NMR Data Analysis Exercise

UAB Metabolomics Training Course

June 14-18, 2015

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NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

NIH Eastern Regional Comprehensive Metabolomics Resource Core at RTI

Support for Experimental Design

Sample Receipt

Entry into BSI II

Sample Preparation

QC Standards

Pooled Samples

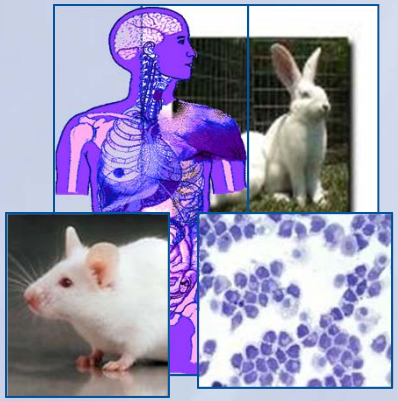
Data Capture & Storage

Data Reduction & Visualization

Empirical & Standards Library Matching

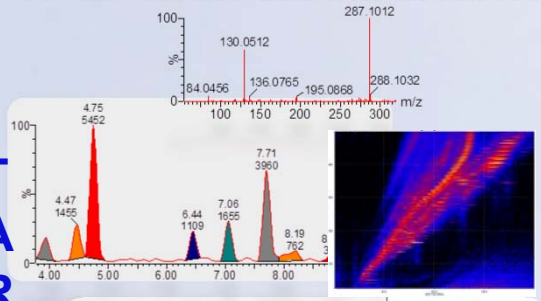
Discovery & Pathway Mapping

Communicating Results

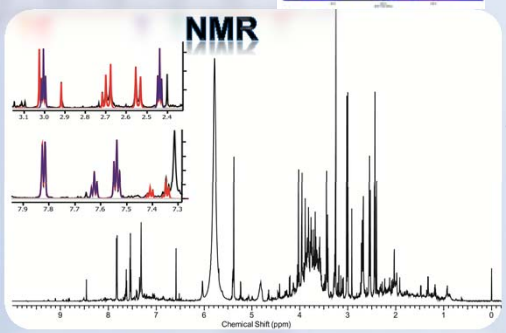


TARGETED

LC-MS, MS/MS and ION-MOBILITY

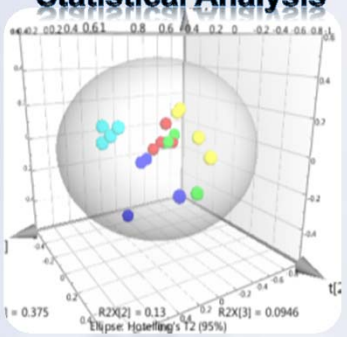


NMR

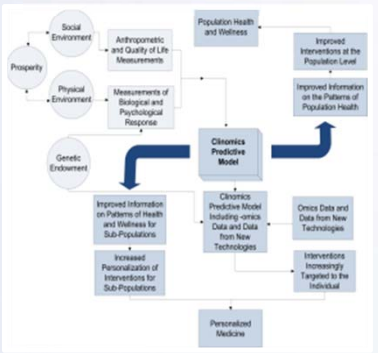


BROAD SPECTRUM

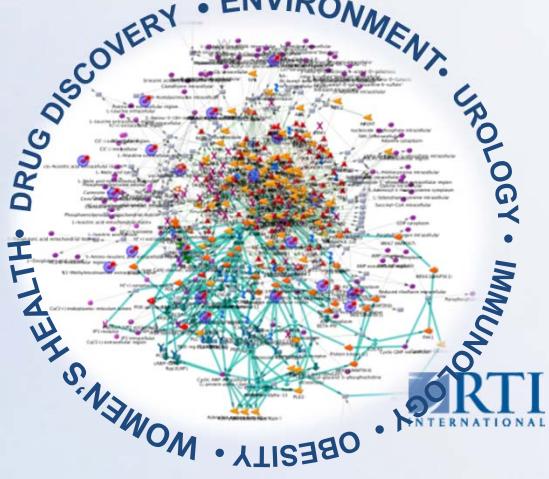
Multivariate and Statistical Analysis



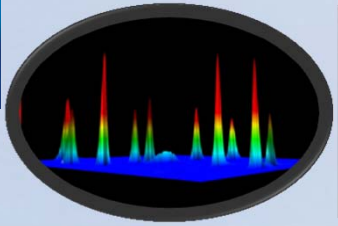
Predictive Modeling



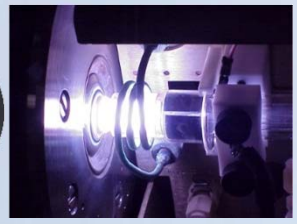
Pathway Mapping Metabolites-Proteins-Genes



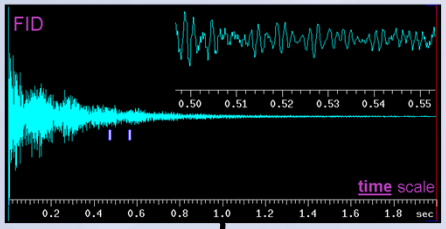
GC-MS

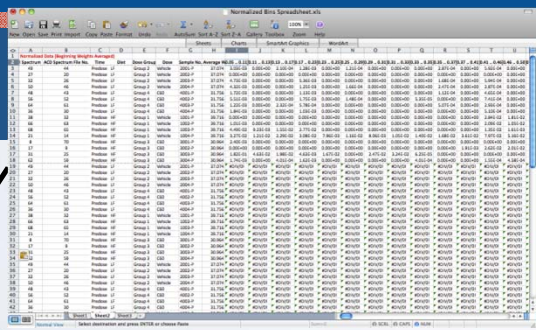


ICP-MS



NMR Metabolomics Workflow

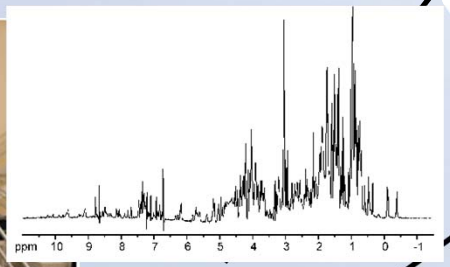


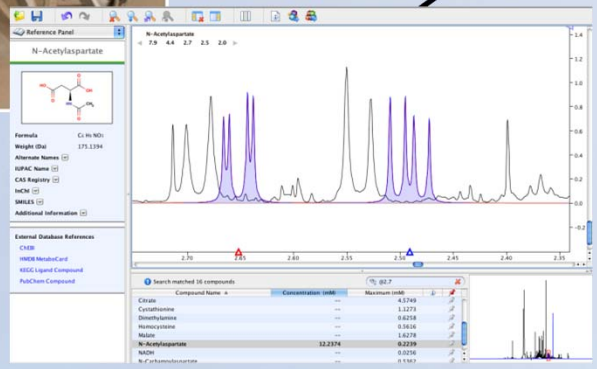


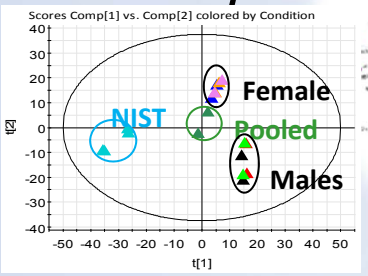
Peak Alignment QC Check → Binned NMR Data

Sample Preparation → NMR Data Acquisition → Raw NMR data (FID) → Fourier Transform Phase and Baseline Correction → Processed NMR Spectrum 1r, cnx, esp, jdx → Multivariate Data Analysis → Statistical Analysis → Pathway Analysis

Processed NMR Spectrum 1r, cnx, esp, jdx → Library Matched Data → Multivariate Data Analysis







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

RTI INTERNATIONAL

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

RTI INTERNATIONAL

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)

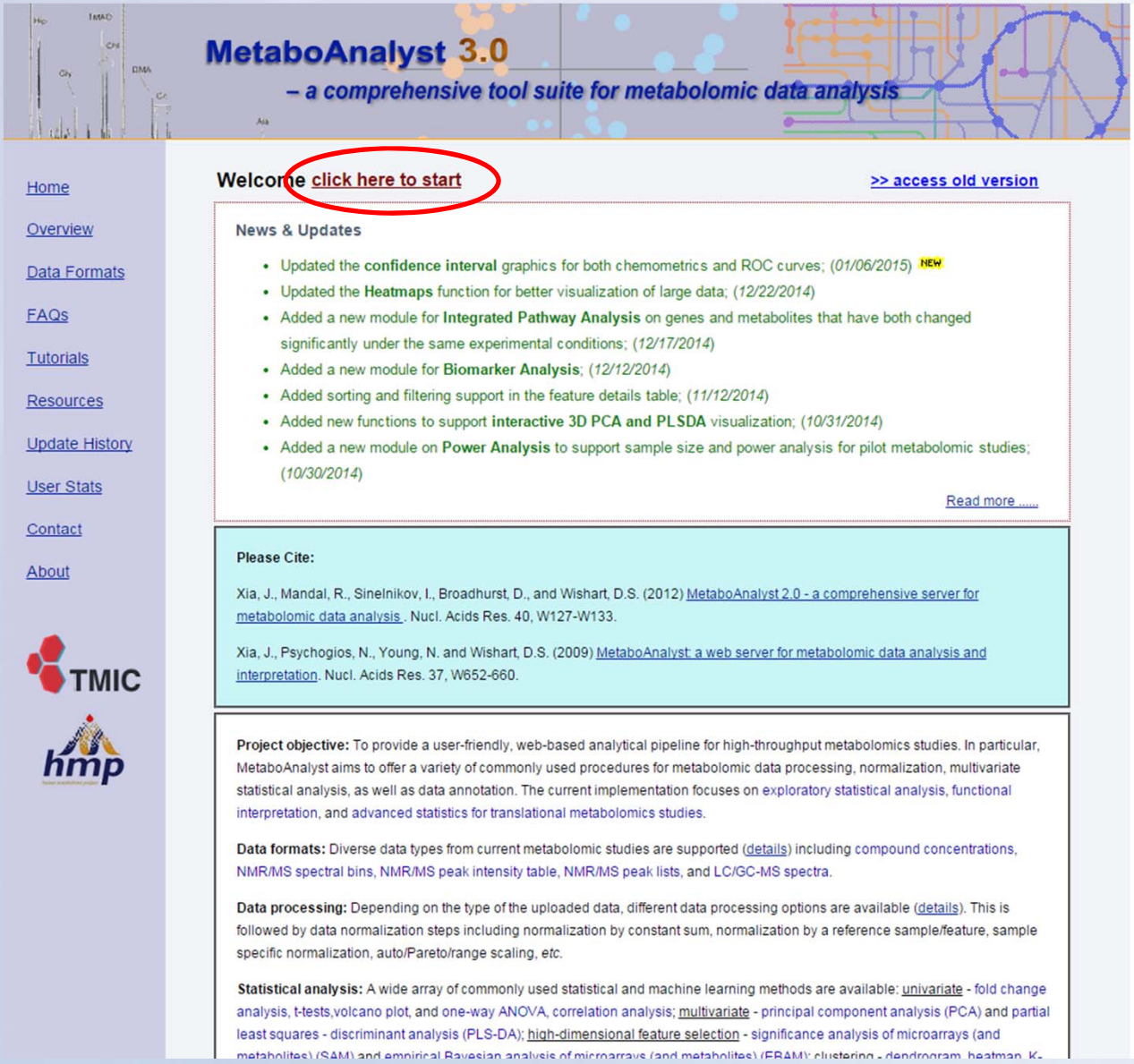
Binned Data

- 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)

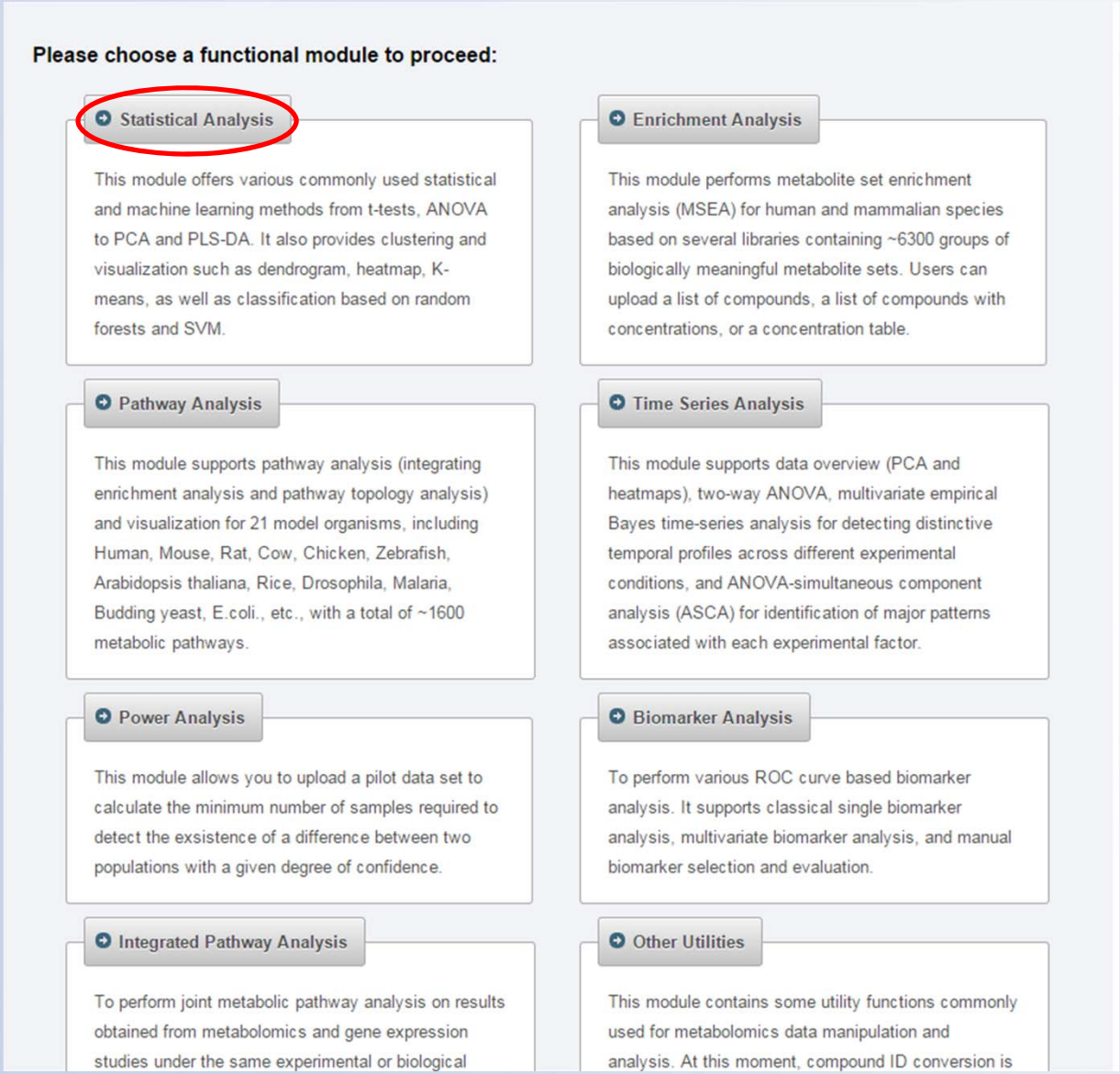
MetaboAnalyst 3.0

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

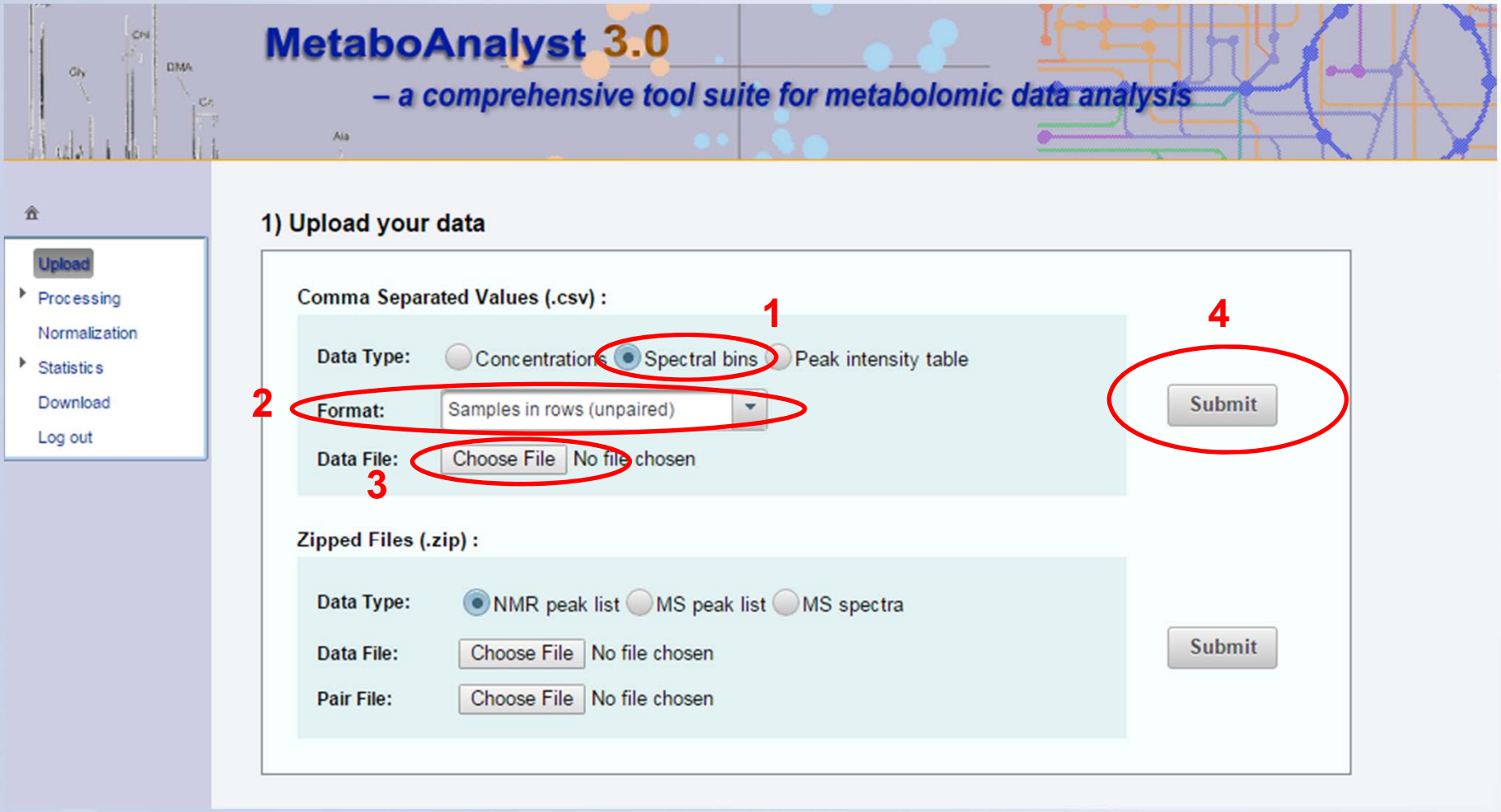


RTI INTERNATIONAL

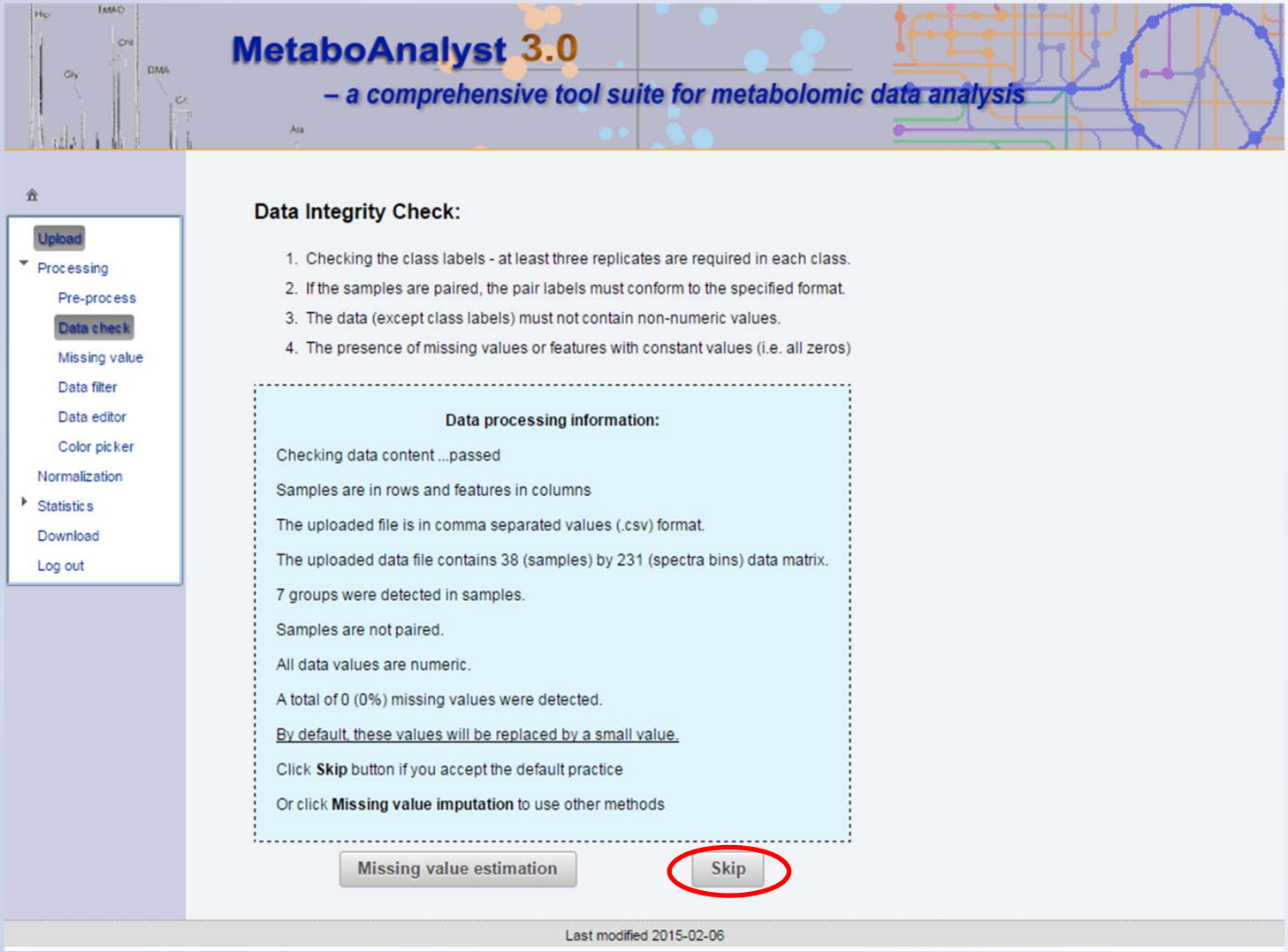
MetaboAnalyst: Functional Modules



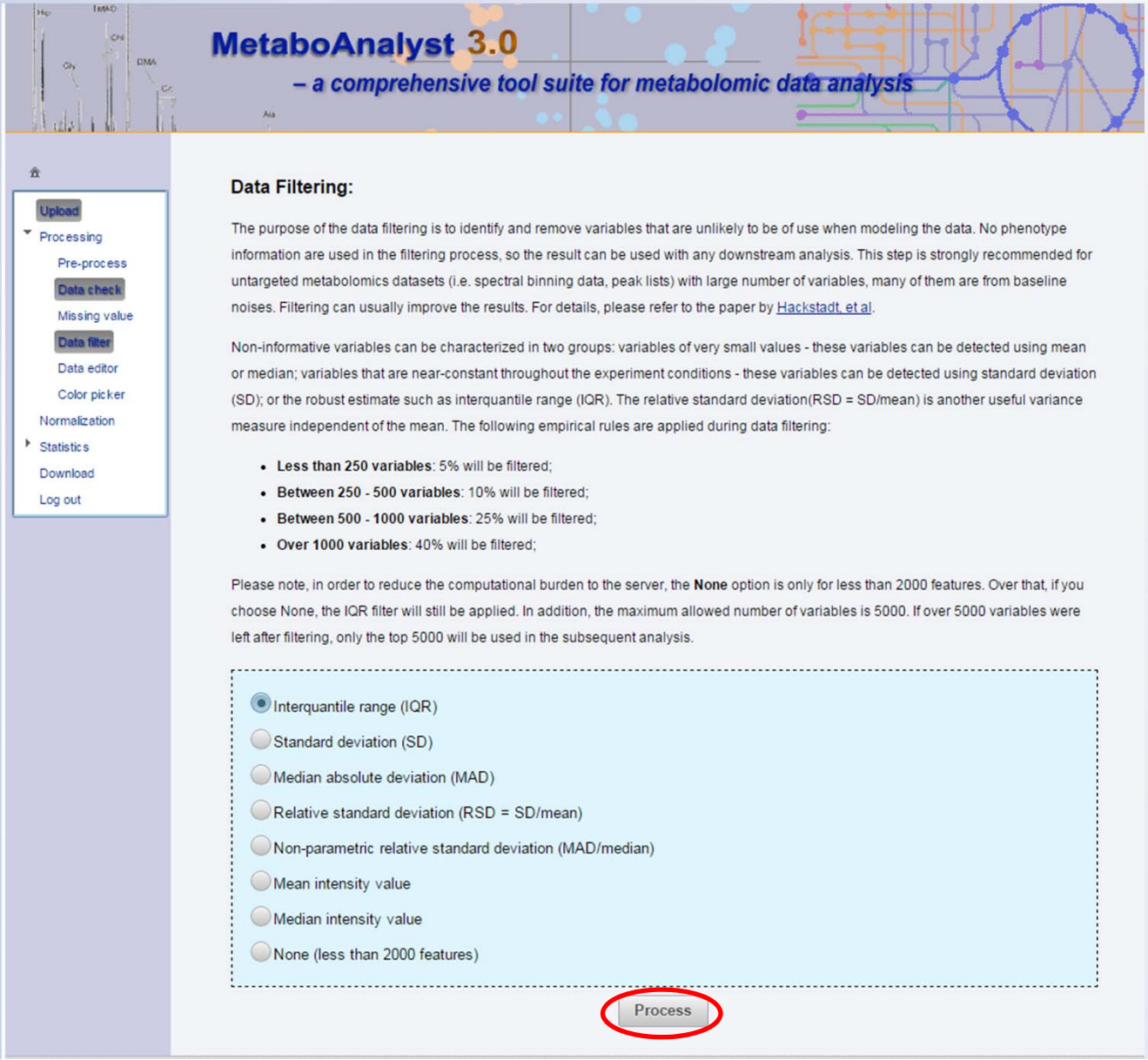
Data Upload


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
1
2
3
4
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

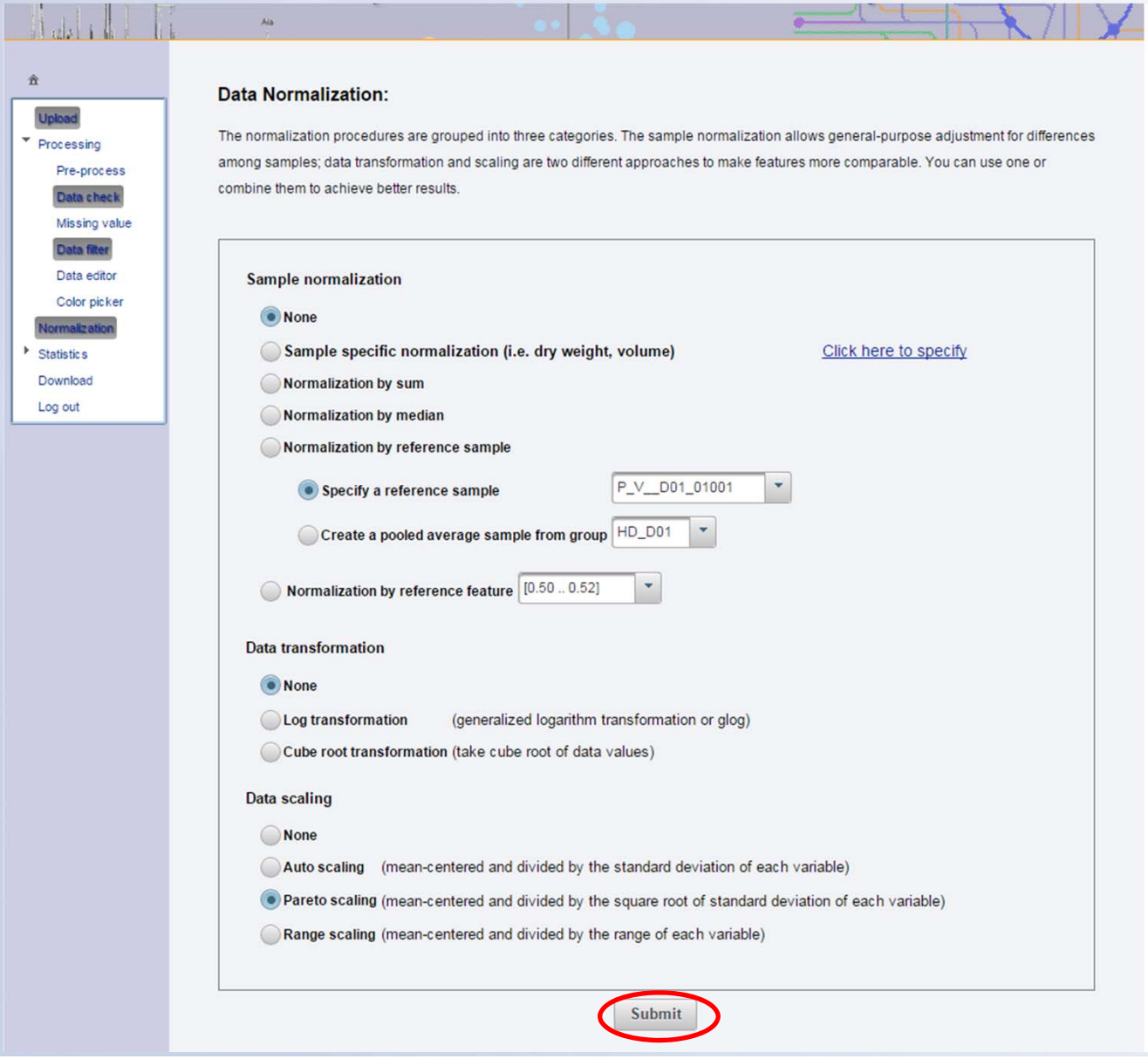
Data Integrity Check



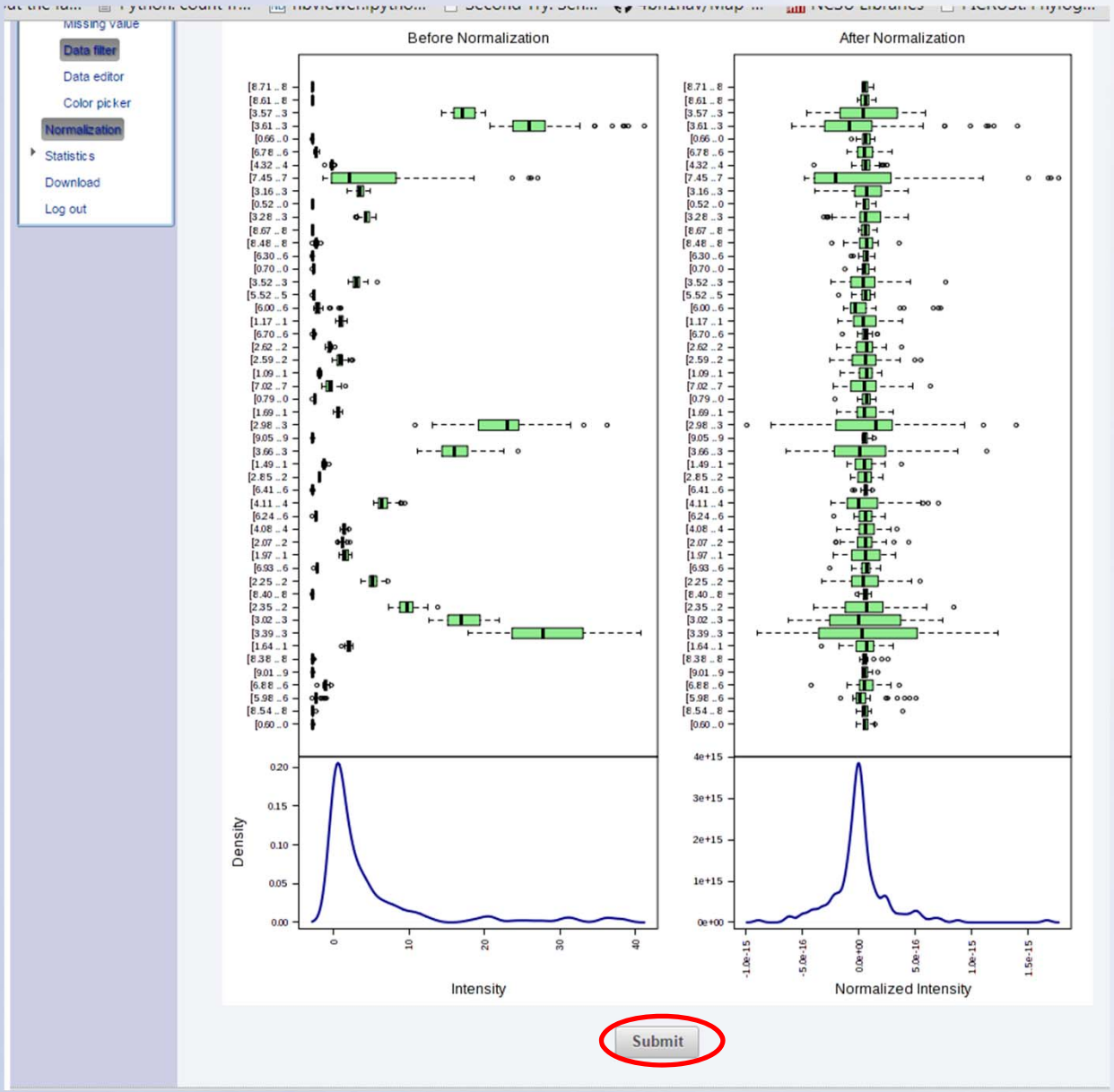
Data Filtering



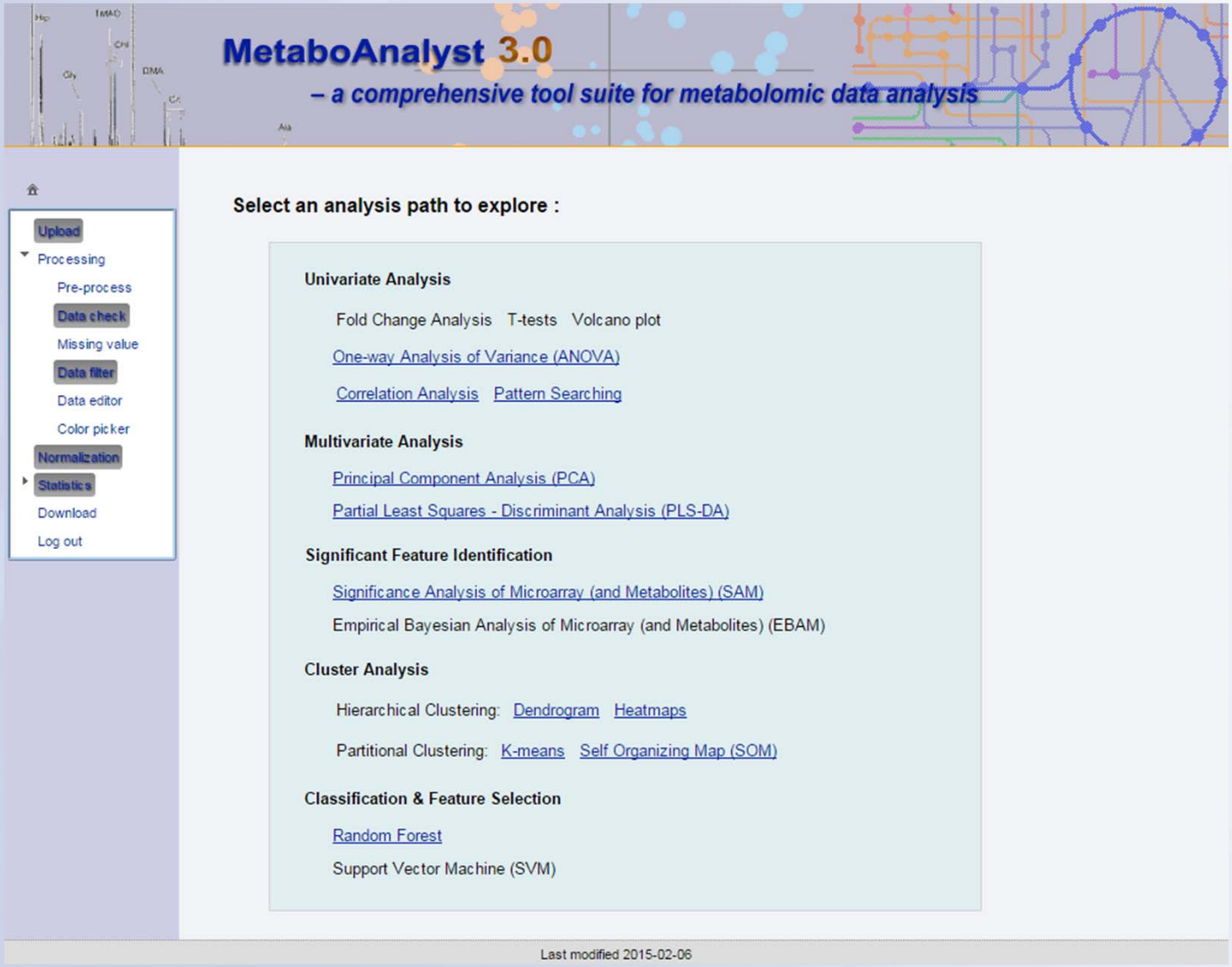
Data Normalization



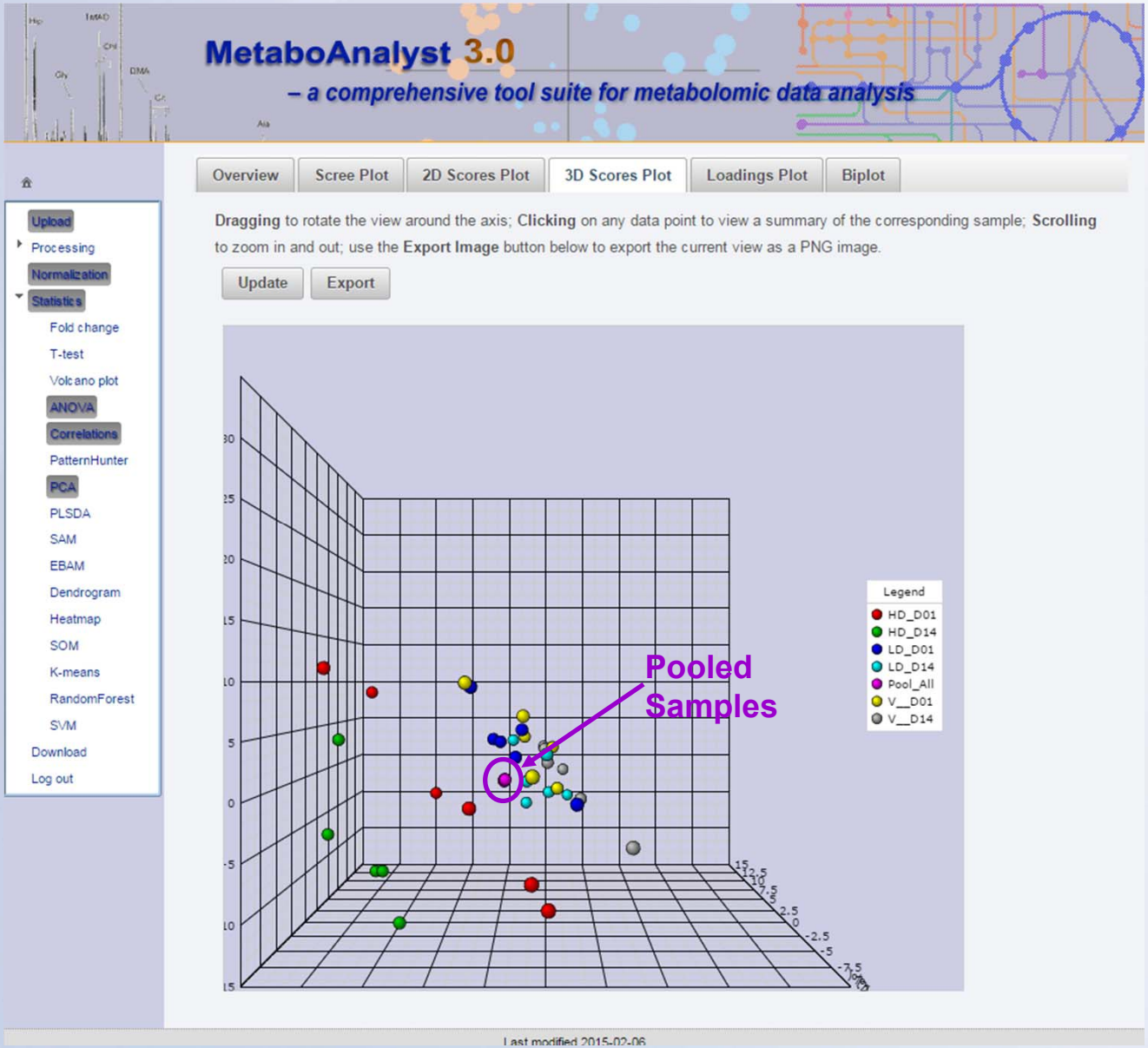
Summary: Normalization



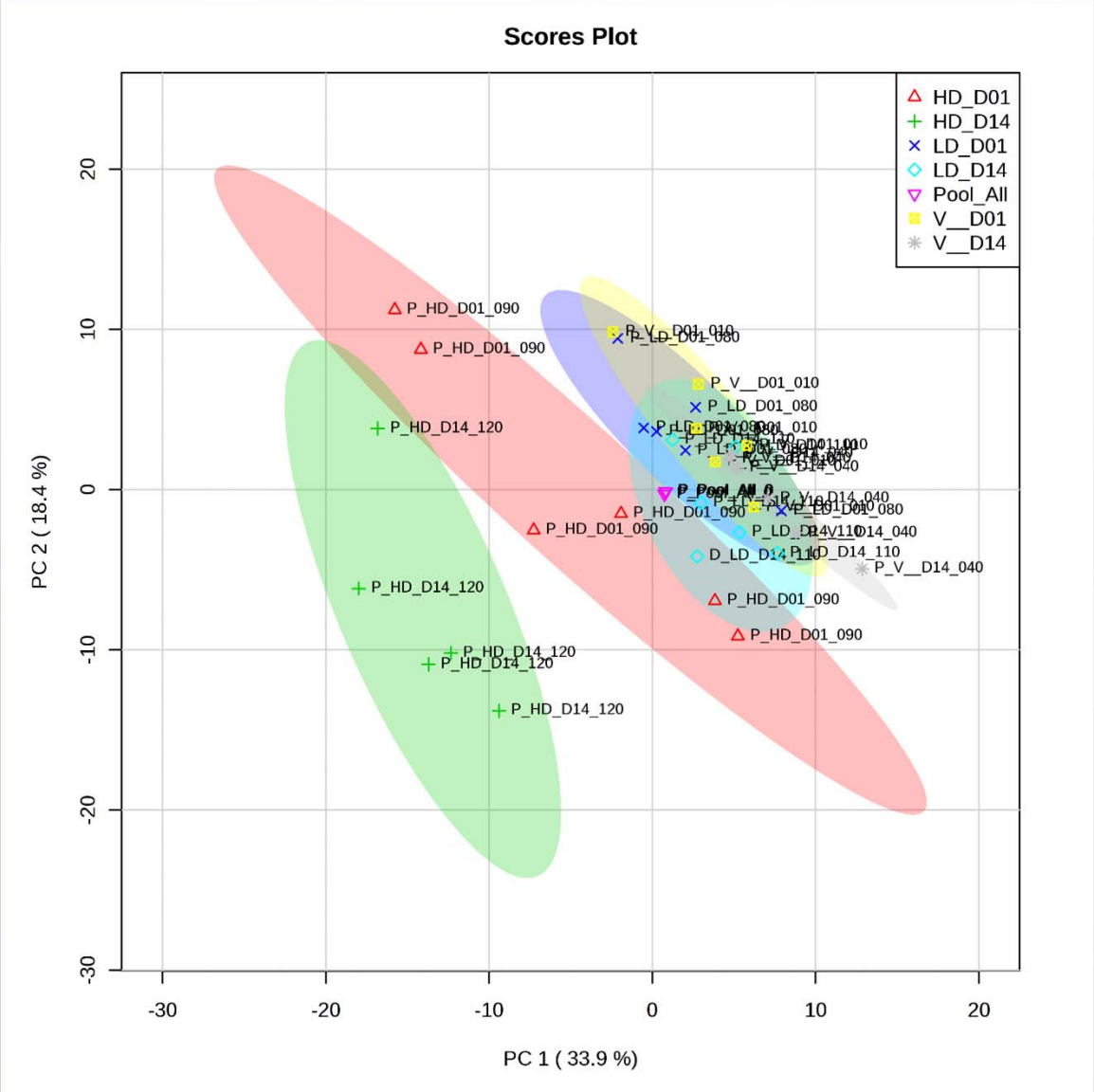
Statistical Analysis



Pooled QC Samples



PCA Day 01 and Day 14



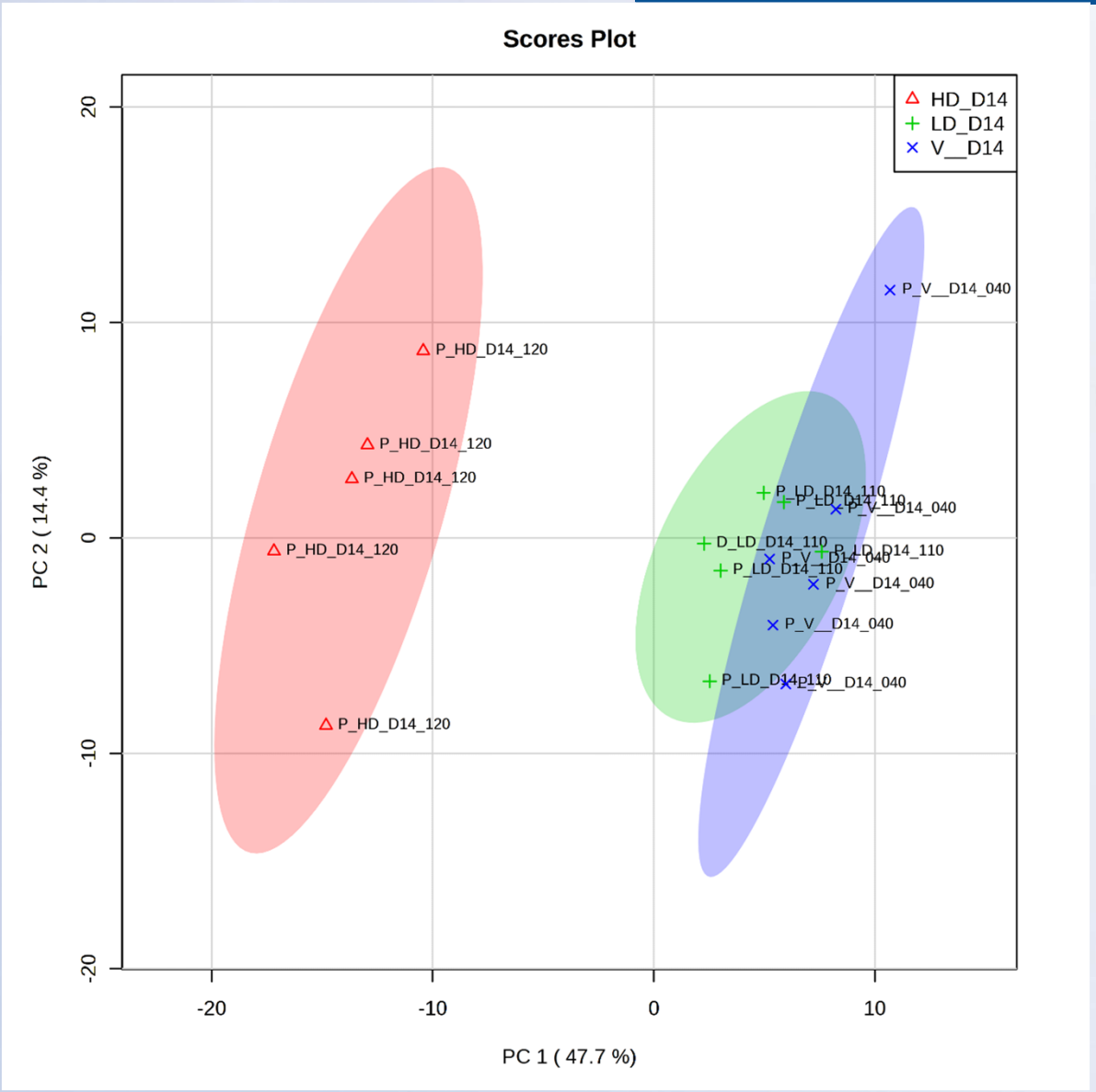
Day 14: Vehicle, Low Dose, and High Dose Groups

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

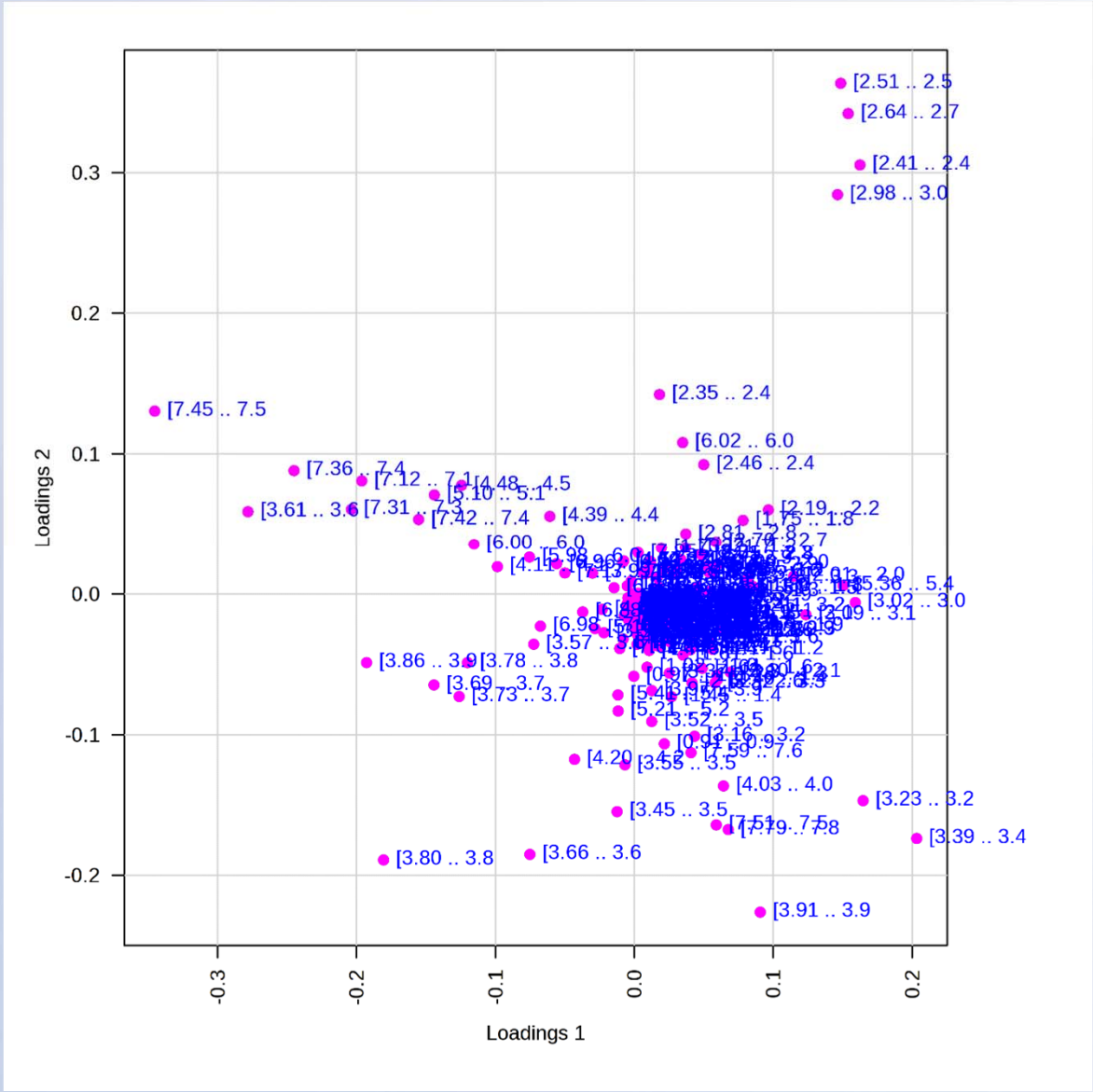
Vehicle, Low Dose, and High Dose groups

Day 14 PCA Scores Plot



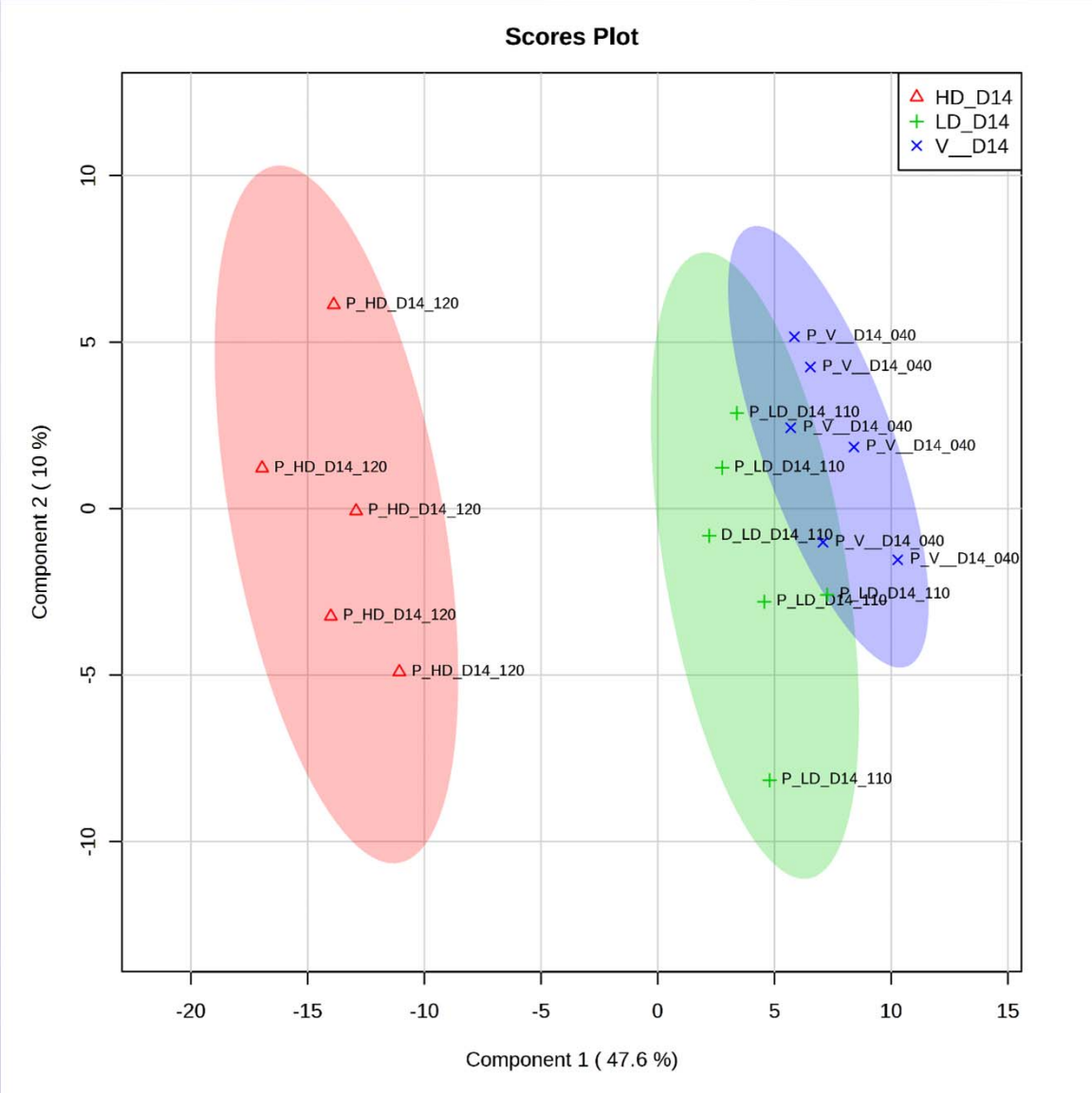
Vehicle, Low Dose, and High Dose groups

PCA Loadings Plot



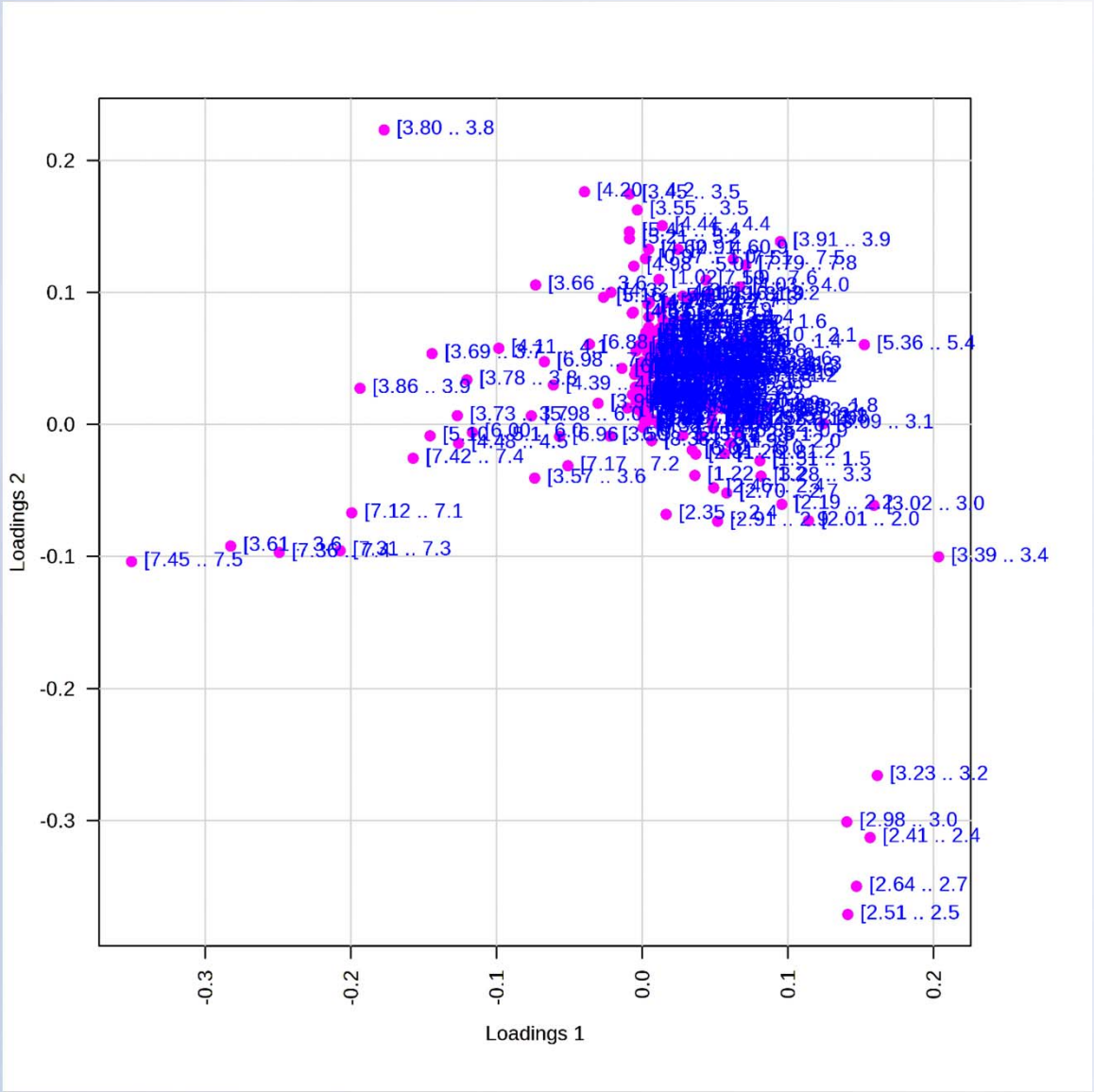
Vehicle, Low Dose, and High Dose groups

PLS-DA Scores Plot

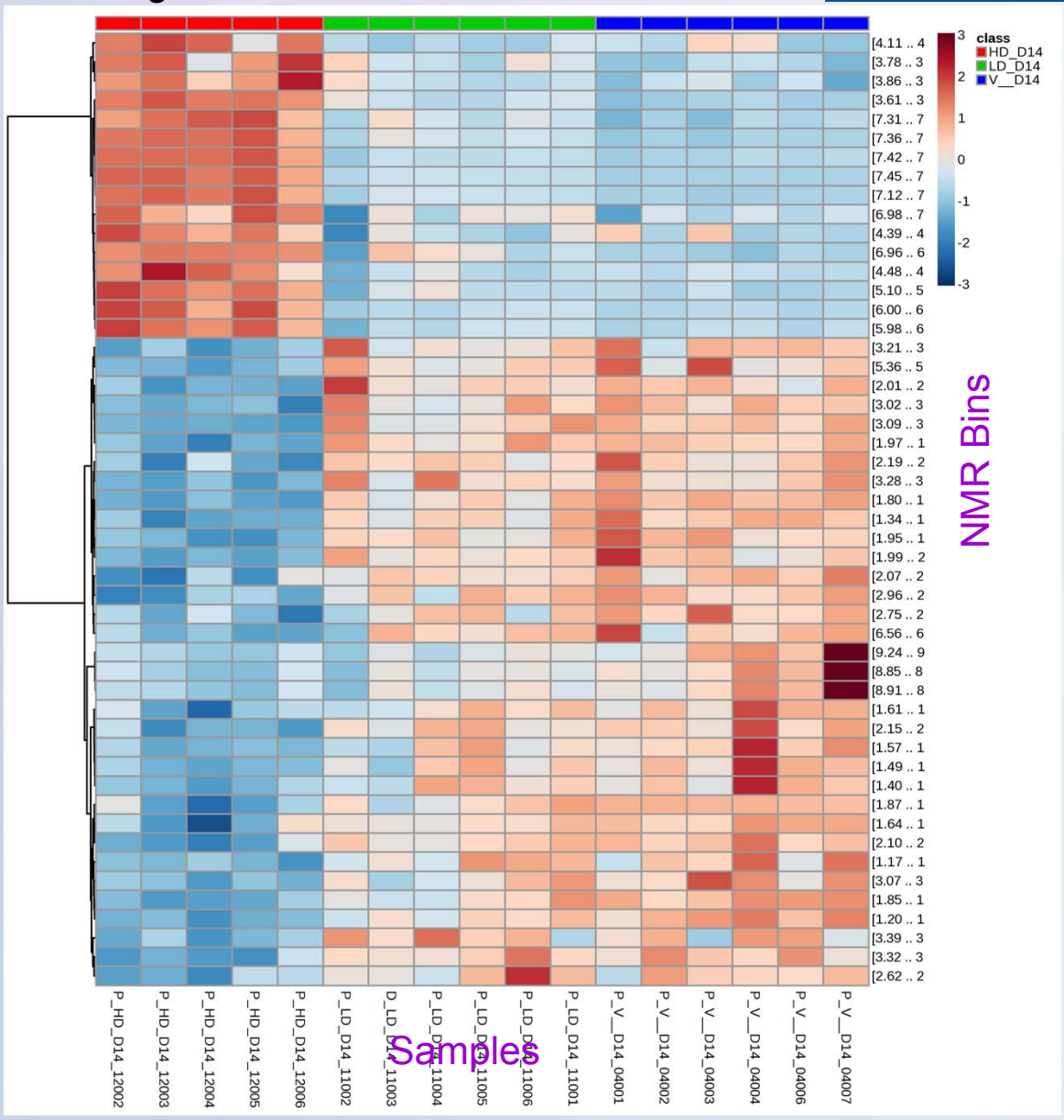


Vehicle, Low Dose, and High Dose groups

PLS-DA Loadings Plot



Day 14 Heat Map

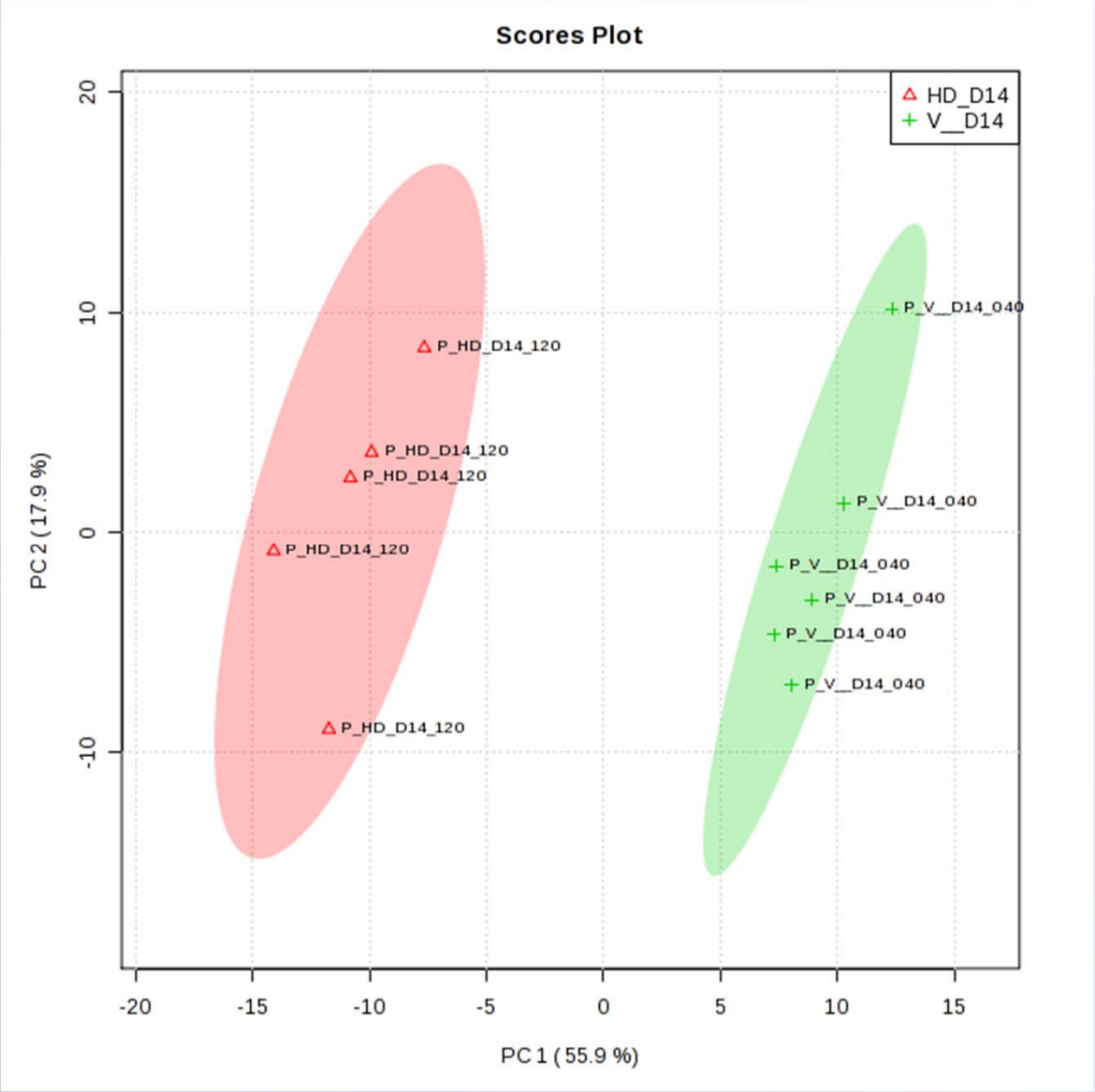


Comparison of Day 14 High Dose and Vehicle

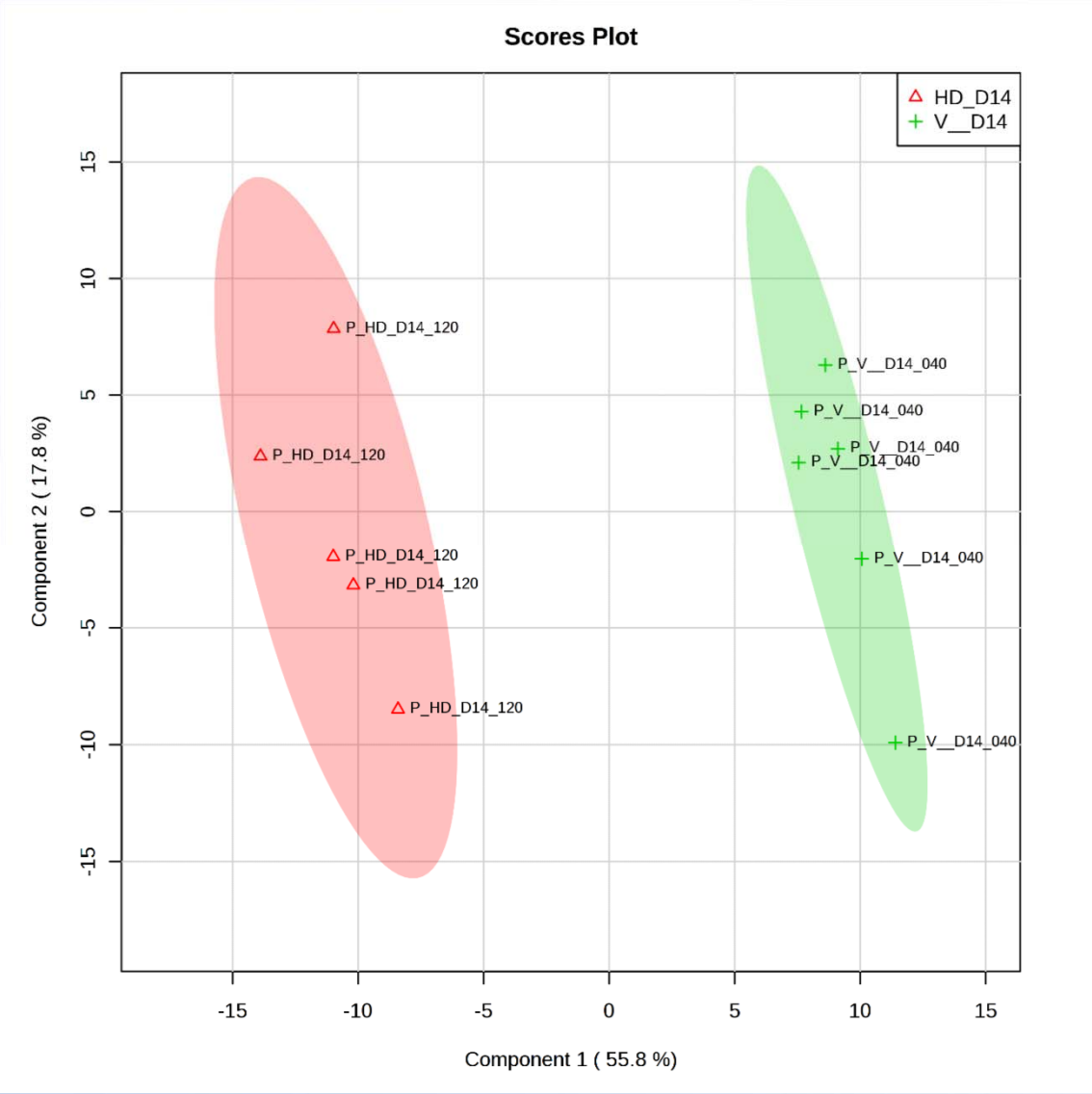
Please start from the start page and upload the data

- 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

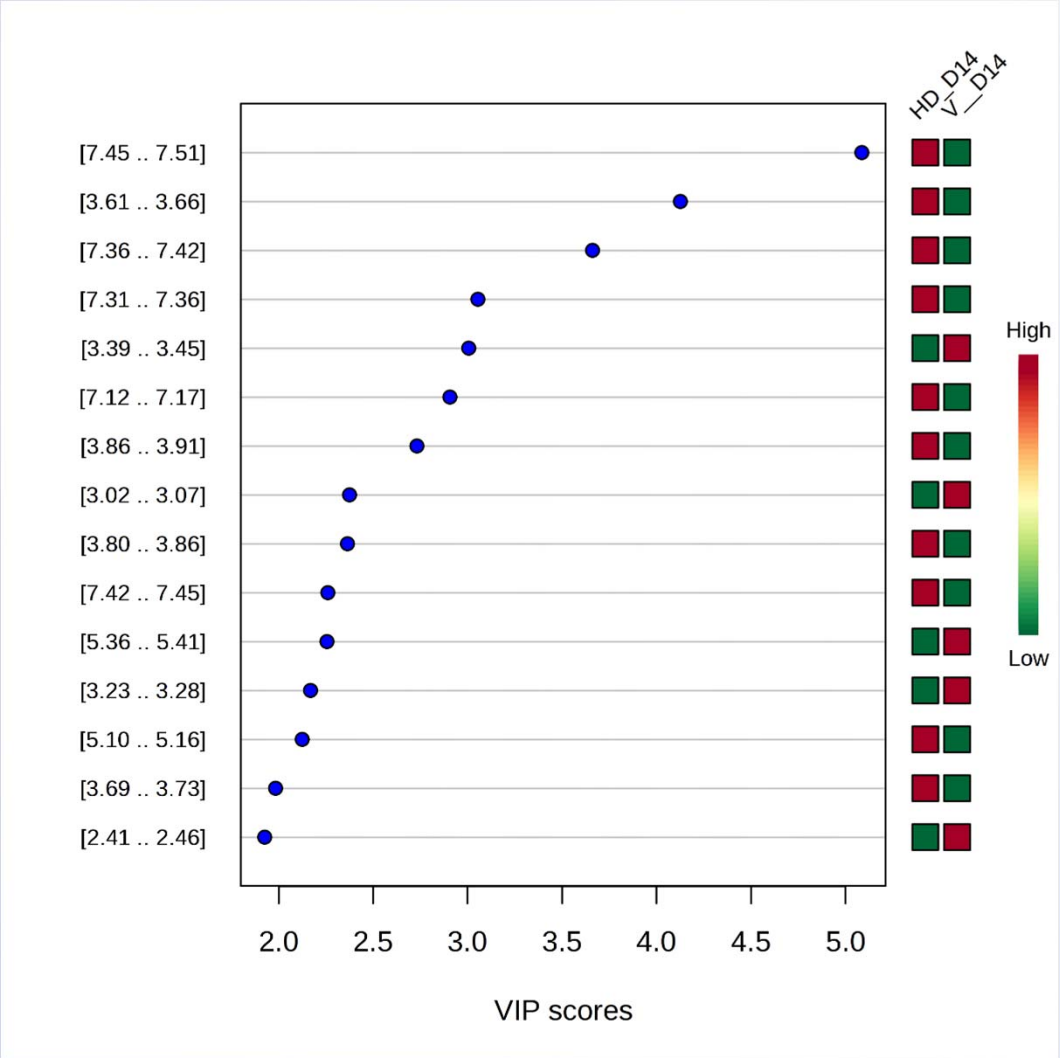
Day 14 PCA Scores Plot: High Dose vs Vehicle



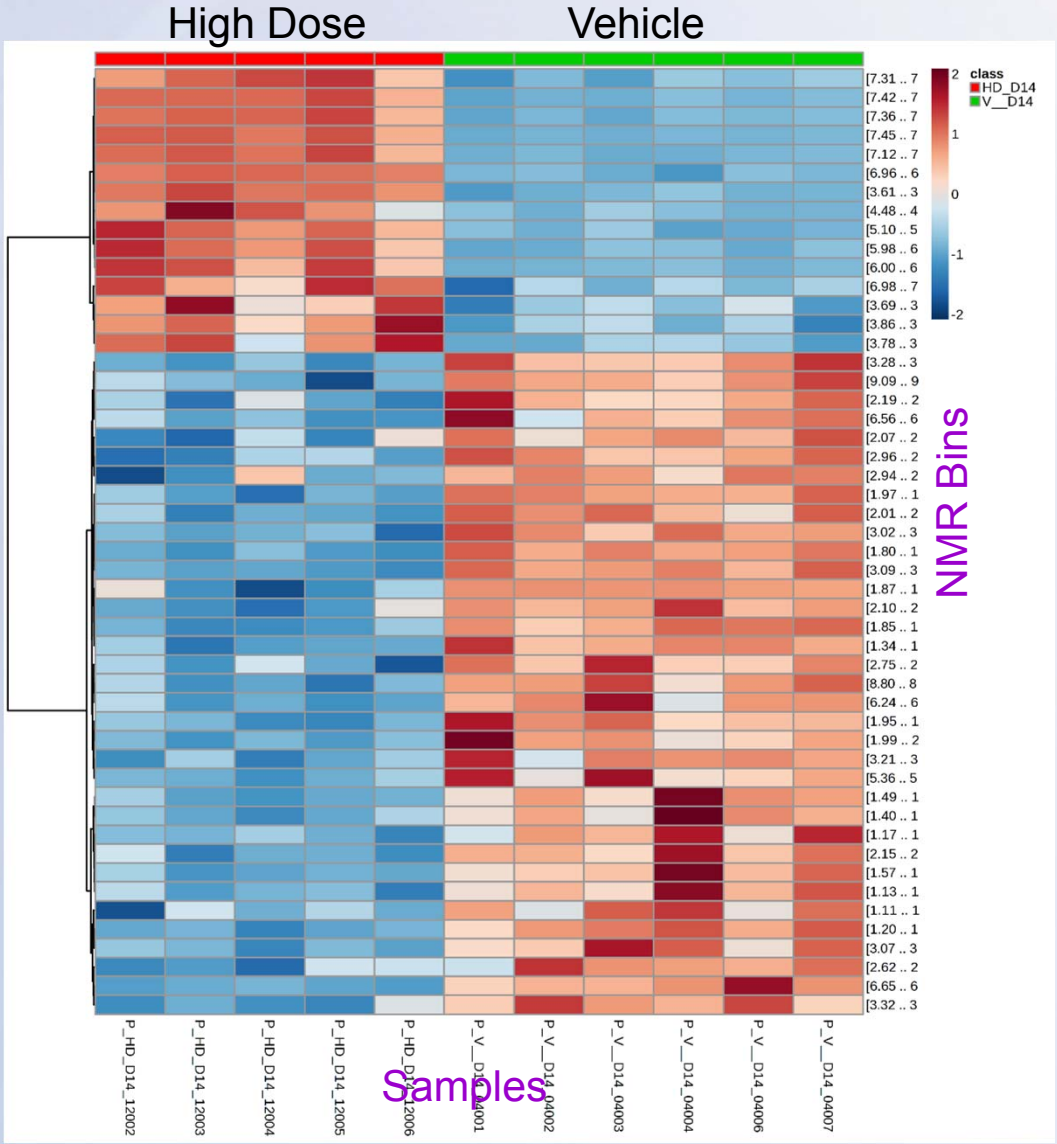
Day 14 PLS-DA Scores Plot: High Dose vs Vehicle



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

Thank You!

If you have any questions, please e-mail me

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Useful link:

Metabolomics Workbench

http://www.metabolomicsworkbench.org/

ACKNOWLEDGEMENTS

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