the few cases where extensive washing, using SDS, with the goal of lowering background is possible. For biotin based pulldowns we elute by digesting proteins off the beads. Elution by digestion is better than 90% for the proteins that can be digested off.

For biotin type experiments, if possible, we often use endogenous biotinylated proteins to access loading. Examples includes pyruvate carboxylase (PC), propionyl-CoA carboxylase alpha chain (PCCA) and methylcrotonoyl-CoA carboxylase subunit alpha (MCC1). – see Figure 1 on page 2.

Guidelines for washing and preparing an APEX experiment for LCMS analysis adapted by J. M. Luna and D. Phua after the protocol reported in *'Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2'* [1].

First Day

1) Pellets lysed in 1000 µl RIPA buffer with quenchers. Incubated on ice for 15 min. Sonicated once for 15 seconds at 4°C. Clarified at max speed in cold centrifuge for 10 minutes.

2) Saved 100µl of lysate from each of the samples.

3) Equilibrate High capacity Neutravidin agarose beads (our users also have had good experience using Pierce™ Streptavidin Magnetic Beads: 88816) in RIPA buffer using 400µl wet volume. Wash each tube 2x with 1,000µl RIPA buffer and spin at 3,000g for 1 min (or pellet with magnetic rack). Do final spin to remove supernatant.

4) Add at least 5mg of protein (approx. 750-1,000 µl lysate) per sample of beads.

5) Let stand on shaker overnight at 4°C.

Next Day – SDS free day:

6) Saved 100 µl of lysate total from diagnostic samples ("Post-IP"), same pooling scheme as above for diagnostic WB.

7) Wash beads 2x with RIPA buffer without SDS, 1x with 1M KCl, 1x with 0.1M Na_2CO_3, 1x with 2M Urea in 10mM Tris-HCl pH 8.0 (prepared fresh). All washes done for 3-5 minutes on shaker with 1ml buffer and 3,000g spins for one minute (or pellet with magnetic rack).

8) Final washes: 3x in 10mM Tris-HCl pH 8.0 changing tubes each time. OR wash very carefully to remove detergent.

9) Sample drop-off:
If Agarose beads: Re-suspend in volume of 10mM TrisHCl pH 8.0 that is 2-3 times the bead volume.

If magnetic beads: Remove last wash and drop off dry.

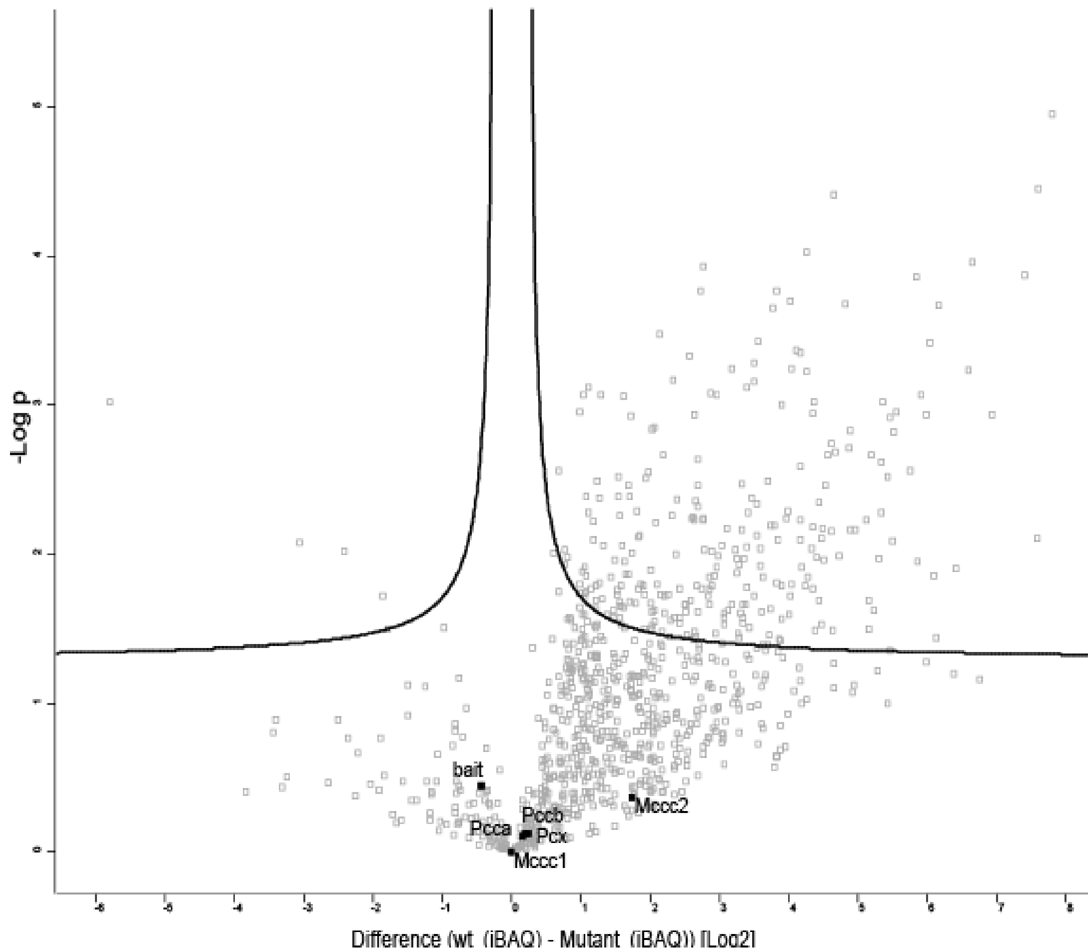

Figure 1. Volcano plot. In black are marked bait and endogenous biotinylated proteins. Neither bait nor biotinylated proteins are showing significant differences.

References

1. Hung, V. et al. Spatially resolved proteomic mapping in living cells with the engineered peroxidase APEX2. *Nat Protoc* 11, 456-475 (2016).
2. Hertz, N.T. et al. Neuronally Enriched RUFY3 Is Required for Caspase-Mediated Axon Degeneration. *Neuron* (2019).