A COMPARATIVE ANALYSIS OF ESTRADIOL AND PPT TREATMENT EFFECTS IN MCF7 CELLS AS PART OF THE HUMAN TOXOME PROJECT USING AGILENT'S GENESPRING MULTI-OMICS ANALYSIS AND INTEGRATION SOLUTION

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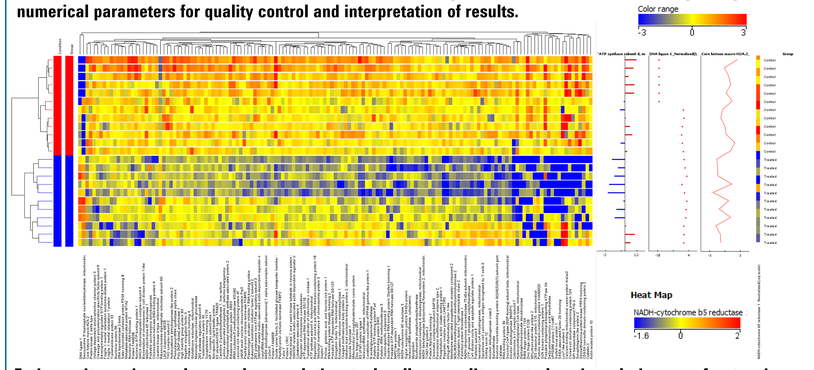
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Abstract

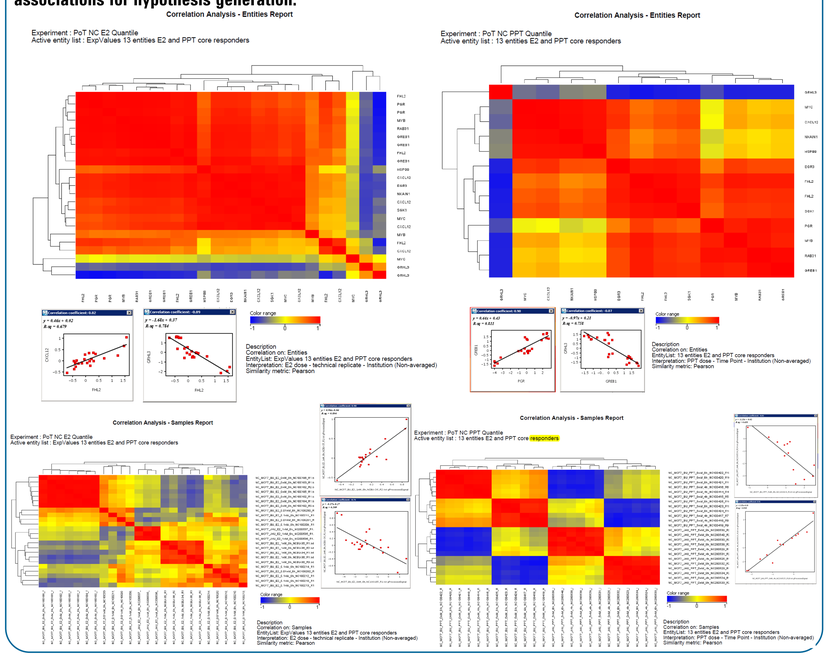
MCF7 cells are a widely used in vitro system to study estrogenic effects. Using Agilent Gene Expression and Comparative Genomic Hybridization (CGH) microarrays we are building a transcriptional network of 17β–estradiol (E2) and propyl pyrazole triol (PPT) responsive genes (RGs) including time and dose dependencies. The core network of estrogen RGs was used to extrapolate responsive pathways. Cell culture work is performed at two independent sites (Johns Hopkins University and Brown University) with extensive protocol synchronization. CGH profiling of MCF7 cells from each site allows for "customization" of network inputs including available transcription factors (TFs) and transcription factor binding sites (TFBSs) present in the genome of MCF7 cancer cell line. We previously presented differences in responsiveness in a subset of differentially expressed (DE) genes between cells cultured at different sites and are working on reproducibility issues. We also reported estrogen response "sentinel" genes, a subset of RGs, which are DE in E2 and PPT treated cells relative to vehicle controls in several experimental conditions including comparisons with relevant publicly available datasets re-analyzed in this study. We created gene lists based on correlated E2 responsiveness and are testing whether their correlation is associated with predicted transcriptional regulators. Using GeneSpring Multi-Omic Analysis (MOA) software we integrated gene expression microarray, RNA-seq, metabolomics and proteomics datasets and identified common RG's between E2 and PPT induced cells. Currently we are in the process of extending estrogen ligand network with this additional information.

Metadata Framework Visualization and Correlation

Metadata framework enables visualization and re-ordering of associated values including categorical and numerical parameters for quality control and interpretation of results.



Entity-entity and sample-sample correlation tools allow quality control and exploring as of yet unknown associations for hypothesis generation.

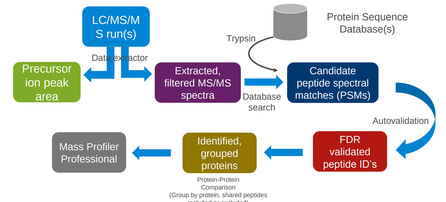


Proteomics

A Jet Stream Proteomics approach was used to collect discovery proteomics data in data dependent acquisition (AutoMSMS) mode on an Agilent 6550 iFunnel Q-TOF using a 100 min gradient. A 25 µg aliquot of trypsin digest was injected on a 2.1 X 250 mm AdvanceBio Peptide Mapping column and peptides were separated at a flow rate of 0.2 mL/min. Four biological replicates were analyzed in triplicate for control and treated (estradiol 24-hour time point only) for this pilot study. Protein identification was performed by database searching in Spectrum Mill software, then then results were exported for analysis in Mass Profiler Professional, a member of the GeneSpring Suite designed specifically for mass spectrometric data.



Proteomics data was collected using the Agilent Jet Stream source interfaced to the Agilent 6550 Q-TOF mass spectrometer.

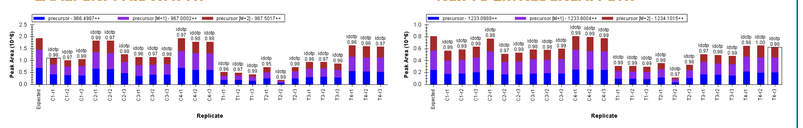


Data processing workflow for protein database searching and protein abundance calculation in Spectrum Mill. Protein abundance results in Spectrum Mill are shown below.



O75947 ATP synthase subunit d, mitochondrial

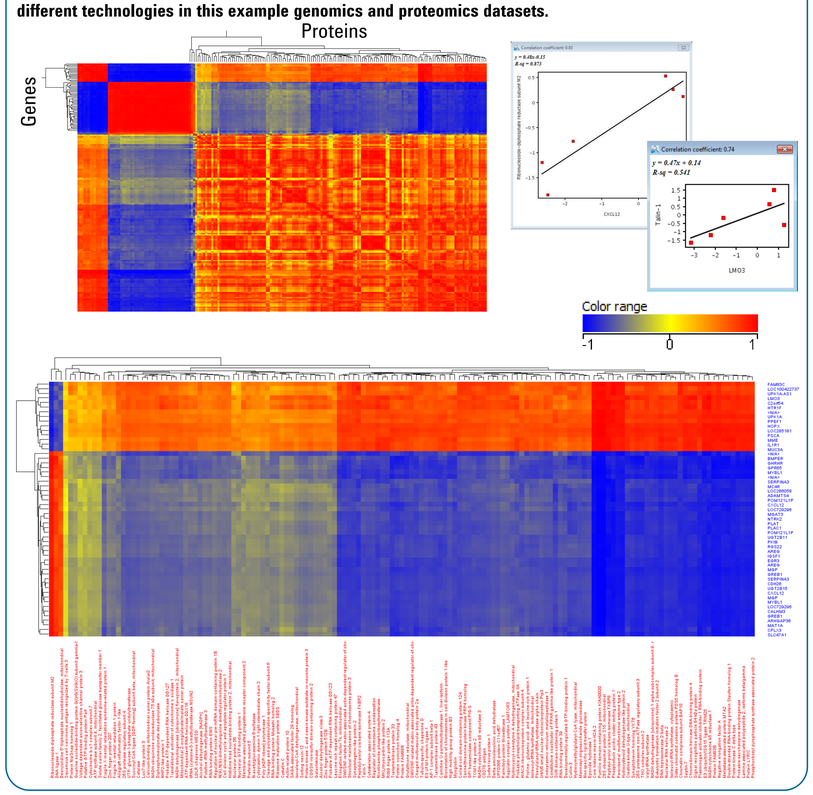
LAALPENPPAIDWAYYK **NLIPFDQMTIEDLNEAFPETK**



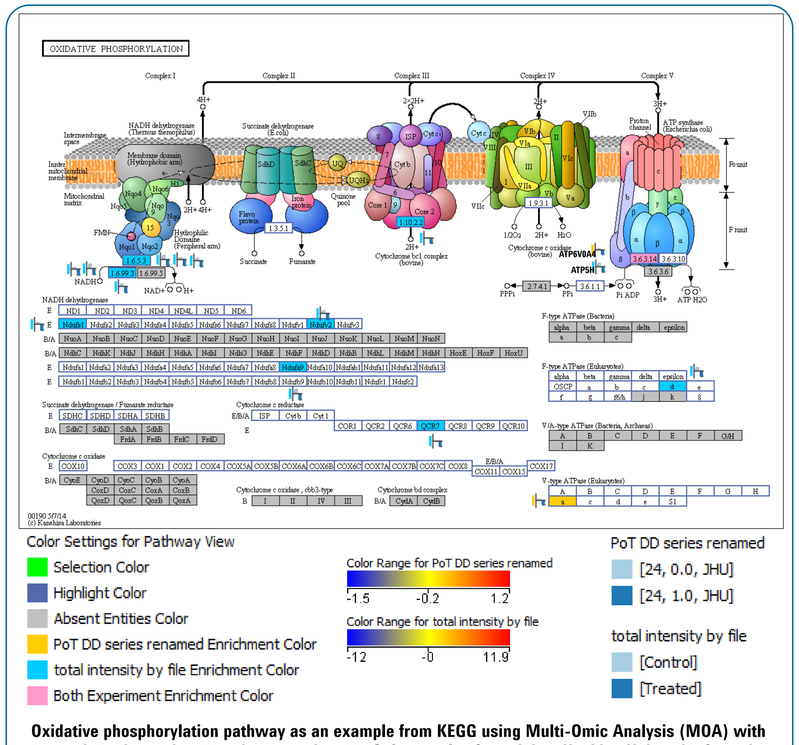
Peak area results were process in Skyline software for two peptides unique to ATP synthase subunit d. MS1 Filtering shows the reproducibility of the technical and biological replicates and indicates down-regulation of this protein.

Multi-Omics Correlation Analysis

Multi-omic analysis (MOA) enables users to compare datasets between any pair of experiments across different technologies in this example genomics and proteomics datasets.



Multi-Omics Pathway Analysis

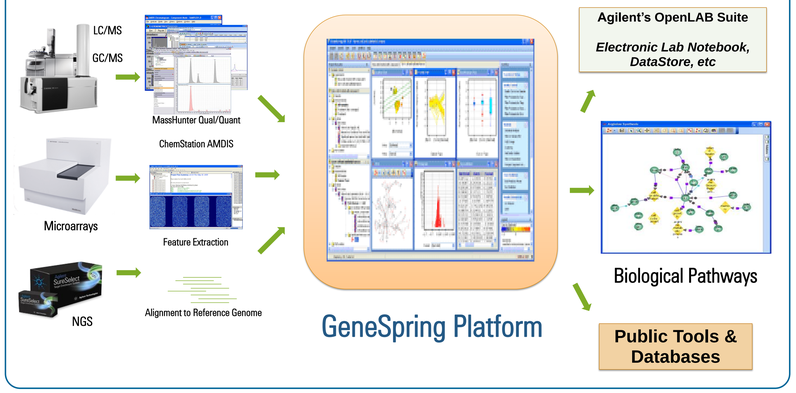


Oxidative phosphorylation pathway as an example from KEGG using Multi-Omic Analysis (MOA) with transcriptomics and proteomics experiments. Only samples from Johns Hopkins University from the 24 hr timepoint were used in this analysis.

GeneSpring and Integrated Biology

Visit www.genespring.com and www.agilent.com for more information.

Agilent's Platform for Integrated Biology



References and Acknowledgements

The human Toxome Project: http://humantoxome.com/

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