INTRODUCTION

Stable isotope labelling can be routinely applied in LC-MS proteomics, for accurate and reproducible quantitative profiling on a large scale. Labels are incorporated metabolically, enzymatically, chemically or by stable isotope labelling. Here we demonstrate a novel informatics processing pipeline for data sets generated using dimethyl chemical labelling, applied in triplic. This method has the advantage of being broadly applicable to any sample type, and has quantitative reproducibility close to that achievable with metabolic labelling. We show the benefits of using software able to incorporate retention time (tR) alignment and profiling, as well as profiling of ion mobility (2M) drift times (tL) to increase confidence in peptide quantification and sensitivity.

METHODS

Multidimensional data acquisition and analysis

Example data are shown in Figure 5, illustrating a two-dimensional representation of the data (intensity vs. m/z) and inset two three-dimensional counterparts of the same set of triplets, showing intensity as a function of tL and tR, and intensity as a function of tL and m/z. Progenesis Q3 for proteins of drill down to correct ion alignment across multiple runs and to correlate ISA preextract and product ions, whereas Proteolabels uses the same information for pair/triplet detection and scoring.

Aquisition and DIA search specificity

The first experimental design shown in Figure 2 was used to access the specificity of the LC-MS-DIA-MS acquisition method and the DIA search algorithm embedded in Progenesis Q3 for proteins. A limited amount of 100 ng (all peptides combined) of labelled samples was analysed using default triplicate DIA search parameters and automatic precursor and product ion search tolerances (5 and 12 ppm, respectively). The results shown in Figure 4 illustrate how the peptide identification FDR was estimated by expressing the incorrectly labeled peptide, as a function of the number of identified peptides and intensity.

RESULTS

QC Metrics

Shown in Figure 6 are Proteolabels QC metric and data summary graphs showing comprehensive metrics and protein coverage to arrive at the best protein quantitation set. These QC tools complement the raw data QC metrics implemented in Progenesis Q3 for proteomics.

Protein inference and quantitation

A protein group step, to account for different proteins supported by the same peptides, is conducted prior to quantitation. “Unique peptides” (assigned to a single protein) and “resolved peptides” (assigned to a single set of proteins) are used for quantitation. Conflicted peptides cannot be uniquely assigned to a protein group, are removed from quantification by default, but can be manually added back in.

Protein level quantification

In order to quantitate changes at the protein level, peptide feature groups (Proteolabels “pair/triplet scoring” for increased confidence), both contributing to increased confidence in peptide quantification by default, but can be manually added back in.

CONCLUSION

• Quantitative functionality of Progenesis Q3 for proteomics and Proteolabels has been shown to include the analysis of dimethyl isotopically labelled samples in triplic for the large scale of LC-MS acquisition.

• High quantitative precision was observed, including reproducible quantitation of the expected ratios according to the spike-in design.

• Co-detection across LC-MS runs and metabolic pair matching afforded ~2 fold gain in both peptide and protein detection/quantification.

• Proteolabels “Weighted averaging” based profiling of peptide feature groups delivers high-level quantitative accuracy at the protein-level.

• The analysis demonstrated that accurate and reliable differential expression could be detected of yeast and E. coli proteins, against a dominant background of unchanging human proteins.

• The Proteolabels QC metrics further enhance accurate quantitation by enabling users to explore numerous aspects of the data, including criteria for three peptides (pairs/triples), and overall trends for peptide combinations.

References


Figure 1. Progenesis Q3 for proteomics/Proteolabels workflow.

Figure 2. Experimental design (top): proteome unique; bottom: HeLa 100:100:100, yeast 50:50:50, E. coli 50:50:50, ‘light’, ‘intermediate’ and ‘heavy’, respectively.

Figure 3. Dimethyl 50:25:10 (yeast) triplet separated by m/z, tL, and tR.

Figure 4. Peptide FDR estimation for the individual proteomes and all peptide searches combined across all species.

Figure 5. Identification of a dimethyl 100:100:100 (HeLa) peptide triplet showing good agreement between the chromatographic, isotope identification and drift properties of the detected and identified peptide.

Figure 6. Analysis of data from the second design – quantified in isolation (single replicates) and on a tandem analysis workflow (two replicates). The analysis demonstrates that accurate and reliable differential expression could be detected of yeast and E. coli proteins, against a dominant background of unchanging human proteins.

Figure 7. Rare, unlabeled dimethyl peptide fold change illustrating observed, i.e. ‘light’ vs. ‘heavy’ conditions/shape comparison for expected 1:1 (Homo sapiens – yeast) and a 1:3:2 (E. coli – represented by the desired line).