
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
 Knowledge that will change your world

Pathway analysis with Metaboanalyst

Stephen Barnes, PhD
 Professor of Pharmacology & Toxicology
sbarnes@uab.edu

Targeted
Metabolomics &
Proteomics
Laboratory

Files in mummichog results folder

mummichog.log	Today, 2:30 PM	16 KB	Log File
result.html	Today, 2:30 PM	261 KB	HTML
sif	Today, 2:30 PM	--	Folder
tsv	Today, 2:34 PM	--	Folder
_tentative_featurem...ch_workshop_data.tsv	Today, 2:30 PM	184 KB	Plain Text
_tentative_featurem...h_workshop_data.xlsx	Today, 2:30 PM	66 KB	Micros...(xlsx)
InspectedNodes_ActivityNetwork.tsv	Today, 2:30 PM	15 KB	Plain Text
mcg_metabolite_wor...t_workshop_data.tsv	Today, 2:30 PM	38 KB	Plain Text
mcg_metabolite_wor...t_workshop_data.xlsx	Today, 2:30 PM	20 KB	Micros...(xlsx)
mcg_modularanalysis_workshop_data.tsv	Today, 2:30 PM	122 KB	Plain Text
mcg_modularanalysis_workshop_data.xlsx	Today, 2:30 PM	36 KB	Micros...(xlsx)
mcg_pathwayanalysis_workshop_data.tsv	Today, 2:30 PM	123 KB	Plain Text
mcg_pathwayanalysis_workshop_data.xlsx	Today, 2:30 PM	44 KB	Micros...(xlsx)
web	Today, 2:18 PM	--	Folder

Open the file. It's on your thumb drive.

Identification of each observed ion

	A	B	C	D	E	F	G	H	I
1	m/z	id	match_form	mz_difference	name	pathway			
2	121.0519	C12455	M+Na-2H[-]	0.0004	5-Aminopentanal				
3	125.0972	C06423	M-H2O-H[-]	0.0001	Octanoic acid; Caprylic acid; Octylic acid	Fatty acid activation			
4	131.0653	C00300	M(C13)-H[-]	-0.0003	Creatine; alpha-Methylguanidino acetic acid; M	Glycine, serine, alanine and threonine metabol			
5	135.0461	C00601	M-H+O[-]	0.001	Phenylacetaldehyde; alpha-Tolualdehyde	Ascorbate (Vitamin C) and Aldarate Metabolisr			
6	135.0461	C01801	M(S34)-H[-]	-0.0003	Deoxyribose; 2-Deoxy-D-erythro-pentose; Thyr	Pyrimidine metabolism\$Pentose phosphate pa			
7	135.0461	C03765	M-H[-]	0.001	4-Hydroxyphenylacetaldehyde; 2-(4-Hydroxyp	Tyrosine metabolism			
8	135.0461	C07086	M-H[-]	0.001	Phenylacetic acid; Benzylformic acid; Phenylac	Tyrosine metabolism			
9	135.0461	CE0520	M(S34)-H[-]	-0.0003	(R)-glycerol 1-acetate	Glycerophospholipid metabolism			
10	135.0464	C00601	M-H+O[-]	0.0013	Phenylacetaldehyde; alpha-Tolualdehyde	Ascorbate (Vitamin C) and Aldarate Metabolisr			
11	135.0464	C01801	M(S34)-H[-]	0	Deoxyribose; 2-Deoxy-D-erythro-pentose; Thyr	Pyrimidine metabolism\$Pentose phosphate pa			
12	135.0464	C03765	M-H[-]	0.0013	4-Hydroxyphenylacetaldehyde; 2-(4-Hydroxyp	Tyrosine metabolism			
13	135.0464	C05649	M(S34)-H[-]	-0.0013	Dihydropteridine; 6,7-Dihydropteridine				
14	135.0464	C07086	M-H[-]	0.0013	Phenylacetic acid; Benzylformic acid; Phenylac	Tyrosine metabolism			
15	135.0464	CE0520	M(S34)-H[-]	0	(R)-glycerol 1-acetate	Glycerophospholipid metabolism			
16	136.0588	C02505	M(C137)-H[-]	0.0005	2-Phenylacetamide	Tyrosine metabolism			
17	136.0588	C02558	M(C137)-H[-]	0.0005	N-Acetylarylamine				
18	137.0251	C00156	M-H[-]	0.0007	4-Hydroxybenzoate; Hydroxybenzoic acid; 4-Hy	Ubiquinone Biosynthesis			
19	137.0251	C00180	M-H+O[-]	0.0007	Benzoate; Benzoic acid; Benzenecarboxylic acid	Alkaloid biosynthesis II			
20	137.0251	C00633	M-H+O[-]	0.0007	4-Hydroxybenzaldehyde; p-Hydroxybenzaldehyde				
21	137.0251	C01620	M(S34)-H[-]	-0.0006		Ascorbate (Vitamin C) and Aldarate Metabolisr			
22	137.0251	C05585	M-H[-]	0.0007	Gentisate aldehyde				
23	137.0251	C12455	M+K-2H[-]	0.0001	5-Aminopentanal				
24	145.0518	C00233	M-H+O[-]	0.0012	4-Methyl-2-oxopentanoate; 2-Oxoisocaproate	Valine, leucine and isoleucine degradation			
25	145.0518	C00671	M-H+O[-]	0.0012	(S)-3-Methyl-2-oxopentanoic acid; (S)-3-Methyl	Valine, leucine and isoleucine degradation			

Select and copy the KEGG IDs

	A	B	C	D	E	F	G	H	I
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Go to Metaboanalyst

Please choose a functional module to proceed:

Statistical Analysis

This module offers various commonly used statistical and machine learning methods including t-tests, ANOVA, PCA, PLS-DA and OPLS-DA. It also provides clustering and visualization tools to create dendrograms and heatmaps as well as to classify based on random forests and SVM.

Pathway Analysis

This module supports pathway analysis (integrating enrichment analysis and pathway topology analysis) and visualization for 21 model organisms, including Human, Mouse, Rat, Cow, Chicken, Zebrafish, Arabidopsis thaliana, Rice, Drosophila, Malaria, S. cerevisiae, E.coli. and others, with a total of ~1600 metabolic pathways.

Enrichment Analysis

This module performs metabolite set enrichment analysis (MSEA) for human and mammalian species based on several libraries containing ~6300 groups of metabolite sets. Users can upload either 1) a list of compounds, 2) a list of compounds with concentrations, or 3) a concentration table.

Time Series Analysis

This module supports temporal and two-factor data analysis including data overview, two-way ANOVA, and empirical Bayes time-series analysis for detecting distinctive temporal profiles. It also supports ANOVA-simultaneous component analysis (ASCA) to identify major patterns associated with each experimental factor.

Please enter a one-column compound list:

```
C12455
C06423
C00300
C00601
C01801
C03765
C07086
CE0520
C00601
C01801
C03765
C05649
C07086
CE0520
C02505
C02558
.....
```

Input Type:

Use our example data

Transfer the KEGG IDs into the box, select the input type and submit

Listed metabolites

Compound Name/ID Standardization:

Please note:

- Greek alphabets are not recognized, they should be replaced by English names (i.e. alpha, beta)
- Query names in normal white indicate exact match - marked by "1" in the download file;
- Query names highlighted indicate **no exact or unique match** - marked by "0" in the downloaded file;
- For **compound name**, you should click the **View** link to perform **approximate search** and manually select the correct match if found;
- For **KEGG ID**, it is possible to have multiple hits, you should click the **View** link to manually select the correct match if found;

Query	Hit	HMDB	PubChem	KEGG	Details
C12455	5-Aminopentanal	HMDB12815	443849	C12455	
C06423	Caprylic acid	HMDB00482	379	C06423	
C00300	Creatine	HMDB00064	586	C00300	
C00601	Phenylacetaldehyde	HMDB06236	998	C00601	
C01801	Deoxyribose	HMDB03224	22833604	C01801	
C03765	4-Hydroxyphenylacetaldehyde	HMDB03767	440113	C03765	
C07086	Phenylacetic acid	HMDB00209	999	C07086	
C05924		-	-	-	View

No records?

Query name: **C05924**

Matched Name	HMDB	PubChem	KEGG
No records found.			

OK

Cancel

Proceed to bottom of the table

C02836		-	-	-	View
C02836		-	-	-	View
C01051	Uroporphyrinogen III	HMDB01086	1179	C01051	
C05766	Uroporphyrinogen I	HMDB02211	440775	C05766	
C05774	Cobinamide	HMDB06902	11528127	C05774	
C05800		-	-	-	View
C05774	Cobinamide	HMDB06902	11528127	C05774	

You can download the result [here](#)

Submit

Choose a species

Please select a pathway library:

- | | |
|-------------|---|
| Mammals | <input type="radio"/> Homo sapiens (human) [80] |
| | <input checked="" type="radio"/> Mus musculus (mouse) [82] |
| | <input type="radio"/> Rattus norvegicus (rat) [81] |
| | <input type="radio"/> Bos taurus (cow) [81] |
| Birds | <input type="radio"/> Gallus gallus (chicken) [78] |
| Fish | <input type="radio"/> Danio rerio (zebrafish) [81] |
| Insects | <input type="radio"/> Drosophila melanogaster (fruit fly) [79] |
| Nematodes | <input type="radio"/> Caenorhabditis elegans (nematode) [78] |
| Fungi | <input type="radio"/> Saccharomyces cerevisiae (yeast) [65] |
| Plants | <input type="radio"/> Oryza sativa japonica (Japanese rice) [83] |
| | <input type="radio"/> Arabidopsis thaliana (thale cress) [87] |
| Parasites | <input type="radio"/> Schistosoma mansoni [69] |
| | <input type="radio"/> Plasmodium falciparum 3D7 (Malaria) [47] |
| | <input type="radio"/> Trypanosoma brucei [54] |
| Prokaryotes | <input type="radio"/> Escherichia coli K-12 MG1655 [87] |
| | <input type="radio"/> Bacillus subtilis [80] |
| | <input type="radio"/> Pseudomonas putida KT2440 [89] |
| | <input type="radio"/> Staphylococcus aureus N315 (MRSA/VSSA) [73] |
| | <input type="radio"/> Thermotoga maritima [57] |
| | <input type="radio"/> Synechococcus elongatus PCC7942 [75] |
| | <input type="radio"/> Mesorhizobium loti [86] |

Statistical analysis selection

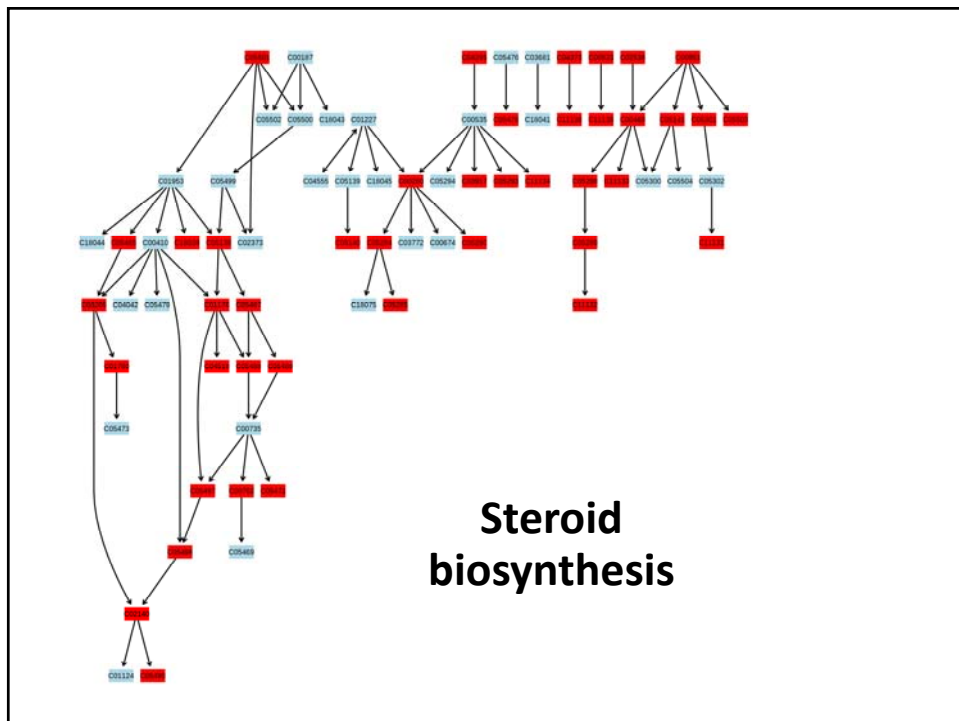
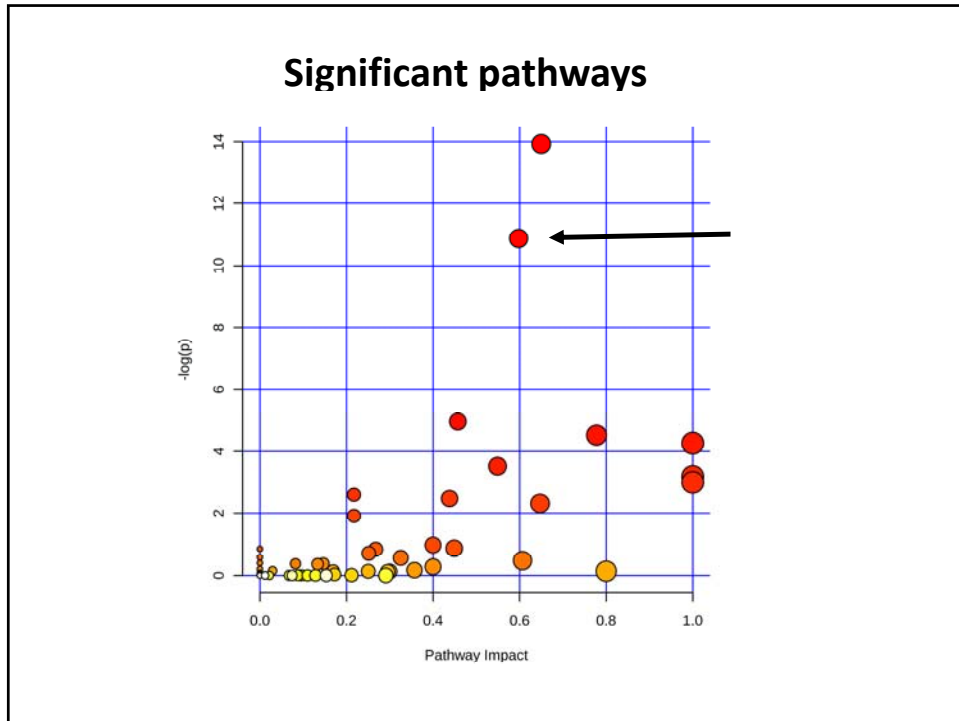
Please specify pathway analysis algorithms:

- | | |
|------------------------------|--|
| Over Representation Analysis | <input checked="" type="radio"/> Hypergeometric Test |
| | <input type="radio"/> Fisher's Exact Test |
| Pathway Topology Analysis | <input checked="" type="radio"/> Relative-betweenness Centrality |
| | <input type="radio"/> Out-degree Centrality |

Please specify a reference metabolome:

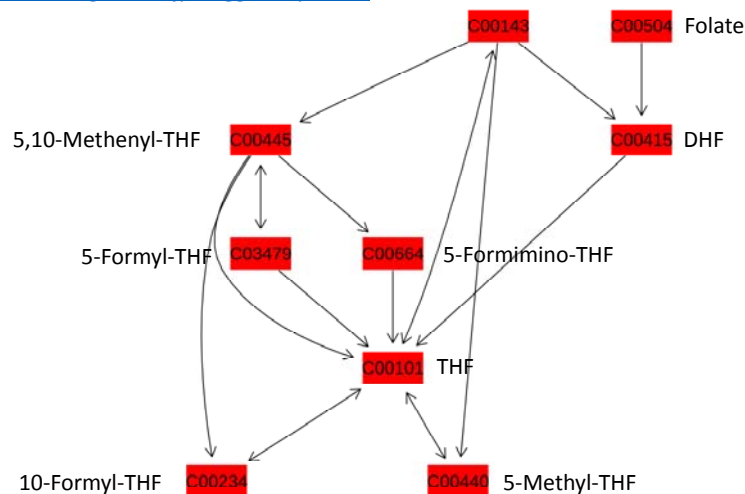
- | |
|--|
| <input checked="" type="radio"/> Use all compounds in the selected pathways |
| <input type="radio"/> Upload a reference metabolome based on your technical platform |

Submit



One carbon metabolism

<http://www.genome.jp/kegg/compound/> 5,10-Methylene-THF



Mummichog pathways Workshop study

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact	Details
Drug metabolism - cytochrome P450	37/56	8.9246E-7	13.929	7.3182E-5	7.3182E-5	0.65003	KEGG
Steroid hormone biosynthesis	42/72	1.8919E-5	10.875	0.0015325	7.7569E-4	0.59781	KEGG
Arachidonic acid metabolism	20/36	0.0070611	4.9532	0.56489	0.193	0.45729	KEGG
Phenylalanine metabolism	8/11	0.010871	4.5216	0.85882	0.22286	0.77778	KEGG
Phenylalanine, tyrosine and tryptophan biosynthesis	4/4	0.014069	4.2638	1.0	0.23073	1.0	KEGG
Tryptophan metabolism	20/40	0.029381	3.5274	1.0	0.40153	0.54903	KEGG
Ubiquinone and other terpenoid-quinone biosynthesis	3/3	0.040932	3.1958	1.0	0.47949	1.0	KEGG
One carbon pool by folate	6/9	0.049559	3.0046	1.0	0.50797	1.0	KEGG

Big omission – no records for the isoflavones that were the prominent ions. Isoflavones are in the diet as β -glucosides. They were hydrolyzed by intestinal wall enzymes, sulfated and glucuronidated and excreted into bile. In the colon, they were differentially metabolized in this experiment. The treatment eliminated the sulfatases and allowed the sulfated genistein and daidzein to accumulate.

Questions?