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Tips for Data Processing of iTRAQ Labeling Quantitative Proteomics Using ProteinPilot and its Background Correction Factor

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Accuracy and precision of protein abundance ratios is the bottleneck of LC-based quantitative proteomics approaches. iTRAQ-labeling, a technique commonly used in global proteomics research has been shown to report compressed ratios towards 1 due to impurities at the reporter ion level and background interference from co-eluting peaks of similar size precursor ions, as well as variations due mainly to low intensity peptides. This phenomenon is observed using a number of different instruments such as Q-TOF and the LTQ-FT mass spectrometers. Bioinformatics tools have been developed and described to improve the ratio accuracy and precision; however, some of them are customized tools that are difficult to utilize at facilities with limited bioinformatics expertise. In this present study, the iTRAQ ratios accuracy and precision were evaluated using ProteinPilot v4 (AB Sciex), a commercial software first introduced to quantitate iTRAQ labeled proteins and more recently upgraded to include a feature called the background correction factor that reduces the ratio compression. A complex mixture of proteins with spiked standards at known concentrations was run onto a LTQ-Orbitrap Velos using only HCD for protein identification and quantification at the peptide level, and the acquired data was processed using ProteinPilot v4. The background correction factor was evaluated for its accuracy and precision spanning a wide dynamic range in fold changes. Additionally, the data was manually inspected to understand the source of the variation in order to improve accuracy. Additional filters and parameters to include in ProteinPilot software are proposed, as a way to improve the confidence of protein identification and quantification. Furthermore, the use of multiple injections of samples to improve protein coverage and precision of quantitative results was also compared. These results will be summarized and discussed.

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A Label-free Quantitative Proteomics Assessment of Stress-responses in Candida albicans

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The fungal pathogen *Candida albicans* survives in humans most commonly as a commensal organism in the flora of the gastrointestinal and urogenital tracts, occasionally causing opportunistic infections, such as thrush. However, in people who are immunocompromised, *C.albicans* can cause severe and life-threatening infections. Stress adaptation is critical for the pathogenicity of *C.albicans*, and hence has been studied in detail at the genomic level to try to assess the mechanisms for host-defence implemented as adaptations to the environments encountered.

This study investigated the proteomic changes between *C.albicans* grown under normal conditions and those experiencing salt-stress in the growth media. Three biological replicates of each condition were proteolysed with trypsin and the resulting peptides analysed by LC-HDMS^E (data-independent acquisition, in ion mobility mode) using a Waters-Synapt G2-S ToF instrument. As little as 100 ng of protein loaded on column, separated over a 90 min linear reversed-phase LC gradient, returned over 1500 protein identifications per run. To each biological replicate digest, 10 fmol/μl phosphorylase b (P00489) digestion standard (Waters) was added and then each was analysed in triplicate. The data was processed using ProteinLynx Global Server software to indentify and quantify the proteins present with the 'Hi3' label-free approach, using the three most intense peptides of the internal standard protein to determine a response factor for quantification of other proteins in the sample. This data was then uploaded to Progenesis LC-MS software (Nonlinear Dynamics) for statistical comparisons of up and down-regulated proteins between the two biological conditions. Initial data interpretation has returned confident expression ratios for over 1000 proteins and indicates that enzymes involved in glycerol metabolism and heat-shock proteins, are some of the most highly up-regulated proteins during the salt-stress in *C.albicans*.

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Quantification of a Model Protein Lysozyme and Peptide MUC5AC-13 in Serum Matrix Using LC-QTOF

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Drug metabolism and phomacokinetics of biologics has been an increasingly interesting topic. LC-MS techniques can potentially provide more accurate understanding of the biotransformation of therapeutic proteins and complimentary information to ELISA methodologies. Although, using LC-MS/MS as the technology for small molecule DMPK has been established in the industry, using LC-MS as a qualititative and quantitative tool for large molecules DMPK have not been widely accepted. In this abstract, a model protein, Lysozyme and model peptide MUC5AC-13 were spiked into human serum. An analog of MUC5AC-13 was spiked in as an internal calibration standard. A high resolution Q-TOF mass spectrometry was used to acquire data. For the peptide, LOQ of 50pg on 2.1mm column and 4pg on 75um HPLC-Chip were achieved. Average RSD was 4.2% with three orders of magnitude linearity. At the same time, MS/MS provided qualitative information for the validation the peptide sequence. For model protein lysozyme, linearity was achieved from 1 – 1000ug/mL in TOF scan mode. These results have shown excellent potential of Q-TOF as a Qual/Quant technique for biologic samples.

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Strategies for Incorporating Novel Post-Translational Modification Enrichment Methods in a Quantitative Proteomics Environment

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