

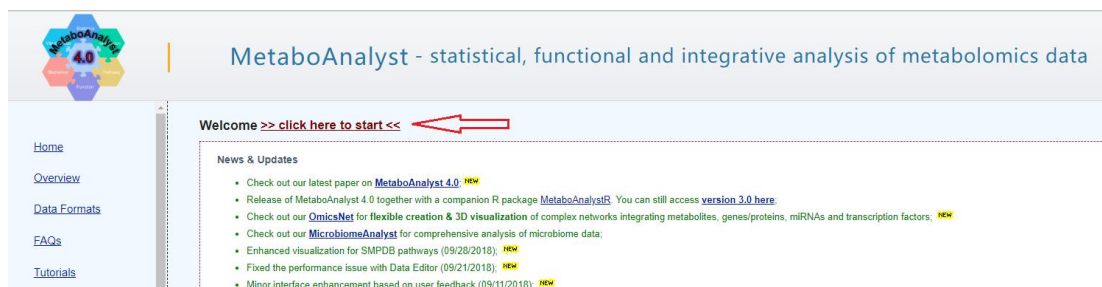
Pathway Analysis

网址：<https://www.metaboanalyst.ca/>

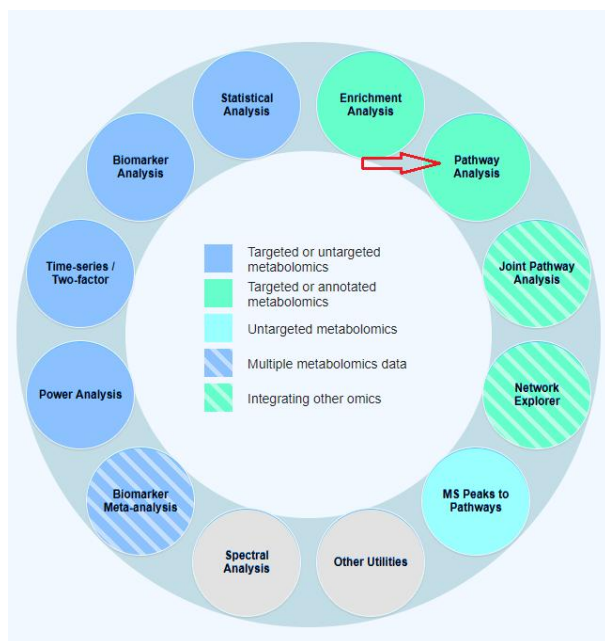
数据上传准备，数据格式可参照：

	A	B	C	D	E	F	G	H	I
1	Patient ID	Muscle loss	1,6-Anhydro-beta-D-glucose	1-Methylnicotinamide	2-Aminobutyrate	2-Hydroxyisobutyrate	2-Oxoglutarate	3-Aminoisobutyrate	3-Hydroxybutyrate
2	PIF_178	cachexic	40.85	65.37	18.73	26.05	71.52	1480.3	56.83
3	PIF_087	cachexic	62.18	340.36	24.29	41.68	67.36	116.75	43.82
4	PIF_090	cachexic	270.43	64.72	12.18	65.37	23.81	14.3	5.64
5	NETL_003_V1	cachexic	154.47	52.98	172.43	74.44	1199.91	555.57	175.91
6	PIF_115	cachexic	22.2	73.7	15.64	83.93	33.12	29.67	76.71
7	PIF_110	cachexic	212.72	31.82	18.26	80.64	47.94	17.46	31.82
8	NETL_019_V1	cachexic	151.41	36.6	8.67	42.52	223.63	56.26	11.59
9	NETCR_014_V1	cachexic	31.5	6.82	4.18	12.94	25.03	8.67	1.73
10	NETCR_014_V2	cachexic	51.42	30.27	7.54	34.81	80.64	17.99	9.03
11	PIF_154	cachexic	117.92	32.46	19.49	72.24	73.7	57.97	26.84
12	NETL_022_V1	cachexic	20.7	221.41	15.18	28.79	357.81	93.69	13.07
13	NETL_022_V2	cachexic	127.74	177.68	12.68	15.03	68.03	105.64	29.08
14	NETL_008_V1	cachexic	59.74	50.91	6.82	46.06	111.05	8.08	17.12
15	PIF_146	cachexic	89.12	32.79	10.38	32.14	32.46	43.38	8.08
16	PIF_119	cachexic	23.57	6.89	2.12	7.85	8.33	2.97	1.7
17	PIF_099	cachexic	41.26	8.67	2.86	7.85	6.89	6.36	3.42
18	PIF_162	cachexic	589.93	21.98	15.18	46.06	32.79	31.82	25.03
19	PIF_160	cachexic	112.17	25.28	15.49	47.94	28.79	16.12	30.27
20	PIF_113	cachexic	167.34	19.89	13.46	31.19	47.94	79.04	11.7
21	PIF_143	cachexic	183.09	90.92	8.94	64.07	20.49	18.73	26.05
22	NETCR_007_V1	cachexic	208.51	53.52	5.26	47.94	212.72	50.4	30.27
23	NETCR_007_V2	cachexic	34.81	95.58	23.57	68.03	287.15	104.58	60.34
24	PIF_137	cachexic	333.62	35.87	7.92	54.6	20.49	63.43	29.96
25	PIF_100	cachexic	32.46	9.68	3.9	11.02	170.72	2.97	6.36
26	NETL_004_V1	cachexic	4.71	11.13	43.38	30.88	104.58	54.05	7.61
27	PIF_094	cachexic	66.72	13.87	12.18	25.03	23.22	72.97	11.47
28	PIF_132	cachexic	214.86	127.74	31.5	33.78	88.23	64.07	54.05
29	PIF_163	cachexic	304.9	25.79	27.11	40.45	70.81	126.47	16.95
30	NETCR_003_V1	cachexic	37.71	10.8	5	8.25	11.7	8.41	6.75

Step 1：在 MetaboAnalyst home 界面，点击 [>> click here to start <<](#)，进入模块选择页面



Step 2：在模块选择页面，选择 Pathway Analysis 模块



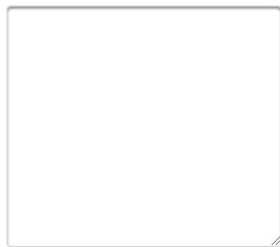
Step 3：上传数据

这里支持 2 种数据类型的输入

- 1) 一列化合物名称或各数据库 ID 号 (HMDB ID KEGG ID) **注意** Input Type 选择。
- 2) 上传自己的浓度数据表 (.txt 文件或.csv 文件, 数据格式如上), 同样需**注意**分组标签是离散型还是连续型, ID 类型和样本在行中还是列中。

参数设置完成后, 点击 **Submit**

Please enter a one-column compound list:



Input Type: -- Please specify

Use our example data

Submit

Or upload a concentration table (.csv or .txt):

Group Label: Discrete (Classification) Continuous (Regression)

ID Type: -- Please specify

Data Format: Samples in rows

Data File: 未选择任何文件

Use the example data

Data Description

Dataset Urinary metabolite concentrations from 77 cancer patients measured by 1H NMR.
Phenotype: N - cachexic; Y - control

Submit

(这里以示例数据为例, 选择第一种, 输入信息为化合物名称)

Step 4: 化合物名称/ID 检查:

- 不识别希腊字母, 应该用英文名称 (即 alpha, beta) 替换;
- 正常白色的查询名称表示完全匹配 - 在下载文件中标记为“1”;
- 突出显示的查询名称表示没有完全匹配项或唯一匹配项 - 在下载的文件中标记为“0”;
- 对于化合物名称, 可单击 “**View**” 链接以执行近似搜索, 并在找到时手动选择正确的匹配项 (示例数据进行此项检查, 将 L-Isolucine 更正为 L-Isoleucine);
- 对于 KEGG ID, 可单击 “**View**” 链接, 在 match 到的情况下以手动选择正确的匹配项。
- 点击 **Submit**

Step 5: 选择通路分析数据库和相关参数

参数设置完成后点击 **Submit**

Please select a pathway library:

Mammals 哺乳动物	<input checked="" type="radio"/> Homo sapiens (KEGG) [80] <input type="radio"/> Homo sapiens (SMPDB) [99] <input type="radio"/> Mus musculus (KEGG) [82] <input type="radio"/> Mus musculus (SMPDB) [99] <input type="radio"/> Rattus norvegicus (rat) [81] <input type="radio"/> Bos taurus (cow) [81]
Birds 鸟类	<input type="radio"/> Gallus gallus (chicken) [78]
Fish 鱼	<input type="radio"/> Danio rerio (zebrafish) [81]
Insects 昆虫	<input type="radio"/> Drosophila melanogaster (fruit fly) [79]
Nematodes 线虫	<input type="radio"/> Caenorhabditis elegans (nematode) [78]
Fungi 菌类	<input type="radio"/> Saccharomyces cerevisiae (yeast) [65]
Plants 植物	<input type="radio"/> Oryza sativa japonica (Japanese rice) [83] <input type="radio"/> Arabidopsis thaliana (thale cress) [87]
Parasites 寄生虫	<input type="radio"/> Schistosoma mansoni [69] <input type="radio"/> Plasmodium falciparum 3D7 (Malaria) [47] <input type="radio"/> Trypanosoma brucei [54]
Prokaryotes 原核生物	<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]

Please specify pathway analysis algorithms:

Over Representation Analysis 过表达分析	<input checked="" type="radio"/> Hypergeometric Test 超几何检验 <input type="radio"/> Fisher's Exact Test 费歇尔精确检验
Pathway Topology Analysis 拓扑分析	<input checked="" type="radio"/> Relative-betweenness Centrality 中介中心性 <input type="radio"/> Out-degree Centrality 出度中心性

Please specify a reference metabolome:

<input checked="" type="radio"/> Use all compounds in the selected pathways 使用被选pathways 中的所有物质
<input type="radio"/> Upload a reference metabolome based on your technical platform 根据自己的技术平台上传参考代谢组

Submit

Step 6 : 结果查看

左侧的代谢组视图是根据通路富集分析的 p 值和通路拓扑分析的 pathway impact values 显示所有匹配到的通路。将鼠标放在每个节点上将显示其通路名称。单击每个节点将在右侧面板上显示对应 pathway。

可以通过单击左侧图像上的相应节点或单击下表中的通路名称来启动该 pathway。请**注意**，每个节点（化合物）都是可点击的，点击该物质将会显示该物质的名称、Importance 和 DB Links 等信息。您可以使用下面的控制按钮放大和缩小图像，然后将图像拖动到合适位置。

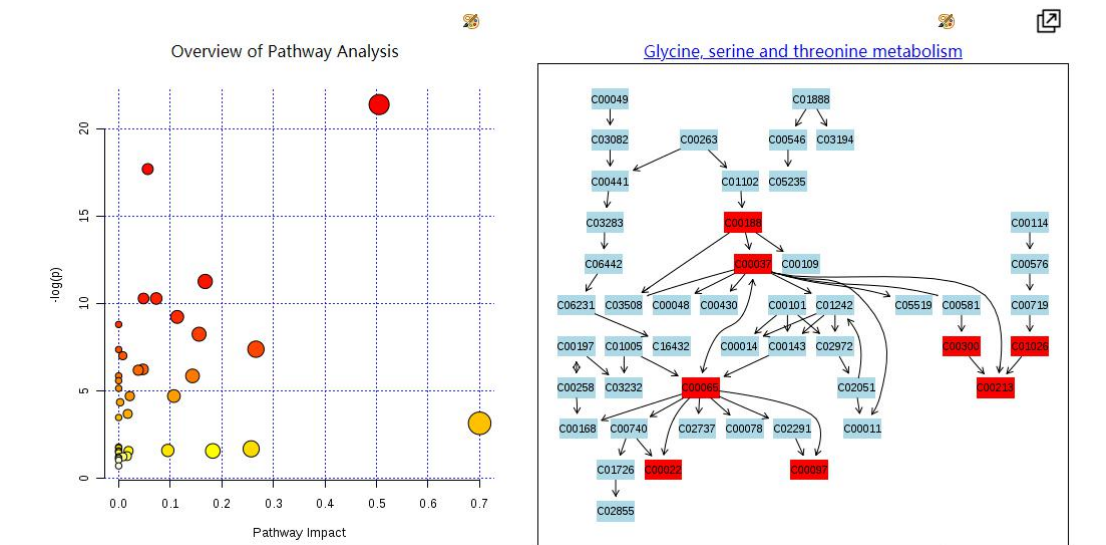
关于通路中的各种颜色：浅蓝色表示这些代谢物仅作为富集分析的背景信息，并未被匹配到；灰色表示该物质既不在您的数据中，富集分析时也不包括这些数据（仅适用于您上传了自定义代谢组谱时）；其他颜色（从黄色到红色不等）表示代谢物在数据中具有不同的显著性水平。

Result View:

The **metabolome view** on the left shows all matched pathways according to the p values from the pathway enrichment analysis and pathway impact values from the pathway topology analysis. Placing your **mouse over** each pathway node will reveal its pathway name. **Clicking each node** will launch the **pathway view** on the right panel.

The pathway can be launched either by clicking the corresponding node on the left image or by clicking the pathway name from the table below. Please note, each node (compound) is clickable. You can **zoom in and out** using the control buttons below, and then **drag** the image to the locations of interest. Placing the **mouse over** each metabolite node will reveal its common name. **Clicking the node** will trigger the **compound view** of the selected compound.

About compound colors within the pathway - **light blue** means those metabolites are not in your data and are used as background for enrichment analysis; **gray** means the metabolite is not in your data and is also excluded from enrichment analysis (only applicable if you have uploaded a custom metabolome profile); other colors (varying from yellow to red) means the metabolites are in the data with different levels of significance.



拓扑图解释：

Y 轴基于 p 值（来自通路富集分析），X 轴基于 pathway impact value（来自通路拓扑分析）。节点颜色基于 p 值，由浅到深，p 值由大到小，节点半径基于其 pathway impact value，由小到大，impact 值由小到大。

Click the corresponding **Pathway Name** to view its graphical presentation; click **Match Status** to view the pathway compounds (with matched ones highlighted).

Pathway Name	Match Status	p	-log(p)	Holm p	FDR	Impact	Details
Glycine, serine and threonine metabolism	8/48	5.1483E-10	21.387	4.1187E-8	4.1187E-8	0.50503	KEGG SMP
Aminoacyl-tRNA biosynthesis	8/75	2.0786E-8	17.689	1.6421E-6	8.3143E-7	0.05634	KEGG
Phenylalanine metabolism	5/45	1.302E-5	11.249	0.0010155	3.4719E-4	0.16781	KEGG SMP
Valine, leucine and isoleucine biosynthesis	4/27	3.4566E-5	10.273	0.0026616	5.5305E-4	0.04823	KEGG SMP
Pantothenate and CoA biosynthesis	4/27	3.4566E-5	10.273	0.0026616	5.5305E-4	0.07286	KEGG SMP
Propanoate metabolism	4/35	9.9329E-5	9.2171	0.0074496	0.0013244	0.11348	KEGG SMP
Nitrogen metabolism	4/39	1.5313E-4	8.7842	0.011332	0.0017501	0.0	KEGG SMP
Sulfur metabolism	3/18	2.6735E-4	8.2269	0.019517	0.0026735	0.1559	KEGG SMP
Cysteine and methionine metabolism	4/56	6.3128E-4	7.3678	0.045452	0.0051579	0.26615	KEGG SMP SMP
Thiamine metabolism	3/24	6.4473E-4	7.3467	0.045776	0.0051579	0.0	KEGG SMP
Phenylalanine, tyrosine and tryptophan biosynthesis	3/27	9.1872E-4	6.9925	0.06431	0.0066816	0.008	KEGG SMP
Tyrosine metabolism	4/76	0.0020068	6.2112	0.13847	0.012965	0.04724	KEGG SMP SMP
Arginine and proline metabolism	4/77	0.0021068	6.1626	0.14326	0.012965	0.03799	KEGG SMP
Valine, leucine and isoleucine degradation	3/40	0.0029196	5.8363	0.19561	0.015571	0.0	KEGG SMP
Butanoate metabolism	3/40	0.0029196	5.8363	0.19561	0.015571	0.14317	KEGG SMP
Nicotinate and nicotinamide metabolism	3/44	0.0038409	5.562	0.24966	0.019205	0.0	KEGG SMP
Cyanoamino acid metabolism	2/16	0.0059587	5.1229	0.38135	0.028041	0.0	KEGG
Taurine and hypotaurine metabolism	2/20	0.0092689	4.6811	0.58394	0.039027	0.02158	KEGG SMP
Citrate cycle (TCA cycle)	2/20	0.0092689	4.6811	0.58394	0.039027	0.10708	KEGG SMP
Alanine, aspartate and glutamate metabolism	2/24	0.013228	4.3254	0.80692	0.052913	0.00285	KEGG SMP SMP SMP

Submit

表格中详细展示了 pathway analysis 的分析结果，Match Status 为富集到的代谢物个数/通路中所有代谢物个数，Holm p 是经 HB 方法矫正后的 p 值，FDR p 是 False Discovery Rate 矫正后的 p 值，Impact 是经通路拓扑分析得出的 pathway impact value。

Step 7：结果下载

所有运行过的程序得到的图表等文件均可在 Download 中下载。

- Upload
- Processing
- Normalization
- Pathway
 - Set parameter
 - View result
 - Download
 - Exit

Result Download

Please download the results (tables and images) below. The **Download.zip** contains all the files in your home directory. You can also generate a PDF analysis report using the button below.

[Generate Report](#)

Download.zip	crop1538184783672.png
Rhistory.R	name_map.csv
Glycine_serine_and_threonine_metabolism.png	zoom1538184783672.png
pathway_results.csv	path_view_0_dot72.png

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