Tutorial to run MetENPWeb

Sonal Choudhary
Metabolomics Workbench
Example 1. Datasets already on Metabolomics Workbench

Steps

Click on Analyze Studies

As of 11/17/20, the National Metabolomics Data Repository (NMDR) contains 1263 publicly available studies. A total of 1471 studies have been processed by the NMDR and the remainder (208) will be made available subject to their embargo dates.

Recently released studies on NMDR

ST001378 - Global metabolomics of COPD2020; Homo sapiens; Seoul National University Hospital

ST001512 - Diel investments in phytoplankton metabolite production influenced by associated heterotrophic bacteria; Thalassiosira pseudonana; University of Georgia

ST001498 - Interday validation of of developed quantitation method; Fecal Bacteria; Helmholtz Centre for Environmental Research - UFZ

NIH Common Fund Stage 2 Metabolomics Consortium Centers

Metabolomics Consortium Coordinating Center (MCC)©
Richard Vost, U. of Florida

Metabolomics Workbench/NMDR ©
Shankar Subramaniam, UC San Diego (this website)

Compound Identification Cores (CIDs)©
Arthur Edison, U. of Georgia
Alexey Nesvizhskii, U. of Michigan
Oliver Pieh, UC Davis
Dean Paul Jones, Emory University
Thomas Metz, Pacific Northwest Nat. Lab.
Choose study

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 Excess... (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

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
- Perform Volcano plot analysis
- Perform ANOVA analysis

MetENP: Metabolite enrichment and species-specific pathway annotation
- MetENPWeb analysis
- MetENP R package

Clustering and correlation
- Perform hierarchical 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
Choose a analysis:

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

Select all
- Phospholipids, Chol. esters and Diacylglycerols
- Sphingolipids
- Dervitized 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:

<table>
<thead>
<tr>
<th>combined_factors</th>
<th>TreatmentGroup</th>
<th>TreatmentDuration</th>
<th>SamplingTimePoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TreatmentGroup:Prednisolone</td>
<td>TreatmentDuration:0d</td>
<td>SamplingTimePoint:before</td>
</tr>
<tr>
<td>2</td>
<td>TreatmentGroup:Prednisolone</td>
<td>TreatmentDuration:4d</td>
<td>SamplingTimePoint:after</td>
</tr>
<tr>
<td>3</td>
<td>TreatmentGroup:Tetracosactide</td>
<td>TreatmentDuration:00w</td>
<td>SamplingTimePoint:before</td>
</tr>
<tr>
<td>4</td>
<td>TreatmentGroup:Tetracosactide</td>
<td>TreatmentDuration:25w</td>
<td>SamplingTimePoint:after</td>
</tr>
</tbody>
</table>

Choose factor column:

Take a hint from the table above
Parameters

Choose factor 1

Choose factor 2

Group:
- Prednisolone
- Tetracosactid

Padjust method: nd
Handle missing data: 50percent

P-value cutoff: 0.05
Log2 fold change cutoff: 0.5

Choose a Metabolite class: cist_class

Minimum if (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: S. am.</doc>
For any difficulty in running the application, contact Sonal Choudhary:
kschoudhary@eng.ucsd.edu or biozonal@gmail.com
Visualization Plots

Metabolite enrichment plot

Pathway - Metabolite network

Class: sub_class

SM
PC
LPC
HexCer
Cer

-log10(p value)
Visualization Plots

- Sphingolipid metabolism
- Retinol metabolism
- Phosphonate and phosphinate metabolism
- Linoleic acid metabolism
- Glycerophospholipid metabolism
- Arachidonic acid metabolism
- alpha-Linolenic acid metabolism

- log10(pathway_HG) axis

- Metabolite name

- Metabolite class

- Car
- HexCar
- LPC
- PC
- SM
Example 2. Custom dataset

Click on Analyze Studies
Perform data analysis on user-uploaded data

- Load and analyze your own dataset

[Charts and graphs]

- Load and analyze your own dataset by MetENP New! Click here
Upload a metabolomics data file (.csv or .txt): Choose File  No file chosen

Please check the data format of your file.

☐ Sample names in 1st column
☐ Metabolites names in 1st column
Start Upload

Upload the metabolomics data.

Click the correct format

Input file structure of metabolomics data file

Metabolomics data file. Please check here for formats of the input file:

- Metabolites names in 1st column
- Sample names in 1st column

The second example file is taken from Metaboanalyst
Example upload

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