Knowledge that will change your world

Peaks to pathways

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THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

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Peaks to pathways in Metaboanalyst

- **This feature was added in 2019 and is part of version 5.0**
- **Necessary to recover the normalized data from the stats analysis**
  - Go to the download zip file and open the data.normalized file
- **In the current version, necessary to transpose the normalized data**
  - Then undo the concatenated format to get m/z and RT values
  - Calculate p-values and t-scores for each of the feature
  - The format and order of the headers is critical
    - m.z, r.t, p.value and t.score

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Steps for today's class (1)

- **Start with the Excel file sent to you (has named +ve metabolites in a study with two groups plus three pooled samples)**
  - Determine the mean, standard deviation and CoV for the values in the three pooled samples
  - Determine the ratio of the mean values to those in the three extraction blanks
  - Where the Ratio is < 10, insert a line space – those that are below this line can be discarded
- **Note that the group numbers are on the second line (keep these)**
- **Delete the lines where a drug has been identified**
- **Sort the data by the *m/z* value (from low to high) – do not include the first two lines**

3

After calculating CoV and ordering by ratio

Alignment ID	Average Rt(r	Average Mz	Metabolite name	Adduct type	Mean	Sd	CoV	Ratio
Group								
19886	4.153	321.13547	Mycophenolic acid_130117	[M+H]+	3,161,416	46,018	1.46	296277.43
50153	4.372	595.34943	Stercobilin	[M+H]+	483,875	25,238	5.22	198003.56
18808	1.998	312.1297	N2,N2-Dimethylguanosine	[M+H]+	882,320	39,569	4.48	189068.64
13477	2.801	265.11954	Phenylacetylglutamine	[M+H]+	7,291,748	183,071	2.51	186607.55
7417	3.313	196.06097	o-Hydroxyhippuric acid; CE0; ONJSZLXSECQRC	[M+H]+	539,202	6,792	1.26	111327.93
8099	0.955	204.12329	Acetylcarnitine	[M]+	8,005,717	137,761	1.72	106270.58
11907	3.656	247.10896	N-ACETYL-D-TRYPTOPHAN	[M+H]+	550,250	27,398	4.98	82996.89
6893	2.531	190.05112	Kynurenic acid; CE10; HCZHHEIFKROPDY-UHF	[M+H]+	555,188	41,376	7.45	73823.28
13819	1.03	269.12537	N-Acetylcarnosine; CE10; BKAYIFDRRZZKNF-\	[M+H]+	417,343	7,755	1.86	56910.36
5181	1.258	169.03677	Uric acid; CE10; LEHOTFFKMJEONL-UHFFFAC	[M+H]+	1,970,036	131,340	6.67	52768.81
15248	1.334	282.12167	6-Methyladenosine	[M+H]+	742,012	22,440	3.02	51768.26
7421	2.281	196.06274	o-Hydroxyhippuric acid; CE0; ONJSZLXSECQRC	[M+H]+	429,817	8,164	1.90	51282.86
7416	2.118	196.06062	4-Hydroxyhippuric acid	[M+H]+	340,199	9,681	2.85	51029.90
10247	0.774	229.11786	Proline-hydroxyproline	[M+H]+	1,259,254	16,491	1.31	38946.01
19888	5.659	321.13635	Mycophenolic acid; LC-ESI-ITFT; MS2; CE	[M+H]+	426,025	22,974	5.39	32731.61
3315	0.675	144.10152	(2R)-6-methylpiperidine-2-carboxylic acid	[M+H]+	4,144,428	66,576	1.61	28714.28
49973	4.336	593.33649	Urobilinogen	[M+H]+	62,569	6,349	10.15	26485.40
8021	0.641	203.15019	N,N-Dimethylarginine	[M+H]+	1,101,976	46,327	4.20	25430.21
7079	2.683	192.0668	5-Hydroxyindole-3-acetic acid; LC-ESI-QTOF; I	[M+H]+	128,203	4,430	3.46	18491.98
6881	0.488	189.16058	NEPSILON,NEPSILON,NEPSILON-TRIMETHYLL	[M+H]+	141,036	5,706	4.05	18396.00
26565	2.939	377.14835	(-)-Riboflavin; LC-ESI-QTOF; MS2; CE	[M+H]+	87,205	4,089	4.69	18070.03
53673	3.906	643.33362	Rubusoside	[M+H]+	23,788	1,280	5.38	17633.20
17063	1.73	298.11536	1-Methylguanosine	[M+H]+	180,181	4,822	2.68	16380.12
2797	1.318	137.04674	Hypoxanthine	[M+H]+	659,647	37,702	5.72	16354.88

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After ordering by *m/z*

Alignment ID	Average Rt(r	Average Mz	Metabolite name	Adduct type	Mean	Sd	CoV	Ratio
Group								
190	0.531	61.03899	Urea	[M+H]+	1,006,985	84,516	8.39	16.00
429	0.545	76.0759	Trimethylamine N-oxide; LC-ESI-QTOF; MS2;	[M+H]+	268,206	5,682	2.12	1319.04
611	1.378	86.09709	Piperidine	[M+H]+	97,335	2,442	2.51	580.53
612	0.867	86.0972	Piperidine	[M+H]+	156,957	4,895	3.12	2924.67
918	1.95	100.0751	2-Piperidone; CE10; XUWHAWMETYGRKB-UH	[M+H]+	38,457	2,417	6.28	393.76
921	1.853	100.07584	2-Piperidone; CE10; XUWHAWMETYGRKB-UH	[M+H]+	111,641	4,819	4.32	289.48
1039	0.525	104.10677	Choline	[M]+	63,484	1,911	3.01	474.94
1229	2.192	110.05899	4-Aminophenol; CE10; PLIKAWJENQZMHA-UH	[M+H]+	43,971	1,283	2.92	825.88
1230	0.767	110.05909	4-Aminophenol; CE10; PLIKAWJENQZMHA-UH	[M+H]+	30,762	1,768	5.75	769.04
1267	2.16	111.04457	Butyric acid	[M+Na]+	49,684	2,459	4.95	112.33
1311	1.141	112.05105	Cytosine	[M+H]+	28,379	2,714	9.56	560.12
1403	2.631	114.06589	Creatinine	[M+H]+	26,675	2,242	8.41	142.38
1451	0.95	115.05032	Dihydrouracil	[M+H]+	83,154	2,323	2.79	758.24
1620	0.551	118.08523	Betaine; CE30; CE10; KWIUHFFTVRNATP-UHF	[M+H]+	436,043	9,373	2.15	215.68
1627	0.735	118.08682	Valine; LC-ESI-ITFT; MS2; CE 50.0 eV; [M+H]+	[M+H]+	106,067	1,353	1.28	37.79
1697	0.658	120.06567	Threonine; LC-ESI-QTOF; MS2; CE	[M+H]+	61,526	277	0.45	1079.40
1768	3.504	121.06445	Tyramine; PlaSMA ID-59	[M+H-NH3]+	131,986	3,048	2.31	818.90
1905	0.845	123.05415	2-Acetylpyrazine; CE10; DBZAKQWXICEWNW	[M+H]+	77,776	1,418	1.82	632.33
1907	1.178	123.05422	NICOTINAMIDE	[M+H]+	17,431	1,798	10.31	156.10
2093	0.675	126.065	5-Methylcytosine	[M+H]+	28,617	460	1.61	520.32
2352	1.203	130.04918	L-5-Oxoproline; LC-ESI-QTOF; MS2; CE	[M+H]+	668,185	30,353	4.54	460.08
2512	0.694	131.11737	N-Acetylputrescine	[M+H]+	77,692	3,704	4.77	2988.15
2549	0.617	132.07646	CREATINE	[M+H]+	213,379	7,765	3.64	1600.35
2564	1.378	132.10159	Isoleucine; LC-ESI-ITFT; MS2; CE 30.0 eV; [M+	[M+H]+	332,327	690	0.21	1789.91

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Next steps

- **Move columns B, C and D to a new file**
- **Add the pooled samples and the individual samples to this new file**
  - You do not need to copy the extraction blanks, mean SD, CoV and ratio columns
  - Save the file (class database)
- **Make a copy of this file and delete those features that are drugs**
  - Save this file, too (class database-2)
- **In the new file, RT is in column A, and *m/z* is in column B**
  - Add two new columns after column B
  - In cell C3, enter a concatenate command as follows
  - =concatenate(round(B3,3),"/",round(A2,2))
    - Copy to the bottom of the column C
    - Copy column C and paste special (values) in column D
    - Delete columns A, B and C

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Class database-2

Average Rt(r	Average Mz	Metabolite n	Pool-1	Pool-2	Pool-3	166	167	192	242	312	404	407
			0	0	0	1	1	2	2	2	2	1
0.531	61.03899	Urea	1024668	915027	1081261	958818	1062631	851022	794976	1265634	1739819	1237578
0.545	76.0759	Trimethylam	261706	272229	270682	110938	304825	103169	36083	138122	81114	469427
1.378	86.09709	Piperidine	94821	99698	97486	76393	35180	53528	54798	17429	142300	167006
0.867	86.0972	Piperidine	152728	155824	162320	67316	26554	17503	154815	12171	8126	674362
1.95	100.0751	2-Piperidone	40936	36108	38328	38579	28989	5189	11233	28658	15094	202169
1.853	100.07584	2-Piperidone	109474	108286	117163	3663	27353	5224	1126	2196	1178	190999
0.525	104.10677	Choline	62562	62208	65681	264843	32896	110624	27860	185761	10605	74866
2.192	110.05899	4-Aminophe	42575	44239	45098	1206	1173	290	354	89	662	7136
0.767	110.05909	4-Aminophe	30353	29234	32698	165439	224	738	558	252	91	672
2.16	111.04457	Butyric acid	49282	47450	52319	2566	4452	2965	25624	1116	6319	399
1.141	112.05105	Cytosine	27702	26068	31368	40639	6516	3374	41663	7444	2324	21013
2.631	114.06589	Creatinine	24662	26271	29092	27790	603	122941	6758	11064	93258	1372
0.95	115.05032	Dihydrouraci	81989	85829	81643	76102	56202	67362	46890	51981	63137	87344
0.551	118.08523	Betaine; CE3	425416	439578	443134	1474854	124239	194713	122123	698172	15394	1865309
0.735	118.08682	Valine; LC-ES	106631	104523	107047	162141	63464	26575	73662	52896	92434	312172
0.658	120.06567	Threonine; L	61735	61212	61630	71358	39418	90177	131539	25665	8332	107739
3.504	121.06445	Tyramine; Pl	132375	134821	128762	3410	3482	186	353500	513	2157	3765
0.845	123.05415	2-Acetylpyra	76197	78191	78941	145867	15402	1691	27217	166286	90898	34028
1.178	123.05422	NICOTINAMI	17592	15558	19143	7902	13179	8731	10594	8683	6729	45395
0.675	126.065	5-Methylcyto	28589	28172	29091	17951	16413	30928	47628	10240	10852	26091
1.203	130.04918	L-5-Oxoprolii	650413	650910	703233	828567	279209	598748	694308	400439	696026	709469
0.694	131.11737	N-Acetylputr	73995	77678	81403	45676	29039	142158	79684	22936	14823	217772
0.617	132.07646	CREATINE	205650	213308	221180	31314	69769	33760	42032	292276	706804	334847
1.378	132.10159	Isoleucine; L	333024	332312	331645	258584	112452	82742	190801	53062	509451	569216

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Getting ready for metaboanalyst

MZ/RT	Pool-1	Pool-2	Pool-3	166	167	192	242	312	404	407
Group	0	0	0	1	1	2	2	2	2	1
61.039/0.53	1024668	915027	1081261	958818	1062631	851022	794976	1265634	1739819	1237578
76.076/0.55	261706	272229	270682	110938	304825	103169	36083	138122	81114	469427
86.097/1.38	94821	99698	97486	76393	35180	53528	54798	17429	142300	167006
86.097/0.87	152728	155824	162320	67316	26554	17503	154815	12171	8126	674362
100.075/1.9	40936	36108	38328	38579	28989	5189	11233	28658	15094	202169
100.076/1.8	109474	108286	117163	3663	27353	5224	1126	2196	1178	190999
104.107/0.5	62562	62208	65681	264843	32896	110624	27860	185761	10605	74866
110.059/2.1	42575	44239	45098	1206	1173	290	354	89	662	7136
110.059/0.7	30353	29234	32698	165439	224	738	558	252	91	672
111.045/2.1	49282	47450	52319	2566	4452	2965	25624	1116	6319	399
112.051/1.1	27702	26068	31368	40639	6516	3374	41663	7444	2324	21013
114.066/2.6	24662	26271	29092	27790	603	122941	6758	11064	93258	1372
115.05/0.95	81989	85829	81643	76102	56202	67362	46890	51981	63137	87344
118.085/0.5	425416	439578	443134	1474854	124239	194713	122123	698172	15394	1865309
118.087/0.7	106631	104523	107047	162141	63464	26575	73662	52896	92434	312172
120.066/0.6	61735	61212	61630	71358	39418	90177	131539	25665	8332	107739
121.064/3.5	132375	134821	128762	3410	3482	186	353500	513	2157	3765
123.054/0.8	76197	78191	78941	145867	15402	1691	27217	166286	90898	34028
123.054/1.1	17592	15558	19143	7902	13179	8731	10594	8683	6729	45395
126.065/0.6	28589	28172	29091	17951	16413	30928	47628	10240	10852	26091
130.049/1.2	650413	650910	703233	828567	279209	598748	694308	400439	696026	709469
131.117/0.6	73995	77678	81403	45676	29039	142158	79684	22936	14823	217772
132.076/0.6	205650	213308	221180	31314	69769	33760	42032	292276	706804	334847
132.102/1.3	333024	332312	331645	258584	112452	82742	190801	53062	509451	569216

Concatenated column A

Now save this file as 'class_metaboanalyst' as a .csv file

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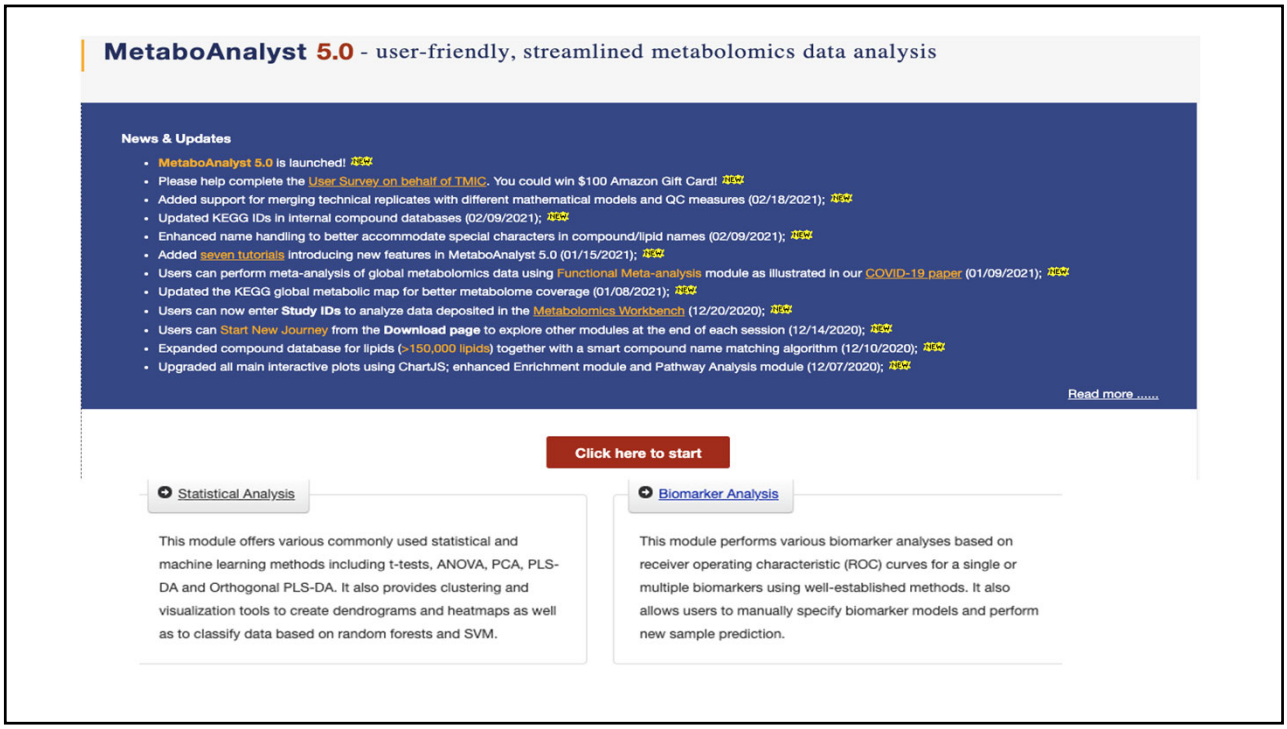
MetaboAnalyst 5.0 - user-friendly, streamlined metabolomics data analysis
News & Updates
MetaboAnalyst 5.0 is launched!
Please help complete the User Survey on behalf of TMIC. You could win $100 Amazon Gift Card!
Added support for merging technical replicates with different mathematical models and QC measures (02/18/2021);
Updated KEGG IDs in internal compound databases (02/09/2021);
Enhanced name handling to better accommodate special characters in compound/lipid names (02/09/2021);
Added seven tutorials introducing new features in MetaboAnalyst 5.0 (01/15/2021);
Users can perform meta-analysis of global metabolomics data using Functional Meta-analysis module as illustrated in our COVID-19 paper (01/09/2021);
Updated the KEGG global metabolic map for better metabolome coverage (01/08/2021);
Users can now enter Study IDs to analyze data deposited in the Metabolomics Workbench (12/20/2020);
Users can Start New Journey from the Download page to explore other modules at the end of each session (12/14/2020);
Expanded compound database for lipids (>150,000 lipids) together with a smart compound name matching algorithm (12/10/2020);
Upgraded all main interactive plots using ChartJS; enhanced Enrichment module and Pathway Analysis module (12/07/2020);
Read more .....
Click here to start
Statistical Analysis
This module offers various commonly used statistical and machine learning methods including t-tests, ANOVA, PCA, PLS-DA and Orthogonal PLS-DA. It also provides clustering and visualization tools to create dendrograms and heatmaps as well as to classify data based on random forests and SVM.
Biomarker Analysis
This module performs various biomarker analyses based on receiver operating characteristic (ROC) curves for a single or multiple biomarkers using well-established methods. It also allows users to manually specify biomarker models and perform new sample prediction.

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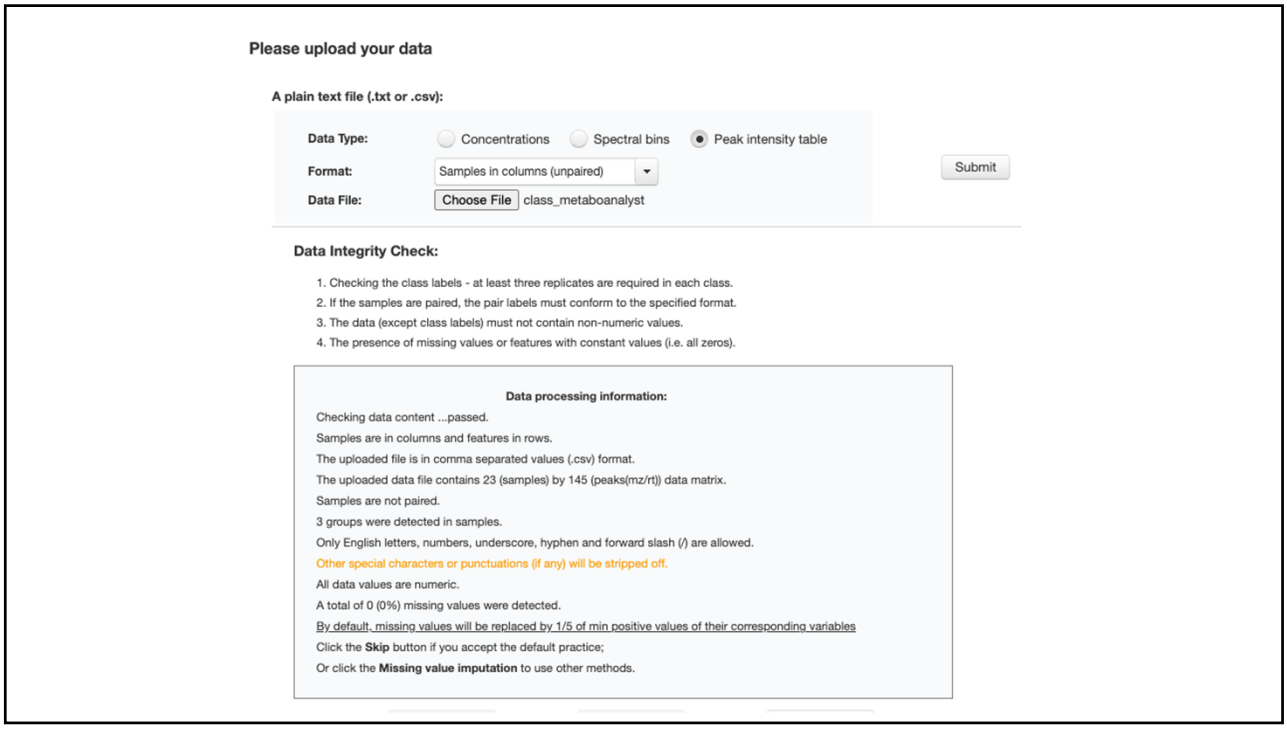
Please upload your data
A plain text file (.txt or .csv):
Data Type:
Concentrations
Spectral bins
Peak intensity table
Format:
Samples in columns (unpaired)
Data File:
Choose File
class_metaboanalyst
Submit
Data Integrity Check:
1. Checking the class labels - at least three replicates are required in each class.
2. If the samples are paired, the pair labels must conform to the specified format.
3. The data (except class labels) must not contain non-numeric values.
4. The presence of missing values or features with constant values (i.e. all zeros).
Data processing information:
Checking data content ...passed.
Samples are in columns and features in rows.
The uploaded file is in comma separated values (.csv) format.
The uploaded data file contains 23 (samples) by 145 (peaks(mz/rt)) data matrix.
Samples are not paired.
3 groups were detected in samples.
Only English letters, numbers, underscore, hyphen and forward slash (/) are allowed.
Other special characters or punctuations (if any) will be stripped off.
All data values are numeric.
A total of 0 (0%) missing values were detected.
By default, missing values will be replaced by 1/5 of min positive values of their corresponding variables
Click the Skip button if you accept the default practice;
Or click the Missing value imputation to use other methods.

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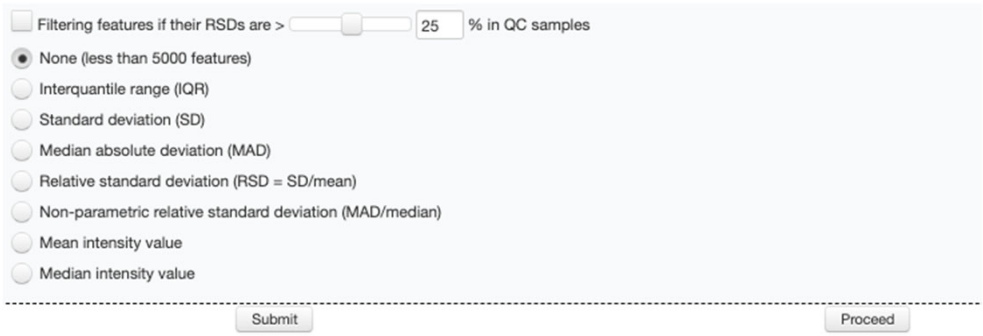
Filtering features if their RSDs are >
25
% in QC samples
None (less than 5000 features)
Interquantile range (IQR)
Standard deviation (SD)
Median absolute deviation (MAD)
Relative standard deviation (RSD = SD/mean)
Non-parametric relative standard deviation (MAD/median)
Mean intensity value
Median intensity value
Submit
Proceed

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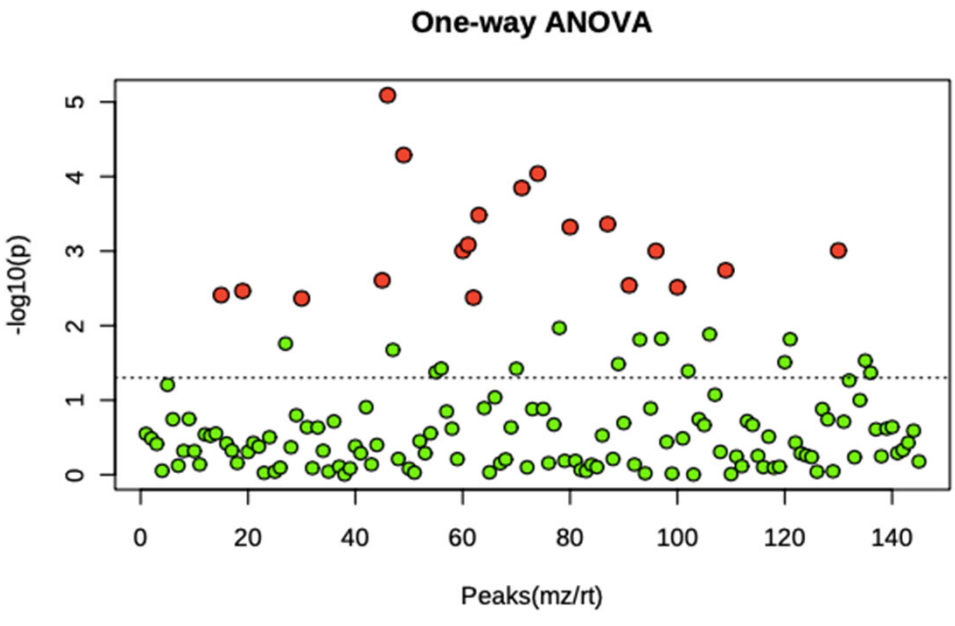
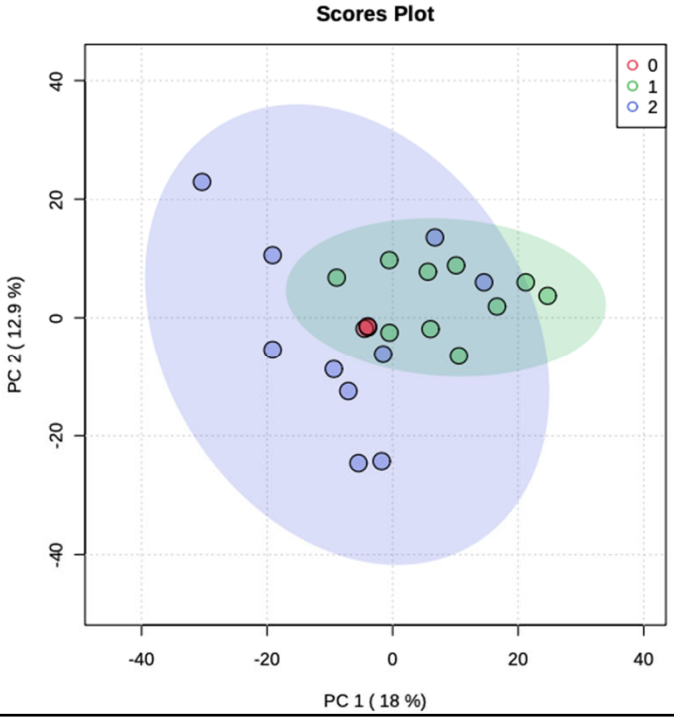
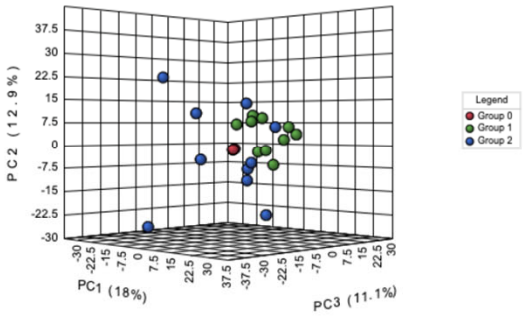

One-way ANOVA
-log10(p)
0
1
2
3
4
5
0
20
40
60
80
100
120
140
Peaks(mz/rt)

12



PCA plots



The green dots (group 1) are more compact than the blue ones and show separation in the third dimension. The red dot is the three pooled samples

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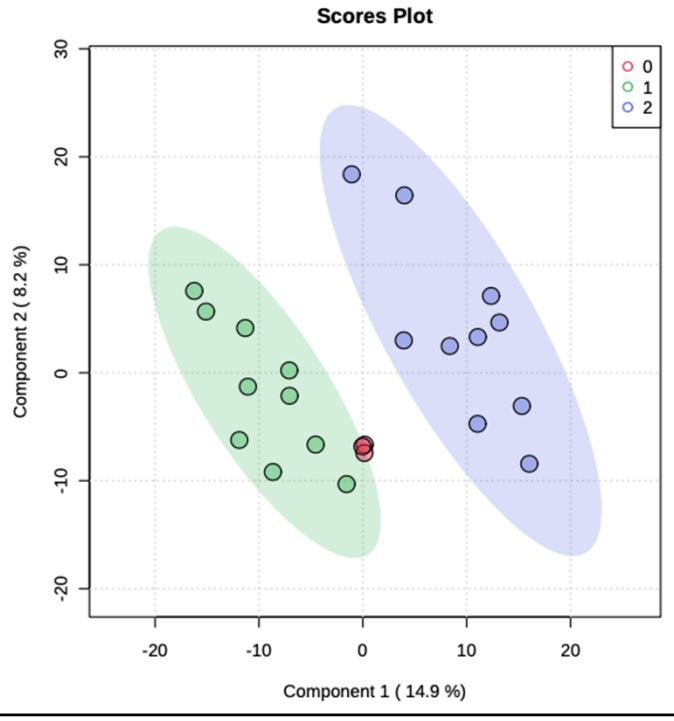
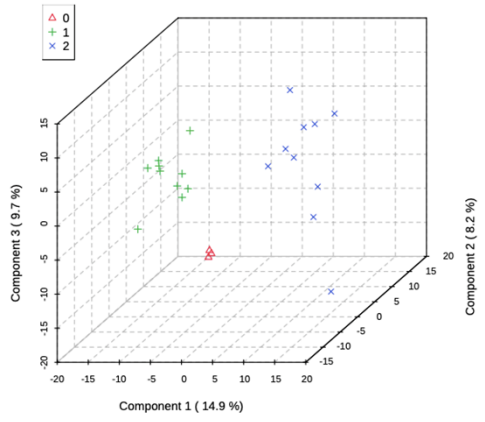


PLSDA plots



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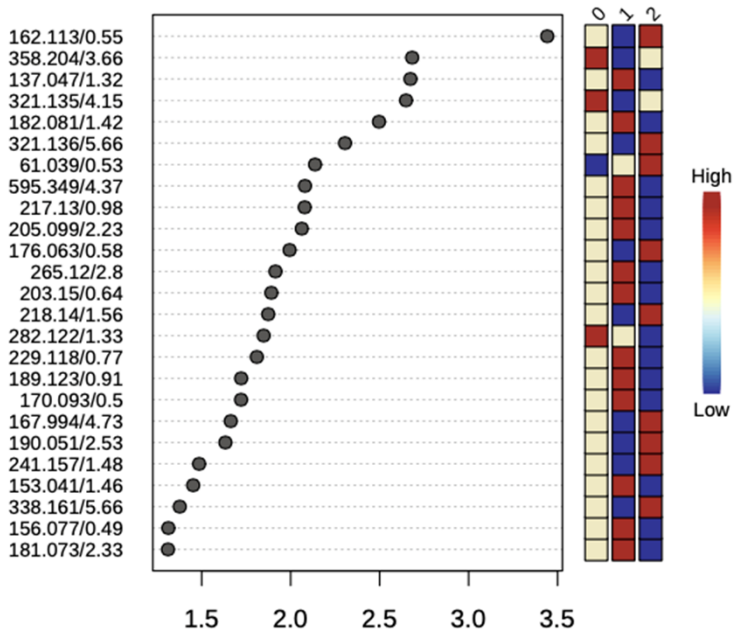
What are these ion features?



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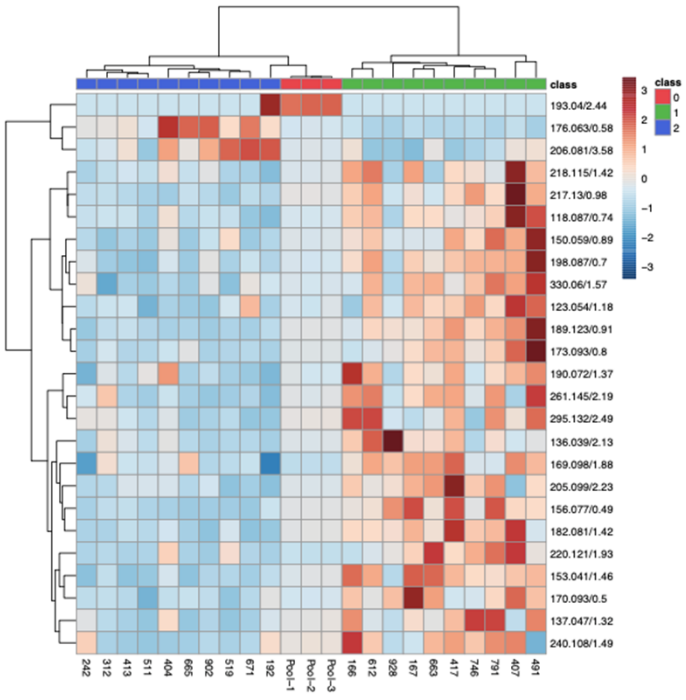
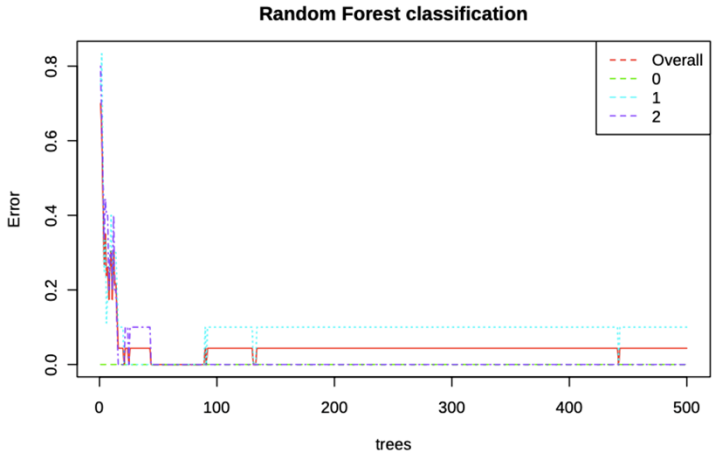
Heat map

There is one ion that is dark in the pooled (red) samples – it's because this feature is very strong in pt 192.

What is it?



16



	0	1	2	class.error
0	3.00	0.00	0.00	0.00
1	0.00	9.00	1.00	0.10
2	0.00	0.00	10.00	0.00

Almost perfect classification of the two patient groups and the pooled samples

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Path to peaks-to-pathways

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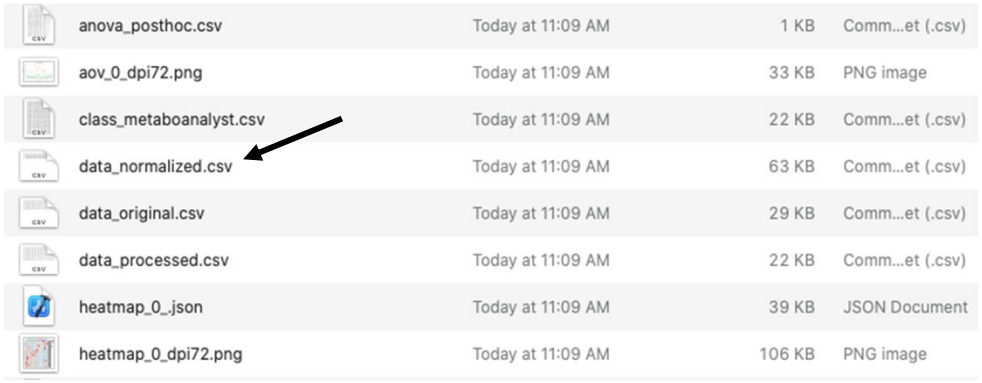
Finish using Metaboanalyst

- **Download the data – unzip the .zip file**



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Normalized and mean centered data

	Label	61.039/0.53	76.076/0.55	86.097/1.38	86.097/0.87	100.075/1.95	100.076/1.85	104.107/0.5	110.059/2.15	110.059/0.7	111.045/2.16	112.051/1.14
Pool-1	0	-2.0300418	-0.4528597	-0.1901459	-0.4759526	-0.3484671	0.09799174	-0.4319723	0.22492664	0.01056621	0.0741049	-0.3371556
Pool-2	0	-2.6369764	-0.3446457	-0.0959993	-0.4435775	-0.4320334	0.09000939	-0.4336023	0.26421063	-0.0070589	0.05367054	-0.3612959
Pool-3	0	-1.7599399	-0.3910471	-0.1576741	-0.4045436	-0.4009194	0.1678586	-0.3939843	0.27047023	0.04674097	0.1061727	-0.2836681
166	1	-1.9628086	-1.7763021	-0.4089437	-1.173963	-0.3348214	-1.0072062	2.90350588	-0.6571749	2.61699372	-0.5034974	-0.0813888
167	1	7.16685577	4.15030606	-0.3277332	-1.235109	0.20174338	-0.3391702	-0.1501459	-0.6229512	-0.5125326	-0.4010209	-0.519463
407	1	0.49246363	2.29253638	1.64892015	5.16101507	3.19051756	1.3201867	-0.0430508	-0.504442	-0.5083022	-0.532077	-0.381621
417	1	-2.4715513	-0.6328306	0.28796475	-1.273846	-0.0380051	0.9464557	-0.9510484	1.86262056	2.29265359	-0.4039781	-0.3056052
491	1	-3.4178752	5.05134779	2.10215803	-0.3669907	1.15377428	0.27627849	0.28902875	1.22415945	-0.4988938	-0.1668353	1.52641296
612	1	-3.3437929	0.39964944	0.64953461	-0.5519948	1.38011918	-0.8353866	1.42069442	-0.6427232	-0.5090189	0.09032839	0.5875081
663	1	1.63521243	1.54308568	-0.1801407	2.69138315	1.27807436	1.49847648	-0.5611193	-0.5591521	2.65907184	-0.3652292	-0.296809
746	1	-0.8333637	-0.9391056	-0.1207277	-1.1429574	-0.0725344	6.93756739	-0.2114757	-0.3271349	-0.4437645	-0.4717863	-0.6449343
791	1	-2.4998618	-0.9871247	0.85230494	-1.5970038	-0.6673003	-0.9980495	-0.2749158	-0.1652195	-0.4529777	0.09734313	2.97056855
928	1	1.41722942	-2.1524583	-1.1692582	-1.3924515	-0.5723493	-0.8704551	-0.6157976	2.10803257	-0.4099796	6.54182028	-0.7448232
192	2	-2.2336361	-1.7763811	-0.7727766	-1.6315488	-0.9709389	-0.985083	0.55220892	-0.6778332	-0.5072132	-0.4951468	-0.7120059
242	2	-2.3463424	-2.4848899	-0.6904293	-0.1612221	-0.8342152	-1.0342969	-0.8582225	-0.6758108	-0.5103258	-0.1498869	0.02700528
312	2	5.33804407	-0.5405165	-1.3154893	-1.6150455	-0.1448992	-1.0066605	3.69464543	-0.6815622	-0.5141894	-0.5126258	-0.5594697
404	2	7.36899691	-1.7350689	2.01292924	-1.700395	-0.6658902	-1.0297656	-1.122378	-0.663349	-0.5198219	-0.4176517	-0.7176668
413	2	-1.5880564	-0.6235056	-0.5402155	1.46312686	1.07477623	-0.7182224	-0.0471327	-0.0367243	-0.5126547	-0.415028	-0.0575571
511	2	0.08509065	-1.1852284	-0.8767441	3.48276641	0.07760579	-0.7054084	-0.5596473	1.19758075	-0.5195076	-0.3787305	-0.4278285
519	2	1.25695651	1.37132272	1.53556663	-1.6551185	-0.9968187	-1.0413948	-0.8624496	-0.1550997	-0.4213988	-0.5101706	-0.7098277
665	2	1.12126039	1.44304541	-0.6111408	1.65648782	-0.6815287	-0.3331026	-0.951229	-0.5825927	-0.5023133	-0.4397177	-0.7247006
671	2	2.39939353	0.81756726	-0.9806258	4.08917173	-0.1734949	0.60840447	-0.9139113	-0.4931265	0.11621404	-0.3778434	-0.7124775
902	2	-1.1572569	-1.0468966	-0.6513343	-1.7222313	-1.0223945	-1.039027	0.52199952	0.29289555	-0.3922872	-0.4222146	3.46680312

The data need to be transposed – highlight all the data and copy to a new file

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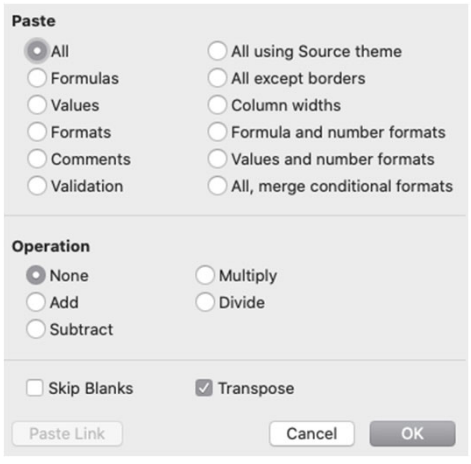


Copy paste special and click "transpose"

Transposed file is now in the original format, but organized by groups

	Pool-1	Pool-2	Pool-3	166	167	407	417	491
Label	0	0	0	1	1	1	1	1
61.039/0.53	-2.0300418	-2.6369764	-1.7599399	-1.9628086	7.16685577	0.49246363	-2.4715513	-3.4178752
76.076/0.55	-0.4528597	-0.3446457	-0.3910471	-1.7763021	4.15030606	2.29253638	-0.6328306	5.05134779
86.097/1.38	-0.1901459	-0.0959993	-0.1576741	-0.4089437	-0.3277332	1.64892015	0.28796475	2.10215803
86.097/0.87	-0.4759526	-0.4435775	-0.4045436	-1.173963	-1.235109	5.16101507	-1.273846	-0.3669907
100.075/1.9	-0.3484671	-0.4320334	-0.4009194	-0.3348214	0.20174338	3.19051756	-0.0380051	1.15377428
100.076/1.8	0.09799174	0.09000939	0.1678586	-1.0072062	-0.3391702	1.3201867	0.9464557	0.27627849
104.107/0.5	-0.4319723	-0.4336023	-0.3939843	2.90350588	-0.1501459	-0.0430508	-0.9510484	0.28902875
110.059/2.1	0.22492664	0.26421063	0.27047023	-0.6571749	-0.6229512	-0.504442	1.86262056	1.22415945
110.059/0.7	0.01056621	-0.0070589	0.04674097	2.61699372	-0.5125326	-0.5083022	2.29265359	-0.4988938
111.045/2.1	0.0741049	0.05367054	0.1061727	-0.5034974	-0.4010209	-0.532077	-0.4039781	-0.1668353
112.051/1.1	-0.3371556	-0.3612959	-0.2836681	-0.0813888	-0.519463	-0.381621	-0.3056052	1.52641296
114.066/2.6	-0.1688743	-0.1342883	-0.0845054	-0.0602678	-0.6368413	-0.6341897	-0.5093872	1.12010567
115.05/0.95	-0.5728945	-0.4670918	-0.5996361	-0.5660081	0.87554024	-0.0300658	-0.1733613	1.25570398
118.085/0.5	-0.6027125	-0.5102198	-0.522012	6.20022534	-1.2880573	9.77669161	-1.8229003	-0.3340745
118.087/0.7	-0.3565503	-0.3824579	-0.3644097	0.69130732	0.41497243	3.6589657	-0.0849264	2.46056749
120.066/0.6	-0.4097631	-0.4150802	-0.4218364	-0.1196823	0.26618913	0.8305284	-0.7529623	0.51260144
121.064/3.5	0.72714822	0.76157663	0.67240368	-0.7614729	-0.7043645	-0.7525416	-0.7702726	-0.7887435
123.054/0.8	-0.2843432	-0.2628436	-0.2651223	0.47674436	-0.6398299	-0.6197456	-0.6658772	-0.5972429

21

Delete the pooled samples and highlight group 1

	166	167	407	417	491	612	663	746	791	928	192
Label	1	1	1	1	1	1	1	1	1	1	2
61.039/0.53	-1.9628086	7.16685577	0.49246363	-2.4715513	-3.4178752	-3.3437929	1.63521243	-0.8333637	-2.4998618	1.41722942	-2.2336361
76.076/0.55	-1.7763021	4.15030606	2.29253638	-0.6328306	5.05134779	0.39964944	1.54308568	-0.9391056	-0.9871247	-2.1524583	-1.7763811
86.097/1.38	-0.4089437	-0.3277332	1.64892015	0.28796475	2.10215803	0.64953461	-0.1801407	-0.1207277	0.85230494	-1.1692582	-0.7727766
86.097/0.87	-1.173963	-1.235109	5.16101507	-1.273846	-0.3669907	-0.5519948	2.69138315	-1.1429574	-1.5970038	-1.3924515	-1.6315488
100.075/1.9	-0.3348214	0.20174338	3.19051756	-0.0380051	1.15377428	1.38011918	1.27807436	-0.0725344	-0.6673003	-0.5723493	-0.9709389
100.076/1.8	-1.0072062	-0.3391702	1.3201867	0.9464557	0.27627849	-0.8353866	1.49847648	6.93756739	-0.9980495	-0.8704551	-0.985083
104.107/0.5	2.90350588	-0.1501459	-0.0430508	-0.9510484	0.28902875	1.42069442	-0.5611193	-0.2114757	-0.2749158	-0.6157976	0.55220892
110.059/2.1	-0.6571749	-0.6229512	-0.504442	1.86262056	1.22415945	-0.6427232	-0.5591521	-0.3271349	-0.1652195	2.10803257	-0.6778332
110.059/0.7	2.61699372	-0.5125326	-0.5083022	2.29265359	-0.4988938	-0.5090189	2.65907184	-0.4437645	-0.4529777	-0.4099796	-0.5072132
111.045/2.1	-0.5034974	-0.4010209	-0.532077	-0.4039781	-0.1668353	0.09032839	-0.3652292	-0.4717863	0.09734313	6.54182028	-0.4951468
112.051/1.1	-0.0813888	-0.519463	-0.381621	-0.3056052	1.52641296	0.5875081	-0.296809	-0.6449343	2.97056855	-0.7448232	-0.7120059
114.066/2.6	-0.0602678	-0.6368413	-0.6341897	-0.5093872	1.12010567	-0.2378366	-0.4987535	-0.4407534	-0.4205634	-0.6279182	2.21907358
115.05/0.95	-0.5660081	0.87554024	-0.0300658	-0.1733613	1.25570398	0.85355243	0.22700668	-0.0101215	0.39214945	-0.965247	-0.6665464
118.085/0.5	6.20022534	-1.2880573	9.77669161	-1.8229003	-0.3340745	1.595961	-1.0509451	-2.0338771	-1.7852496	2.51491725	-1.7616339
118.087/0.7	0.69130732	0.41497243	3.6589657	-0.0849264	2.46056749	1.00555167	0.40955755	0.20734588	0.61832391	-0.9376889	-1.5129969
120.066/0.6	-0.1196823	0.26618913	0.8305284	-0.7529623	0.51260144	0.70556017	0.36514005	1.51313458	0.33213576	-0.5388814	0.40168095
121.064/3.5	-0.7614729	-0.7043645	-0.7525416	-0.7702726	-0.7887435	-0.7744166	-0.7812246	-0.6598286	2.53440057	-0.7984226	-0.80161
123.054/0.8	0.47674436	-0.6398299	-0.6197456	-0.6658772	-0.5972429	-0.763299	-0.3731572	8.32879908	-0.8433333	-0.2708186	-0.9777681
123.054/1.1	-0.4819091	0.41130268	1.19402006	0.27413044	0.89143793	0.39292529	0.20116799	0.6063571	0.20255571	-0.1842195	-0.4223725
126.065/0.6	-0.4536081	0.1923432	-0.1042001	-0.3925917	1.70711201	0.35871556	-0.0643252	0.11927854	1.01182416	-0.671773	0.04943032
130.049/1.2	0.58225793	-1.2822128	0.07497799	-0.1621044	0.67237276	0.0239536	4.0743261	-0.3991949	0.05743305	-2.2337254	-1.241557
131.117/0.6	-0.8167347	-0.4601022	2.48194384	-0.7962912	1.15696216	-0.5930404	2.66253138	0.51275626	0.14450913	-0.6716096	1.01623953
132.076/0.6	-1.4727235	-0.3660258	1.41601379	-1.4363628	5.47476854	-1.0461392	-0.7758016	-0.7547954	-1.2741808	-1.5338346	-1.4297938

22

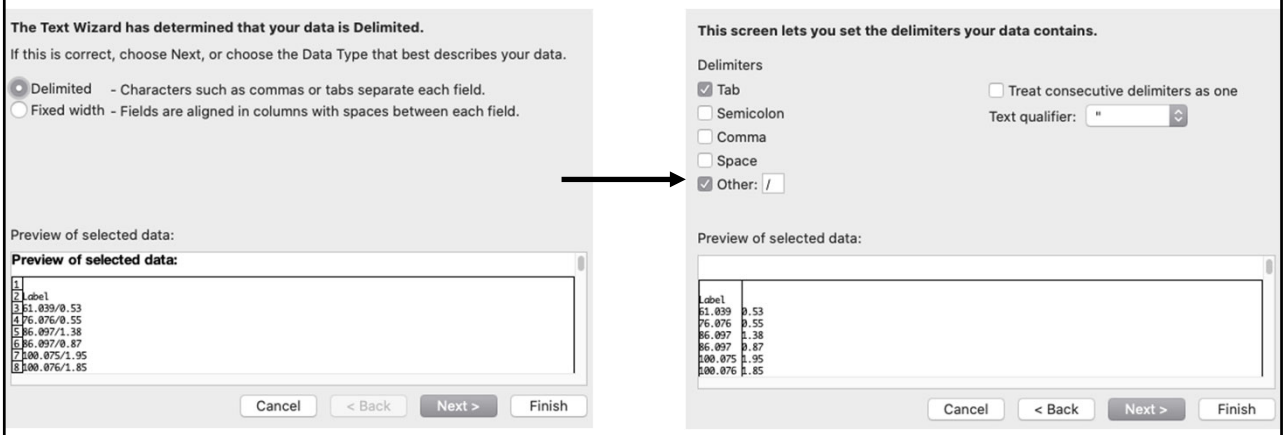
De-concatenate row A

Add two columns - highlight column A – go to the Data tab and select “text to columns”

A	B	C	D	E	F	G	H	I
			166	167	407	417	491	612
Label			1	1	1	1	1	1
61.039/0.53			-1.9628086	7.16685577	0.49246363	-2.4715513	-3.4178752	-3.3437929
76.076/0.55			-1.7763021	4.15030606	2.29253638	-0.6328306	5.05134779	0.39964944
86.097/1.38			-0.4089437	-0.3277332	1.64892015	0.28796475	2.10215803	0.64953461
86.097/0.87			-1.173963	-1.235109	5.16101507	-1.273846	-0.3669907	-0.5519948
100.075/1.9			-0.3348214	0.20174338	3.19051756	-0.0380051	1.15377428	1.38011918
100.076/1.8			-1.0072062	-0.3391702	1.3201867	0.9464557	0.27627849	-0.8353866
104.107/0.5			2.90350588	-0.1501459	-0.0430508	-0.9510484	0.28902875	1.42069442
110.059/2.1			-0.6571749	-0.6229512	-0.504442	1.86262056	1.22415945	-0.6427232
110.059/0.7			2.61699372	-0.5125326	-0.5083022	2.29265359	-0.4988938	-0.5090189
111.045/2.1			-0.5034974	-0.4010209	-0.532077	-0.4039781	-0.1668353	0.09032839
112.051/1.1			-0.0813888	-0.519463	-0.381621	-0.3056052	1.52641296	0.5875081
114.066/2.6			-0.0602678	-0.6368413	-0.6341897	-0.5093872	1.12010567	-0.2378366
115.05/0.95			-0.5660081	0.87554024	-0.0300658	-0.1733613	1.25570398	0.85355243
118.085/0.5			6.20022534	-1.2880573	9.77669161	-1.8229003	-0.3340745	1.595961
118.087/0.7			0.69130732	0.41497243	3.6589657	-0.0849264	2.46056749	1.00555167
120.066/0.6			-0.1196823	0.26618913	0.8305284	-0.7529623	0.51260144	0.70556017
121.064/3.5			-0.7614729	-0.7043645	-0.7525416	-0.7702726	-0.7887435	-0.7744166
123.054/0.8			0.47674436	-0.6398299	-0.6197456	-0.6658772	-0.5972429	-0.763299
123.054/1.1			-0.4819091	0.41130268	1.19402006	0.27413044	0.89143793	0.39292529

23

Text to columns



24

Create workspace – add columns and headings

A	B	C	D	E	F	G	H	I	J	K
m.z	r.t		p.value	t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53								-1.9628086	7.16685577
76.076	0.55								-1.7763021	4.15030606
86.097	1.38								-0.4089437	-0.3277332
86.097	0.87								-1.173963	-1.235109
100.075	1.95								-0.3348214	0.20174338
100.076	1.85								-1.0072062	-0.3391702
104.107	0.53								2.90350588	-0.1501459
110.059	2.19								-0.6571749	-0.6229512
110.059	0.77								2.61699372	-0.5125326
111.045	2.16								-0.5034974	-0.4010209
112.051	1.14								-0.0813888	-0.519463
114.066	2.63								-0.0602678	-0.6368413
115.05	0.95								-0.5660081	0.87554024
118.085	0.55								6.20022534	-1.2880573
118.087	0.74								0.69130732	0.41497243
120.066	0.66								-0.1196823	0.26618913
121.064	3.5								-0.7614729	-0.7043645
123.054	0.85								0.47674436	-0.6398299
123.054	1.18								-0.4819091	0.41130268
126.065	0.68								-0.4536081	0.1923432
130.049	1.2								0.58225793	-1.2822128
131.117	0.69								-0.8167347	-0.4601022

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Calculating p-values – then copy to the rest of the column

=TTEST(J3:S3,T3:AC3,2,2)

A	B	C	D	E	F	G	H	I	J	K
m.z	r.t		p.value	t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53		0.34578926						-1.9628086	7.16685577
76.076	0.55								-1.7763021	4.15030606
86.097	1.38								-0.4089437	-0.3277332
86.097	0.87								-1.173963	-1.235109
100.075	1.95								-0.3348214	0.20174338
100.076	1.85								-1.0072062	-0.3391702
104.107	0.53								2.90350588	-0.1501459
110.059	2.19								-0.6571749	-0.6229512
110.059	0.77								2.61699372	-0.5125326
111.045	2.16								-0.5034974	-0.4010209
112.051	1.14								-0.0813888	-0.519463

m.z	r.t		p.value	t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53		0.34578926						-1.9628086	7.16685577
76.076	0.55		0.17407701						-1.7763021	4.15030606
86.097	1.38		0.2048154						-0.4089437	-0.3277332
86.097	0.87		0.76318588						-1.173963	-1.235109
100.075	1.95		0.03515709						-0.3348214	0.20174338
100.076	1.85		0.0839821						-1.0072062	-0.3391702
104.107	0.53		0.69307737						2.90350588	-0.1501459
110.059	2.19		0.30834535						-0.6571749	-0.6229512
110.059	0.77		0.08267023						2.61699372	-0.5125326

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Transfer p-values to column C

m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926							-1.9628086	7.16685577
76.076	0.55	0.17407701							-1.7763021	4.15030606
86.097	1.38	0.2048154							-0.4089437	-0.3277332
86.097	0.87	0.76318588							-1.173963	-1.235109
100.075	1.95	0.03515709							-0.3348214	0.20174338
100.076	1.85	0.0839821							-1.0072062	-0.3391702
104.107	0.53	0.69307737							2.90350588	-0.1501459
110.059	2.19	0.30834535							-0.6571749	-0.6229512
110.059	0.77	0.08267023							2.61699372	-0.5125326
111.045	2.16	0.26011112							-0.5034974	-0.4010209
112.051	1.14	0.56590116							-0.0813888	-0.519463
114.066	2.63	0.14519116							-0.0602678	-0.6368413
115.05	0.95	0.53612435							-0.5660081	0.87554024
118.085	0.55	0.14263056							6.20022534	-1.2880573

Copy column D – paste special (values) to column C, then delete column D

27

Calculate Delta

m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926			=average(j3:s3)-average(t3:ac3)				-1.9628086	7.16685577
76.076	0.55	0.17407701							-1.7763021	4.15030606
86.097	1.38	0.2048154							-0.4089437	-0.3277332
86.097	0.87	0.76318588							-1.173963	-1.235109
100.075	1.95	0.03515709							-0.3348214	0.20174338
100.076	1.85	0.0839821							-1.0072062	-0.3391702
104.107	0.53	0.69307737							2.90350588	-0.1501459
110.059	2.19	0.30834535							-0.6571749	-0.6229512
110.059	0.77	0.08267023							2.61699372	-0.5125326
111.045	2.16	0.26011112							-0.5034974	-0.4010209
112.051	1.14	0.56590116							-0.0813888	-0.519463
114.066	2.63	0.14519116							-0.0602678	-0.6368413
115.05	0.95	0.53612435							-0.5660081	0.87554024
118.085	0.55	0.14263056							6.20022534	-1.2880573
118.087	0.74	0.00210732							0.69130732	0.41497243
120.066	0.66	0.28132246							-0.1196823	0.26618913
121.064	3.5	0.40089129							-0.7614729	-0.7043645
123.054	0.85	0.44666741							0.47674436	-0.6398299
123.054	1.18	0.00185509							-0.4819091	0.41130268

28

Inserting variance terms for groups 1 and 2

=((STDEV(J3:S3))^2)/10

A	B	C	D	E	F	G	H	I	J	K
m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926			-1.4061943	=((STDEV(J3:S3))^2)/10			-1.9628086	7.16685577
76.076	0.55	0.17407701							-1.7763021	4.15030606
86.097	1.38	0.2048154							-0.4089437	-0.3277332
86.097	0.87	0.76318588							-1.173963	-1.235109
100.075	1.95	0.03515709							-0.3348214	0.20174338
100.076	1.85	0.0839821							-1.0072062	-0.3391702
104.107	0.53	0.69307737							2.90350588	-0.1501459
110.059	2.19	0.30834535							-0.6571749	-0.6229512
110.059	0.77	0.08267023							2.61699372	-0.5125326
111.045	2.16	0.26011112							-0.5034974	-0.4010209
112.051	1.14	0.56590116							-0.0813888	-0.519463
114.066	2.63	0.14519116							-0.0602678	-0.6368413

=((STDEV(S3:AC3))^2)/10

A	B	C	D	E	F	G	H	I	J	K
m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926			-1.4061943	1.05	1.05843408		-1.9628086	7.16685577
76.076	0.55	0.17407701							-1.7763021	4.15030606
86.097	1.38	0.2048154							-0.4089437	-0.3277332
86.097	0.87	0.76318588							-1.173963	-1.235109
100.075	1.95	0.03515709							-0.3348214	0.20174338
100.076	1.85	0.0839821							-1.0072062	-0.3391702
104.107	0.53	0.69307737							2.90350588	-0.1501459
110.059	2.19	0.30834535							-0.6571749	-0.6229512
110.059	0.77	0.08267023							2.61699372	-0.5125326
111.045	2.16	0.26011112							-0.5034974	-0.4010209

29

Calculate SQRT and then the t-score

copy to the rest of the file

m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926		-0.9681702	-1.4061943	1.05110317	1.05843408	1.45242461	-1.9628086	7.16685577
76.076	0.55	0.17407701							-1.7763021	4.15030606
86.097	1.38	0.2048154							-0.4089437	-0.3277332
86.097	0.87	0.76318588							-1.173963	-1.235109
100.075	1.95	0.03515709							-0.3348214	0.20174338
100.076	1.85	0.0839821							-1.0072062	-0.3391702
104.107	0.53	0.69307737							2.90350588	-0.1501459
110.059	2.19	0.30834535							-0.6571749	-0.6229512
110.059	0.77	0.08267023							2.61699372	-0.5125326

m.z	r.t	p.value		t.score	Delta	SD1	SD2	SQRT	166	167
Label									1	1
61.039	0.53	0.34578926		-0.9681702	-1.4061943	1.05110317	1.05843408	1.45242461	-1.9628086	7.16685577
76.076	0.55	0.17407701		1.41520477	1.27096556	0.61948713	0.18705861	0.89807891	-1.7763021	4.15030606
86.097	1.38	0.2048154		1.31561393	0.62243389	0.09940528	0.12443052	0.47311287	-0.4089437	-0.3277332
86.097	0.87	0.76318588		-0.3059061	-0.308791	0.49514759	0.52380218	1.00943042	-1.173963	-1.235109
100.075	1.95	0.03515709		2.27799498	0.98570165	0.14372231	0.043512	0.4327058	-0.3348214	0.20174338
100.076	1.85	0.0839821		1.82922659	1.42132539	0.57634174	0.02740114	0.77700893	-1.0072062	-0.3391702
104.107	0.53	0.69307737		0.40108701	0.23517922	0.13277181	0.21103993	0.58635462	2.90350588	-0.1501459
110.059	2.19	0.30834535		1.0483619	0.41916369	0.12279689	0.03706496	0.39982728	-0.6571749	-0.6229512
110.059	0.77	0.08267023		1.83770686	0.85167477	0.21091994	0.00386068	0.4634443	2.61699372	-0.5125326

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Copy t.score values in column E -> then paste special (values) into column D -> then delete other columns

m.z	r.t	p.value	t.score
Label			
61.039	0.53	0.34578926	-0.9681702
76.076	0.55	0.17407701	1.41520477
86.097	1.38	0.2048154	1.31561393
86.097	0.87	0.76318588	-0.3059061
100.075	1.95	0.03515709	2.27799498
100.076	1.85	0.0839821	1.82922659
104.107	0.53	0.69307737	0.40108701
110.059	2.19	0.30834535	1.0483619
110.059	0.77	0.08267023	1.83770686
111.045	2.16	0.26011112	1.1627604
112.051	1.14	0.56590116	0.58486089
114.066	2.63	0.14519116	-1.5227706
115.05	0.95	0.53612435	0.63076068
118.085	0.55	0.14263056	1.53313599

This file saved in a .csv format is what we need to run peaks-to-pathways

Class_p2p

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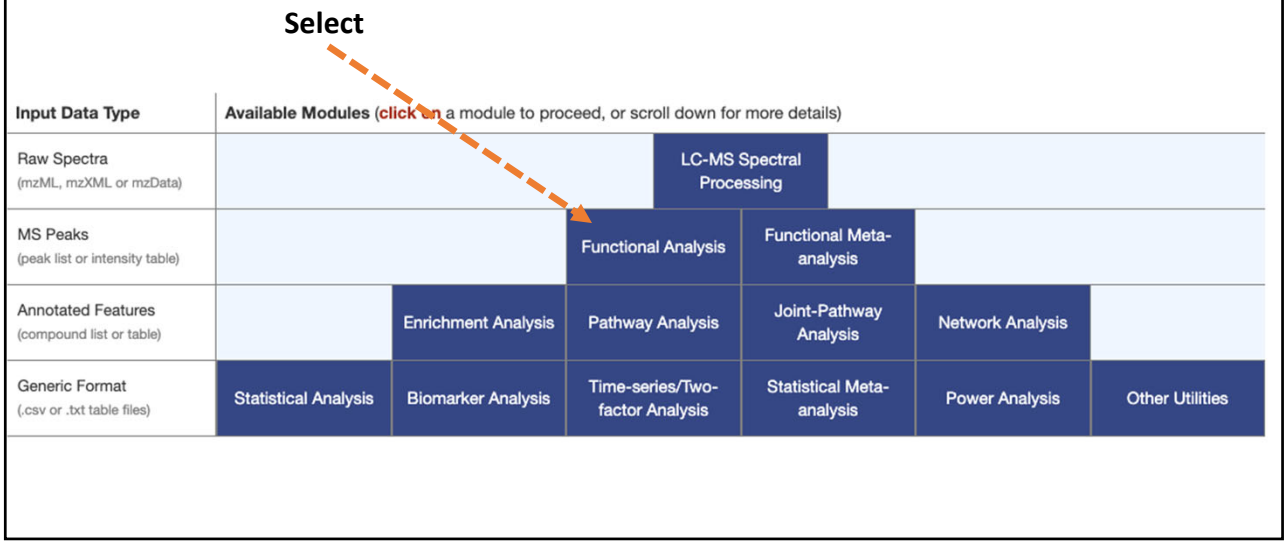
Go to metaboanalyst

Select



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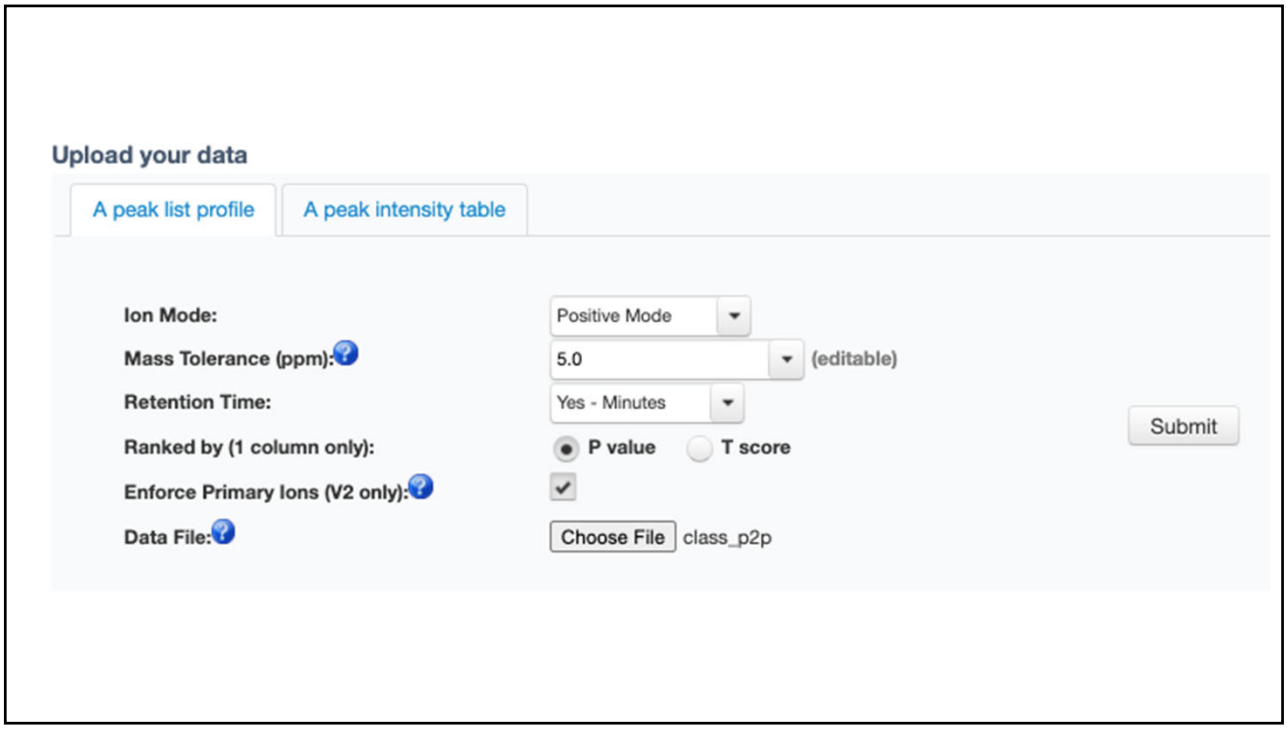
Upload your data
A peak list profile
A peak intensity table
Ion Mode:
Positive Mode
Mass Tolerance (ppm):
5.0
(editable)
Retention Time:
Yes - Minutes
Ranked by (1 column only):
P value
T score
Submit
Enforce Primary Ions (V2 only):
Data File:
Choose File
class_p2p

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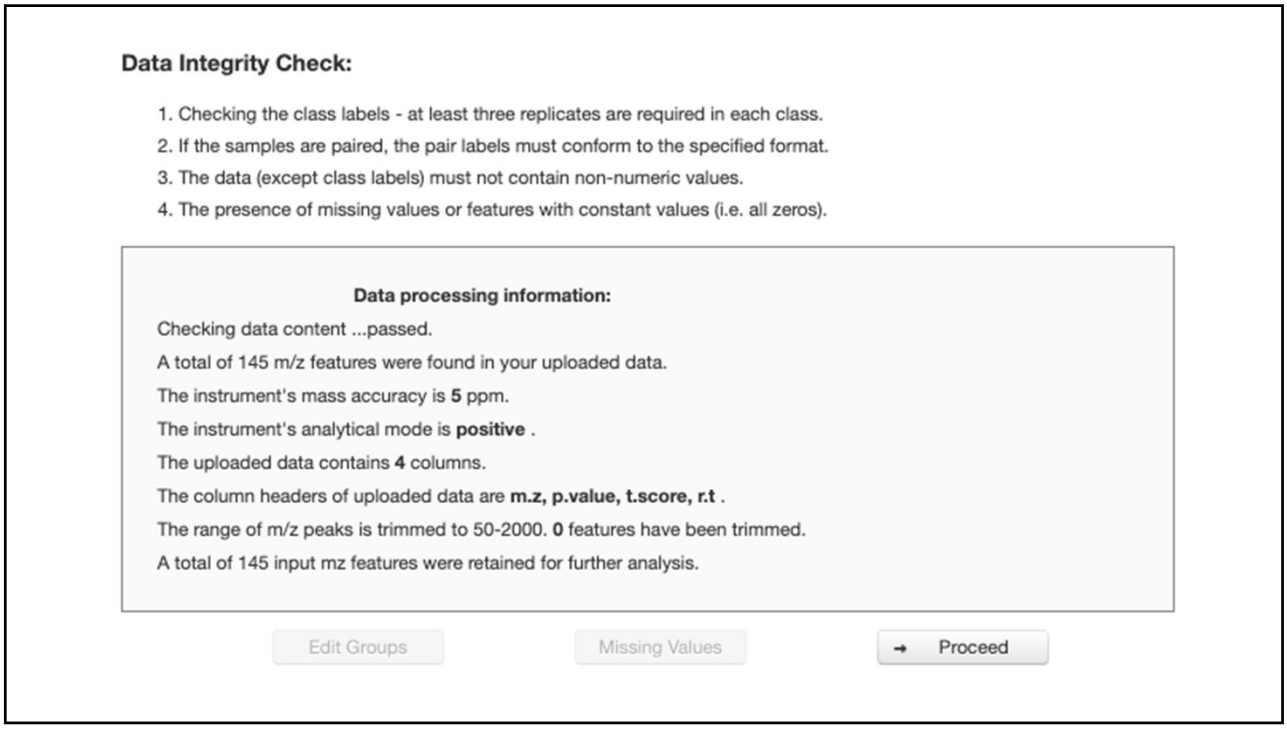
Data Integrity Check:
1. Checking the class labels - at least three replicates are required in each class.
2. If the samples are paired, the pair labels must conform to the specified format.
3. The data (except class labels) must not contain non-numeric values.
4. The presence of missing values or features with constant values (i.e. all zeros).
Data processing information:
Checking data content ...passed.
A total of 145 m/z features were found in your uploaded data.
The instrument's mass accuracy is 5 ppm.
The instrument's analytical mode is positive .
The uploaded data contains 4 columns.
The column headers of uploaded data are m.z, p.value, t.score, r.t .
The range of m/z peaks is trimmed to 50-2000. 0 features have been trimmed.
A total of 145 input mz features were retained for further analysis.
Edit Groups
Missing Values
Proceed

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Specify analysis parameters:

Algorithms	☑ Mummichog — P-value cutoff: 0.05 (default top 10% peaks); Which version: ◉ 2.0 ○ 1.0 ☐ GSEA (using the overall rank based on t.score)
Visual analytics:	◉ Scatter plot (Test significant peaks) ○ Heatmaps (Test peaks within manually selected patterns)
Advanced options	Edit Currency Metabolites Edit Adducts

Select a pathway library: *(KEGG pathway info were obtained in Oct. 2019)*

Mammals	◉ Homo sapiens (human) [MFN] ○ Homo sapiens (human) [BioCyc] ○ Homo sapiens (human) [KEGG] ○ Mus musculus (mouse) [BioCyc] ○ Mus musculus (mouse) [KEGG] ○ Rattus norvegicus (rat) [KEGG] ○ Bos taurus (cow) [KEGG]

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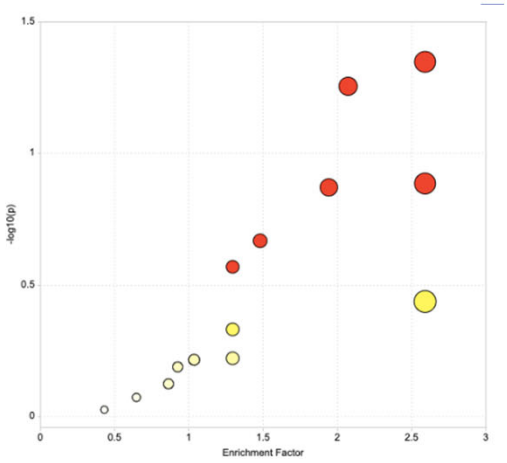
Metabolite Sets	○ Disease-associated Metabolite Sets (Blood) ○ Disease-associated Metabolite Sets (CSF) ○ Disease-associated Metabolite Sets (Urine) ○ SNP-associated Metabolite Sets ○ Location-based Metabolite Sets ○ Predicted Metabolite Sets

☑ Only use pathways / metabolite sets containing at least 3 entries

Submit



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Detailed result table

Mouse over the **Help** icon to find more information on each columns

Pathway Name	Total	Hits (all)	Hits (sig.)	Expected	P-value	Gamma P	Details
Biopterin metabolism	3	3	3	1.1579	0.045003	7.8792E-4	View
Purine metabolism	5	5	4	1.9298	0.055718	8.2323E-4	View
Glycolysis and Gluconeogenesis	2	2	2	0.77193	0.13051	0.0011207	View
Selenoamino acid metabolism	2	2	2	0.77193	0.13051	0.0011207	View
Methionine and cysteine metabolism	4	4	3	1.5439	0.13501	0.0011418	View
Glycine, serine, alanine and threonine metabolism	7	7	4	2.7018	0.2155	0.0016006	View
Tyrosine metabolism	10	10	5	3.8596	0.27069	0.0020253	View

The colored compounds indicate potential matches from the user's input, with red colors indicating significant hits and blue colors indicating non-significant hits. Note for users running V2, the number of hits listed in the results table will be lower than the number of compounds shown below. This is because the analysis is performed in the Empirical Compound space, and for the sake of interpretability, we show which compounds were matched instead. For more information, please refer to the FAQs.

Pathway	Metabolites
Biopterin metabolism	CE1951; C00143; CE6511; C05923; C05922; C06148; C00536; C00272; C04895; C03684; C03279; C04874; C00058; CE2705; C00079; **thbpt4acam**; **C04244**; CE5236; **C00268**; **C00082**; C00440; C00044

Pathway Hits | Compound Hits | Network Explorer

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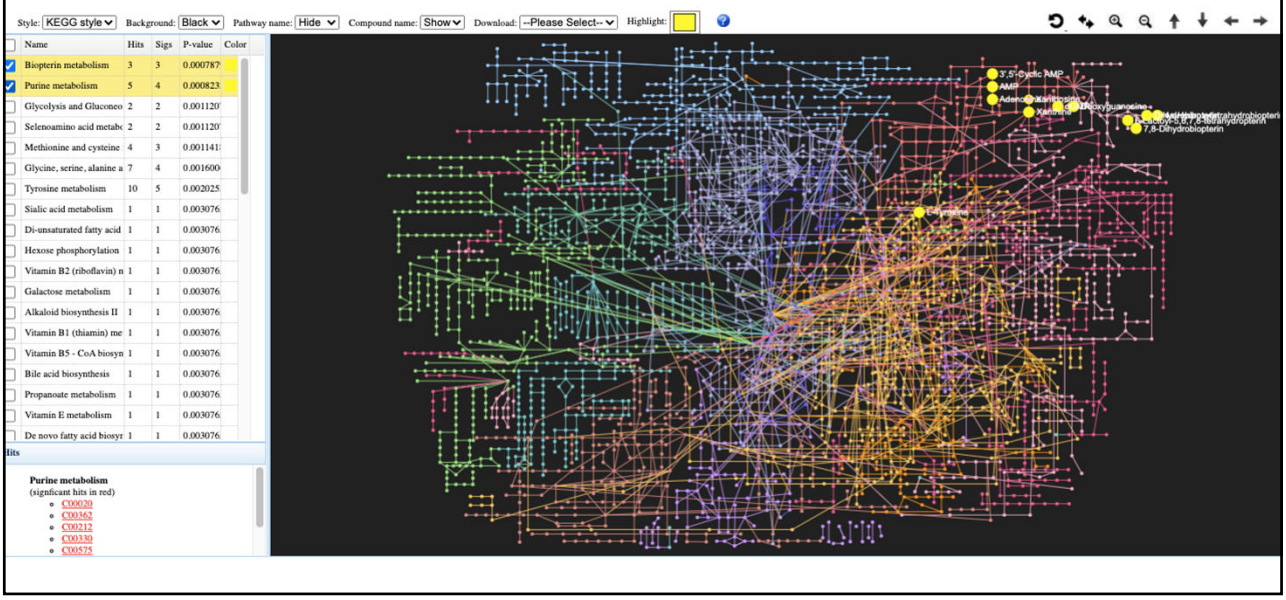
Network visualization



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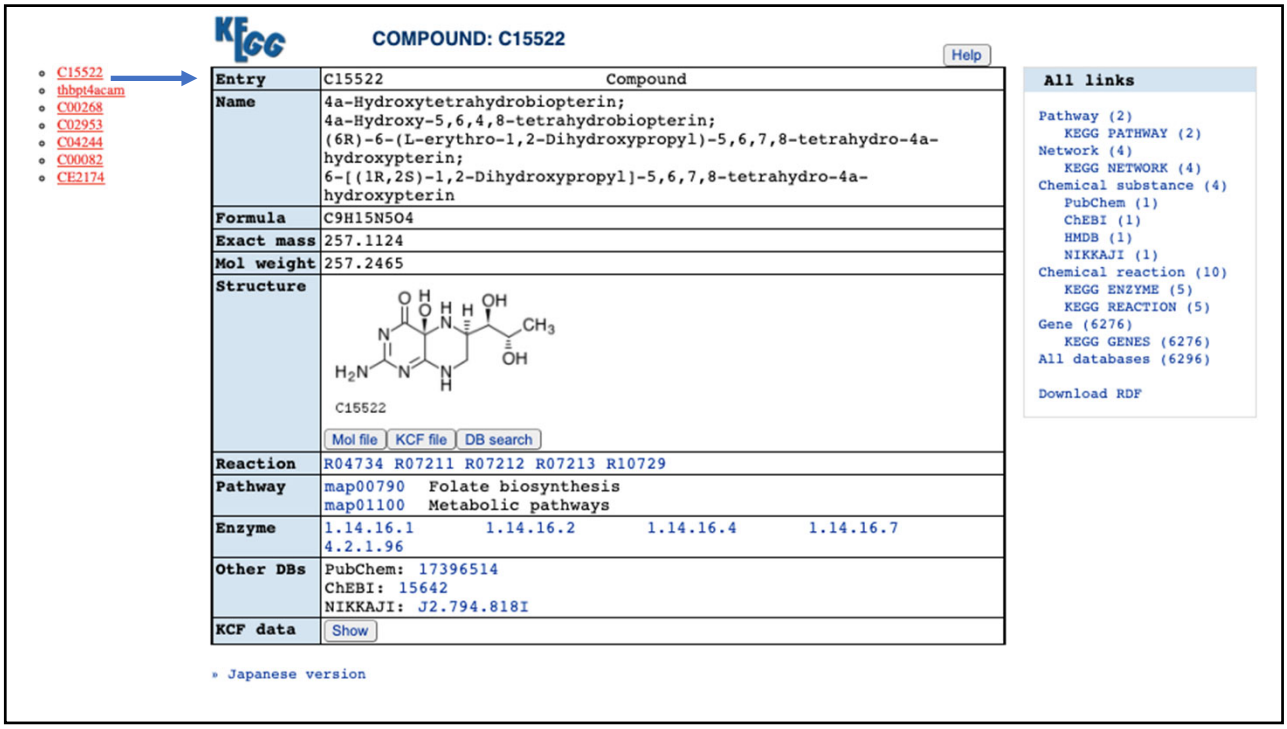

- C15522
- thbpt4acam
- C00268
- C02953
- C04244
- C00082
- CE2174

KEGG

COMPOUND: C15522

Help

Entry	C15522 Compound
Name	4a-Hydroxytetrahydrobiopterin; 4a-Hydroxy-5,6,4,8-tetrahydrobiopterin; (6R)-6-(L-erythro-1,2-Dihydroxypropyl)-5,6,7,8-tetrahydro-4a-hydroxypterin; 6-[(1R,2S)-1,2-Dihydroxypropyl]-5,6,7,8-tetrahydro-4a-hydroxypterin
Formula	C9H15N5O4
Exact mass	257.1124
Mol weight	257.2465
Structure	C15522 Mol file KCF file DB search
Reaction	R04734 R07211 R07212 R07213 R10729
Pathway	map00790 Folate biosynthesis map01100 Metabolic pathways
Enzyme	1.14.16.1 1.14.16.2 1.14.16.4 1.14.16.7 4.2.1.96
Other DBs	PubChem: 17396514 ChEBI: 15642 NIKKAJI: J2.794.818I
KCF data	Show

» Japanese version

All links

Pathway (2)
KEGG PATHWAY (2)
Network (4)
KEGG NETWORK (4)
Chemical substance (4)
PubChem (1)
ChEBI (1)
HMDB (1)
NIKKAJI (1)
Chemical reaction (10)
KEGG ENZYME (5)
KEGG REACTION (5)
Gene (6276)
KEGG GENES (6276)
All databases (6296)

Download RDF

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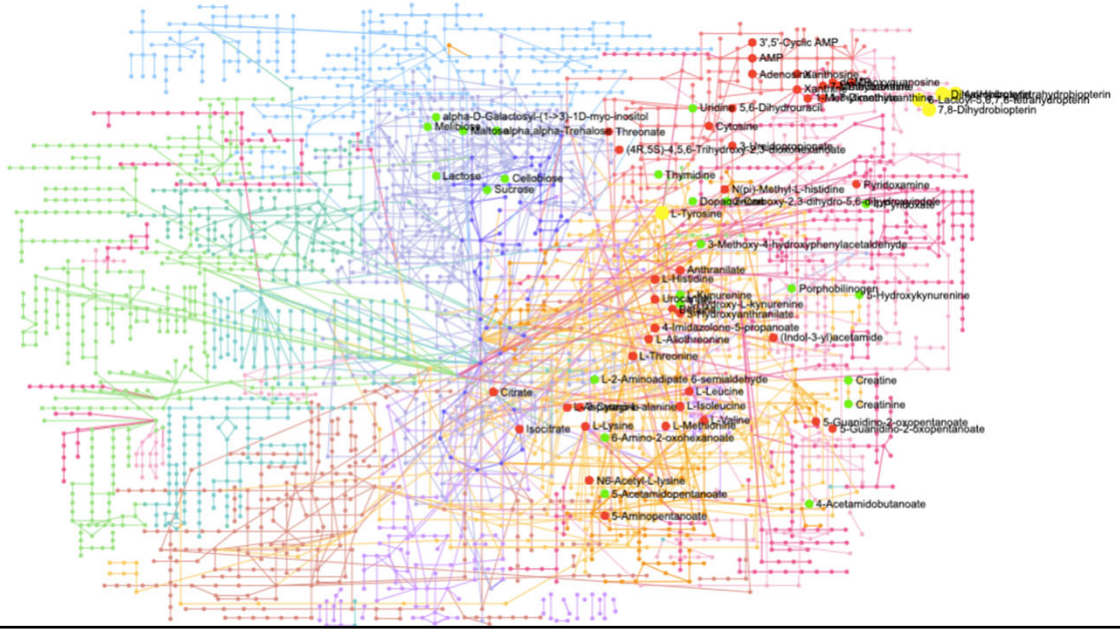
Changing the background to white and using red/green for “expression”



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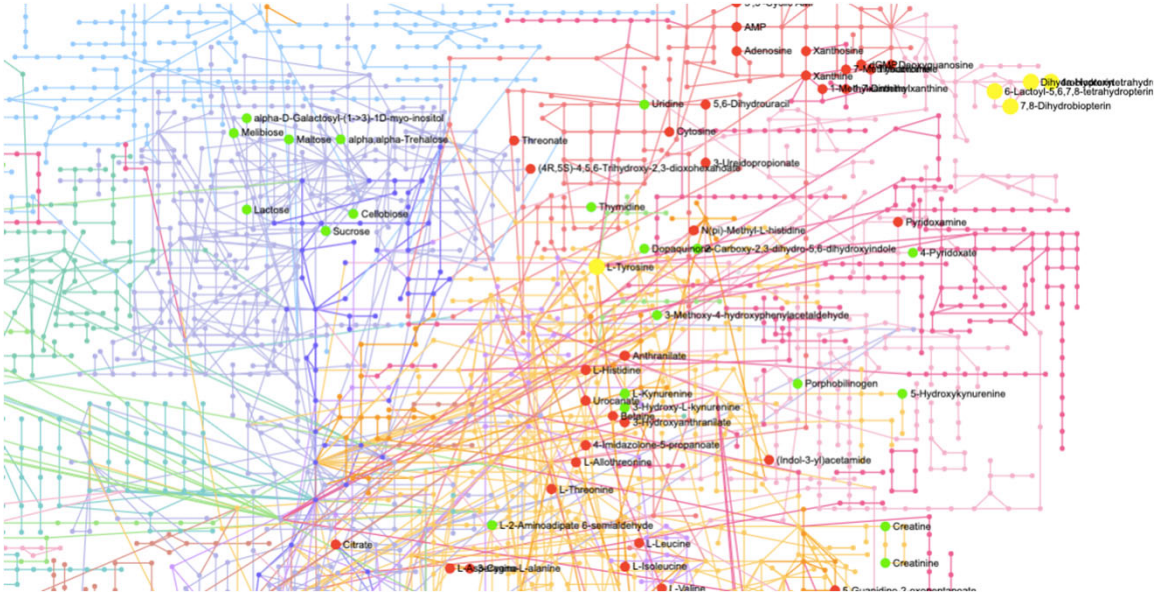
Magnifying the previous slide



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How could this package be improved?

- What we submitted were the *m/z* and RT values of "known" compounds
- There is a risk that since the *m/z* value does not have accompanying MSMS data as submitted, the interpretation of that value may be erroneous
- It would be better if the name of the metabolite could be used instead
- MZ-tab may be the answer

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