NMR Data Analysis Exercise

UAB Metabolomics Training Course
July 17-21, 2017

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Department of Nutrition – Nutrition Research Institute
University of North Carolina at Chapel Hill

NIH Eastern Regional Comprehensive Metabolomics Resource Core at NRI

Support for Experimental Design

Sample Receipt

Entry into BSI II

Sample Preparation

QC Standards

Pooled Samples

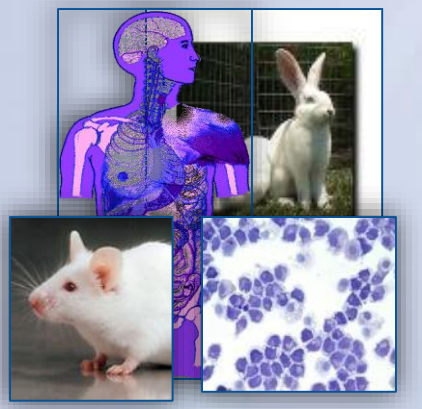
Data Capture & Storage

Data Reduction & Visualization

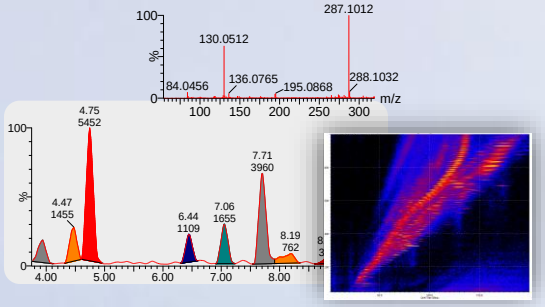
Empirical & Standards Library Matching

Discovery & Pathway Mapping

Communicating Results



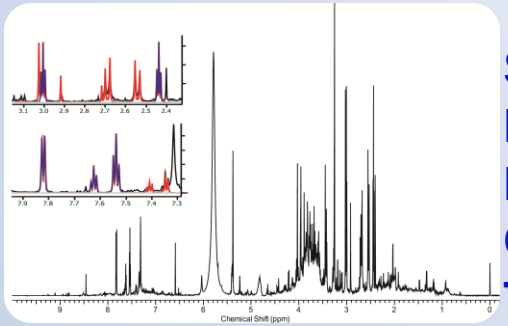
LC-MS, MS/MS and ION-MOBILITY



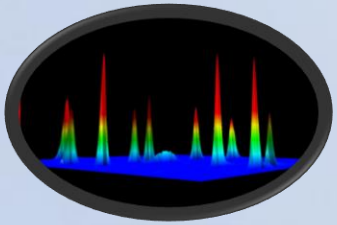
TARGETED

BROAD SPECTRUM

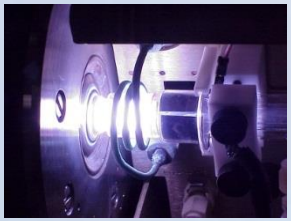
NMR



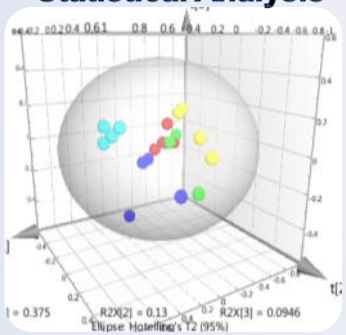
GC-MS



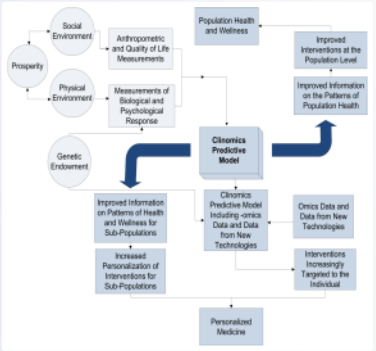
ICP-MS



Multivariate and Statistical Analysis

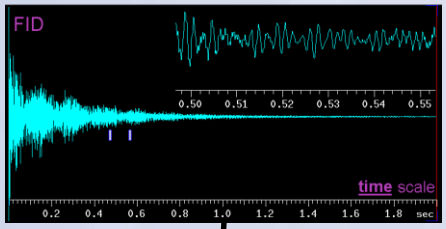


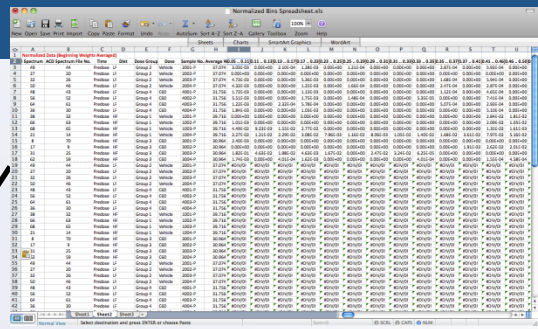
Predictive Modeling



Pathway Mapping Metabolites-Proteins-Genes

NMR Metabolomics Workflow





Sample Preparation

NMR Data Acquisition

Raw NMR data (FID)

Fourier Transform Phase and Baseline Correction

Processed NMR Spectrum 1r, cnx, esp, jdx

Peak Alignment QC Check

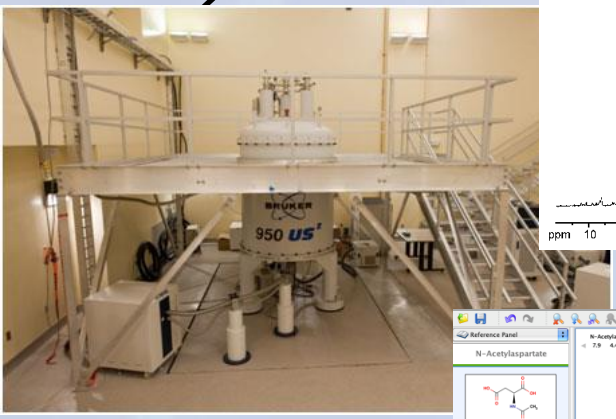
Binned NMR Data

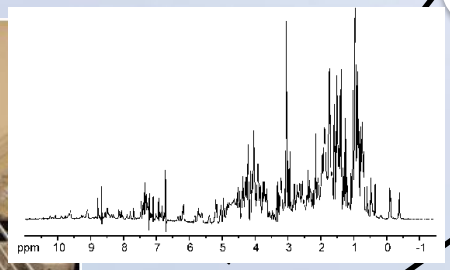
Multivariate Data Analysis

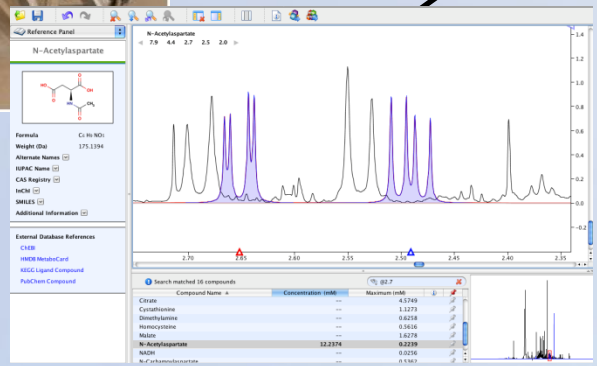
Statistical Analysis

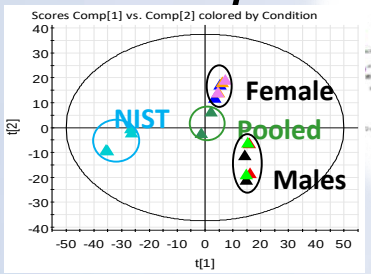
Pathway Analysis

Library Matched Data









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

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, MetScape 3 for Cytoscape, metaP-Server,, KEGG, IMPALA

Also available through www.metabolomicsworkbench.org

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)

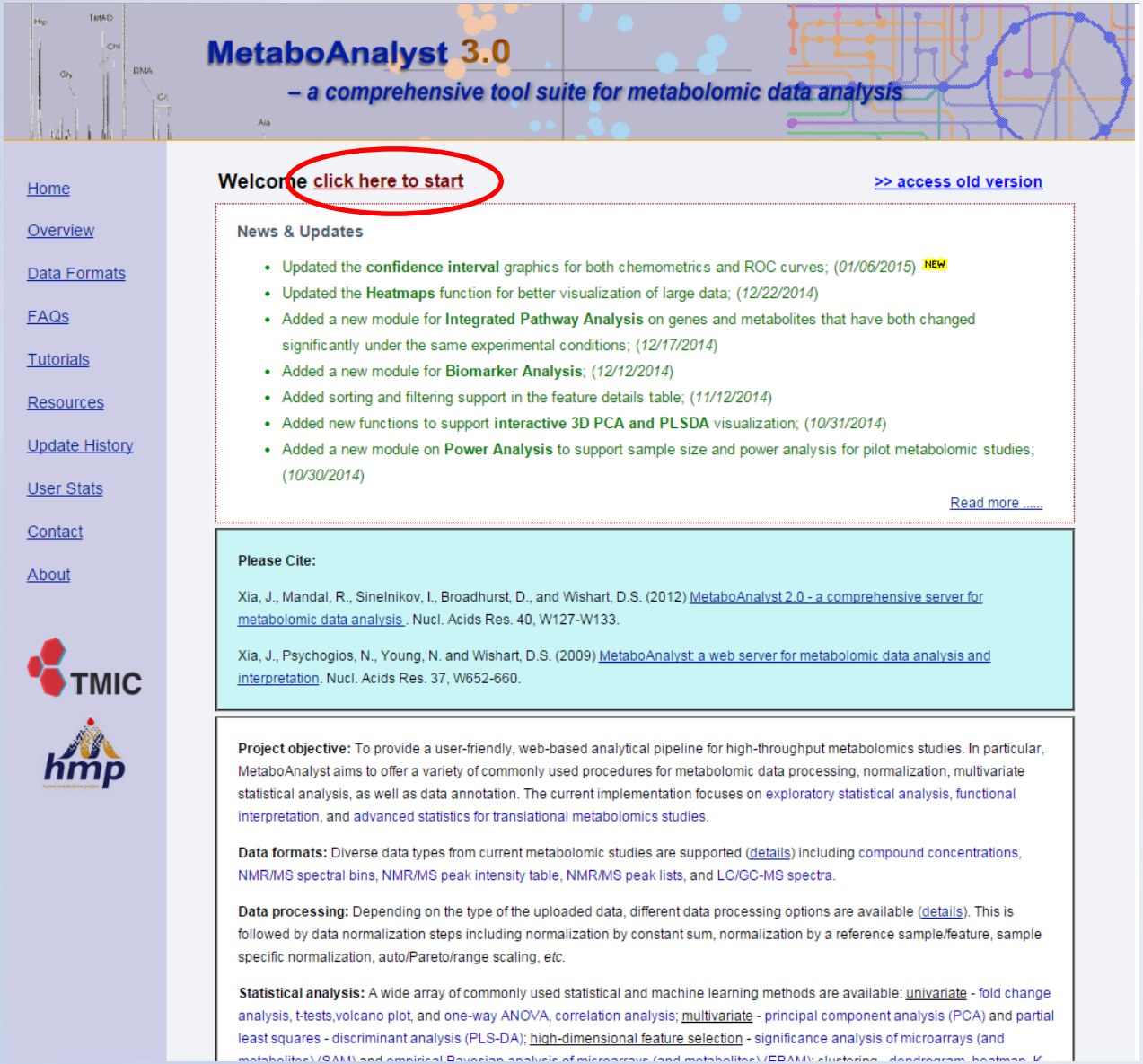
Binned Data

- 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

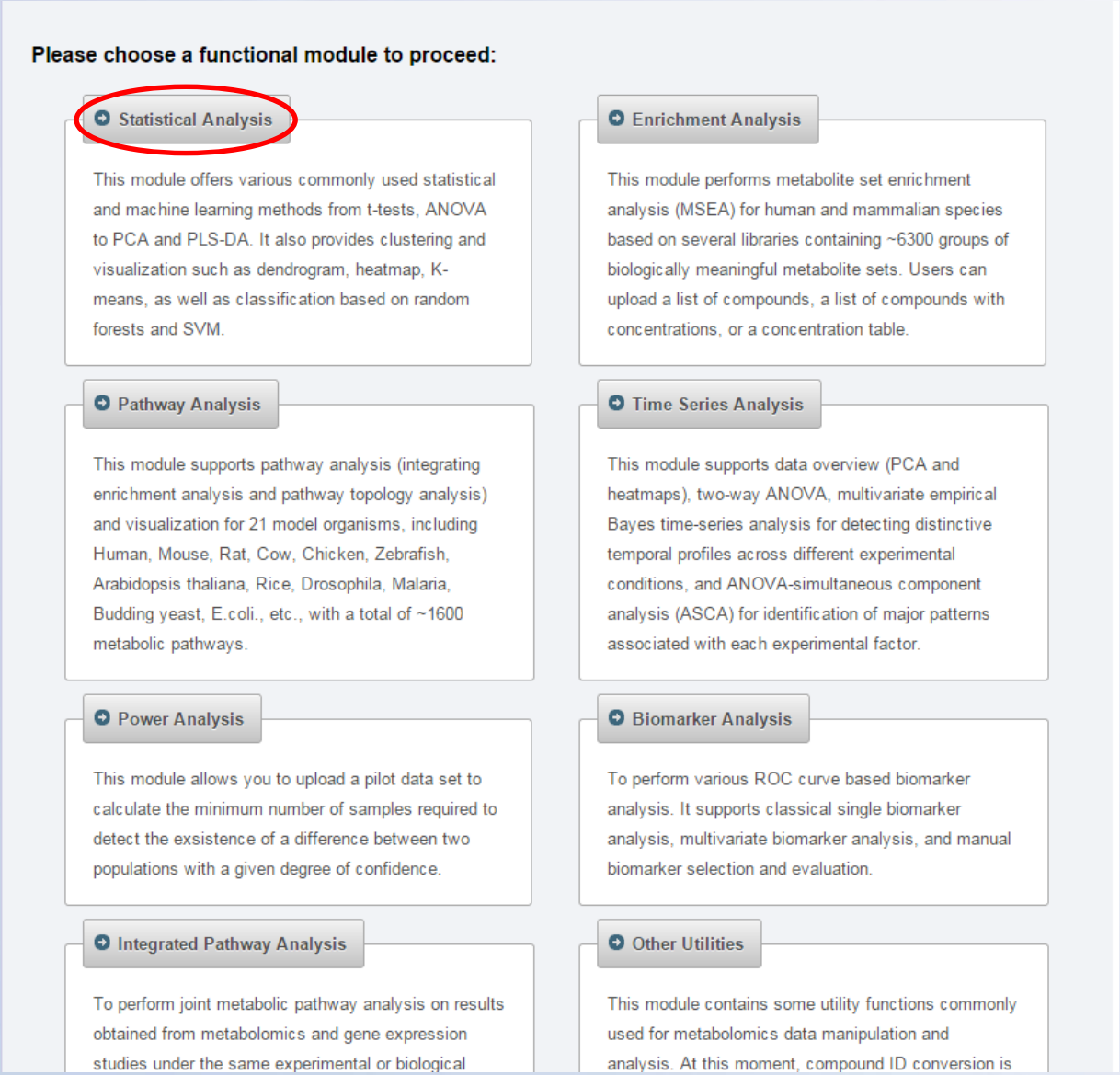
- Spreadsheets 2-3 were derived from the initial spreadsheet no. 1 (for easy upload into MetaboAnalyst in the subsequent analyses)

MetaboAnalyst 3.0

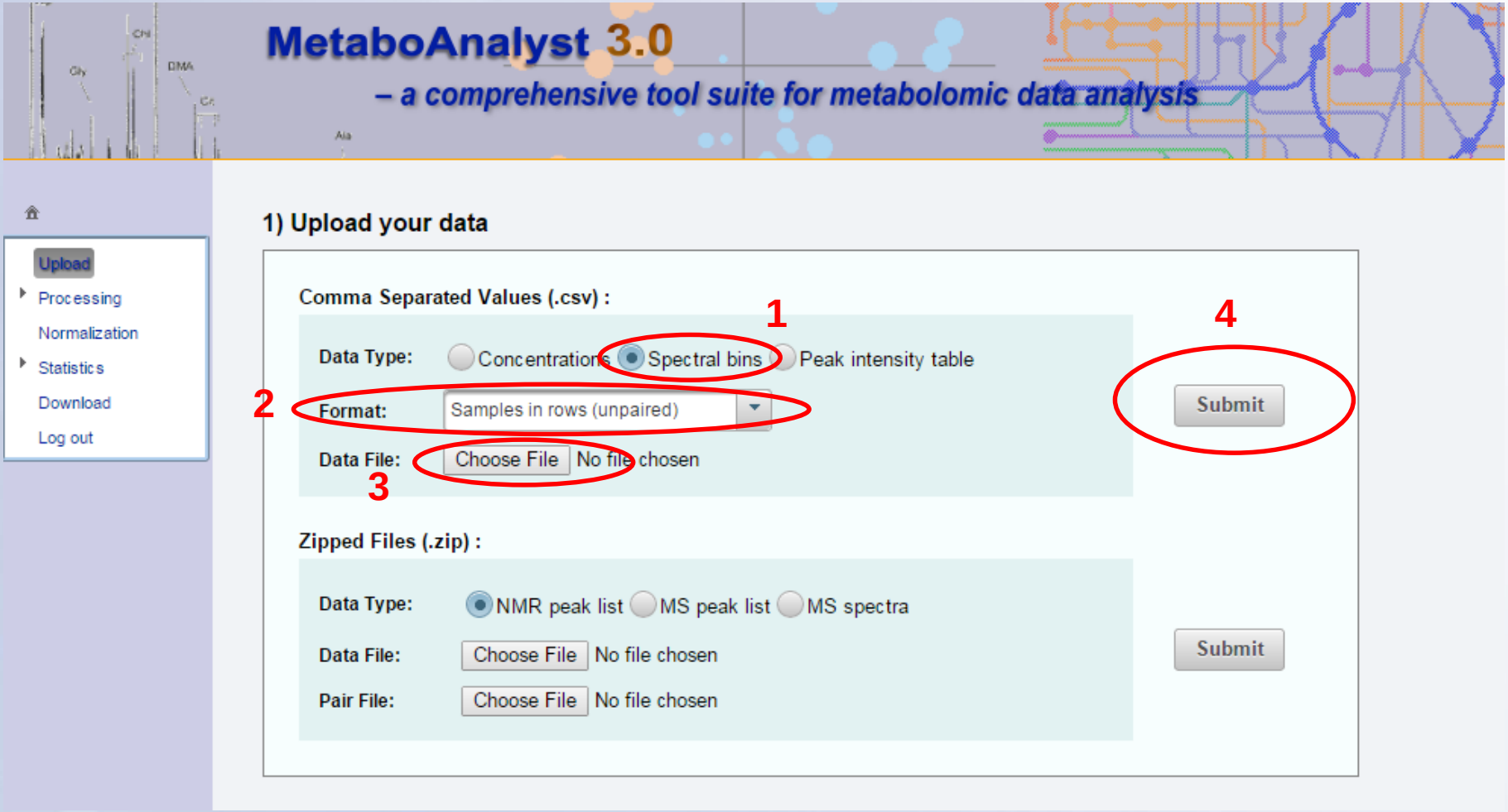
Please go to the webpage: http://www.metaboanalyst.ca/MetaboAnalyst/



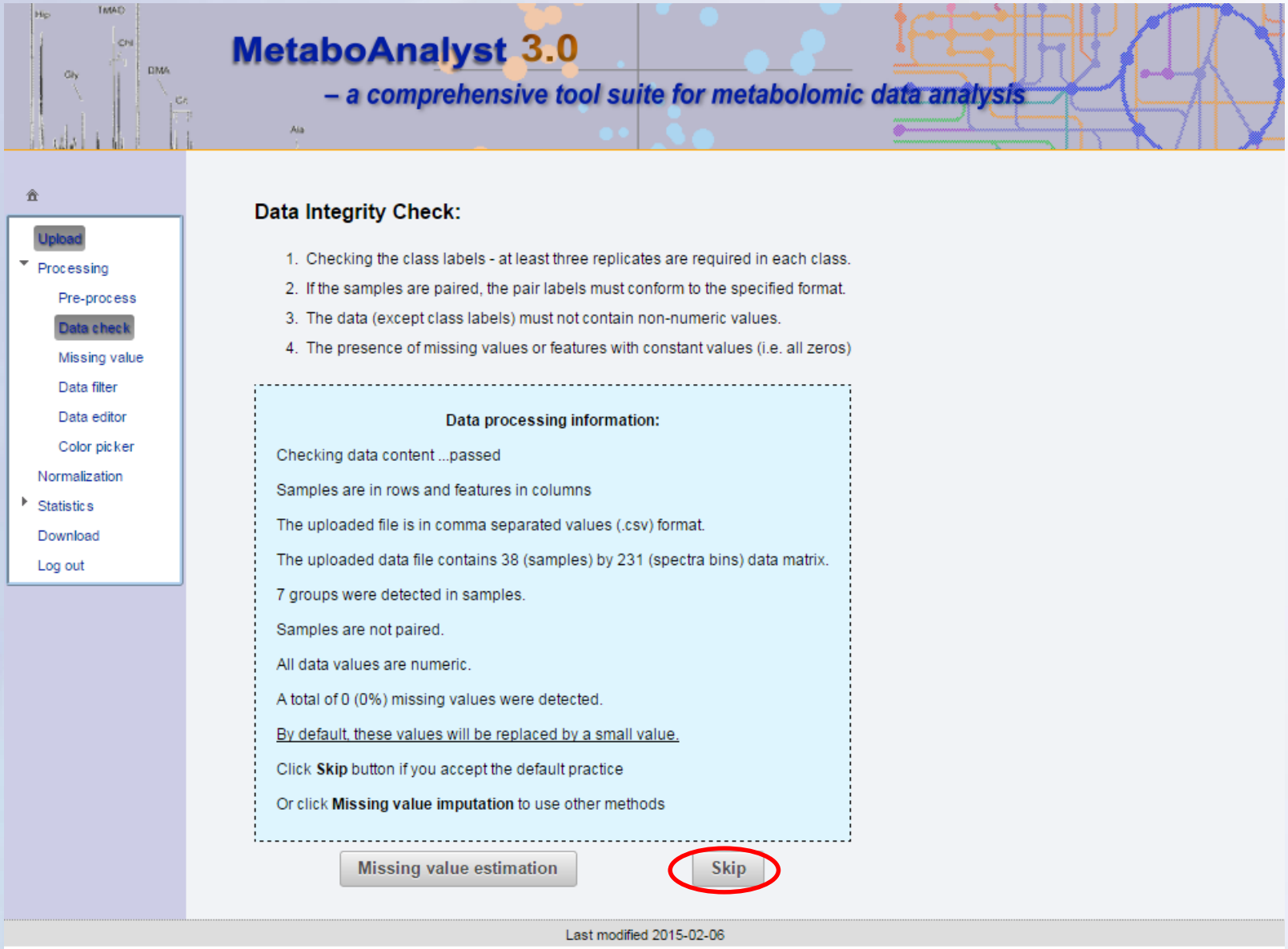
MetaboAnalyst: Functional Modules



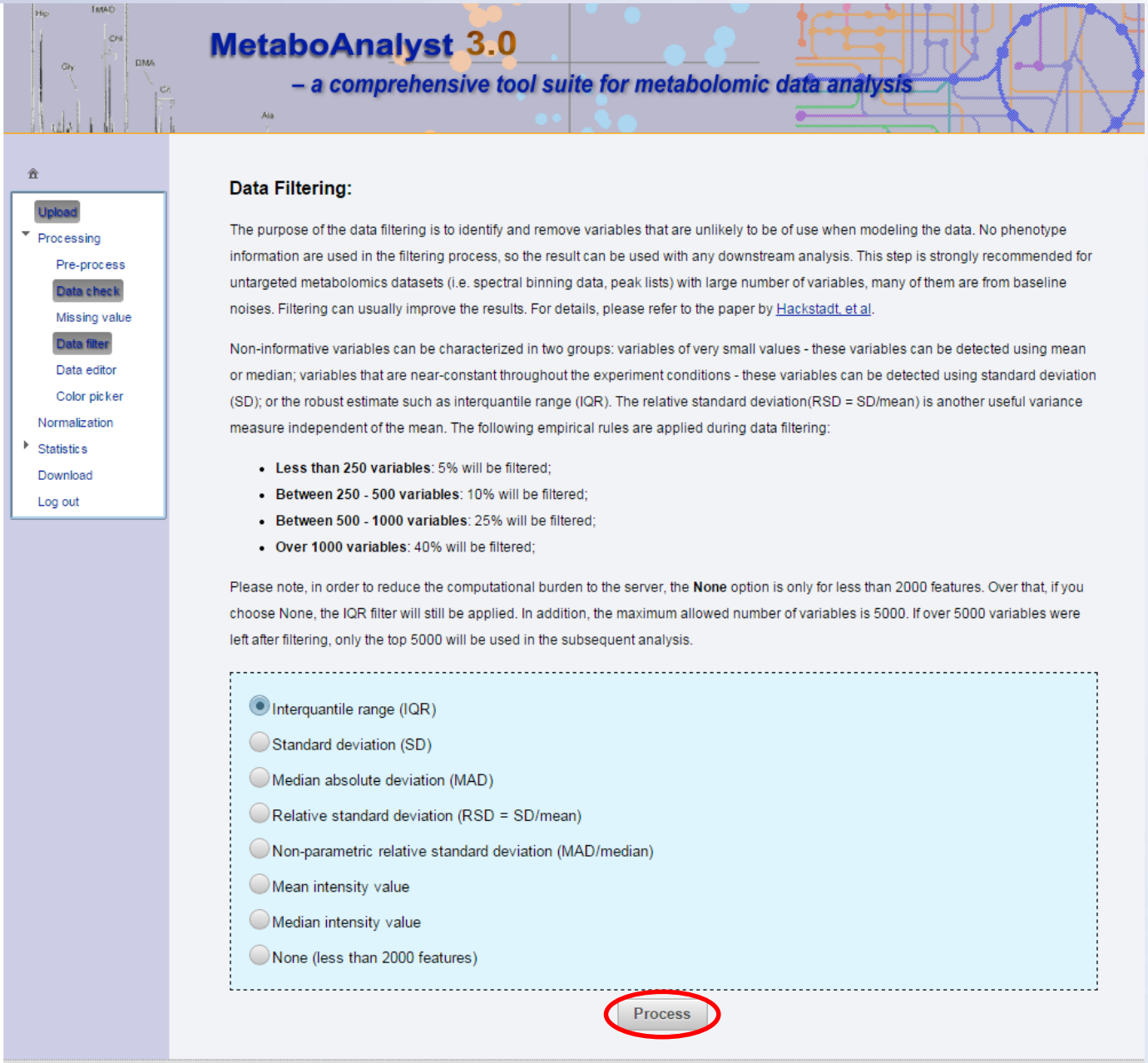
Data Upload


MetaboAnalyst 3.0
– a comprehensive tool suite for metabolomic data analysis
Upload
Processing
Normalization
Statistics
Download
Log out
1) Upload your data
Comma Separated Values (.csv) :
Data Type:
Concentrations
Spectral bins
Peak intensity table
Format:
Samples in rows (unpaired)
Data File:
Choose File
No file chosen
Submit
Zipped Files (.zip) :
Data Type:
NMR peak list
MS peak list
MS spectra
Data File:
Choose File
No file chosen
Pair File:
Choose File
No file chosen
Submit
1
2
3
4

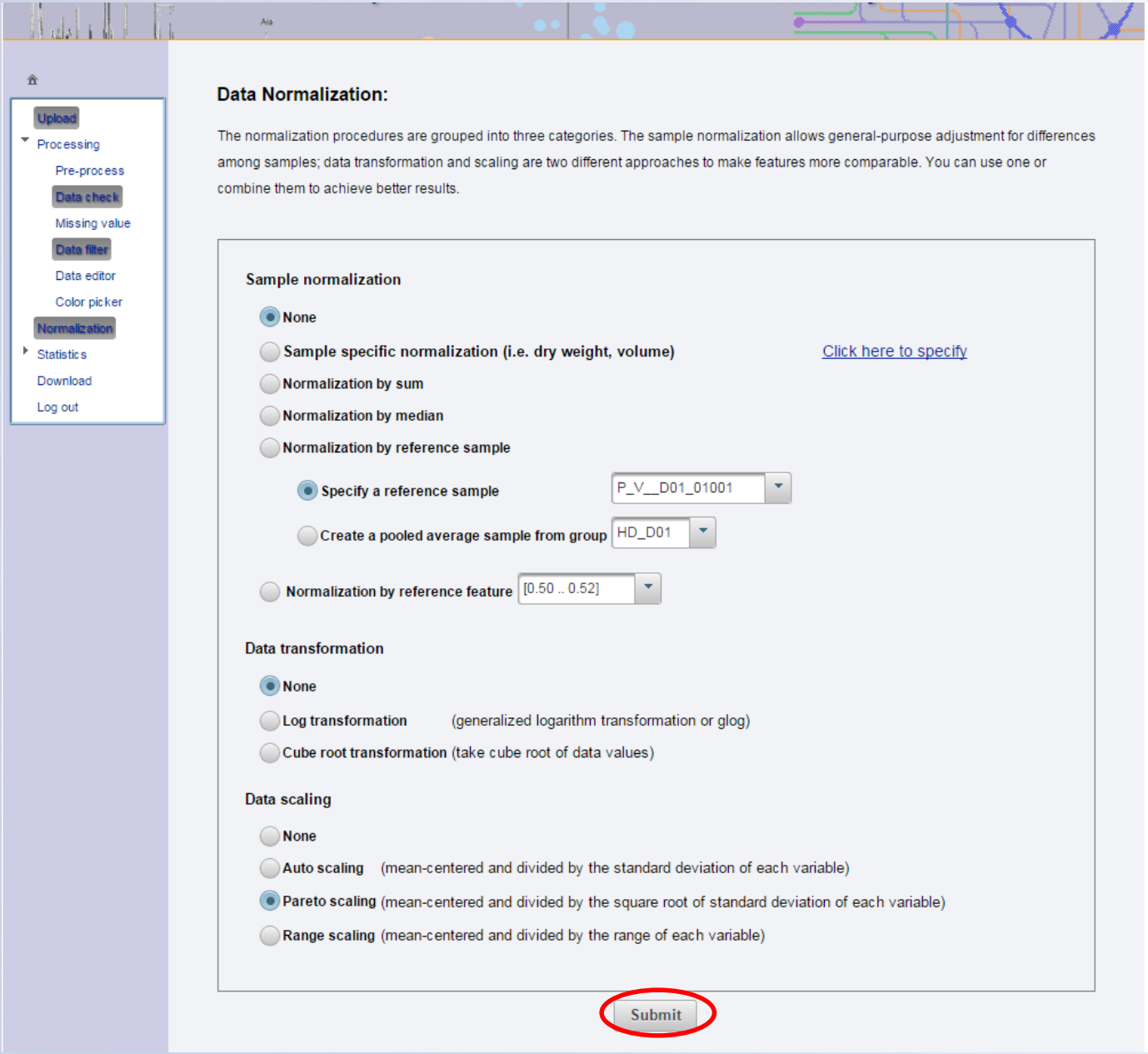
Data Integrity Check



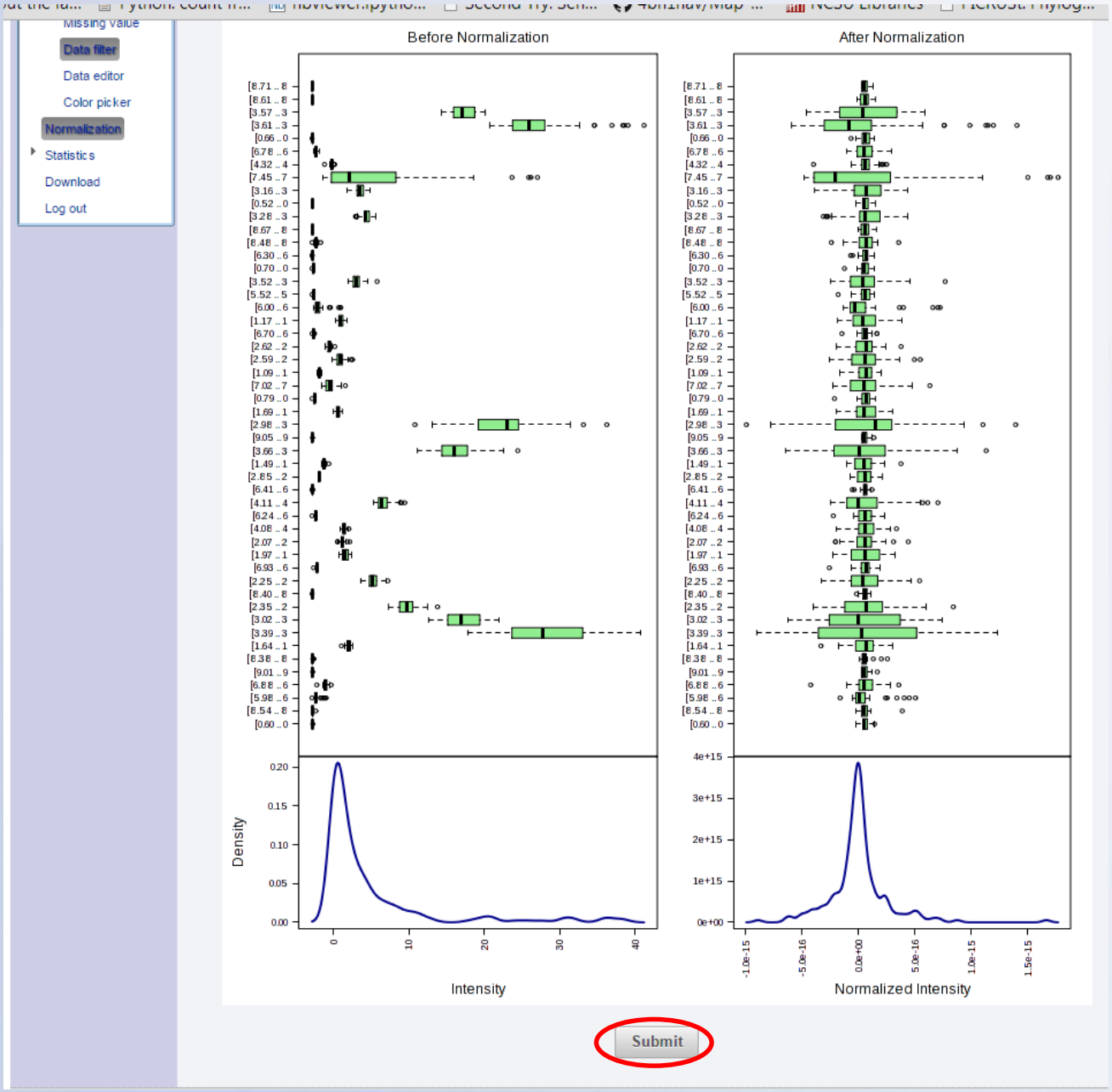
Data Filtering



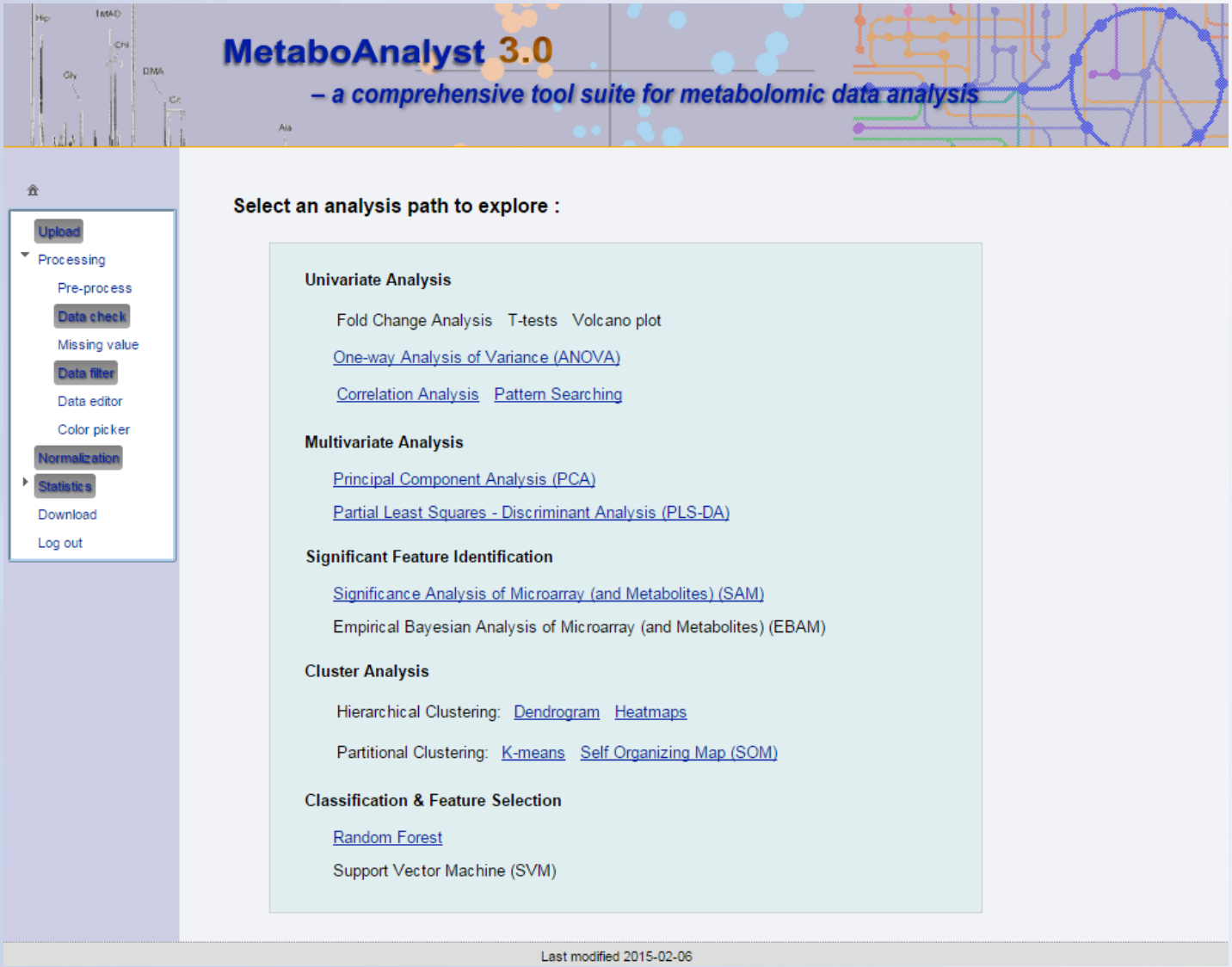
Data Normalization



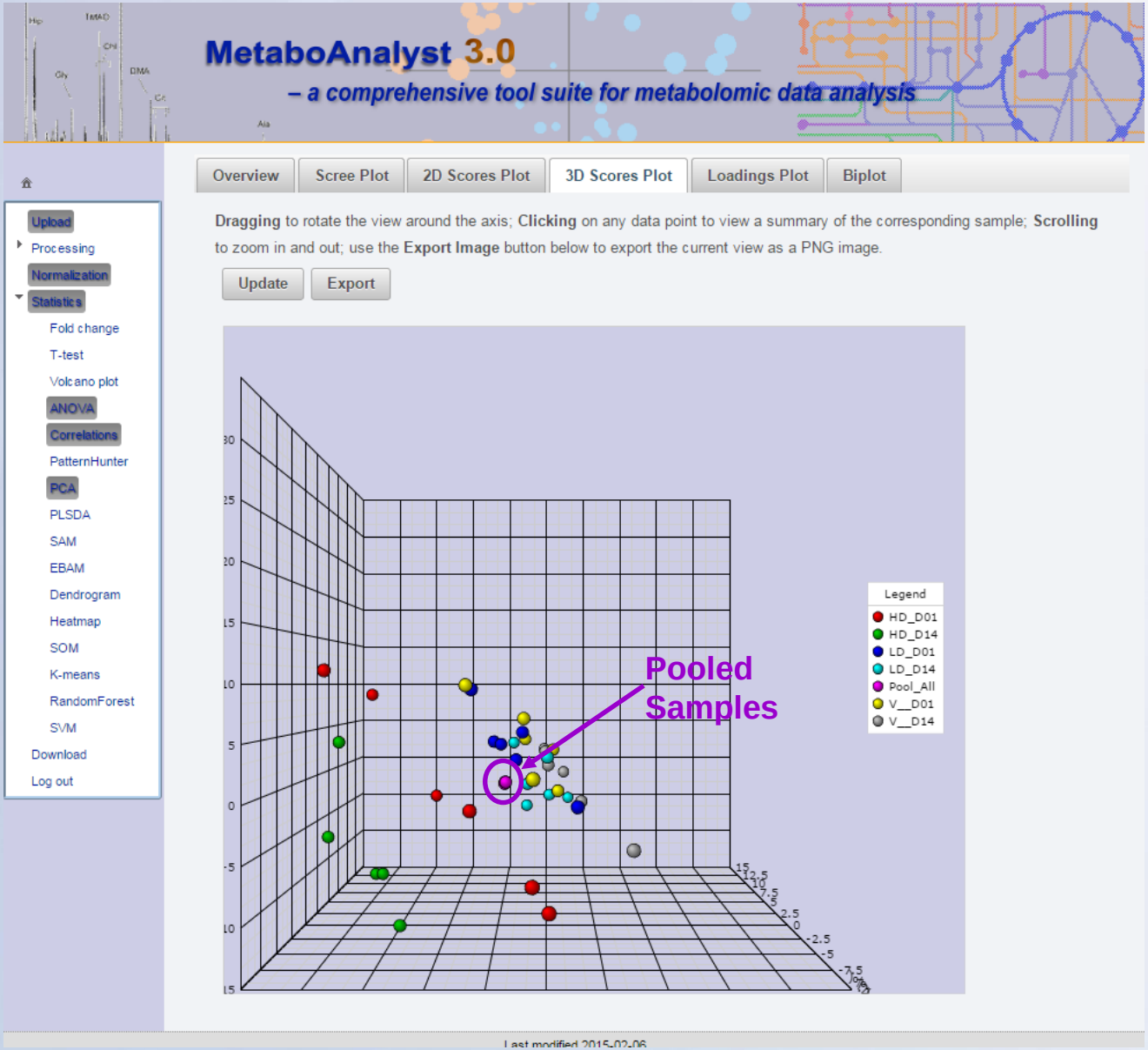
Summary: Normalization



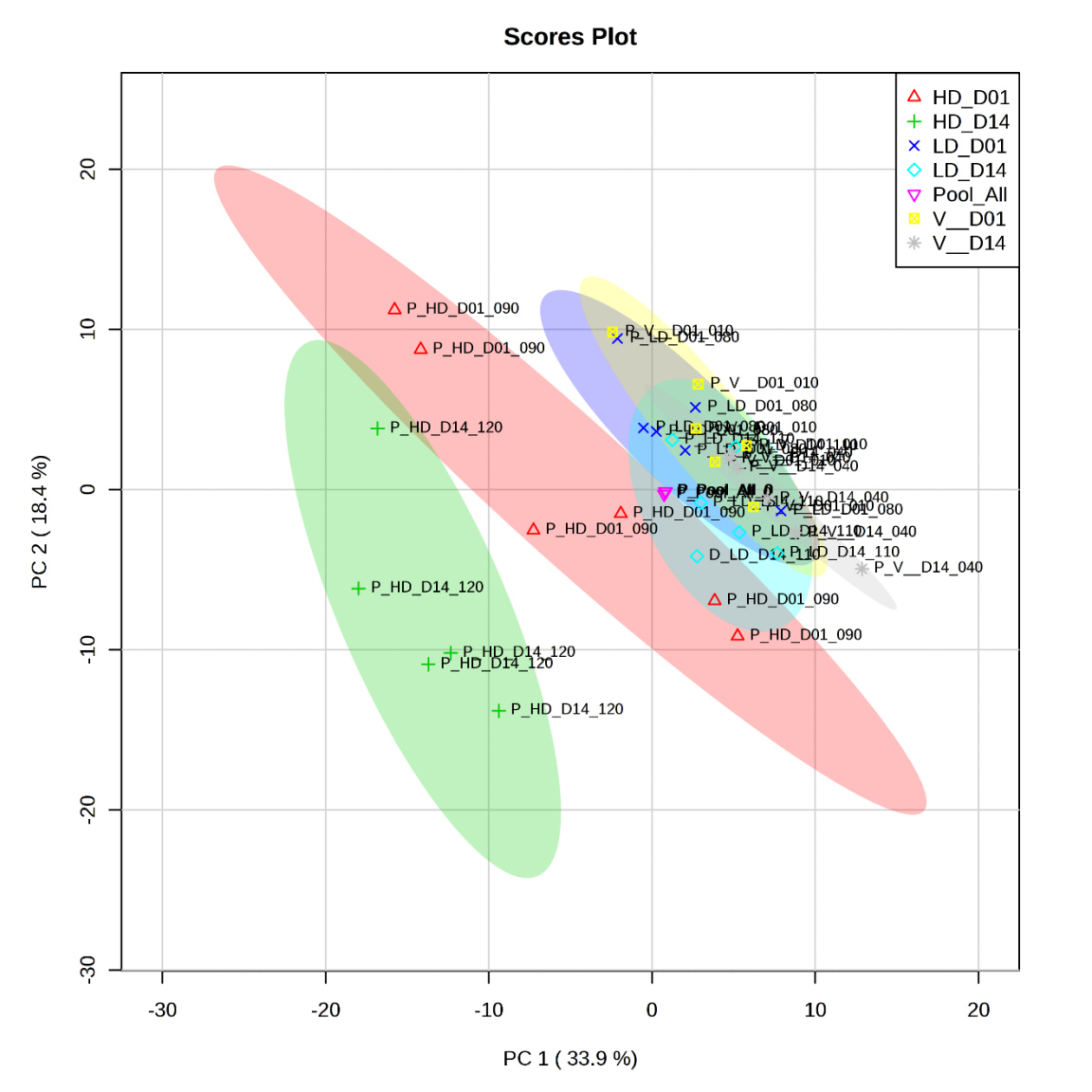
Statistical Analysis



Pooled QC Samples



PCA Day 01 and Day 14



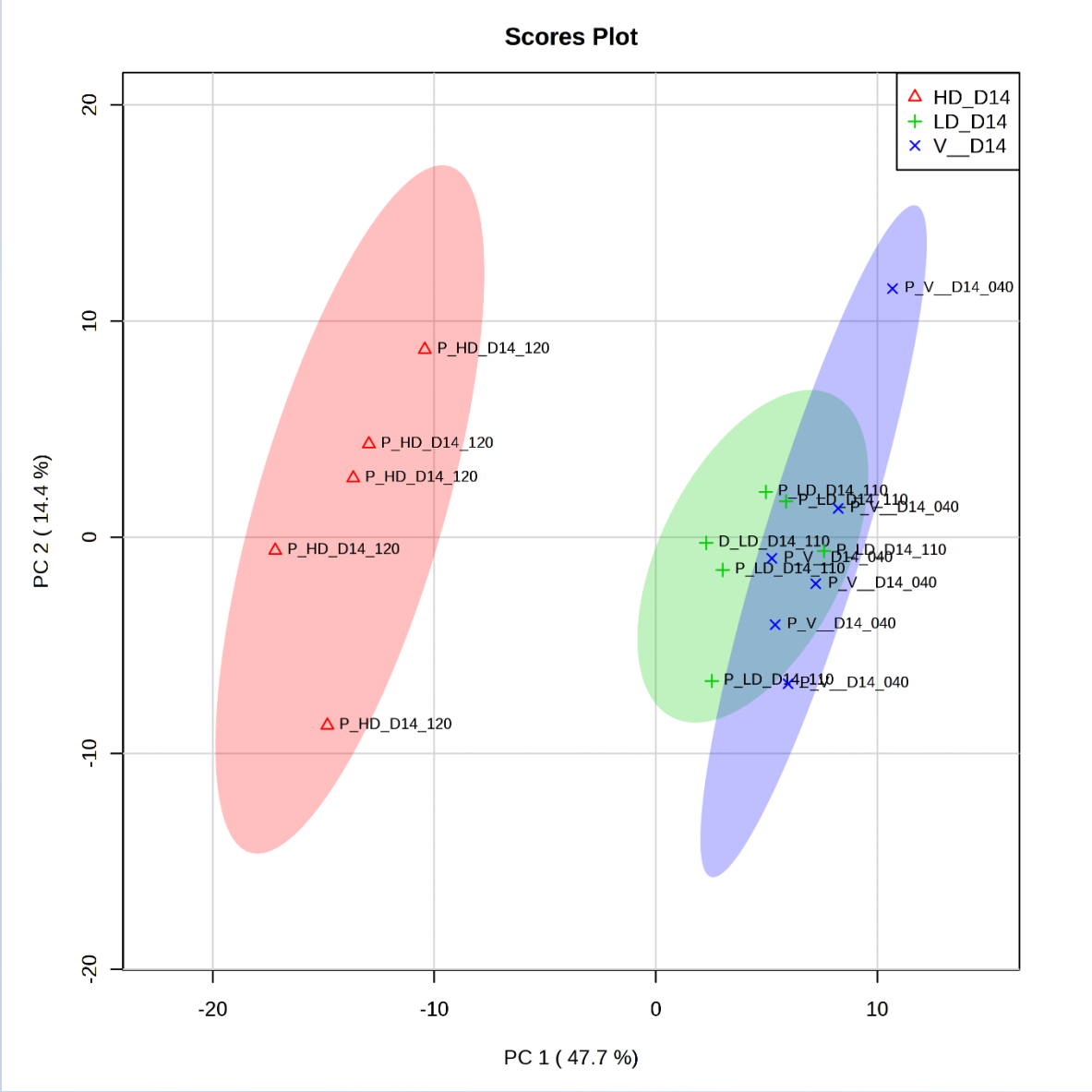
Day 14: Vehicle, Low Dose, and High Dose Groups

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

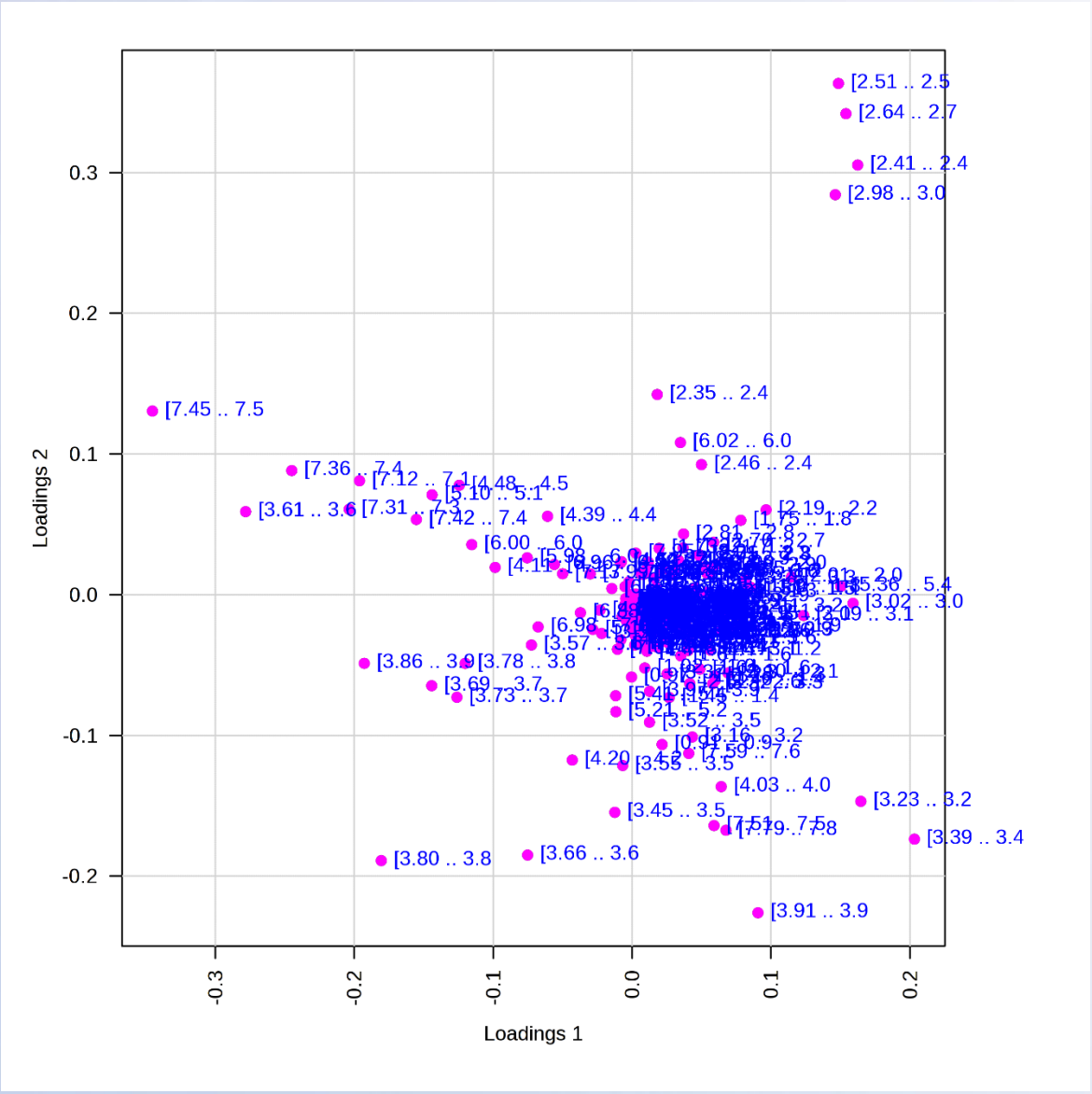
Day 14 PCA Scores Plot

Vehicle, Low Dose, and High Dose groups



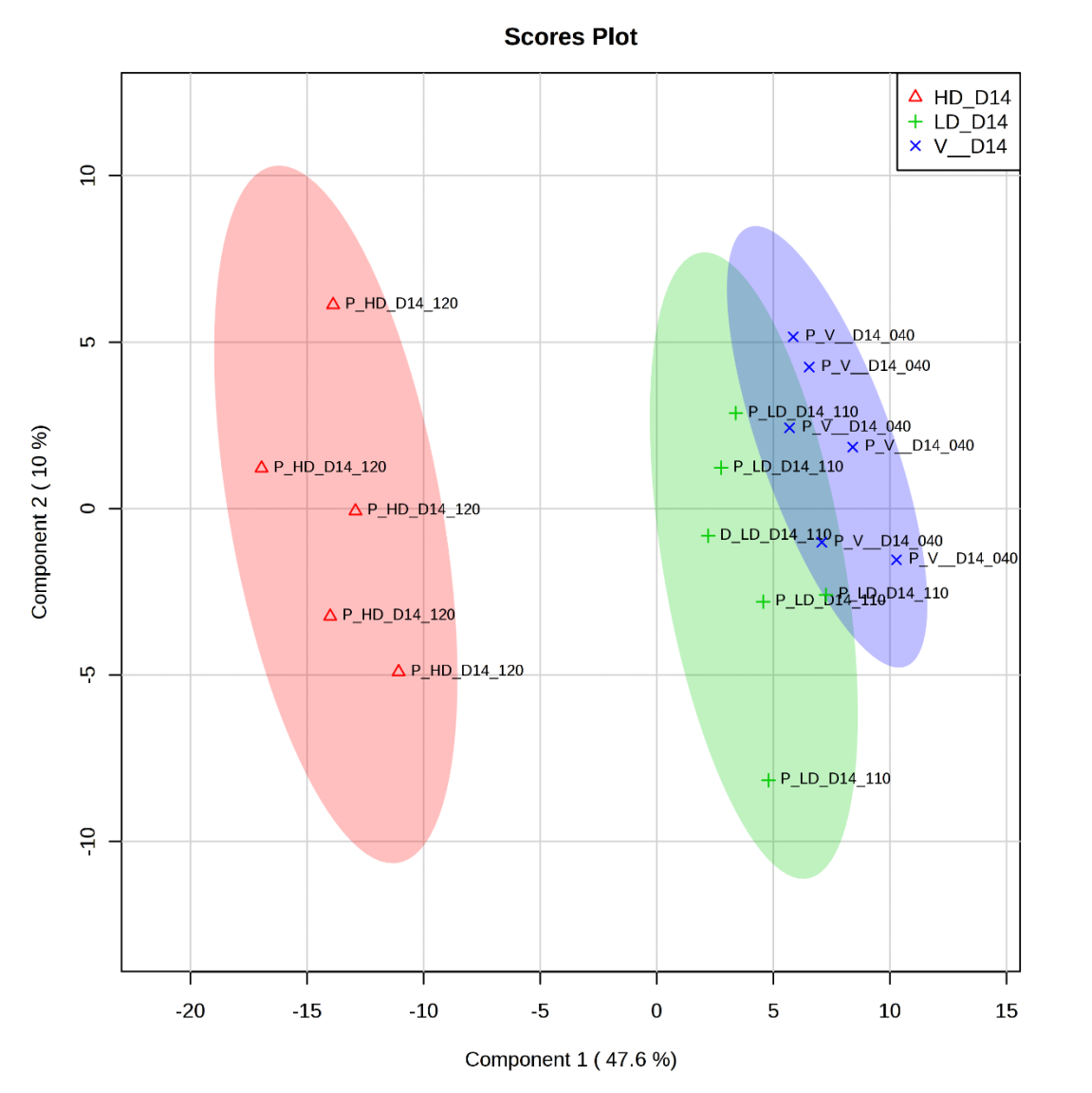
PCA Loadings Plot

Vehicle, Low Dose, and High Dose groups



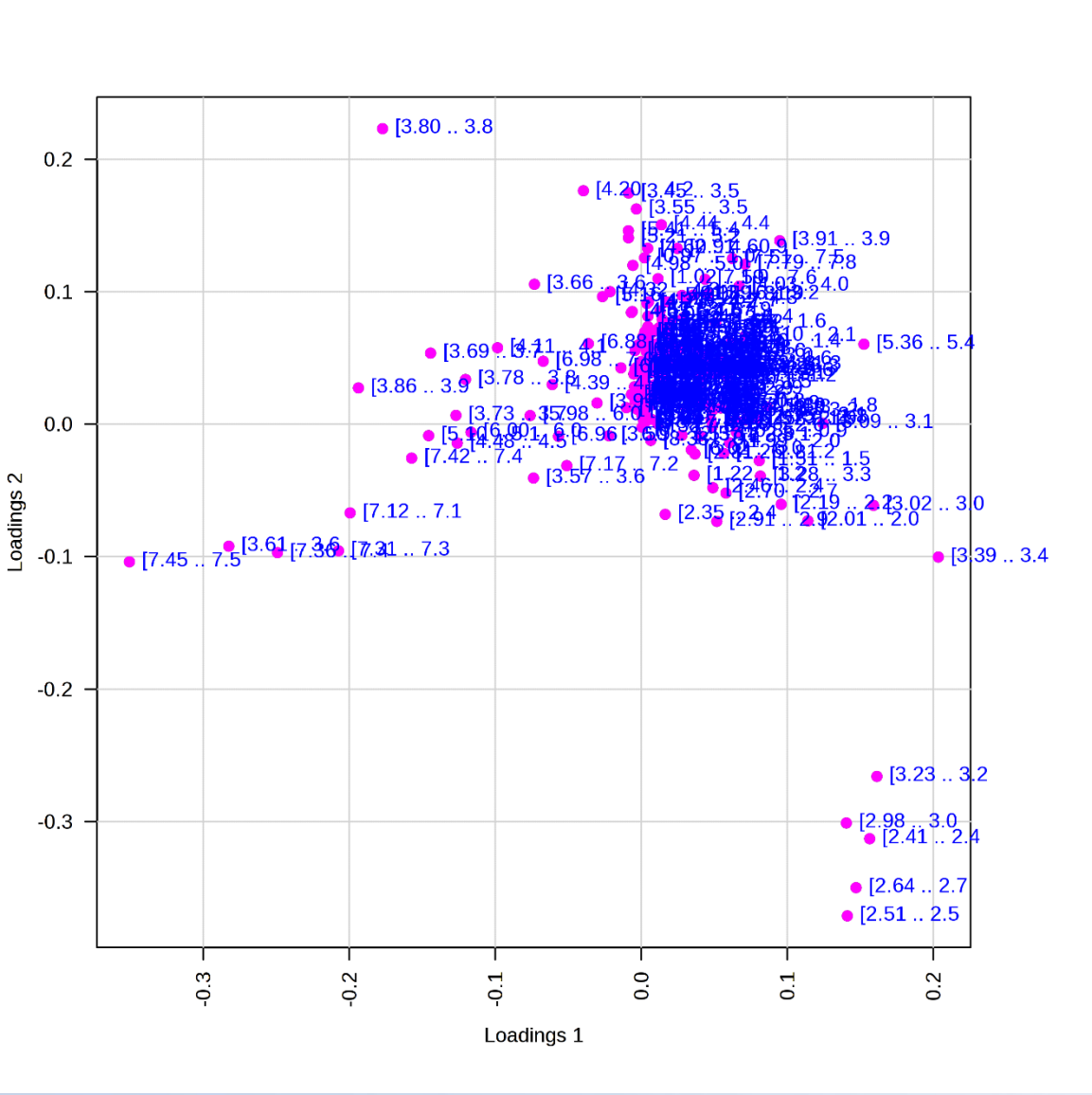
PLS-DA Scores Plot

Vehicle, Low Dose, and High Dose groups

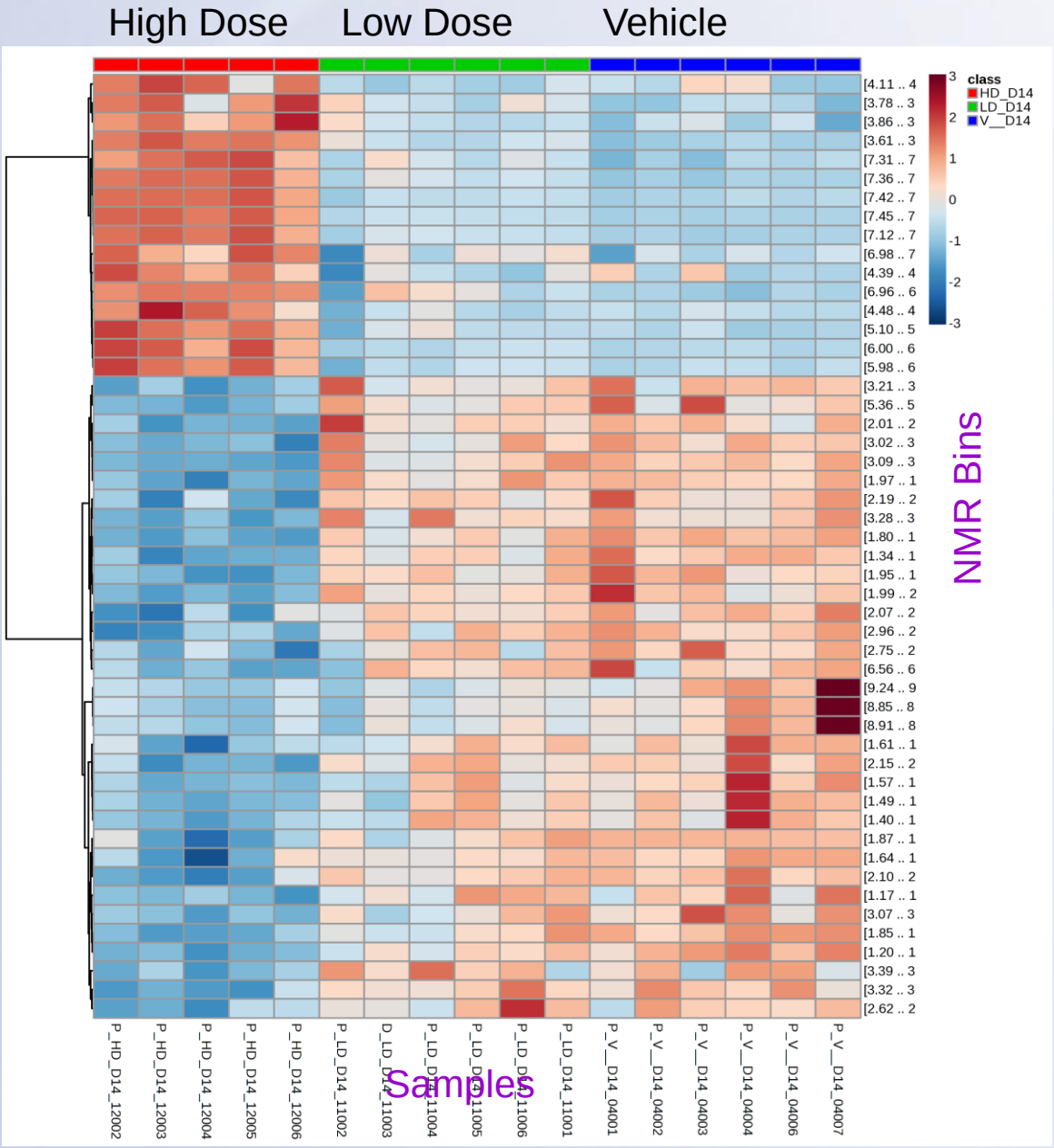


PLS-DA Loadings Plot

Vehicle, Low Dose, and High Dose groups



Day 14 Heat Map

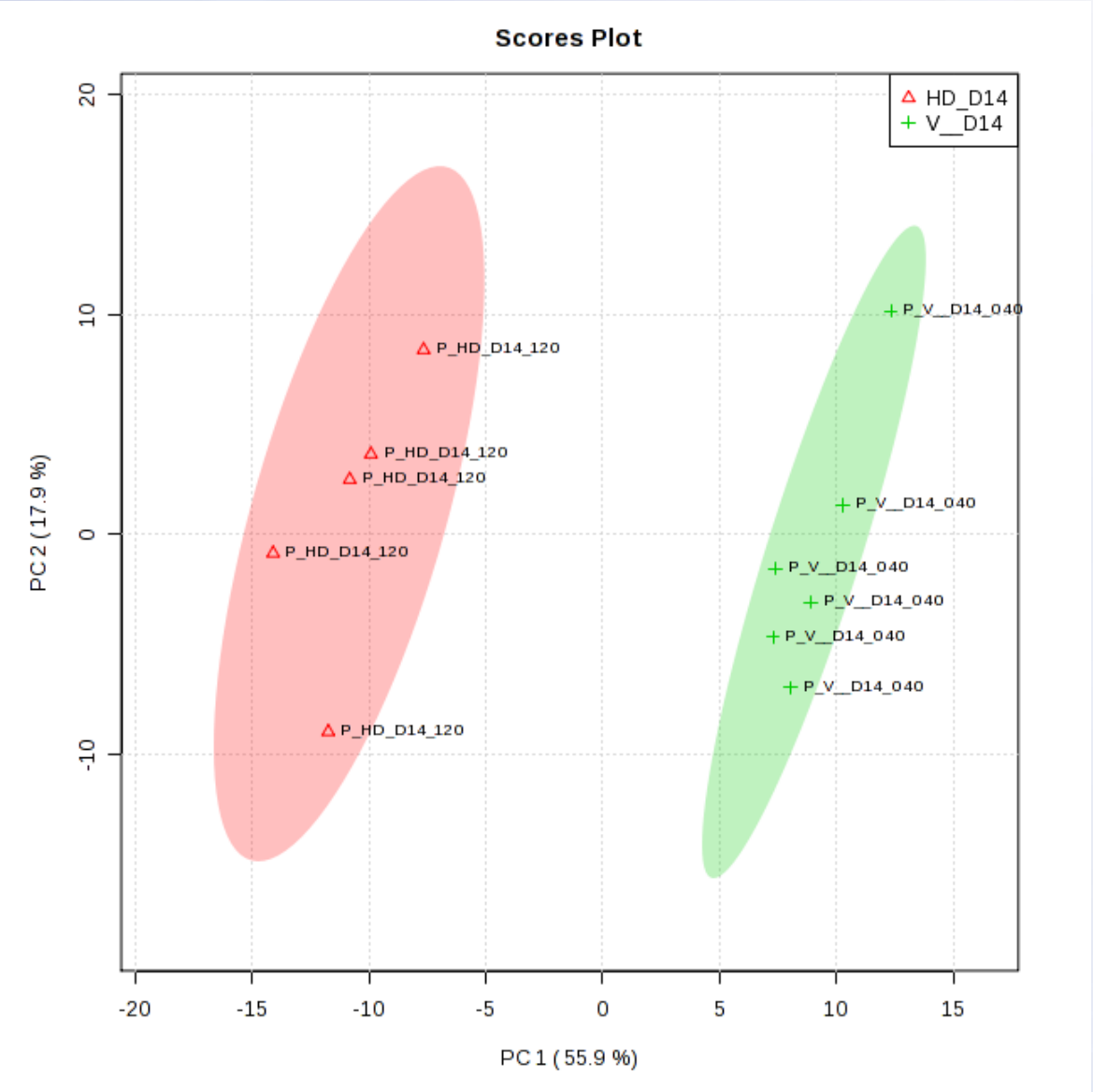


Comparison of Day 14 High Dose and Vehicle

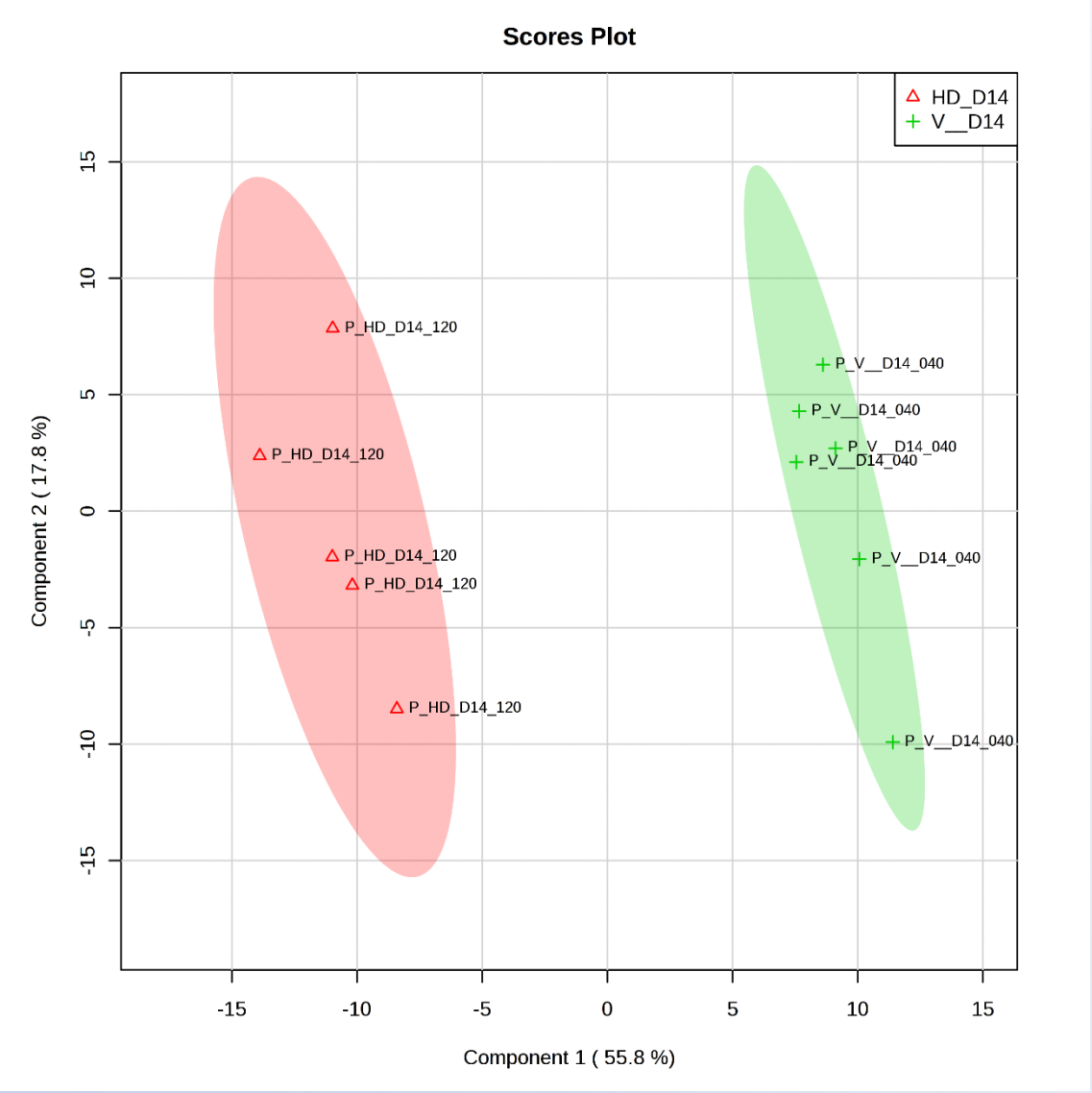
Please start from the start page and upload the data

- 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

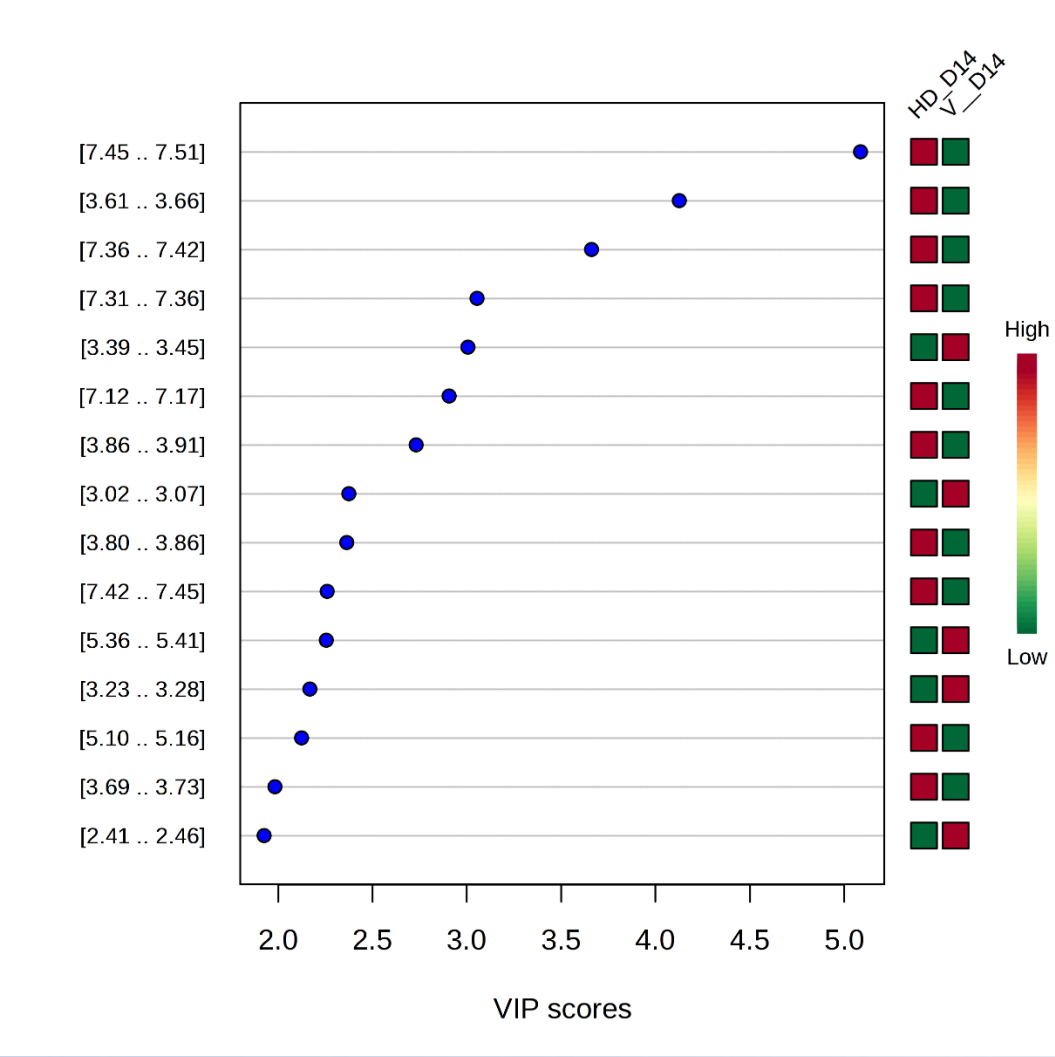
Day 14 PCA Scores Plot: High Dose vs Vehicle



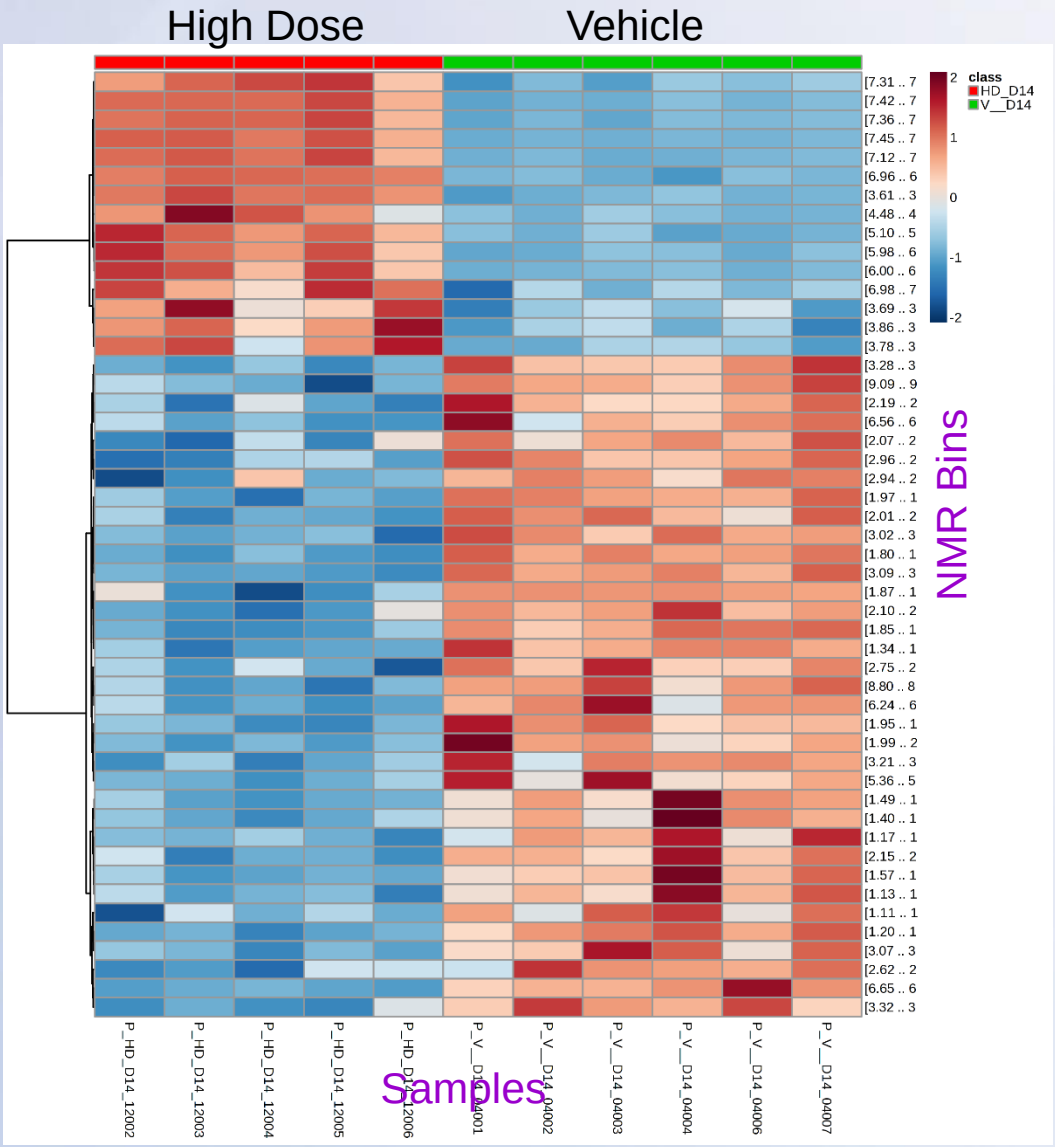
Day 14 PLS-DA Scores Plot: High Dose vs Vehicle



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



Day 14 Heat Map: High Dose vs Vehicle



Top 50 bins in the VIP Plot

ERCMRC at UNC Chapel Hill

UNC NUTRITION OBESITY RESEARCH CENTER

Yuanyuan Li
LC-MS/MS
LC-TOF-MS

Wimal Pathmasiri
NMR & GC-TOF-MS

Delisha Stewart
NMR and LC-TOF-MS

Maria Moreno
NMR and LC-MS/MS

Reza Ghanbari
Postdoctoral Fellow

Rose Ewald
Graduate Studies

Susan Sumner
PI, ERCMRC

Susan McRitchie
Program Coordinator
Data Analysis

NCRC

Nick Gillitt
Dole
700 MHz NMR
6500 Sciex LC-MS

Debby Reed
GC-MS
GC-TOF-MS

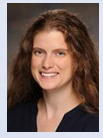
Stephen Orena
LC-MS/MS

Martin Kohlmeier
Training

Tim Fennell
Director,
Analytical Chemistry &
Pharmaceutics

RTI

Yan Lan Yueh
LC-MS



Jessica Gooding
LC-MS

Rod Snyder
NMR and LC-MS

Courtney Whitaker
LC-MS

Scott Watson
Neurotransmitter
LC/MS

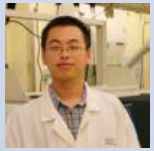
Colin Kaye
NCSU
6500 Sciex
Triple Quad

DHMRI

Kevin Knagge
700 and 950 MHz NMR

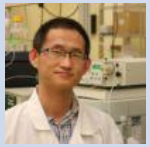
Jason Winnike
NMR
2D-GC-TOF-MS

David Kirchner
LC-MS/MS



Huadong Chen
LC-MS
LC-TOF-MS

Huiyuan Chen
GC-MS
GC-TOF-MS



UNC Charlotte Bioinformatics

Owen Myers

XiuXia Du

Aleksandr
Smirnov

UNC-G
Q-Exactive

Thank You!

If you have any questions, please e-mail me

wimal_pathmasiri@unc.edu

Useful link:

Metabolomics Workbench

http://www.metabolomicsworkbench.org/