University of Michigan
Center for Translational Pathology

Metabolomics: Techniques and Applications

ABRF

Sacramento, CA

March 23, 2010

Overview

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- Metabolomics Definitions
- Representative Project
  - Sarcosine, a prostatic cancer biomarker
- Technical overview/ How we do it.

Unbiased Metabolomics

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- Seeks to identify all chemical entities on a biological sample and comment on their relevance.
  - All chemical entities are characterized irrespective of their identity, and indeed many are unknown.
  - Statistical relevance does not require chemical identity
  - Biochemical relevance does require chemical identity

Metabolomics

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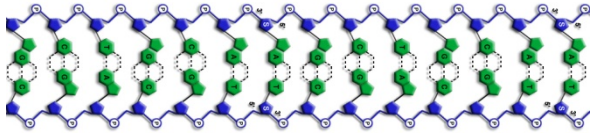
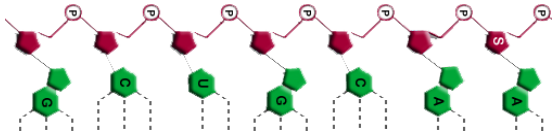
DNA



Genomics – 25,000 Genes

RNA

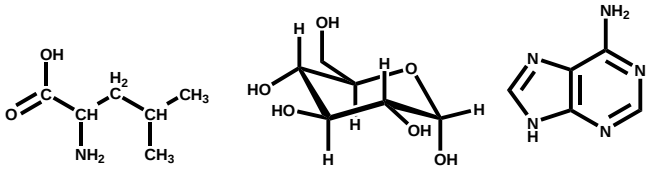


Transcriptomics – 100,000 Transcripts

Protein

Proteomics – 1,000,000 Proteins

Biochemicals
(Metabolites)



Metabolomics – 1,800 Compounds

Chemical Ecology of a Human Cell

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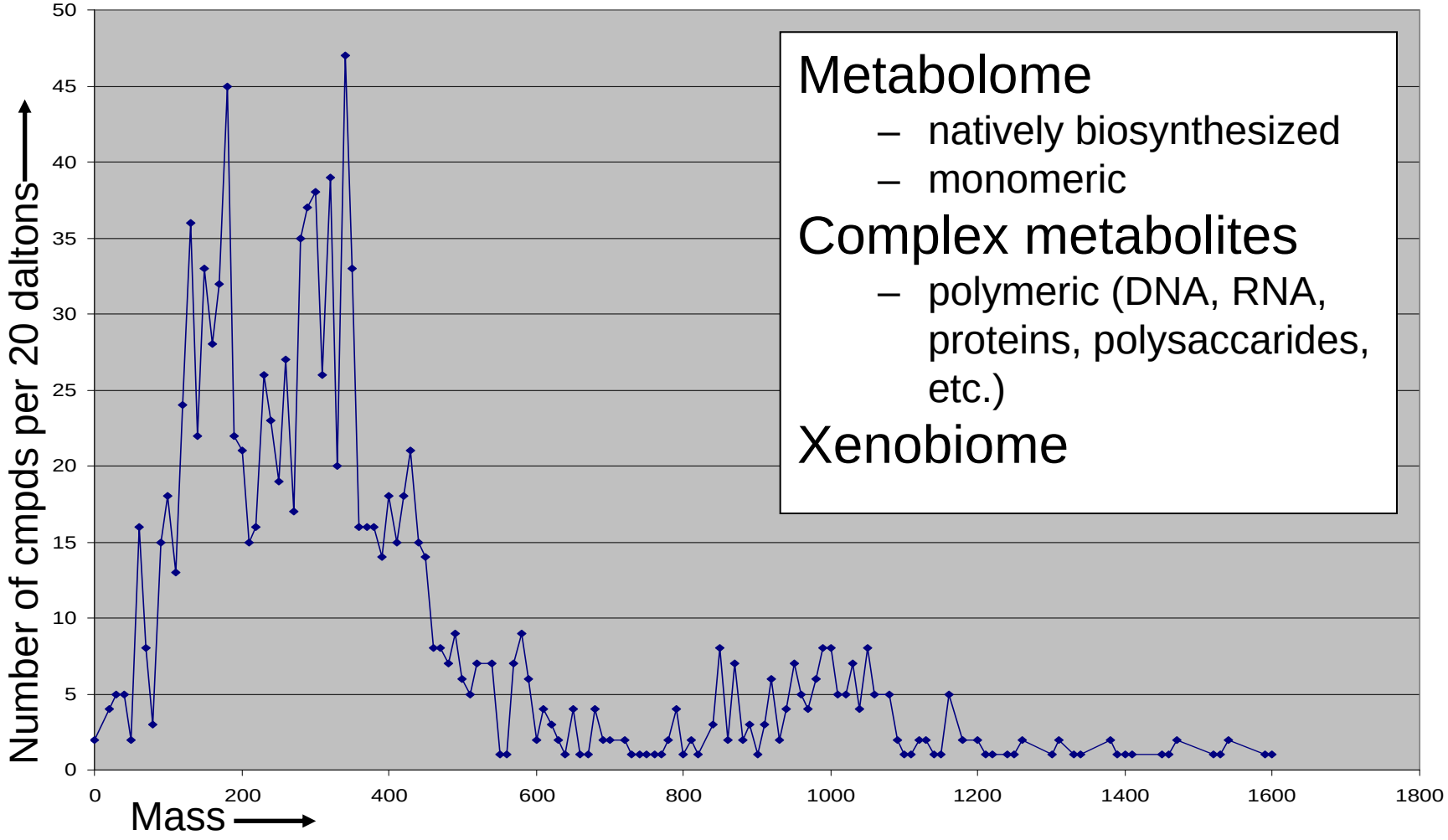

Metabolome

- natively biosynthesized
- monomeric

Complex metabolites

- polymeric (DNA, RNA, proteins, polysaccarides, etc.)

Xenobiome

Experimental design

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	Prostate Tissues (n=20/class) Benign Low Grade PCA High Grade PCA	Plasma (n=50/class) Biopsy Negative Controls Low Grade PCA High Grade PCA	Post-DRE Urine (n=50/class) Biopsy Negative Controls Low Grade PCA High Grade PCA
Genomics	Transcriptomic/cDNA arrays SNP Chips microRNAs ETS gene fusion analysis (QRT-PCR, FISH) Differential Methylation Hybridization	Will not be done for this biospecimen	ETS gene fusion analysis (QRT-PCR, FISH) QRT-PCR of other candidates
Proteomics	High-throughput immunoblot analysis 2-D liquid phase fractionation/mass spec	Autoantibody Signatures ELISAs	Immunoblot Analysis ELISAs
Metabolomics	Full Platform	Full Platform	Full Platform

Results

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- Using a combination of high throughput liquid and gas chromatography-based mass spectrometry, we profiled more than 1265 metabolites across 262 clinical samples related to prostate cancer (tissue, urine, and plasma).

*Funded by the Early Detection Research Network (EDRN) /NCI

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Chemical Variation

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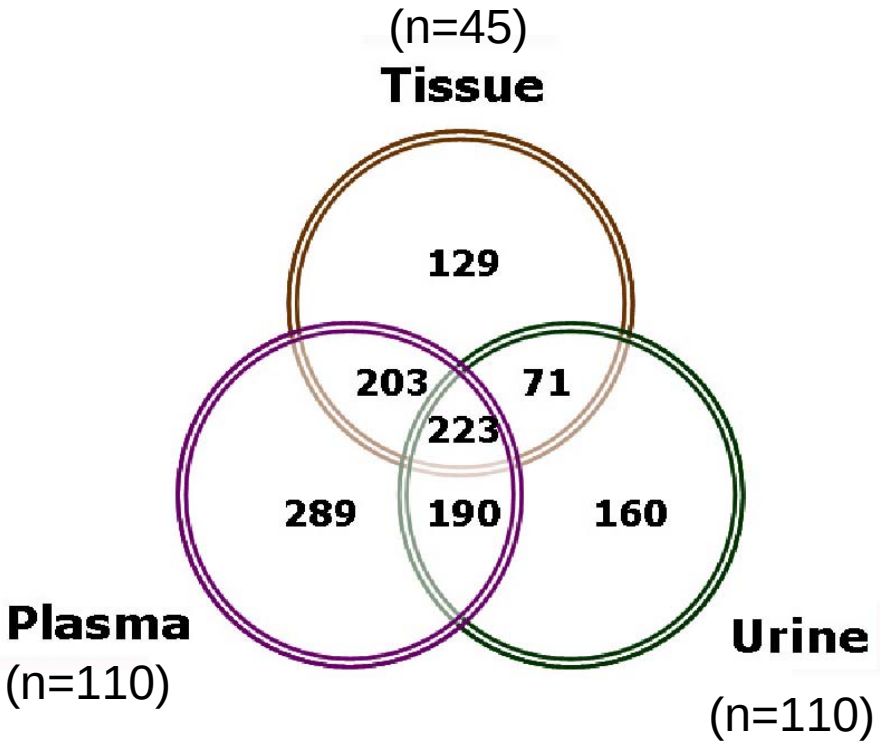
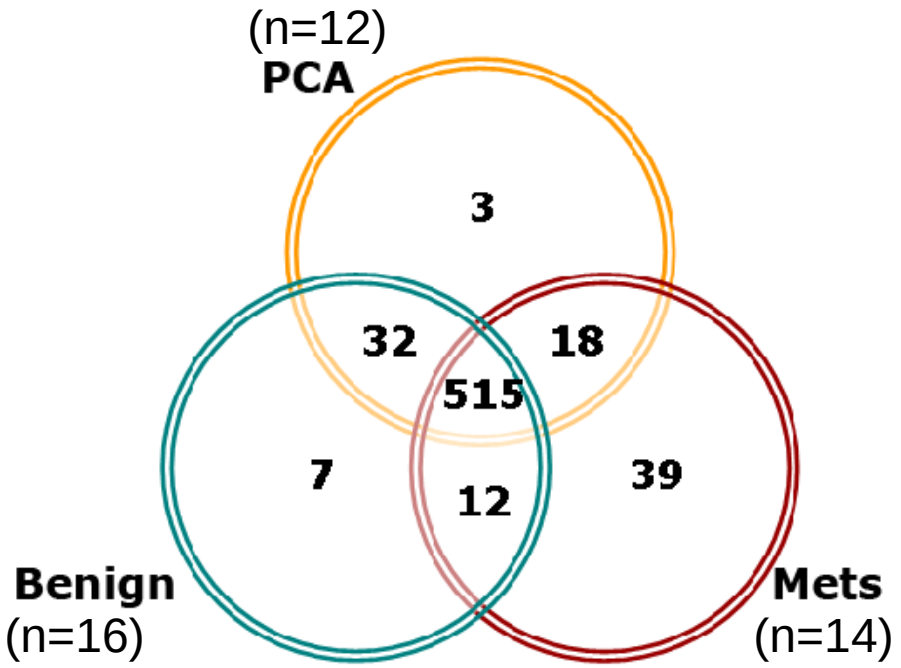
Total = 1265 compounds



Total = 626 compounds



Statistical Differences

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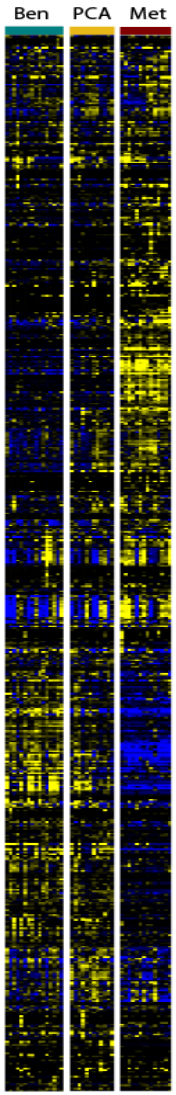

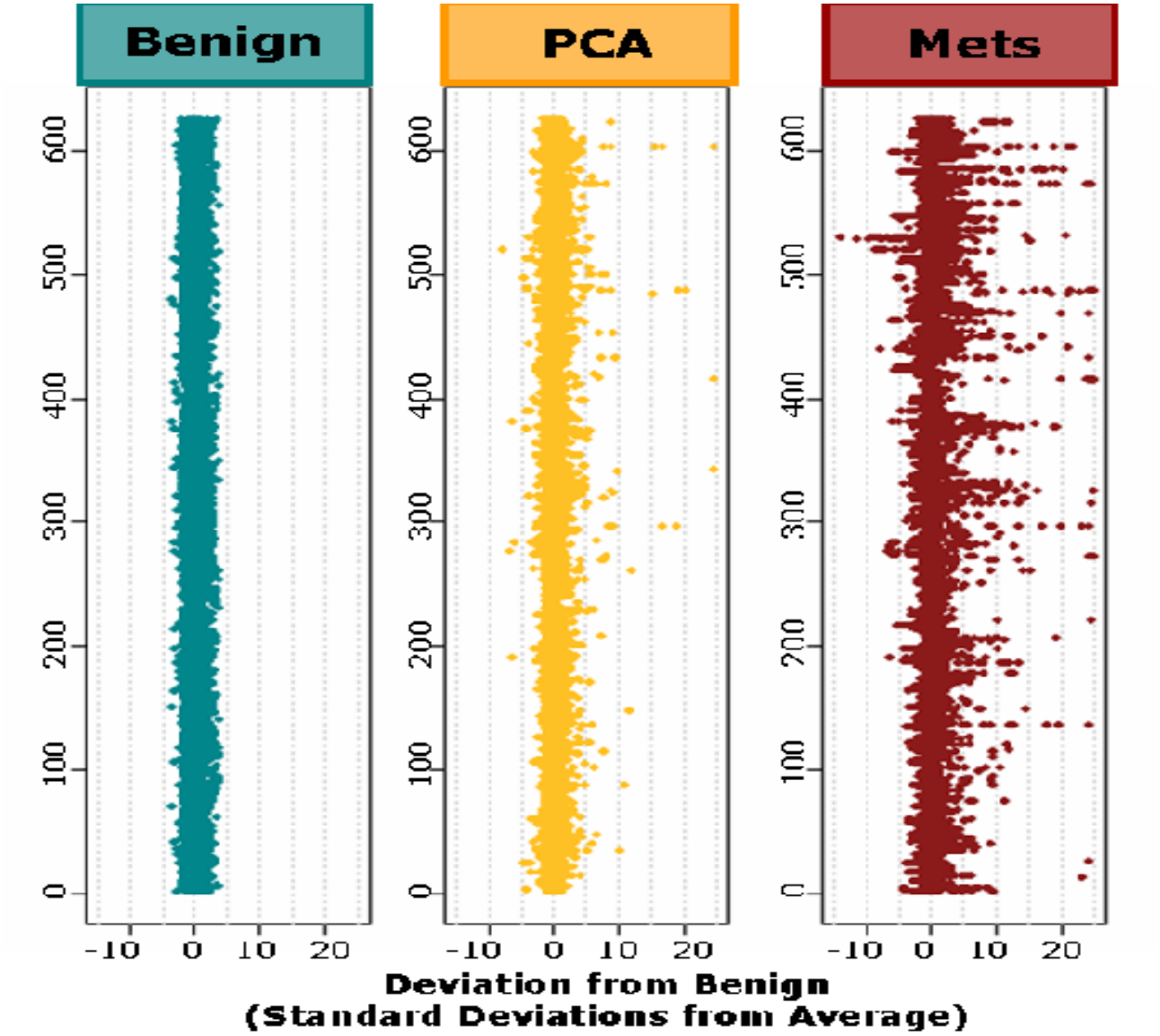

Principle Components Analysis

(all compounds / total data set)

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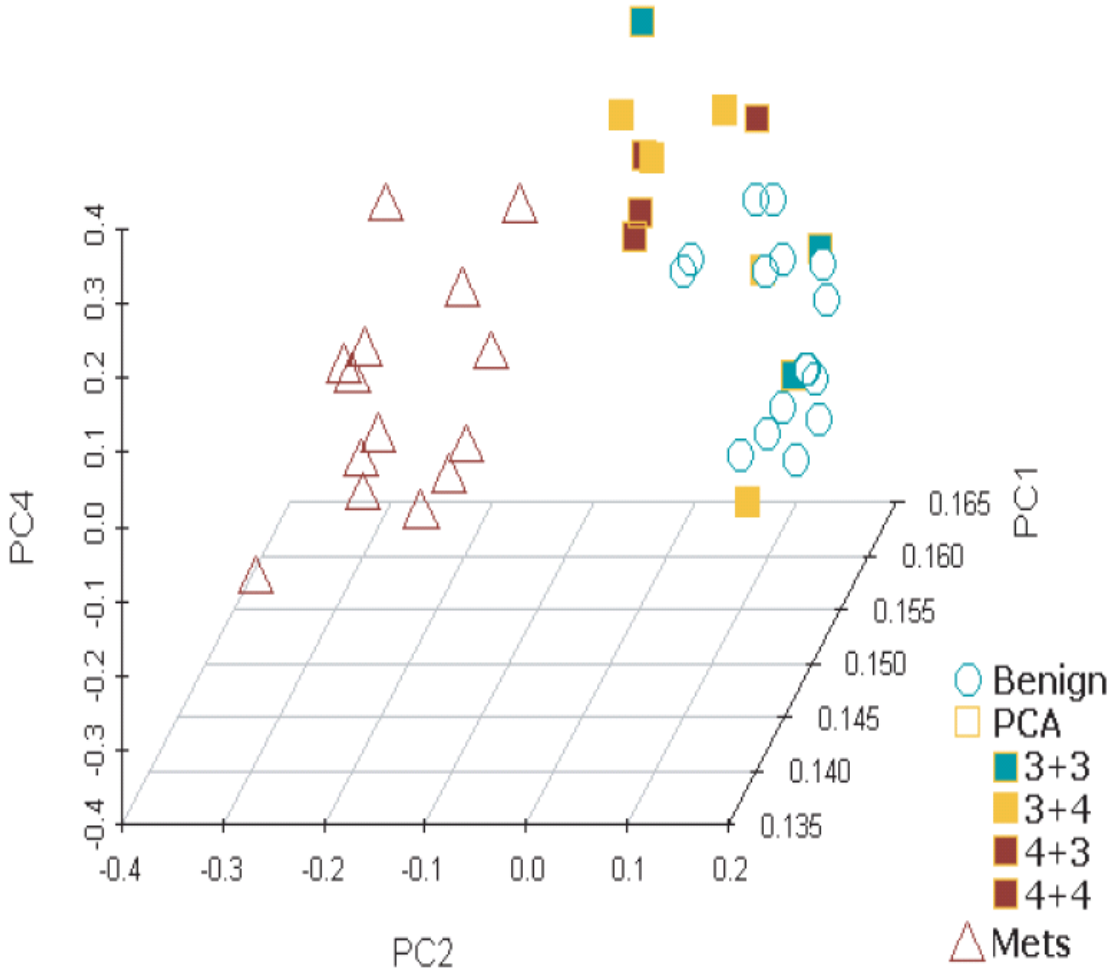

Metabolic Alterations

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ALANINE AND ASPARTATE METABOLISM

ARGININE AND PROLINE METABOLISM

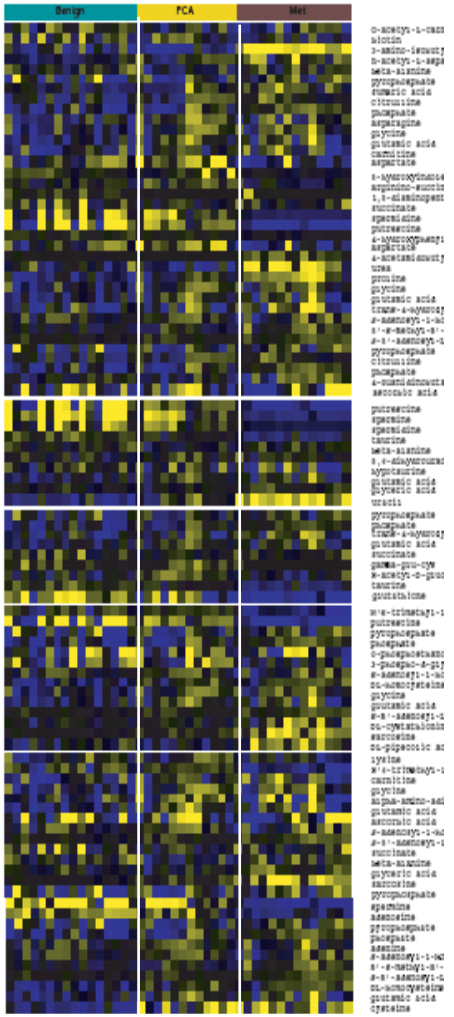
BETA ALANINIE METABOLISM

GLUTAMATE METABOLISM

GLYCINE,THREONINE,SERINE METABOLISM

LYSINE METABOLISM

METHIONINE METABOLISM



NICOTINATE METABOLISM

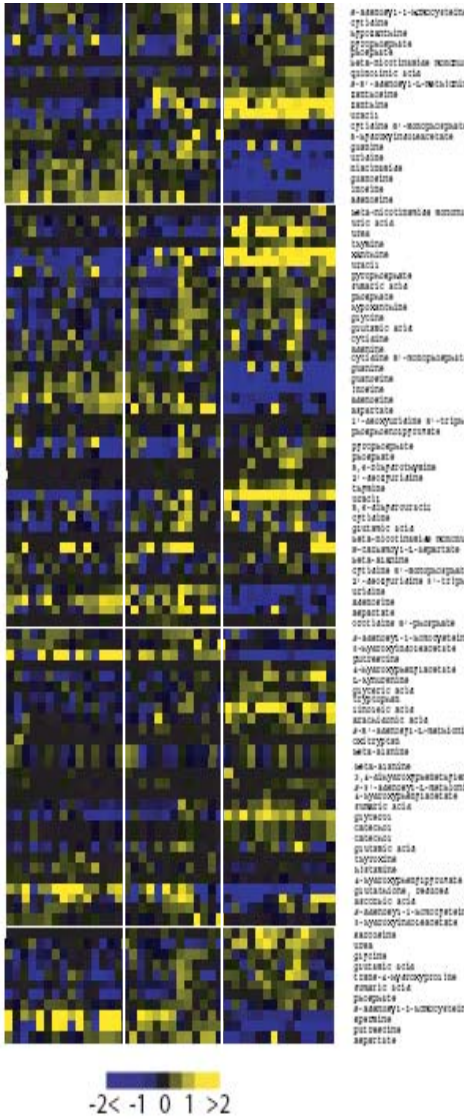
PURINE METABOLISM

PYRIMIDINE METABOLISM

TRYTOPHAN METABOLISM

TYROSINE METABOLISM

UREA CYCLE



Molecular Concepts Mapping

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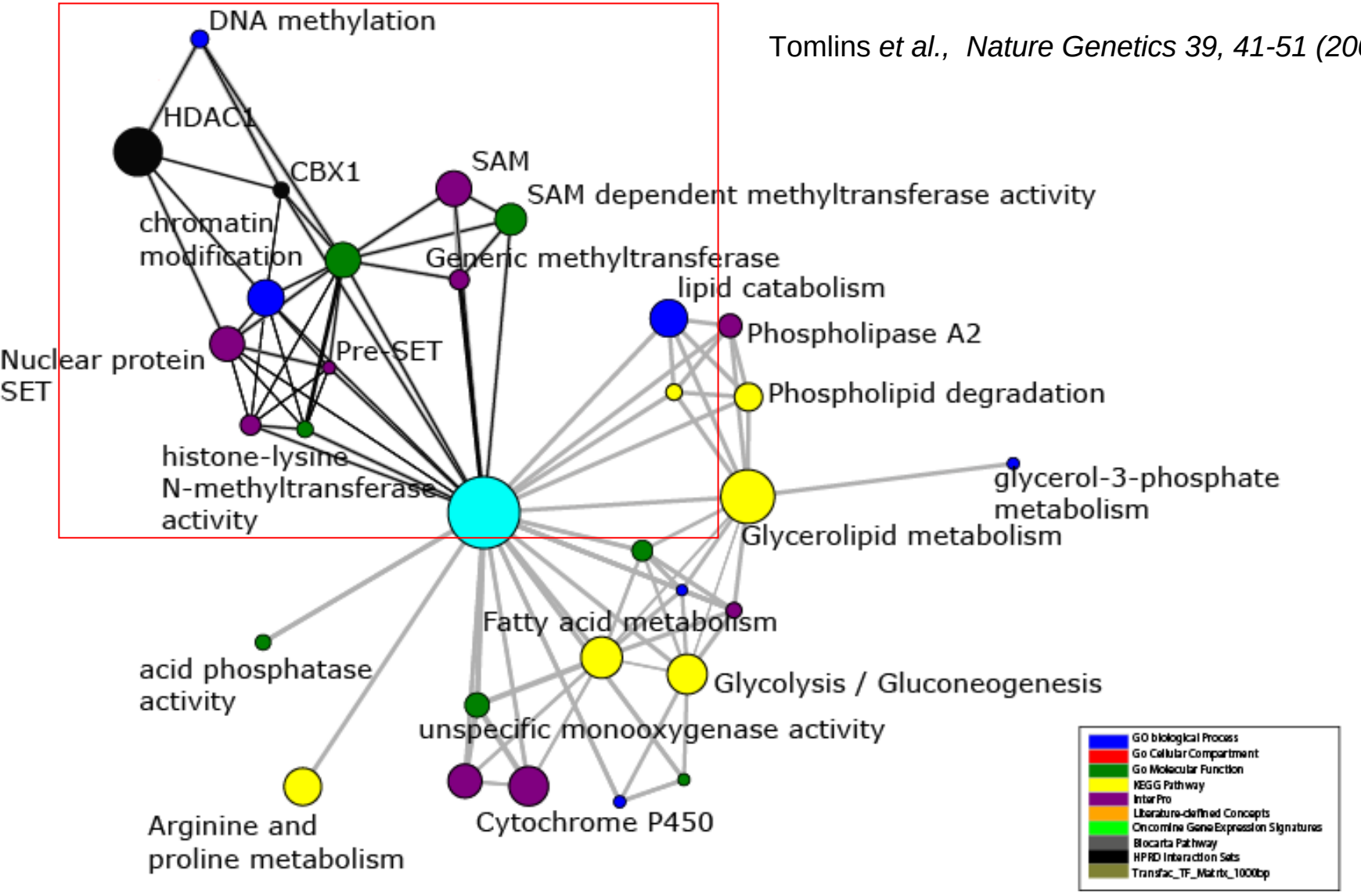
Tomlins *et al.*, *Nature Genetics 39, 41-51 (2007)*



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METABOLOMIC SIGNATURE OF METASTATIC SAMPLES COMPARED TO PCa

FATTY ACID INTERMEDIATES

KETONE BODIES

CoA PRECURSORS

S-ADENOSYL METHIONINE

AMINO ACIDS

NUCLEIC ACID PRECURSORS

Sarcosine: A Methylated derivative of Glycine is elevated in Metastatic Prostate cancer

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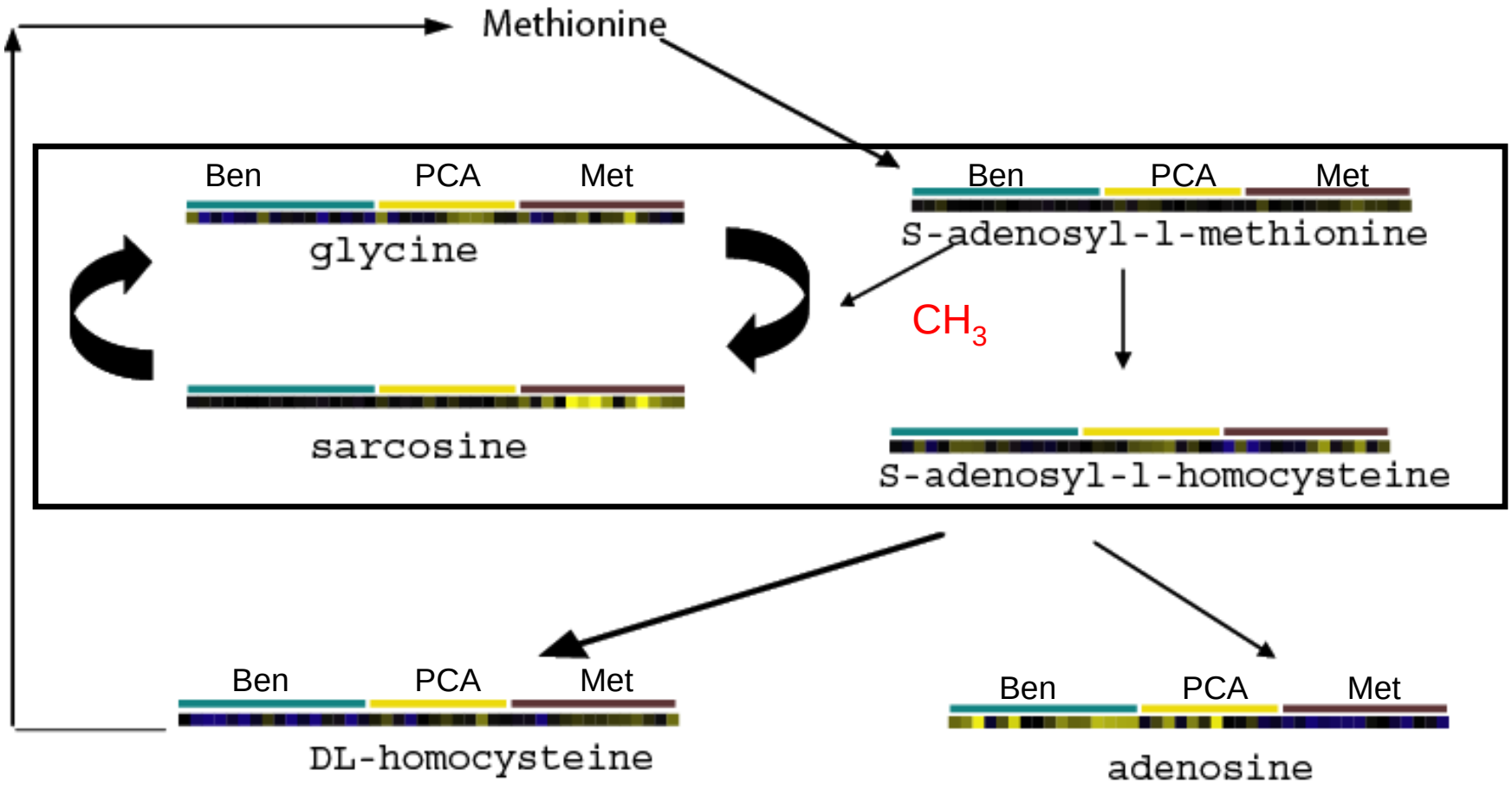

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Validation of Sarcosine

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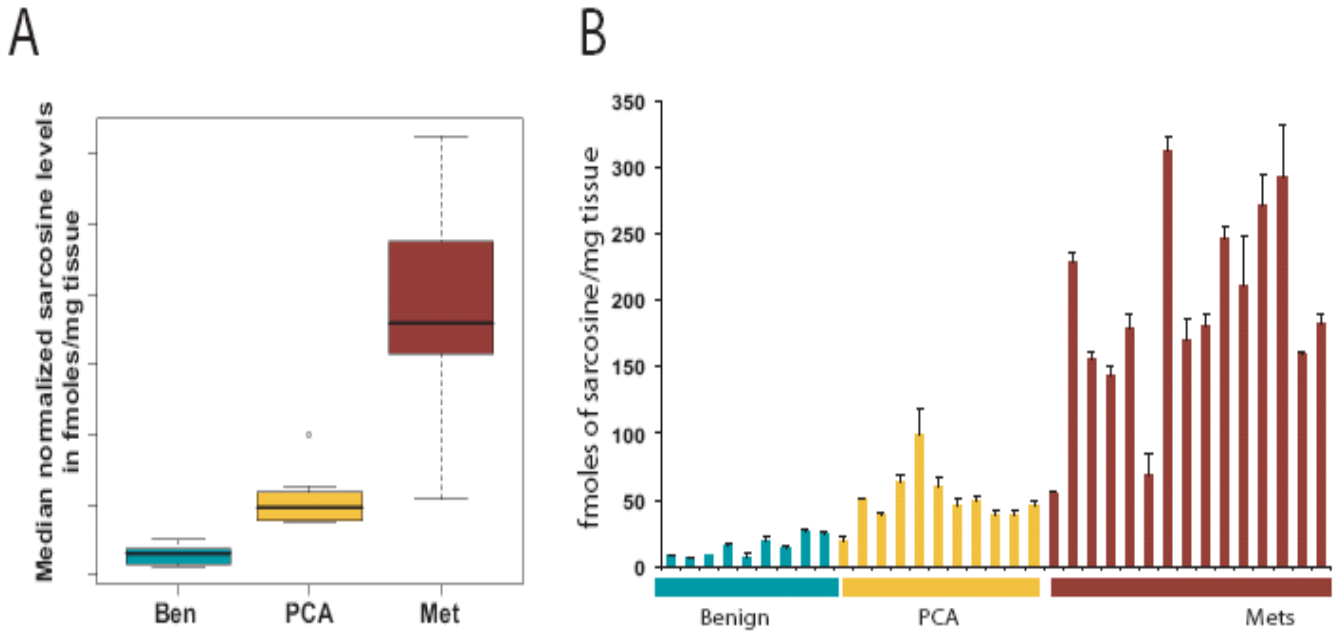

Using an Independent GC/MS (“targeted”) Assay
<u>on the same sample set.</u>

Revalidation of Sarcosine

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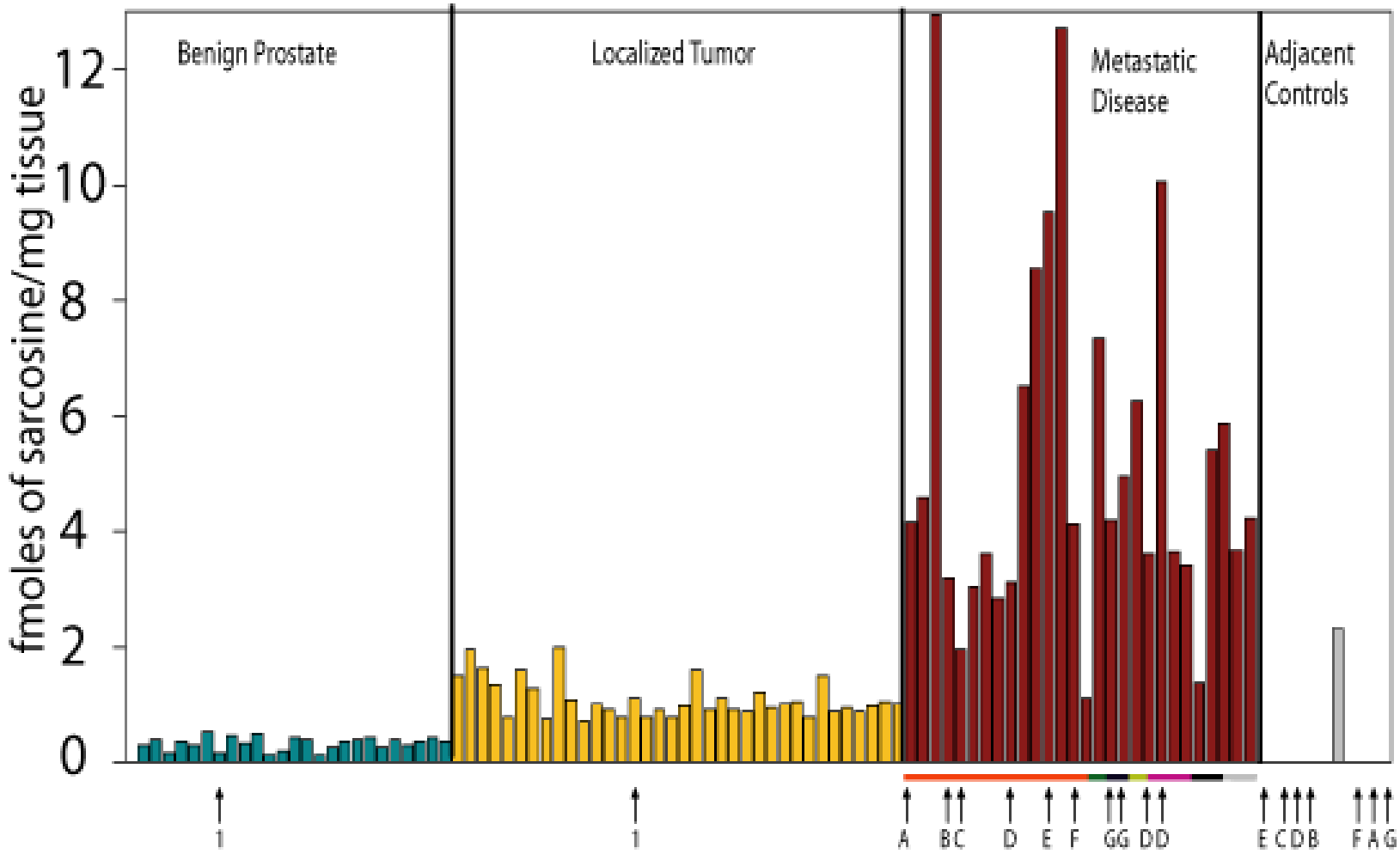

Using an Independent GC/MS (“targeted”) Assay
on additional samples.

Characteristics

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- Chemocentric platform
- Unbiased analyses
- Library based compound identification
- Quantitative
- Robust
- Reproducible
- CVs < 10%

Platform

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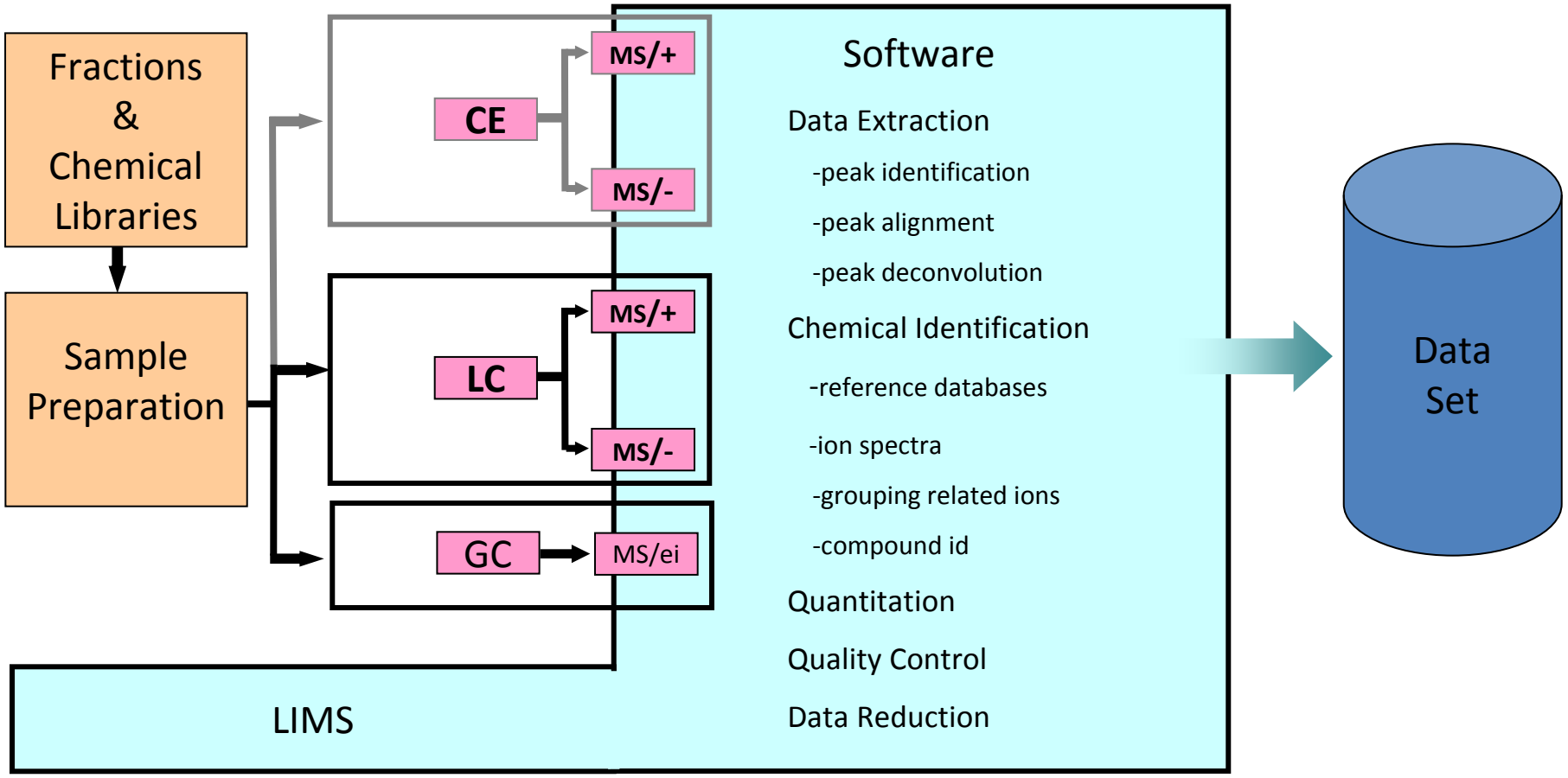

Preparation Analysis Informatics

MS Dimensionality

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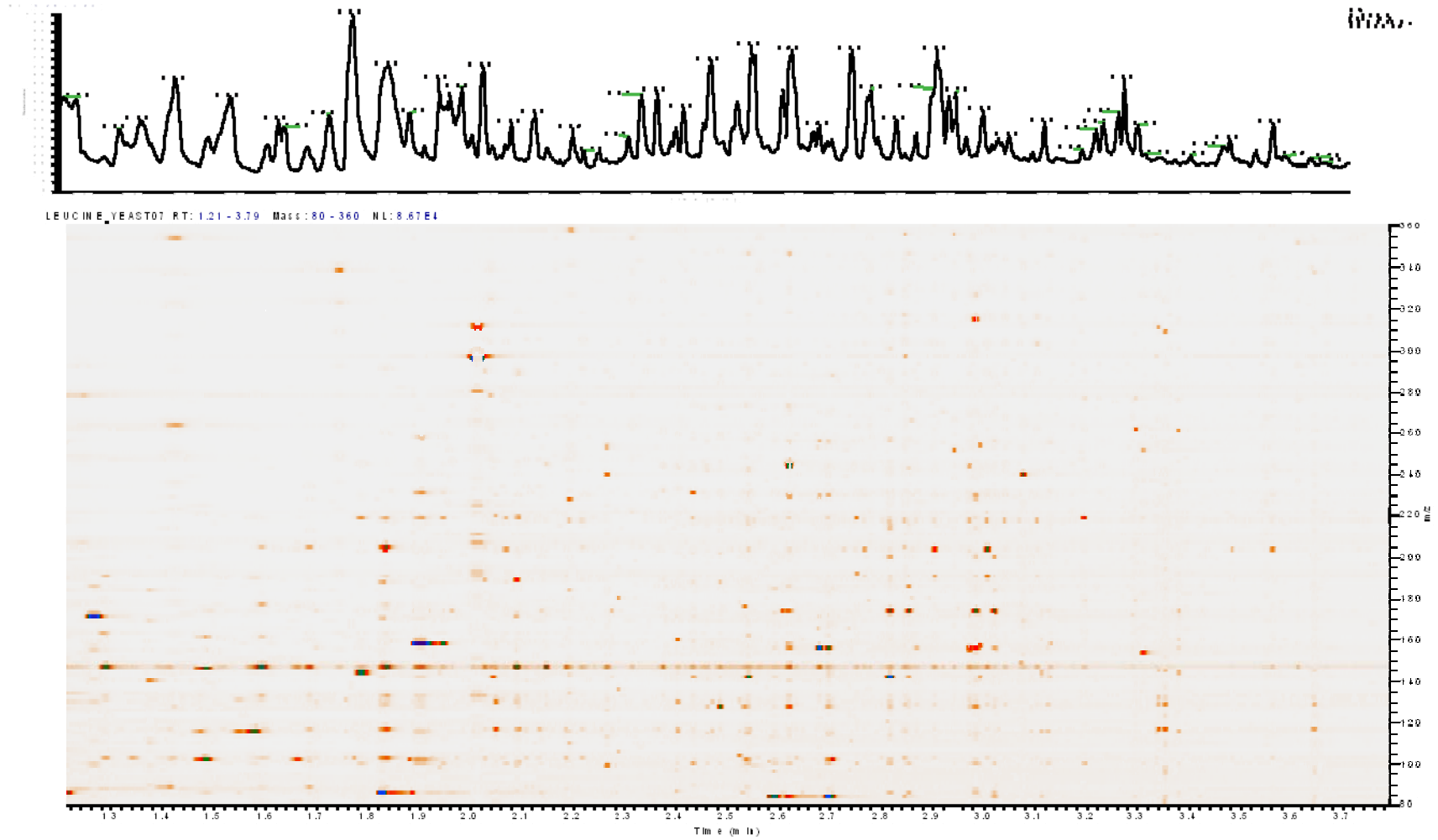

UPLC/QToF

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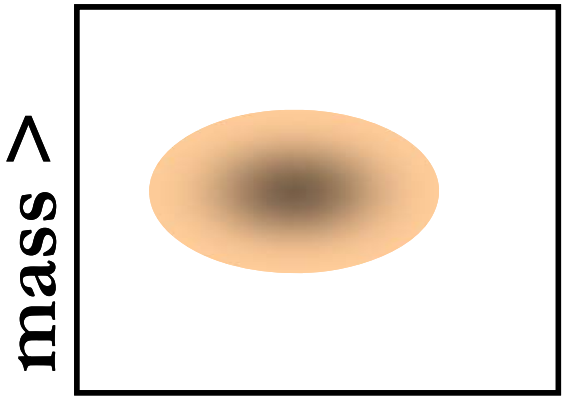
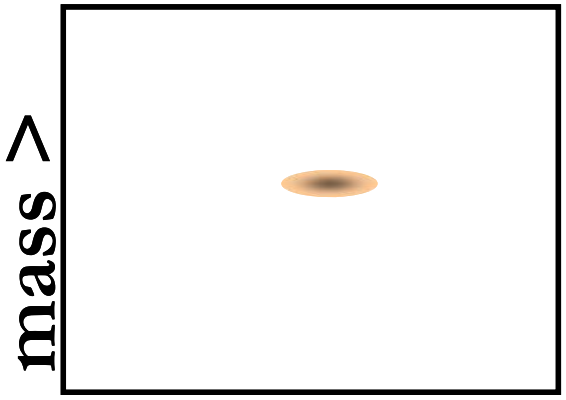
	Previous	Current
		
time	20 sec	2 sec
mass	+/-0.3 amu	+/-0.0003 amu
concentration	1x	20x
Characterization	Library comparison only, may add fragmentation	Formula based, may add fragmentation

Reproducibility

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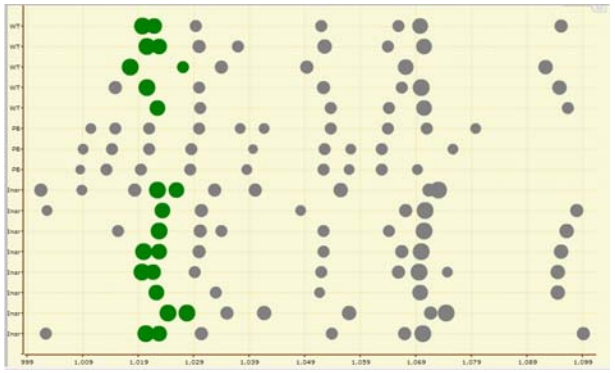

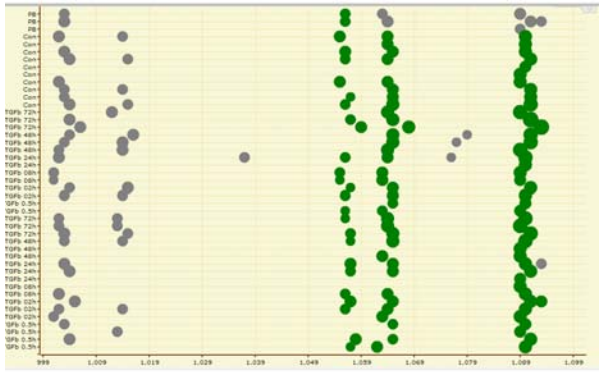

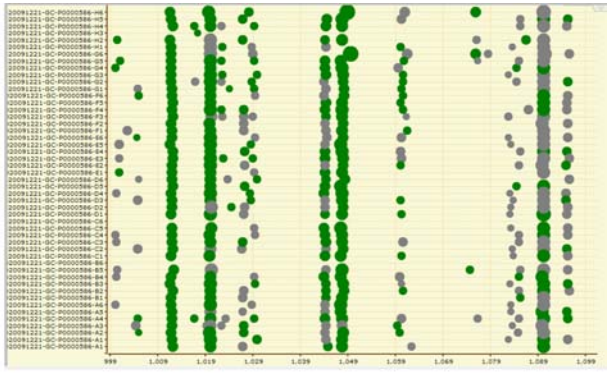

Unit mass resolution on ion masses, and fragmentation of molecular ions requires very high reproducibility to accurately name compounds.

May 2009

August 2009

December 2009

Reproducibility (2)

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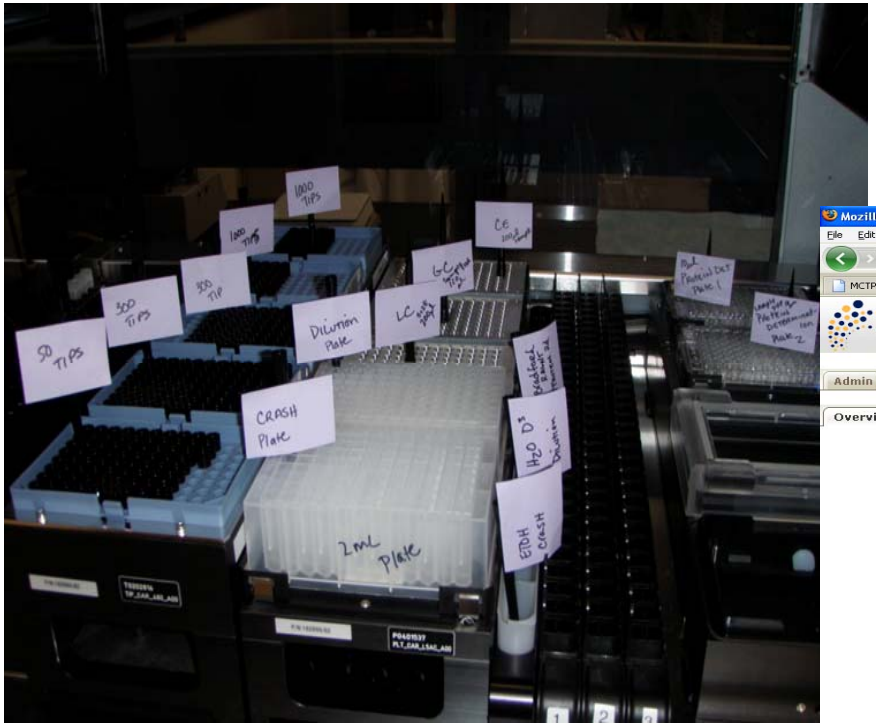
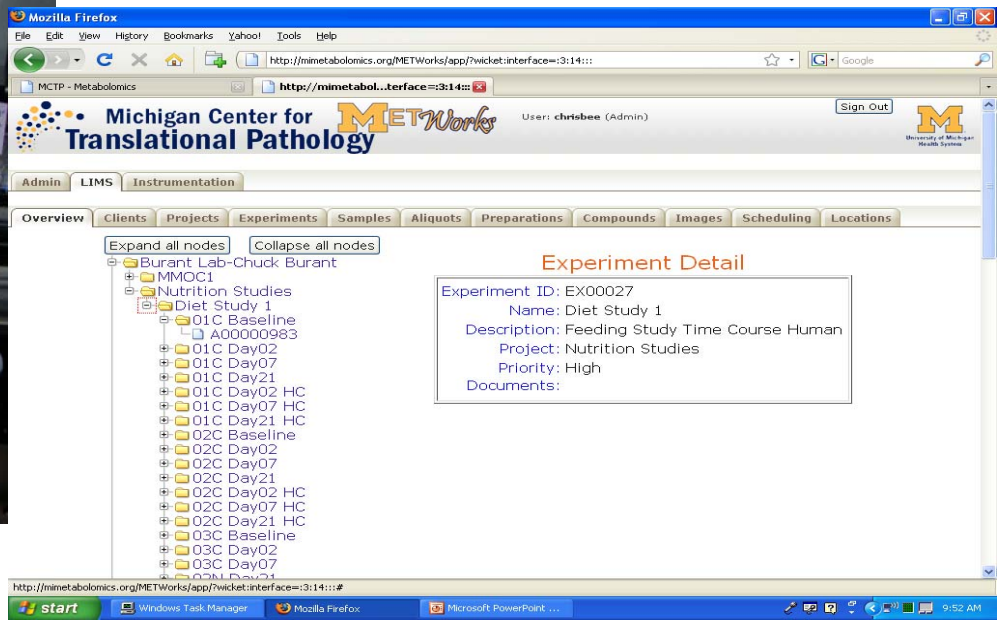
- The use of LIMS, robotics & barcodes is required to achieve reproducible sample prep, and data.





Integration through LIMS

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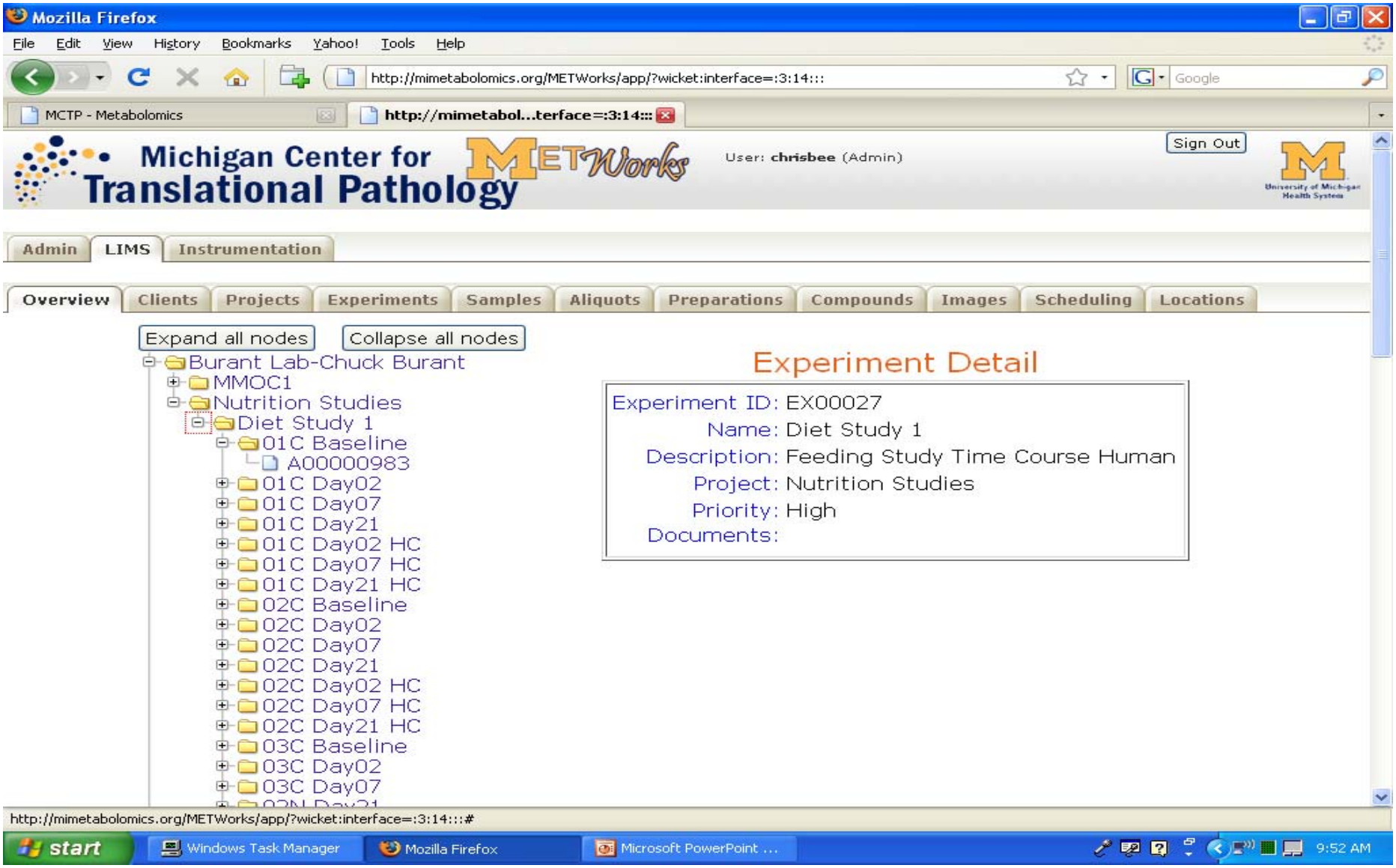

Metabolomic Library coverage

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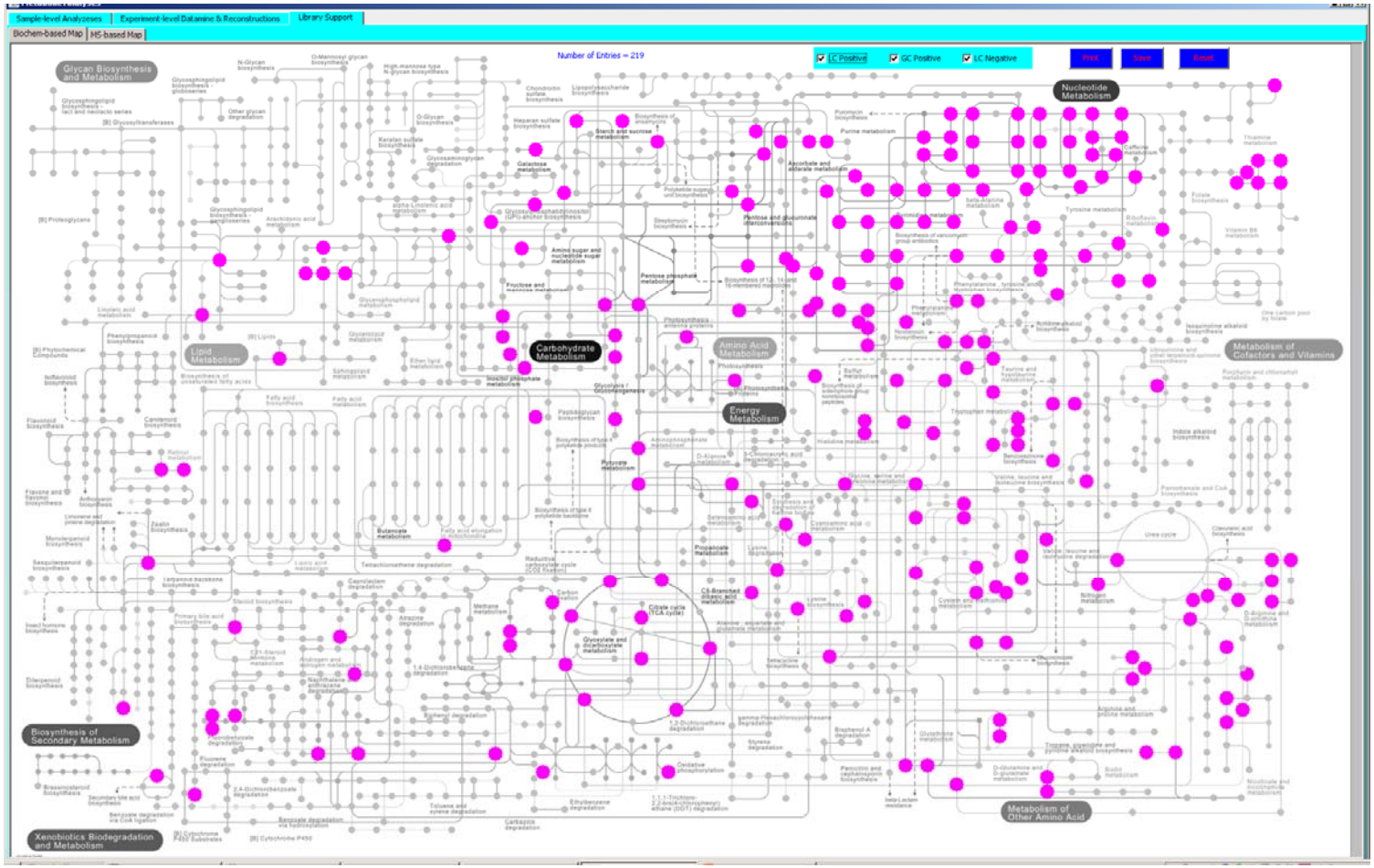
- Our current library has 950 compounds and is growing. Approximately 250 are shown below.



Variance Analyses

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- Run periodically to test ourselves
- 50 aliquots from a single pooled plasma sample
- Processed individually with simple pooling pattern
- Final dataset examined statistically to identify sources of sample prep induced variance.

- (There are many variants to this experiment)

Variance Analyses

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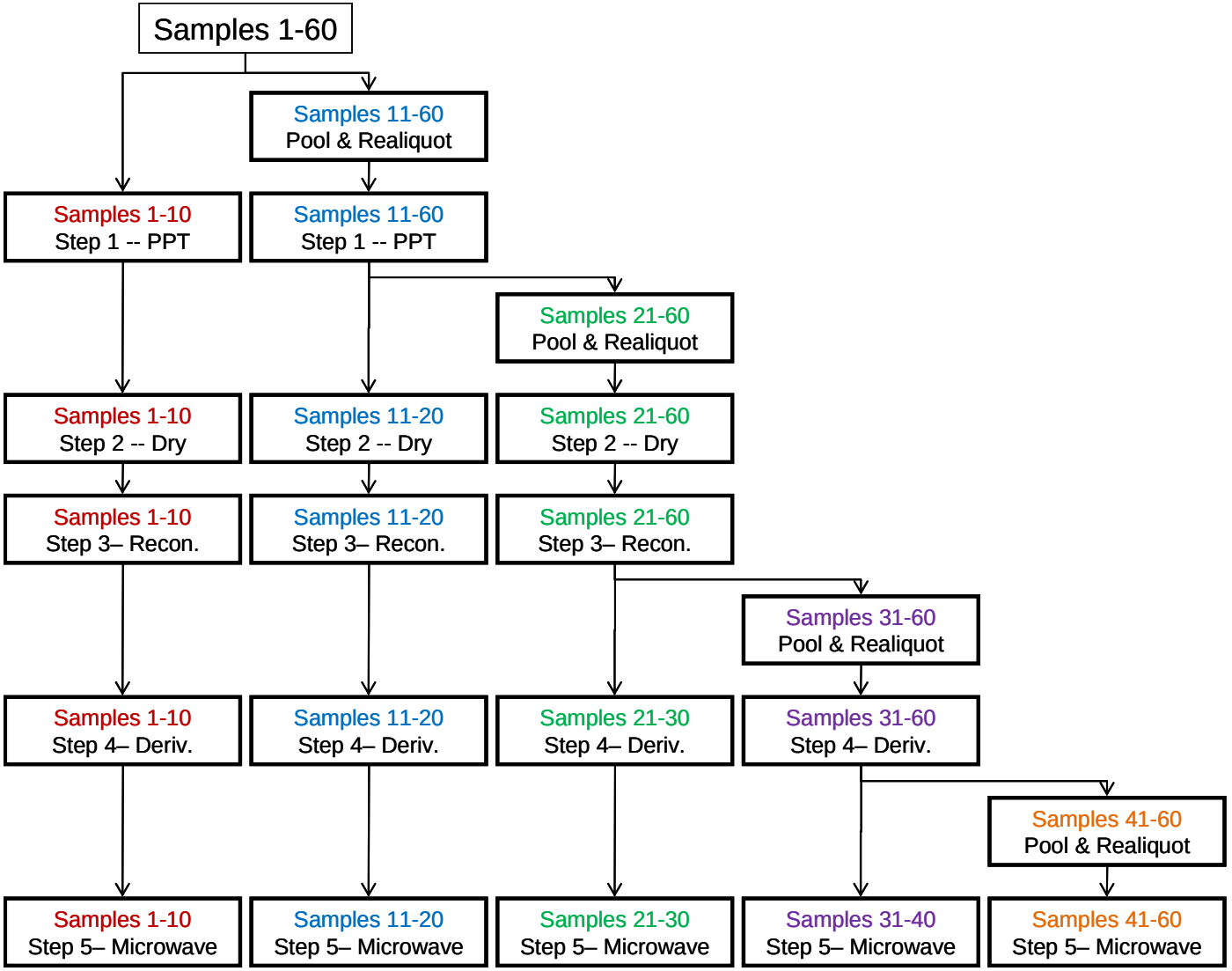

Summary

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- It is possible to create a highly reproducible, accurate, and sensitive Metabolomics platform that can provide significant biological insight.
- It requires:
  - continuous attention to detail
  - constant repetition
  - library of authentic standards
- Given the above it does not require the latest and greatest equipment

Thank You

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Hierarchical Analysis

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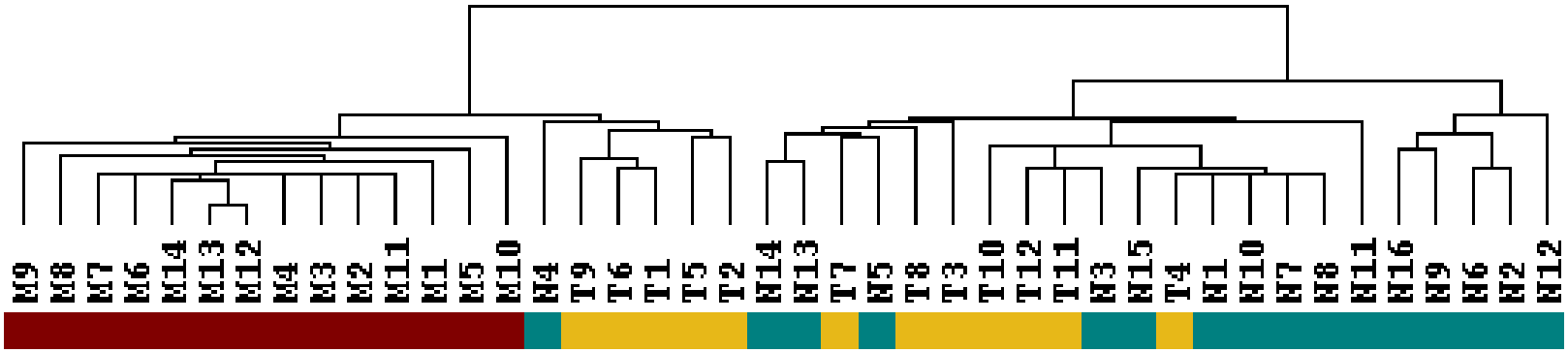

Benign

Localized PCa

Metastatic Disease