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**Pulldowns of RNA interacting protein complexes for Mass Spectrometry-based identification**

RNA binding proteins play a crucial role in various processes in healthy cells and are associated with various diseases. Several methods have been developed to study these interactions. We present a workflow that allows for an unbiased isolation of RNA binding proteins and protein-protein complexes for the analysis via mass spectrometry. The method relies on the incorporation of the unnatural nucleotide 5-Ethynyl-Uridine into nascent RNA. We use aldehyde cross-linking in order to capture RNA-protein interactions. Labelled complexes are immobilized by using click chemistry and subjected to liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. Our approach is able to isolate and enrich known RNA associated proteins as well as vast number of DNA binding proteins as well as new putative RNA binding proteins and complexes.

An example of a typical volcano plot visualizing the proteins extracted from MCF7 under our optimized conditions is shown. Out of 791 proteins identified, 112 were considered significantly enriched under these conditions. Known RBPs are depicted as green X, proteins so far not considered to be RNA associated are depicted as blue O.

We compared the RBPs that we enriched from the LCC2 and MCF7 to a published data set of the full proteomes for these two cell lines. In total 8276 gene symbols were included in this comparative analysis. 846 genes were exclusive to MCF7’s full proteome, 532 to LCC2 and 6170 were detected in both lines, but not in the pulldowns. 186 genes were enriched only in the pulldowns from MCF7 but present in both cell lines when analyzing their full proteomes. For 241 genes this is the case for LCC2 pulldowns. 16 genes could only be detected in the pulldowns, but not in the full proteomes. We evaluated 1641 genes of RNA binding proteins from different workflows for the enrichment of RBPs. The pan cell line comparison is depicted in the right panel. Only 59 genes (4 %) overlap in all five cell lines. 431 genes (28 %) are exclusive to the CCCP MS gene lists from LCC2 and MCF7 described in this publication and 501 genes (31 %) were exclusive to the polyT-based pulldowns, leaving 709 genes (43 %) that were detected at least once in pulldowns originating from either workflow.

We probed our list of enriched RBPs against a set of RBPs, transcription factors (TFs) and epigenetic regulators (ERs) that were characterized across 15 human cancers: DNA binding proteins that we are isolating play an important role in BC. The interaction between histones and RNA-binding proteins has been validated in a targeted pulldown MS experiment recently, too.

32 proteins that are altered when the two BC cell line are treated with 4-Hydroxytamoxifen (4-OHT) were captured by CCCP. We are able to isolate several members of the MCM complex and PCNA. These proteins have been shown to play a crucial role in BC which further emphasizes the value of CCCP MS as a tool to study disease-relevant RBPs and their interaction with DNA and DNA-binding proteins.

**References:**