



MetaboAnalyst 5.0

A Web-based Tool for streamlined
metabolomics data analysis

2022.07.12

2. Functional Analysis

The **Functional Analysis** module of MetaboAnalyst has undergone several major updates since its introduction in Version 4. First, it includes a modified Gene Set Enrichment Analysis method, which considers the overall ranks of uploaded peaks and is capable of detecting more subtle and consistent changes than the original mummichog algorithm (Li et al. 2013). Second, it supports the inclusion of retention time when performing functional analysis to increase the confidence and robustness of putative compound annotation. Finally, MetaboAnalyst 5.0 has included an interactive heatmap visualization of a user's peak intensity table to help users perform functional interpretation of manually identified patterns of interest.

Other Highlights:

- Users can upload either a peak intensity table (generic or MZMine) or peak list.
 - Heatmap based pattern specific functional analysis is available.
 - Added support for pathway analysis of 26 organisms including human, mouse, zebrafish, *C. elegans*, among other species.
 - Added ~9, 000 metabolite sets (e.g. Disease-associated sets, chemical classes) to be used for functional interpretation.
- 
- A decorative network diagram in the bottom right corner of the slide. It consists of numerous white dots of varying sizes connected by thin white lines, forming a complex web of interconnected nodes and edges. The background is a solid teal color.

2.0 Knowledge & Background

- Mass spectrometry based untargeted metabolomics traditionally require metabolites to be identified before any biological meaning can be drawn from the data. Metabolite identification is a challenging and low throughput process, therefore becomes the bottleneck of the field. [Li et al.](#) report here a novel approach to predict biological activity directly from mass spectrometry data without a priori identification of metabolites by unifying network analysis and metabolite prediction under the same computational framework. (version 1)
- The algorithm has been further enhanced to version 2 by considering the retention time information for more accuracy by introducing empirical compounds. Empirical Compounds are intermediaries between m/z features and compounds. The steps for how they are formed are as follows:

First, all m/z features are matched to potential compounds considering different adducts. Then, per compound, all matching m/z features are split into Empirical Compounds based on whether they match within an expected retention time window. The retention time window (in seconds) is calculated as the maximum retention time * 0.02. This results in the initial Empirical Compounds list.

Next, Empirical Compounds are merged if they have the same m/z, matched form/ion, and retention time. This results in the merged Empirical Compounds list.

Then, if primary ions are enforced, only Empirical Compounds containing at least 1 primary ion are kept. Primary ions considered are 'M+H[1+]', 'M+Na[1+]', 'M-H₂O+H[1+]', 'M-H[-]', 'M-2H[2-]', 'M-H₂O-H[-]', 'M+H [1+]', 'M+Na [1+]', 'M-H₂O+H [1+]', 'M-H [1-]', 'M-2H [2-]', and 'M-H₂O-H [1-]'. This results in the final Empirical Compounds list.

Finally, pathway libraries are converted from "Compound" space to "Empirical Compound" space. This is done by converting all compounds in each pathway to all Empirical Compound matches. Then the mummichog/GSEA algorithms work as before to calculate pathway enrichment.

2.1 Start Functional Analysis



MetaboAnalyst 5.0 - user-friendly, streamlined metabolomics data analysis

[Home](#)

[Data Formats](#)

[Tutorials](#)

[OmicsForum](#)

[APIs](#)

[Update History](#)

[MetaboAnalystR](#)

[Contact](#)

[User Stats](#)

[Publications](#)

[COVID-19 Data](#)

[About](#)

Module Overview

Click here to start

Input Data Type	Available Modules (click on a module to roll down for more details)					
Raw Spectra (mzML, mzXML or mzData)				LC-MS Spectra Processing		
MS Peaks (peak list or intensity table)			Functional Analysis	Functional Meta-analysis		
Annotated Features (compound list or table)		Enrichment Analysis	Pathway Analysis	Joint-Pathway Analysis	Network Analysis	
Generic Format (.csv or .txt table files)	Statistical Analysis [one factor]	Statistical Analysis [metadata table]	Biomarker Analysis	Statistical Meta-analysis	Power Analysis	Other Utilities

2.2 Starting from a list

From peak list to pathways



2.2.1 Peak Uploading – peak list

TIP1 : Multiple examples are provided here. Please try to download one and follow the style of the example rigorously. Please make sure that the list header is consistently same as the example.

1. Switch the different uploading data type from here (peak list or table)

Please upload your data

This module supports functional analysis of untargeted metabolomics data generated from high-resolution mass spectrometry (HRMS). The basic assumption is that putative annotation at individual compound level can collectively predict changes at functional levels as defined by metabolite sets or pathways. This is because changes at group level rely on "collective behavior" which is more tolerant to random errors in compound annotation as demonstrated by [Li et al.](#) To use this approach,

- The input peak list or peak table must contain the complete data, not just significant data - we need the complete data to estimate the null model (background);
- [Required] Feature or peak names must be their numeric mass (m/z) values for putative annotation;
- [Optional] You can also provide retention time (RT) to further improve peak annotation

A peak list profile A peak intensity table

Upload a peak list file

Ion Mode:

Mass Tolerance (ppm): (editable)

Retention Time:

Ranked by (1 column only): P values T scores

Enforce Primary Ions (V2 only):

Data File:

2. Set parameters (mass error and ion mode) based your instrument

3. Click submit to continue

Try our example datasets

Data	Format
<input checked="" type="radio"/> IBD	Three columns (m.z, p.value, t.score) <small>(controls) obtained using a Q-Exactive Plus Orbitrap (negative ion mode) from the Integrative Human Microbiome Project (IHMP).</small>
<input type="radio"/> IBD_2	Four columns (m.z, p.value, t.score, rt) Same as above

2.2.2 Data Integrity Check

Data Integrity Check:

1. Checking the class labels - at least three replicates are required in each class.
2. If the samples are paired, the pair labels must conform to the specified format.
3. The data (except class labels) must not contain non-numeric values.
4. The presence of missing values or features with constant values (i.e. all zeros).

Data processing information:

Checking data content ...passed.

A total of 4187 m/z features were found in your uploaded data.

The instrument's mass accuracy is 5 ppm.

The instrument's analytical mode is **negative**.

The uploaded data contains **3** columns.

The column headers of uploaded data are **m.z, p.value, t.score**.

The range of m/z peaks is trimmed to 50-2000. **0** features have been trimmed.

A total of 4187 input mz features were retained for further analysis.

[Edit Groups](#) [Missing Values](#) [Proceed](#)

1. Check **Data Integrity Result** to make sure correct

2. Click **Proceed** to continue

2.2.3 Set Parameters

Upload

Processing

Data check

Set corresponding parameters/Library

Exit

Specify analysis parameters:

Algorithms	<input checked="" type="checkbox"/> Mummichog P-value cutoff: <input type="text" value="0.2"/> (default top 10% peaks) <input type="checkbox"/> GSEA (using the overall rank based on t.score)
Visual analytics:	<input checked="" type="radio"/> Scatter plot - test significant peaks <input type="radio"/> Heatmaps - test peaks in a visual pattern (good for multiple groups)
Advanced options	Edit Currency Metabolites Edit Adducts

Select a pathway library: (KEGG pathway info were obtained in Oct. 2019)

Mammals

- Homo sapiens (human) [MFN]
- Homo sapiens (human) [BioCyc]
- Homo sapiens (human) [KEGG]
- Mus musculus (mouse) [BioCyc]
- Mus musculus (mouse) [KEGG]
- Rattus norvegicus (rat) [KEGG]

TIP1 : You can manually customize the abundant substances as 'Currency Metabolites' and adducts for not considering at the pathway analysis.

TIP2 : Heatmap analysis only works if users upload a peak table If you want to do the heatmap based analysis, please see [6.3](#).

Currency Metabolite Customization

Use the panels below to select metabolites to include as currency:

Available	Include
Acetoacetyl CoA (C00332)	Water (C00001)
Acetyl coenzyme A (C00024)	Proton (C00080)
Adenosine diphosphate (C00008)	Oxygen (C00007)
Adenosine monophosphate (C00020)	NADPH (C00005)
Carbon monoxide (C00237)	NADP (C00096)
Coenzyme A (C00010)	NADH (C00004)
Flavin adenine dinucleotide (C00016)	NAD (C00003)
FADH2 (C00016)	Adenosine triphosphate (C00002)
Guanosine triphosphate (C00044)	Pyrophosphate (C00013)
Guanosine diphosphate (C00035)	Phosphate (C00009)
Guanosine monophosphate (C00144)	Carbon dioxide (C00011)
Hydrogen (C00282)	
Hydrogen peroxide (C00027)	
Carbonic acid (C01353)	

Submit

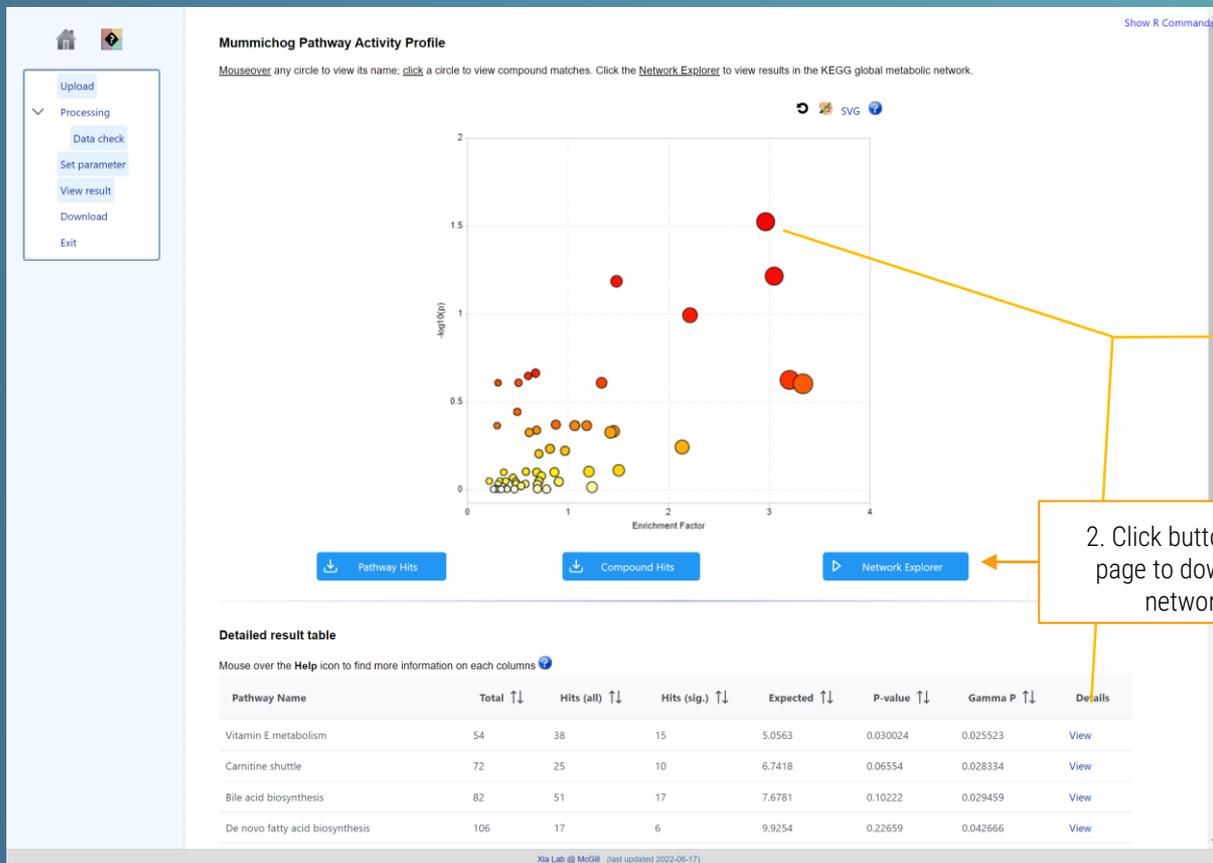
Adduct Customization

Use the panels below to select adducts to consider:

Available	Include
M-3H [3-]	M-H [1-]
M+FA-H [1-]	M-2H [2-]
M+Hac-H [1-]	M-H2O-H [1-]
M+TFA-H [1-]	M-H+O [1-]
2M-H [1-]	M+K-2H [1-]
2M+FA-H [1-]	M+Na-2H [1-]
2M+Hac-H [1-]	M+Cl [1-]
3M-H [1-]	M+Cl37 [1-]
	M+Br [1-]
	M+Br81 [1-]
	M+ACN-H [1-]
	M+HCOO [1-]
	M+CH3COO [1-]
	M(C13)-H [1-]

Submit

2.2.4 Pathway analysis results



1. The compounds/empirical compounds hits in this pathway

The colored compounds/empirical compounds indicate potential matches from the user's input, with red colors indicating significant hits and blue colors indicating non-significant hits.

Pathway	Metabolites
Vitamin E metabolism	CE5849 ; C00024 ; C00027 ; CE0812 ; CE5643 ; C00020 ; C00100 ; CE5948 ; CE5841 ; CE5840 ; CE5843 ; CE5842 ; CE5845 ; CE5721 ; CE5847 ; CE5723 ; CE6000 ; CE6219 ; CE5022 ; C11378 ; CE5021 ; CE5844 ; C02477 ; CE5838 ; CE7144 ; CE7145 ; C00601 ; C14153 ; CE7047 ; C00010 ; CE5986 ; CE5835 ; CE5837 ; CE5719 ; CE5718 ; CE5850 ; CE5851 ; CE5856 ; CE5855 ; CE1926 ; CE5899 ; CE1924 ; CE1925 ; CE5017 ; CE5846 ; CE4898 ; CE1928 ; CE7101 ; CE5655 ; C00088 ; CE7072 ; CE7073 ; CE7074 ; CE5639

2. Click buttons at the bottom of this page to download results or go the network exploration page

2.3 Starting from a table



2.3.1 Peak uploading – peak table

The screenshot shows the 'Please upload your data' section of the MetaboAnalyst web application. On the left is a navigation menu with 'Upload' selected. The main content area has two tabs: 'A peak list profile' and 'A peak intensity table', with the latter being active. Below the tabs is a form titled 'Upload a peak intensity table' with various input fields and a 'Submit' button. At the bottom, there is a 'Try our example datasets' section with a table of example data.

1. Switch to uploading peak intensity table tab

2. Set parameters (mass error and ion mode) based your instrument

3. Click submit to continue

Please upload your data

This module supports functional analysis of untargeted metabolomics data generated from high-resolution mass spectrometry (HRMS). The basic assumption is that putative annotation at individual compound level can collectively predict changes at functional levels as defined by metabolite sets or pathways. This is because changes at group level rely on "collective behavior" which is more tolerant to random errors in compound annotation as demonstrated by [Li et al.](#) To use this approach,

- The input peak list or peak table must contain the complete data, not just significant data - we need the complete data to estimate the null model (background);
- [Required] Feature or peak names must be their numeric mass (m/z) values for putative annotation;
- [Optional] You can also provide retention time (RT) to further improve peak annotation

A peak list profile A peak intensity table

Upload a peak intensity table

Ion Mode: Negative Mode

Mass Tolerance (ppm): 5.0 (editable)

Retention Time: Not present

Data Source: Generic

Data Format: Samples in columns

Data File: Choose File No file chosen

Submit

Try our example datasets

Data	Format
<input checked="" type="radio"/> Immune Cell	Generic peak intensity table with retention time cells treated in DSS.
<input type="radio"/> Covid-19	Peak intensity table with retention time Peak intensity table of COVID-19 global metabolomics study , with over 9,000 peaks.

TIP1 : Currently, 2 types of peaks are supported (MetaboAnalyst generic and MZmine style). Users could coerce you table manually to make it applicable here.

2.3.2 Peak uploading – Preprocessing

Data Integrity Check:

1. Checking the class labels - at least three replicates are required in each class.
2. If the samples are paired, the pair labels must conform to the specified format.
3. The data (except class labels) must not contain non-numeric values.
4. The presence of missing values or features with constant values (i.e. all zeros).

Data processing information:

Checking data content - passed
Samples are in columns and features in rows.
The uploaded file is in comma separated values (.csv) format.
The uploaded data file contains 12 (samples) by 4353 (peaks(mz/rt)) data matrix.
Samples are not paired.
4 groups were detected in samples.
Only English letters, numbers, underscore, hyphen and forward slash (/) are allowed.
Other special characters or punctuations (if any) will be stripped off.
All data values are numeric.
404 features with a constant or single value across samples were found and deleted.
A total of 1869 (3.9%) missing values were detected.
By default, missing values will be replaced by 1/5 of min positive values of their corresponding variables
Click the Skip button if you accept the default practice.
Or click the Missing value imputation to use other methods.

Edit Groups

Missing Values

Proceed

Data Filtering:

The purpose of the data filtering is to identify and remove variables that are unlikely to be of use when modeling the data. No phenotype information are used in the filtering process, so the result can be used with any downstream analysis. This step is strongly recommended for untargeted metabolomics datasets (i.e. spectral binning data, peak lists) with large number of variables, many of them are from baseline noises. Filtering can usually improve the results. For details, please refer to the paper by [Hochberg et al.](#)

Non-informative variables can be characterized in three groups: 1) variables of **very small values** (close to baseline or detection limit) - these variables can be detected using mean or median; 2) variables that are **near-constant values** throughout the experiment conditions (housekeeping or homeostasis) - these variables can be detected using standard deviation (SD) or the robust estimate such as interquartile range (IQR); and 3) variables that show **low repeatability** - this can be measured using QC samples using the relative standard deviation (RSD = SD/mean). Features with high percent RSD should be removed from the subsequent analysis (the suggested threshold is 20% for LC-MS and 30% for GC-MS). For data filtering based on the first two categories, the following empirical rules are applied during data filtering:

- **Less than 250 variables:** 5% will be filtered.
- **Between 250 - 500 variables:** 10% will be filtered.
- **Between 500 - 1000 variables:** 25% will be filtered.
- **Over 1000 variables:** 40% will be filtered.

Please note, in order to reduce the computational burden to the server, the **None** option is only for less than 5000 features. The maximum allowed number of variables is 5000. [For more details, visit the max number is 2500](#) to improve power and to control computing time. Over that, the IQR filter will still be applied to keep only top maximum features, even if you choose **None** option.

- Filtering features if their RSDs are > % in QC samples
- None (less than 5000 features)
 - Interquartile range (IQR)
 - Standard deviation (SD)
 - Median absolute deviation (MAD)
 - Relative standard deviation (RSD = SD/mean)
 - Non-parametric relative standard deviation (MAD/median)
 - Mean intensity value
 - Median intensity value

Submit

Proceed

Normalization overview:

The normalization procedures are grouped into three categories. The sample normalization allows general-purpose adjustment for differences among your sample, data transformation and scaling are two different approaches to make individual features more comparable. You can use one or combine them to achieve better results.

Sample Normalization

- None
- Sample-specific normalization (i.e. weight, volume) [Specify](#)
- Normalization by sum
- Normalization by median
- Normalization by reference sample (PGN) [Specify](#)
- Normalization by a pooled sample from group [Specify](#)
- Normalization by reference feature [Specify](#)
- Quantile normalization

Data transformation

- None
- Log transformation (generalized logarithm transformation or glog)
- Cube root transformation (takes the cube root of data values)

Data scaling

- None
- Mean centering (mean-centered only)
- Auto scaling (mean-centered and divided by the standard deviation of each variable)
- Pareto scaling (mean-centered and divided by the square root of the standard deviation of each variable)
- Range scaling (mean-centered and divided by the range of each variable)

1. Perform Data Integrity Check

2. Perform Data Filtering

3. Perform Data Normalization

2.3.3 Set parameters



MetaboAnalyst 5.0 - user-friendly, end-to-end metabolomics data analysis



Upload

Processing

Data check

Missing value

Data filter

Data editor

Normalization

Set parameter

View result

Metabolic network

Heatmap viewer

Download

Exit

Specify analysis parameters:

Algorithms	<input checked="" type="checkbox"/> Mummichog P-value cutoff: <input type="text" value="1.0E-5"/> (default top 10% peaks) <input type="checkbox"/> GSEA (using the overall rank based on t.score)
View options:	<input checked="" type="radio"/> Scatter plot (Test significant features) <input type="radio"/> Heatmaps (Test manually selected patterns)
Advanced options ?	Edit Currency Metabolites Edit Adducts

Select a pathway library: (KEGG pathway info were obtained in Oct. 2019)

Mammals	<input checked="" type="radio"/> Homo sapiens (human) [MFN] ? <input type="radio"/> Homo sapiens (human) [BioCyc] <input type="radio"/> Homo sapiens (human) [KEGG] <input type="radio"/> Mus musculus (mouse) [BioCyc] <input type="radio"/> Mus musculus (mouse) [KEGG] <input type="radio"/> Rattus norvegicus (rat) [KEGG] <input type="radio"/> Bos taurus (cow) [KEGG]
Birds	<input type="radio"/> Gallus gallus (chicken) [KEGG]
Fish	<input type="radio"/> Danio rerio (zebrafish) [KEGG] <input type="radio"/> Danio rerio (zebrafish) [MTF] ?
Insects	<input type="radio"/> Drosophila melanogaster (fruit fly) [KEGG] <input type="radio"/> Drosophila melanogaster (fruit fly) [BioCyc]
Nematodes	<input type="radio"/> Caenorhabditis elegans (nematode) [KEGG]
Fungi	<input type="radio"/> Saccharomyces cerevisiae (yeast) [KEGG] <input type="radio"/> Saccharomyces cerevisiae (yeast) [BioCyc]

Set corresponding parameters/Library

TIPS : Most parameters are same as the ones used for processing the peak list, as described in 6.2.3. The only difference is that peak table allow the heatmap based pattern specific functional analysis.

2.3.4 Heatmap based pattern specific analysis

TIPs : The scatter plot and its corresponding functions for peak table uploading is totally same the one of peak list uploading. Please refer to [6.2.4](#).

MetaboAnalyst 5.0 - user-friendly, end-to-end metabolomics data analysis

Specify analysis parameters:

Algorithms	<input checked="" type="checkbox"/> Mummichog P-value cutoff: <input type="text" value="5.0E-5"/> (default top 10% peaks) <input type="checkbox"/> GSEA (using the overall rank based on t.score)
View options:	<input type="radio"/> Scatter plot (Test significant features) <input checked="" type="radio"/> Heatmaps (Test manually selected patterns)
Advanced options ?	Edit Currency Metabolites Edit Adducts

Select a pathway library: (KEGG pathway info were obtained in Oct. 2019)

Mammals	<input checked="" type="radio"/> Homo sapiens (human) [MFN] ? <input type="radio"/> Homo sapiens (human) [BioCyc] <input type="radio"/> Homo sapiens (human) [KEGG] <input type="radio"/> Mus musculus (mouse) [BioCyc] <input type="radio"/> Mus musculus (mouse) [KEGG] <input type="radio"/> Rattus norvegicus (rat) [KEGG] <input type="radio"/> Bos taurus (cow) [KEGG]
Birds	<input type="radio"/> Gallus gallus (chicken) [KEGG]
Fish	<input type="radio"/> Danio rerio (zebrafish) [KEGG] <input type="radio"/> Danio rerio (zebrafish) [MTF] ?
Insects	<input type="radio"/> Drosophila melanogaster (fruit fly) [KEGG] <input type="radio"/> Drosophila melanogaster (fruit fly) [BioCyc]
Nematodes	<input type="radio"/> Caenorhabditis elegans (nematode) [KEGG] <input type="radio"/> Saccharomyces cerevisiae (yeast) [KEGG]

1. Select **Heatmaps** radio to start !

2.3.5 Heatmap based pattern specific analysis - result

This section maybe too complicated to easily understand/follow for beginners, why not watch a [video](#) first?

Resolution: Medium Colors: navy-white-firebrick Border: Default Cluster peaks: P value Download: --Please Select-- Builder

Overview [Select all](#) Focus View

Condition

128.04824
128.05116
128.05168
128.05262
128.05380
128.05432
128.05484
128.05536
128.05588
128.05640
128.05692
128.05744
128.05796
128.05848
128.05900
128.05952
128.06004
128.06056
128.06108
128.06160
128.06212
128.06264
128.06316
128.06368
128.06420
128.06472
128.06524
128.06576
128.06628
128.06680
128.06732
128.06784
128.06836
128.06888
128.06940
128.06992
128.07044
128.07096
128.07148
128.07200
128.07252
128.07304
128.07356
128.07408
128.07460
128.07512
128.07564
128.07616
128.07668
128.07720
128.07772
128.07824
128.07876
128.07928
128.07980
128.08032
128.08084
128.08136
128.08188
128.08240
128.08292
128.08344
128.08396
128.08448
128.08500
128.08552
128.08604
128.08656
128.08708
128.08760
128.08812
128.08864
128.08916
128.08968
128.09020
128.09072
128.09124
128.09176
128.09228
128.09280
128.09332
128.09384
128.09436
128.09488
128.09540
128.09592
128.09644
128.09696
128.09748
128.09800
128.09852
128.09904
128.09956
128.10008
128.10060
128.10112
128.10164
128.10216
128.10268
128.10320
128.10372
128.10424
128.10476
128.10528
128.10580
128.10632
128.10684
128.10736
128.10788
128.10840
128.10892
128.10944
128.10996
128.11048
128.11100
128.11152
128.11204
128.11256
128.11308
128.11360
128.11412
128.11464
128.11516
128.11568
128.11620
128.11672
128.11724
128.11776
128.11828
128.11880
128.11932
128.11984
128.12036
128.12088
128.12140
128.12192
128.12244
128.12296
128.12348
128.12400
128.12452
128.12504
128.12556
128.12608
128.12660
128.12712
128.12764
128.12816
128.12868
128.12920
128.12972
128.13024
128.13076
128.13128
128.13180
128.13232
128.13284
128.13336
128.13388
128.13440
128.13492
128.13544
128.13596
128.13648
128.13700
128.13752
128.13804
128.13856
128.13908
128.13960
128.14012
128.14064
128.14116
128.14168
128.14220
128.14272
128.14324
128.14376
128.14428
128.14480
128.14532
128.14584
128.14636
128.14688
128.14740
128.14792
128.14844
128.14896
128.14948
128.15000
128.15052
128.15104
128.15156
128.15208
128.15260
128.15312
128.15364
128.15416
128.15468
128.15520
128.15572
128.15624
128.15676
128.15728
128.15780
128.15832
128.15884
128.15936
128.15988
128.16040
128.16092
128.16144
128.16196
128.16248
128.16300
128.16352
128.16404
128.16456
128.16508
128.16560
128.16612
128.16664
128.16716
128.16768
128.16820
128.16872
128.16924
128.16976
128.17028
128.17080
128.17132
128.17184
128.17236
128.17288
128.17340
128.17392
128.17444
128.17496
128.17548
128.17600
128.17652
128.17704
128.17756
128.17808
128.17860
128.17912
128.17964
128.18016
128.18068
128.18120
128.18172
128.18224
128.18276
128.18328
128.18380
128.18432
128.18484
128.18536
128.18588
128.18640
128.18692
128.18744
128.18796
128.18848
128.18900
128.18952
128.19004
128.19056
128.19108
128.19160
128.19212
128.19264
128.19316
128.19368
128.19420
128.19472
128.19524
128.19576
128.19628
128.19680
128.19732
128.19784
128.19836
128.19888
128.19940
128.19992
129.00044
129.00096
129.00148
129.00200
129.00252
129.00304
129.00356
129.00408
129.00460
129.00512
129.00564
129.00616
129.00668
129.00720
129.00772
129.00824
129.00876
129.00928
129.00980
129.01032
129.01084
129.01136
129.01188
129.01240
129.01292
129.01344
129.01396
129.01448
129.01500
129.01552
129.01604
129.01656
129.01708
129.01760
129.01812
129.01864
129.01916
129.01968
129.02020
129.02072
129.02124
129.02176
129.02228
129.02280
129.02332
129.02384
129.02436
129.02488
129.02540
129.02592
129.02644
129.02696
129.02748
129.02800
129.02852
129.02904
129.02956
129.03008
129.03060
129.03112
129.03164
129.03216
129.03268
129.03320
129.03372
129.03424
129.03476
129.03528
129.03580
129.03632
129.03684
129.03736
129.03788
129.03840
129.03892
129.03944
129.03996
129.04048
129.04100
129.04152
129.04204
129.04256
129.04308
129.04360
129.04412
129.04464
129.04516
129.04568
129.04620
129.04672
129.04724
129.04776
129.04828
129.04880
129.04932
129.04984
129.05036
129.05088
129.05140
129.05192
129.05244
129.05296
129.05348
129.05400
129.05452
129.05504
129.05556
129.05608
129.05660
129.05712
129.05764
129.05816
129.05868
129.05920
129.05972
129.06024
129.06076
129.06128
129.06180
129.06232
129.06284
129.06336
129.06388
129.06440
129.06492
129.06544
129.06596
129.06648
129.06700
129.06752
129.06804
129.06856
129.06908
129.06960
129.07012
129.07064
129.07116
129.07168
129.07220
129.07272
129.07324
129.07376
129.07428
129.07480
129.07532
129.07584
129.07636
129.07688
129.07740
129.07792
129.07844
129.07896
129.07948
129.08000
129.08052
129.08104
129.08156
129.08208
129.08260
129.08312
129.08364
129.08416
129.08468
129.08520
129.08572
129.08624
129.08676
129.08728
129.08780
129.08832
129.08884
129.08936
129.08988
129.09040
129.09092
129.09144
129.09196
129.09248
129.09300
129.09352
129.09404
129.09456
129.09508
129.09560
129.09612
129.09664
129.09716
129.09768
129.09820
129.09872
129.09924
129.09976
130.00028
130.00080
130.00132
130.00184
130.00236
130.00288
130.00340
130.00392
130.00444
130.00496
130.00548
130.00600
130.00652
130.00704
130.00756
130.00808
130.00860
130.00912
130.00964
130.01016
130.01068
130.01120
130.01172
130.01224
130.01276
130.01328
130.01380
130.01432
130.01484
130.01536
130.01588
130.01640
130.01692
130.01744
130.01796
130.01848
130.01900
130.01952
130.02004
130.02056
130.02108
130.02160
130.02212
130.02264
130.02316
130.02368
130.02420
130.02472
130.02524
130.02576
130.02628
130.02680
130.02732
130.02784
130.02836
130.02888
130.02940
130.02992
130.03044
130.03096
130.03148
130.03200
130.03252
130.03304
130.03356
130.03408
130.03460
130.03512
130.03564
130.03616
130.03668
130.03720
130.03772
130.03824
130.03876
130.03928
130.03980
130.04032
130.04084
130.04136
130.04188
130.04240
130.04292
130.04344
130.04396
130.04448
130.04500
130.04552
130.04604
130.04656
130.04708
130.04760
130.04812
130.04864
130.04916
130.04968
130.05020
130.05072
130.05124
130.05176
130.05228
130.05280
130.05332
130.05384
130.05436
130.05488
130.05540
130.05592
130.05644
130.05696
130.05748
130.05800
130.05852
130.05904
130.05956
130.06008
130.06060
130.06112
130.06164
130.06216
130.06268
130.06320
130.06372
130.06424
130.06476
130.06528
130.06580
130.06632
130.06684
130.06736
130.06788
130.06840
130.06892
130.06944
130.06996
130.07048
130.07100
130.07152
130.07204
130.07256
130.07308
130.07360
130.07412
130.07464
130.07516
130.07568
130.07620
130.07672
130.07724
130.07776
130.07828
130.07880
130.07932
130.07984
130.08036
130.08088
130.08140
130.08192
130.08244
130.08296
130.08348
130.08400
130.08452
130.08504
130.08556
130.08608
130.08660
130.08712
130.08764
130.08816
130.08868
130.08920
130.08972
130.09024
130.09076
130.09128
130.09180
130.09232
130.09284
130.09336
130.09388
130.09440
130.09492
130.09544
130.09596
130.09648
130.09700
130.09752
130.09804
130.09856
130.09908
130.09960
131.00004
131.00056
131.00108
131.00160
131.00212
131.00264
131.00316
131.00368
131.00420
131.00472
131.00524
131.00576
131.00628
131.00680
131.00732
131.00784
131.00836
131.00888
131.00940
131.00992
131.01044
131.01096
131.01148
131.01200
131.01252
131.01304
131.01356
131.01408
131.01460
131.01512
131.01564
131.01616
131.01668
131.01720
131.01772
131.01824
131.01876
131.01928
131.01980
131.02032
131.02084
131.02136
131.02188
131.02240
131.02292
131.02344
131.02396
131.02448
131.02500
131.02552
131.02604
131.02656
131.02708
131.02760
131.02812
131.02864
131.02916
131.02968
131.03020
131.03072
131.03124
131.03176
131.03228
131.03280
131.03332
131.03384
131.03436
131.03488
131.03540
131.03592
131.03644
131.03696
131.03748
131.03800
131.03852
131.03904
131.03956
131.04008
131.04060
131.04112
131.04164
131.04216
131.04268
131.04320
131.04372
131.04424
131.04476
131.04528
131.04580
131.04632
131.04684
131.04736
131.04788
131.04840
131.04892
131.04944
131.04996
131.05048
131.05100
131.05152
131.05204
131.05256
131.05308
131.05360
131.05412
131.05464
131.05516
131.05568
131.05620
131.05672
131.05724
131.05776
131.05828
131.05880
131.05932
131.05984
131.06036
131.06088
131.06140
131.06192
131.06244
131.06296
131.06348
131.06400
131.06452
131.06504
131.06556
131.06608
131.06660
131.06712
131.06764
131.06816
131.06868
131.06920
131.06972
131.07024
131.07076
131.07128
131.07180
131.07232
131.07284
131.07336
131.07388
131.07440
131.07492
131.07544
131.07596
131.07648
131.07700
131.07752
131.07804
131.07856
131.07908
131.07960
131.08012
131.08064
131.08116
131.08168
131.08220
131.08272
131.08324
131.08376
131.08428
131.08480
131.08532
131.08584
131.08636
131.08688
131.08740
131.08792
131.08844
131.08896
131.08948
131.09000
131.09052
131.09104
131.09156
131.09208
131.09260
131.09312
131.09364
131.09416
131.09468
131.09520
131.09572
131.09624
131.09676
131.09728
131.09780
131.09832
131.09884
131.09936
131.09988
132.00008
132.00060
132.00112
132.00164
132.00216
132.00268
132.00320
132.00372
132.00424
132.00476
132.00528
132.00580
132.00632
132.00684
132.00736
132.00788
132.00840
132.00892
132.00944
132.00996
132.01048
132.01100
132.01152
132.01204
132.01256
132.01308
132.01360
132.01412
132.01464
132.01516
132.01568
132.01620
132.01672
132.01724
132.01776
132.01828
132.01880
132.01932
132.01984
132.02036
132.02088
132.02140
132.02192
132.02244
132.02296
132.02348
132.02400
132.02452
132.02504
132.02556
132.02608
132.02660
132.02712
132.02764
132.02816
132.02868
132.02920
132.02972
132.03024
132.03076
132.03128
132.03180
132.03232
132.03284
132.03336
132.03388
132.03440
132.03492
132.03544
132.03596
132.03648
132.03700
132.03752
132.03804
132.03856
132.03908
132.03960
132.04012
132.04064
132.04116
132.04168
132.04220
132.04272
132.04324
132.04376
132.04428
132.04480
132.04532
132.04584
132.04636
132.04688
132.04740
132.04792
132.04844
132.04896
132.04948
132.05000
132.05052
132.05104
132.05156
132.05208
132.05260
132.05312
132.05364
132.05416
132.05468
132.05520
132.05572
132.05624
132.05676
132.05728
132.05780
132.05832
132.05884
132.05936
132.05988
132.06040
132.06092
132.06144
132.06196
132.06248
132.06300
132.06352
132.06404
132.06456
132.06508
132.06560
132.06612
132.06664
132.06716
132.06768
132.06820
132.06872
132.06924
132.06976
132.07028
132.07080
132.07132
132.07184
132.07236
132.07288
132.07340
132.07392
132.07444
132.07496
132.07548
132.07600
132.07652
132.07704
132.07756
132.07808
132.07860
132.07912
132.07964
132.08016
132.08068
132.08120
132.08172
132.08224
132.08276
132.08328
132.08380
132.08432
132.08484
132.08536
132.08588
132.08640
132.08692
132.08744
132.08796
132.08848
132.08900
132.08952
132.09004
132.09056
132.09108
132.09160
132.09212
132.09264
132.09316
132.09368
132.09420
132.09472
132.09524
132.09576
132.09628
132.09680
132.09732
132.09784
132.09836
132.09888
132.09940
132.09992
133.00004
133.00056
133.00108
133.00160
133.00212
133.00264
133.00316
133.00368
133.00420
133.00472
133.00524
133.00576
133.00628
133.00680
133.00732
133.00784
133.00836
133.00888
133.00940
133.00992
133.01044
133.01096
133.01148
133.01200

2.3.6 Heatmap interface introduction

The screenshot shows the Heatmap Viewer interface with several callout boxes:

- Controller panel to adjust parameters for clustering.** Points to the top navigation bar containing: Resolution: Medium, Colors: navy-white-firebrick, Border: Default, Cluster peaks: P value, Download: --Please Select--, and a Builder button.
- Overview of the peak across the spectrum. Default is clustered based on p value.** Points to the Overview panel on the left, which shows a vertical heatmap.
- Focus view of the specific peak pattern from the whole spectrum. Default is the top 50 peaks.** Points to the Focus View panel in the center, which shows a larger heatmap with a list of 50 peak IDs on the right.
- Pattern based Enrichment analysis panel.** Points to the Enrichment Analysis panel on the right, which includes options for Operation Mode (Annotate/Extract), Database (KEGG), and a table with columns: Name, Hits, Sigs, Gamma-p, Color.
- Dynamic Display panel, used to show the peak/sample information dynamically.** Points to the bottom right panel, which displays sample and metadata information: Sample: VT_140521_045, Metadata: Condition, Label: DSS_WT_dDC.
- Sample Names View panel.** Points to the bottom left panel, which lists sample names: 100 T2000 T1A, 100 T2000 T1A.

Xia Lab @ McGill (last updated 2021-01-09)

2.3.7 Peak patterns' stitch -1

Resolution: Medium Colors: navy-white-firebrick Border: Default Cluster peaks: Ward's method Download: --Please Select-- **Builder**

Overview Select all Focus View

Enrichment Analysis

Operation Mode Database: KEGG Tip: click a row Name

1. If you want to stitch the different peak patterns. Click the **Builder** Button and the stitch tools appears.

To Focusview
Add separator
Edit samples

ID: 182.0813
P-val: 0.00013
T-stat: 27.840

Xia Lab @ McGill (last updated 2021-01-09)

Resolution: Medium Colors: navy-white-firebrick Border: Default Cluster peaks: Ward's method Download: --Please Select-- **Builder**

Overview Select all Focus View

Enrichment Analysis

Operation Mode Database: KEGG Tip: click a row Name

2. Hold the left click of your mouse and move over the whole area as the selected part. The selected part will appear at the bottom panel.

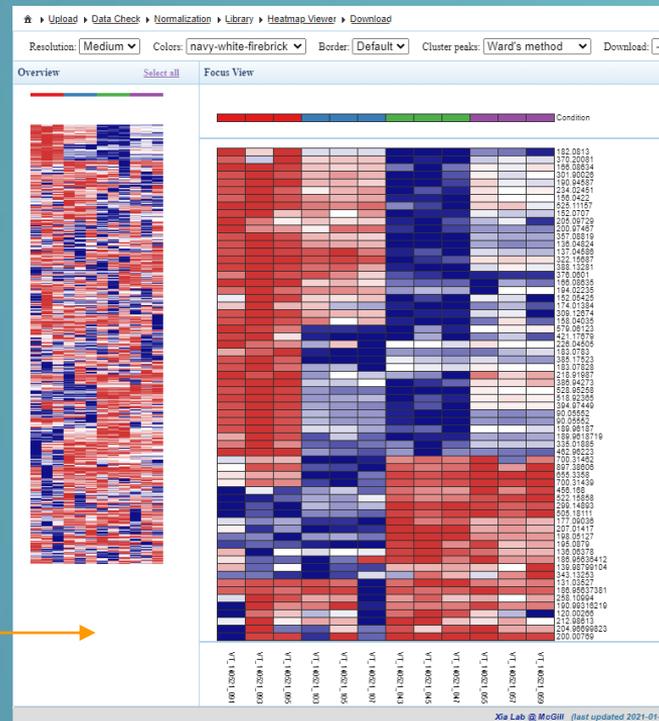
To Focusview
Add separator
Edit samples

ID: 182.0813
P-val: 0.00013
T-stat: 27.840

Xia Lab @ McGill (last updated 2021-01-09)

Continue at the next page

2.3.7 Peak patterns' stitch -2



2.3.8 Enrichment Analysis -1

Home > Upload > Data Check > Normalization > Library > Heatmap_Viewer > Download

Resolution: Medium Colors: navy-white-firebrick Border: Default Cluster peaks: Ward's method Download: --Please Select-- Builder

Overview **Select all** Focus View

Condition

Enrichment Analysis

Operation Mode: Annotate Extract

Database: **KEGG** Submit

Tip: click a row to view corresponding annotation

Name	Hits	Sigs	
<input type="checkbox"/> Nicotinate and nicotinamide	4	4	
<input type="checkbox"/> Phenylalanine metabolism	6	4	
<input type="checkbox"/> Tyrosine metabolism	4	3	
<input type="checkbox"/> Aminoacyl-tRNA biosynthes	4	3	
<input type="checkbox"/> Glycolysis / Gluconeogenesis	5	4	0.041719
<input type="checkbox"/> Arginine and proline metabo	5	3	0.041719
<input type="checkbox"/> Glyoxylate and dicarboxylate	5	5	0.041719
<input type="checkbox"/> Pantothenate and CoA biosy	5	4	0.041719
<input type="checkbox"/> Glycerolipid metabolism	2	2	0.042528
<input type="checkbox"/> Pentose phosphate pathway	6	3	0.052185
<input type="checkbox"/> beta-Alanine metabolism	3	2	0.058117
<input type="checkbox"/> Fatty acid biosynthesis	4	2	0.074959
<input type="checkbox"/> Steroid hormone biosynthesis	9	4	0.09083
<input type="checkbox"/> Fructose and mannose metab	5	2	0.092589
<input type="checkbox"/> Pyrimidine metabolism	5	2	0.092589
<input type="checkbox"/> Drug metabolism - cytochro	5	2	0.092589
<input type="checkbox"/> Glycine, serine and threonine	7	4	0.12894
<input type="checkbox"/> Inositol phosphate metabolis	7	2	0.12894
<input type="checkbox"/> Purine metabolism	12	4	0.13585
<input type="checkbox"/> Cysteine and methionine met	8	3	0.14725
<input type="checkbox"/> Biosynthesis of unsaturated	4	2	0.16548

ID: 388.13281
P-val: 2.4438289969452853e-7
T-stat: 147.2839

Xia Lab @ McGill (last updated 2021-01-09)

1. Select database and submit to do the enrichment analysis

TIP1 : Operation Mode could show the hits in different way. Annotate will annotate directly on the heatmap, while the 'Extract' will extract the ions hits and hide other non-hits.

Continue at the next page

2.3.8 Enrichment Analysis -2

Resolution: Medium Colors: navy-white-firebrick Border: Default Cluster peaks: Ward's method Download: --Please Select-- Builder

Overview **Select all** Focus View

Enrichment Analysis

Operation Mode: Annotate Extract
Database: KEGG Submit Reset Save
Tip: click a row to view corresponding annotations or to extract

Name	Hits	Sigs	Gamma-p	Color
<input checked="" type="checkbox"/> Nicotinate and nicotinamide	4	4	0.019178	P1
<input checked="" type="checkbox"/> Phenylalanine metabolism	6	4	0.027558	P1
<input checked="" type="checkbox"/> Tyrosine metabolism	4	3	0.031897	P2
<input checked="" type="checkbox"/> Aminoacyl-tRNA biosynthe	4	3	0.031897	P3
<input checked="" type="checkbox"/> Glycolysis / Gluconeogenesi	5	4	0.041229	P4
<input checked="" type="checkbox"/> Arginine and proline metabo	5	3	0.041229	P5
<input checked="" type="checkbox"/> Glyoxylate and dicarboxylat	5	5	0.041229	P6
<input type="checkbox"/> Pentothionate and CoA biosyn	5	1	0.041229	
<input type="checkbox"/> Glycerolipid metabolism	2	2	0.04151	
<input type="checkbox"/> Pentose phosphate pathway	6	3	0.052271	
<input type="checkbox"/> beta-Alanine metabolism	3	2	0.057704	
<input type="checkbox"/>

2. Select the pathways you are interested in, then the corresponding hits will appear at the right side of the Focus view panel.

Sample: VT_140521_059
Metadata: Condition
Label: DSS_WT_Epi

Xia Lab @ McGill (last updated 2021-01-09)

2.3.9 Result Download

Resolution: Medium Colors

Overview Low Medium High Select all

navy-white-firebrick Border: Default Cluster peaks: Ward's method Download: --Please Select-- Builder

Focus View

Condition

M-H=O[+] | C00311 + 2 more

M-H=C | C00851
M-C[+] | C00695

Enrichment Analysis

Operation Mode: Annotate Extract

Database: KEGG Submit Reset Save

Tip: click a row to view corresponding annotations or to extract

Name	Hits	Sigs	Gamma-p	Color
<input checked="" type="checkbox"/> Nicotinate and nicotinamide	4	4	0.019178	P0
<input checked="" type="checkbox"/> Phenylalanine metabolism	6	4	0.027558	P1
<input checked="" type="checkbox"/> Tyrosine metabolism	4	3	0.031897	P2
<input checked="" type="checkbox"/> Aminoacyl-tRNA biosynthesis	4	3	0.031897	P3
<input checked="" type="checkbox"/> Glycolysis / Gluconeogenesis	5	4	0.041229	P4
<input checked="" type="checkbox"/> Arginine and proline metabo	5	3	0.041229	P5
<input checked="" type="checkbox"/> Glyoxylate and dicarboxylat	5	5	0.041229	P6
<input type="checkbox"/> Pantothenate and CoA biosy	5	4	0.041229	
<input type="checkbox"/> Glycerolipid metabolism	2	2	0.04151	
<input type="checkbox"/> Pentose phosphate pathway	6	3	0.052271	
<input type="checkbox"/> beta-Alanine metabolism	3	2	0.057704	
<input type="checkbox"/> Fatty acid biosynthesis	4	2	0.075283	
<input type="checkbox"/> Histidine metabolism	4	3	0.075283	
<input type="checkbox"/> Steroid hormone biosynthesis	9	4	0.093129	
<input type="checkbox"/> Fructose and mannose metab	5	2	0.093719	
<input type="checkbox"/> Pyrimidine metabolism	5	2	0.093719	
<input type="checkbox"/> Drug metabolism - cytochro	5	2	0.093719	
<input type="checkbox"/> Glycine, serine and threoni	7	4	0.13172	
<input type="checkbox"/> Inositol phosphate metaboli	7	2	0.13172	
<input type="checkbox"/> Purine metabolism	12	4	0.14056	
<input type="checkbox"/> Cysteine and methionine me	8	3	0.15084	
<input type="checkbox"/> Biosynthesis of unsaturated	1	9	0.16985	
<input type="checkbox"/> Pentose and glucuronate inte	10	5	0.18869	

• Sample_VT_140521_057
• Metadata_Condition
• Label_DSS_WT_Epi

Xia Lab @ McGill (last updated 2021-01-09)

1. Set the resolution of the image in Focus view

2. Download the images from Download menu and click to download it!

2.3.9 Result Download



MetaboAnalyst 5.0 - user-friendly, end-to-end metabolomics data analysis



- Upload
- Processing
 - Data check
 - Missing value
 - Data filter
 - Data editor
 - Normalization
 - Set parameter

Click the **“Generate Report”** to download a pdf report summarizing your analysis.

Download Results & Start New Journey

Please download the results (tables and images) from the **Results Download** tab below. The **Download.zip** contains all the files in your home directory. You can also generate a PDF **analysis report** using the button. Finally, you can continue to explore other compatible modules using the **Start New Journey** tab.

Results Download Start New Journey

Generate Report

Download zip	data_original.csv
Rhistory.R	mummichog_enrichment_3.csv
data_processed.csv	mummichog_matched_compound_all.csv
snorm_1_dpi72.png	norm_1_dpi72.png
mummichog_enrichment_3.json	metaboanalyst_heatmap_2.json

Logout

Thanks

*If you have any questions please read through the FAQs or contact us at
[Zhiqiang.pang\[at\]xialab.ca](mailto:Zhiqiang.pang@xialab.ca) or [Jeff.xia\[at\]xialab.ca](mailto:Jeff.xia@xialab.ca)*

