Deep Plasma Proteomics at Scale with Proteograph Product Suite: A Performance Evaluation with Label-free and TMT Multiplexing Methods

Mass Spectrometry

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Human blood plasma is a widely accessible sample for assessing individual health status. However, the large dynamic range of circulating proteins combined with the diversities of proteoforms present in plasma have limited the comprehensive characterization of the plasma proteome in a high throughput manner. To address such challenges, current plasma proteomics workflows combine immunodepletion of high abundance proteins, peptide fractionation and sample multiplexing approaches such as tandem mass tags (TMT). Recent advancement in sample preparation (Seer's Proteograph Product Suite), coupled with improved mass spectrometry instrument sensitivity and speed, enable the quantification of thousands of proteins from plasma without compromising throughput or reproducibility, creating a unique opportunity to detect robust protein biomarkers for complex diseases. Here we evaluate the performance of label-free and TMT multiplexing methods with a set of control plasma samples processed with Proteograph Product Suite for deep plasma proteomic analysis.

Pooled control human plasma samples were processed with Proteograph in 4 batches prepared on 4 different days. Tryptic peptides were either directly analyzed by LC-MS/MS or labeled with one of the TMTpro reagents followed by peptide fractionation (high pH RP) and LC-MS/MS analysis.

LC-MS analysis were performed with 40-48-hours workflow (LFQ and TMTpro 16plex). TMT with peptide fractionation, resulted in ~3,000 protein groups. Approximately 86% of the peptide features are detected across all 4 batches, with a median CV < 20%, (PSM level). Label-free performance across four plates run on two different instrument and two different days were evaluated with protein group intensity for most NPs with CVs < 20%, enabling large scale plasma proteomics without compromising depth or precision. We detected plasma proteins spanning 9 orders of magnitude including 40 cytokine activity proteins and several members of TNF superfamily.

This study evaluates performance of the Proteograph platform combined with label-free or TMT technology mass spectrometry analysis.