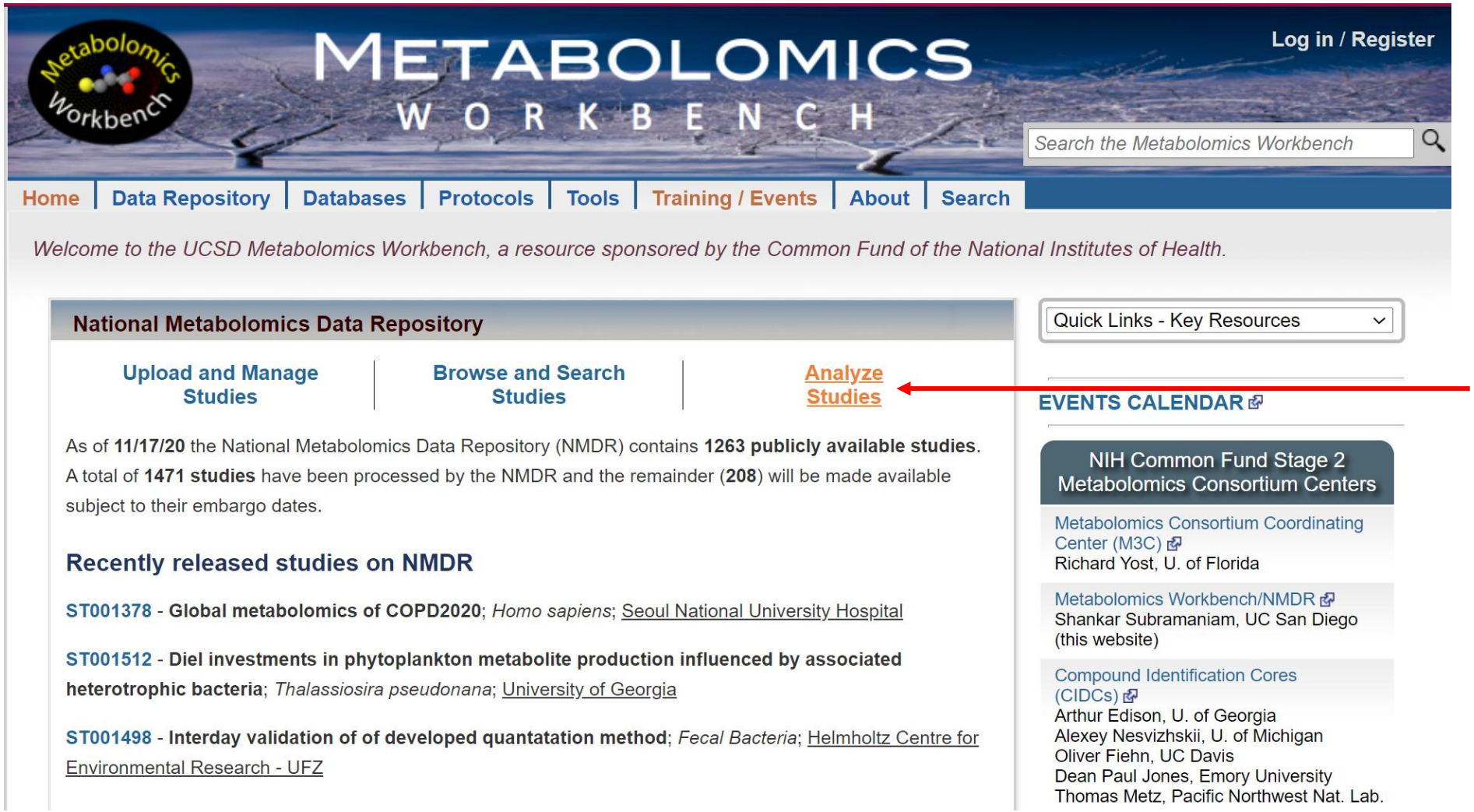
Tutorial to run MetENPWeb

Sonal Choudhary
Metabolomics Workbench

Steps

Example 1. Datasets already on Metabolomics Workbench



Click on Analyze Studies

Study selection

Analyze Studies

Analyze studies using **Jupyter Notebooks** or the following online tools.

MS/NMR studies identifying named metabolites

Select a study for analysis:

ST001140: Changes in the Canine Plasma Lipidome after Short- and Long-Term Exces... (Life Sciences Institute, National University of Singapore)

Submit

Analysis tools may also be accessed from within each **study page** using the 'Perform statistical analysis' link

Choose study

Comparative analysis across studies

- **Perform meta-analysis on selected studies (compare ratios of 2 selected metabolites)**
- **Compare list of metabolites in 2 selected studies (all analyses)**
- **Compare list of metabolites in 2 selected studies (individual analyses)**

MS untargeted experiments containing unidentified ions

- **Search Untargeted MS data by m/z, retention time, instrumentation**
- **Superimpose unknown m/z on RefMet mass defect plot**

Perform data analysis on user-uploaded data

- **Load and analyze your own dataset**

Select MetENP

Metabolite classes (all analyses combined)

- Pie chart of metabolite super classes
- Pie chart of metabolite main classes
- Pie chart of metabolite sub classes

Normalization and averaging

- Show Metabolite averages per experimental factor
- Perform normalization on data
- Create Relative log abundance plots

Univariate analysis

- Perform multi-condition dot plot analysis New!
- Perform Volcano plot analysis
- Perform ANOVA analysis

MetENP: Metabolite enrichment and species-specific pathway annotation New!

- MetENPWeb analysis
- MetENP R package

Clustering and correlation

- Perform hierarchial or heatmap cluster analysis
- Perform Clustered correlation analysis
- Perform Network analysis on correlated metabolites

Multivariate analysis

- Perform Principal component analysis
- Perform Linear discriminant analysis
- Perform Partial least-squares discriminant analysis (PLS-DA)

Classification and feature analysis

- Perform OPLS-DA and VIP projection
- Random Forest and VIP projection

Mapping metabolites to human biochemical pathways

- Map study metabolites to HMDB and KEGG pathways
- Map study metabolites to pathways with ratio/t-test data

Select analysis and factor column

Choose a analysis:

Hint: You can either select all, or use ctrl + select to choose multiple

Select all
Phospholipids, Chol. esters and Diacylglycerols
Sphingolipids
Derivatized Spingosine-1-phosphates

Check the experimental factors of this study in the table below. The first column is grouped (combined) factors and subsequent columns are individual factors

	combined_factors	TreatmentGroup	TreatmentDuration	SamplingTimePoint
1	TreatmentGroup:Prednisolone | TreatmentDuration:0d | SamplingTimePoint:before	Prednisolone	0d	before
2	TreatmentGroup:Prednisolone | TreatmentDuration:4d | SamplingTimePoint:after	Prednisolone	4d	after
3	TreatmentGroup:Tetracosactide | TreatmentDuration:00w | SamplingTimePoint:before	Tetracosactide	00w	before
4	TreatmentGroup:Tetracosactide | TreatmentDuration:25w | SamplingTimePoint:after	Tetracosactide	25w	after

Choose factor column:

Take a hint from the table above

combined_factors

- combined_factors
- TreatmentGroup
- TreatmentDuration
- SamplingTimePoint

Parameters

Choose among these factors as Group 1 and Group 2. Multiple factors can be choosen for one group

Group1:

Prednisolone
Tetracosactide

Choose factor 1

Group2:

Prednisolone
Tetracosactide

Choose factor 2

Padjust method: fdr

Handle missing data: 50percent

Hint:

- **half_of_min:** where the NAs are replaced by half of the min values in the data.
- **remove_NAs:** where metabolites with NAs values are removed and
- **50percent:** where metabolites with more than 50% NAs values are removed.

P-value cutoff: 0.05

Log2 fold change cutoff: 0.5

Choose a Metabolite class: sub_class

Minimum # of (most significant) metabolites per class to use in group calculation: 3

Kegg id of species is automatically filled. For bacterial species, please check our notes below

Kegg id of species: cfa Species -Dog

Check this box only if you want your pathway enrichment analysis based on all the KEGG compounds associated to pathways.

- (•) Not applicable
- ( ) All pathways

Do you want to associate genes to pathways?

If you select 'Perform gene analysis' option, please be aware it may take longer than 5 mins to analyze the study

(•) **Don't perform gene analysis** ( ) **Perform gene analysis**

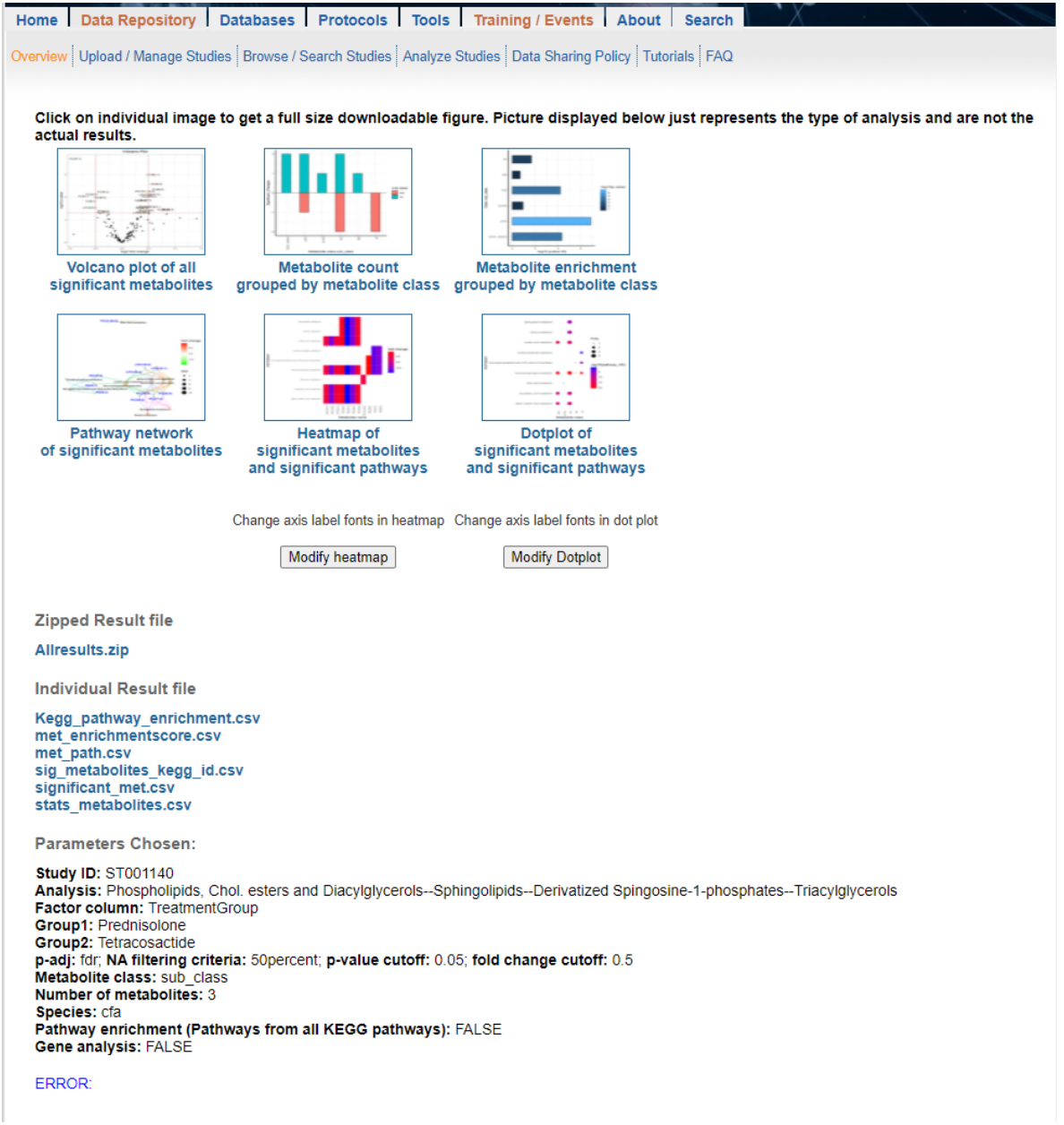
Submit

The analysis may take 2-5 minutes, please wait and do not close the window. Longer datasets may take even longer. You may want to run MetENP package on the R commandline for quicker results

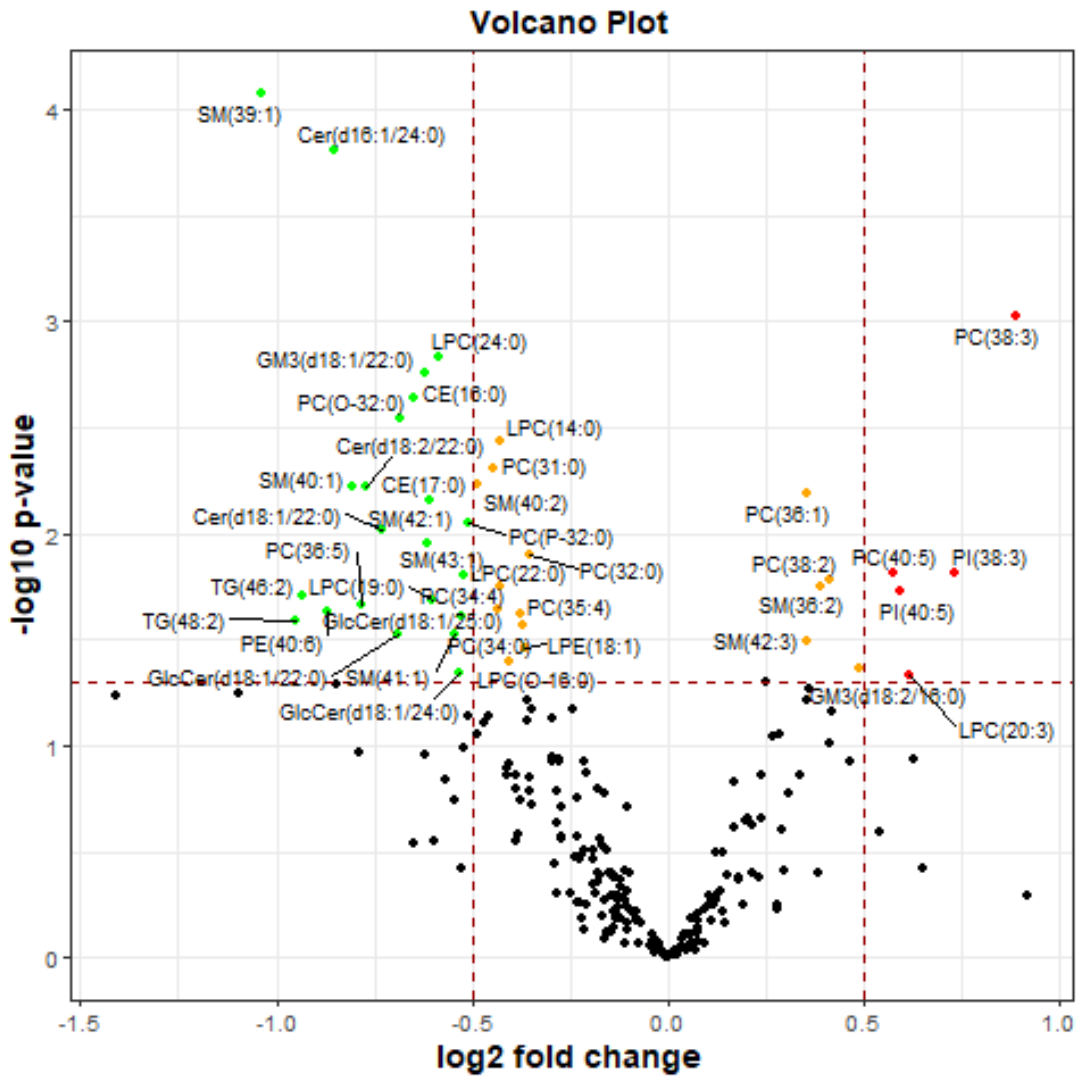
Please note that some bacterial species have different strain name in kegg and hence different kegg codes, e.g, : Salmonella enterica subsp. enterica serovar Typhimurium has 'seo' for Salmonella enterica subsp. enterica serovar Typhimurium 14028S but 'sev' for Salmonella enterica subsp. enterica serovar Typhimurium D23580, in such cases, if study in metabolomcis workbench has the name Salmonella enterica subsp. enterica serovar Typhimurium, it may not be able to pick up the kegg code. Please check the correct kegg code for your analysis.

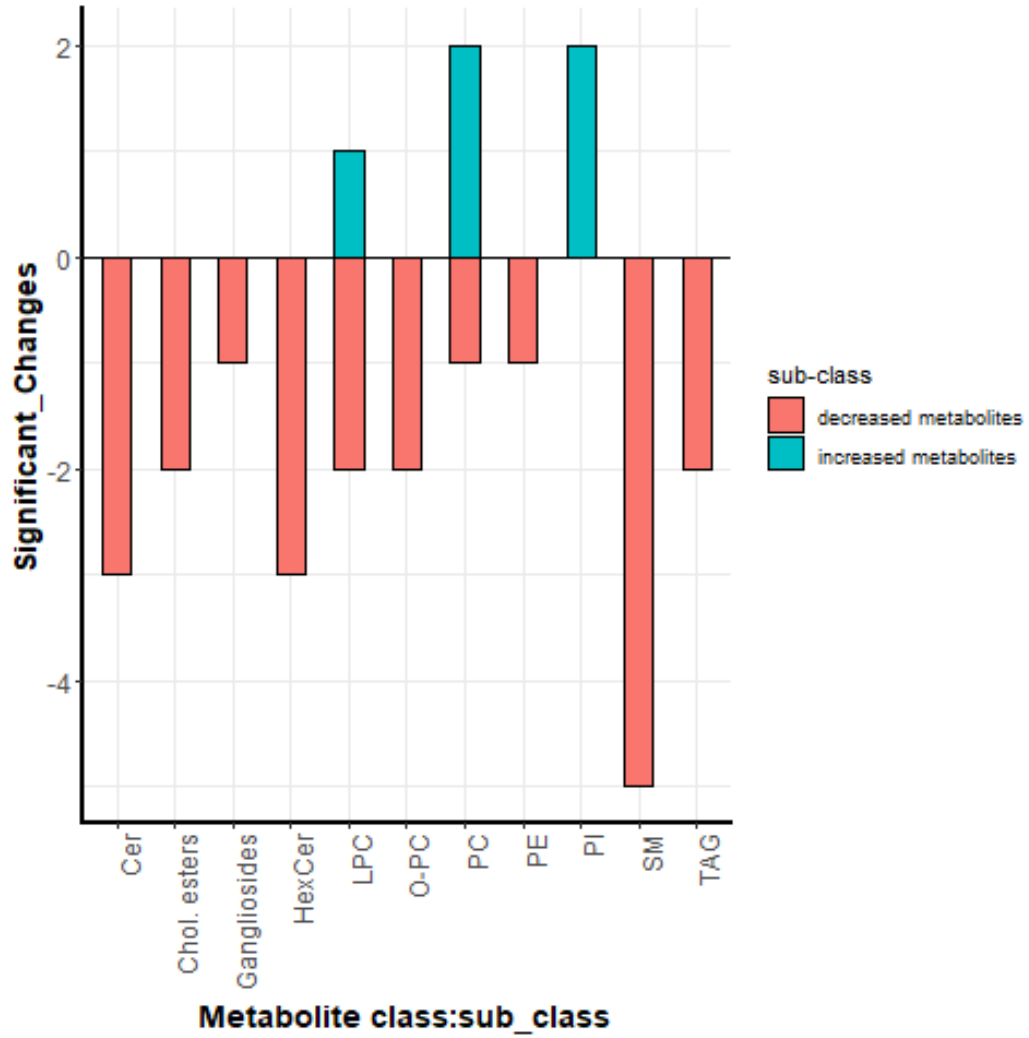
Results

For any difficulty in running the application, contact Sonal Choudhary: kschoudhary@eng.ucsd.edu or biozonal@gmail.com



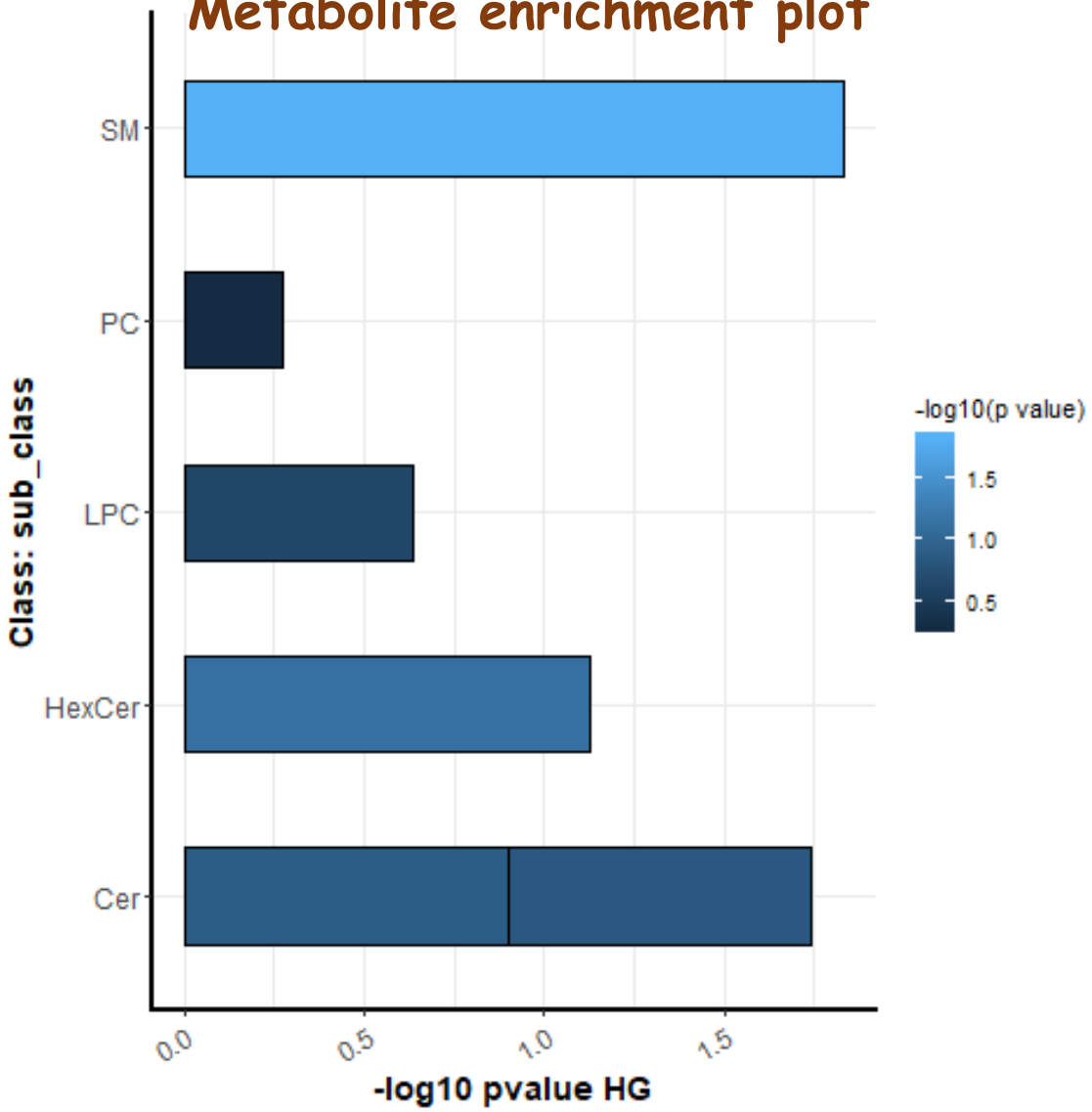
Visualization Plots



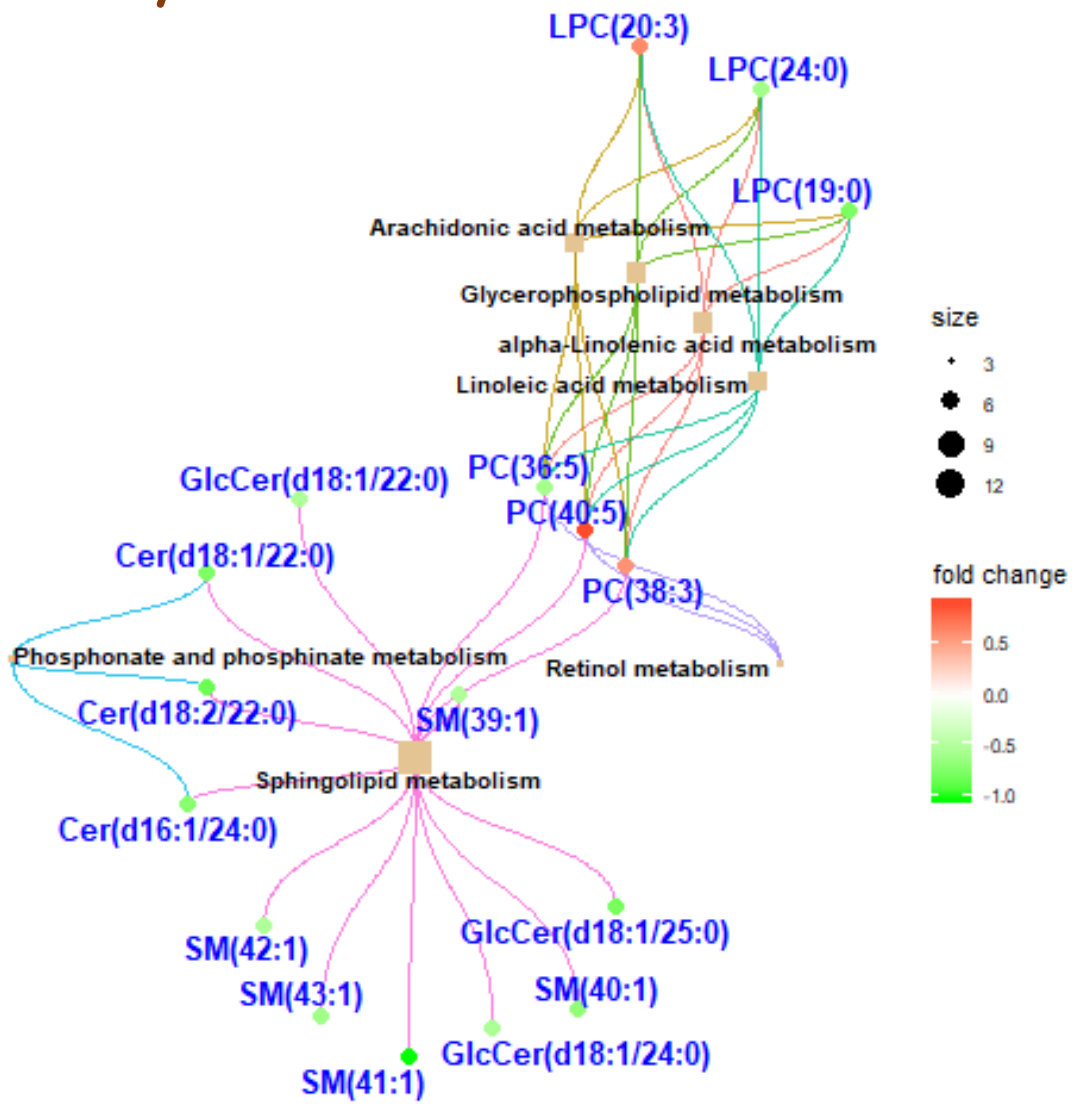


Visualization Plots

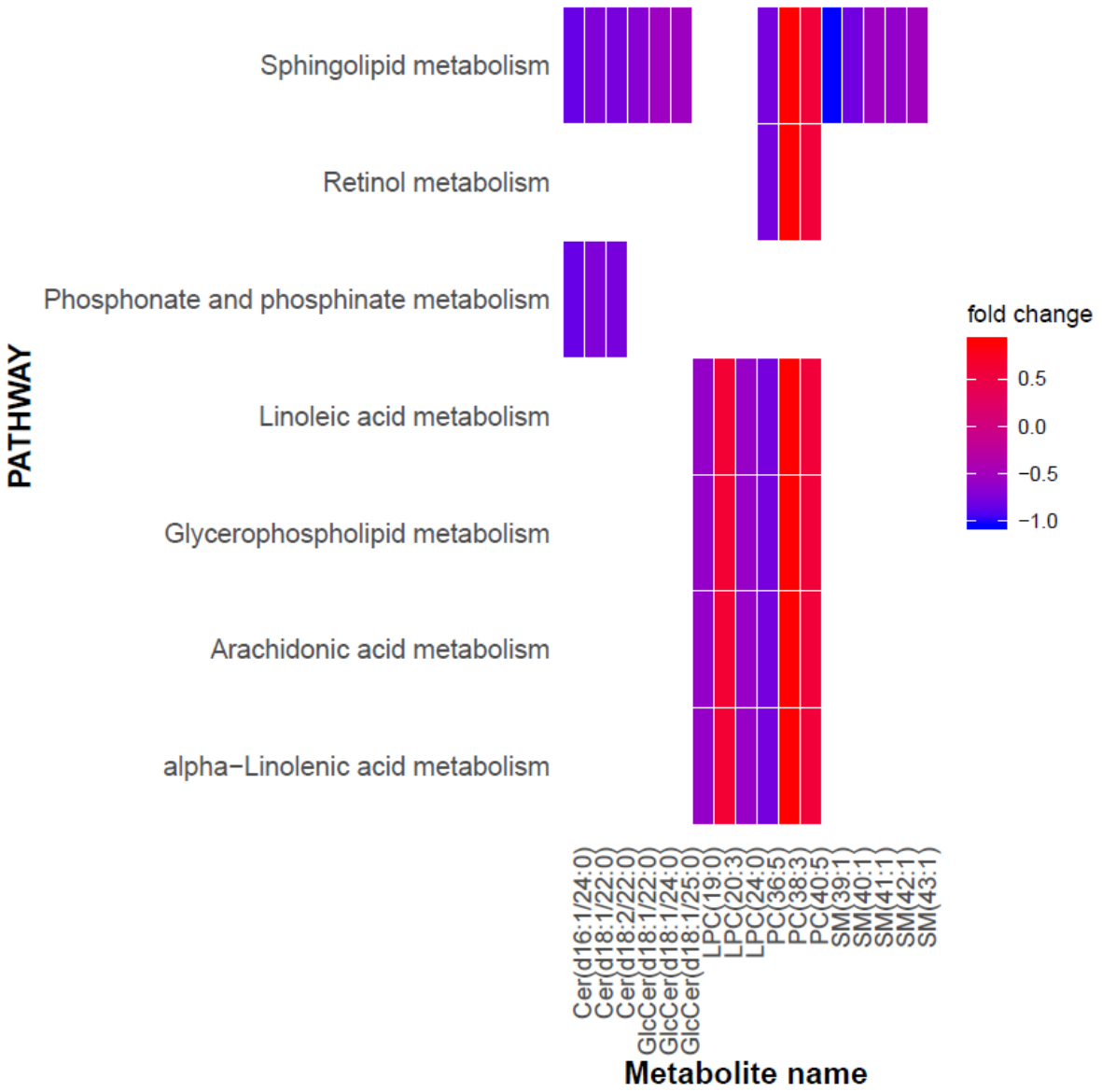
Metabolite enrichment plot

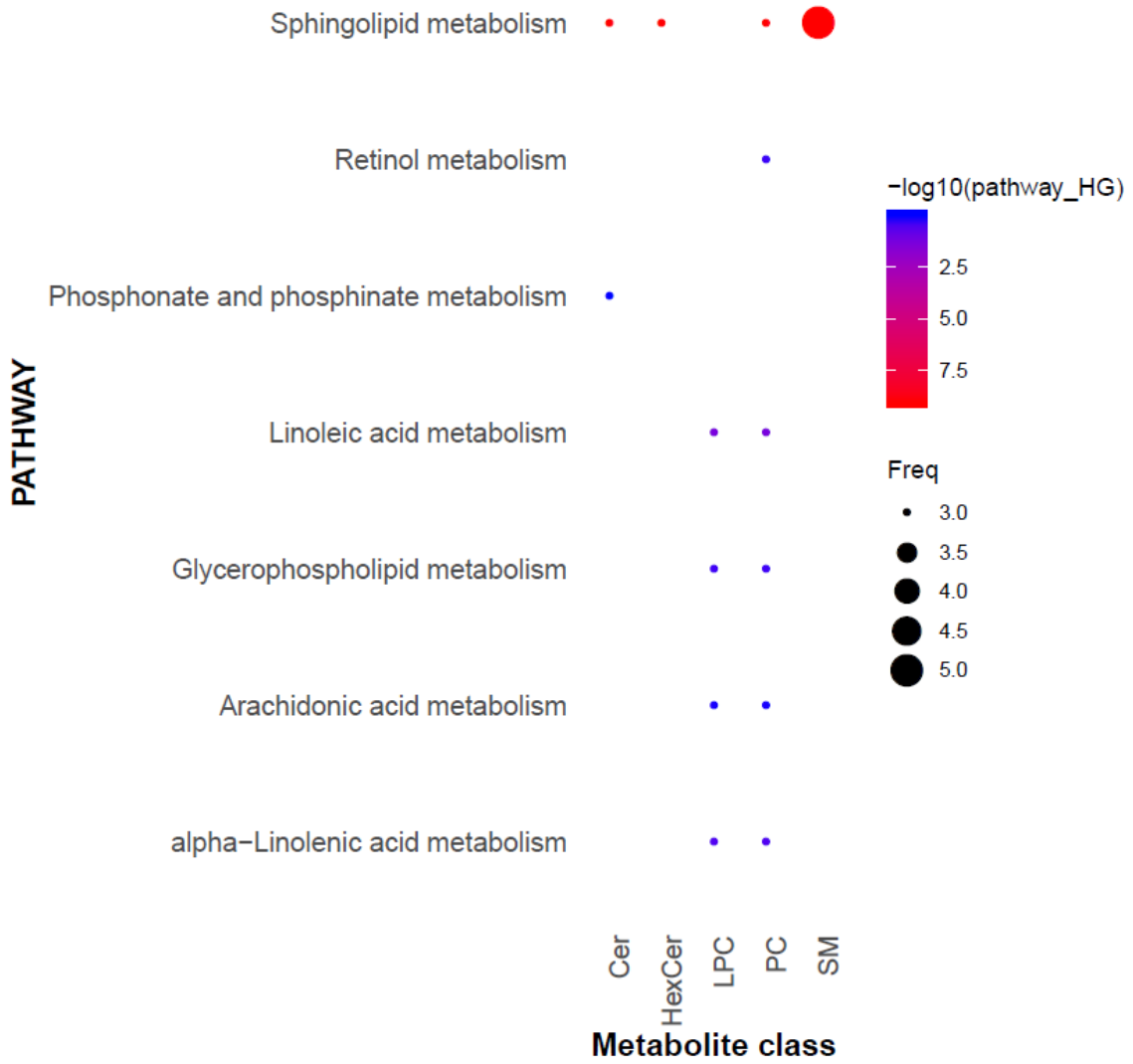


Pathway- Metabolite network



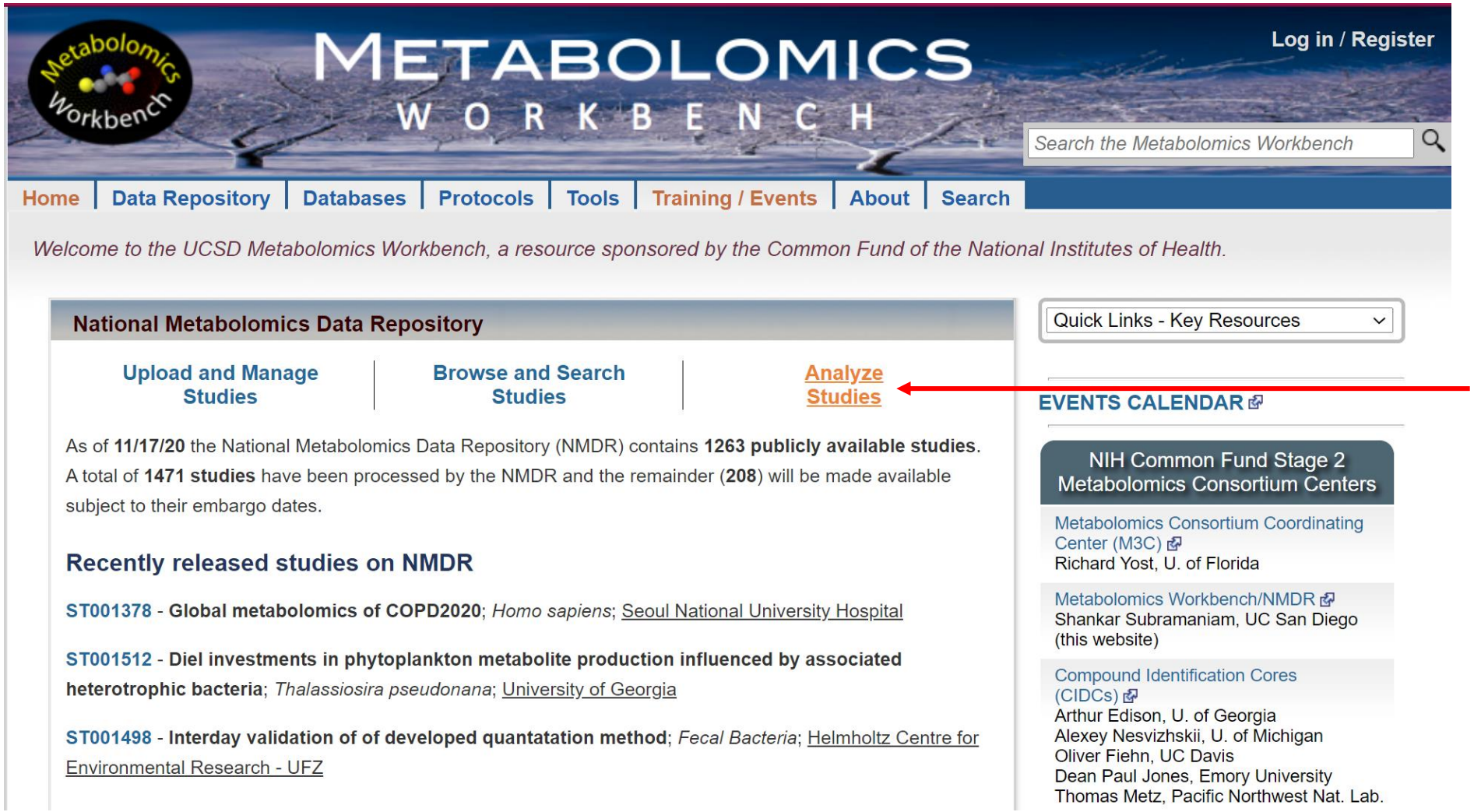
Visualization Plots





User dataset

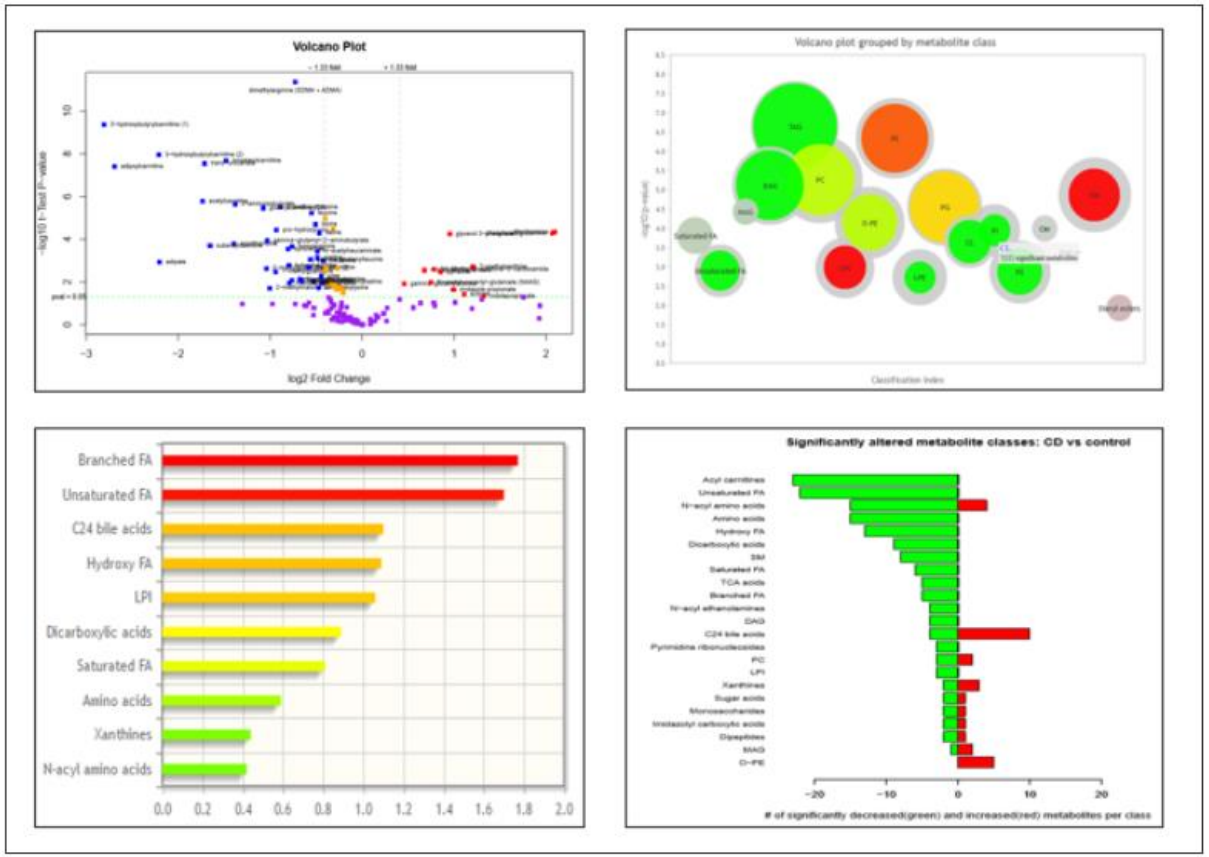
Example 2. Custom dataset



Click on Analyze Studies

Perform data analysis on user-uploaded data

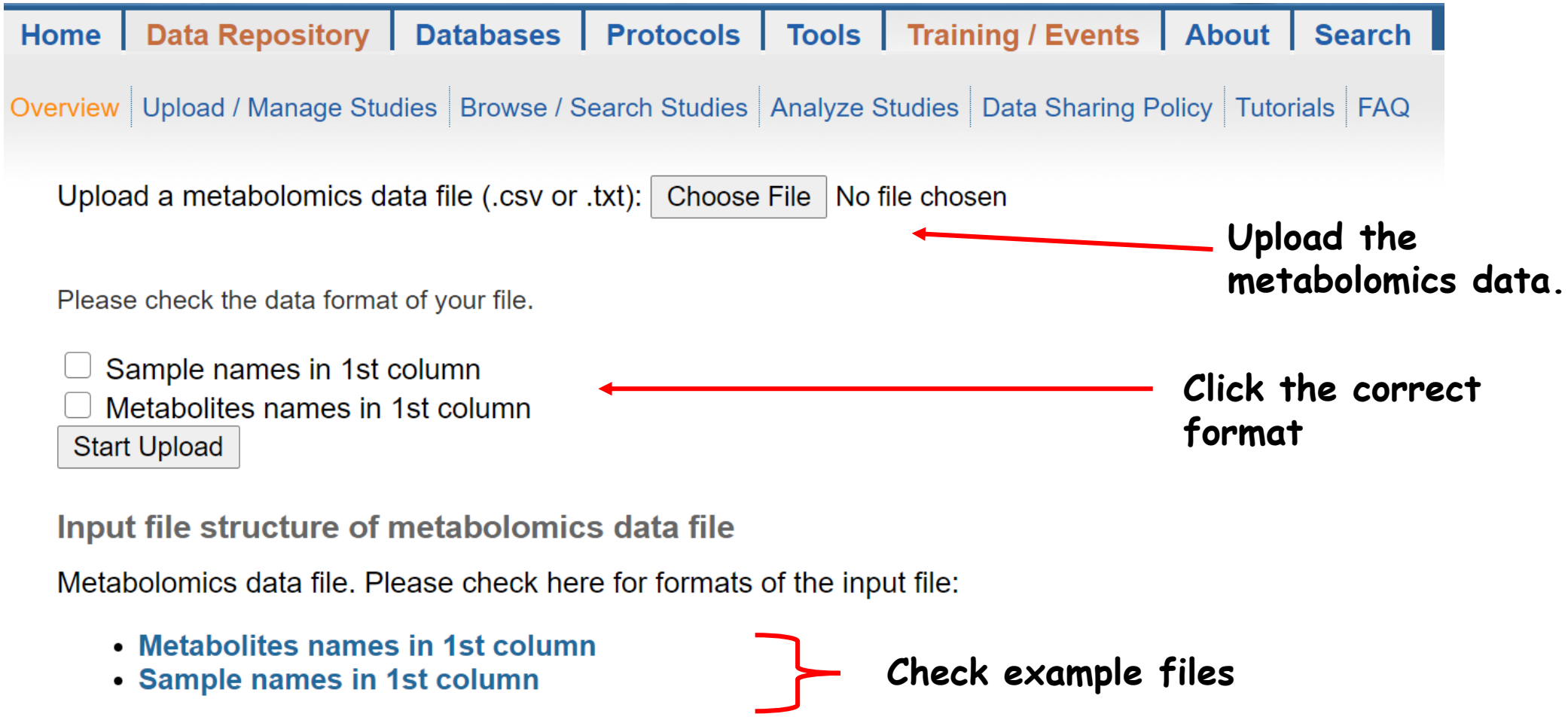
- Load and analyze your own dataset



- Load and analyze your own dataset by MetENP New!

Click here

Data Upload



Upload the metabolomics data.

Click the correct format

Check example files

Example upload

After you hit 'Start Upload' button, you can run the whole analysis like in Example 1