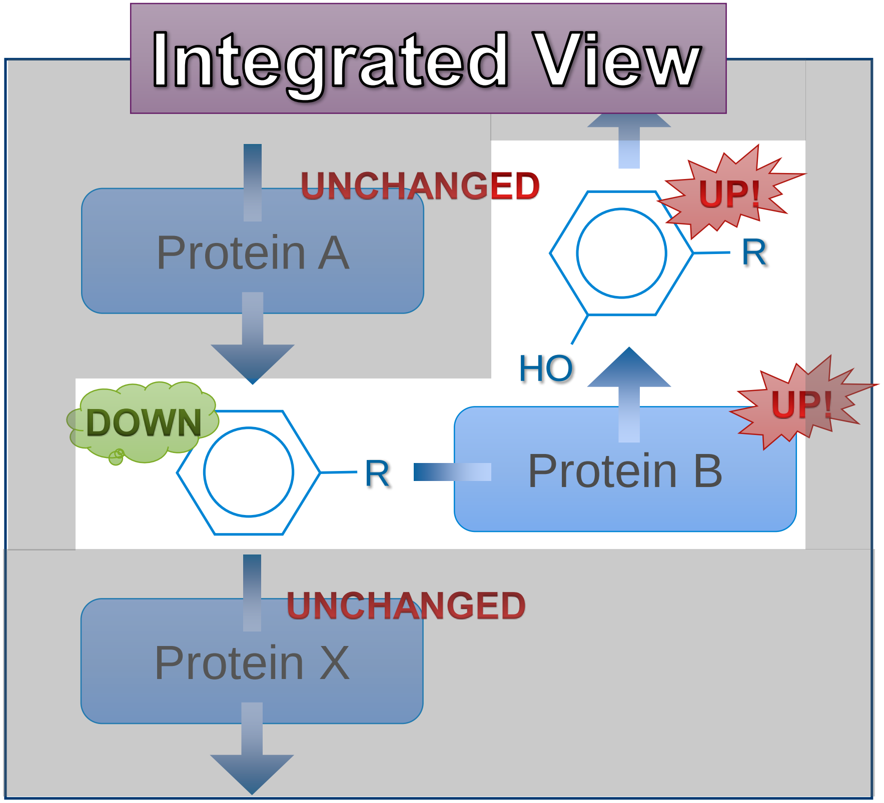
Metabolomic and Proteomics Solutions for Integrated Biology

Christine Miller

Omics Market Manager

ASMS 2015

Integrating Biological Analysis Using Pathways



- Identifies *why* the pathway is active
- Suggests follow-on experiments

Agilent Instrumentation For Metabolomics

5977A GC/MS

7000C GC/QQQ

7100 CE

Hi-DEF Q-TOF 6500 series

Q-TOF 6500 series

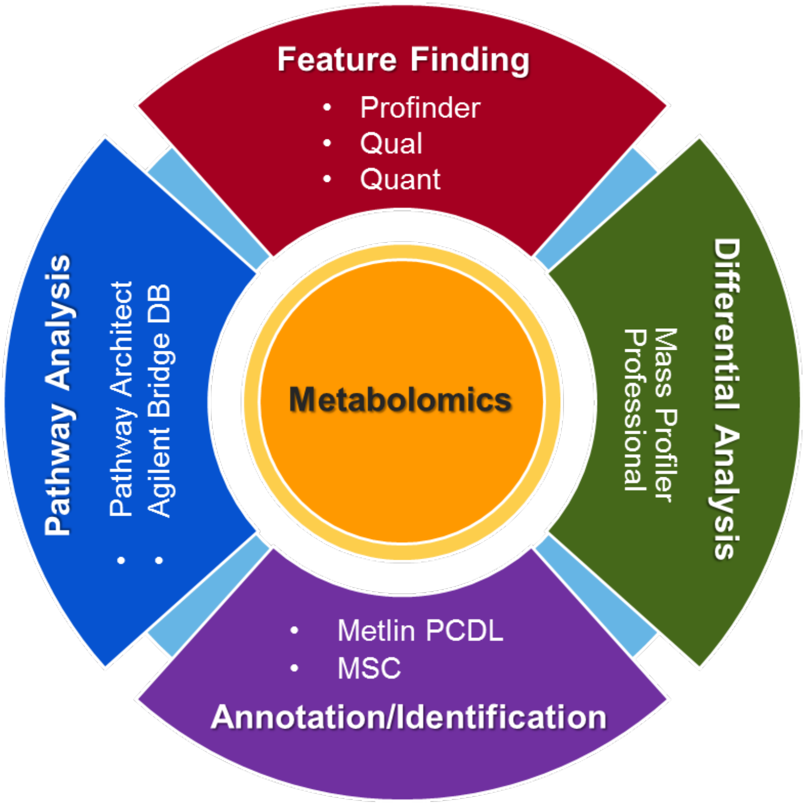
7200B GC/Q-TOF

1290 Infinity II UHPLC

QQQ 6400 Series

TOF 6200 series

Most Extensive Software Portfolio for Metabolomics Data Analysis



Feature Finding

- Profinder
- Qual
- Quant

Differential Analysis

Mass Profiler Professional

Annotation/Identification

- Metlin PCDL
- MSC

Pathway Analysis

- Pathway Architect
- Agilent Bridge DB

Metabolomics

Find features across many complex data files

Align extraction results from many files

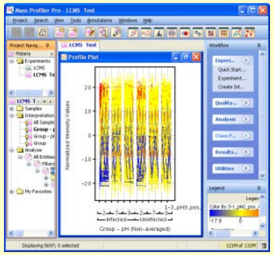
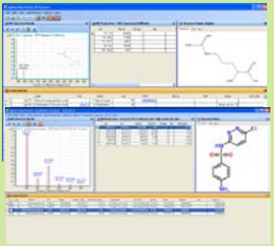
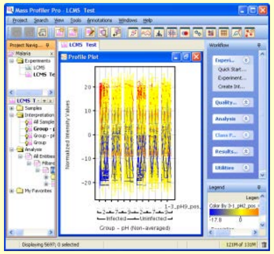
Analyze extracted data for statistically different features

Annotate and identify interesting features

Search and map annotated metabolites to find interesting pathways

Visualize results on pathways

Discovery & Pathway Directed Data Analysis

	Separate & Detect	Feature Finding	Alignment & Statistics	Identify	Pathways
Discovery	 LC-TOF/QTOF	 Profinder	 Mass Profiler Professional	 ID Browser	 Pathway Architect
Pathway Directed	 LC-TOF/QTOF	 Profinder	 Mass Profiler Professional		 Pathway Architect

Lipidomics: The Systematic Study Of The Entire Lipid Profile Of A Cell/Tissue/Organ/Organism

Shotgun lipidomics using QQQ

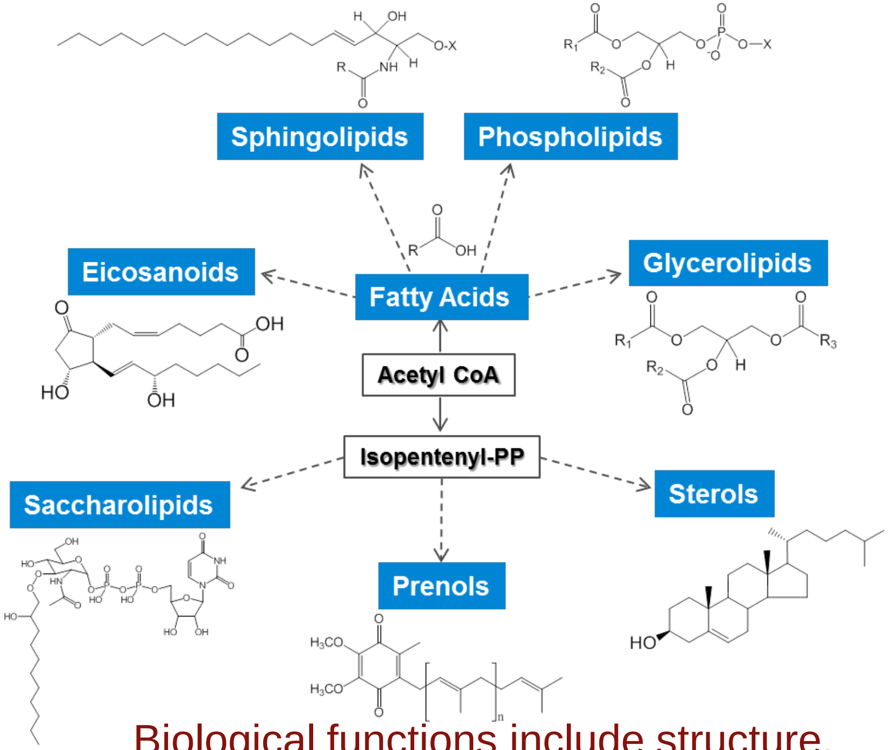
- Infusion based
- Uses mixed scan functions
- Quantitative using class internal stds
- Class and composition info

Shotgun lipidomics using QTOF

- Infusion based, MS/MS product ion
- Quantitative using class internal stds
- Class, composition and R-group info

Profiling lipidomics

- LC/MS on accurate mass instrument
- MS or MS/MS used for identification
- Class, composition, R-group and **isomer**



Sphingolipids

Phospholipids

Eicosanoids

Fatty Acids

Glycerolipids

Acetyl CoA

Isopentenyl-PP

Saccharolipids

Sterols

Prenols

Biological functions include structure, storage, energy and signaling

Agilent Instrumentation For Lipidomics

5977A GC/MS

7000C GC/QQQ

1260 SFC

Hi-DEF Q-TOF 6500 series

Q-TOF 6500 series

7200B GC/Q-TOF

1290 Infinity II UHPLC

QQQ 6400 Series

TOF 6200 series

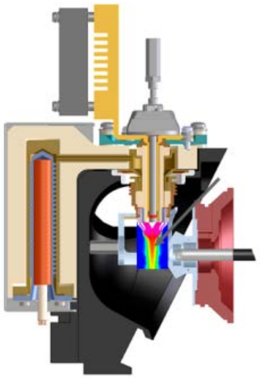
Overview Of Different Chromatographic Separations For Various Lipid Classes

Lipid Category	GC/MS	LC/MS	SFC/MS
Fatty acids (acyls)	***	**	**
Glycerolipids (triglycerides)	*	***	**
Glycerophospholipids		***	**
Sphingolipids		***	**
Sterol lipids	***	**	**
Prenol lipids		**	**
Saccharolipids		***	**
Terpenes (plants)	***	**	**
Polyketides	*	**	**

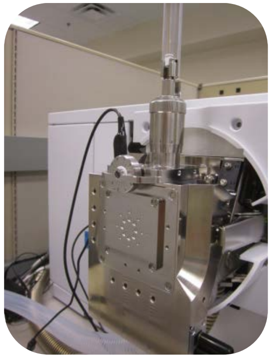
*** is the best choice – chromatography impacts class of lipids resolved and detected!

Agilent Instrumentation For Proteomics

Agilent
AssayMAP
Bravo



Agilent Jet
Stream
source

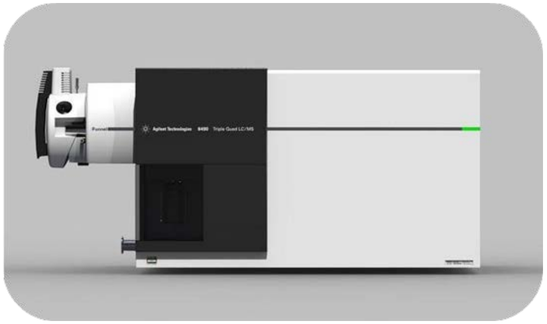


G1992A
Nanospray source

HPLC-Chip

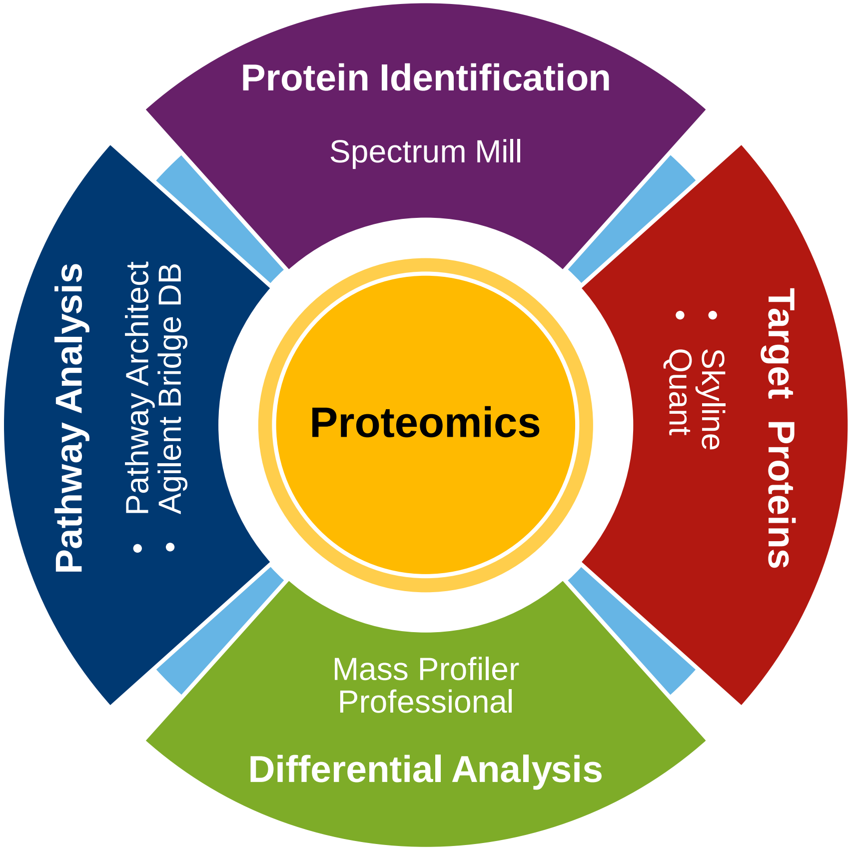
Q-TOF
6500 series

1290 Infinity
UHPLC



QQQ
6400 Series

Most Extensive Software Portfolio for Proteomics Data Analysis



Identify proteins from discovery data-dependent analysis

and/or

Target peptides for proteins of interest

Analyze data for statistically different features

Search and map annotated proteins to find interesting pathways

Visualize results on pathways

Discovery ProteomicsWorkflow

Separate & Detect

Identify or Feature Find

Statistics & Visualization

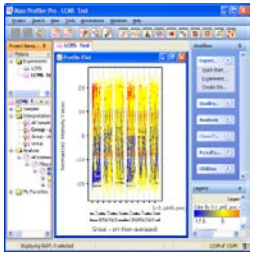
Pathway Analysis

Agilent LCMS

Q-TOF

Spectrum Mill
Skyline
Profinder

Mass Profiler Professional and Pathway Architect



Statistics and visualization

Pathway Architect

Omics data can be analyzed together in the same project

Targeted Proteomics Workflow

Separate & Detect

Target and quantitate

Statistics & Visualization

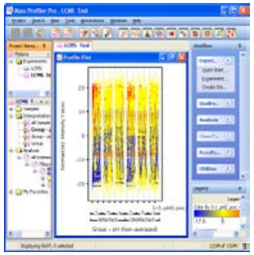
Pathway Analysis

Agilent LCMS

Q-TOF, QQQ

MassHunter Quant
Spectrum Mill
Skyline

Mass Profiler Professional and Pathway Architect

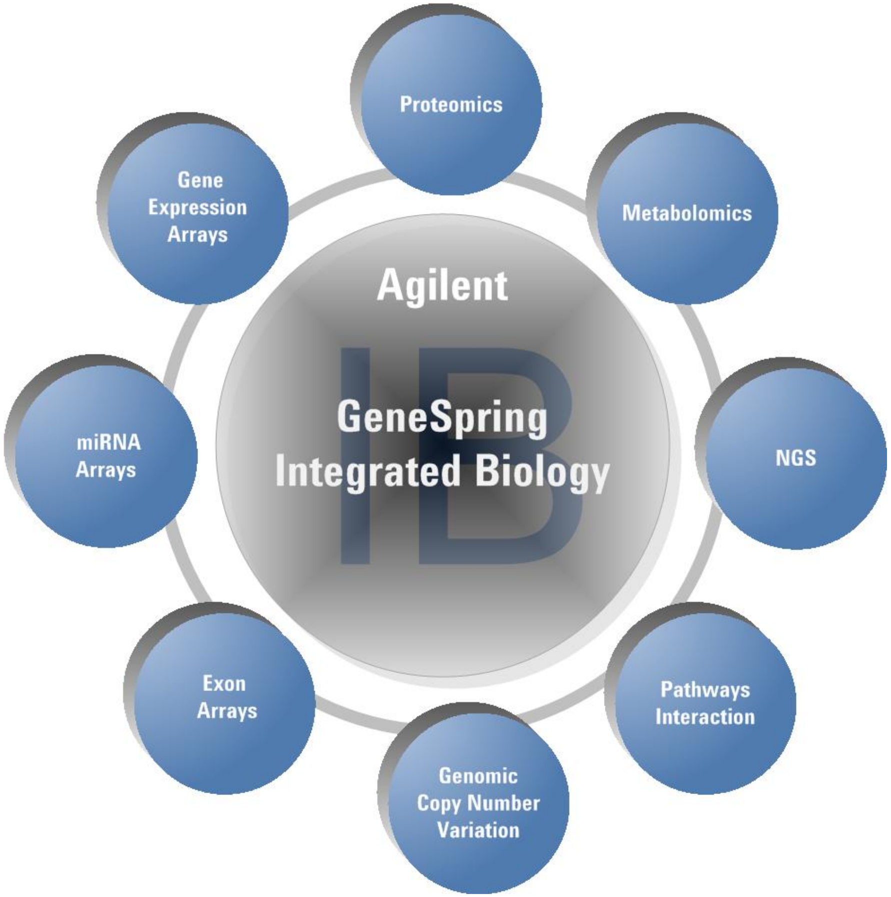


Statistics and visualization

Pathway Architect

Omics data can be analyzed together in the same project

Integrated Biology Software
GeneSpring Suite



GX
- mRNA expression
- Alternative Splicing
- microRNA expression
- Genome-wide association
- Copy Number Variation

Strand NGS
- DNA-Seq, RNA-Seq
- ChIP-Seq, Methyl-Seq,
- small RNA-Seq &
- SureSelect QC

Mass Profiler Professional
- Proteomics
- Metabolomics

Pathway Architect
- Multi-Omic Pathway Analysis
- Canonical and NL Pathways

LC/MS Data Mining

MassHunter Profinder Batch Processor

Single software for untargeted and targeted feature extraction

Designed specifically for the needs of the metabolomics user

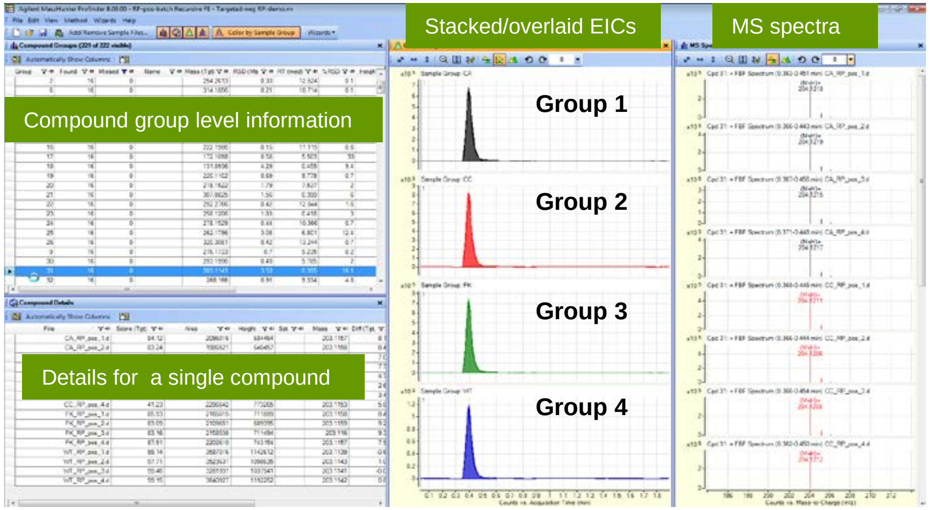
Processes many samples!

Minimizes false positive and negative results

Compound Centric: review and manual editing functionalities

Fast processing

MassHunter Profinder Main Window



Statistics and Pathway Analysis

Mass Profiler Professional (MPP)

Designed for MS data

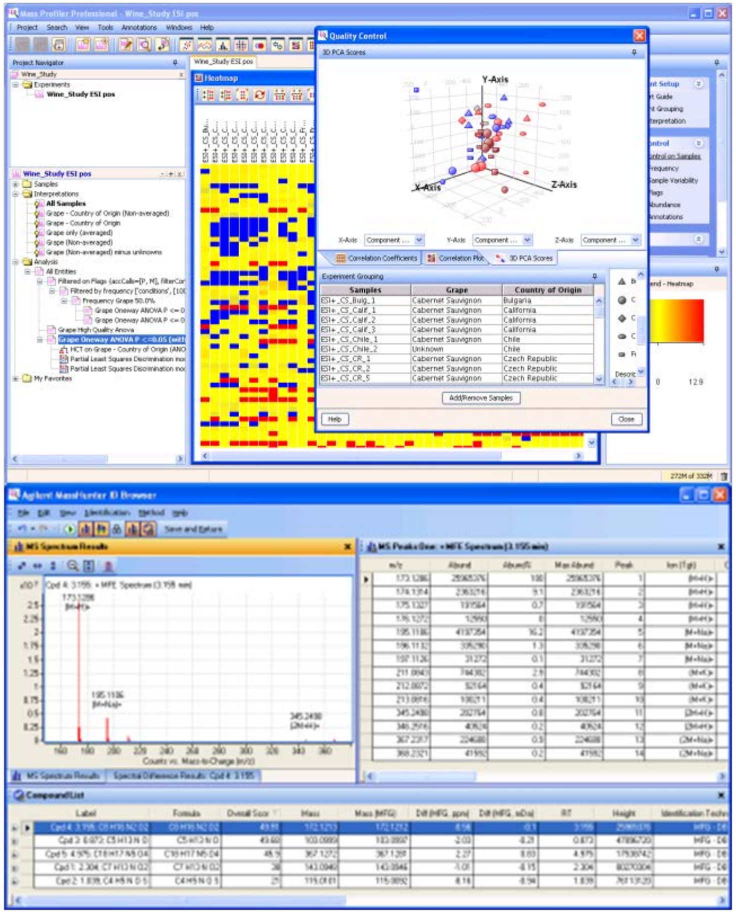
Performs many types of statistical analysis

- ANOVA, clustering, PCA, class prediction tools, correlation

ID Browser for compound annotation and identification

Export MS/MS target list

Pathway Architect for biological context



Samples	Grape	Country of Origin
ESI+_CS_Bulg_1	Cabernet Sauvignon	Bulgaria
ESI+_CS_Calif_1	Cabernet Sauvignon	California
ESI+_CS_Calif_2	Cabernet Sauvignon	California
ESI+_CS_Calif_3	Cabernet Sauvignon	California
ESI+_CS_Chile_1	Cabernet Sauvignon	Chile
ESI+_CS_Chile_2	Unknown	Chile
ESI+_CS_CR_1	Cabernet Sauvignon	Czech Republic
ESI+_CS_CR_2	Cabernet Sauvignon	Czech Republic
ESI+_CS_CR_5	Cabernet Sauvignon	Czech Republic

What’s Coming for Integrated Biology Workflows?

Peptide-level proteomics capability

- New output file format for Skyline and Spectrum Mill
  - Detailed peptide information exported for proteins
  - Agilent only capability

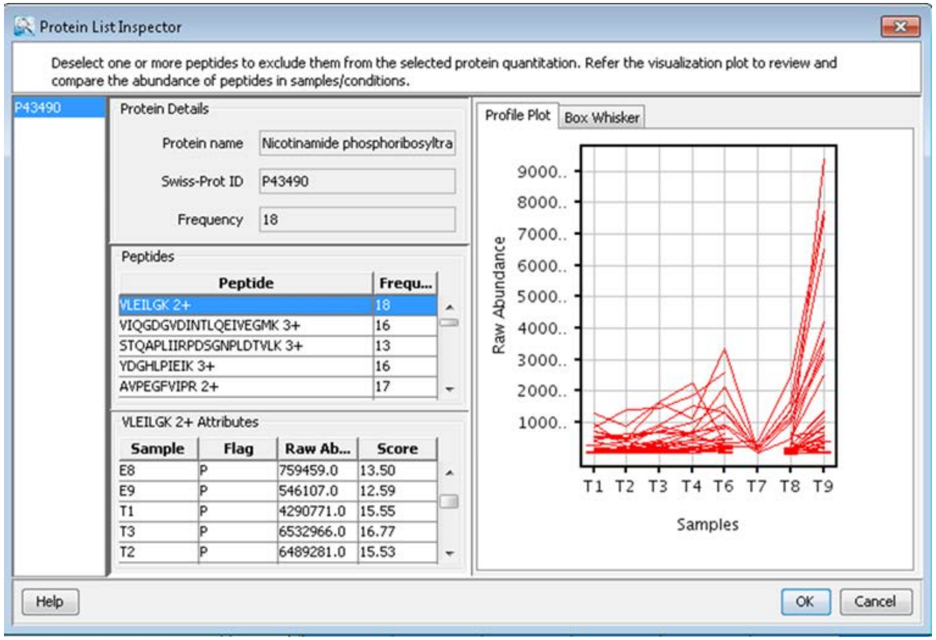
MassProfiler Professional 14.0

- Supports new peptide-level export
- Enables peptide-level queries, filters and visualization

Profinder 8.0

- Faster (multi-threaded)
- GC/MS support

Peptide-Level Export Empowers Deeper Analysis



Deselect one or more peptides to exclude them from the selected protein quantitation. Refer the visualization plot to review and compare the abundance of peptides in samples/conditions.

Protein name: Nicotinamide phosphoribosyltra
Swiss-Prot ID: P43490
Frequency: 18

Peptide	Frequ...
VLEILGK 2+	18
VIQGDGVDINTLQEIVEGMK 3+	16
STQAPLIIRPDSGNPLDTVLK 3+	13
YDGHLPIEIK 3+	16
AVPEGFVIPR 2+	17

Sample	Flag	Raw Ab...	Score
E8	P	759459.0	13.50
E9	P	546107.0	12.59
T1	P	4290771.0	15.55
T3	P	6532966.0	16.77
T2	P	6489281.0	15.53

Filter on peptides by

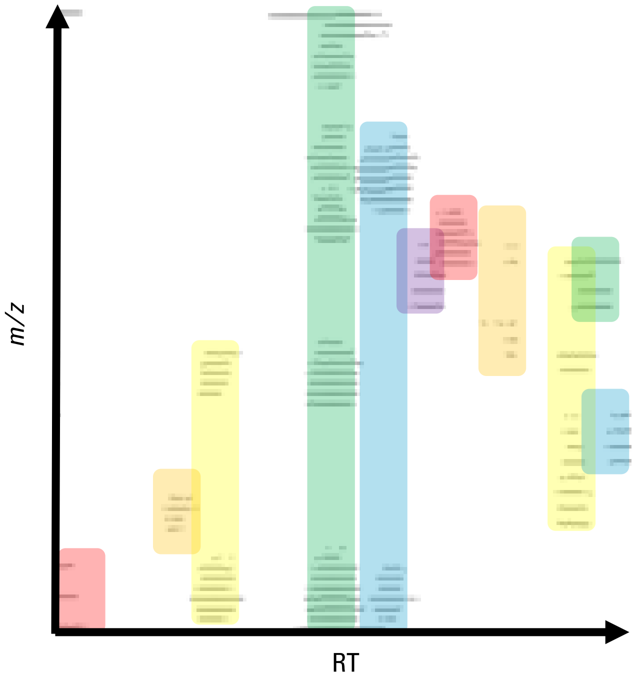
- Sequence
- PTMs
- Reproducibility

Statistical and correlation analysis at the peptide level

Visualize peptide results

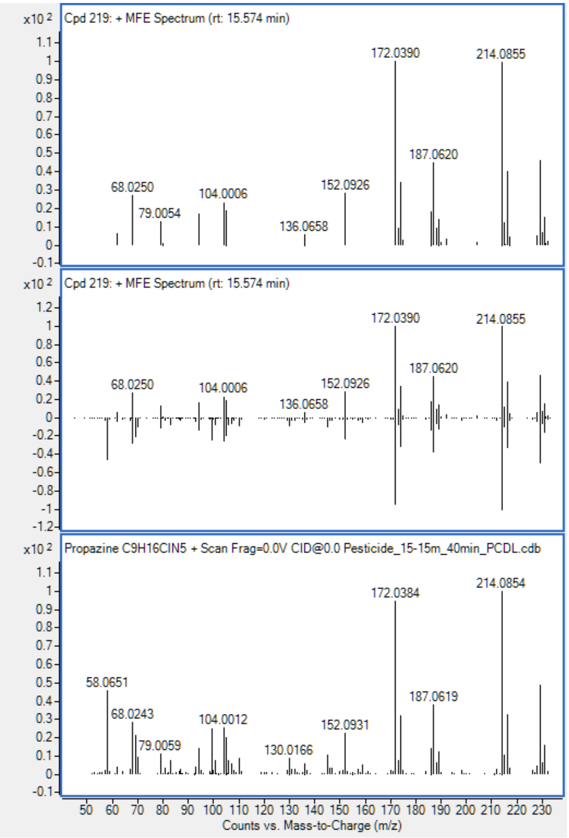
- For proteins across samples
- On pathways

Profinder 8.0: Scalable Performance and GC/MS Support



GC/MS by MFE:

- Signals corresponding to different ions elute at the same retention time
- Each colored box = a unique co-elution group = a compound



MFE Spectrum

Spectral match 98.54

Library Spectrum

Library matching of MFE component spectrum

Brain Tumor Study: Background

Immunohistochemistry (protein) is the most commonly used method for patient sample evaluation

Using genomic signatures, glioblastoma multiforme (GBM) tumors have been classified into the following subtypes:

- Classical
- Mesenchymal
- Neural
- Proneural

Brain Tumor Study: Strategy

Research questions

- Can we separate tumors profiled into subgroups using genomic signatures?
- Can we reduce the number of genes in signature without losing classifier performance?

Approach

- Use cancer genome atlas (TCGA) genomic signatures capable of classifying GBM subtypes
- Acquire discovery proteomics data from grade IV GBM tumors without corresponding genomic information as well as control brain samples collected from epilepsy surgeries
- Leverage proteomics results to improve genomics classification

Brain Tumor Study: Workflow

Genomics

- Use hierarchical clustering of mRNA profiles to confirm genomic signatures
- Assess data quality and bias using meta data framework analysis
- Convert gene id to protein accession number

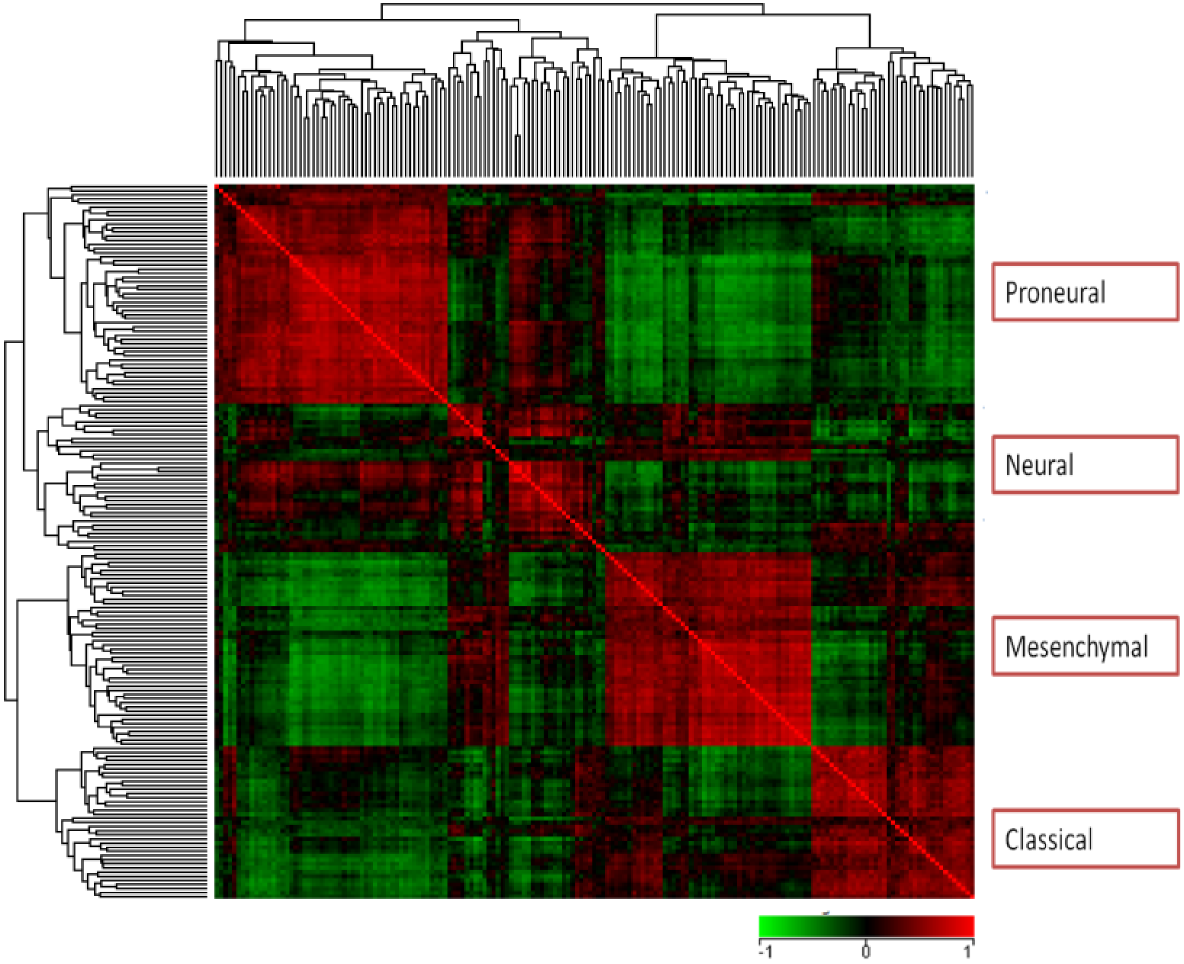
Proteomics

- Use PCA of discovery proteomics data to confirm tumor vs. control could be distinguished
- Identify a set of differential proteins for the tumor samples
- Use sample-sample correlation to cluster for QC and subtyping in proteomics experiment

Multi-omics

- Perform Venn analysis of differential proteins and genes
- Find reduced set of genes for classifying tumor sub-types

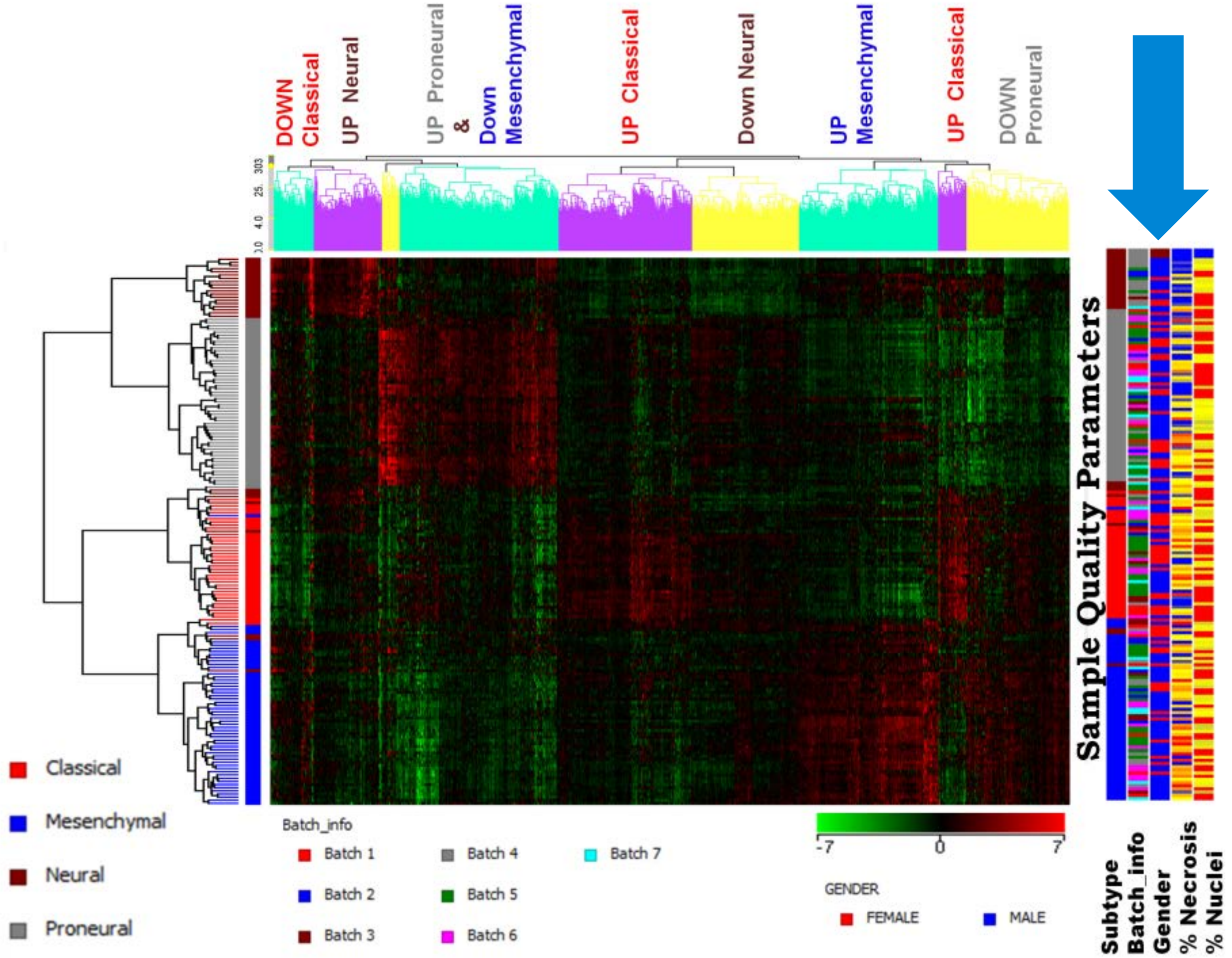
Brain Tumor Study: Hierarchical Clustering of Genomic Signatures



Hierarchical clustering of 840 mRNA profiles from 173 GBM tumors

The four GBM subtypes were re-created

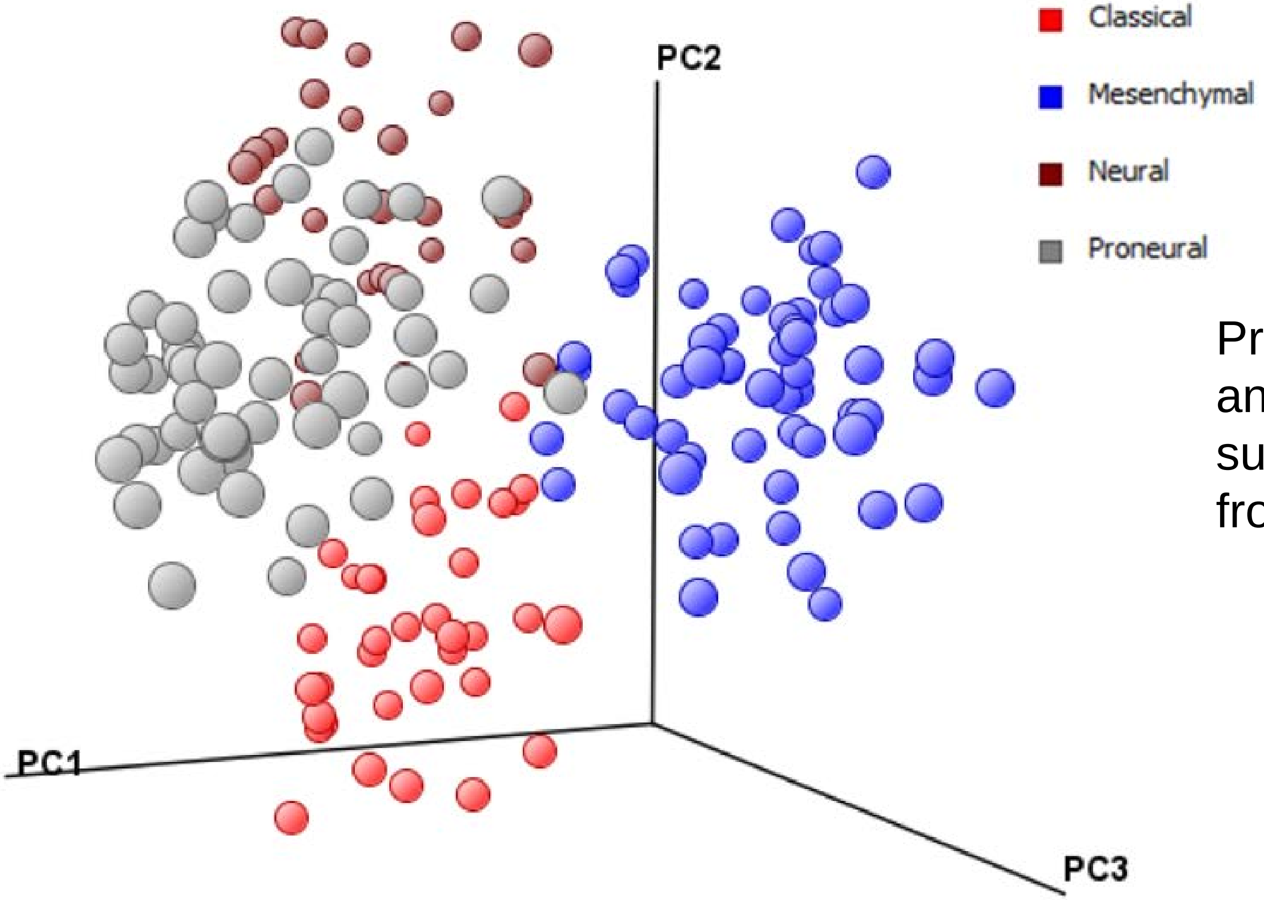
Brain Tumor Study: Metadata Analysis of Genomic Signatures



Metadata obtained from TCGA was imported into the project

Metadata framework analysis of batch and sex was used to assess batch affects or bias

Brain Tumor Study: Principal Component Analysis of Genomic Signatures

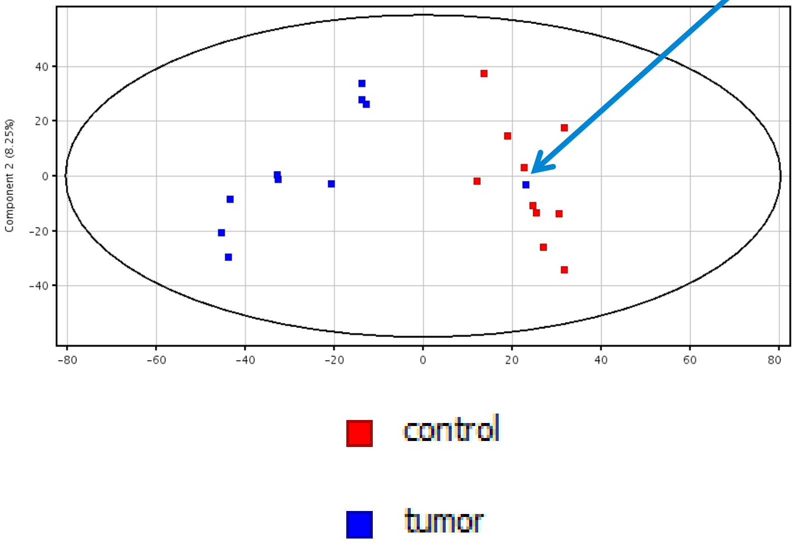


Principal component analysis of confirms subtypes in GBM samples from TCGA

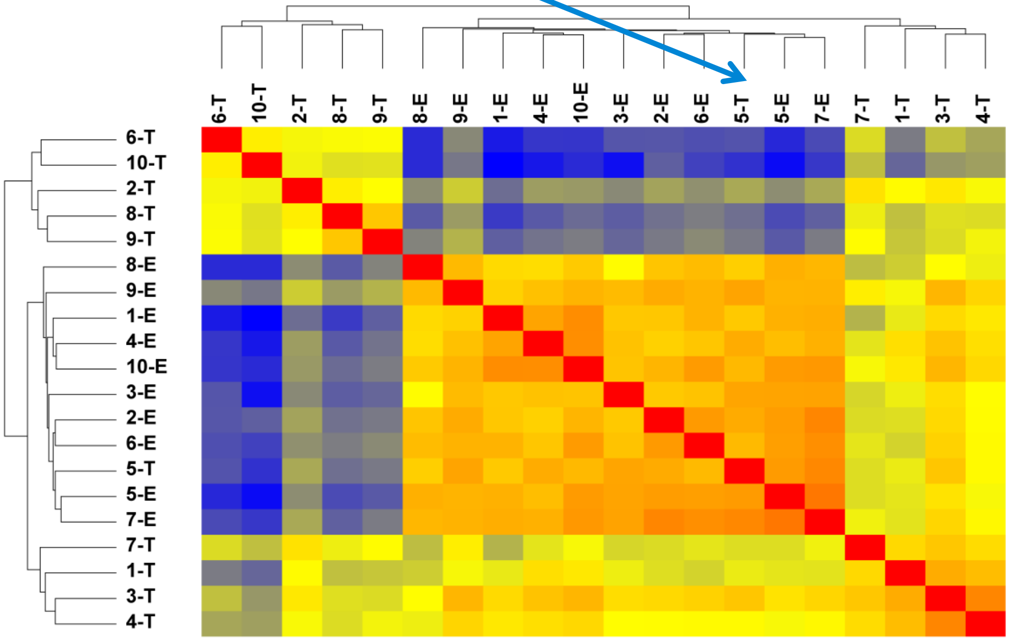
Brain Tumor Study: Quality Control of Discovery Proteomics Data

Sample 5-T

Principle Component Analysis Plot



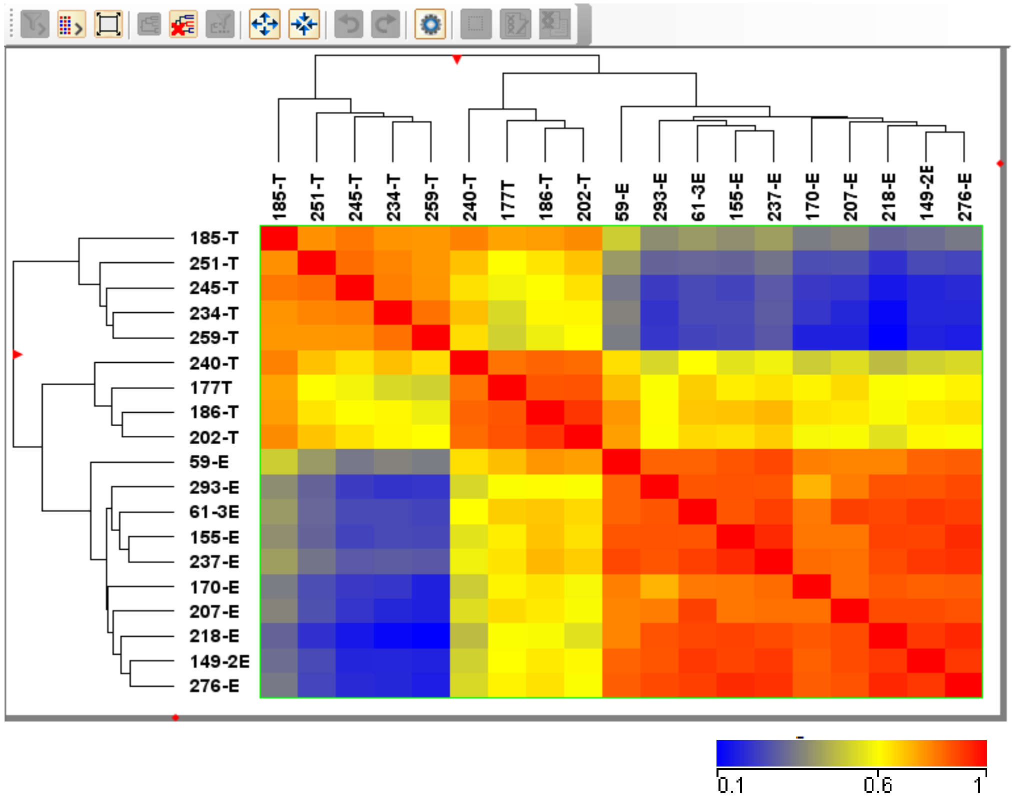
Sample Clustered Correlation Plot

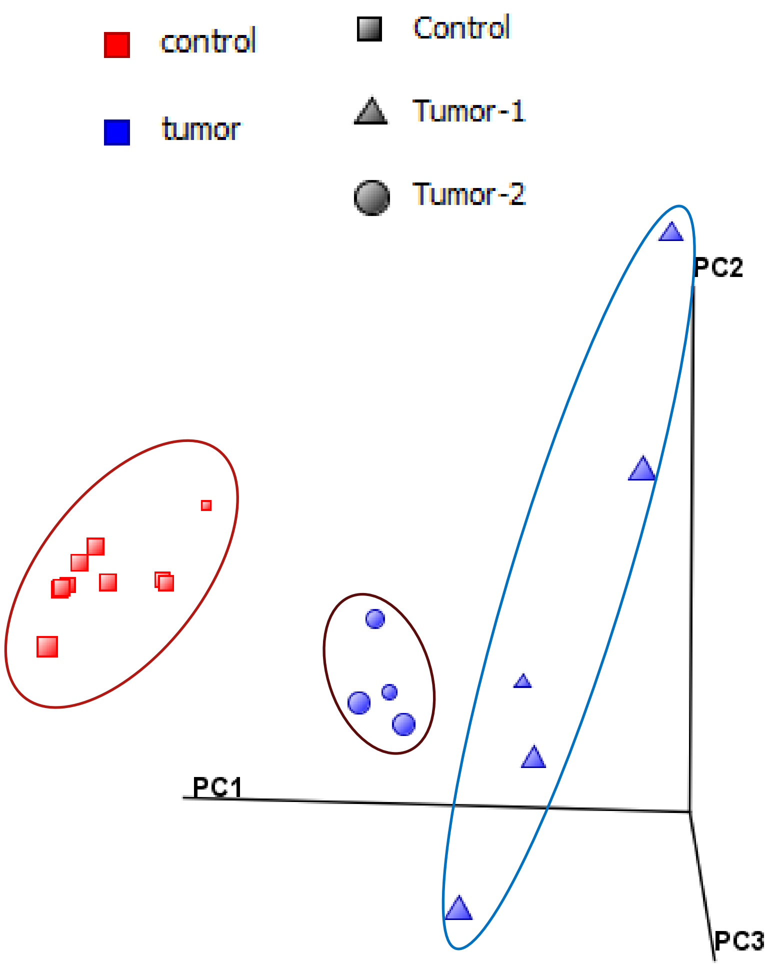


T – Tumor samples E – Epilepsy samples

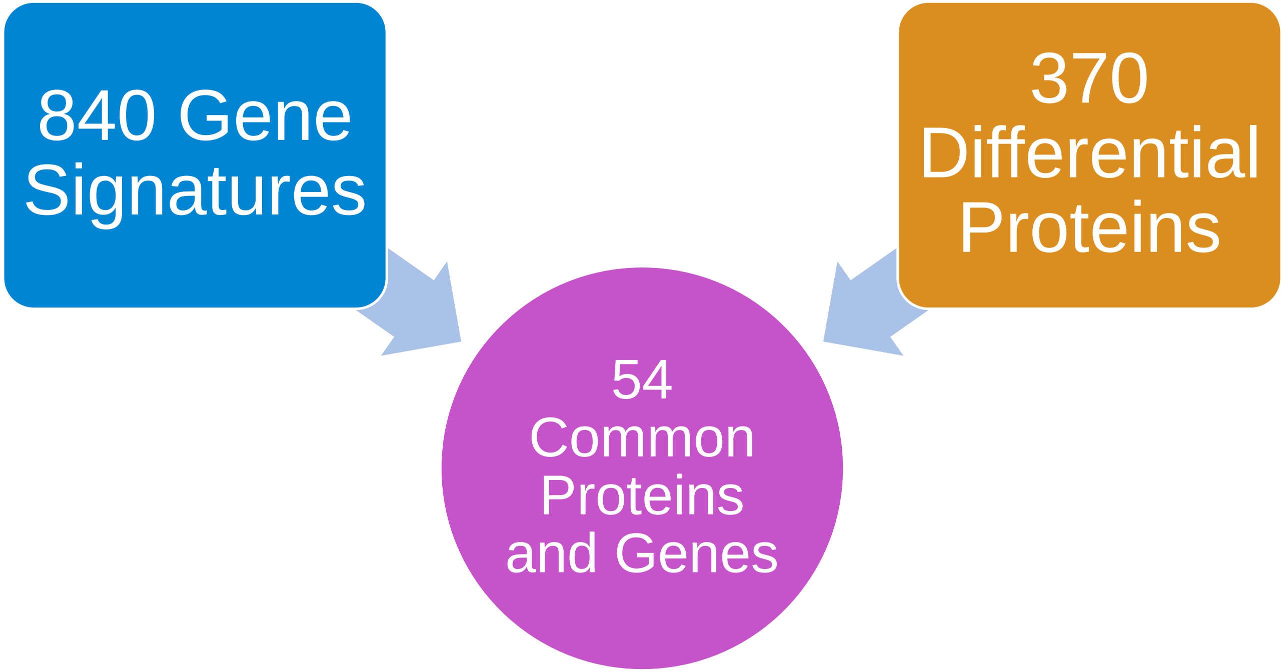
Brain Tumor Study: Sample-sample Correlation and PCA of Discovery Proteomics Data

Tumor subgroups demonstrated, using **370 differential proteins,** in both PCA and sample correlation showing the sample heterogeneity





Brain Tumor Study: Venn Analysis of Genomic and Proteomic Data Set



840 Gene Signatures

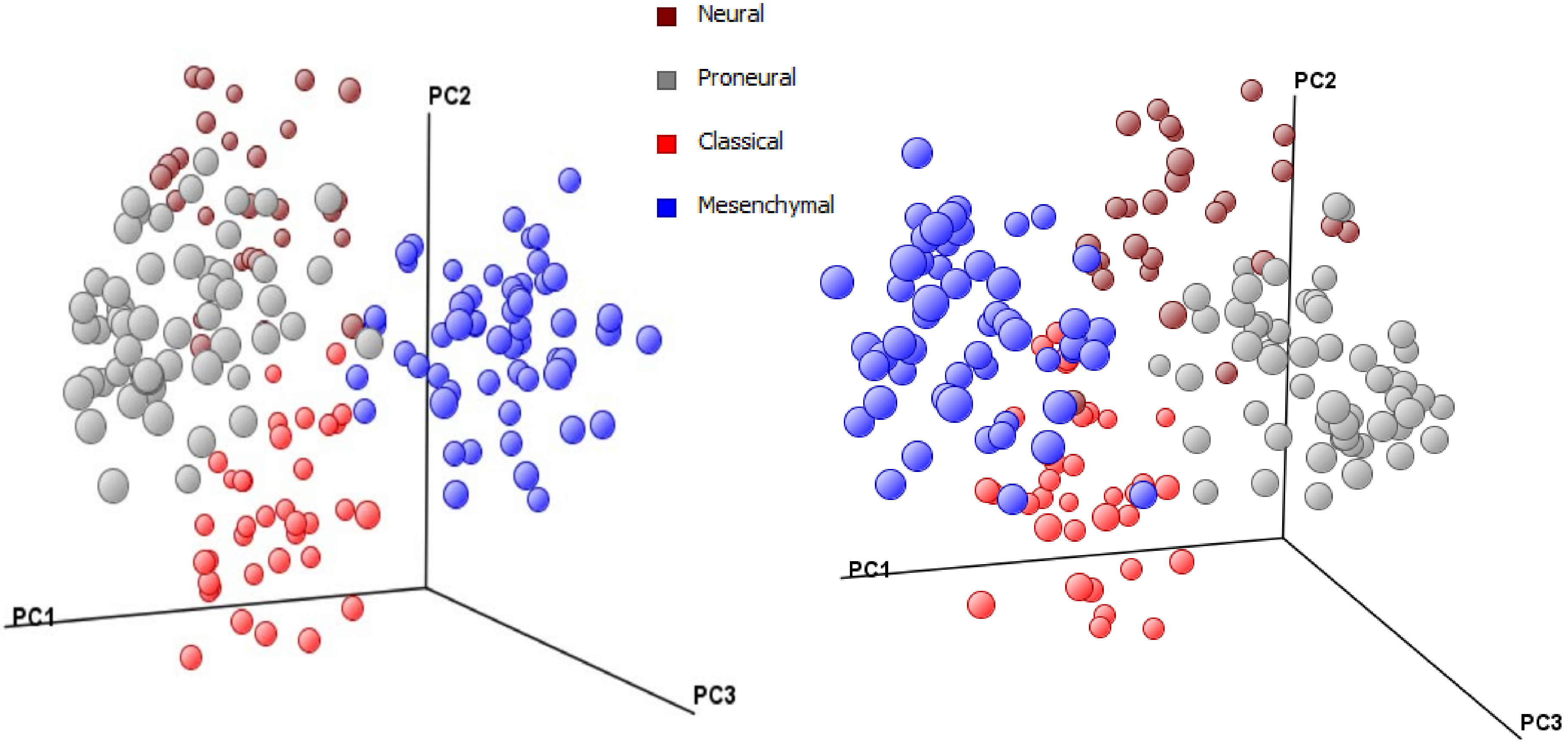
370 Differential Proteins

54 Common Proteins and Genes

Brain Tumor Study: Comparison of 54 Genes Identified Using Proteomics Integration to Original Signature

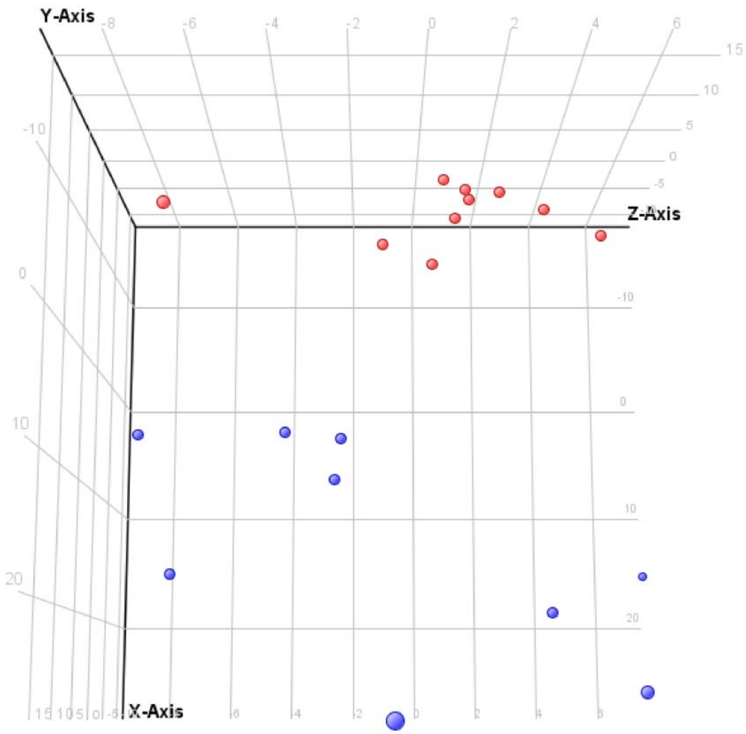
Original Genomic Signature

Genomic-Proteomic Signature

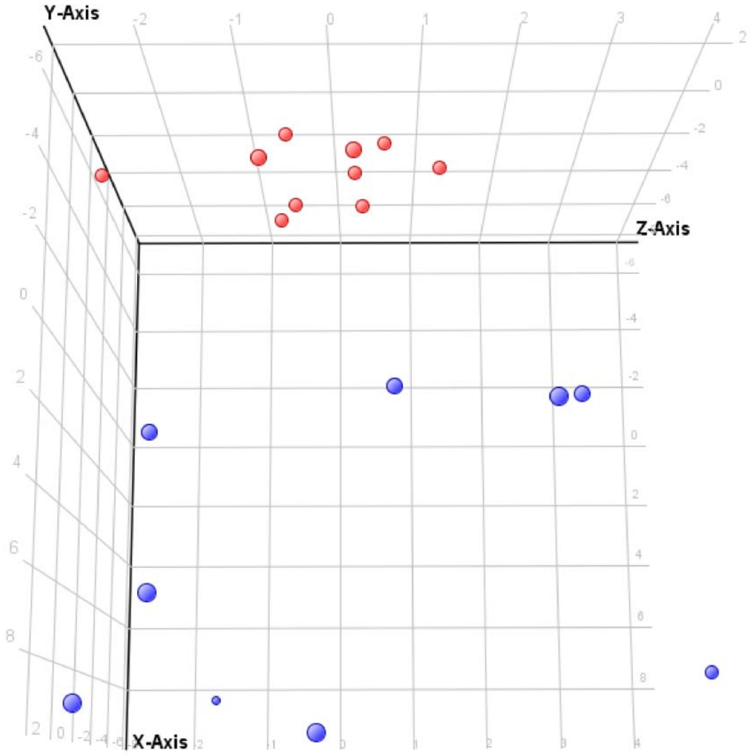


Brain Tumor Study: Comparison of Original 370 Proteins to 54 Proteins Identified Using Integration

Original 370 Protein List



54 Protein List after Integration



Color by Tissue type

🟥 control

🟦 tumor

Integrated Biology Workflow Enhancements From Agilent to Accelerate Your Research

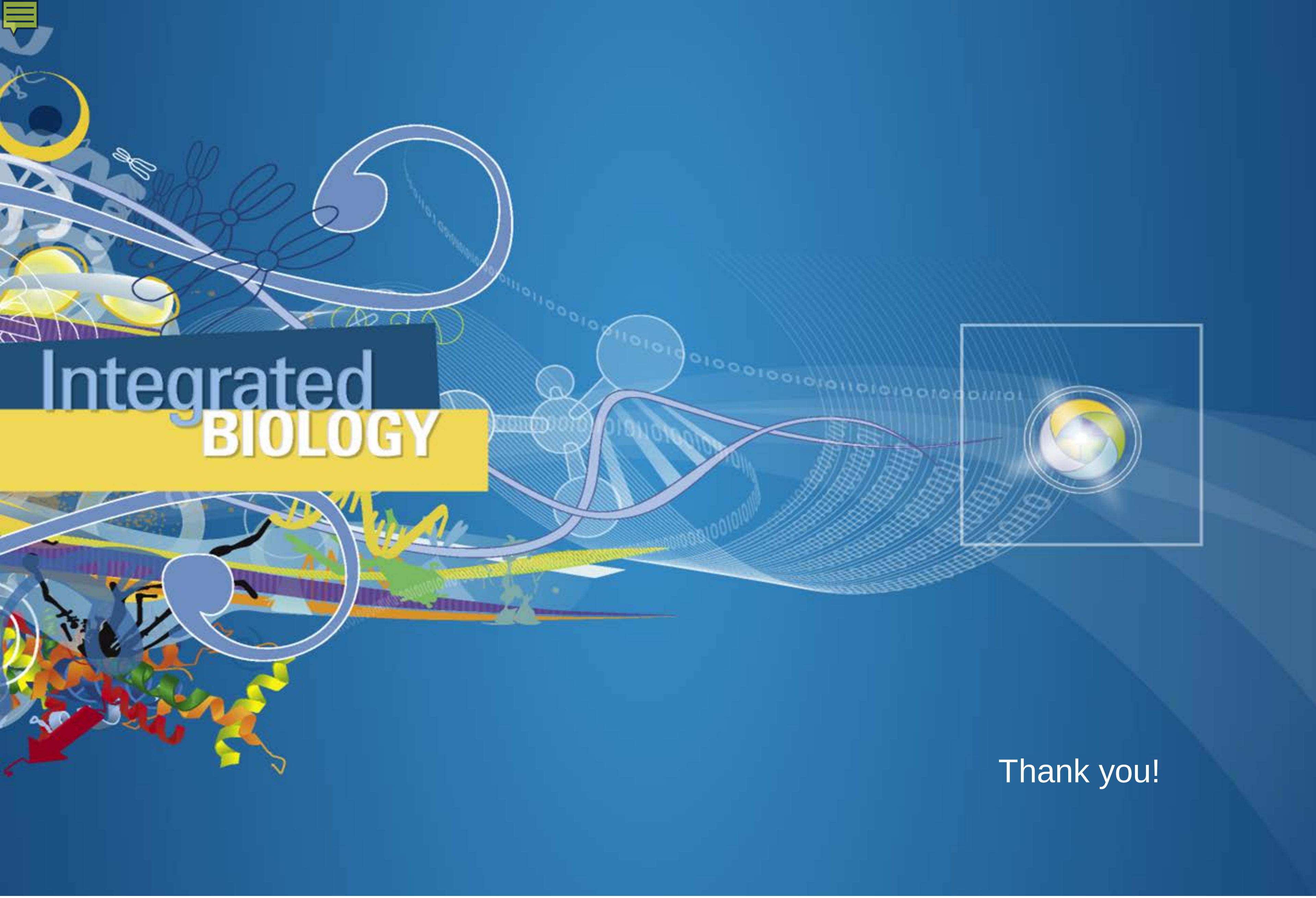
Comprehensive -omics workflows for metabolomics, proteomics, and genomics

Multi-variate software tools for integrated biology workflows

Pathway Architect provides data visualization directly on pathways

Peptide-level export and analysis coming in the Fall!

Profinder for GC/MS and enhanced speed coming in the Fall!



Thank you!