Stable isotope labelling can be routinely applied to LC-MS proteomics, allowing for faster and more reproducible quantification profiling on a large scale. Labels are incorporated in the samples, at the N-terminus, enzymatically, chemically or by stable isotope labeling. Here we demonstrate a novel informatics processing pipeline for data sets generated using dimethyl chemical labeling, applied in tripleplex. This method has the advantage of being broadly applicable to any sample type, and has quantitative reproducibility close to that achievable with metabolic labeling. We demonstrate the benefit of using software able to accommodate the simultaneous analysis and integration of multiple peptide datasets, as well as profiling of ion mobility (IM) drift times (tD) to increase confidence peptide quantification and sensitivity.

**INTRODUCTION**

Stable isotope labelling can be routinely applied to LC-MS proteomics, allowing for faster and more reproducible quantification profiling on a large scale. Labels are incorporated in the samples, at the N-terminus, enzymatically, chemically or by stable isotope labeling. Here we demonstrate a novel informatics processing pipeline for data sets generated using dimethyl chemical labeling, applied in tripleplex. This method has the advantage of being broadly applicable to any sample type, and has quantitative reproducibility close to that achievable with metabolic labeling. We demonstrate the benefit of using software able to accommodate the simultaneous analysis and integration of multiple peptide datasets, as well as profiling of ion mobility (IM) drift times (tD) to increase confidence peptide quantification and sensitivity.

**METHODS**

Sample preparation
Triple dimethyl samples were prepared as previously described and labeled with the peptide-based isotopes (light, medium and heavy) as shown in Figure 1. LC-MS conditions
Nonlinear LC separation of tryptic peptides was conducted with a capillary configuration using a 9-µm-bore column and a 50 or 120-mm gradient from 5% to 90% acetonitrile in 0.1% formic acid over 80-120 min

**RESULTS**

Multidimensional data acquisition and analysis
Example data are shown in Figure 2, illustrating a two-dimensional representation of the data (intensity vs. m/z) and a three-dimensional representation of the tryptic peptides, showing intensity as a function of tD and m/z, and intensity as a function of tD and m/z. Progenesis QI for proteomics Q3D workflow is shown to illustrate the performance of the tripleplex, and is manufactured in addition to Proteolabels (generated using dimethyl chemical labeling, applied in tripleplex). This method has the advantage of being broadly applicable to any sample type, and has quantitative reproducibility close to that achievable with metabolic labeling. We demonstrate the benefit of using software able to accommodate the simultaneous analysis and integration of multiple peptide datasets, as well as profiling of ion mobility (IM) drift times (tD) to increase confidence peptide quantification and sensitivity.

**ACQUISITION AND DATA SEARCH**

The first experimental design shown in Figure 2 was used to assess the specificity of the LC-MS/MS acquisition method and the Q3D search algorithm embedded in Proteolabels Q3D for proteomics. A limited amount of 100 ng (all proteomes combined) of sample complex was analyzed using default triplex quantitation. Shown in Figure 3 is a representative example of the data acquired using tripleplex quantitation. In Figure 4 (a) is the peptide detection/quantification. Co-identification and drift properties of the de-identified and identified peptides. In Figure 5 (b) is the detected mass shift distributions (c), and m/z vs. drift with tD. Shown in Figure 6 (a) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown). Shown in Figure 6 (b) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown).

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**CONCLUSION**

- Quantitative functionality of Proteolabels to detect protein fold change as a function of regulation probability (Student’s T test) was illustrated in Figure 7, a log2 fold change and p value. As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification.
- Co-detection across LC-MS and metabolic pair/peptide mixing/labeling. 2-fold gain in both peptide and protein protein detection/quantification
- Progenesis “Weighted averaging” based profiling of peptide features: protein group, protein high-throughput peptide identification and drift properties of the de-identified and identified peptides. Co-identification and drift properties of the de-identified and identified peptides. In Figure 5 (b) is the detected mass shift distributions (c), and m/z vs. drift with tD. Shown in Figure 6 (a) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown). Shown in Figure 6 (b) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown).

**REFERENCES**


**Figure 4.** Peptide FDR estimation for the individual proteomes and protein coverage. Note that the number of peptides (an increase in peptide quantity is performed in Proteolabels using a novel “Weighted average” system, by which the overall abundance level quantification value, but while allowing other plausible peptide feature groups to contribute in a system-wide way. High quantitative precision was observed, including reliable quantitation of the expected ratios according to the spike-in design. The analysis demonstrated that accurate and reliable differential expression could be detected of yeast and E. coli proteins, against a dominant background of unchanged human proteins. The Proteolabels QC matrix further enhance accurate quantitation by modeling stable isotope labeling with triplex quantification and protein expression. Protein expression was determined using the.song for expected 1:1 (Homo sapiens against yeast) and a 1.8:1 (Escherichia coli to human) ratio. As a reference, dashed two vertical lines are shown indicating a false positive level determined by the pair scoring/detection of protein abundance (Student’s T test) was illustrated in Figure 7, a log2 fold change and p value. As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification.

**Figure 5.** Peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown). Shown in Figure 6 (b) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown).

**Figure 6.** Peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown). Shown in Figure 6 (b) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown).

**Figure 7.** Weighted average normalized protein fold change illustrating potential changes in the proteomes (changing in abundance) from the HeLa (blue, human) cells (not changing in abundance) and the yeast (green) and yeast (red) proteomes (changing in abundance) from the Hela (blue, human) cells (not changing in abundance). As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification. Protein expression was determined using the.song for expected 1:1 (Homo sapiens against yeast) and a 1.8:1 (Escherichia coli to human) ratio. As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification.

**Figure 8.** Weighted average normalized protein fold change illustrating potential changes in the proteomes (changing in abundance) from the HeLa (blue, human) cells (not changing in abundance) and the yeast (green) and yeast (red) proteomes (changing in abundance) from the Hela (blue, human) cells (not changing in abundance). As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification.

**Figure 3.** Dimethyl 50:25:10 (yeast) triplet separated by m/z (red) proteomes (changing in abundance) from the Hela (blue, human) cells (not changing in abundance) and the yeast (green) and yeast (red) proteomes (changing in abundance) from the Hela (blue, human) cells (not changing in abundance). As a reference, dashed two horizontal lines are shown indicating a false positive level determined by the protein detection/quantification.

**Figure 2.** Experimental design (top, proteome unique; bottom, HeLa 100:100:100, yeast 50:25:10, and E. coli 50:75:90, ‘light’ : ‘intermediate’ : ‘heavy’, respectively). The first experimental design shown in Figure 2 was used to assess the specificity of the LC-MS/MS acquisition method and the Q3D search algorithm embedded in Proteolabels Q3D for proteomics. A limited amount of 100 ng (all proteomes combined) of sample complex was analyzed using default triplex quantitation. Shown in Figure 3 is a representative example of the data acquired using tripleplex quantitation. In Figure 4 (a) is the peptide detection/quantification. Co-identification and drift properties of the de-identified and identified peptides. In Figure 5 (b) is the detected mass shift distributions (c), and m/z vs. drift with tD. Shown in Figure 6 (a) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown). Shown in Figure 6 (b) is the peptide FDR estimation for the individual proteomes and protein grouping step, to account for different proteins supplemented in Progenesis QI for proteomics (not shown).