Progenesis LC-MS : Quantitative label free workflow for the differential analysis of complex samples

M. O'Gorman, I. Morns, D. Bramwell, A. Borthwick, M. Bennett. Nonlinear Dynamics, Newcastle-upon-Tyne, UK

MS based quantitative differential proteomics issues: •Per sample run times can be prohibitively long - a 40 fraction offline / 3 hour online separation strategy can require as many as 5 days data capture per sample • Researchers forced to make experiment design compromises - at 5 days a sample there can be real time pressures on collecting the amount of data that the researcher desires •Greater exposure to technical variation- with data capture spread across many weeks the chances of a significant change in the properties of the chromatography system are greatly increased. •Quantitation strategies rely heavily on protein identification - with the data spread across so many runs, protein identifications are usually required to reassemble the complete data set. This means that the quality of data is highly dependent on the success of identifying the proteins. Studies have shown that successful identification in MUDPIT runs is proportional to abundance. [e.g. Liu et al, Anal Chem 2004, 76, 4193-4201] Workflow details Progenesis LC-MS is a workflow-driven software designed to optimise the analysis of label-free comparative data sets. An example workflow is shown in below. The green squares represent functionality provided by Progenesis LC-MS and the blue squares are external operations or functionality. Sample Alignment Peptide Detection Technical variation in experimental procedures can Using the aggregated peak data, peptides are automatically identified creating a map of all peptides result in data sets that are misaligned in retention time. Alignment of data sets allows successful on all samples. An associated charge and mass is matching of peptide data across samples. Alignment is Eurther LC-MS/MS Experime assigned to each peptide. Detection uses isotope landmark-driven and uses all peak data for validation. profiles to confidently build pentides utilising three Landmarks can be manually identified by the user via dimensional data an easy-to-use interface, or automatically generated using the software. Data Generation Feature Identification Data Exploratio Easily configurable and flexible reporting of all of the peptides identified as from the sa nrotein Further Analysis using MS/MS data Aggregate Data Set Peak Modelling Online LC-MS can generate very large data sets. For To ensure consistent pentide detection and matching across all data files, an aggregate data set is created this reason data is often centroided, resulting in a loss This contains all peak information from all sample of valuable information. To overcome these files, allowing the detection of a single peptide map limitations and allow the handling of large numbers that can then be applied to each individual sample. of samples, we have developed an intelligent peak-modelling algorithm that can reduce data files by an This powerful approach ensures 100% matching of peptide features and thus enables the application of order of magnitude. Using a wavelet based approach, multivariate statistical tools to explore pentide data peaks are identified and peak models created that Differential analysis can also be successfully applied. retain all relevant quantitation and positional Peak data is combined in such a way as to maintain information peptide shape properties Review and select from any MS/MS data Further explore the results using Here is a second example of multiple Advanced data processing handles associated with the detected features. multivariate statistics. Here we confirm that overlapping peptides allowing more peptide ions of a protein where they all the differential expression of all of the Create a custom database search file complex sample injections. Charge states behave in a similar manner but in an peptide ions that matched the same protein Import and review the search results are also determined and color coder Group the peptide ions by search result behave in the same way.

Progenesis LC-MS:

- Handles complex samples reducing the need to offline pre-fractionate resulting in huge time savings
 Is designed to handle large numbers of replicates
- •Gives the researcher additional options to allow more optimal experiment designs
- •Rapid sample acquisition reduces the chances of drift or equipment failure influencing the results •Protein identification is supported but not necessary for quantitative analysis
- Visit booth #73 for a live demonstration www.nonlinear.com/LC-MS
- not necessary for quantitative analysis