LABEL-FREE DIFFERENTIAL QUANTITATIVE PROTEOMICS OF GRAPEVINE CELL CULTURES: PERFORMANCE OF TWO DIFFERENT MASS SPECTROMETRIC PLATFORMS

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MS technology is not intrinsically quantitative because ionization efficiency and precursor selection sampling may vary from experiment to experiment. To overcome this problem, several *in vivo* and *in vitro* stableisotopic labelling (SIL) techniques have been developed for relative quantification based on MS or MS/MS intensities. However, the management of large sets of samples and replicates to achieve robust statistical quantification becomes a major bottleneck for SIL as the workflows for data analysis are still underdeveloped. Label-free (LF) is a growing trend in MS-based differential quantitative proteomics. Unlike SIL, in LF no covalent or isotopic modifications are carried out on the sample, neither at protein nor at peptide level. The precursor intensity has proved to be the most robust variable to be used for LF quantification. Morever, the inter-experiment variability can be overcome by data pre-normalization and a pre-analytical alignment of the chromatographic drift. All these resources together with an appropriate data management workflow and statistical multivariate analysis are implemented in Progenesis LC-MS (Nonlinear Dynamics).

Critical points in the proteome coverage of LC-MS-based LF experiments are due to instrument performance such as resolution, mass accuracy and m/z scan speed. To compare the performance of high and low resolution instruments in LF differential quantitative proteomics we have analyzed the same sample in two mass spectrometric platforms: nLC-MS/MS XCTplus Ion Trap and nLC-MS/MS LTQ-Orbitrap XL. The sample consists of five replicates of whole protein extracts from two grapevine (*Vitis vinifera*) cell lines in liquid culture: the intensely red pigmented cv. Gamay and the albino cv. Monastrell. The proteome and differential proteome coverage, the accuracy and reproducibility in the quantifications is compared between both platforms. Also, a DIGE experiment was carried out to assess the extent of the differential proteome at the polypeptide level as compared to that detected by the LF technology.