

Metabolomics: Techniques and Applications

ABRF

Sacramento, CA

March 23, 2010

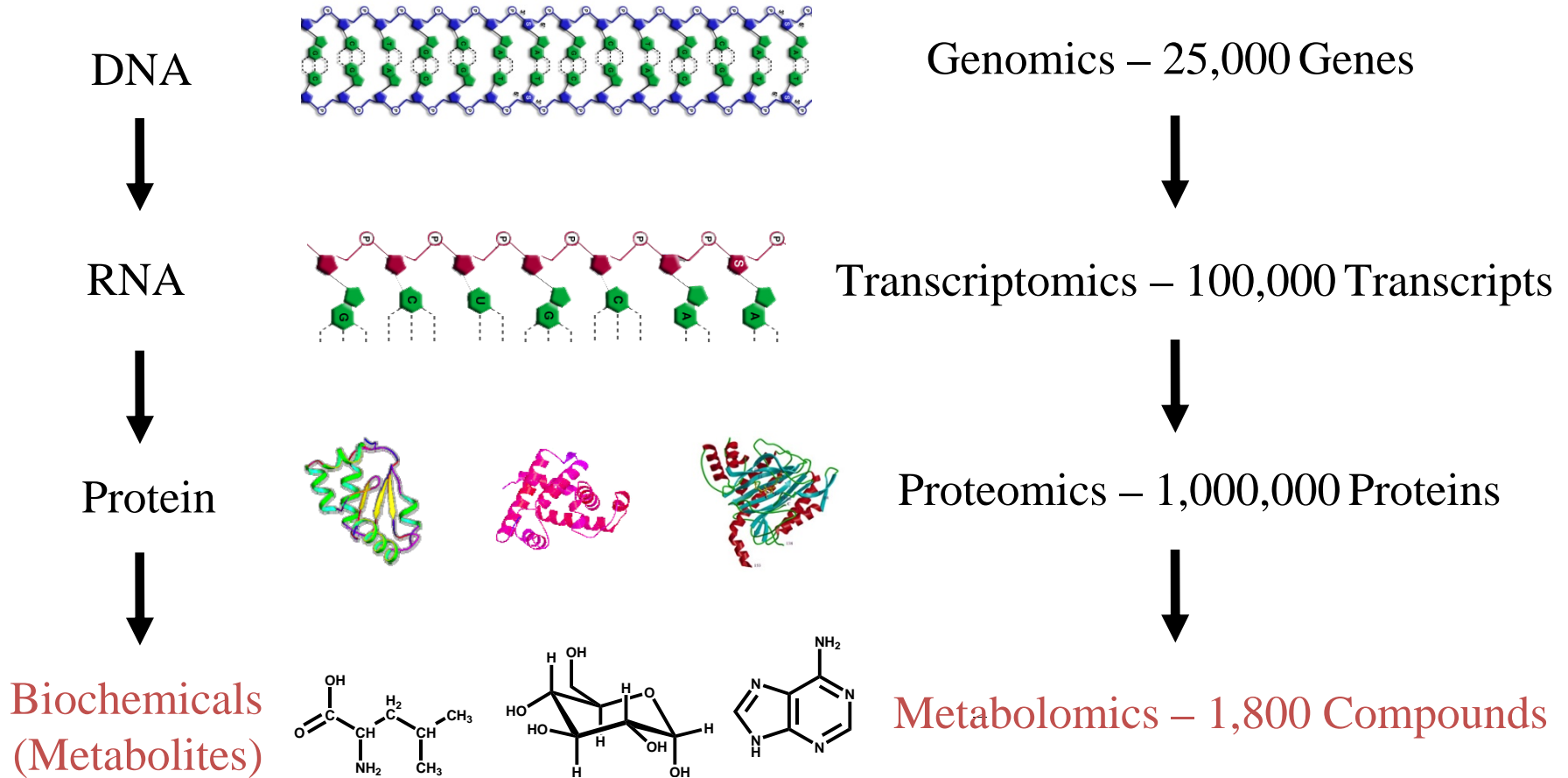
Overview

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

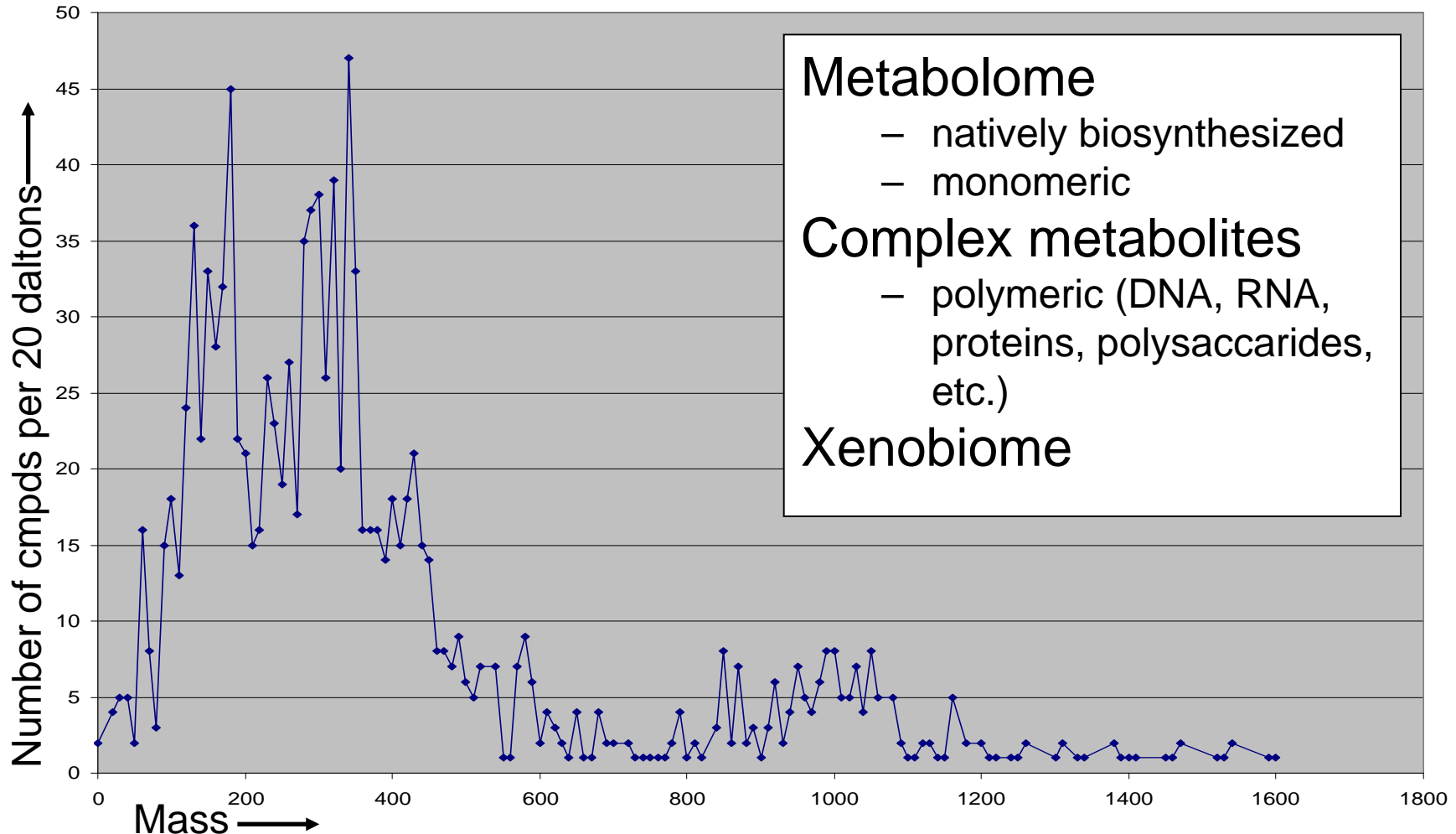
Unbiased Metabolomics

- 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



Chemical Ecology of a Human Cell



Experimental design

	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

- 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).

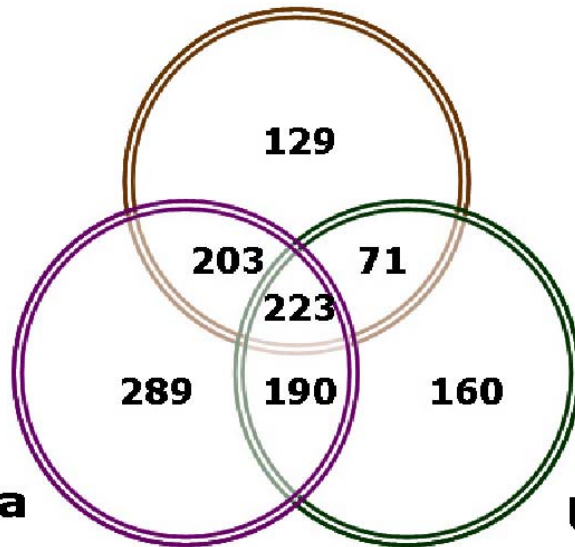
*Funded by the Early Detection Research Network (EDRN) /NCI

Chemical Variation

Total = 1265 compounds

Total = 626 compounds

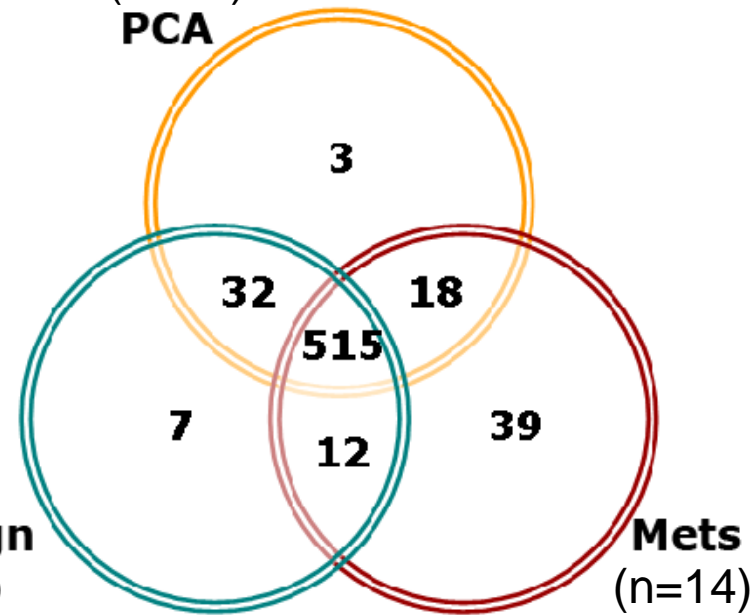
(n=45)
Tissue



Plasma
(n=110)

Urine
(n=110)

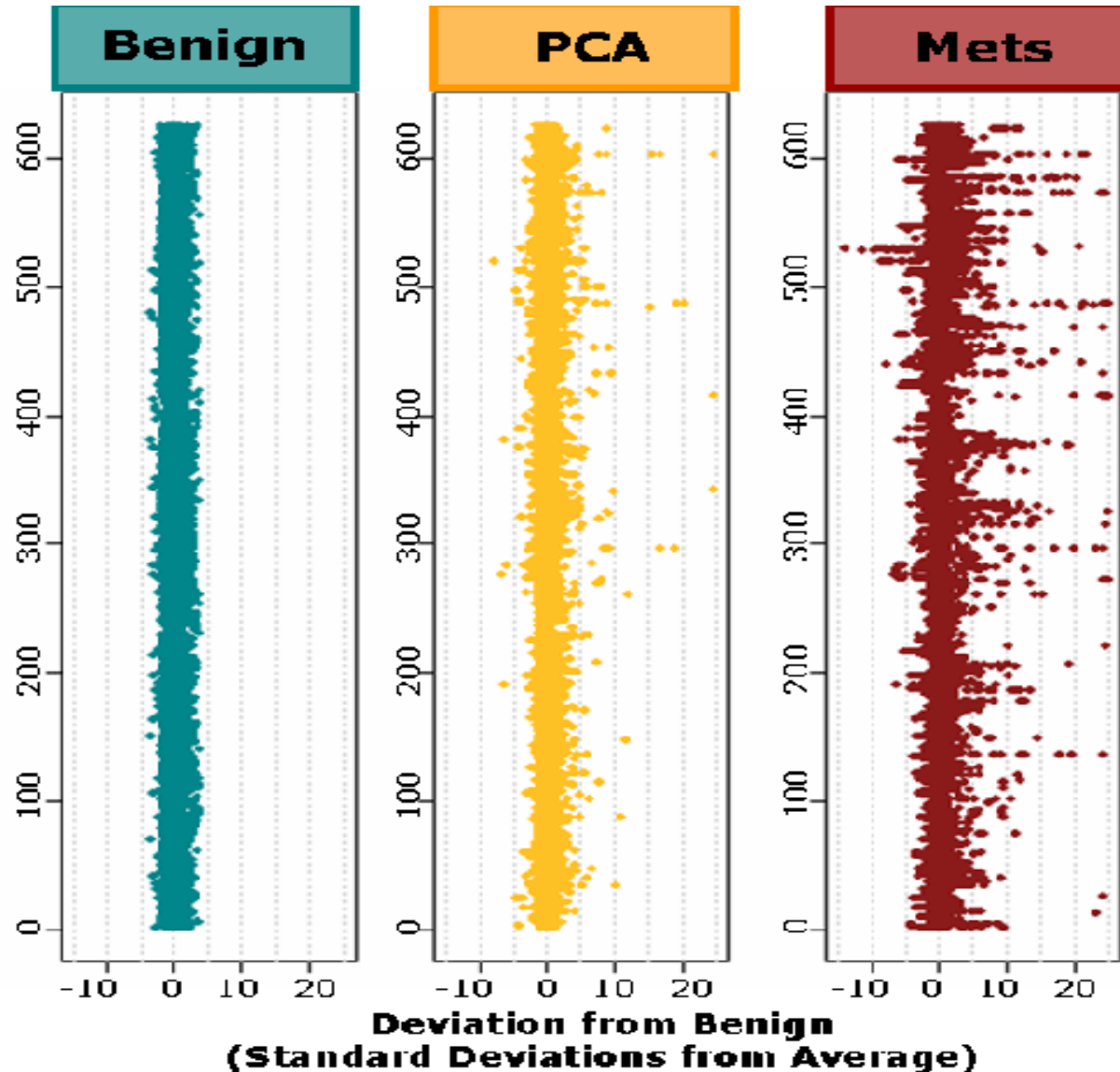
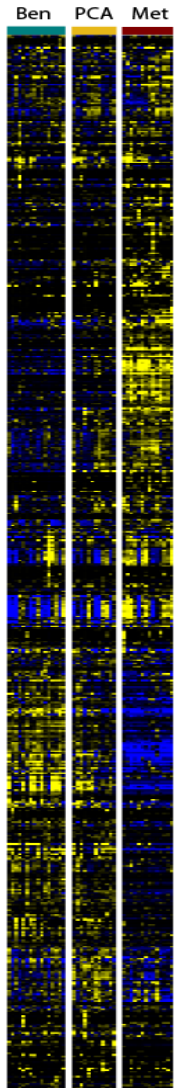
(n=12)
PCA



Benign
(n=16)

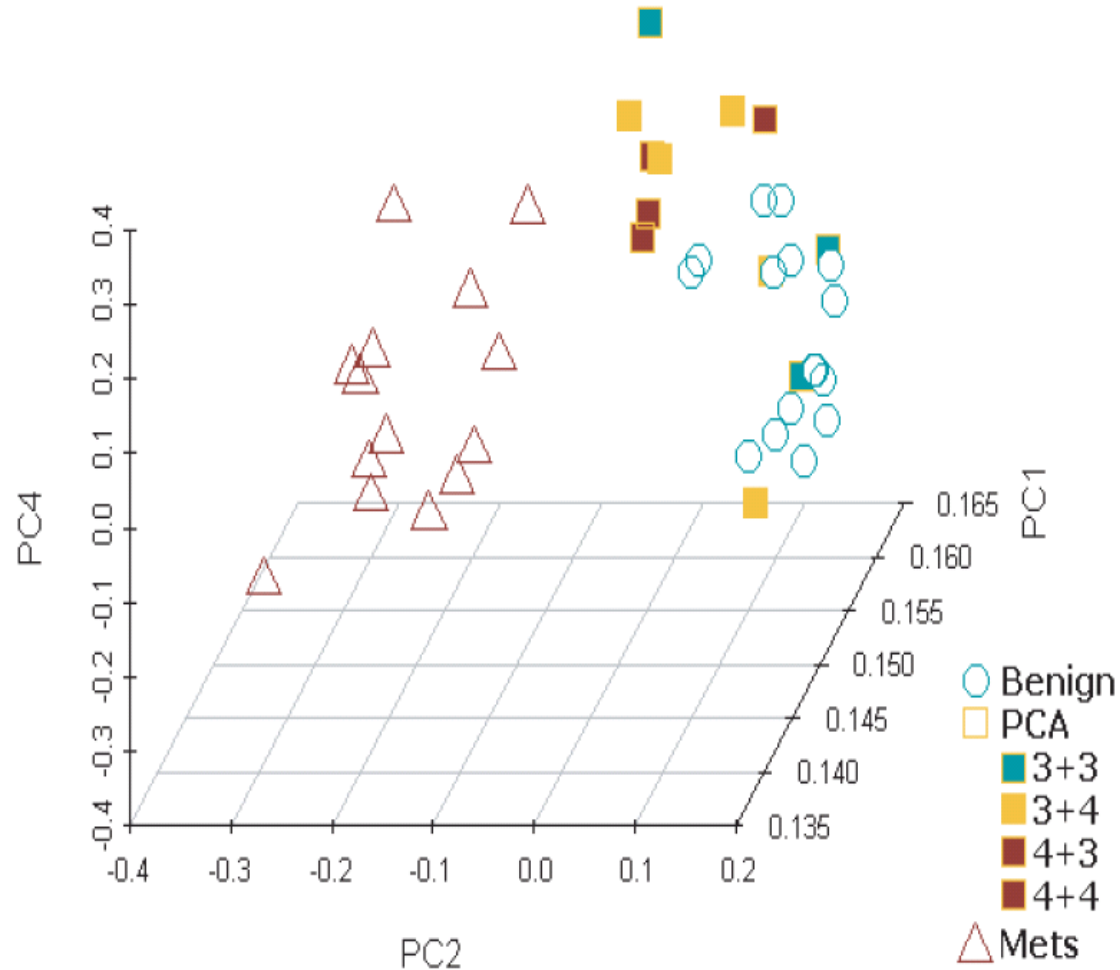
Mets
(n=14)

Statistical Differences



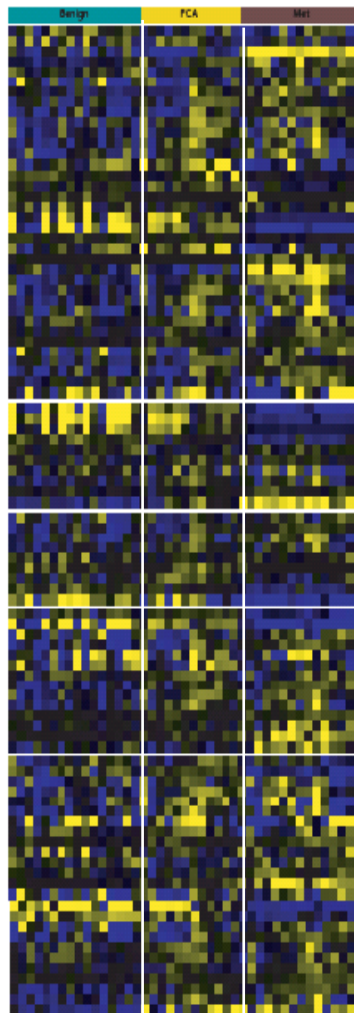
Principle Components Analysis

(all compounds / total data set)

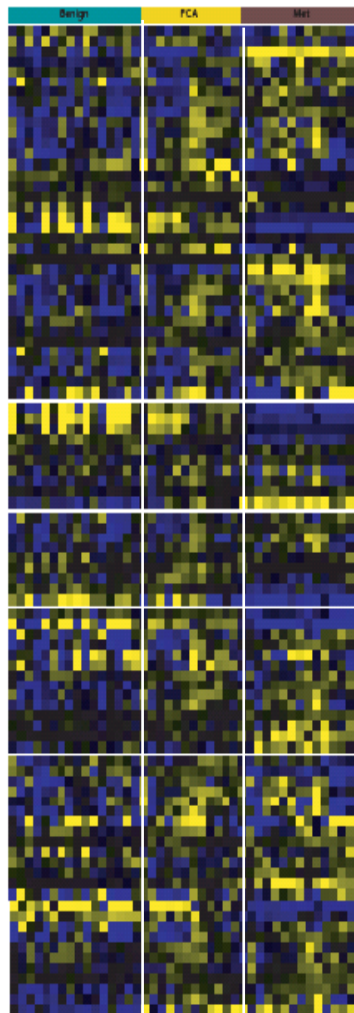


Metabolic Alterations

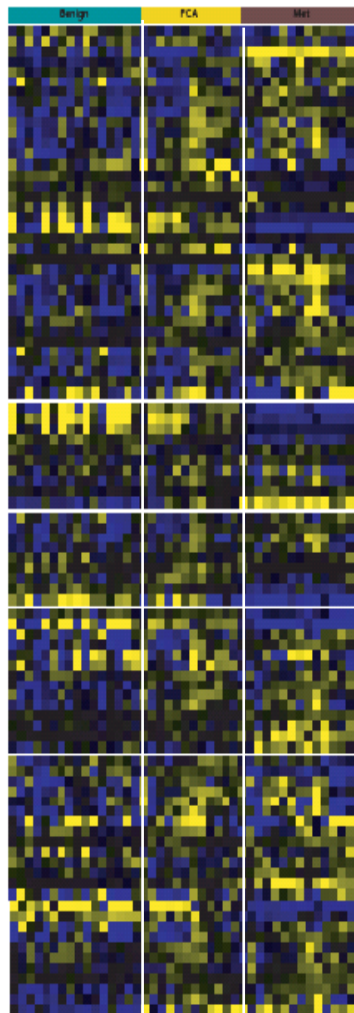
ALANINE AND ASPARTATE METABOLISM



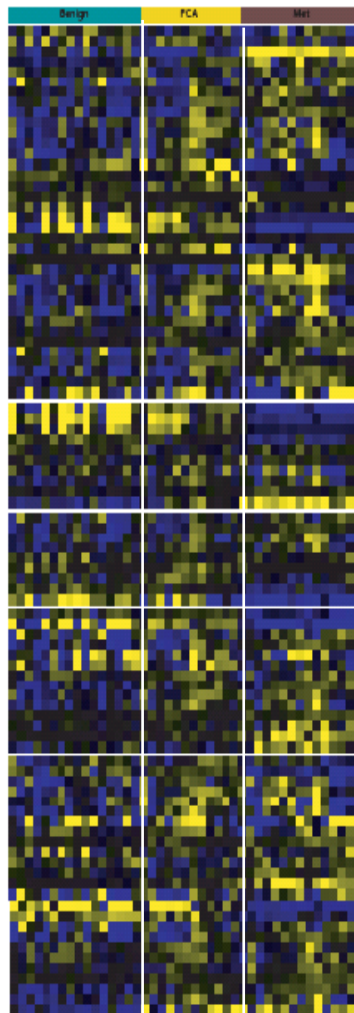
ARGININE AND PROLINE METABOLISM



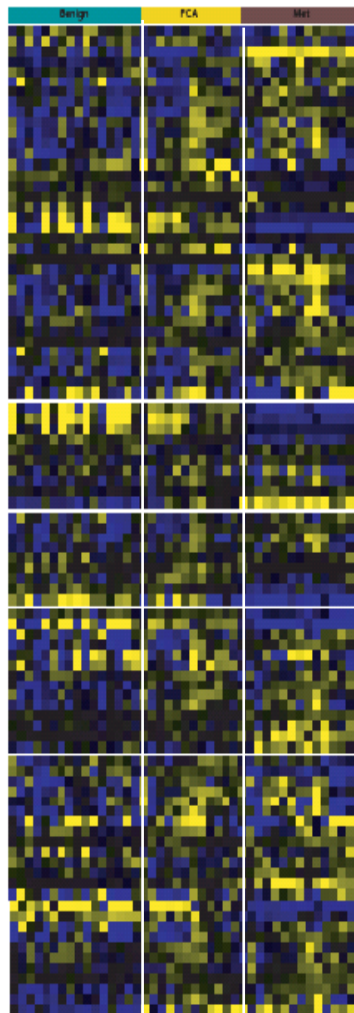
BETA ALANINE METABOLISM



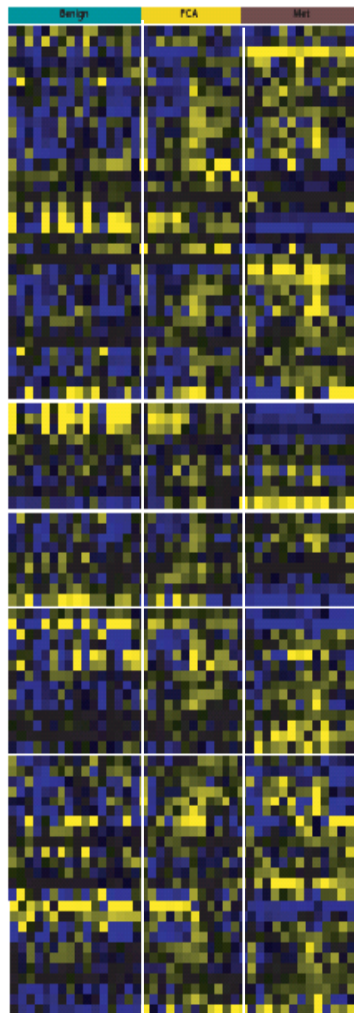
GLUTAMATE METABOLISM



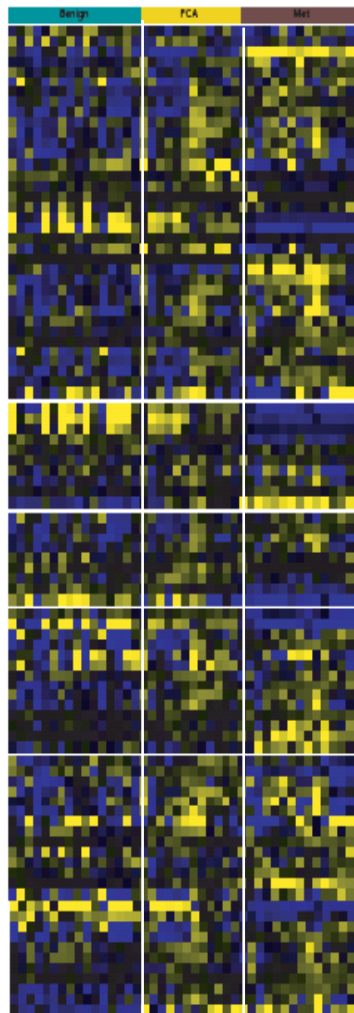
GLYCINE, THREONINE, SERINE METABOLISM



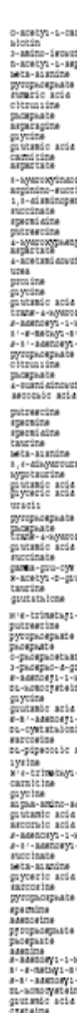
LYSINE METABOLISM



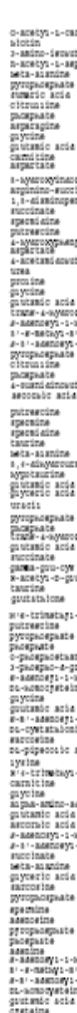
METHIONINE METABOLISM



NICOTINATE METABOLISM



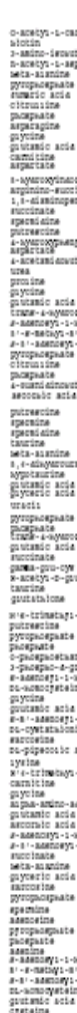
PURINE METABOLISM



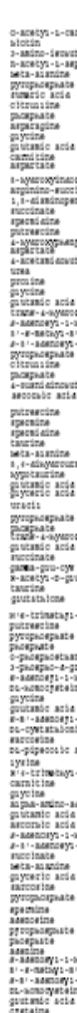
PYRIMIDINE METABOLISM



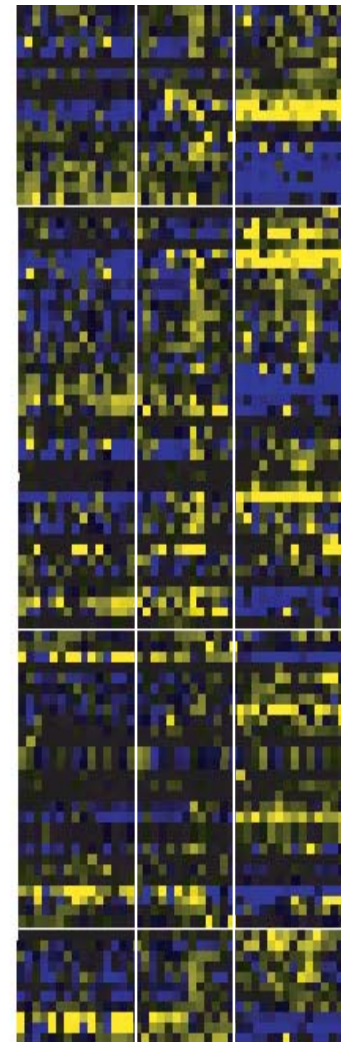
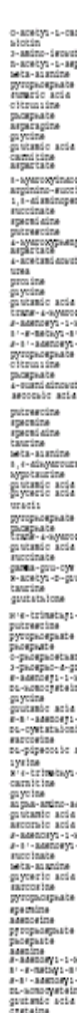
TRYPTOPHAN METABOLISM



TYROSINE METABOLISM

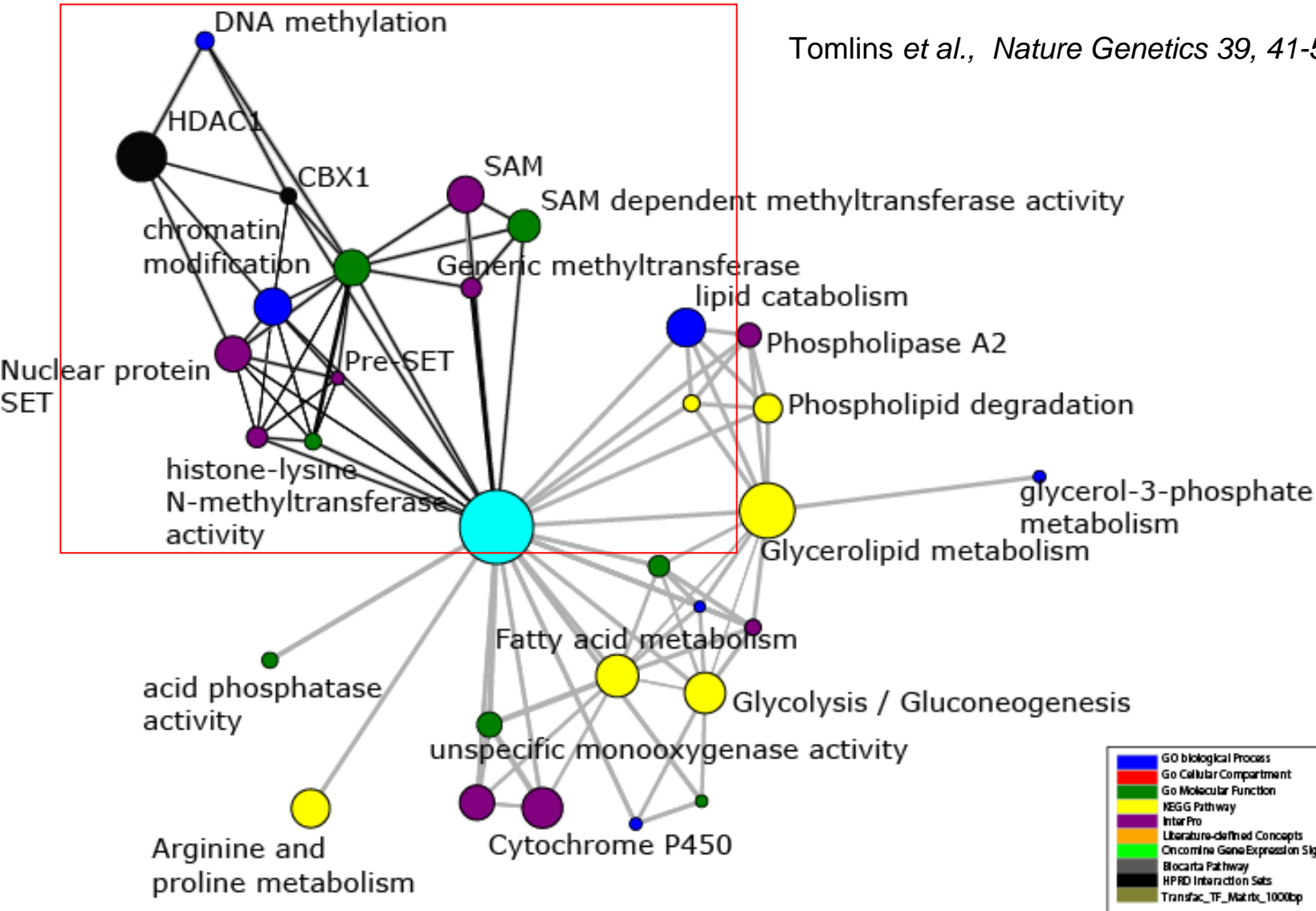


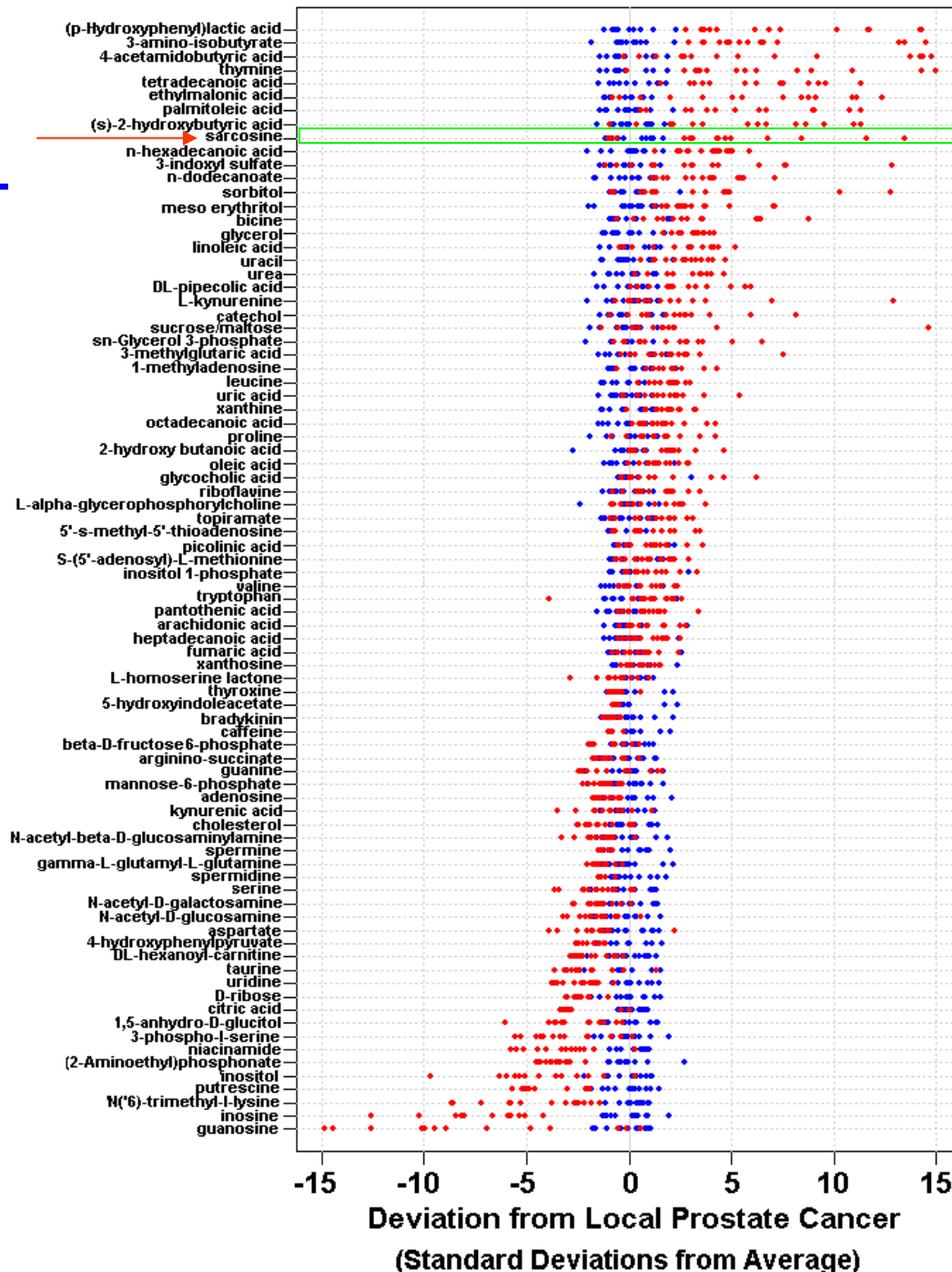
UREA CYCLE



Molecular Concepts Mapping

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





METABOLOMIC SIGNATURE OF METASTATIC SAMPLES COMPARED TO PCa

FATTY ACID INTERMEDIATES

KETONE BODIES

CoA PRECURSORS

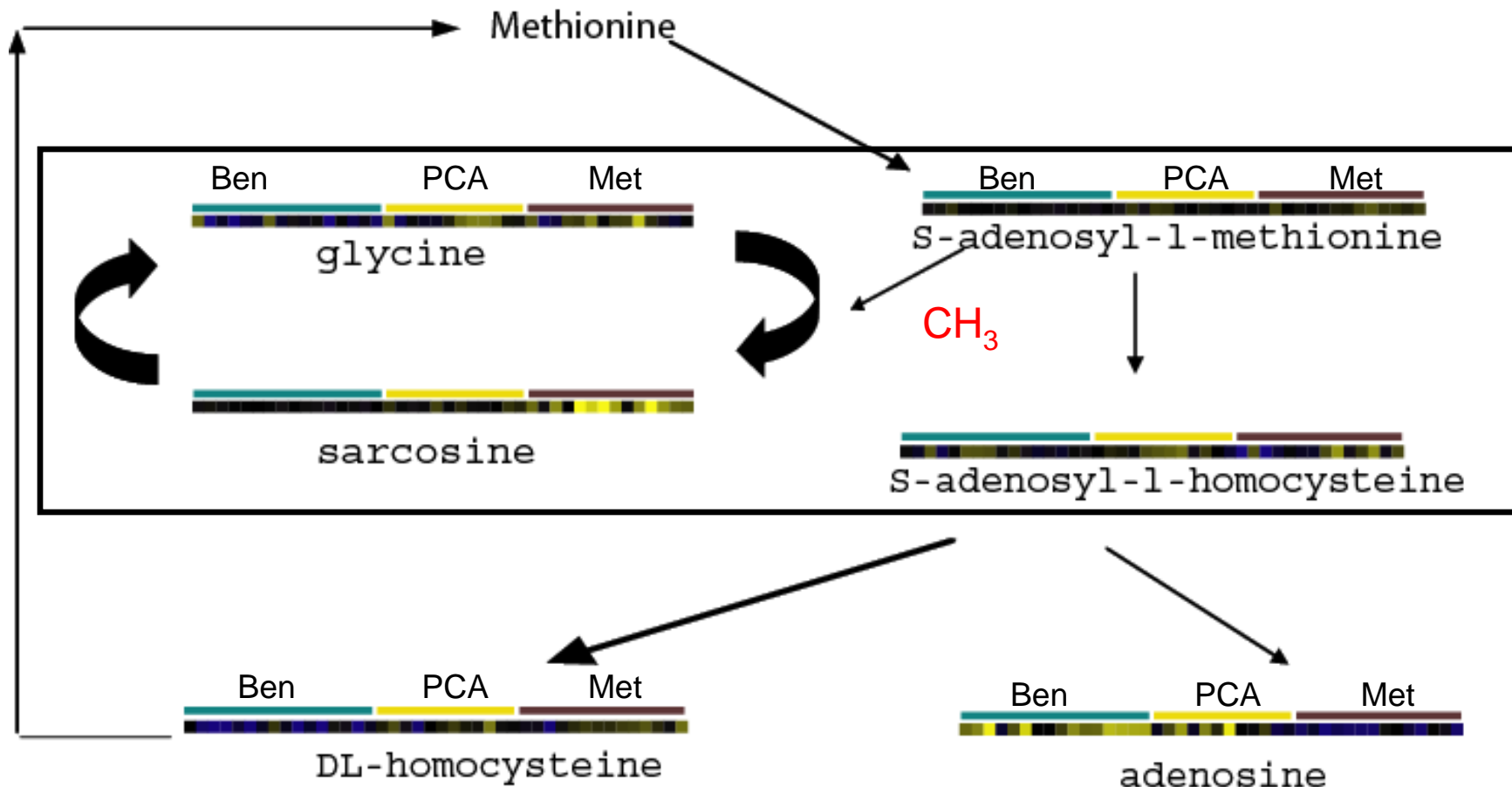
S-ADENOSYL METHIONINE

AMINO ACIDS

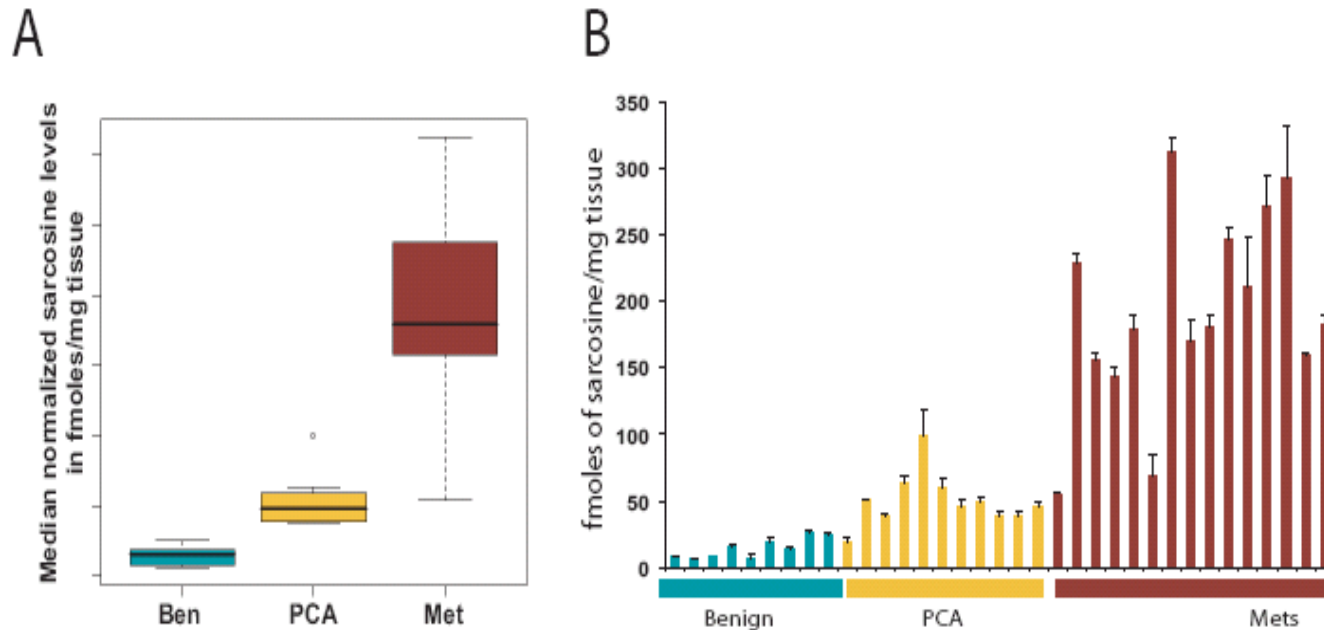
NUCLEIC ACID PRECURSORS

Deviation from Local Prostate Cancer
(Standard Deviations from Average)

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

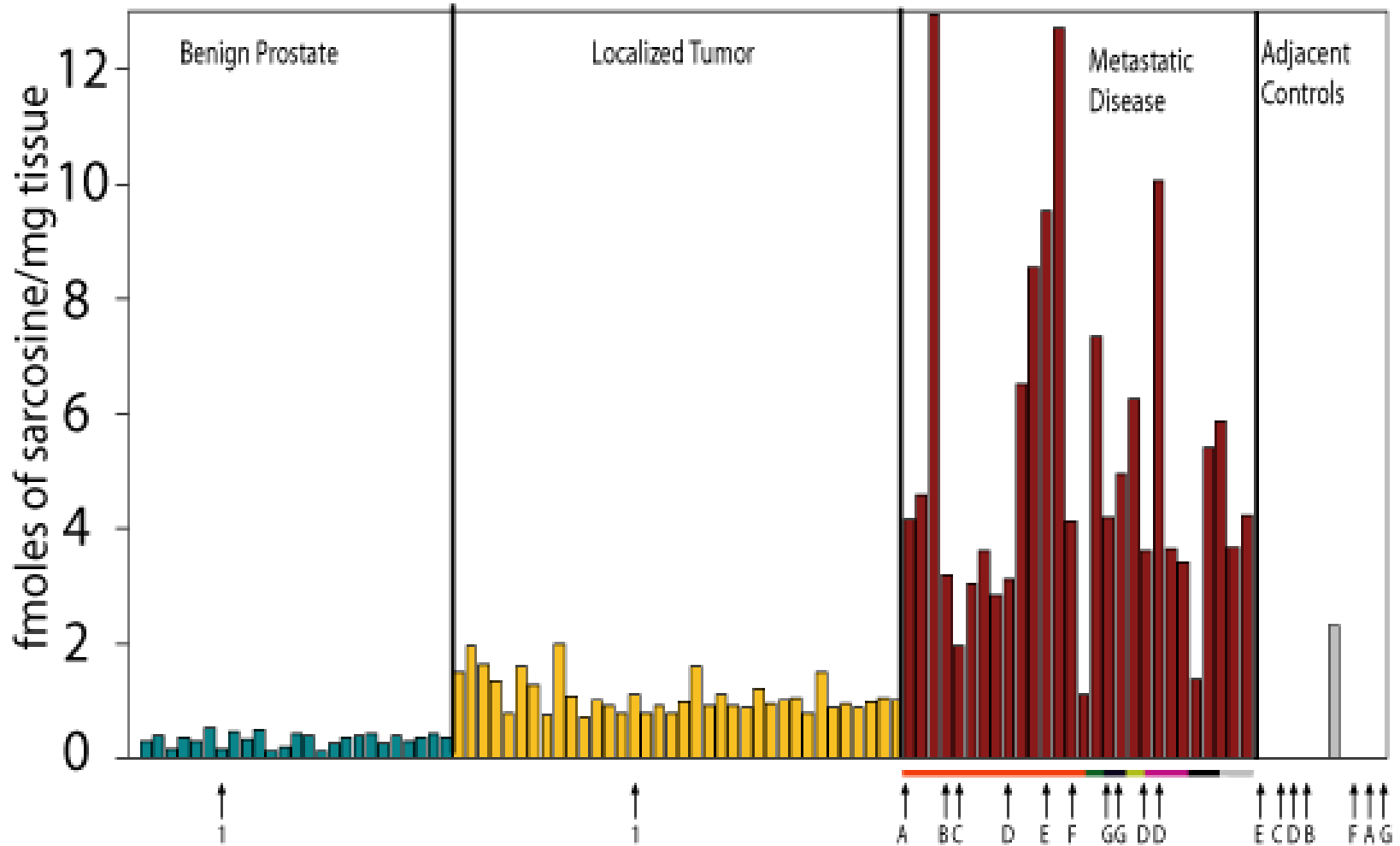


Validation of Sarcosine



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

Revalidation of Sarcosine

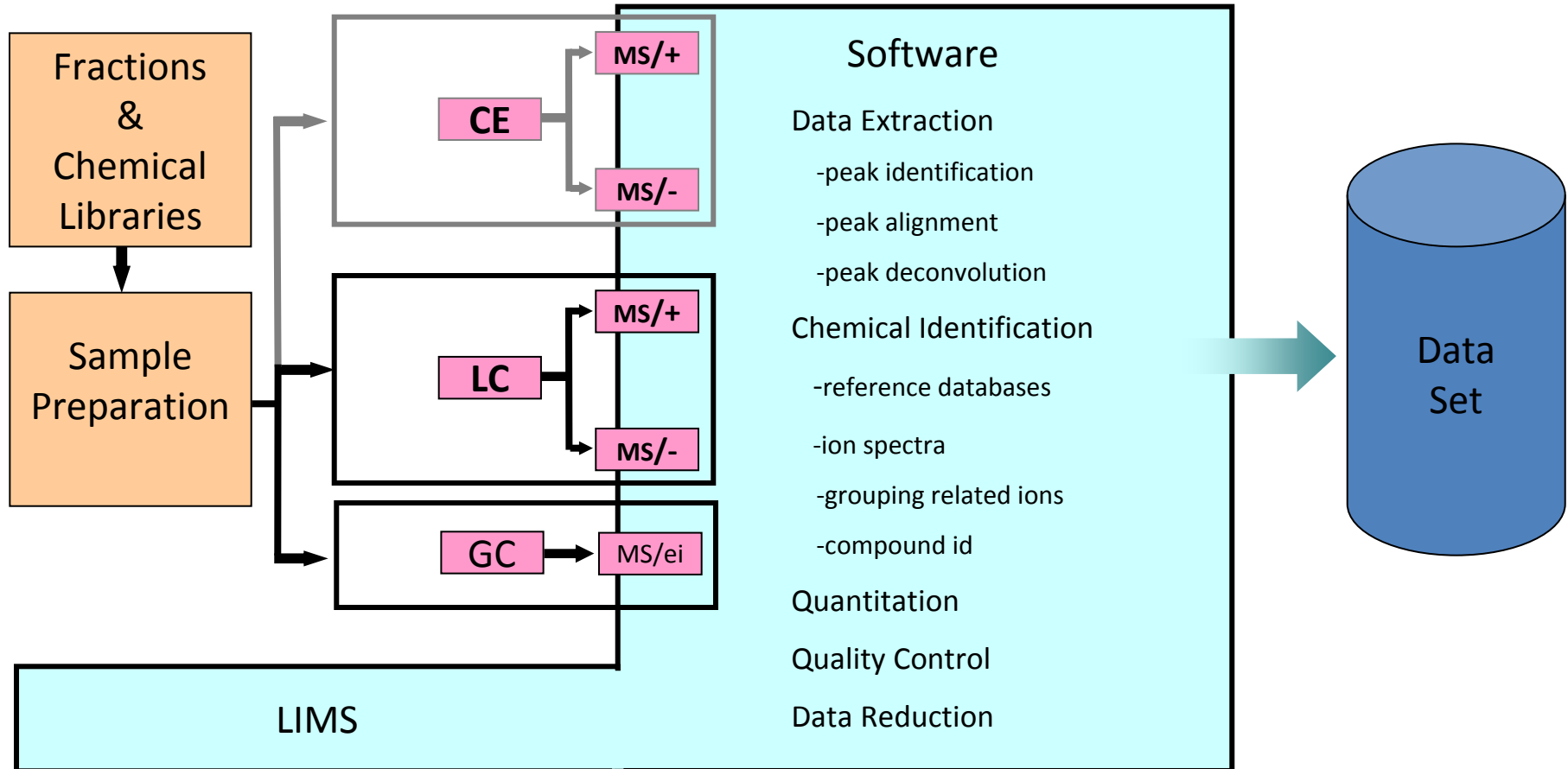


Using an Independent GC/MS (“targeted”) Assay
on additional samples.

Characteristics

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

Platform

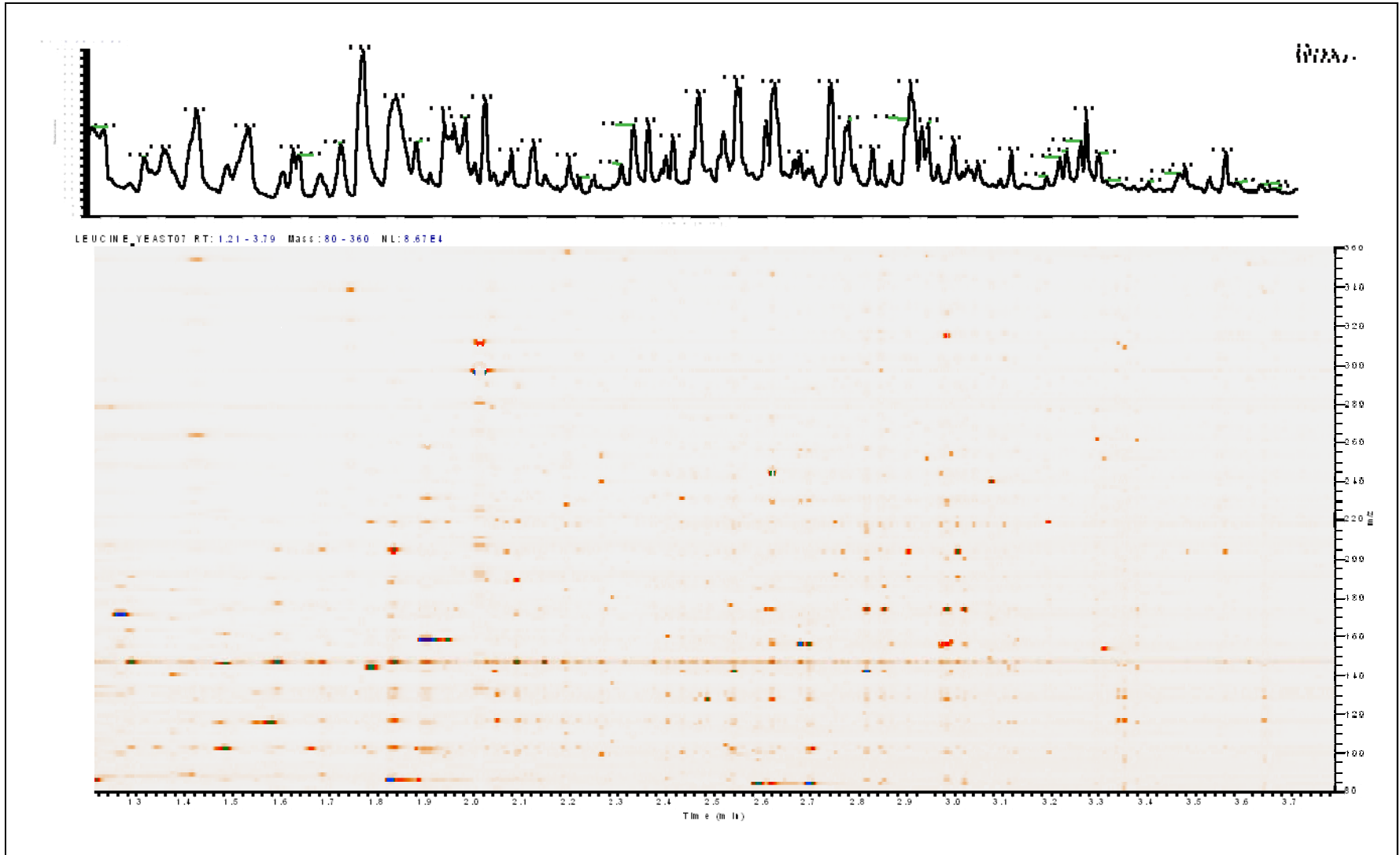


Preparation

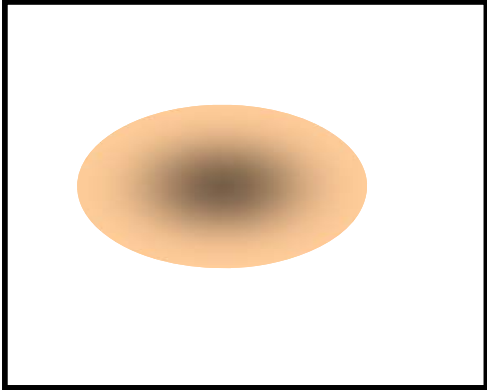
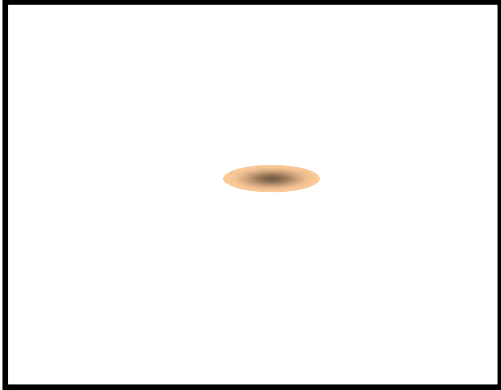
Analysis

Informatics

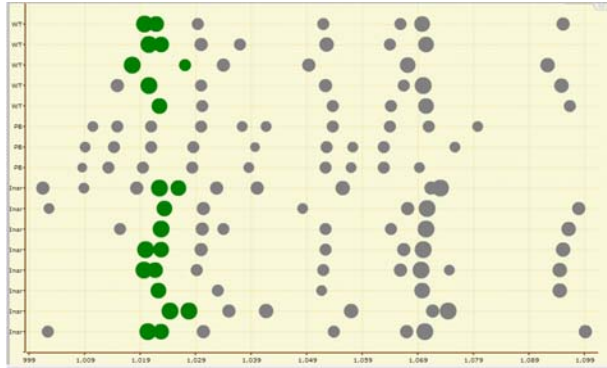
MS Dimensionality



UPLC/QToF

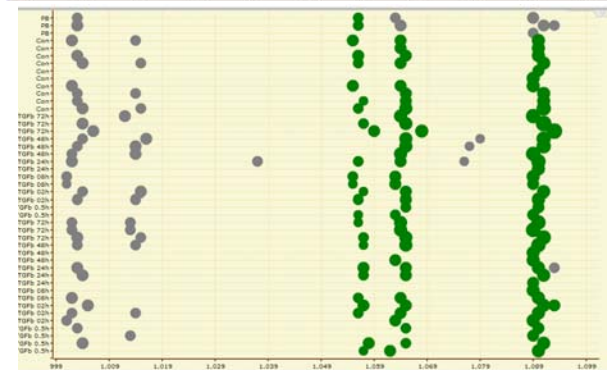
	Previous	Current
	 <p>mass \wedge time \triangleright</p>	 <p>mass \wedge time \triangleright</p>
time	20 sec	2 sec
mass	+/-0.3 amu	+/-0.0003 amu
concentration	1x	20x
Characterization	Library comparison only, may add fragmentation	Formula based, may add fragmentation

Reproducibility

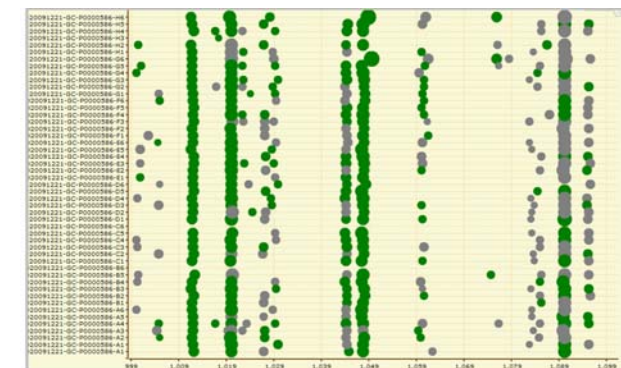


May 2009

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



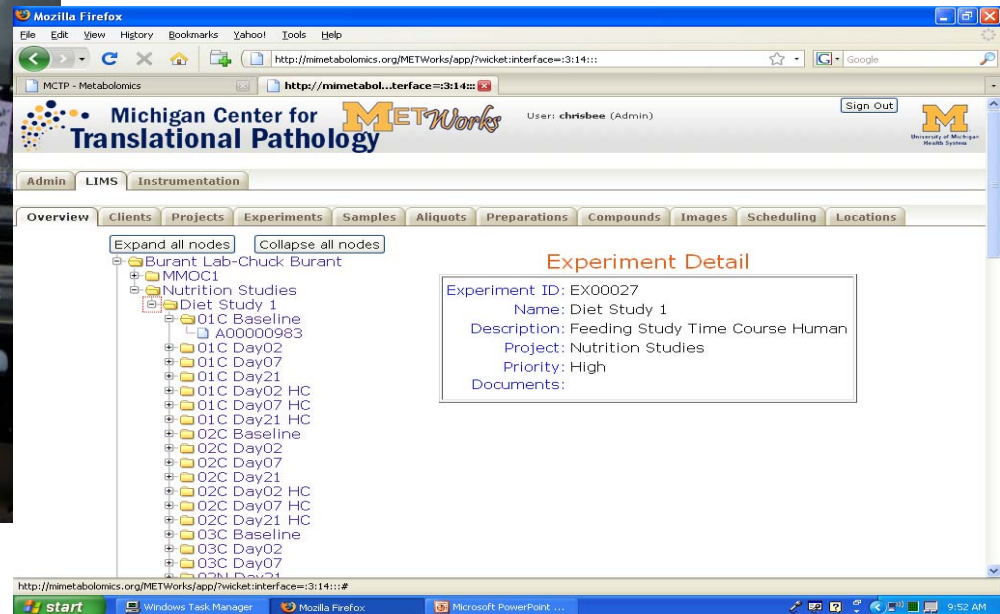
August 2009



December 2009

Reproducibility (2)

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



Michigan Center for Translational Pathology METWorks

Admin LIMS Instrumentation

Overview Clients Projects Experiments Samples Aliquots Preparations Compounds Images Scheduling Locations

Expand all nodes Collapse all nodes

Burant Lab-Chuck Burant

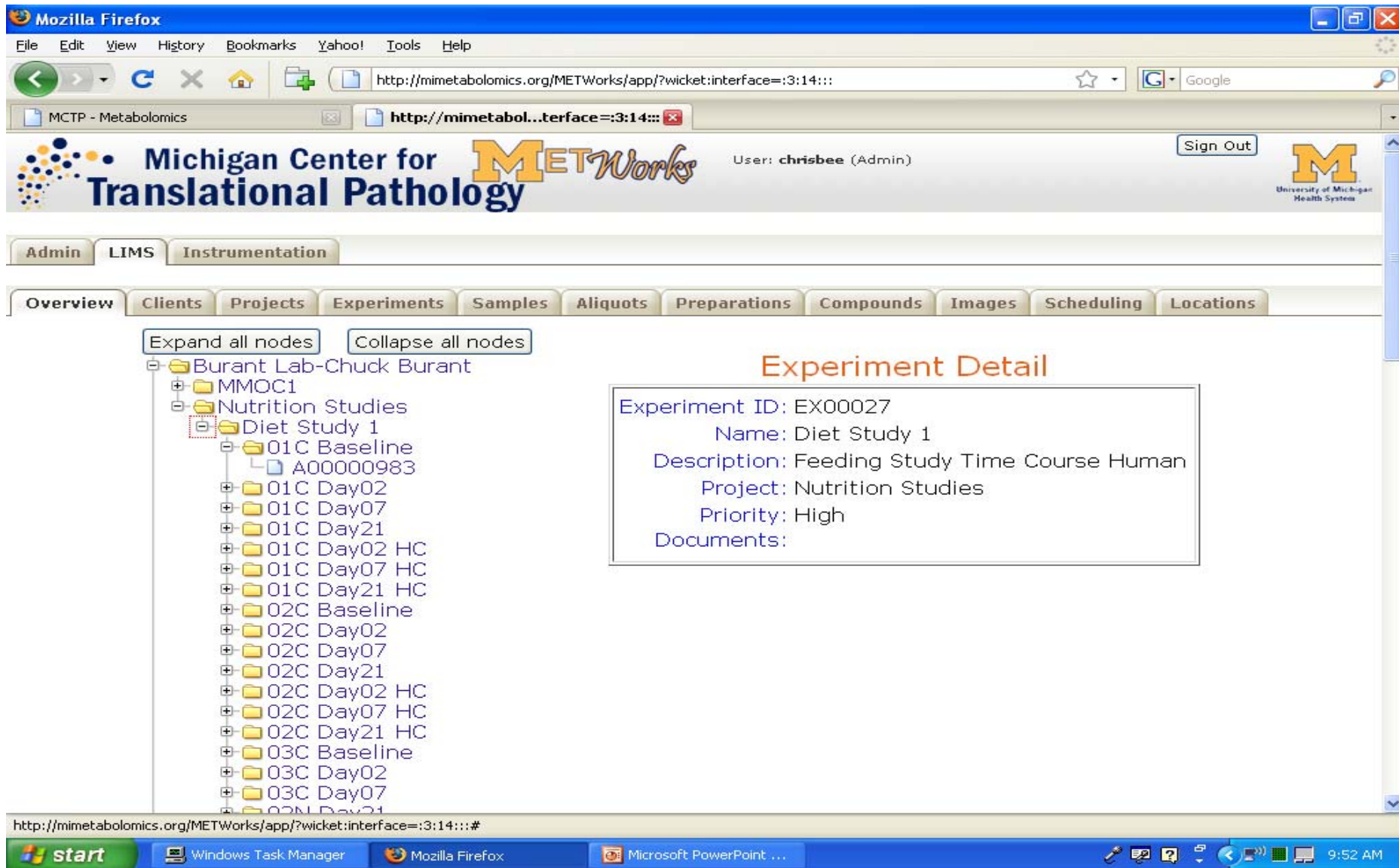
- MMOC1
 - Nutrition Studies
 - Diet Study 1
 - 01C Baseline
 - A0000983
 - 01C Day02
 - 01C Day07
 - 01C Day21
 - 01C Day02 HC
 - 01C Day07 HC
 - 01C Day21 HC
 - 02C Baseline
 - 02C Day02
 - 02C Day07
 - 02C Day21
 - 02C Day02 HC
 - 02C Day07 HC
 - 02C Day21 HC
 - 03C Baseline
 - 03C Day02
 - 03C Day07
 - 03C Day21

Experiment Detail

Experiment ID: EX00027
Name: Diet Study 1
Description: Feeding Study Time Course Human
Project: Nutrition Studies
Priority: High
Documents:

start Windows Task Manager Mozilla Firefox Microsoft PowerPoint ... 9:52 AM

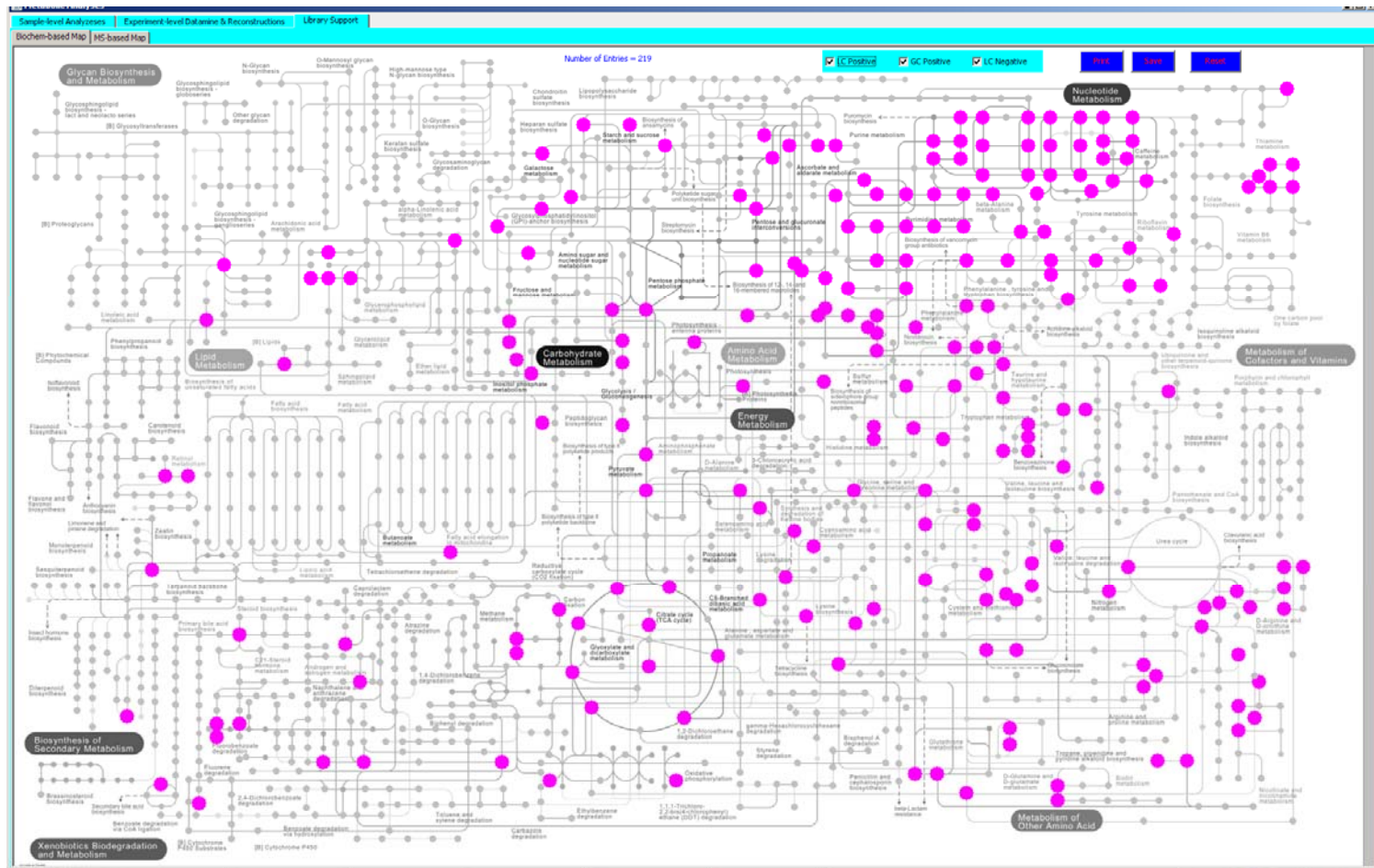
Integration through LIMS



The screenshot shows the METWorks LIMS interface within a Mozilla Firefox browser window. The browser's address bar displays the URL: `http://mimetabolomics.org/METWorks/app/?wicket:interface=:3:14:::`. The page header includes the Michigan Center for Translational Pathology logo, the METWorks logo, the user name "User: chrisbee (Admin)", and a "Sign Out" button. Below the header is a navigation menu with tabs for "Admin", "LIMS", and "Instrumentation". The "LIMS" tab is active, and a sub-menu shows various options: "Overview", "Clients", "Projects", "Experiments", "Samples", "Aliquots", "Preparations", "Compounds", "Images", "Scheduling", and "Locations". The "Experiments" option is selected, leading to a tree view of experiment data. The tree view shows a hierarchy starting with "Burant Lab-Chuck Burant", followed by "MMOC1", "Nutrition Studies", and "Diet Study 1". Under "Diet Study 1", there are several sub-folders for different time points and conditions, such as "01C Baseline", "01C Day02", "01C Day07", "01C Day21", "01C Day02 HC", "01C Day07 HC", "01C Day21 HC", "02C Baseline", "02C Day02", "02C Day07", "02C Day21", "02C Day02 HC", "02C Day07 HC", "02C Day21 HC", "03C Baseline", "03C Day02", and "03C Day07". A specific experiment, "A00000983", is highlighted under the "01C Baseline" folder. To the right of the tree view, an "Experiment Detail" box provides information for the selected experiment: "Experiment ID: EX00027", "Name: Diet Study 1", "Description: Feeding Study Time Course Human", "Project: Nutrition Studies", "Priority: High", and "Documents:". The browser's status bar at the bottom shows the URL and the system tray with the time "9:52 AM".

Metabolomic Library coverage

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

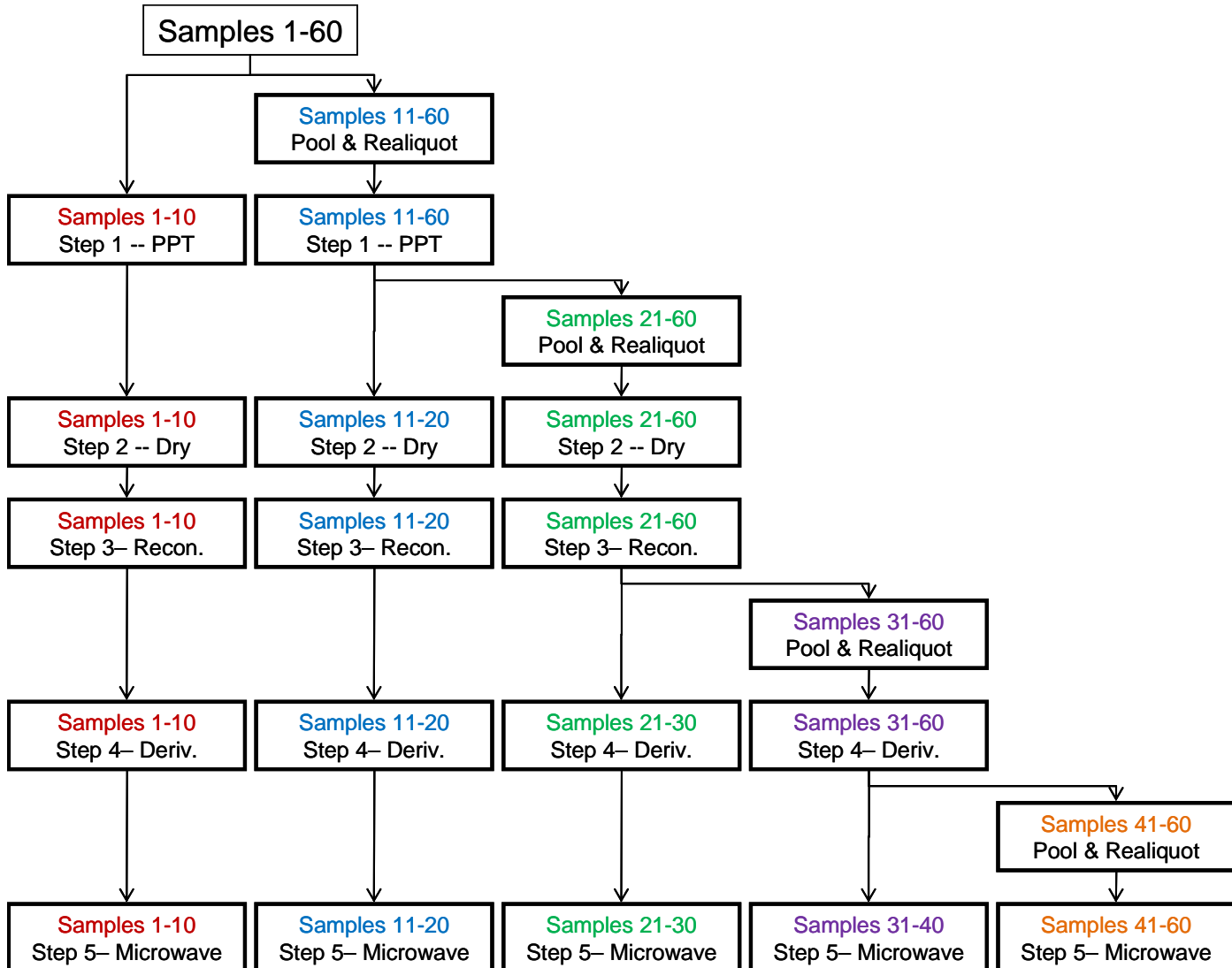


Variance Analyses

- 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



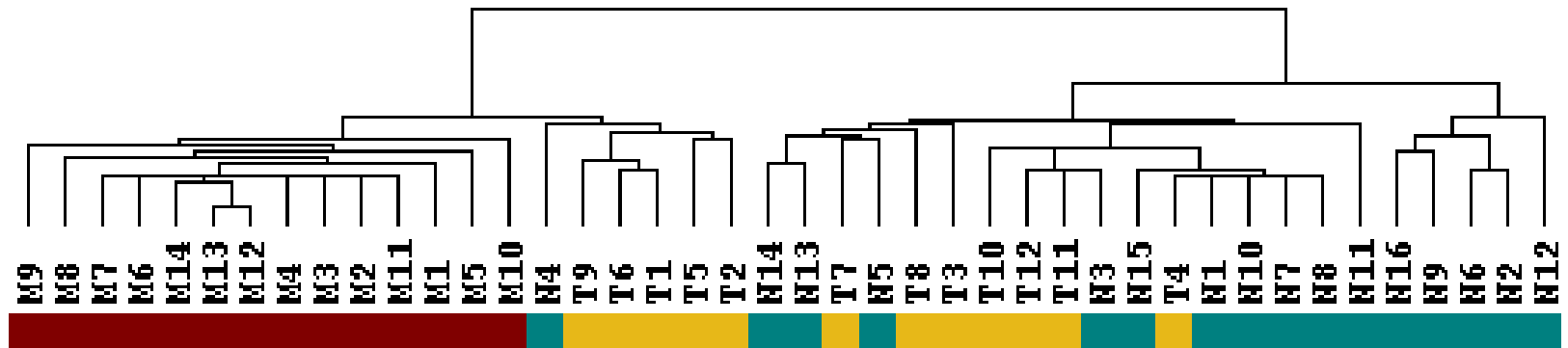
Summary

- 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



Hierarchical Analysis



-  Benign
-  Localized PCa
-  Metastatic Disease