

## **Specific and Reproducible Protein Sample Fractionation for Focused Proteomics**

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In the fast developing field of proteomics, it has become apparent during the past decade that simple analysis of a total cell or tissue lysate gives insufficient information for most studies on a given proteome. The reasons for this lie mainly in the high complexity and huge dynamic range of the proteins present in cells. For example, studies on cell lysates separated by 2D-PAGE often ended up in the identification of a number of highly expressed house-keeping or cytoskeletal proteins, and never enabled the detection of low-copy-number proteins such as regulatory proteins. To overcome these limitations, great efforts have been made to develop protocols for the reduction of sample complexity. Typically, these techniques comprise subfractionation of proteins: by purification of cellular organelles or by purification of protein according to their different biophysical properties. In this talk, various solutions for protein fractionation prior to subsequent downstream applications will be discussed. The covered topics include the purification of different cell organelles - with a focus on the purification of mitochondria from mammalian tissue - and the separation of proteins due to their specific solubilization properties or posttranslational modifications (e.g., phosphorylation or glycosylation). A number of assays (enzymatic tests, western blot panels of marker proteins, 2D-PAGE/MS) used to monitor the performance of the protein separation steps will be introduced. In addition, the possibility of extracting proteins from formalin-fixed paraffin-embedded (FFPE) tissue for further downstream proteomic analyses will be demonstrated.