

# Metabolomic Data Analysis with MetaboAnalyst 3.0

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## 1 Background

The Pathway Analysis module combines results from powerful pathway enrichment analysis with the pathway topology analysis to help researchers identify the most relevant pathways involved in the conditions under study.

There are many commercial pathway analysis software tools, such as Pathway Studio, MetaCore, or Ingenuity Pathway Analysis (IPA), etc. Compared to them, the pathway analysis module was specially developed for metabolomics studies. It uses the high-quality KEGG metabolic pathways as the backend knowledgebase. It integrates many well-established (i.e. univariate analysis, over-representation analysis) methods, as well as novel algorithms and concepts (i.e. Global Test, GlobalAncova, network topology analysis) into pathway analysis. Another feature is a Google-Map style interactive visualization system to deliver the analysis results in an intuitive manner.

## 2 Data Input

Pathway Analysis accepts either a list of compound labels (common names, HMDB IDs or KEGG IDs) with one compound per row, or a compound concentration table with samples in rows and compounds in columns. The second column must be phenotype labels (binary, multi-group, or continuous). The table is uploaded as comma separated values (.csv).

## 3 Compound Name Matching

The first step is to standardize the compound labels used in user uploaded data. This is a necessary step since these compounds will be subsequently compared with compounds contained in the pathway library. There are three outcomes from the step - exact match, approximate match (for common names only), and no match. Users should click the textbfView button from the approximate matched results to manually select the correct one. Compounds without match will be excluded from the subsequently pathway analysis.

**Table 1** shows the conversion results. Note: *1* indicates exact match, *2* indicates approximate match, and *0* indicates no match. A text file contain the result can be found the downloaded file *name\_map.csv*

Table 1: Result from Compound Name Mapping

	Query	Match	HMDB	PubChem	KEGG	Comment
1	1-linoleoyl-GPE (18:2)	NA	NA	NA	NA	0
2	1-palmitoyl-2-linoleoyl-GPG (16:0/18:2)	NA	NA	NA	NA	0
3	1-palmitoyl-GPE (16:0)	NA	NA	NA	NA	0
4	arabonate/xylonate	NA	NA	NA	NA	0
5	cytidine-5'-diphosphoethanolamine	NA	NA	NA	NA	0
6	glycerophosphoinositol	NA	NA	NA	NA	0
7	N1-methyladenosine	1-Methyladenosine	HMDB03331	27476	C02494	1
8	N6-carbamoylthreonyl-adenosine	N6-Carbamoyl-L-threonyl-adenosine	HMDB41623	161466		1
9	xanthosine	Xanthosine	HMDB00299	64959	C01762	1

10	5-methyltetrahydrofolate (5MeTHF)	NA	NA	NA	NA	0
11	erythronate	Erythronic acid	HMDB00613	2781043		1
12	pantothenate	Pantothenic acid	HMDB00210	988	C00864	1
13	stearidonate (18:4n3)	NA	NA	NA	NA	0
14	1-palmitoyl-GPI (16:0)	NA	NA	NA	NA	0
15	allantoin	Allantoin	HMDB00462	204	C01551	1
16	choline	Choline	HMDB00097	305	C00114	1
17	hypotaurine	Hypotaurine	HMDB00965	107812	C00519	1
18	palmitoylcholine	NA	NA	NA	NA	0
19	tricosanoyl sphingomyelin (d18:1/23:0)	NA	NA	NA	NA	0
20	2-palmitoleoyl-GPC (16:1)	NA	NA	NA	NA	0
21	cytidine 5'-diphosphocholine	Citicoline	HMDB01413	13804	C00307	1
22	4-hydroxy-nonenal-glutathione	NA	NA	NA	NA	0
23	isoleucine	(+/-)-erythro-Isoleucine	HMDB33923	791	C16434	1
24	pipecolate	Pipecolic acid	HMDB00070	849	C00408	1
25	retinal	Retinal	HMDB01358	638015	C00376	1

## 4 Pathway Analysis

In this step, users are asked to select a pathway library, as well as specify the algorithms for pathway enrichment analysis and pathway topology analysis.

### 4.1 Pathway Library

There are 15 pathway libraries currently supported, with a total of 1173 pathways :

- Homo sapiens (human) [80]
- Mus musculus (mouse) [82]
- Rattus norvegicus (rat) [81]
- Bos taurus (cow) [81]
- Danio rerio (zebrafish) [81]
- Drosophila melanogaster (fruit fly) [79]
- Caenorhabditis elegans (nematode) [78]
- Saccharomyces cerevisiae (yeast) [65]
- Oryza sativa japonica (Japanese rice) [83]
- Arabidopsis thaliana (thale cress) [87]
- Escherichia coli K-12 MG1655 [87]
- Bacillus subtilis [80]
- Pseudomonas putida KT2440 [89]
- Staphylococcus aureus N315 (MRSA/VSSA)[73]
- Thermotoga maritima [57]

Your selected pathway library code is **mmu** (KEGG organisms abbreviation).

### 4.2 Over Representation Analysis

Over-representation analysis is to test if a particular group of compounds is represented more than expected by chance within the user uploaded compound list. In the context of pathway analysis, we are testing if compounds involved in a particular pathway is enriched compared by random hits. MetPA offers two most commonly used methods for over-representation analysis:

- Fishers'Exact test
- Hypergeometric Test

*Please note, MetPA uses one-tailed Fisher's exact test which will give essentially the same result as the result calculated by the hypergeometric test.*

The selected over-representation analysis method is **Hypergeometric test**.

### 4.3 Pathway Topology Analysis

The structure of biological pathways represent our knowledge about the complex relationships among molecules within a cell or a living organism. However, most pathway analysis algorithms fail to take the structural information into consideration when estimating which pathways are significantly changed under conditions of study. It is well-known that changes in more important positions of a network will trigger a more severe impact on the pathway than changes occurred in marginal or relatively isolated positions.

The pathway topology analysis uses two well-established node centrality measures to estimate node importance - **degree centrality** and **betweenness centrality**. Degree centrality is defined as the number of links occurred upon a node. For directed graph, there are two types of degree: in-degree for links come from other nodes, and out-degree for links initiated from the current node. Metabolic networks are directed graph. Here we only consider the out-degree for node importance measure. It is assumed that nodes in upstream will have regulatory roles for the downstream nodes, not vice versa. The betweenness centrality measures number of shortest paths going through the node. Since metabolic network is directed, we use relative betweenness centrality for metabolite importance measure. The degree centrality measures focus more on local connectivities, while the betweenness centrality measures focus more on global network topology. For more detailed discussions on various graph-based methods for analysing biological networks, please refer to the article by Tero Aittokallio, T. et al. <sup>1</sup>

*Please note, for comparison among different pathways, the node importance values calculated from centrality measures are further normalized by the sum of the importance of the pathway. Therefore, the total/maximum importance of each pathway is 1; the importance measure of each metabolite node is actually the percentage w.r.t the total pathway importance, and the pathway impact value is the cumulative percentage from the matched metabolite nodes.*

Your selected node importance measure for topological analysis is **relative betweenness centrality**.

## 5 Pathway Analysis Result

The results from pathway analysis are presented graphically as well as in a detailed table.

A Google-map style interactive visualization system was implemented to facilitate data exploration. The graphical output contains three levels of view: **metabolome view**, **pathway view**, and **compound view**. Only the metabolome view is shown below. Pathway views and compound views are generated dynamically based on your interactions with the visualization system. They are available in your downloaded files.

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<sup>1</sup>Tero Aittokallio and Benno Schwikowski. *Graph-based methods for analysing networks in cell biology*, Briefings in Bioinformatics 2006 7(3):243-255

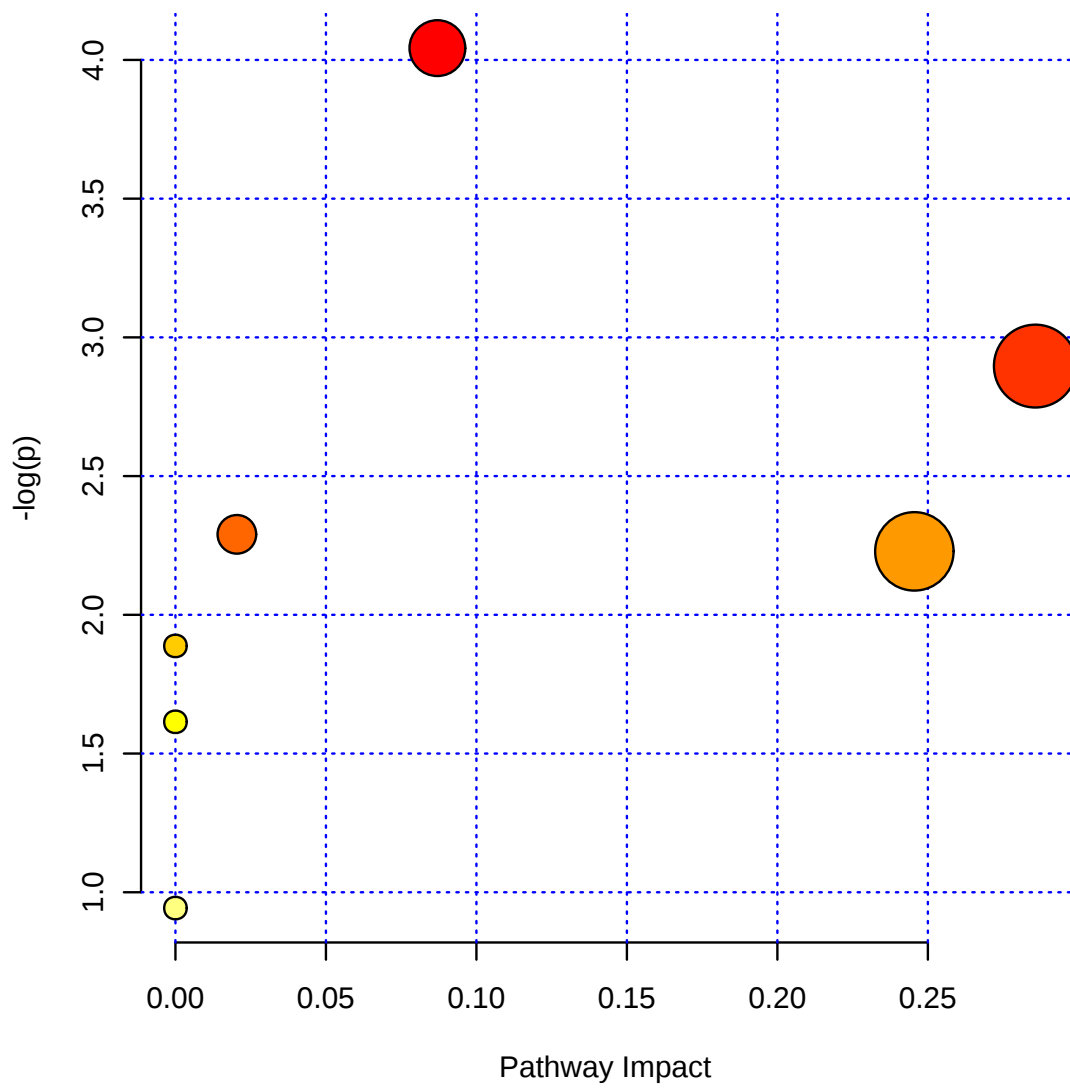


Figure 1: Summary of Pathway Analysis

The table below shows the detailed results from the pathway analysis. Since we are testing many pathways at the same time, the statistical p values from enrichment analysis are further adjusted for multiple testings. In particular, the **Total** is the total number of compounds in the pathway; the **Hits** is the actually matched number from the user uploaded data; the **Raw p** is the original p value calculated from the enrichment analysis; the **Holm p** is the p value adjusted by Holm-Bonferroni method; the **FDR p** is the p value adjusted using False Discovery Rate; the **Impact** is the pathway impact value calculated from pathway topology analysis.

Table 2: Result from Pathway Analysis

	Total	Expected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Glycerophospholipid metabolism	30	0.21	2	1.76E-02	4.04E+00	1.00E+00	1.00E+00	0.09
Taurine and hypotaurine metabolism	8	0.06	1	5.52E-02	2.90E+00	1.00E+00	1.00E+00	0.29
Pantothenate and CoA biosynthesis	15	0.11	1	1.01E-01	2.29E+00	1.00E+00	1.00E+00	0.02
Retinol metabolism	16	0.11	1	1.08E-01	2.23E+00	1.00E+00	1.00E+00	0.25
Lysine degradation	23	0.16	1	1.51E-01	1.89E+00	1.00E+00	1.00E+00	0.00
Glycine, serine and threonine metabolism	31	0.22	1	1.99E-01	1.61E+00	1.00E+00	1.00E+00	0.00
Purine metabolism	68	0.48	1	3.89E-01	9.43E-01	1.00E+00	1.00E+00	0.00

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The report was generated on Fri Aug 28 08:36:57 2015 with R version 3.2.0 (2015-04-16). Thank you for using MetaboAnalyst! For suggestions and feedback please contact Jeff Xia ([jeff.xia@mcgill.ca](mailto:jeff.xia@mcgill.ca)).