INTRODUCTION

The requirement for rapid and robust high-throughput sample preparation workflows has become a necessity in clinical proteomics research. These workflows enable processing of large sample volumes with throughput, robustness, and reproducibility required for a rapid laboratory setting. This work details a new automated workflow for proteins and phosphopeptides, allowing for efficient bead dissociation and phosphopeptide enrichment in mass spectrometry analysis, allowing for parallel processing of up to 96 samples in less than 6 hours (excluding digest time). Magnetic beads are automated despite their ease as they are easy to handle, simple to automate, highly available, and high throughput compatible on a single range of magnetic bead handling stations. The automation of phosphopeptide enrichment was originally illustrated by Hopp et al. in 2014, and coupling to automated upfront clean-up and digestion was recently reported by Nakamura et al., in 2020. The current workflow adapts the protein aggregation capture (PAC) workflow described by Hopp et al. in 2014 to automate an at-plate™ 480 magnetic bead handling system, and couples it to phosphopeptide enrichment using new prototype Ti-IMAC and BioMAX HP (high performance) magnetic beads, and data analysis by Spectronaut™ (ThermoFisher).

Tissue heat inactivation & homogenization of 12 tissues from 3 replicates

SPECTRAL LIBRARY

DIA WORKFLOW

DIA PHOSPHO WORKFLOW

PAC digestion

Off-line High pH fractionation

500 ng peptide digest

200 samples/day DIA FAIMS CV-45

60 samples/day DIA FAIMS CV-45

14 hours

18 days

1500 injections (12 tissues with 4 technical replicates)

60 samples (12 tissues with 5 technical replicates)

500 ng peptide digest

250 ng

60 samples/day DIA FAIMS CV-45

Spectral library DIA

directDIA

60 samples/day DIA FAIMS CV-45

14 hours

Prophosphopeptide Elution 200 µg peptide

RESULTS

Above images reproduced from Bekker et al., 2019. Number of peptides (above left) and proteins (above right) quantified from each tissue library. Box-plot four high groups: liver, spleen, testis, and brain, and proteins (yellow). Protein quantification of phosphopeptides (green) processed using directDIA (Spectronaut™ 480). Data was processed using project specific spectral libraries. Number of phosphopeptides (green) and phosphopeptides (orange) for each tissue were digested in 60 samples/day DIA analysis with Orbitrap HF-480 data acquired using directDIA. Phosphopeptides with FAIMS CV-45 enrichment analysis for enrichment of 200µg peptide, with 500ng peptide digest. Data was acquired using same high performance magnetic beads and data analysis by Spectronaut™ 480.

REFERENCES