Spen-KD HYD

Metabolomic Data Analysis with MetaboAnalyst 4.0

Name: guest5687566664954251710

March 19, 2019

1 Background

MSEA or Metabolite Set Enrichment Analysis is a way to identify biologically meaningful patterns that are significantly enriched in quantitative metabolomic data. In conventional approaches, metabolites are evaluated individually for their significance under conditions of study. Those compounds that have passed certain significance level are then combined to see if any meaningful patterns can be discerned. In contrast, MSEA directly investigates if a set of functionally related metabolites without the need to preselect compounds based on some arbitrary cut-off threshold. It has the potential to identify subtle but consistent changes among a group of related compounds, which may go undetected with the conventional approaches.

Essentially, MSEA is a metabolomic version of the popular GSEA (Gene Set Enrichment Analysis) software with its own collection of metabolite set libraries as well as an implementation of user-friendly web-interfaces. GSEA is widely used in genomics data analysis and has proven to be a powerful alternative to conventional approaches. For more information, please refer to the original paper by Subramanian A, and a nice review paper by Nam D, Kim SY. [1]. [2]

2 MSEA Overview

Metabolite set enrichment analysis consists of four steps - data input, data processing, data analysis, and results download. Different analysis procedures are performed based on different input types. In addition, users can also browse and search the metabolite set libraries as well as upload their self-defined metabolite sets for enrichment analysis. Users can also perform metabolite name mapping between a variety of compound names, synonyms, and major database identifiers.

3 Data Input

There are three enrichment analysis algorithms offered by MSEA. Accordingly, three different types of data inputs are required by these three approaches:

- A list of important compound names - entered as a one column data (*Over Representation Analysis (ORA)*);
- A single measured biofluid (urine, blood, CSF) sample- entered as tab separated two-column data with the first column for compound name, and the second for concentration values (*Single Sample Profiling (SSP)*);

[1] Subramanian *Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.*, Proc Natl Acad Sci USA. 2005 102(43): 15545-50

[2] Nam D, Kim SY. *Gene-set approach for expression pattern analysis*, Briefings in Bioinformatics. 2008 9(3): 189-197.

- A compound concentration table - entered as a comma separated (.csv) file with the each sample per row and each metabolite concentration per column. The first column is sample names and the second column for sample phenotype labels (*Quantitative Enrichment Analysis (QEA)*)

You selected Over Representation Analysis (ORA) which requires a list of compound names as input.

4 Data Process

The first step is to standardize the compound labels. It is an essential step since the compound labels will be subsequently compared with compounds contained in the metabolite set library. MSEA has a built-in tool to convert between compound common names, synonyms, identifiers used in HMDB ID, PubChem, ChEBI, BiGG, METLIN, KEGG, or Reactome. **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	C00134	Putrescine	HMDB0001414	1045	C00134	1
2	C01005	Phosphoserine	HMDB0000272	68841	C01005	1
3	C02352	1,4-beta-D-Xylan	METPA0277		C02352	1
4	C00186	L-Lactic acid	HMDB0000190	107689	C00186	1
5	C05635	5-Hydroxyindoleacetic acid	HMDB0000763	1826	C05635	1
6	C02220	2-Aminomuconic acid	HMDB0001241	5280499	C02220	1
7	C00245	Taurine	HMDB0000251	1123	C00245	1
8	C03453	(Z)-5-Oxohex-2-enedioate	METPA0394		C03453	1
9	C00042	Succinic acid	HMDB0000254	1110	C00042	1
10	C01602	L-Ornithine monochlorohydrate/ornithine	HMDB32455	389	C01602	1
11	C00366	Uric acid	HMDB0000289	1175	C00366	1
12	C00047	L-Lysine	HMDB0000182	5962	C00047	1
13	C01571	Capric acid	HMDB0000511	2969	C01571	1
14	C00329	Glucosamine	HMDB0001514	439213	C00329	1
15	C02930	NA	NA	NA	NA	0
16	C02354	2',3'-Cyclic CMP	METPA0278		C02354	1
17	C01157	4-Hydroxyproline	HMDB0000725	5810	C01157	1
18	C02679	Dodecanoic acid	HMDB0000638	3893	C02679	1
19	C11821	5-Hydroxyisourate	HMDB0030097	250388	C11821	1
20	C00022	Pyruvic acid	HMDB0000243	1060	C00022	1
21	C01026	Dimethylglycine	HMDB0000092	673	C01026	1
22	C08480	NA	NA	NA	NA	0
23	C00031	D-Glucose	HMDB0000122	5793	C00031	1
24	C00079	L-Phenylalanine	HMDB0000159	6140	C00079	1
25	C02589	NA	NA	NA	NA	0

The second step is to check concentration values. For SSP analysis, the concentration must be measured in *umol* for blood and CSF samples. The urinary concentrations must be first converted to *umol/ mmol creatinine* in order to compare with reported concentrations in literature. No missing or negative values are allowed in SSP analysis. The concentration data for QEA analysis is more flexible. Users can upload either the original concentration data or normalized data. Missing or negative values are allowed (coded as *NA*) for QEA.

5 Selection of Metabolite Set Library

Before proceeding to enrichment analysis, a metabolite set library has to be chosen. There are seven built-in libraries offered by MSEA:

- Metabolic pathway associated metabolite sets (*currently contains 99 entries*);
- Disease associated metabolite sets (reported in blood) (*currently contains 344 entries*);
- Disease associated metabolite sets (reported in urine) (*currently contains 384 entries*)
- Disease associated metabolite sets (reported in CSF) (*currently contains 166 entries*)
- Metabolite sets associated with SNPs (*currently contains 4598 entries*)
- Predicted metabolite sets based on computational enzyme knockout model (*currently contains 912 entries*)
- Metabolite sets based on locations (*currently contains 73 entries*)
- Drug pathway associated metabolite sets (*currently contains 461 entries*)

In addition, MSEA also allows user-defined metabolite sets to be uploaded to perform enrichment analysis on arbitrary groups of compounds which researchers want to test. The metabolite set library is simply a two-column comma separated text file with the first column for metabolite set names and the second column for its compound names (**must use HMDB compound name**) separated by ";". Please note, the built-in libraries are mainly from human studies. The functional grouping of metabolites may not be valid. Therefore, for data from subjects other than human being, users are suggested to upload their self-defined metabolite set libraries for enrichment analysis.

6 Enrichment Analysis

Over Representation Analysis (ORA) is performed when a list of compound names is provided. The list of compound list can be obtained through conventional feature selection methods, or from a clustering algorithm, or from the compounds with abnormal concentrations detected in SSP, to investigate if some biologically meaningful patterns can be identified.

ORA was implemented using the *hypergeometric test* to evaluate whether a particular metabolite set is represented more than expected by chance within the given compound list. One-tailed p values are provided after adjusting for multiple testing. **Figure 2** below summarizes the result.

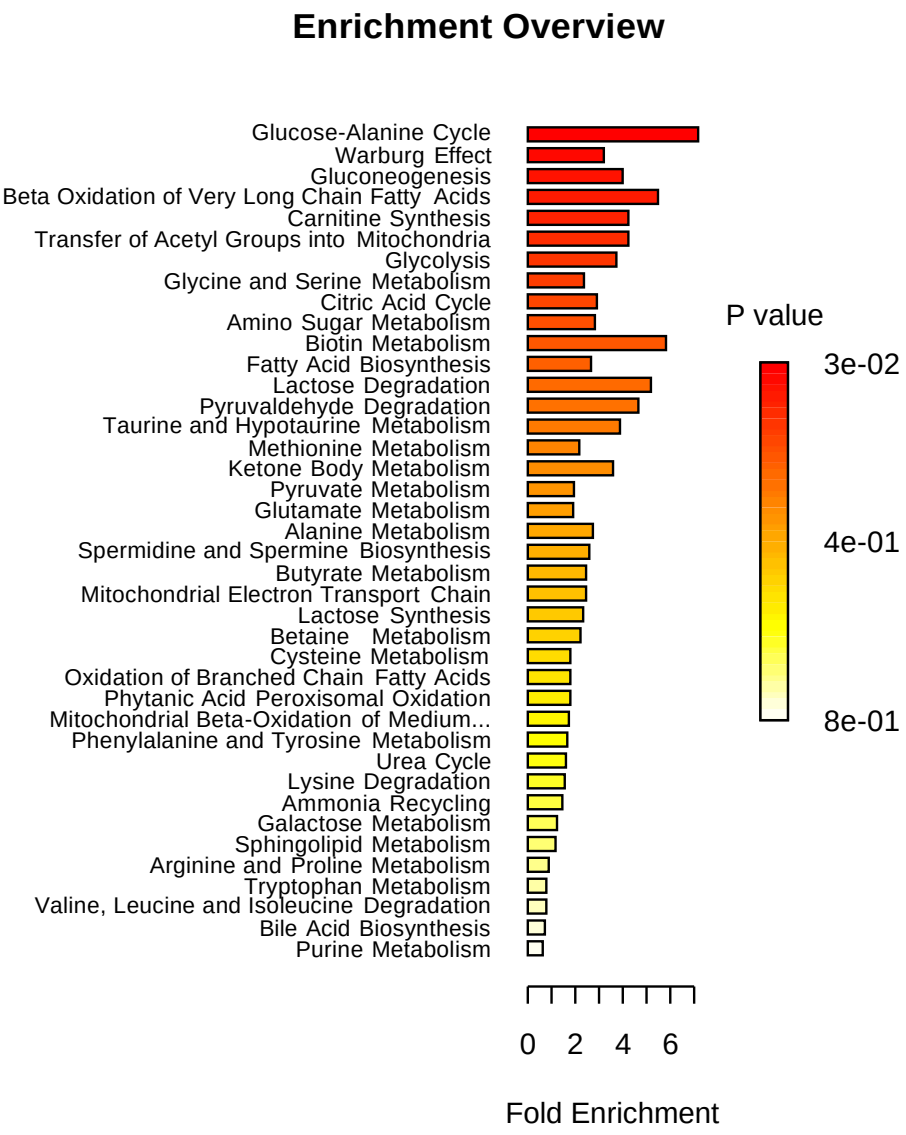

Figure 1: Summary Plot for Over Representation Analysis (ORA)

Table 2: Result from Over Representation Analysis

	total	expected	hits	Raw p	Holm p	FDR
Glucose-Alanine Cycle	13	0.28	2	2.98E-02	1.00E+00	1.00E+00
Warburg Effect	58	1.25	4	3.15E-02	1.00E+00	1.00E+00
Gluconeogenesis	35	0.75	3	3.61E-02	1.00E+00	1.00E+00
Beta Oxidation of Very Long Chain Fatty Acids	17	0.36	2	4.93E-02	1.00E+00	1.00E+00
Carnitine Synthesis	22	0.47	2	7.85E-02	1.00E+00	1.00E+00
Transfer of Acetyl Groups into Mitochondria	22	0.47	2	7.85E-02	1.00E+00	1.00E+00
Glycolysis	25	0.54	2	9.81E-02	1.00E+00	1.00E+00
Glycine and Serine Metabolism	59	1.27	3	1.28E-01	1.00E+00	1.00E+00
Citric Acid Cycle	32	0.69	2	1.48E-01	1.00E+00	1.00E+00
Amino Sugar Metabolism	33	0.71	2	1.56E-01	1.00E+00	1.00E+00
Biotin Metabolism	8	0.17	1	1.60E-01	1.00E+00	1.00E+00
Fatty Acid Biosynthesis	35	0.75	2	1.71E-01	1.00E+00	1.00E+00
Lactose Degradation	9	0.19	1	1.78E-01	1.00E+00	1.00E+00
Pyruvaldehyde Degradation	10	0.21	1	1.96E-01	1.00E+00	1.00E+00
Taurine and Hypotaurine Metabolism	12	0.26	1	2.31E-01	1.00E+00	1.00E+00
Methionine Metabolism	43	0.92	2	2.35E-01	1.00E+00	1.00E+00
Ketone Body Metabolism	13	0.28	1	2.47E-01	1.00E+00	1.00E+00
Pyruvate Metabolism	48	1.03	2	2.76E-01	1.00E+00	1.00E+00
Glutamate Metabolism	49	1.05	2	2.84E-01	1.00E+00	1.00E+00
Alanine Metabolism	17	0.36	1	3.11E-01	1.00E+00	1.00E+00
Spermidine and Spermine Biosynthesis	18	0.39	1	3.26E-01	1.00E+00	1.00E+00
Butyrate Metabolism	19	0.41	1	3.41E-01	1.00E+00	1.00E+00
Mitochondrial Electron Transport Chain	19	0.41	1	3.41E-01	1.00E+00	1.00E+00
Lactose Synthesis	20	0.43	1	3.55E-01	1.00E+00	1.00E+00
Betaine Metabolism	21	0.45	1	3.69E-01	1.00E+00	1.00E+00
Cysteine Metabolism	26	0.56	1	4.35E-01	1.00E+00	1.00E+00
Oxidation of Branched Chain Fatty Acids	26	0.56	1	4.35E-01	1.00E+00	1.00E+00
Phytanic Acid Peroxisomal Oxidation	26	0.56	1	4.35E-01	1.00E+00	1.00E+00
Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids	27	0.58	1	4.48E-01	1.00E+00	1.00E+00
Phenylalanine and Tyrosine Metabolism	28	0.60	1	4.60E-01	1.00E+00	1.00E+00
Urea Cycle	29	0.62	1	4.72E-01	1.00E+00	1.00E+00
Lysine Degradation	30	0.65	1	4.84E-01	1.00E+00	1.00E+00
Ammonia Recycling	32	0.69	1	5.06E-01	1.00E+00	1.00E+00
Galactose Metabolism	38	0.82	1	5.69E-01	1.00E+00	1.00E+00
Sphingolipid Metabolism	40	0.86	1	5.88E-01	1.00E+00	1.00E+00
Arginine and Proline Metabolism	53	1.14	1	6.93E-01	1.00E+00	1.00E+00
Tryptophan Metabolism	60	1.29	1	7.39E-01	1.00E+00	1.00E+00
Valine, Leucine and Isoleucine Degradation	60	1.29	1	7.39E-01	1.00E+00	1.00E+00
Bile Acid Biosynthesis	65	1.40	1	7.67E-01	1.00E+00	1.00E+00
Purine Metabolism	74	1.59	1	8.11E-01	1.00E+00	1.00E+00

7 Appendix: R Command History

```
 [1] "mSet<-InitDataObjects(\"conc\", \"msetora\", FALSE)"
 [2] "cmpd.vec<-c(\"C00134\",\"C01005\",\"C02352\",\"C00186\",\"C05635\",\"C02220\",
 \"C00245\",\"C03453\",\" C00042\",\" C01602\",\" C00366\", C00047\",\" C01571\",
 \" C00329\",\" C02930\",\" C02354\",\" C01157\",\" C02679\",\" C02821\",\" C00022\",
 \" C01026\",\" C08480\",\" C00031\",\" C00079\",\" C02589\")
 [3] "mSet<-Setup.MapData(mSet, cmpd.vec);"
 [4] "mSet<-CrossReferencing(mSet, \"kegg\");"
 [5] "mSet<-CreateMappingResultTable(mSet)"
 [6] "mSet<-SetMetabolomeFilter(mSet, F);"
 [7] "mSet<-SetCurrentMsetLib(mSet, \"pathway\", 2);"
 [8] "mSet<-CalculateHyperScore(mSet)"
 [9] "mSet<-PlotORA(mSet, \"ora_0_\", \"net\", \"png\", 72, width=NA)"
[10] "mSet<-CalculateHyperScore(mSet)"
[11] "mSet<-PlotORA(mSet, \"ora_1_\", \"net\", \"png\", 72, width=NA)"
[12] "mSet<-SaveTransformedData(mSet)"
[13] "mSet<-PreparePDFReport(mSet, \"guest5687566664954251710\")\n"
```

The report was generated on Tue Mar 19 19:05:33 2019 with R version 3.5.1 (2018-07-02).

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7	C01026	Dimethylglycine	HMDB0000092	673	C01026	1
8	C01996	Acetylcholine	HMDB0000895	187	C01996	1
9	C00065	L-Serine	HMDB0000187	5951	C00065	1
10	C00041	L-Alanine	HMDB0000161	5950	C00041	1
11	C00346	O-Phosphoethanolamine	HMDB0000224	1015	C00346	1
12	C05283	N2-gamma-Glutamylglutamine	HMDB0011738	150914	C05283	1
13	C03738	NA	NA	NA	NA	0
14	C00022	Pyruvic acid	HMDB0000243	1060	C00022	1
15	C02589	NA	NA	NA	NA	0
16	C06423	Caprylic acid	HMDB0000482	379	C06423	1
17	C00315	Spermidine	HMDB0001257	1102	C00315	1
18	C00245	Taurine	HMDB0000251	1123	C00245	1
19	C00475	Cytidine	HMDB0000089	6175	C00475	1
20	C00135	L-Histidine	HMDB0000177	6274	C00135	1
21	C01904	D-Arabitol	HMDB0000568	827	C01904	1
22	C01233	Glycerylphosphorylethanolamine	HMDB0000114	22833510	C01233	1
23	C01571	Capric acid	HMDB0000511	2969	C01571	1
24	C00037	Glycine	HMDB0000123	750	C00037	1
25	C01210	N-Methylethanolamine phosphate	METPA0145		C01210	1
26	C03153	NA	NA	NA	NA	0
27	C00279	D-Erythrose 4-phosphate	HMDB0001321	122357	C00279	1
28	C08435	NA	NA	NA	NA	0
29	C00669	gamma-Glutamylcysteine	HMDB0001049	123938	C00669	1
30	C00319	Sphingosine	HMDB0000252	5353955	C00319	1
31	C00906	Dihydrothymine	HMDB0000079	93556	C00906	1
32	C00183	L-Valine	HMDB0000883	6287	C00183	1
33	C00148	L-Proline	HMDB0000162	145742	C00148	1
34	C00061	Flavin Mononucleotide	HMDB0001520	643976	C00061	1
35	C06231	Ectoine	METPA0797		C06231	1
36	C08362	Palmitoleic acid	HMDB0003229	445638	C08362	1
37	C06156	NA	NA	NA	NA	0
38	C01432	NA	NA	NA	NA	0
39	C00042	Succinic acid	HMDB0000254	1110	C00042	1
40	C00328	L-Kynurenine	HMDB0000684	161166	C00328	1
41	C00318	L-Carnitine	HMDB0000062	2724480	C00318	1
42	C05635	5-Hydroxyindoleacetic acid	HMDB0000763	1826	C05635	1
43	C00049	L-Aspartic acid	HMDB0000191	5960	C00049	1
44	C00062	L-Arginine	HMDB0000517	6322	C00062	1
45	C00020	Adenosine monophosphate	HMDB0000045	6083	C00020	1
46	C00620	Ribose 1-phosphate	HMDB0001489	439236	C00620	1
47	C08480	NA	NA	NA	NA	0
48	C00385	Xanthine	HMDB0000292	1188	C00385	1
49	C00542	Allocystathionine	HMDB0000455	10104953	C00542	1
50	C00118	NA	NA	NA	NA	0
51	C00327	Citrulline	HMDB0000904	9750	C00327	1
52	C00078	L-Tryptophan	HMDB0000929	6305	C00078	1
53	C00064	L-Glutamine	HMDB0000641	5961	C00064	1
54	C00079	L-Phenylalanine	HMDB0000159	6140	C00079	1
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56	C02728	N(6)-Methyllysine	HMDB0002038	164795	C02728	1
57	C00127	Oxidized glutathione	HMDB0003337	975	C00127	1
58	C00249	Palmitic acid	HMDB0000220	985	C00249	1
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61	C00009	Phosphate	HMDB0001429	1061	C00009	1
62	C00712	Oleic acid	HMDB0000207	445639	C00712	1
63	C00637	Indoleacetaldehyde	HMDB0001190	800	C00637	1
64	C01585	Caproic acid	HMDB0000535	8892	C01585	1
65	C01879	Pyroglutamic acid	HMDB0000267	7405	C01879	1
66	C00178	Thymine	HMDB0000262	1135	C00178	1
67	C03453	(Z)-5-Oxohex-2-enedioate	METPA0394		C03453	1
68	C00072	Ascorbic acid	HMDB0000044	54670067	C00072	1
69	C02532	NA	NA	NA	NA	0
70	C01595	Linoleic acid	HMDB0000673	5280450	C01595	1
71	C06424	Myristic acid	HMDB0000806	11005	C06424	1
72	C00013	Pyrophosphate	HMDB0000250	644102	C00013	1
73	C02630	NA	NA	NA	NA	0
74	C02989	L-Methionine S-oxide	METPA0344		C02989	1
75	C00490	Itaconic acid	HMDB0002092	811	C00490	1
76	C00114	Choline	HMDB0000097	305	C00114	1
77	C01835	Maltotriose	HMDB0001262	439586	C01835	1
78	C06738	cis-p-Coumaric acid	HMDB0030677	1549106	C06738	1
79	C06427	Alpha-Linolenic acid	HMDB0001388	5280934	C06427	1
80	C06124	Sphingosine 1-phosphate	HMDB0000277	5353956	C06124	1
81	C02930	NA	NA	NA	NA	0
82	C00499	Allantoic acid	HMDB0001209	203	C00499	1
83	C00082	L-Tyrosine	HMDB0000158	6057	C00082	1
84	C05834	3-Methyldioxyindole	HMDB0004186	151066	C05834	1

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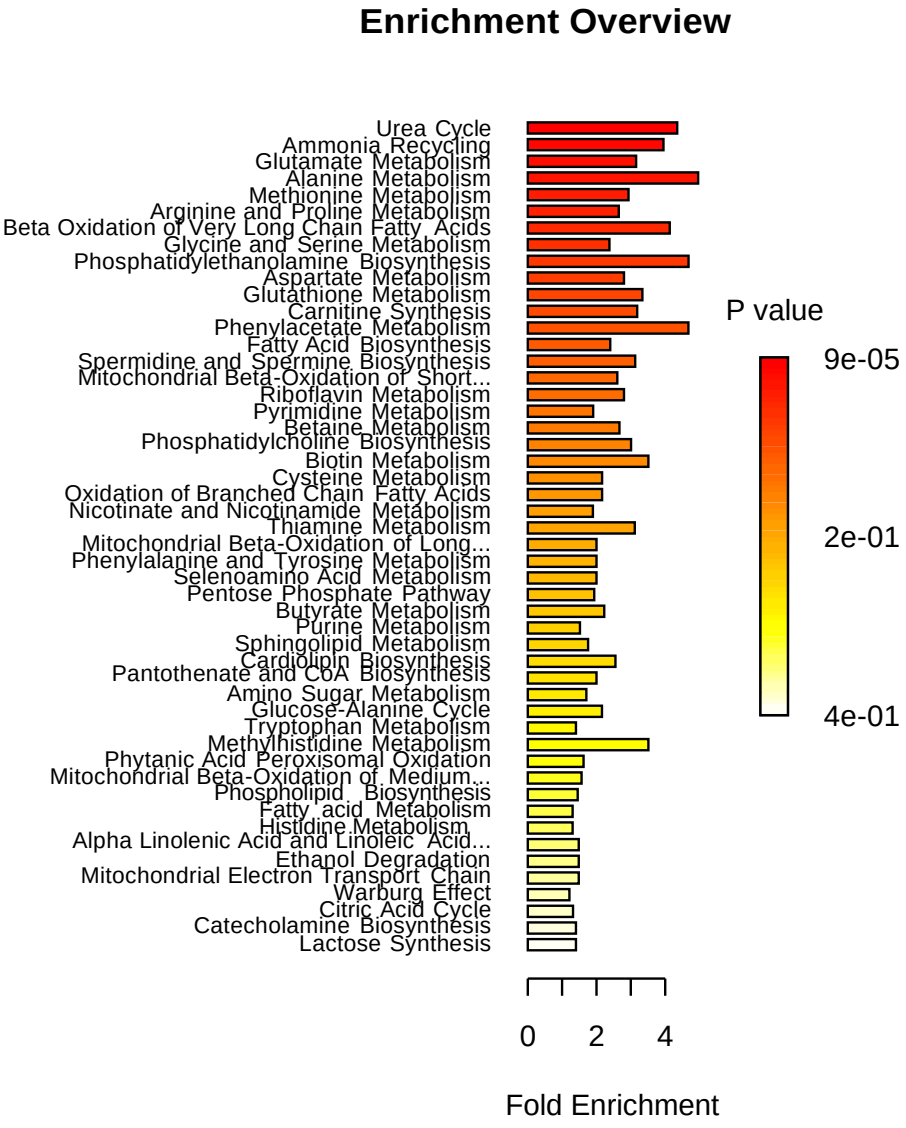

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Table 2: Result from Over Representation Analysis

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Ammonia Recycling	32	2.28	9	2.15E-04	2.09E-02	1.05E-02
Glutamate Metabolism	49	3.49	11	3.68E-04	3.53E-02	1.20E-02
Alanine Metabolism	17	1.21	6	7.06E-04	6.71E-02	1.73E-02
Methionine Metabolism	43	3.07	9	2.27E-03	2.13E-01	4.44E-02
Arginine and Proline Metabolism	53	3.78	10	2.92E-03	2.72E-01	4.78E-02
Beta Oxidation of Very Long Chain Fatty Acids	17	1.21	5	5.05E-03	4.65E-01	7.07E-02
Glycine and Serine Metabolism	59	4.21	10	6.62E-03	6.02E-01	8.11E-02
Phosphatidylethanolamine Biosynthesis	12	0.85	4	7.60E-03	6.84E-01	8.28E-02
Aspartate Metabolism	35	2.50	7	9.34E-03	8.31E-01	9.15E-02
Glutathione Metabolism	21	1.50	5	1.33E-02	1.00E+00	1.18E-01
Carnitine Synthesis	22	1.57	5	1.62E-02	1.00E+00	1.32E-01
Phenylacetate Metabolism	9	0.64	3	2.14E-02	1.00E+00	1.61E-01
Fatty Acid Biosynthesis	35	2.50	6	3.30E-02	1.00E+00	2.21E-01
Spermidine and Spermine Biosynthesis	18	1.28	4	3.39E-02	1.00E+00	2.21E-01
Mitochondrial Beta-Oxidation of Short Chain Saturated Fatty Acids	27	1.92	5	3.76E-02	1.00E+00	2.30E-01
Riboflavin Metabolism	20	1.43	4	4.81E-02	1.00E+00	2.77E-01
Pyrimidine Metabolism	59	4.21	8	5.18E-02	1.00E+00	2.82E-01
Betaine Metabolism	21	1.50	4	5.63E-02	1.00E+00	2.90E-01
Phosphatidylcholine Biosynthesis	14	1.00	3	7.16E-02	1.00E+00	3.51E-01
Biotin Metabolism	8	0.57	2	1.06E-01	1.00E+00	4.58E-01
Cysteine Metabolism	26	1.85	4	1.08E-01	1.00E+00	4.58E-01
Oxidation of Branched Chain Fatty Acids	26	1.85	4	1.08E-01	1.00E+00	4.58E-01
Nicotinate and Nicotinamide Metabolism	37	2.64	5	1.17E-01	1.00E+00	4.58E-01
Thiamine Metabolism	9	0.64	2	1.30E-01	1.00E+00	4.58E-01
Mitochondrial Beta-Oxidation of Long Chain Saturated Fatty Acids	28	2.00	4	1.33E-01	1.00E+00	4.58E-01
Phenylalanine and Tyrosine Metabolism	28	2.00	4	1.33E-01	1.00E+00	4.58E-01
Selenoamino Acid Metabolism	28	2.00	4	1.33E-01	1.00E+00	4.58E-01
Pentose Phosphate Pathway	29	2.07	4	1.46E-01	1.00E+00	4.58E-01
Butyrate Metabolism	19	1.35	3	1.48E-01	1.00E+00	4.58E-01
Purine Metabolism	74	5.28	8	1.48E-01	1.00E+00	4.58E-01
Sphingolipid Metabolism	40	2.85	5	1.50E-01	1.00E+00	4.58E-01
Cardiolipin Biosynthesis	11	0.78	2	1.82E-01	1.00E+00	5.30E-01
Pantothenate and CoA Biosynthesis	21	1.50	3	1.84E-01	1.00E+00	5.30E-01
Amino Sugar Metabolism	33	2.35	4	2.04E-01	1.00E+00	5.71E-01
Glucose-Alanine Cycle	13	0.93	2	2.36E-01	1.00E+00	6.42E-01
Tryptophan Metabolism	60	4.28	6	2.51E-01	1.00E+00	6.61E-01
Methylhistidine Metabolism	4	0.28	1	2.56E-01	1.00E+00	6.61E-01
Phytanic Acid Peroxisomal Oxidation	26	1.85	3	2.81E-01	1.00E+00	7.07E-01
Mitochondrial Beta-Oxidation of Medium Chain Saturated Fatty Acids	27	1.92	3	3.02E-01	1.00E+00	7.39E-01
Phospholipid Biosynthesis	29	2.07	3	3.42E-01	1.00E+00	8.18E-01
Fatty acid Metabolism	43	3.07	4	3.68E-01	1.00E+00	8.22E-01
Histidine Metabolism	43	3.07	4	3.68E-01	1.00E+00	8.22E-01
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	1.35	2	3.98E-01	1.00E+00	8.22E-01
Ethanol Degradation	19	1.35	2	3.98E-01	1.00E+00	8.22E-01
Mitochondrial Electron Transport Chain	19	1.35	2	3.98E-01	1.00E+00	8.22E-01
Warburg Effect	58	4.13	5	3.99E-01	1.00E+00	8.22E-01
Citric Acid Cycle	32	2.28	3	4.03E-01	1.00E+00	8.22E-01
Catecholamine Biosynthesis	20	1.43	2	4.24E-01	1.00E+00	8.30E-01
Lactose Synthesis	20	1.43	2	4.24E-01	1.00E+00	8.30E-01
Beta-Alanine Metabolism	34	2.42	3	4.42E-01	1.00E+00	8.38E-01
Pyruvate Metabolism	48	3.42	4	4.52E-01	1.00E+00	8.38E-01
Sulfate/Sulfite Metabolism	22	1.57	2	4.73E-01	1.00E+00	8.38E-01
Transfer of Acetyl Groups into Mitochondria	22	1.57	2	4.73E-01	1.00E+00	8.38E-01
De Novo Triacylglycerol Biosynthesis	9	0.64	1	4.87E-01	1.00E+00	8.38E-01
Homocysteine Degradation	9	0.64	1	4.87E-01	1.00E+00	8.38E-01
Lactose Degradation	9	0.64	1	4.87E-01	1.00E+00	8.38E-01
Galactose Metabolism	38	2.71	3	5.18E-01	1.00E+00	8.56E-01
Malate-Aspartate Shuttle	10	0.71	1	5.24E-01	1.00E+00	8.56E-01
Pyruvaldehyde Degradation	10	0.71	1	5.24E-01	1.00E+00	8.56E-01
Glycerolipid Metabolism	25	1.78	2	5.43E-01	1.00E+00	8.59E-01
Glycolysis	25	1.78	2	5.43E-01	1.00E+00	8.59E-01
Trehalose Degradation	11	0.78	1	5.59E-01	1.00E+00	8.65E-01
Plasmalogen Synthesis	26	1.85	2	5.65E-01	1.00E+00	8.65E-01
Propanoate Metabolism	42	2.99	3	5.88E-01	1.00E+00	8.77E-01
Taurine and Hypotaurine Metabolism	12	0.85	1	5.90E-01	1.00E+00	8.77E-01
Ketone Body Metabolism	13	0.93	1	6.20E-01	1.00E+00	8.93E-01
Thyroid hormone synthesis	13	0.93	1	6.20E-01	1.00E+00	8.93E-01
Starch and Sucrose Metabolism	31	2.21	2	6.63E-01	1.00E+00	9.37E-01
Fructose and Mannose Degradation	32	2.28	2	6.80E-01	1.00E+00	9.37E-01
Steroid Biosynthesis	48	3.42	3	6.81E-01	1.00E+00	9.37E-01
Inositol Metabolism	33	2.35	2	6.97E-01	1.00E+00	9.37E-01
Bile Acid Biosynthesis	65	4.63	4	6.98E-01	1.00E+00	9.37E-01
Phosphatidylinositol Phosphate Metabolism	17	1.21	1	7.18E-01	1.00E+00	9.52E-01
Gluconeogenesis	35	2.50	2	7.28E-01	1.00E+00	9.52E-01
Nucleotide Sugars Metabolism	20	1.43	1	7.75E-01	1.00E+00	9.62E-01

Threonine and 2-Oxobutanoate Degradation	20	1.43	1	7.75E-01	1.00E+00	9.62E-01
Ubiquinone Biosynthesis	20	1.43	1	7.75E-01	1.00E+00	9.62E-01
Vitamin B6 Metabolism	20	1.43	1	7.75E-01	1.00E+00	9.62E-01
Valine, Leucine and Isoleucine Degradation	60	4.28	3	8.19E-01	1.00E+00	1.00E+00
Inositol Phosphate Metabolism	26	1.85	1	8.57E-01	1.00E+00	1.00E+00
Folate Metabolism	29	2.07	1	8.86E-01	1.00E+00	1.00E+00
Lysine Degradation	30	2.14	1	8.95E-01	1.00E+00	1.00E+00
Tyrosine Metabolism	72	5.13	3	9.03E-01	1.00E+00	1.00E+00
Fatty Acid Elongation In Mitochondria	35	2.50	1	9.28E-01	1.00E+00	1.00E+00
Porphyrin Metabolism	40	2.85	1	9.51E-01	1.00E+00	1.00E+00
Arachidonic Acid Metabolism	69	4.92	1	9.95E-01	1.00E+00	1.00E+00

7 Appendix: R Command History

```
 [1] "mSet<-InitDataObjects(\"conc\", \"msetora\", FALSE)"
 [2] "cmpd.vec<-c(\"C00134\",\"C02352\",\"C00047\",\"C01157\",\"C00188\",\"C01602\",
 \"C01026\",\"C01996\",\"C00065\",\"C00041\",\"C00346\",\"C05283\",\"C03738\",\"C00022\",
 \"C02589\",\"C06423\",\"C00315\",\"C00245\",\"C00475\",\"C00135\",\"C01904\",\"C01233\",
 \"C01571\",\"C00037\",\"C01210\",\"C03153\",\"C00279\",\"C08435\",\"C00669\",\"C00319\",
 \"C00906\",\"C00183\",\"C00148\",\"C00061\",\"C06231\",\"C08362\",\"C06156\",\"C01432\",
 \"C00042\",\"C00328\",\"C00318\",\"C05635\",\"C00049\",\"C00062\",\"C00020\",\"C00620\",
 \"C08480\",\"C00385\",\"C00542\",\"C00118\",\"C00327\",\"C00078\",\"C00064\",\"C00079\",
 \"C02679\",\"C00127\",\"C00249\",\"C02354\",\"C01530\",\"C00009\",\"C00712\",\"C00637\",
 \"C01585\",\"C01879\",\"C00178\",\"C03453\",\"C00072\",\"C02532\",\"C01595\",\"C06424\",
 \"C00013\",\"C02630\",\"C02989\",\"C00490\",\"C00114\",\"C01835\",\"C06427\",\"C06124\",
 \"C02930\",\"C00499\",\"C00082\",\"C05834\" )
 [3] "mSet<-Setup.MapData(mSet, cmpd.vec);"
 [4] "mSet<-CrossReferencing(mSet, \"kegg\");"
 [5] "mSet<-CreateMappingResultTable(mSet)"
 [6] "mSet<-SetMetabolomeFilter(mSet, F);"
 [7] "mSet<-SetCurrentMsetLib(mSet, \"pathway\", 2);"
 [8] "mSet<-CalculateHyperScore(mSet)"
 [9] "mSet<-PlotORA(mSet, \"ora_0_\", \"net\", \"png\", 72, width=NA)"
[10] "mSet<-CalculateHyperScore(mSet)"
[11] "mSet<-PlotORA(mSet, \"ora_1_\", \"net\", \"png\", 72, width=NA)"
[12] "mSet<-SaveTransformedData(mSet)"
[13] "mSet<-PreparePDFReport(mSet, \"guest7643961153718848615\")\n"
```

The report was generated on Tue Mar 19 18:45:58 2019 with R version 3.5.1 (2018-07-02).