Pathway Analysis Using Progenesis QI for Proteomics Combined with PANTHER

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GOAL

Data resulting from perturbed mouse liver proteomes are used to demonstrate the pathway analysis functionality and benefit of Progenesis® QI for proteomics in combination with PANTHER Classification System.

BACKGROUND

Large scale (multi-) omic studies often generate complex datasets that require comprehensive bioinformatic tools to process, search and rationalize outputs into a biological context. This 'systems approach' and the ability to understand the relationships between elements within a biological system and the factors that can perturb the system are becoming increasingly important. Pathway analysis tools allow molecular mechanisms to be investigated and test the association between various molecular pathways using sophisticated algorithms. Results from the SYNAPT® G2-S*i* for perturbed mouse liver proteomes are used to demonstrate the application of the computational and pathway tools of Progenesis QI for proteomics and PANTHER respectively for data independent HDMS[E] experiments.

THE SOLUTION

A protein extract originating from mouse liver was digested and 100 ng injected on-column. Peptides were separated and analyzed using a nanoACQUITY® System coupled with a

Pathway analysis tools allow molecular mechanisms to be investigated and test the association between various biochemical pathways.
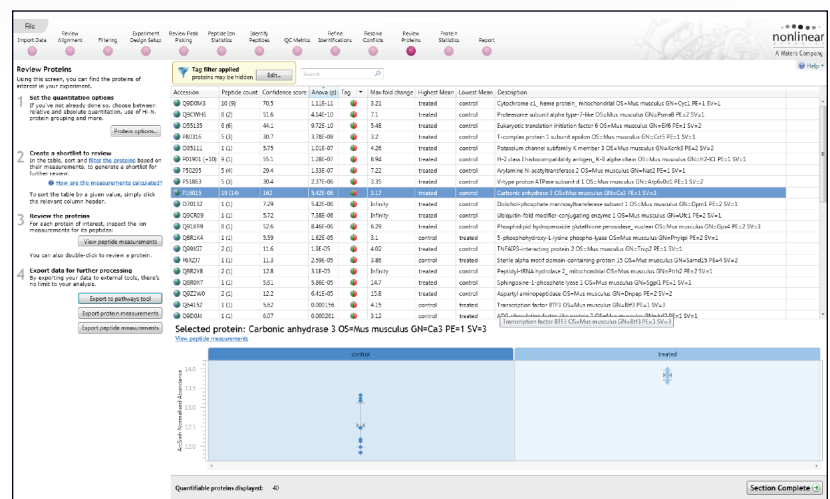

Figure 1. Progenesis QI for proteomics export tool to automatically create a PANTHER-compatible entry list. Protein biomarkers found to be significantly different between groups were submitted for over-representation analysis using PANTHER.

SYNAPT G2-S*i* operating at a precursor and product ion mass resolution of >20,000 FWHM and data acquired in LC/HDMS[E] scanning mode. Searching and quantification were conducted with Progenesis QI for proteomics before being exported for pathway analysis using PANTHER Classification System (Figure 1).

Figure 2 shows the PANTHER browser window, providing the facility to upload exported Progenesis QI for proteomics search results and specify other parameters such as organism and required analysis. In this example, Mus musculus was specified as the organism and statistical enrichment selected as the analysis type. For statistical enrichment, the Wilcoxon Rank-Sum Test is applied to determine a likelihood value that the data submitted is random compared with the overall distribution. On selecting the analysis of choice, a variety of additional enrichment search options become available regarding PANTHER pathways and ontology. Following analysis, results can be displayed in a number of ways, including graphs, lists and pathway maps. Figure 3 is an example genomic distribution displaying matched gene ontology (GO) terms with 47% of identified proteins being related to metabolic processes. Additional detail can be extracted from the chart including gene lists, percentage of genes classified for export. Protein entries are mapped to specific pathways and can be interrogated further. Figure 4 indicates a number of proteins that are associated with the inflammation pathway and are highlighted as blue boxes. Upstream or downstream components can be reviewed, in addition to detailed information of the genes involved and associated PANTHER ontology terms and families.
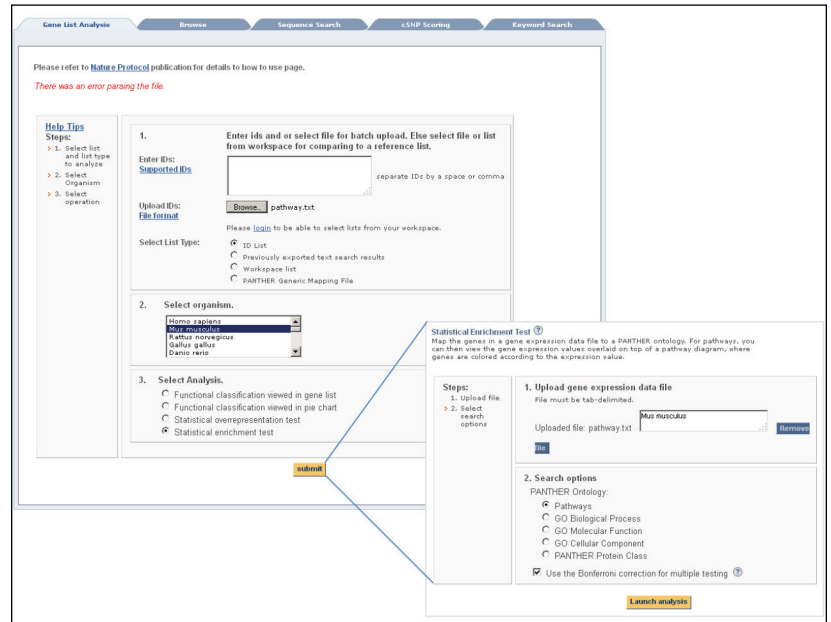
SUMMARY

Pathway analysis tools were used to review the HDMS^E datasets, providing a understanding of the underlying biology of differentially expressed proteins. Significant pathways implicated included inflammation, lipid metabolism and molecular transport.

Figure 2. PANTHER gene list analysis page and associated enrichment test tool page (based on statistical enrichment test).

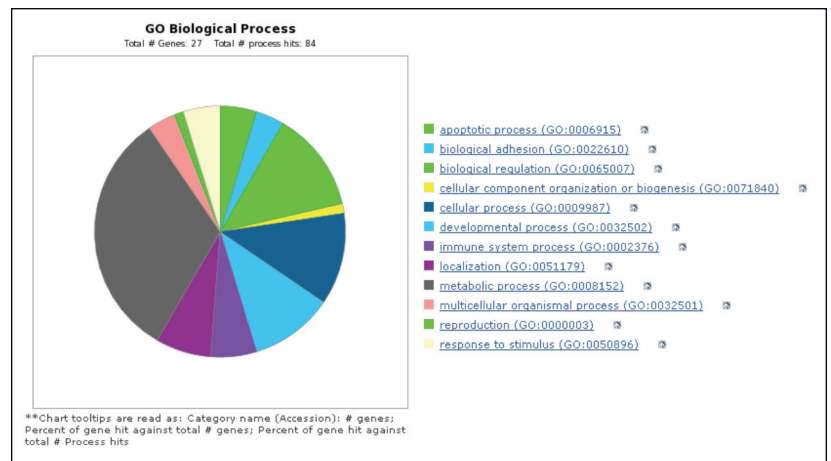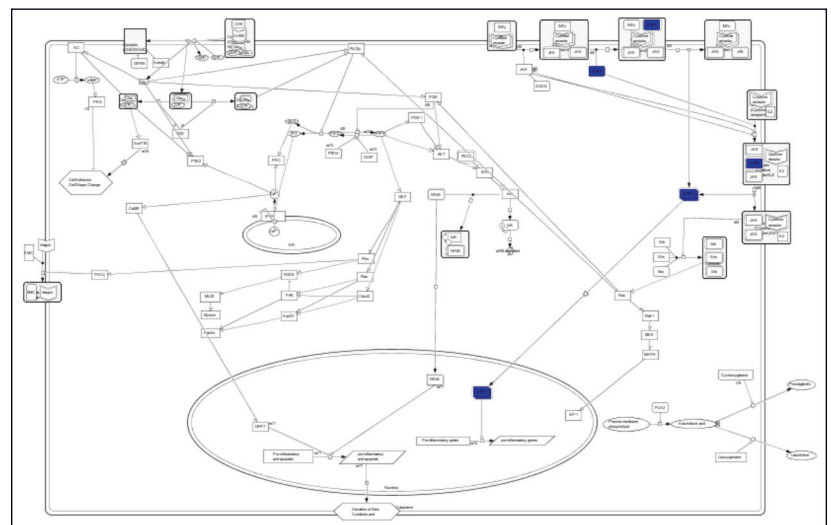Figure 3. Mus musculus protein entries matched to particular GO biological processes.

Figure 4. Inflammation pathway generated by PANTHER. Genes highlighted in blue signify protein entries mapped to the pathway.

References

1. Mi et al. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res., 2013; 41:D377-86.

Waters

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