Metabolomic Data Analysis with MetaboAnalyst 4.0

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1 Background

The Pathway Analysis module combines results from powerful pathway enrichment analysis with 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 these commercial tools, the pathway analysis module was specifically developed for metabolomics studies. It uses high-quality KEGG metabolic pathways as the backend knowledgebase. This module 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

The Pathway Analysis module 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:

	Query	Match	HMDB	PubChem	KEGG	SMILES
1	C00337	4,5-Dihydroorotic acid	HMDB0000528	648	C00337	C1C(NC(=O)NC1=O)C(
2	C00212	Adenosine	HMDB0000050	60961	C00212	C1=NC2=C(C(=N1)N)N
3	C00620	Ribose 1-phosphate	HMDB0001489	439236	C00620	C([C@@H]1[C@H]([C@H]
4	C00079	L-Phenylalanine	HMDB0000159	6140	C00079	C1=CC=C(C=C1)C[C@
5	C00089	Sucrose	HMDB0000258	5988	C00089	C([C@@H]1[C@H]([C@@
6	C00072	Ascorbic acid	HMDB0000044	54670067	C00072	C([C@@H]([C@@H]1C(=
7	C00438	Ureidosuccinic acid	HMDB0000828	93072	C00438	C([C@@H](C(=O)O)NC(
8	C00475	Cytidine	HMDB0000089	6175	C00475	C1=CN(C(=O)N=C1N)[
9	C11434	2-C-Methyl-D-erythritol 4-phosphate	METPA1027		C11434	

10	C00186	L-Lactic acid	HMDB0000190	107689	C00186	C[C@@H](C(=O)O)O
11	C00256	D-Lactic acid	HMDB0001311	61503	C00256	C[C@H](C(=O)O)O
12	C00152	L-Asparagine	HMDB0000168	6267	C00152	C([C@@H](C(=O)O)N)C
13	C00037	Glycine	HMDB0000123	750	C00037	C(C(=O)O)N
14	C00188	L-Threonine	HMDB0000167	6288	C00188	C[C@H]([C@@H](C(=O)O
15	C11453	2-C-Methyl-D-erythritol 2,4-cyclodiphosphate	METPA1030		C11453	
16	C00065	L-Serine	HMDB0000187	5951	C00065	C([C@@H](C(=O)O)N)O
17	C00387	Guanosine	HMDB0000133	6802	C00387	C1=NC2=C(N1[C@H]3[C
18	C00031	D-Glucose	HMDB0000122	5793	C00031	C([C@@H]1[C@H]([C@@H
19	C00064	L-Glutamine	HMDB0000641	5961	C00064	C(CC(=O)N)[C@@H](C(
20	C01551	Allantoin	HMDB0000462	204	C01551	C1(C(=O)NC(=O)N1)NC
21	C00026	Oxoglutaric acid	HMDB0000208	51	C00026	C(CC(=O)O)C(=O)C(=O
22	C00299	Uridine	HMDB0000296	6029	C00299	C1=CN(C(=O)NC1=O)[
23	C00295	Orotic acid	HMDB0000226	967	C00295	C1=C(NC(=O)NC1=O)C
24	C00078	L-Tryptophan	HMDB0000929	6305	C00078	C1=CC=C2C(=C1)C(=C
25	C02642	Ureidopropionic acid	HMDB0000026	111	C02642	C(CNC(=O)N)C(=O)O
26	C00062	L-Arginine	HMDB0000517	6322	C00062	C(C[C@@H](C(=O)O)N)
27	C00135	L-Histidine	HMDB0000177	6274	C00135	C1=C(NC=N1)C[C@@H]
28	C00493	Shikimic acid	HMDB0003070	8742	C00493	C1[C@H]([C@@H]([C@@H
29	C00122	Fumaric acid	HMDB0000134	444972	C00122	C(=C/C(=O)O)\C(=O)O
30	C00103	Glucose 1-phosphate	HMDB0001586	439165	C00103	C([C@@H]1[C@H]([C@@H
31	C00047	L-Lysine	HMDB0000182	5962	C00047	C(CCN)C[C@@H](C(=O
32	C00082	L-Tyrosine	HMDB0000158	6057	C00082	C1=CC(=CC=C1C[C@@
33	C01204	Myo-inositol hexakisphosphate	HMDB0003502	890	C01204	C1(C(C(C(C(C1OP(=O)
34	C00077	Ornithine	HMDB0000214	6262	C00077	C(C[C@@H](C(=O)O)N)
35	C11436	2-Phospho-4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol	METPA1029		C11436	
36	C00942	Cyclic GMP	HMDB0001314	24316	C00942	C1[C@@H]2[C@H]([C@H
37	C00061	Flavin Mononucleotide	HMDB0001520	643976	C00061	CC1=CC2=C(C=C1C)N
38	C00199	D-Ribulose 5-phosphate	HMDB0000618	439184	C00199	C([C@H]([C@H](C(=O)C
39	C00117	D-Ribose 5-phosphate	HMDB0001548	439167	C00117	C([C@@H]1[C@H]([C@H]
40	C00588	Phosphorylcholine	HMDB0001565	1014	C00588	C[N+](C)(C)CCOP(=O)
41	C00025	L-Glutamic acid	HMDB0000148	33032	C00025	C(CC(=O)O)[C@@H](C(
42	C00281	D-Sedoheptulose 7-phosphate	HMDB0001068	22833559	C00281	C([C@@H]1[C@H]([C@H]
43	C00279	D-Erythrose 4-phosphate	HMDB0001321	122357	C00279	C([C@H]([C@H](C=O)O)
44	C00311	Isocitric acid	HMDB0000193	1198	C00311	C(C(C(C(=O)O)O)C(=O
45	C11174	1-Diphosinositol pentakisphosphate	HMDB0012494		C11174	OP(O)(=O)O[C@H]1[C@
46	C00049	L-Aspartic acid	HMDB0000191	5960	C00049	C([C@@H](C(=O)O)N)C
47	C00655	Xanthylic acid	HMDB0001554	73323	C00655	C1=NC2=C(N1[C@H]3[C
48	C00158	Citric acid	HMDB0000094	311	C00158	C(C(=O)O)C(CC(=O)O)
49	C00417	cis-Aconitic acid	HMDB0000072	643757	C00417	C(/C(=C/C(=O)O)/C(=
50	C02341	trans-Aconitic acid	HMDB0000958	444212	C02341	C(/C(=C\C(=O)O)/C(=
51	C00497	D-Malic acid	HMDB0031518	92824	C00497	C([C@H](C(=O)O)O)C(=
52	C00035	Guanosine diphosphate	HMDB0001201	8977	C00035	C1=NC2=C(N1[C@H]3[C
53	C00029	Uridine diphosphate glucose	HMDB0000286	53477679	C00029	C1=CN(C(=O)NC1=O)[
54	C00044	Guanosine triphosphate	HMDB0001273	6830	C00044	C1=NC2=C(N1[C@H]3[C
55	C03794	Adenylsuccinic acid	HMDB0000536	440122	C03794	C1=NC2=C(C(=N1)NC(
56	C00010	Coenzyme A	HMDB0001423	6816	C00010	CC(C)(COP(=O)(O)OP(
57	C00020	Adenosine monophosphate	HMDB0000045	6083	C00020	C1=NC2=C(C(=N1)N)N
58	C00021	S-Adenosylhomocysteine	HMDB0000939	439155	C00021	C1=NC2=C(C(=N1)N)N
59	C00016	FAD	HMDB0001248	643975	C00016	CC1=CC2=C(C=C1C)N
60	C00498	ADP-glucose	HMDB0006557	16500	C00498	C1=NC2=C(C(=N1)N)N
61	C00363	dTDP	HMDB0001274	164628	C00363	CC1=CN(C(=O)NC1=O
62	C00301	Adenosine diphosphate ribose	HMDB0001178	192	C00301	C1=NC2=C(C(=N1)N)N
63	C00352	Glucosamine 6-phosphate	HMDB0001254	439217	C00352	C([C@@H]1[C@H]([C@@H
64	C01185	Nicotinic acid mononucleotide	HMDB0001132	53477721	C01185	C1C(C=CC=[N+]1[C@H
65	C00051	Glutathione	HMDB0000125	124886	C00051	C(CC(=O)N[C@@H](CS)
66	C00459	Thymidine 5'-triphosphate	HMDB0001342	64968	C00459	CC1=CN(C(=O)NC1=O
67	C00597	3-Phosphoglyceric acid	HMDB0000807	724	C00597	C(C(C(=O)O)O)OP(=O)
68	C06746	Quinic acid	HMDB0003072		C06746	OC1C[C@@](O)(C[C@@H
69	C00055	Cytidine monophosphate	HMDB0000095	6131	C00055	C1=CN(C(=O)N=C1N)[
70	C00004	NADH	HMDB0001487	928	C00004	C1C=CN(C=C1C(=O)N
71	C01272	Inositol 1,3,4,5-tetraphosphate	HMDB0001059	107758	C01272	[C@H]1([C@H](C([C@H](
72	C00024	Acetyl-CoA	HMDB0001206	444493	C00024	CC(=O)SCCNC(=O)CC
73	C00345	6-Phosphogluconic acid	HMDB0001316	91493	C00345	C([C@H]([C@H]([C@@H]
74	C00093	Glycerol 3-phosphate	HMDB0000126	439162	C00093	C([C@H](COP(=O)(O)O
75	C06156	NA	NA	NA	NA	NA
76	C00119	Phosphoribosyl pyrophosphate	HMDB0000280	7339	C00119	C([C@@H]1[C@H]([C@H]
77	C00085	Fructose 6-phosphate	HMDB0000124	69507	C00085	C([C@H]([C@H]([C@@H]
78	C00008	ADP	HMDB0001341	6022	C00008	C1=NC2=C(C(=N1)N)N
79	C00356	3-Hydroxy-3-methylglutaryl-CoA	HMDB0001375	439218	C00356	C[C@](CC(=O)O)(CC(=
80	C01245	Inositol 1,4,5-trisphosphate	HMDB0001498	439456	C01245	[C@H]1([C@@H]([C@H](
81	C00127	Oxidized glutathione	HMDB0003337	975	C00127	C(CC(=O)NC(CSSCC(C
82	C00002	Adenosine triphosphate	HMDB0000538	5957	C00002	C1=NC2=C(C(=N1)N)N
83	C00105	Uridine 5'-monophosphate	HMDB0000288	6030	C00105	C1=CN(C(=O)NC1=O)[
84	C00668	Glucose 6-phosphate	HMDB0001401	5958	C00668	C([C@@H]1[C@H]([C@@H
85	C00005	NADPH	HMDB0000221	22833512	C00005	C1C=CN(C=C1C(=O)N
86	C00112	CDP	HMDB0001546	6132	C00112	C1=CN(C(=O)N=C1N)[
87	C00063	Cytidine triphosphate	HMDB0000082	6176	C00063	C1=CN(C(=O)N=C1N)[
88	C00003	NAD	HMDB0000902	5893	C00003	C1=CC(=C[N+](=C1)[C
89	C00075	Uridine triphosphate	HMDB0000285	6133	C00075	C1=CN(C(=O)NC1=O)[
90	C00015	Uridine 5'-diphosphate	HMDB0000295	6031	C00015	C1=CN(C(=O)NC1=O)[

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 **ath** (KEGG organisms abbreviation).

4.2 Pathway Enrichment Analysis

Pathway enrichment analysis usually refers to quantitative enrichment analysis directly using the compound concentration values, as compared to compound lists used by over-representation analysis. As a result, it is more sensitive and has the potential to identify **subtle but consistent** changes amongst compounds involved in the same biological pathway.

Many procedures have been developed in the last decade for quantitative enrichment analysis, the most famous being the Gene Set Enrichment Analysis. Many new and improved methods have been implemented since. The enrichment analysis is based on GlobalTest and GlobalAncova. Both methods support enrichment analysis with binary, multi-group, as well as continuous phenotypes. The p-values can be approximated based on the asymptotic distribution without using permutations which is computationally very intensive and is not suitable for web applications. Please note, when sample sizes are small, the approximated p values may be slightly less accurate compared to p values obtained by using a permutation-based method (for details, please refer to the paper by Goeman, J.J. et al. [1] and by

[1] Jelle J. Goeman and Peter Buhlmann. *Analyzing gene expression data in terms of gene sets: methodological issues*, Bioinformatics 2007 23(8):980-987

Hummel, M. et al. [2]) However, since our focus is to identify the most relevant pathways within the pathways in the library, we are more interested in the rank of the pathway, not its absolute p-value. Therefore, this disadvantage may be tolerated.

The selected pathway enrichment analysis method is **Globaltest**.

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 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 a 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 upstream will have regulatory roles for the downstream nodes, not vice versa. The betweenness centrality measures the number of shortest paths going through the node. Since the metabolic network is directed, we use the relative betweenness centrality for a metabolite as the importance measure. The degree centrality measure focuses more on local connectivities, while the betweenness centrality measure focuses more on global network topology. For more detailed discussions on various graph-based methods for analyzing biological networks, please refer to the article by Tero Aittokallio, T. et al. [3]

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.

[2]Manuela Hummel, Reinhard Meister and Ulrich Mansmann. *GlobalANCOVA: exploration and assessment of gene group effects*, Bioinformatics 2008 24(1):78-85

[3]Tero Aittokallio and Benno Schwikowski. *Graph-based methods for analyzing networks in cell biology*, Briefings in Bioinformatics 2006 7(3):243-255

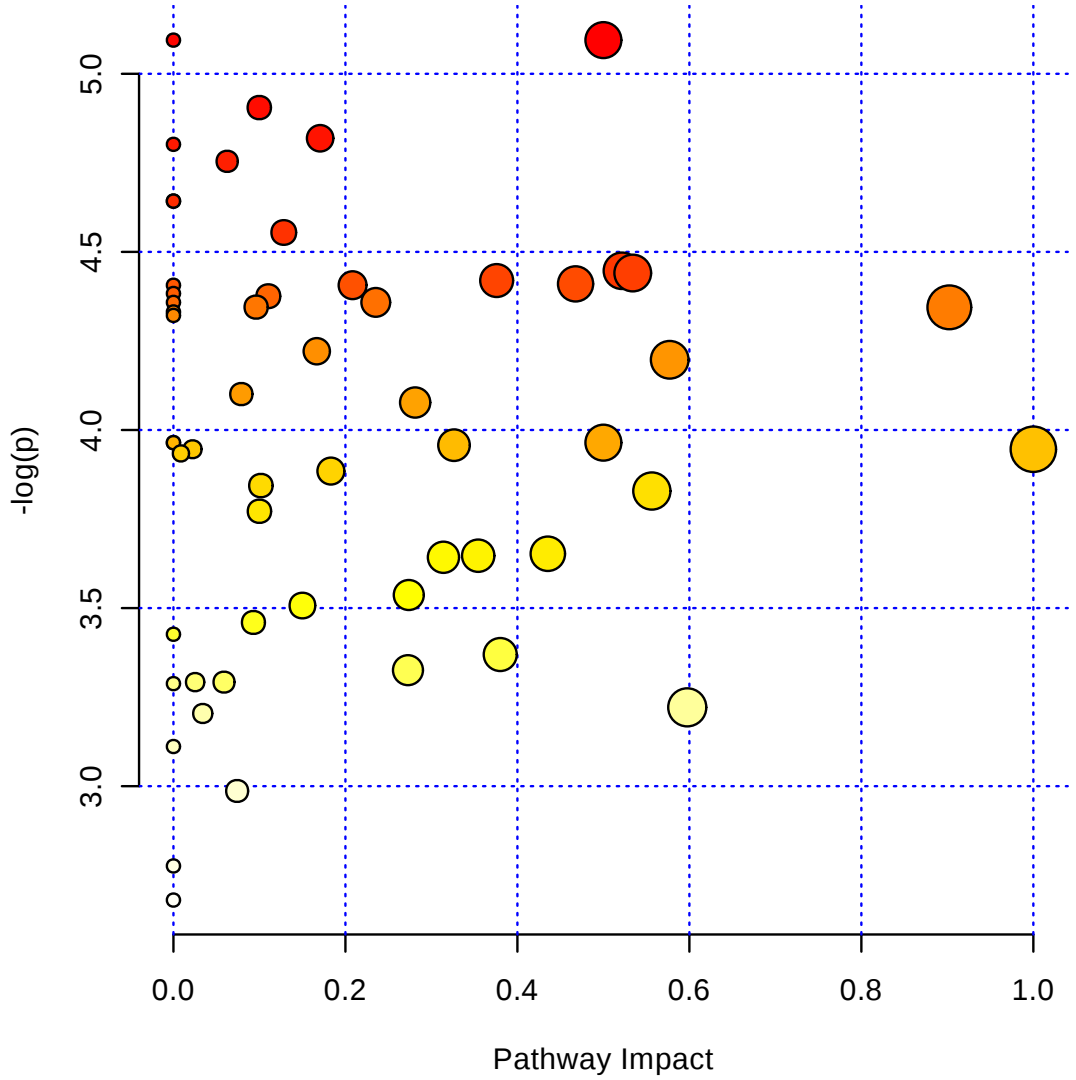

Figure 1: Summary of Pathway Analysis

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 Cmpd	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Isoquinoline alkaloid biosynthesis	6	1	6.13E-03	5.09E+00	3.43E-01	3.22E-02	0.50
Ubiquinone and other terpenoid-quinone biosynthesis	23	1	6.13E-03	5.09E+00	3.43E-01	3.22E-02	0.00
Phenylalanine, tyrosine and tryptophan biosynthesis	21	6	7.41E-03	4.91E+00	4.00E-01	3.22E-02	0.10
Tryptophan metabolism	27	2	8.07E-03	4.82E+00	4.28E-01	3.22E-02	0.17
Indole alkaloid biosynthesis	7	1	8.21E-03	4.80E+00	4.28E-01	3.22E-02	0.00
Histidine metabolism	16	2	8.61E-03	4.75E+00	4.39E-01	3.22E-02	0.06
Sphingolipid metabolism	13	1	9.63E-03	4.64E+00	4.82E-01	3.22E-02	0.00
Sulfur metabolism	12	1	9.63E-03	4.64E+00	4.82E-01	3.22E-02	0.00
Glycerophospholipid metabolism	25	2	1.05E-02	4.55E+00	5.05E-01	3.22E-02	0.13
Pyrimidine metabolism	38	16	1.17E-02	4.45E+00	5.51E-01	3.22E-02	0.52
Glycine, serine and threonine metabolism	30	5	1.18E-02	4.44E+00	5.51E-01	3.22E-02	0.53
Arginine and proline metabolism	38	6	1.20E-02	4.42E+00	5.51E-01	3.22E-02	0.38
Nicotinate and nicotinamide metabolism	12	3	1.22E-02	4.41E+00	5.51E-01	3.22E-02	0.47
beta-Alanine metabolism	12	2	1.22E-02	4.41E+00	5.51E-01	3.22E-02	0.21
Cyanoamino acid metabolism	11	3	1.22E-02	4.41E+00	5.51E-01	3.22E-02	0.00
Nitrogen metabolism	15	4	1.25E-02	4.38E+00	5.51E-01	3.22E-02	0.00
Carbon fixation in photosynthetic organisms	21	5	1.26E-02	4.38E+00	5.51E-01	3.22E-02	0.11
Porphyrin and chlorophyll metabolism	29	1	1.28E-02	4.36E+00	5.51E-01	3.22E-02	0.00
Butanoate metabolism	18	3	1.28E-02	4.36E+00	5.51E-01	3.22E-02	0.24
Cysteine and methionine metabolism	34	3	1.30E-02	4.34E+00	5.51E-01	3.22E-02	0.10
Alanine, aspartate and glutamate metabolism	22	9	1.30E-02	4.34E+00	5.51E-01	3.22E-02	0.90
Glucosinolate biosynthesis	54	2	1.31E-02	4.33E+00	5.51E-01	3.22E-02	0.00
Folate biosynthesis	16	1	1.33E-02	4.32E+00	5.51E-01	3.22E-02	0.00
Methane metabolism	11	2	1.47E-02	4.22E+00	5.51E-01	3.22E-02	0.17
Glutathione metabolism	26	6	1.50E-02	4.20E+00	5.51E-01	3.22E-02	0.58
Glycerolipid metabolism	13	2	1.66E-02	4.10E+00	5.51E-01	3.22E-02	0.08
Terpenoid backbone biosynthesis	25	5	1.70E-02	4.08E+00	5.51E-01	3.22E-02	0.28
Phenylalanine metabolism	8	1	1.90E-02	3.96E+00	5.51E-01	3.22E-02	0.50
Phenylpropanoid biosynthesis	45	1	1.90E-02	3.96E+00	5.51E-01	3.22E-02	0.00
Tropane, piperidine and pyridine alkaloid biosynthesis	8	1	1.90E-02	3.96E+00	5.51E-01	3.22E-02	0.00
Purine metabolism	61	12	1.91E-02	3.96E+00	5.51E-01	3.22E-02	0.33
Synthesis and degradation of ketone bodies	4	2	1.93E-02	3.95E+00	5.51E-01	3.22E-02	1.00
Valine, leucine and isoleucine degradation	34	2	1.93E-02	3.95E+00	5.51E-01	3.22E-02	0.02
Zeatin biosynthesis	19	5	1.96E-02	3.93E+00	5.51E-01	3.22E-02	0.01
Ascorbate and aldarate metabolism	15	2	2.06E-02	3.88E+00	5.51E-01	3.29E-02	0.18
Galactose metabolism	26	5	2.14E-02	3.84E+00	5.51E-01	3.29E-02	0.10
Starch and sucrose metabolism	30	6	2.17E-02	3.83E+00	5.51E-01	3.29E-02	0.56
Pentose and glucuronate interconversions	12	3	2.30E-02	3.77E+00	5.51E-01	3.39E-02	0.10
Glyoxylate and dicarboxylate metabolism	17	4	2.59E-02	3.65E+00	5.51E-01	3.58E-02	0.44
Citrate cycle (TCA cycle)	20	6	2.61E-02	3.65E+00	5.51E-01	3.58E-02	0.35
Fatty acid metabolism	34	2	2.62E-02	3.64E+00	5.51E-01	3.58E-02	0.31
Pyruvate metabolism	21	2	2.91E-02	3.54E+00	5.51E-01	3.88E-02	0.27
Pantothenate and CoA biosynthesis	14	2	3.00E-02	3.51E+00	5.51E-01	3.90E-02	0.15
Aminoacyl-tRNA biosynthesis	67	13	3.14E-02	3.46E+00	5.51E-01	4.00E-02	0.09
Inositol phosphate metabolism	24	3	3.25E-02	3.43E+00	5.51E-01	4.04E-02	0.00
Amino sugar and nucleotide sugar metabolism	41	6	3.44E-02	3.37E+00	5.51E-01	4.18E-02	0.38
Tyrosine metabolism	18	2	3.59E-02	3.33E+00	5.51E-01	4.18E-02	0.27
Propanoate metabolism	15	1	3.72E-02	3.29E+00	5.51E-01	4.18E-02	0.06
Fatty acid biosynthesis	49	1	3.72E-02	3.29E+00	5.51E-01	4.18E-02	0.03
Riboflavin metabolism	10	3	3.73E-02	3.29E+00	5.51E-01	4.18E-02	0.00
Pentose phosphate pathway	18	7	3.99E-02	3.22E+00	5.51E-01	4.37E-02	0.60
Glycolysis or Gluconeogenesis	25	4	4.06E-02	3.20E+00	5.51E-01	4.37E-02	0.03
Vitamin B6 metabolism	11	1	4.45E-02	3.11E+00	5.51E-01	4.71E-02	0.00
Lysine biosynthesis	10	2	5.05E-02	2.99E+00	5.51E-01	5.23E-02	0.07
Valine, leucine and isoleucine biosynthesis	26	1	6.23E-02	2.78E+00	5.51E-01	6.34E-02	0.00
Lysine degradation	17	2	6.85E-02	2.68E+00	5.51E-01	6.85E-02	0.00

6 Appendix: R Command History

```
 [1] "mSet<-InitDataObjects(\"conc\", \"pathqea\", FALSE)"
 [2] "mSet<-Read.TextData(mSet, \"Replacing_with_your_file_path\", \"rowu\", \"disc\");"
 [3] "mSet<-CrossReferencing(mSet, \"kegg\");"
 [4] "mSet<-CreateMappingResultTable(mSet)"
 [5] "mSet<-SanityCheckData(mSet)"
 [6] "mSet<-ImputeVar(mSet, method=\"min\")"
 [7] "mSet<-PreparePrenormData(mSet)"
 [8] "mSet<-Normalization(mSet, \"NULL\", \"NULL\", \"ParetoNorm\", ratio=FALSE, ratioNum=20)"
 [9] "mSet<-PlotNormSummary(mSet, \"norm_0_\", \"png\", 72, width=NA)"
[10] "mSet<-PlotSampleNormSummary(mSet, \"snorm_0_\", \"png\", 72, width=NA)"
[11] "mSet<-SetKEGG.PathLib(mSet, \"ath\")"
[12] "mSet<-SetMetabolomeFilter(mSet, F);"
[13] "mSet<-CalculateQeaScore(mSet, \"rbc\", \"gt\")"
[14] "mSet<-PlotPathSummary(mSet, \"path_view_0_\", \"png\", 72, width=NA)"
[15] "mSet<-PlotPathSummary(mSet, \"path_view_0_\", \"tiff\", 600, width=NA)"
[16] "mSet<-PlotKEGGPath(mSet, \"Isoquinoline alkaloid biosynthesis\",528, 480, \"png\", NULL)"
[17] "mSet<-RerenderMetPAGraph(mSet, \"zoom1570632080785.png\",528.0, 480.0, 100.0)"
[18] "mSet<-PlotKEGGPath(mSet, \"Ubiquinone and other terpenoid-quinone biosynthesis\",528, 480, \"
[19] "mSet<-PlotKEGGPath(mSet, \"Phenylalanine, tyrosine and tryptophan biosynthesis\",528, 480, \"
[20] "mSet<-PlotKEGGPath(mSet, \"Tryptophan metabolism\",528, 480, \"png\", NULL)"
[21] "mSet<-PlotKEGGPath(mSet, \"Histidine metabolism\",528, 480, \"png\", NULL)"
[22] "mSet<-PlotKEGGPath(mSet, \"Indole alkaloid biosynthesis\",528, 480, \"png\", NULL)"
[23] "mSet<-PlotKEGGPath(mSet, \"Sphingolipid metabolism\",528, 480, \"png\", NULL)"
[24] "mSet<-PlotKEGGPath(mSet, \"Sulfur metabolism\",528, 480, \"png\", NULL)"
[25] "mSet<-PlotKEGGPath(mSet, \"Glycerophospholipid metabolism\",528, 480, \"png\", NULL)"
[26] "mSet<-PlotKEGGPath(mSet, \"Alanine, aspartate and glutamate metabolism\",528, 480, \"png\", NU
[27] "mSet<-PlotKEGGPath(mSet, \"Glycine, serine and threonine metabolism\",528, 480, \"png\", NULL
[28] "mSet<-PlotKEGGPath(mSet, \"Pyrimidine metabolism\",528, 480, \"png\", NULL)"
[29] "mSet<-PlotKEGGPath(mSet, \"Nicotinate and nicotinamide metabolism\",528, 480, \"png\", NULL)"
[30] "mSet<-PlotKEGGPath(mSet, \"Arginine and proline metabolism\",528, 480, \"png\", NULL)"
[31] "mSet<-PlotKEGGPath(mSet, \"Butanoate metabolism\",528, 480, \"png\", NULL)"
[32] "mSet<-PlotKEGGPath(mSet, \"beta-Alanine metabolism\",528, 480, \"png\", NULL)"
[33] "mSet<-PlotKEGGPath(mSet, \"Carbon fixation in photosynthetic organisms\",528, 480, \"png\", NU
[34] "mSet<-PlotKEGGPath(mSet, \"Cysteine and methionine metabolism\",528, 480, \"png\", NULL)"
[35] "mSet<-PlotKEGGPath(mSet, \"Cyanoamino acid metabolism\",528, 480, \"png\", NULL)"
[36] "mSet<-PlotKEGGPath(mSet, \"Nitrogen metabolism\",528, 480, \"png\", NULL)"
[37] "mSet<-PlotKEGGPath(mSet, \"Porphyrin and chlorophyll metabolism\",528, 480, \"png\", NULL)"
[38] "mSet<-PlotKEGGPath(mSet, \"Glucosinolate biosynthesis\",528, 480, \"png\", NULL)"
[39] "mSet<-PlotKEGGPath(mSet, \"Folate biosynthesis\",528, 480, \"png\", NULL)"
[40] "mSet<-PlotKEGGPath(mSet, \"Methane metabolism\",528, 480, \"png\", NULL)"
[41] "mSet<-PlotKEGGPath(mSet, \"Terpenoid backbone biosynthesis\",528, 480, \"png\", NULL)"
[42] "mSet<-PlotKEGGPath(mSet, \"Glutathione metabolism\",528, 480, \"png\", NULL)"
[43] "mSet<-PlotKEGGPath(mSet, \"Synthesis and degradation of ketone bodies\",528, 480, \"png\", NU
[44] "mSet<-PlotKEGGPath(mSet, \"Phenylalanine metabolism\",528, 480, \"png\", NULL)"
[45] "mSet<-PlotKEGGPath(mSet, \"Purine metabolism\",528, 480, \"png\", NULL)"
[46] "mSet<-PlotKEGGPath(mSet, \"Ascorbate and aldarate metabolism\",528, 480, \"png\", NULL)"
[47] "mSet<-PlotKEGGPath(mSet, \"Galactose metabolism\",528, 480, \"png\", NULL)"
[48] "mSet<-PlotKEGGPath(mSet, \"Pentose and glucuronate interconversions\",528, 480, \"png\", NULL
[49] "mSet<-PlotKEGGPath(mSet, \"Valine, leucine and isoleucine degradation\",528, 480, \"png\", NU
[50] "mSet<-PlotKEGGPath(mSet, \"Zeatin biosynthesis\",528, 480, \"png\", NULL)"
[51] "mSet<-PlotKEGGPath(mSet, \"Phenylpropanoid biosynthesis\",528, 480, \"png\", NULL)"
[52] "mSet<-PlotKEGGPath(mSet, \"Tropane, piperidine and pyridine alkaloid biosynthesis\",528, 480,
[53] "mSet<-PlotKEGGPath(mSet, \"Glyoxylate and dicarboxylate metabolism\",528, 480, \"png\", NULL)
[54] "mSet<-PlotKEGGPath(mSet, \"Citrate cycle (TCA cycle)\",528, 480, \"png\", NULL)"
[55] "mSet<-PlotKEGGPath(mSet, \"Fatty acid metabolism\",528, 480, \"png\", NULL)"
[56] "mSet<-PlotKEGGPath(mSet, \"Pyruvate metabolism\",528, 480, \"png\", NULL)"
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 [57] "mSet<-PlotKEGGPath(mSet, \"Pentose phosphate pathway\",528, 480, \"png\", NULL)"
 [58] "mSet<-PlotKEGGPath(mSet, \"Amino sugar and nucleotide sugar metabolism\",528, 480, \"png\", NU
 [59] "mSet<-PlotKEGGPath(mSet, \"Tyrosine metabolism\",528, 480, \"png\", NULL)"
 [60] "mSet<-PlotKEGGPath(mSet, \"Pantothenate and CoA biosynthesis\",528, 480, \"png\", NULL)"
 [61] "mSet<-PlotKEGGPath(mSet, \"Aminoacyl-tRNA biosynthesis\",528, 480, \"png\", NULL)"
 [62] "mSet<-PlotKEGGPath(mSet, \"Inositol phosphate metabolism\",528, 480, \"png\", NULL)"
 [63] "mSet<-PlotKEGGPath(mSet, \"Propanoate metabolism\",528, 480, \"png\", NULL)"
 [64] "mSet<-PlotKEGGPath(mSet, \"Fatty acid biosynthesis\",528, 480, \"png\", NULL)"
 [65] "mSet<-PlotKEGGPath(mSet, \"Riboflavin metabolism\",528, 480, \"png\", NULL)"
 [66] "mSet<-PlotKEGGPath(mSet, \"Glycolysis or Gluconeogenesis\",528, 480, \"png\", NULL)"
 [67] "mSet<-PlotKEGGPath(mSet, \"Vitamin B6 metabolism\",528, 480, \"png\", NULL)"
 [68] "mSet<-PlotKEGGPath(mSet, \"Lysine biosynthesis\",528, 480, \"png\", NULL)"
 [69] "mSet<-PlotKEGGPath(mSet, \"Valine, leucine and isoleucine biosynthesis\",528, 480, \"png\", NU
 [70] "mSet<-PlotKEGGPath(mSet, \"Lysine degradation\",528, 480, \"png\", NULL)"
 [71] "mSet<-PlotKEGGPath(mSet, \"Isoquinoline alkaloid biosynthesis\",528, 480, \"png\", NULL)"
 [72] "mSet<-PlotKEGGPath(mSet, \"Ubiquinone and other terpenoid-quinone biosynthesis\",528, 480, \"p
 [73] "mSet<-PlotKEGGPath(mSet, \"Phenylalanine, tyrosine and tryptophan biosynthesis\",528, 480, \"p
 [74] "mSet<-PlotKEGGPath(mSet, \"Tryptophan metabolism\",528, 480, \"png\", NULL)"
 [75] "mSet<-PlotKEGGPath(mSet, \"Indole alkaloid biosynthesis\",528, 480, \"png\", NULL)"
 [76] "mSet<-PlotKEGGPath(mSet, \"Histidine metabolism\",528, 480, \"png\", NULL)"
 [77] "mSet<-PlotKEGGPath(mSet, \"Sphingolipid metabolism\",528, 480, \"png\", NULL)"
 [78] "mSet<-PlotKEGGPath(mSet, \"Sulfur metabolism\",528, 480, \"png\", NULL)"
 [79] "mSet<-PlotKEGGPath(mSet, \"Glycerophospholipid metabolism\",528, 480, \"png\", NULL)"
 [80] "mSet<-PlotKEGGPath(mSet, \"Pyrimidine metabolism\",528, 480, \"png\", NULL)"
 [81] "mSet<-PlotKEGGPath(mSet, \"Glycine, serine and threonine metabolism\",528, 480, \"png\", NULL
 [82] "mSet<-PlotKEGGPath(mSet, \"Isoquinoline alkaloid biosynthesis\",528, 480, \"png\", NULL)"
 [83] "mSet<-PlotKEGGPath(mSet, \"Indole alkaloid biosynthesis\",528, 480, \"png\", NULL)"
 [84] "mSet<-PlotKEGGPath(mSet, \"Histidine metabolism\",528, 480, \"png\", NULL)"
 [85] "mSet<-PlotKEGGPath(mSet, \"Sphingolipid metabolism\",528, 480, \"png\", NULL)"
 [86] "mSet<-PlotKEGGPath(mSet, \"Sulfur metabolism\",528, 480, \"png\", NULL)"
 [87] "mSet<-PlotKEGGPath(mSet, \"Glycerophospholipid metabolism\",528, 480, \"png\", NULL)"
 [88] "mSet<-PlotKEGGPath(mSet, \"Pyrimidine metabolism\",528, 480, \"png\", NULL)"
 [89] "mSet<-PlotKEGGPath(mSet, \"Arginine and proline metabolism\",528, 480, \"png\", NULL)"
 [90] "mSet<-PlotKEGGPath(mSet, \"Nicotinate and nicotinamide metabolism\",528, 480, \"png\", NULL)"
 [91] "mSet<-PlotKEGGPath(mSet, \"beta-Alanine metabolism\",528, 480, \"png\", NULL)"
 [92] "mSet<-PlotKEGGPath(mSet, \"Cyanoamino acid metabolism\",528, 480, \"png\", NULL)"
 [93] "mSet<-PlotKEGGPath(mSet, \"Nitrogen metabolism\",528, 480, \"png\", NULL)"
 [94] "mSet<-PlotKEGGPath(mSet, \"Carbon fixation in photosynthetic organisms\",528, 480, \"png\", NU
 [95] "mSet<-PlotKEGGPath(mSet, \"Porphyrin and chlorophyll metabolism\",528, 480, \"png\", NULL)"
 [96] "mSet<-PlotKEGGPath(mSet, \"Butanoate metabolism\",528, 480, \"png\", NULL)"
 [97] "mSet<-PlotKEGGPath(mSet, \"Cysteine and methionine metabolism\",528, 480, \"png\", NULL)"
 [98] "mSet<-PlotKEGGPath(mSet, \"Alanine, aspartate and glutamate metabolism\",528, 480, \"png\", NU
 [99] "mSet<-PlotKEGGPath(mSet, \"Methane metabolism\",528, 480, \"png\", NULL)"
[100] "mSet<-PlotKEGGPath(mSet, \"Glucosinolate biosynthesis\",528, 480, \"png\", NULL)"
[101] "mSet<-PlotKEGGPath(mSet, \"Folate biosynthesis\",528, 480, \"png\", NULL)"
[102] "mSet<-PlotKEGGPath(mSet, \"Methane metabolism\",528, 480, \"png\", NULL)"
[103] "mSet<-PlotKEGGPath(mSet, \"Glutathione metabolism\",528, 480, \"png\", NULL)"
[104] "mSet<-PlotKEGGPath(mSet, \"Terpenoid backbone biosynthesis\",528, 480, \"png\", NULL)"
[105] "mSet<-PlotKEGGPath(mSet, \"Glycerolipid metabolism\",528, 480, \"png\", NULL)"
[106] "mSet<-PlotKEGGPath(mSet, \"Phenylalanine metabolism\",528, 480, \"png\", NULL)"
[107] "mSet<-PlotKEGGPath(mSet, \"Phenylpropanoid biosynthesis\",528, 480, \"png\", NULL)"
[108] "mSet<-PlotKEGGPath(mSet, \"Tropane, piperidine and pyridine alkaloid biosynthesis\",528, 480,
[109] "mSet<-PlotKEGGPath(mSet, \"Valine, leucine and isoleucine degradation\",528, 480, \"png\", NU
[110] "mSet<-PlotKEGGPath(mSet, \"Ascorbate and aldarate metabolism\",528, 480, \"png\", NULL)"
[111] "mSet<-PlotKEGGPath(mSet, \"Galactose metabolism\",528, 480, \"png\", NULL)"
[112] "mSet<-PlotKEGGPath(mSet, \"Starch and sucrose metabolism\",528, 480, \"png\", NULL)"
[113] "mSet<-PlotKEGGPath(mSet, \"Pentose and glucuronate interconversions\",528, 480, \"png\", NULL
[114] "mSet<-PlotKEGGPath(mSet, \"Glyoxylate and dicarboxylate metabolism\",528, 480, \"png\", NULL)
```

```
[115] "mSet<-PlotKEGGPath(mSet, \"Fatty acid metabolism\",528, 480, \"png\", NULL)"
[116] "mSet<-PlotKEGGPath(mSet, \"Pyruvate metabolism\",528, 480, \"png\", NULL)"
[117] "mSet<-PlotKEGGPath(mSet, \"Pantothenate and CoA biosynthesis\",528, 480, \"png\", NULL)"
[118] "mSet<-PlotKEGGPath(mSet, \"Aminoacyl-tRNA biosynthesis\",528, 480, \"png\", NULL)"
[119] "mSet<-PlotKEGGPath(mSet, \"Inositol phosphate metabolism\",528, 480, \"png\", NULL)"
[120] "mSet<-PlotKEGGPath(mSet, \"Amino sugar and nucleotide sugar metabolism\",528, 480, \"png\", NU
[121] "mSet<-PlotKEGGPath(mSet, \"Tyrosine metabolism\",528, 480, \"png\", NULL)"
[122] "mSet<-PlotKEGGPath(mSet, \"Propanoate metabolism\",528, 480, \"png\", NULL)"
[123] "mSet<-PlotKEGGPath(mSet, \"Fatty acid biosynthesis\",528, 480, \"png\", NULL)"
[124] "mSet<-PlotKEGGPath(mSet, \"Riboflavin metabolism\",528, 480, \"png\", NULL)"
[125] "mSet<-PlotKEGGPath(mSet, \"Pentose phosphate pathway\",528, 480, \"png\", NULL)"
[126] "mSet<-PlotKEGGPath(mSet, \"Glycolysis or Gluconeogenesis\",528, 480, \"png\", NULL)"
[127] "mSet<-PlotKEGGPath(mSet, \"Vitamin B6 metabolism\",528, 480, \"png\", NULL)"
[128] "mSet<-PlotKEGGPath(mSet, \"Lysine biosynthesis\",528, 480, \"png\", NULL)"
[129] "mSet<-PlotKEGGPath(mSet, \"Valine, leucine and isoleucine biosynthesis\",528, 480, \"png\", NU
[130] "mSet<-PlotKEGGPath(mSet, \"Lysine degradation\",528, 480, \"png\", NULL)"
[131] "mSet<-SaveTransformedData(mSet)"
[132] "mSet<-PreparePDFReport(mSet, \"guest8842274631122909247\")\n"
```

The report was generated on Wed Oct 9 15:04:54 2019 with R version 3.5.1 (2018-07-02).