

# Subcellular fractionation strategies for the proteomic analysis of tissue samples

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In order for proteomics to reach its full analytical potential it is required to establish subcellular fractionation strategies for the individual compartments of a eukaryotic cell. The reason for this is mainly the low expression of certain proteins that play a key role in the metabolism. Two compartments are of special interest in the light of biomarker identification by proteomic methods: the cell nucleus and the cellular membranes. In this respect, the proteomic analysis of subcellular fractions originating from frozen tissues is of great interest. However, the enrichment of such fractions from frozen human or animal material requires special protocols.

We have established two nuclear extractions and one total membrane isolation protocol for the isolation of the desired subcellular fractions from frozen mouse livers. The quality of the applied protocols was assessed by a gel-free proteomic approach (MudPIT) and by Western blots with cellular marker proteins. Both nuclear fractions are shown to be highly enriched in their corresponding proteins (46% and 54% nuclear proteins vs. 6% nuclear proteins in the total cell lysate). The fraction designated as the membrane fraction contained 61% membrane proteins vs. 9% in the total cell lysate.

The subcellular fractionation strategies presented here are crucial steps to further enhance the application range of our ProteoSHOP toolkit of gel-based and gel-free proteomics technologies.