Metabolomic Data Analysis with MetaboAnalyst 5.0

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May 24, 2021

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: Resu

	Query	Match	HMDB	PubChem
1	HMDB0059844	NA	NA	NA
2	HMDB0000642	Erythronilic acid	HMDB0000642	12313370
3	HMDB0041603	NA	NA	NA
4	HMDB0040735	NA	NA	NA
5	HMDB0002011	4-Hydroxyisovaleric acid	HMDB0002011	131760
6	HMDB0001863	2-Hydroxyvaleric acid	HMDB0001863	98009
7	HMDB0000754	3-Hydroxyisovaleric acid	HMDB0000754	69362
8	HMDB0000531	3-Hydroxyvaleric acid	HMDB0000531	107802
9	HMDB0000410	3-Hydroxy-2-methyl-[S-(R,R)]-butanoic acid	HMDB0000410	12313369
10	HMDB0000407	2-Hydroxy-3-methylbutyric acid	HMDB0000407	99823
11	HMDB0000396	2-Ethylhydracrylic acid	HMDB0000396	188979
12	HMDB0000354	2-Methyl-3-hydroxybutyric acid	HMDB0000354	160471
13	HMDB0000351	3-Hydroxy-2-methyl-[R-(R,S)]-butanoic acid	HMDB0000351	12313370
14	HMDB0172069	NA	NA	NA
15	HMDB0172068	NA	NA	NA
16	HMDB0172067	NA	NA	NA
17	HMDB0161206	NA	NA	NA
18	HMDB0161204	NA	NA	NA
19	HMDB0161205	NA	NA	NA
20	HMDB0062584	NA	NA	NA
21	HMDB0062057	NA	NA	NA
22	HMDB0061927	NA	NA	NA
23	HMDB0001987	2-Hydroxy-2-methylbutyric acid	HMDB0001987	95433
24	HMDB0130836	NA	NA	NA
25	HMDB0029599	Glutaral	HMDB0029599	3485
26	HMDB0031648	2,4-Pentanedione	HMDB0031648	31261
27	HMDB0031602	4-Pentenoic acid	HMDB0031602	61138
28	HMDB0029608	Angelic acid	HMDB0029608	6656
29	HMDB0035143	Dihydro-3-methyl-2(3H)-furanone	HMDB0035143	98323
30	HMDB0032385	Methyl methacrylate	HMDB0032385	6658
31	HMDB0032351	Isopropenyl acetate	HMDB0032351	7916
32	HMDB0032459	2-Pentenoic acid	HMDB0032459	638122
33	HMDB0031178	Dihydro-2-methyl-3(2H)-furanone	HMDB0031178	18522
34	HMDB0033840	Dihydro-5-methyl-2(3H)-furanone	HMDB0033840	7921
35	HMDB0031598	2,3-Pentanedione	HMDB0031598	11747
36	HMDB0033978	Ethyl acrylate	HMDB0033978	8821
37	HMDB0002167	3-Methylbutyrolactone	HMDB0002167	98451
38	HMDB0001862	2-Ethylacrylic acid	HMDB0001862	19166
39	HMDB0000509	Senecioic acid	HMDB0000509	10931
40	HMDB0001470	Tiglic acid	HMDB0001470	125468
41	HMDB0129418	NA	NA	NA
42	HMDB0163713	NA	NA	NA
43	HMDB0163714	NA	NA	NA
44	HMDB0163712	NA	NA	NA
45	HMDB0130837	NA	NA	NA
46	HMDB0170858	NA	NA	NA
47	HMDB0180166	NA	NA	NA
48	HMDB0132474	NA	NA	NA
49	HMDB0149276	NA	NA	NA
50	HMDB0029726	2-Vinylthiophene	HMDB0029726	519642
51	HMDB0033746	Benzenethiol	HMDB0033746	7969
52	HMDB0029730	2-Ethyl-4-methylthiazole	HMDB0029730	27440
53	HMDB0040062	NA	NA	NA
54	HMDB0040096	NA	NA	NA
55	HMDB0033155	Trimethylthiazole	HMDB0033155	61653
56	HMDB0031337	Thiiranebutanenitrile	HMDB0031337	148822
57	HMDB0029731	4-Ethyl-2-methylthiazole	HMDB0029731	520568
58	HMDB0029732	5-Ethyl-4-methylthiazole	HMDB0029732	40380
59	HMDB0034300	5-Isothiocyanato-1-pentene	HMDB0034300	87436

60	HMDB0172134	NA	NA	NA
61	HMDB0172135	NA	NA	NA
62	HMDB0172136	NA	NA	NA
63	HMDB0165771	NA	NA	NA
64	HMDB0165773	NA	NA	NA
65	HMDB0165770	NA	NA	NA
66	HMDB0165772	NA	NA	NA
67	HMDB0240308	NA	NA	NA
68	HMDB0029737	1H-Indole-3-carboxaldehyde	HMDB0029737	10256
69	HMDB0181148	NA	NA	NA
70	HMDB0004186	3-Methyldioxyindole	HMDB0004186	151066
71	HMDB0001424	4-(3-Pyridyl)-3-butenoic acid	HMDB0001424	5478892
72	HMDB0062406	NA	NA	NA
73	HMDB0029300	p-Anisidine	HMDB0029300	7732
74	HMDB0167379	NA	NA	NA
75	HMDB0032578	4-Hydroxybenzylamine	HMDB0032578	97472
76	HMDB0040360	NA	NA	NA
77	HMDB0036058	NA	NA	NA
78	HMDB0167378	NA	NA	NA
79	HMDB0032492	2-Propionylpyrrole	HMDB0032492	61260
80	HMDB0240327	NA	NA	NA
81	HMDB0159478	NA	NA	NA
82	HMDB0159477	NA	NA	NA
83	HMDB0158747	NA	NA	NA
84	HMDB0158748	NA	NA	NA
85	HMDB0158745	NA	NA	NA
86	HMDB0158746	NA	NA	NA
87	HMDB0159480	NA	NA	NA
88	HMDB0159479	NA	NA	NA
89	HMDB0161124	NA	NA	NA
90	HMDB0161123	NA	NA	NA
91	HMDB0161125	NA	NA	NA
92	HMDB0155922	NA	NA	NA
93	HMDB0061930	NA	NA	NA
94	HMDB0033837	Dimethyl succinate	HMDB0033837	7820
95	HMDB0006900	(S)-2-Aceto-2-hydroxybutanoic acid	HMDB0006900	440875
96	HMDB0002173	Solerol	HMDB0002173	21252275
97	HMDB0002074	2,2-Dimethylsuccinic acid	HMDB0002074	11701
98	HMDB0000858	Monomethyl glutaric acid	HMDB0000858	73917
99	HMDB0000752	Methylglutaric acid	HMDB0000752	12284
100	HMDB0000422	2-Methylglutaric acid	HMDB0000422	12046
101	HMDB0000448	Adipic acid	HMDB0000448	196
102	HMDB0062489	NA	NA	NA
103	HMDB0010207	L-Rhamnulose	HMDB0010207	439330
104	HMDB0033821	2-O-Methyl-D-xylose	HMDB0033821	560157
105	HMDB0062477	NA	NA	NA
106	HMDB0062498	NA	NA	NA
107	HMDB0062499	NA	NA	NA
108	HMDB0060267	L-Fuculose	HMDB0060267	6857362
109	HMDB0005876	3-Deoxyfructose	HMDB0005876	3080627
110	HMDB0012327	2-Deoxygalactopyranose	HMDB0012327	439804
111	HMDB0002712	1,5-Anhydrosorbitol	HMDB0002712	64960
112	HMDB0059625	NA	NA	NA
113	HMDB0029196	D-Fucose	HMDB0029196	444200
114	HMDB0003081	Beta-D-Fucose	HMDB0003081	439650
115	HMDB0000174	L-Fucose	HMDB0000174	25310
116	HMDB0059624	NA	NA	NA
117	HMDB0000849	Rhamnose	HMDB0000849	25310
118	HMDB0124735	NA	NA	NA
119	HMDB0240304	NA	NA	NA
120	HMDB0003584	Taurocyamine	HMDB0003584	68340
121	HMDB0060537	NA	NA	NA
122	HMDB0060826	NA	NA	NA
123	HMDB0004185	5-Hydroxyindoleacetylglycine	HMDB0004185	440806
124	HMDB0003066	Chalcone	HMDB0003066	637760
125	HMDB0135598	NA	NA	NA
126	HMDB0060323	NA	NA	NA
127	HMDB0032357	N-Lactoyl ethanolamine phosphate	HMDB0032357	11550267
128	HMDB0155289	NA	NA	NA
129	HMDB0060757	NA	NA	NA
130	HMDB0000684	L-Kynurenine	HMDB0000684	161166
131	HMDB0012948	Formyl-5-hydroxykynurenamine	HMDB0012948	440743
132	HMDB0014350	Pyrimethamine	HMDB0014350	4993
133	HMDB0060321	NA	NA	NA
134	HMDB0040367	NA	NA	NA
135	HMDB0037521	NA	NA	NA
136	HMDB0014883	Modafinil	HMDB0014883	4236
137	HMDB0160041	NA	NA	NA
138	HMDB0163698	NA	NA	NA
139	HMDB0165052	NA	NA	NA
140	HMDB0165051	NA	NA	NA
141	HMDB0165056	NA	NA	NA
142	HMDB0165053	NA	NA	NA
143	HMDB0165054	NA	NA	NA
144	HMDB0165057	NA	NA	NA
145	HMDB0165060	NA	NA	NA
146	HMDB0165059	NA	NA	NA

147	HMDB0171223	NA	NA	NA
148	HMDB0163699	NA	NA	NA
149	HMDB0163695	NA	NA	NA
150	HMDB0160042	NA	NA	NA
151	HMDB0160039	NA	NA	NA
152	HMDB0160038	NA	NA	NA
153	HMDB0158762	NA	NA	NA
154	HMDB0158761	NA	NA	NA
155	HMDB0156882	NA	NA	NA
156	HMDB0156881	NA	NA	NA
157	HMDB0160699	NA	NA	NA
158	HMDB0160700	NA	NA	NA
159	HMDB0163691	NA	NA	NA
160	HMDB0163694	NA	NA	NA
161	HMDB0163687	NA	NA	NA
162	HMDB0163686	NA	NA	NA
163	HMDB0163690	NA	NA	NA
164	HMDB0161121	NA	NA	NA
165	HMDB0161119	NA	NA	NA
166	HMDB0161120	NA	NA	NA
167	HMDB0161118	NA	NA	NA
168	HMDB0167299	NA	NA	NA
169	HMDB0012267	N-Succinyl-L,L-2,6-diaminopimelate	HMDB0012267	25202447
170	HMDB0032992	Furaneol 4-glucoside	HMDB0032992	14259114
171	HMDB0179393	NA	NA	NA
172	HMDB0179378	NA	NA	NA
173	HMDB0179374	NA	NA	NA
174	HMDB0179371	NA	NA	NA
175	HMDB0179368	NA	NA	NA
176	HMDB0179370	NA	NA	NA
177	HMDB0179366	NA	NA	NA
178	HMDB0031304	Osmundalin	HMDB0031304	5249536
179	HMDB0179364	NA	NA	NA
180	HMDB0163718	NA	NA	NA
181	HMDB0169674	NA	NA	NA
182	HMDB0169701	NA	NA	NA
183	HMDB0163365	NA	NA	NA
184	HMDB0163366	NA	NA	NA
185	HMDB0163368	NA	NA	NA
186	HMDB0163372	NA	NA	NA
187	HMDB0165598	NA	NA	NA
188	HMDB0165599	NA	NA	NA
189	HMDB0165602	NA	NA	NA
190	HMDB0165606	NA	NA	NA
191	HMDB0183795	NA	NA	NA
192	HMDB0183794	NA	NA	NA
193	HMDB0183797	NA	NA	NA
194	HMDB0183799	NA	NA	NA
195	HMDB0183798	NA	NA	NA
196	HMDB0183796	NA	NA	NA
197	HMDB0183800	NA	NA	NA
198	HMDB0183802	NA	NA	NA
199	HMDB0183801	NA	NA	NA
200	HMDB0186752	NA	NA	NA
201	HMDB0186756	NA	NA	NA
202	HMDB0186754	NA	NA	NA
203	HMDB0183792	NA	NA	NA
204	HMDB0183793	NA	NA	NA
205	HMDB0137406	NA	NA	NA
206	HMDB0157378	NA	NA	NA
207	HMDB0183791	NA	NA	NA
208	HMDB0183790	NA	NA	NA
209	HMDB0186755	NA	NA	NA
210	HMDB0186753	NA	NA	NA
211	HMDB0186758	NA	NA	NA
212	HMDB0186759	NA	NA	NA
213	HMDB0186757	NA	NA	NA
214	HMDB0062411	NA	NA	NA
215	HMDB0062412	NA	NA	NA
216	HMDB0039484	Gibberellin A12 7-aldehyde	HMDB0039484	5253705
217	HMDB0179080	NA	NA	NA
218	HMDB0041057	NA	NA	NA
219	HMDB0034630	(4Z,9a)-9-(3-Methyl-2-butenoyloxy)-4,10(14)-oplopadien-3-one	HMDB0034630	131751595
220	HMDB0036725	NA	NA	NA
221	HMDB0036747	NA	NA	NA
222	HMDB0038885	NA	NA	NA
223	HMDB0036812	NA	NA	NA
224	HMDB0012452	all-trans-18-Hydroxyretinoic acid	HMDB0012452	6506224
225	HMDB0061095	NA	NA	NA
226	HMDB0012451	all-trans-5,6-Epoxyretinoic acid	HMDB0012451	5363137
227	HMDB0060093	NA	NA	NA
228	HMDB0006254	4-Hydroxyretinoic acid	HMDB0006254	6438629
229	HMDB0005832	6b-Hydroxymethandienone	HMDB0005832	13241205
230	HMDB0005079	15-Deoxy-d-12,14-PGJ2	HMDB0005079	5311211
231	HMDB0000344	2-Methoxyestradiol-3-methylether	HMDB0000344	11301534
232	HMDB0156417	NA	NA	NA
233	HMDB0031773	Phytocassane E	HMDB0031773	85169423

234	HMDB0039442	NA	NA	NA
235	HMDB0034691	Yucalexin B9	HMDB0034691	291684
236	HMDB0034631	(4Z,9a)-9-Angeloyloxy-4,10(14)-oplopadien-3-one	HMDB0034631	131751596
237	HMDB0037233	NA	NA	NA
238	HMDB0036147	NA	NA	NA
239	HMDB0036750	NA	NA	NA
240	HMDB0035710	Cafestol	HMDB0035710	2516
241	HMDB0036762	NA	NA	NA
242	HMDB0041056	NA	NA	NA
243	HMDB0014855	Norethindrone	HMDB0014855	6230
244	HMDB0012794	4-Oxoretinal	HMDB0012794	53481523
245	HMDB0060092	NA	NA	NA
246	HMDB0138135	NA	NA	NA
247	HMDB0040644	NA	NA	NA
248	HMDB0002697	19-Norandrosterone	HMDB0002697	128251
249	HMDB0005886	19-Noretiocholanolone	HMDB0005886	9548753
250	HMDB0004590	19-Nor-5-androstenediol	HMDB0004590	9900246
251	HMDB0032672	4,8,12,15-Octadecatetraenoic acid	HMDB0032672	131751278
252	HMDB0034382	(E,E)-11,13-Octadecadien-9-ynoic acid	HMDB0034382	131751554
253	HMDB0006547	Stearidonic acid	HMDB0006547	5312508
254	HMDB0032464	Phenethyl decanoate	HMDB0032464	112733
255	HMDB0184957	NA	NA	NA
256	HMDB0184936	NA	NA	NA
257	HMDB0186509	NA	NA	NA
258	HMDB0062677	NA	NA	NA
259	HMDB0179595	NA	NA	NA
260	HMDB0062408	NA	NA	NA
261	HMDB0062296	NA	NA	NA
262	HMDB0062293	NA	NA	NA
263	HMDB0062222	NA	NA	NA
264	HMDB0183807	NA	NA	NA
265	HMDB0183804	NA	NA	NA
266	HMDB0183803	NA	NA	NA
267	HMDB0179598	NA	NA	NA
268	HMDB0179596	NA	NA	NA
269	HMDB0179597	NA	NA	NA
270	HMDB0179593	NA	NA	NA
271	HMDB0179594	NA	NA	NA
272	HMDB0137428	NA	NA	NA
273	HMDB0183806	NA	NA	NA
274	HMDB0183808	NA	NA	NA
275	HMDB0183816	NA	NA	NA
276	HMDB0183815	NA	NA	NA
277	HMDB0183814	NA	NA	NA
278	HMDB0183812	NA	NA	NA
279	HMDB0183813	NA	NA	NA
280	HMDB0183811	NA	NA	NA
281	HMDB0183810	NA	NA	NA
282	HMDB0183809	NA	NA	NA
283	HMDB0137419	NA	NA	NA
284	HMDB0012611	18R-HEPE	HMDB0012611	53481496
285	HMDB0012534	11R-HEPE	HMDB0012534	5283188
286	HMDB0013633	12-KETE	HMDB0013633	5283162
287	HMDB0060053	NA	NA	NA
288	HMDB0061114	NA	NA	NA
289	HMDB0037523	NA	NA	NA
290	HMDB0036706	NA	NA	NA
291	HMDB0038521	NA	NA	NA
292	HMDB0036712	NA	NA	NA
293	HMDB0041058	NA	NA	NA
294	HMDB0010217	5-KETE	HMDB0010217	5283159
295	HMDB0010212	17,18-EpETE	HMDB0010212	5282277
296	HMDB0010209	15-HEPE	HMDB0010209	53480357
297	HMDB0010210	15-KETE	HMDB0010210	5280701
298	HMDB0010205	14,15-EpETE	HMDB0010205	16061088
299	HMDB0010202	12-HEPE	HMDB0010202	10041593
300	HMDB0006027	Oxymesterone	HMDB0006027	72061
301	HMDB0005081	5-HEPE	HMDB0005081	6439678
302	HMDB0001337	Leukotriene A4	HMDB0001337	5280383
303	HMDB0036752	NA	NA	NA
304	HMDB0036713	NA	NA	NA
305	HMDB0036698	NA	NA	NA
306	HMDB0039012	NA	NA	NA
307	HMDB0035311	Galanal A	HMDB0035311	14330184
308	HMDB0036724	NA	NA	NA
309	HMDB0036930	NA	NA	NA
310	HMDB0030101	Methyl [8]-Shogaol	HMDB0030101	91721121
311	HMDB0036839	NA	NA	NA
312	HMDB0036776	NA	NA	NA
313	HMDB0036806	NA	NA	NA
314	HMDB0035116	(ent-7alpha)-7-Hydroxy-8(14),15-pimaradien-19-oic acid	HMDB0035116	14864259
315	HMDB0039716	NA	NA	NA
316	HMDB0179600	NA	NA	NA
317	HMDB0002369	9-cis-Retinoic acid	HMDB0002369	449171
318	HMDB0036710	NA	NA	NA
319	HMDB0061925	NA	NA	NA
320	HMDB0012788	4-OH-Retinal	HMDB0012788	14015984

321	HMDB0036564	NA	NA	NA
322	HMDB0006219	13-cis-Retinoic acid	HMDB0006219	5282379
323	HMDB0012329	4-Oxoretinol	HMDB0012329	5289090
324	HMDB0038702	NA	NA	NA
325	HMDB0036711	NA	NA	NA
326	HMDB0036697	NA	NA	NA
327	HMDB0012874	9,13-cis-Retinoate	HMDB0012874	6419708
328	HMDB0041925	NA	NA	NA
329	HMDB0156407	NA	NA	NA
330	HMDB0156406	NA	NA	NA
331	HMDB0156405	NA	NA	NA
332	HMDB0156404	NA	NA	NA
333	HMDB0156409	NA	NA	NA
334	HMDB0156408	NA	NA	NA
335	HMDB0156411	NA	NA	NA
336	HMDB0156410	NA	NA	NA
337	HMDB0156413	NA	NA	NA
338	HMDB0156418	NA	NA	NA
339	HMDB0156401	NA	NA	NA
340	HMDB0156403	NA	NA	NA
341	HMDB0156402	NA	NA	NA
342	HMDB0001852	All-trans-retinoic acid	HMDB0001852	444795
343	HMDB0156412	NA	NA	NA
344	HMDB0183805	NA	NA	NA
345	HMDB0030963	Punicic acid	HMDB0030963	12309425
346	HMDB0175408	NA	NA	NA
347	HMDB0030964	Linolenelaidic acid	HMDB0030964	5282822
348	HMDB0003073	Gamma-Linolenic acid	HMDB0003073	5280933
349	HMDB0030962	Calendic acid	HMDB0030962	5282818
350	HMDB0001388	Alpha-Linolenic acid	HMDB0001388	5280934
351	HMDB0160594	NA	NA	NA
352	HMDB0173892	NA	NA	NA
353	HMDB0175402	NA	NA	NA
354	HMDB0175410	NA	NA	NA
355	HMDB0175403	NA	NA	NA
356	HMDB0175411	NA	NA	NA
357	HMDB0175404	NA	NA	NA
358	HMDB0175405	NA	NA	NA
359	HMDB0175407	NA	NA	NA
360	HMDB0175406	NA	NA	NA
361	HMDB0175412	NA	NA	NA
362	HMDB0175413	NA	NA	NA
363	HMDB0175415	NA	NA	NA
364	HMDB0175414	NA	NA	NA
365	HMDB0175409	NA	NA	NA
366	HMDB0060994	NA	NA	NA
367	HMDB0013854	N4-Acetylsulfamethoxazole	HMDB0013854	80641
368	HMDB0167783	NA	NA	NA
369	HMDB0167780	NA	NA	NA
370	HMDB0034058	Quinacridone	HMDB0034058	13976
371	HMDB0178297	NA	NA	NA
372	HMDB0178295	NA	NA	NA
373	HMDB0128029	NA	NA	NA
374	HMDB0171101	NA	NA	NA
375	HMDB0151544	NA	NA	NA
376	HMDB0151545	NA	NA	NA
377	HMDB0131260	NA	NA	NA
378	HMDB0240531	NA	NA	NA
379	HMDB0240529	NA	NA	NA
380	HMDB0131259	NA	NA	NA
381	HMDB0131258	NA	NA	NA
382	HMDB0128028	NA	NA	NA
383	HMDB0040621	NA	NA	NA
384	HMDB0180003	NA	NA	NA
385	HMDB0126429	NA	NA	NA
386	HMDB0124977	NA	NA	NA
387	HMDB0124976	NA	NA	NA
388	HMDB0126430	NA	NA	NA
389	HMDB0183406	NA	NA	NA
390	HMDB0167831	NA	NA	NA
391	HMDB0167833	NA	NA	NA
392	HMDB0140804	NA	NA	NA
393	HMDB0128031	NA	NA	NA
394	HMDB0062575	NA	NA	NA
395	HMDB0001235	5-Aminoimidazole ribonucleotide	HMDB0001235	161500
396	HMDB0134938	NA	NA	NA
397	HMDB0013189	3-Indole carboxylic acid glucuronide	HMDB0013189	53481645
398	HMDB0011658	2,8-Dihydroxyquinoline-beta-D-glucuronide	HMDB0011658	53481014
399	HMDB0131931	NA	NA	NA
400	HMDB0131929	NA	NA	NA
401	HMDB0137816	NA	NA	NA
402	HMDB0137819	NA	NA	NA
403	HMDB0137821	NA	NA	NA
404	HMDB0140538	NA	NA	NA
405	HMDB0126563	NA	NA	NA
406	HMDB0125037	NA	NA	NA
407	HMDB0126566	NA	NA	NA

408	HMDB0131932	NA	NA	NA
409	HMDB0126564	NA	NA	NA
410	HMDB0126567	NA	NA	NA
411	HMDB0126570	NA	NA	NA
412	HMDB0125219	NA	NA	NA
413	HMDB0125216	NA	NA	NA
414	HMDB0125207	NA	NA	NA
415	HMDB0125079	NA	NA	NA
416	HMDB0125067	NA	NA	NA
417	HMDB0125052	NA	NA	NA
418	HMDB0126569	NA	NA	NA
419	HMDB0128213	NA	NA	NA
420	HMDB0131759	NA	NA	NA
421	HMDB0131760	NA	NA	NA
422	HMDB0131925	NA	NA	NA
423	HMDB0131928	NA	NA	NA
424	HMDB0140542	NA	NA	NA
425	HMDB0140556	NA	NA	NA
426	HMDB0167579	NA	NA	NA
427	HMDB0152484	NA	NA	NA
428	HMDB0167582	NA	NA	NA
429	HMDB0167580	NA	NA	NA
430	HMDB0167581	NA	NA	NA
431	HMDB0030835	trans-3,3',4',5,5',7-Hexahydroxyflavanone	HMDB0030835	5153580
432	HMDB0152029	NA	NA	NA
433	HMDB0152031	NA	NA	NA
434	HMDB0152038	NA	NA	NA
435	HMDB0152035	NA	NA	NA
436	HMDB0152036	NA	NA	NA
437	HMDB0152037	NA	NA	NA
438	HMDB0152402	NA	NA	NA
439	HMDB0152479	NA	NA	NA
440	HMDB0039815	NA	NA	NA
441	HMDB0127839	NA	NA	NA
442	HMDB0128709	NA	NA	NA
443	HMDB0125826	NA	NA	NA
444	HMDB0125820	NA	NA	NA
445	HMDB0127778	NA	NA	NA
446	HMDB0127840	NA	NA	NA
447	HMDB0127779	NA	NA	NA
448	HMDB0125825	NA	NA	NA
449	HMDB0172923	NA	NA	NA
450	HMDB0172926	NA	NA	NA
451	HMDB0141622	NA	NA	NA
452	HMDB0034767	(1R*,3R*,3'S*)-1,2,3,4-Tetrahydro-1-(2-thio-3-pyrrolidinyl)-beta-carboline-3-carboxylic acid	HMDB0034767	3004297
453	HMDB0142252	NA	NA	NA
454	HMDB0172514	NA	NA	NA
455	HMDB0172522	NA	NA	NA
456	HMDB0158878	NA	NA	NA
457	HMDB0166871	NA	NA	NA
458	HMDB0166874	NA	NA	NA
459	HMDB0166876	NA	NA	NA
460	HMDB0166877	NA	NA	NA
461	HMDB0158883	NA	NA	NA
462	HMDB0158879	NA	NA	NA
463	HMDB0166873	NA	NA	NA
464	HMDB0158880	NA	NA	NA
465	HMDB0158886	NA	NA	NA
466	HMDB0158885	NA	NA	NA
467	HMDB0160786	NA	NA	NA
468	HMDB0160787	NA	NA	NA
469	HMDB0166859	NA	NA	NA
470	HMDB0166870	NA	NA	NA
471	HMDB0158882	NA	NA	NA
472	HMDB0158881	NA	NA	NA
473	HMDB0158887	NA	NA	NA
474	HMDB0157435	NA	NA	NA
475	HMDB0157434	NA	NA	NA
476	HMDB0156934	NA	NA	NA
477	HMDB0156933	NA	NA	NA
478	HMDB0157433	NA	NA	NA
479	HMDB0156932	NA	NA	NA
480	HMDB0152126	NA	NA	NA
481	HMDB0152125	NA	NA	NA
482	HMDB0160788	NA	NA	NA
483	HMDB0039724	NA	NA	NA
484	HMDB0039726	NA	NA	NA
485	HMDB0029938	Acaciabiuronic acid	HMDB0029938	14160009
486	HMDB0039722	NA	NA	NA
487	HMDB0155824	NA	NA	NA
488	HMDB0156218	NA	NA	NA
489	HMDB0157438	NA	NA	NA
490	HMDB0157437	NA	NA	NA
491	HMDB0157439	NA	NA	NA
492	HMDB0157441	NA	NA	NA
493	HMDB0157440	NA	NA	NA
494	HMDB0166391	NA	NA	NA

495	HMDB0166390	NA	NA	NA
496	HMDB0166392	NA	NA	NA
497	HMDB0156220	NA	NA	NA
498	HMDB0156219	NA	NA	NA
499	HMDB0155322	NA	NA	NA
500	HMDB0125610	NA	NA	NA
501	HMDB0125611	NA	NA	NA
502	HMDB0125612	NA	NA	NA
503	HMDB0125609	NA	NA	NA
504	HMDB0156221	NA	NA	NA
505	HMDB0155320	NA	NA	NA
506	HMDB0155318	NA	NA	NA
507	HMDB0155319	NA	NA	NA
508	HMDB0155321	NA	NA	NA
509	HMDB0166393	NA	NA	NA
510	HMDB0005011	Clopidogrel	HMDB0005011	60606
511	HMDB0175192	NA	NA	NA
512	HMDB0128893	NA	NA	NA
513	HMDB0062467	NA	NA	NA
514	HMDB0128890	NA	NA	NA
515	HMDB0128891	NA	NA	NA
516	HMDB0149084	NA	NA	NA
517	HMDB0164007	NA	NA	NA
518	HMDB0149086	NA	NA	NA
519	HMDB0033659	Dihydrohydroxy-O-methylsterigmatocystin	HMDB0033659	10760703
520	HMDB0030589	O-Methylsterigmatocystin	HMDB0030589	10871462
521	HMDB0148696	NA	NA	NA
522	HMDB0148232	NA	NA	NA
523	HMDB0030619	Ovalitenone	HMDB0030619	627910
524	HMDB0163589	NA	NA	NA
525	HMDB0014743	Sulindac	HMDB0014743	1548887
526	HMDB0147815	NA	NA	NA
527	HMDB0147744	NA	NA	NA
528	HMDB0147742	NA	NA	NA
529	HMDB0147651	NA	NA	NA
530	HMDB0150253	NA	NA	NA
531	HMDB0147649	NA	NA	NA
532	HMDB0150255	NA	NA	NA
533	HMDB0147648	NA	NA	NA
534	HMDB0033298	3-(3,4-Dihydroxybenzyl)-7-hydroxy-5-methoxy-4-chromanone	HMDB0033298	21676257
535	HMDB0148509	NA	NA	NA
536	HMDB0148510	NA	NA	NA
537	HMDB0148508	NA	NA	NA
538	HMDB0148507	NA	NA	NA
539	HMDB0148512	NA	NA	NA
540	HMDB0147889	NA	NA	NA
541	HMDB0148511	NA	NA	NA
542	HMDB0147535	NA	NA	NA
543	HMDB0147529	NA	NA	NA
544	HMDB0138112	NA	NA	NA
545	HMDB0137444	NA	NA	NA
546	HMDB0137443	NA	NA	NA
547	HMDB0137442	NA	NA	NA
548	HMDB0135788	NA	NA	NA
549	HMDB0133961	NA	NA	NA
550	HMDB0133962	NA	NA	NA
551	HMDB0133959	NA	NA	NA
552	HMDB0133957	NA	NA	NA
553	HMDB0133958	NA	NA	NA
554	HMDB0133295	NA	NA	NA
555	HMDB0138110	NA	NA	NA
556	HMDB0138111	NA	NA	NA
557	HMDB0146399	NA	NA	NA
558	HMDB0146753	NA	NA	NA
559	HMDB0146751	NA	NA	NA
560	HMDB0146573	NA	NA	NA
561	HMDB0146572	NA	NA	NA
562	HMDB0146426	NA	NA	NA
563	HMDB0146422	NA	NA	NA
564	HMDB0146412	NA	NA	NA
565	HMDB0140872	NA	NA	NA
566	HMDB0138632	NA	NA	NA
567	HMDB0133294	NA	NA	NA
568	HMDB0151836	NA	NA	NA
569	HMDB0037749	NA	NA	NA
570	HMDB0033797	Olivin	HMDB0033797	131751488
571	HMDB0033924	Cajanol	HMDB0033924	442670
572	HMDB0030111	Homoferreirin	HMDB0030111	442788
573	HMDB0041790	NA	NA	NA
574	HMDB0037481	NA	NA	NA
575	HMDB0033692	Melilotocarpan D	HMDB0033692	5319351
576	HMDB0037477	NA	NA	NA
577	HMDB0152003	NA	NA	NA
578	HMDB0152002	NA	NA	NA
579	HMDB0033683	Melilotocarpan E	HMDB0033683	44257462
580	HMDB0151832	NA	NA	NA
581	HMDB0151833	NA	NA	NA

582	HMDB0151834	NA	NA	NA
583	HMDB0151632	NA	NA	NA
584	HMDB0151630	NA	NA	NA
585	HMDB0151631	NA	NA	NA
586	HMDB0152601	NA	NA	NA
587	HMDB0153237	NA	NA	NA
588	HMDB0037251	NA	NA	NA
589	HMDB0154307	NA	NA	NA
590	HMDB0153788	NA	NA	NA
591	HMDB0041234	NA	NA	NA
592	HMDB0153512	NA	NA	NA
593	HMDB0153415	NA	NA	NA
594	HMDB0151417	NA	NA	NA
595	HMDB0146221	NA	NA	NA
596	HMDB0146754	NA	NA	NA
597	HMDB0147650	NA	NA	NA
598	HMDB0138109	NA	NA	NA
599	HMDB0147743	NA	NA	NA
600	HMDB0132897	NA	NA	NA
601	HMDB0128591	NA	NA	NA
602	HMDB0128590	NA	NA	NA
603	HMDB0128197	NA	NA	NA
604	HMDB0126060	NA	NA	NA
605	HMDB0124872	NA	NA	NA
606	HMDB0130367	NA	NA	NA
607	HMDB0132894	NA	NA	NA
608	HMDB0132895	NA	NA	NA
609	HMDB0132890	NA	NA	NA
610	HMDB0132891	NA	NA	NA
611	HMDB0132883	NA	NA	NA
612	HMDB0132866	NA	NA	NA
613	HMDB0131742	NA	NA	NA
614	HMDB0131738	NA	NA	NA
615	HMDB0130897	NA	NA	NA
616	HMDB0130669	NA	NA	NA
617	HMDB0130668	NA	NA	NA
618	HMDB0130395	NA	NA	NA
619	HMDB0130391	NA	NA	NA
620	HMDB0146752	NA	NA	NA
621	HMDB0162087	NA	NA	NA
622	HMDB0164047	NA	NA	NA
623	HMDB0162089	NA	NA	NA
624	HMDB0185204	NA	NA	NA
625	HMDB0185205	NA	NA	NA
626	HMDB0155721	NA	NA	NA
627	HMDB0240402	NA	NA	NA
628	HMDB0240215	NA	NA	NA
629	HMDB0037263	NA	NA	NA
630	HMDB0029833	Cyclodopa glucoside	HMDB0029833	74413859
631	HMDB0164046	NA	NA	NA
632	HMDB0010362	6-Hydroxy-5-methoxyindole glucuronide	HMDB0010362	196513
633	HMDB0010363	5-Hydroxy-6-methoxyindole glucuronide	HMDB0010363	196512
634	HMDB0183679	NA	NA	NA
635	HMDB0152039	NA	NA	NA
636	HMDB0137817	NA	NA	NA
637	HMDB0137820	NA	NA	NA
638	HMDB0137822	NA	NA	NA
639	HMDB0140545	NA	NA	NA
640	HMDB0150750	NA	NA	NA
641	HMDB0150752	NA	NA	NA
642	HMDB0150751	NA	NA	NA
643	HMDB0136090	NA	NA	NA
644	HMDB0181157	NA	NA	NA
645	HMDB0181159	NA	NA	NA
646	HMDB0040829	NA	NA	NA
647	HMDB0005034	Topiramate	HMDB0005034	5284627
648	HMDB0038515	NA	NA	NA
649	HMDB0141625	NA	NA	NA
650	HMDB0142281	NA	NA	NA
651	HMDB0128445	NA	NA	NA
652	HMDB0128454	NA	NA	NA
653	HMDB0128439	NA	NA	NA
654	HMDB0128457	NA	NA	NA
655	HMDB0128441	NA	NA	NA
656	HMDB0128456	NA	NA	NA
657	HMDB0128453	NA	NA	NA
658	HMDB0128449	NA	NA	NA
659	HMDB0128450	NA	NA	NA
660	HMDB0128452	NA	NA	NA
661	HMDB0128448	NA	NA	NA
662	HMDB0128446	NA	NA	NA
663	HMDB0128442	NA	NA	NA
664	HMDB0128444	NA	NA	NA
665	HMDB0128438	NA	NA	NA
666	HMDB0128437	NA	NA	NA
667	HMDB0128458	NA	NA	NA
668	HMDB0177036	NA	NA	NA

669	HMDB0059612	NA	NA	NA
670	HMDB0177043	NA	NA	NA
671	HMDB0147189	NA	NA	NA
672	HMDB0147188	NA	NA	NA
673	HMDB0148992	NA	NA	NA
674	HMDB0177490	NA	NA	NA
675	HMDB0147184	NA	NA	NA
676	HMDB0140356	NA	NA	NA
677	HMDB0138063	NA	NA	NA
678	HMDB0128976	NA	NA	NA
679	HMDB0128981	NA	NA	NA
680	HMDB0128983	NA	NA	NA
681	HMDB0131634	NA	NA	NA
682	HMDB0131637	NA	NA	NA
683	HMDB0138062	NA	NA	NA
684	HMDB0147185	NA	NA	NA
685	HMDB0158633	NA	NA	NA
686	HMDB0158234	NA	NA	NA
687	HMDB0158235	NA	NA	NA
688	HMDB0158642	NA	NA	NA
689	HMDB0158238	NA	NA	NA
690	HMDB0158621	NA	NA	NA
691	HMDB0158636	NA	NA	NA
692	HMDB0158629	NA	NA	NA
693	HMDB0158625	NA	NA	NA
694	HMDB0158226	NA	NA	NA
695	HMDB0158231	NA	NA	NA
696	HMDB0158228	NA	NA	NA
697	HMDB0157580	NA	NA	NA
698	HMDB0158210	NA	NA	NA
699	HMDB0158213	NA	NA	NA
700	HMDB0158219	NA	NA	NA
701	HMDB0158216	NA	NA	NA
702	HMDB0158221	NA	NA	NA
703	HMDB0158223	NA	NA	NA
704	HMDB0158225	NA	NA	NA
705	HMDB0158639	NA	NA	NA
706	HMDB0158646	NA	NA	NA
707	HMDB0158643	NA	NA	NA
708	HMDB0169111	NA	NA	NA
709	HMDB0171254	NA	NA	NA
710	HMDB0171269	NA	NA	NA
711	HMDB0171253	NA	NA	NA
712	HMDB0171257	NA	NA	NA
713	HMDB0171258	NA	NA	NA
714	HMDB0171264	NA	NA	NA
715	HMDB0171268	NA	NA	NA
716	HMDB0171273	NA	NA	NA
717	HMDB0171272	NA	NA	NA
718	HMDB0169110	NA	NA	NA
719	HMDB0169105	NA	NA	NA
720	HMDB0169108	NA	NA	NA
721	HMDB0158650	NA	NA	NA
722	HMDB0158654	NA	NA	NA
723	HMDB0158656	NA	NA	NA
724	HMDB0158660	NA	NA	NA
725	HMDB0169095	NA	NA	NA
726	HMDB0169099	NA	NA	NA
727	HMDB0169097	NA	NA	NA
728	HMDB0169101	NA	NA	NA
729	HMDB0169103	NA	NA	NA
730	HMDB0169107	NA	NA	NA
731	HMDB0033412	Gibberellin A20 13-glucoside	HMDB0033412	14258563
732	HMDB0037084	NA	NA	NA
733	HMDB0038610	NA	NA	NA
734	HMDB0171261	NA	NA	NA
735	HMDB0015181	Fluocinonide	HMDB0015181	9642
736	HMDB0140090	NA	NA	NA
737	HMDB0033177	Sapiol	HMDB0033177	185639
738	HMDB0038738	NA	NA	NA
739	HMDB0157582	NA	NA	NA
740	HMDB0179952	NA	NA	NA
741	HMDB0179953	NA	NA	NA
742	HMDB0174900	NA	NA	NA
743	HMDB0167227	NA	NA	NA
744	HMDB0167180	NA	NA	NA
745	HMDB0035700	Lucidenic acid J	HMDB0035700	131751851
746	HMDB0167212	NA	NA	NA
747	HMDB0167174	NA	NA	NA
748	HMDB0167200	NA	NA	NA
749	HMDB0167223	NA	NA	NA
750	HMDB0167220	NA	NA	NA
751	HMDB0167217	NA	NA	NA
752	HMDB0167216	NA	NA	NA
753	HMDB0167195	NA	NA	NA
754	HMDB0167213	NA	NA	NA
755	HMDB0167206	NA	NA	NA

756	HMDB0167209	NA	NA	NA
757	HMDB0167203	NA	NA	NA
758	HMDB0167186	NA	NA	NA
759	HMDB0167183	NA	NA	NA
760	HMDB0167177	NA	NA	NA
761	HMDB0167189	NA	NA	NA
762	HMDB0167192	NA	NA	NA
763	HMDB0167196	NA	NA	NA
764	HMDB0167197	NA	NA	NA
765	HMDB0173045	NA	NA	NA
766	HMDB0148903	NA	NA	NA
767	HMDB0148904	NA	NA	NA
768	HMDB0167769	NA	NA	NA
769	HMDB0167768	NA	NA	NA
770	HMDB0173103	NA	NA	NA
771	HMDB0169616	NA	NA	NA
772	HMDB0165411	NA	NA	NA
773	HMDB0173104	NA	NA	NA
774	HMDB0164420	NA	NA	NA
775	HMDB0164423	NA	NA	NA
776	HMDB0164424	NA	NA	NA
777	HMDB0164421	NA	NA	NA
778	HMDB0164422	NA	NA	NA
779	HMDB0164419	NA	NA	NA
780	HMDB0164418	NA	NA	NA

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.

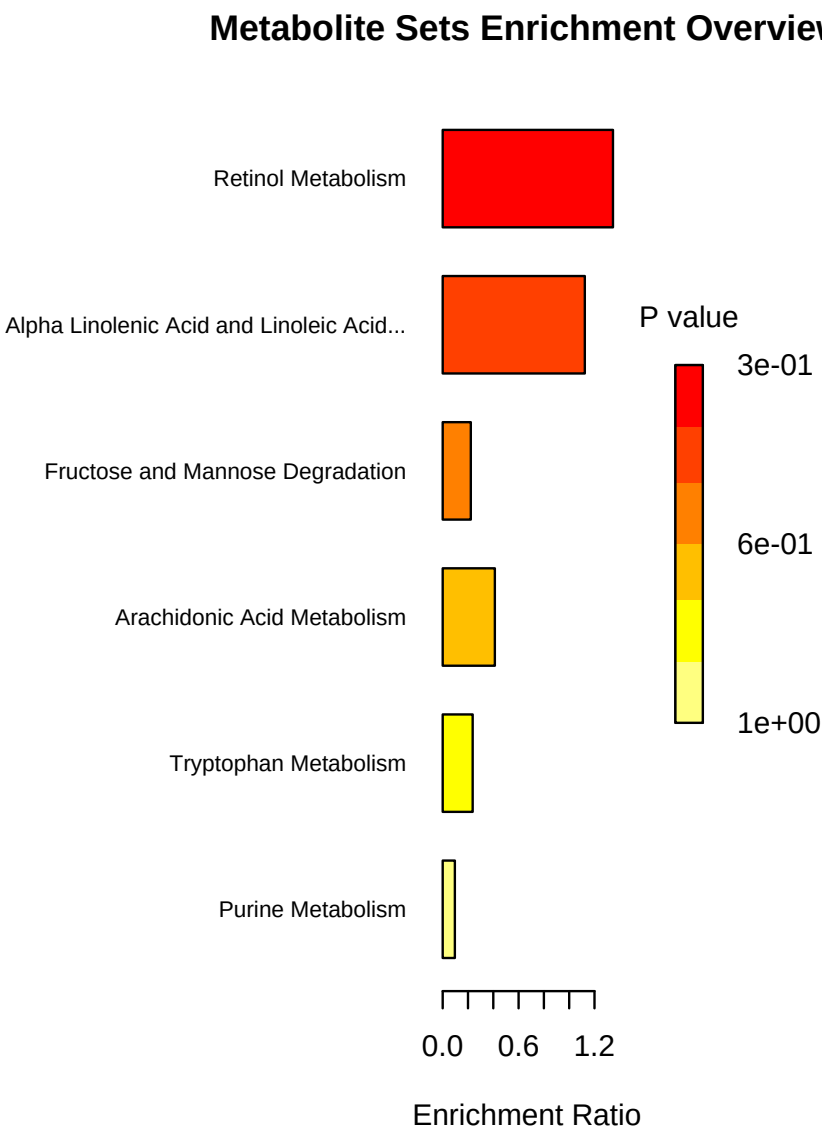

Figure 1: Summary Plot for Over Representation Analysis (ORA)

Table 2: Result from Over Representation Analysis

	total	expected	hits	Raw p	Holm p	FDR
Retinol Metabolism	37	5.20	7	2.55E-01	1.00E+00	1.00E+00
Alpha Linolenic Acid and Linoleic Acid Metabolism	19	2.67	3	5.13E-01	1.00E+00	1.00E+00
Fructose and Mannose Degradation	32	4.50	1	9.93E-01	1.00E+00	1.00E+00
Arachidonic Acid Metabolism	69	9.70	4	9.93E-01	1.00E+00	1.00E+00
Tryptophan Metabolism	60	8.44	2	9.99E-01	1.00E+00	1.00E+00
Purine Metabolism	74	10.40	1	1.00E+00	1.00E+00	1.00E+00

7 Appendix: R Command History

```
 [1] "mSet<-InitDataObjects(\"conc\", \"pathora\", FALSE)"
 [2] "cmpd.vec<-c(\"HMDB0059844\",\"HMDB0000642\",\"HMDB0041603\",\"HMDB0040735\",\"HMDB0002011\",\"
 [3] "mSet<-Setup.MapData(mSet, cmpd.vec);"
 [4] "mSet<-CrossReferencing(mSet, \"hmdb\");"
 [5] "mSet<-CreateMappingResultTable(mSet)"
 [6] "mSet<-SetKEGG.PathLib(mSet, \"hsa\", \"current\")"
 [7] "mSet<-SetMetabolomeFilter(mSet, F);"
 [8] "mSet<-CalculateOraScore(mSet, \"rbc\", \"hyperg\")"
 [9] "mSet<-PlotPathSummary(mSet, F, \"path_view_0_\", \"png\", 72, width=NA)"
[10] "mSet<-SaveTransformedData(mSet)"
[11] "UpdateDataObjects(\"conc\", \"msetora\", FALSE)"
[12] "mSet<-SetMetabolomeFilter(mSet, F);"
[13] "mSet<-SetCurrentMsetLib(mSet, \"smpdb_pathway\", 2);"
[14] "mSet<-CalculateHyperScore(mSet)"
[15] "mSet<-PlotORA(mSet, \"ora_0_\", \"net\", \"png\", 72, width=NA)"
[16] "mSet<-PlotEnrichDotPlot(mSet, \"ora\", \"ora_dot_0_\", \"png\", 72, width=NA)"
[17] "mSet<-CalculateHyperScore(mSet)"
[18] "mSet<-PlotORA(mSet, \"ora_1_\", \"net\", \"png\", 72, width=NA)"
[19] "mSet<-PlotEnrichDotPlot(mSet, \"ora\", \"ora_dot_1_\", \"png\", 72, width=NA)"
[20] "mSet<-SaveTransformedData(mSet)"
[21] "mSet<-PreparePDFReport(mSet, \"guest12058908726568660277\")\n"
```

The report was generated on Mon May 24 15:01:29 2021 with R version 4.0.2 (2020-06-22).