Fractionated Identification Transfer Workflows for SILAC and Label Free Quantification of Large-Scale Data Dependent Analysis

Mass Spectrometry

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To maintain pace with the advancement of new depths of proteomic analysis, we previously introduced PEAKS Online X, a multi-user, cluster based, high-throughput protein sequencing software solution based on the well-known PEAKS de novo sequencing algorithm, PEAKS Online offers a deployment specifically designed for these projects, and is able to search and quantify >28 million MS2 spectra in less than a day.

The main drawback to performing these large-scale studies is the required amount of sample preparation and mass spectrometry time to perform deep fractionation on every sample. To alleviate this, we have developed a workflow called Fraction Assisted Identification Transfer Quantification. In this workflow, only one sample needs to be deeply fractionated, while other samples can be run in single runs. Identifications from the fractionated sample, in, will be transferred between samples for quantification. This workflow works well for both label free quantification as well as SILAC labelled samples.

With data generated from Hela Lysate run on a Bruker TimsTOF instrument and, we tested the ID transfer by analyzing a fractionated data set of 24 Hela fractions alongside 3 single shot runs. Using this workflow, we were able to increase the total number of quantified identified protein groups to 6645 from 6152 without using the fractionated sample. This workflow also resulted in an increase from ?89k feature IDs to ?96k feature IDs. Correspondingly the number of overall feature vectors being transferred from the fractionated sample to the single samples averages 538.

In summary, our new workflow in PEAKS Online provides an even greater depth of discovery while allowing researchers to reduce the preparation and mass spectrometry time required to perform deep MS1 based quantification proteomics experiments.