NMR Data Analysis Exercise

UAB Metabolomics Training Course
July 17-21, 2016

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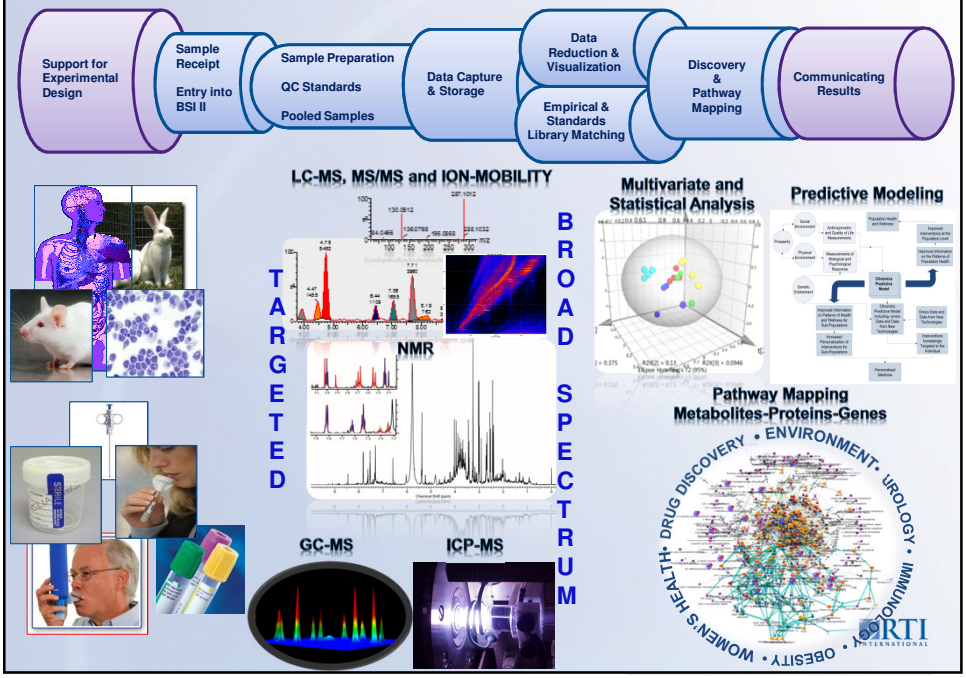
NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

www.rti.org

NIH Eastern Regional Comprehensive Metabolomics Resource Core at RTI



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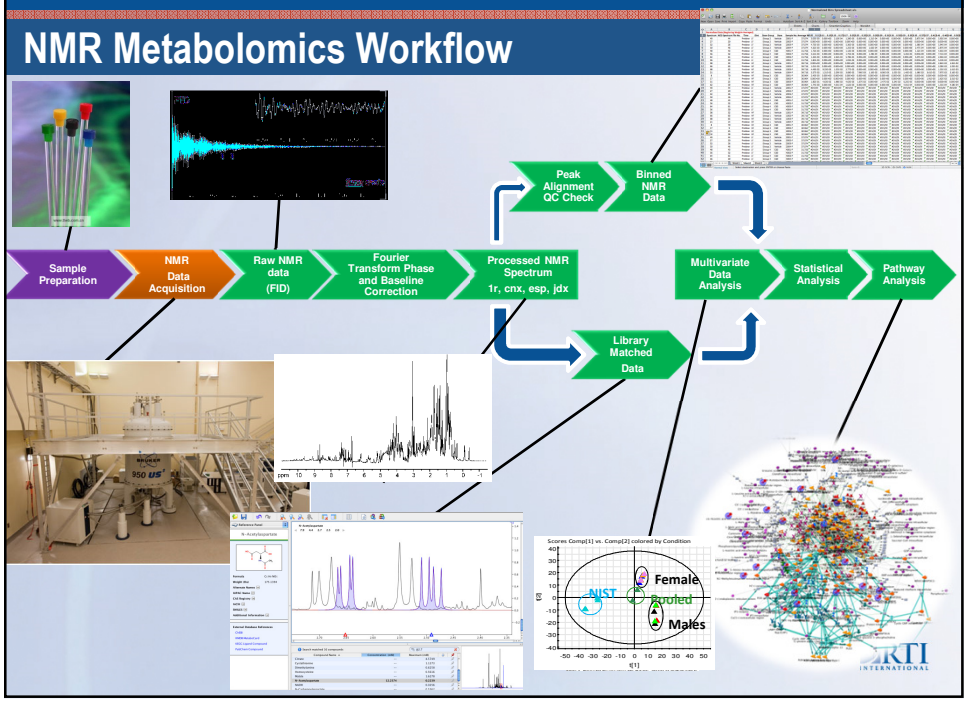
NMR Metabolomics Workflow



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NMR Metabolomics

- Broad Spectrum
  - High throughput
  - NMR Binning
  - Multivariate analysis and other statistics
  - Identifying bins important for separating study groups
  - Library matching of bins to metabolites
- Targeted Metabolomics
  - Identifying a set of metabolites
  - Quantifying metabolites
  - Multivariate analysis and other statistics
- Pathway analysis
  - Use identified metabolites
  - Use other omics data for integrated analysis

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Some Software available for NMR Based Metabolomics

FREE

- NMR Data Processing
  - ACD Software for Academics (ACD Labs, Toronto, Canada)
- Multivariate data analysis
  - MetaboAnalyst 3.0 (http://www.metaboanalyst.ca)
  - MetATT (http://metatt.metabolomics.ca/MetATT/)
  - MUMA (http://www.biomolnmr.org/software.html)
  - Other R-packages
- Library matching and Identification
  - BATMAN
  - Use of databases
    - Birmingham Metabolite library, HMDB, BMRB
- Pathway analysis
  - Metaboanalyst, metaP Server, Met-PA, Cytoscape, KEGG, IMPALA

Also available through www.metabolomicsworkbench.org

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Some Software Available for NMR Based Metabolomics

COMMERCIAL

- NMR Data-preprocessing
  - ACD Software (ACD Labs, Toronto, Canada)
  - Chenomx NMR Suite 8.1 Professional
- Multivariate data analysis
  - SIMCA 14
- Other statistical analysis
  - SAS, SPSS
- Library matching and quantification
  - Chenomx NMR Suite 8.1 Professional
- Pathway analysis
  - GeneGo (MetaCore Module)
  - Ingenuity Pathway Analysis (IPA)

NMR Hands On Exercise

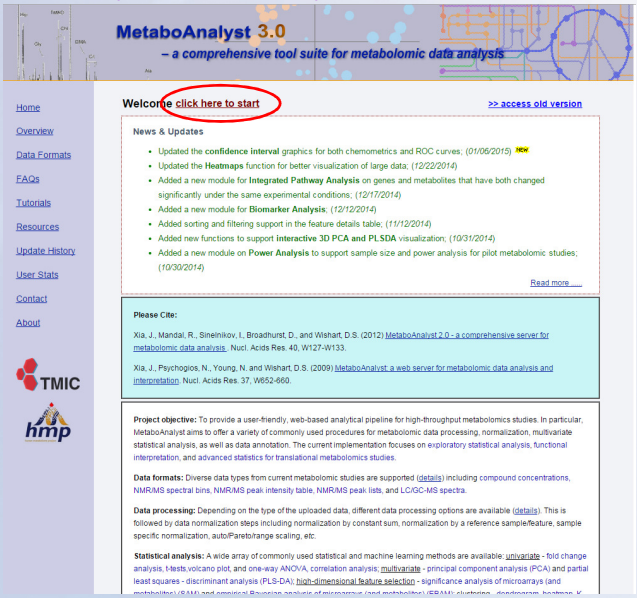
- Drug Induced Liver Injury (DILI) Study using Rat Model
- 3 Study groups and 2 time points
  - Vehicle Control (time matched)
  - Low Dose ("no effect" level, Day 01 and Day 14)
  - High Dose (Day 01 and Day 14)
- 24h Urine collected
- Samples prepared by mixing an aliquot of urine with Phosphate buffer + Chenomx ISTD (DSS, D_2O, and Imidazole)
  - DSS (Chemical shift and line shape reference)
  - Imidazole (pH reference)

Binned Data

- Three (3) Spreadsheets provided
  1. UAB_RFA_Metaboanalyst.csv
  2. UAB_RFA_Metaboanalyst_D14_NoPools.csv
  3. UAB_RFA_Metaboanalyst_D14_Vehicle_vs_HighDose.csv
- Spreadsheets 2-3 were derived from the initial spreadsheet no. 1 (for easy upload into Metaboanalyst in the subsequent analyses)

MetaboAnalyst 3.0

Please go to the webpage:
http://www.metaboanalyst.ca/MetaboAnalyst/



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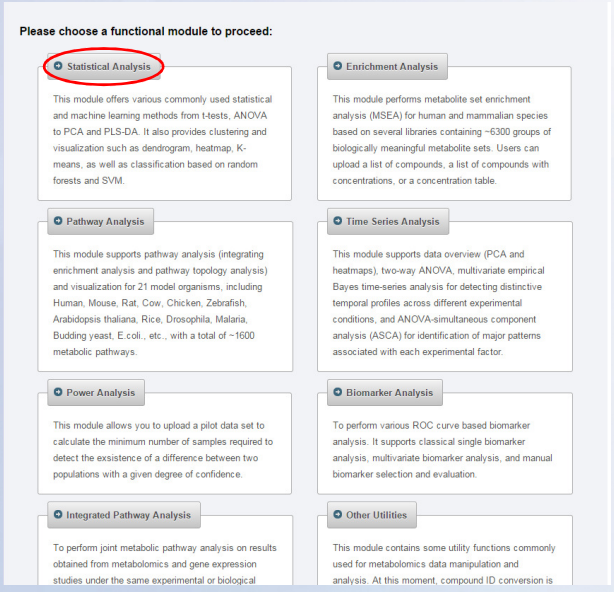
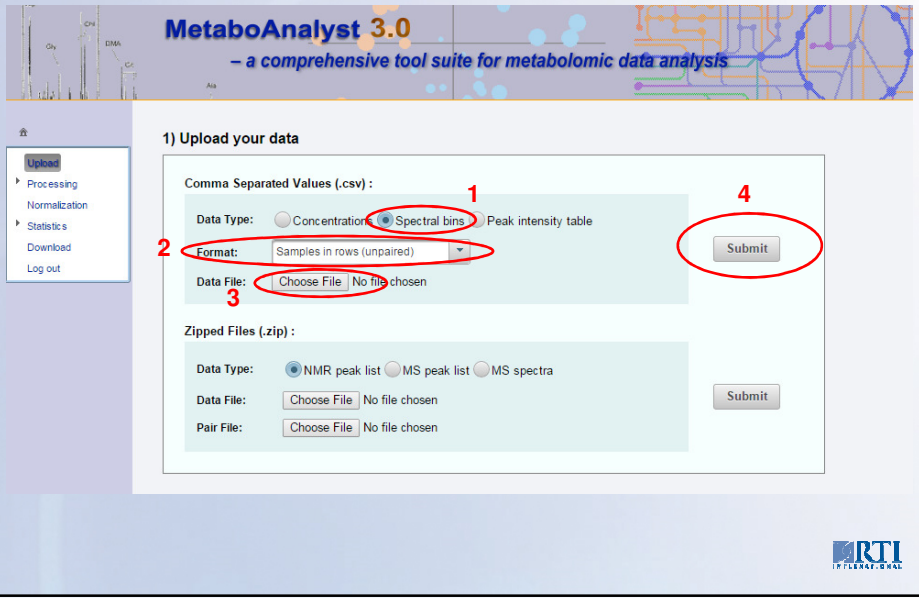
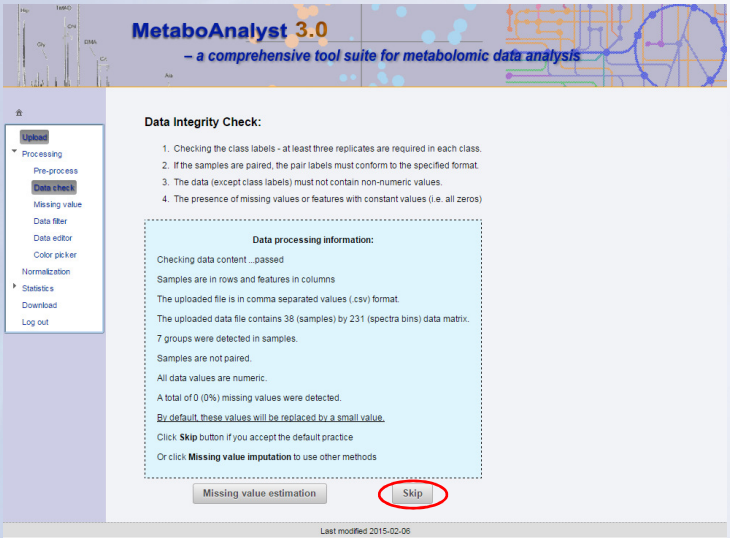
MetaboAnalyst: Functional Modules



Data Upload



Data Integrity Check



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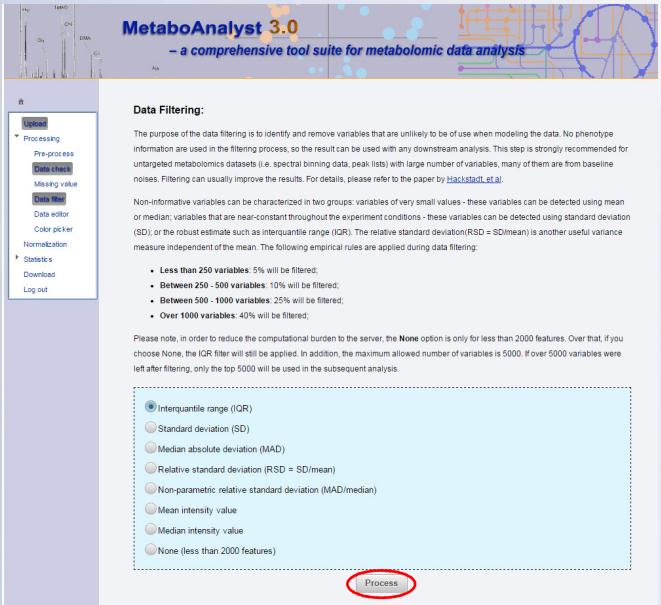
Data Filtering



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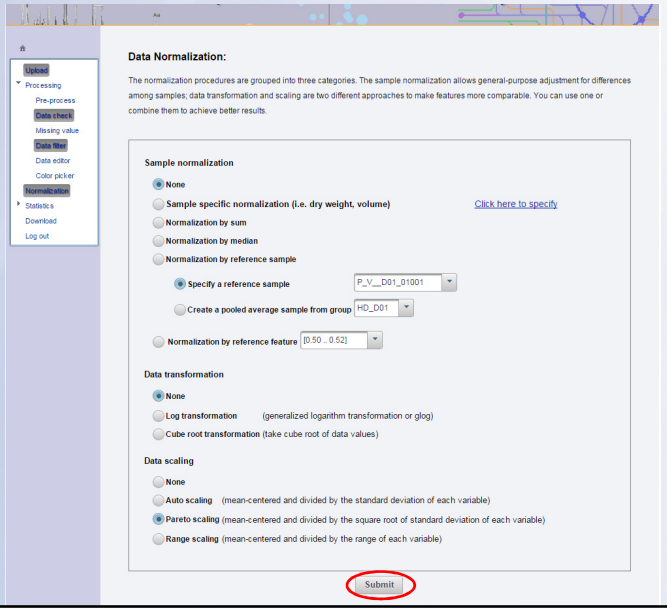
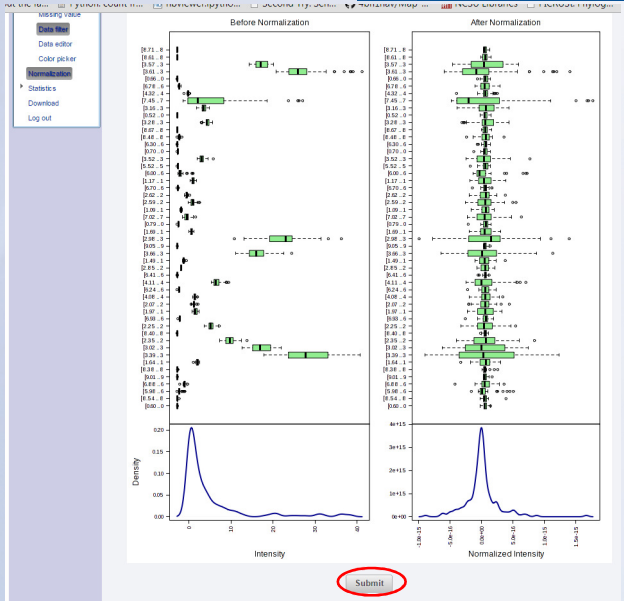
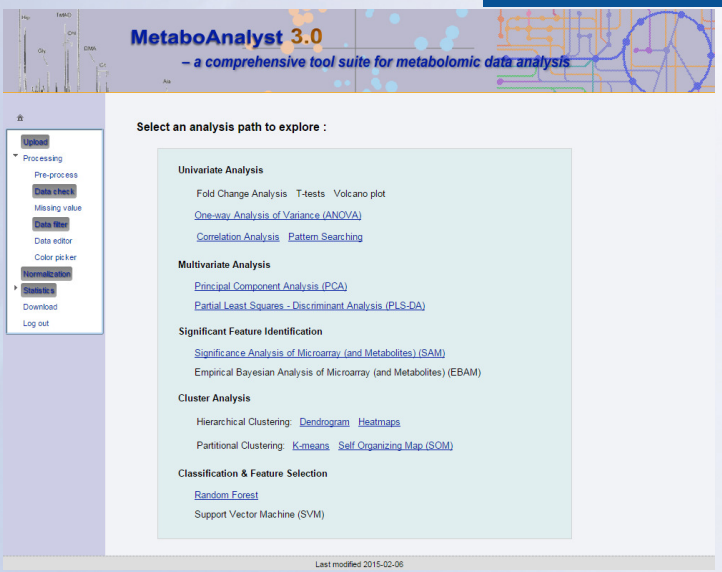
Data Normalization



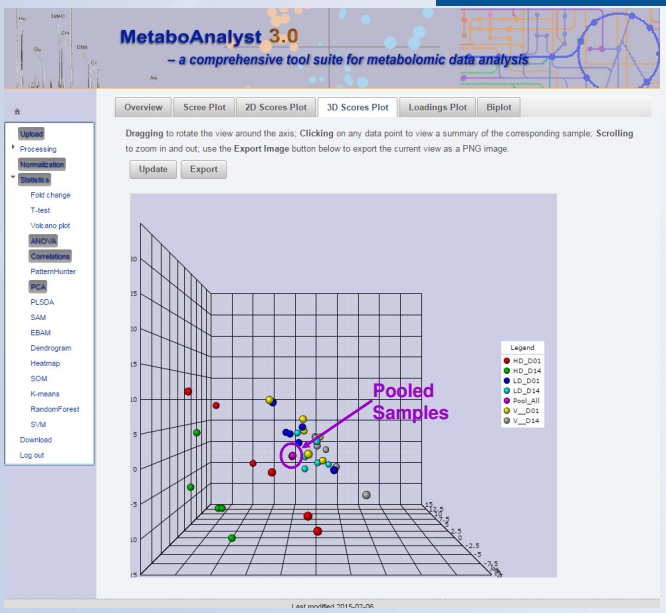
Summary: Normalization



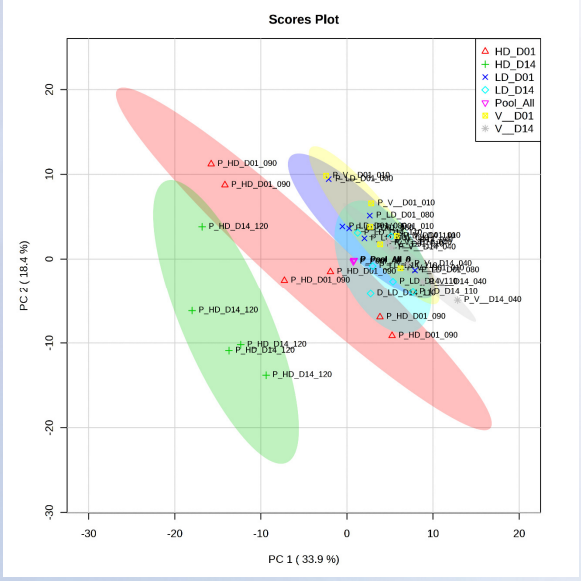
Statistical Analysis



Pooled QC Samples



PCA Day 01 and Day 14



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Day 14: Vehicle, Low Dose, and High Dose Groups

Please go back to the start page and upload the data

- We will compare high dose vs vehicle
  - 2. UAB_RFA_Metaboanalyst_D14_NoPools.csv
- Perform PCA
- Perform PLS-DA
- Heat map

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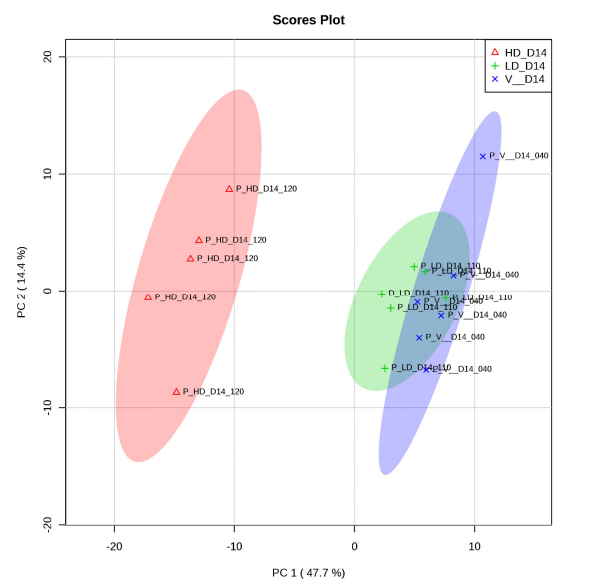
Vehicle, Low Dose, and High Dose groups

Day 14 PCA Scores Plot



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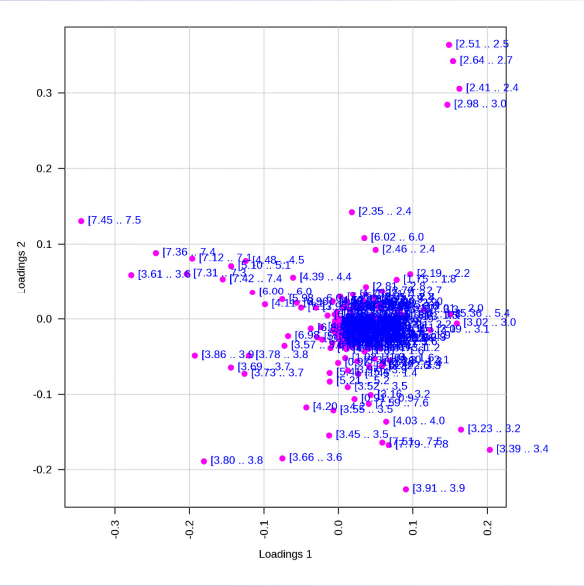
Vehicle, Low Dose, and High Dose groups

PCA Loadings Plot



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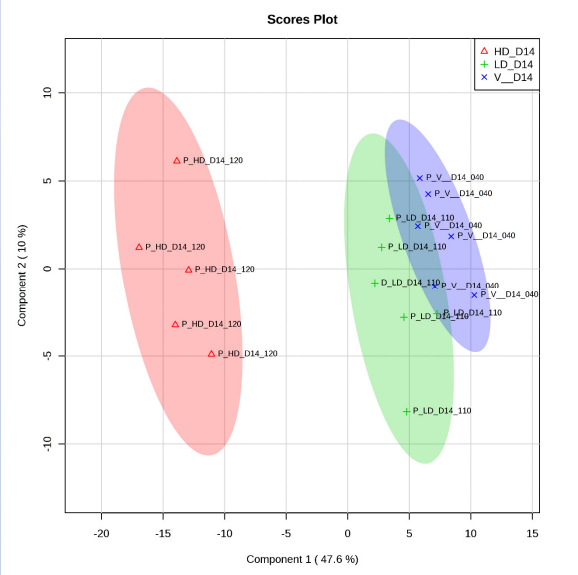
Vehicle, Low Dose, and High Dose groups

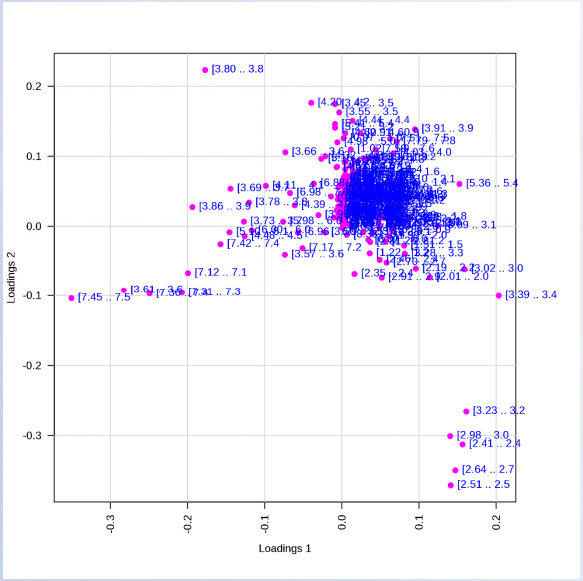
PLS-DA Scores Plot



RTI International

Vehicle, Low Dose, and High Dose groups

PLS-DA Loadings Plot



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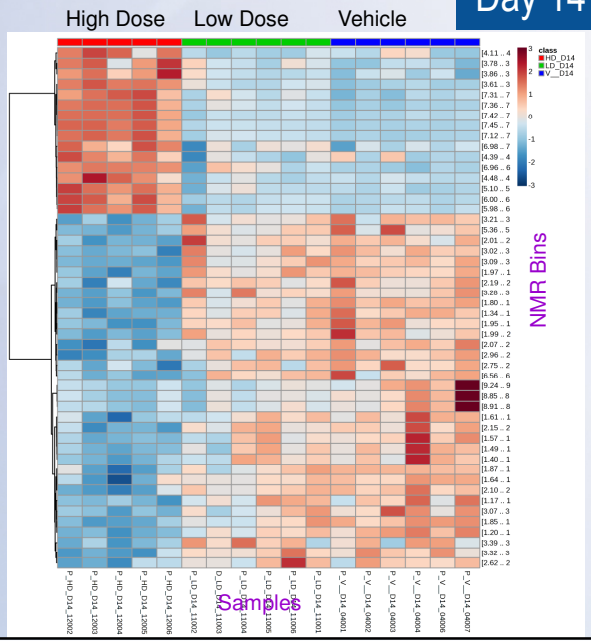
Day 14 Heat Map

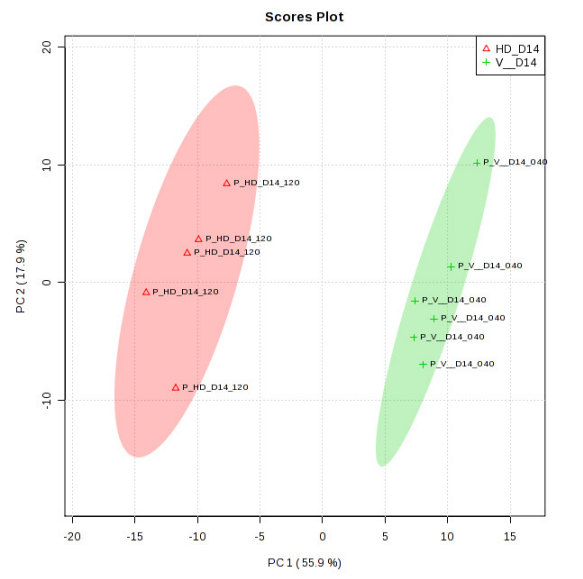


Comparison of Day 14 High Dose and Vehicle

Please start from the start page and upload the data

- We will compare high dose vs vehicle
  - 3. UAB_RFA_Metaboanalyst_D14_Vehicle_vs_HighDose.csv
- Perform PCA
- Perform PLS-DA
- VIP Plot
- Heat map

Day 14 PCA Scores Plot: High Dose vs Vehicle



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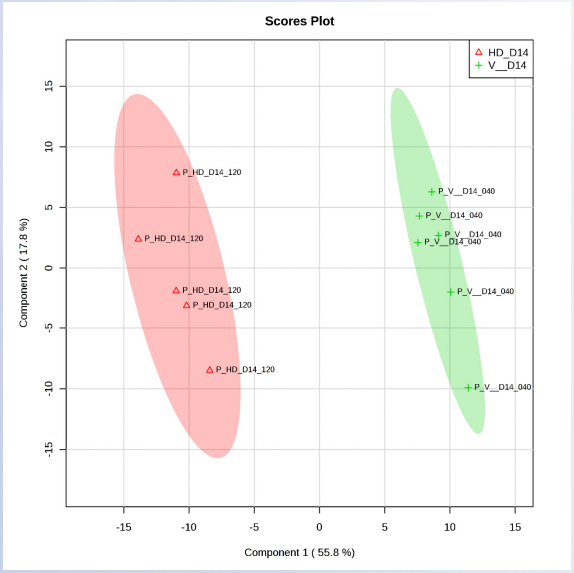
Day 14 PLS-DA Scores Plot: High Dose vs Vehicle



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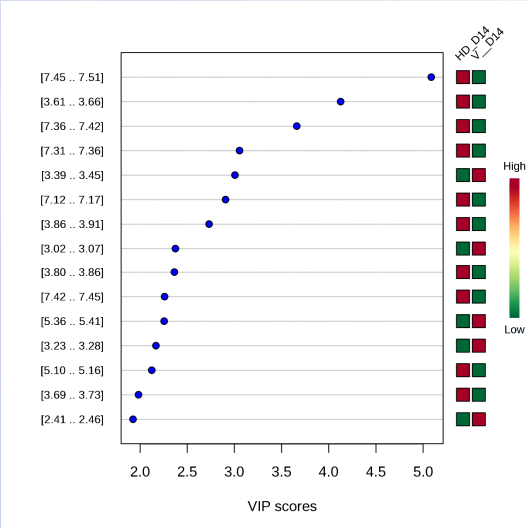
Day 14 PLS-DA VIP Plot: High Dose vs Vehicle



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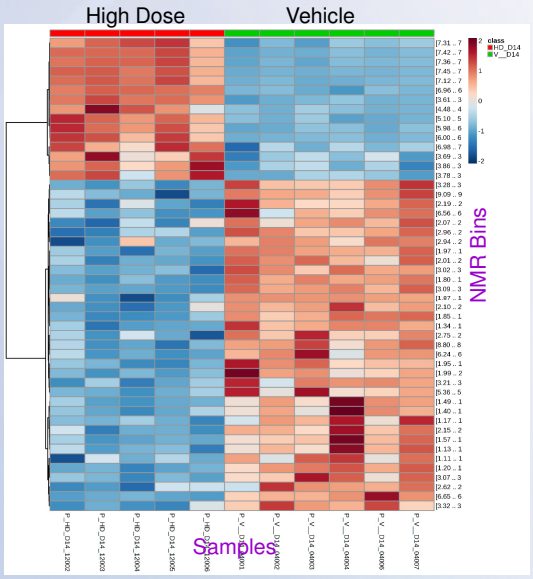
Day 14 Heat Map: High Dose vs Vehicle



Top 50 bins in the VIP Plot

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STS Center


Susan Sumner


Wimal Pathmasiri NMR & GC-MS
Jim Carlson LC- and GC-MS
Jessica Gooding LC-MS
Kelly Mercier NMR


Susan McRitchie Data Analysis


Zach Acuff Biostatistics


Bob Clark Genetics


Jason Burgess Program Coordinator

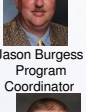

Andrew Novokhatny NMR and QC
Aurora Cabrera LC-MS/MS
Jocelin Spruill GC-MS Neurotransmitter


Tammy Cavallo Biology and QC


Delisha Stewart Cell Biology


Ninell Mortensen Microbiology


Maria Moreno Biology


Keith Levine Metallomics

Rose Ewald intern


Tim Fennell Metabolism


Yuanyuan Li LC-MS


Rod Snyder LC-MS


Sherry Black *In vivo and in vitro* Metabolism

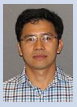

Scott Watson Neurotranmitter LC/MS


Skip Gaudette Systems


Puvi Patel In vitro metabolsm


Yan Lan Yueh LC-MS


Sue Clark *Administrative Support*


Hieu, Vu LC-MS

Melody Markley Model Systems

Courtney Whitaker LC/MS

Thank You!

If you have any questions, please e-mail me

wpathmasiri@rti.org

Useful link:
Metabolomics Workbench
http://www.metabolomicsworkbench.org/