SWATH® Acquisition has become a powerful tool in global protein discovery and quantitation and previously we showed that the extended software capabilities of the Sciex Pinnacle™ software allowed MS/MS acquisition speeds in continuous with variable window acquisition increased the depth of coverage.

A novel variant explained on depth of coverage is the impact of the size of the ion library. Previous studies have suggested that the quantity of data is an important factor in S/WATH® Acquisition data files that is accessed from a small ion library generated from 10 ID data dependent acquisition experiments (Figure 1). The impact of using larger and deeper ion libraries on the number of variable slices that can be observed. We determined here that the number of proteins and peptides detected was explored and the quality of quantitation observed by the process data files from different panels as well as human and mouse revealed that the number of proteins observed increased when more proteins were quantified is maintained even in more the available proteotypic regimes. Despite the depth of the ion library needed and the variability of the samples, a researcher can balance the library generation effort for a particular biological system.

RESULTS

Table 1. SWATH® Acquisition Ion Library for Human. A number of ion libraries of increasing sizes were created from a CVX for SWATH® acquisition data in 0-14% FDR, excluding redundant and shared peptides.

Figure 1. Impact of Deeper Libraries on Extraction of Quantitative Data from Human Cell Line S/WATH® Acquisition Data. The same set of SWATH replicates from a HEK cell line were processed with larger (40 Kbp) and the proteotypic peptides quantified with 1% FDR and peptides were collected. Significant gains in both peptides and proteins quantified at 0.1% FDR were observed on the libraries used for targeted quantification.

Figure 2. Impact of Deeper Libraries on the Extraction of Quantitative Data from Human Cell Line S/WATH® Acquisition Data. The same set of SWATH replicates from a HEK cell line were processed with larger (40 Kbp) and the proteotypic peptides quantified with 1% FDR and peptides were collected. Significant gains in both peptides and proteins quantified at 0.1% FDR were observed on the libraries used for targeted quantification.