



*Knowledge that will change your world*

## Evaluating XCMS analysis

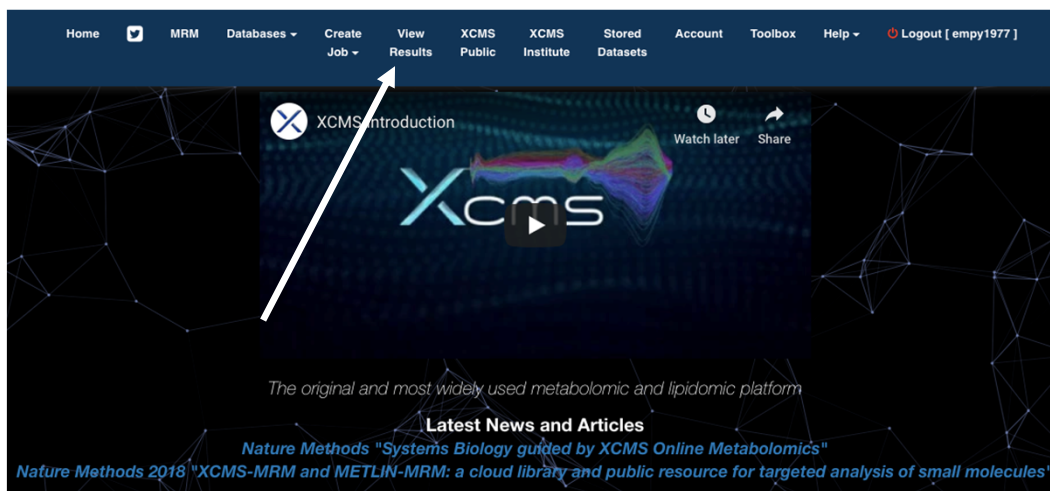
Stephen Barnes, PhD

### The experiment

- These are mouse urines
- **Germ-free mice were inoculated with fecal organisms from three breast cancer patients being treated with chemotherapy**
  - The drugs used were doxorubicin and docotaxel
  - Fecal samples were collected before and 2-4 weeks after therapy
- **Mice on the GE diet were fed a special corn oil customized diet from Teklad/Envigo (TD. 140534-GE 0.25 g/ Kg)**
- **The control mice were on the same diet, but without genistein**

## Upload of data files to XCMS

- We uploaded on Wednesday files C1-C3 and G1-G3.
- Now go to XCMS – logon with your ID/Password



## Results page

The screenshot shows the XCMS Results page. The navigation bar at the top includes: Home, MRM, Databases, Create Job, View Results, XCMS Public, XCMS Institute, Stored Datasets, Account, Toolbox, Help, and Logout [empty1977]. Below the navigation bar, there are buttons for "Show 15 rows", "Share", "Resubmit", "Job Grouping", and "Delete". A search bar is on the right. The main content is a table with columns: EXPTYPE, Status, JobID Filter, Progress, JobName, Datasets / Sources, Created, Parameters (ID#), Group Filter, Share, and Delete. A red arrow points to the "VIEW" button in the first row.

EXPTYPE	Status	JobID Filter	Progress	JobName	Datasets / Sources	Created	Parameters (ID#)	Group Filter	Share	Delete
PAIR	VIEW	1286073	job complete	P_2019-01-27_20:05	Control_ne (#378284) Gen_neg (#378285)	2019-01-27 20:05:51	33859		🔄	✖
PAIR	VIEW	1282886	job complete	P_2019-01-12_16:20	Miller_Mus (#375098) Miller_Mus (#375097)	2019-01-12 16:20:28	21944		🔄	✖
PAIR	VIEW	1281467	job complete	Miller_Muscle_PosMode WT vs FAP KO_010719	Miller_Mus (#373748) Miller_Mus (#373749)	2019-01-07 09:53:10	21618		🔄	✖
PAIR	VIEW	1271165	job complete	Zdenek_Negative_081518	Zdenek_Neg (#364430) Zdenek_Neg	2018-11-14 12:39:50	21944		🔄	✖

Submit Date	Finish Date	Total Aligned Features	Parameter ID#	Log	Shared	Download Results
2019-01-27 21:36:37	2019-01-28 12:50:27	7301	Tripletof_LandonWitt (33859)	<a href="#">View Log</a>	NOT SHARED	hash: dc11e65b5909fe92ddf393d9f056a6

The finished job has the following notes:

2019-01-28 12:35:19 : iHeatMap data prep, memory requires limiting to top 1000 features <0.27793 p-values

**Citation Links**

[Watson](#)

[Results Table](#)

[Metabolomic Cloud Plot](#)

[Interactive Heatmap](#)

[IPCA](#)

[Activity Network \(Connections\)](#)

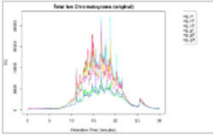
[Systems Biology Results](#)

[Pathway Cloud Plot](#)

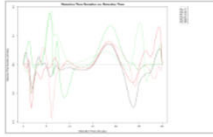
**Multi-Omics Data**

**Datasets Used:**

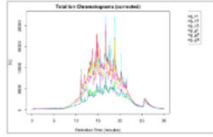
- Control\_neg (378284)
- Gen\_neg (378285)



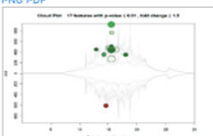
[PNG PDF](#)



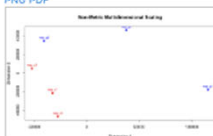
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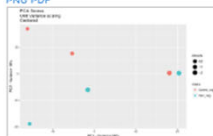
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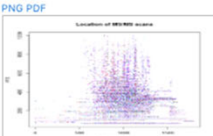
[PNG PDF](#)



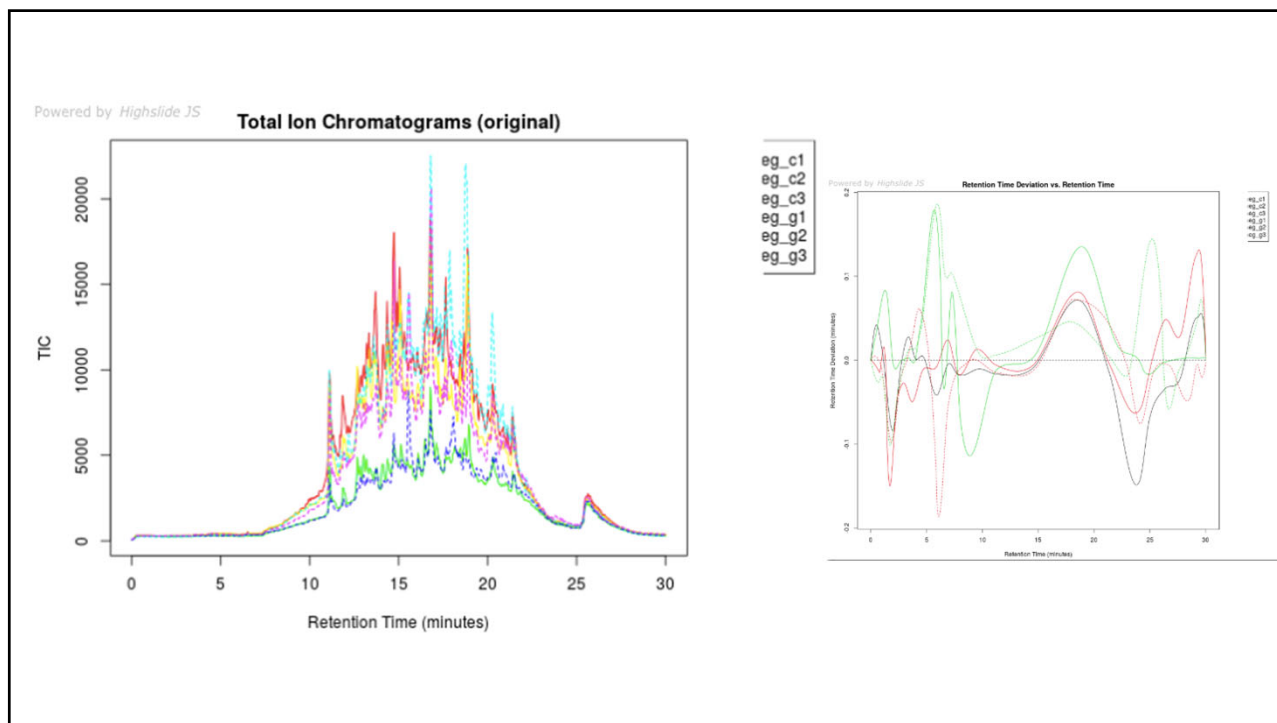
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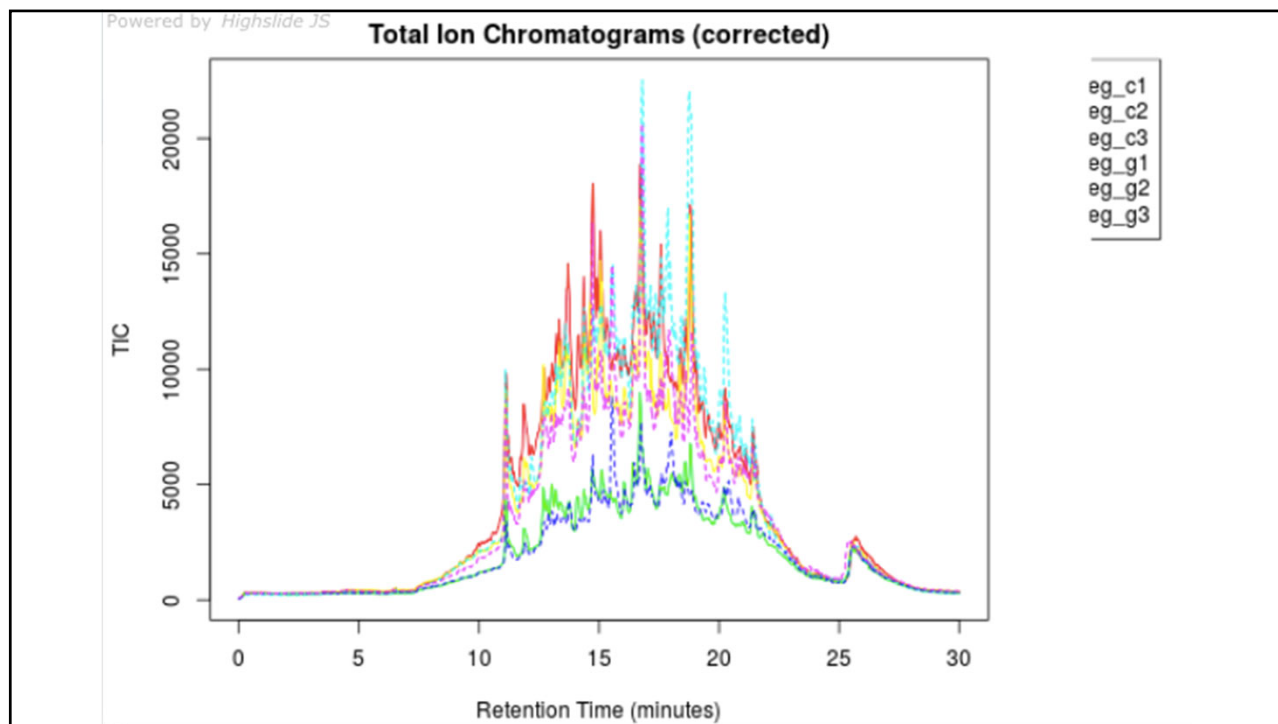


[PNG PDF](#)



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[Citation Links](#)

 Watson

[Results Table](#)

[Metabolomic Cloud Plot](#)

[Interactive Heatmap](#)

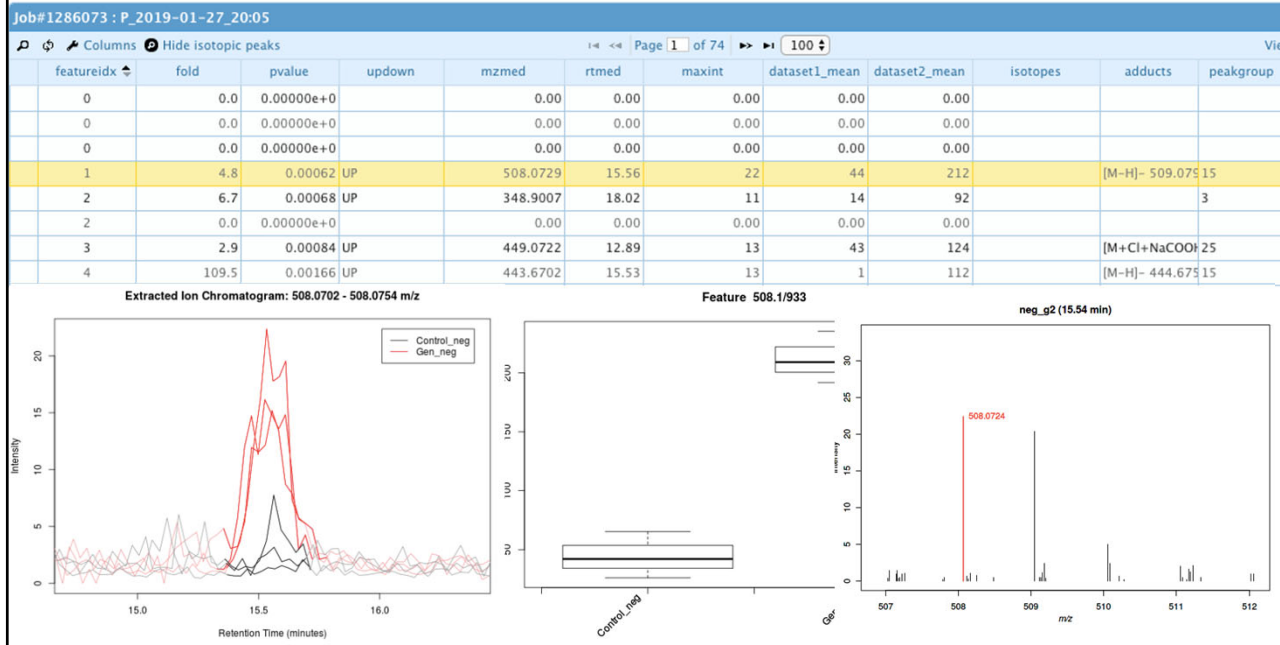
[iPCA](#)

[Activity Network \(Connections\)](#)

[Systems Biology Results](#)

[Pathway Cloud Plot](#)

## Results table



## Modifying the Results table

- The default is to order by the p-value (smallest at the top)
- Alternatives:
  - By featureIDX
  - By m/z
  - By retention time
  - By peak group

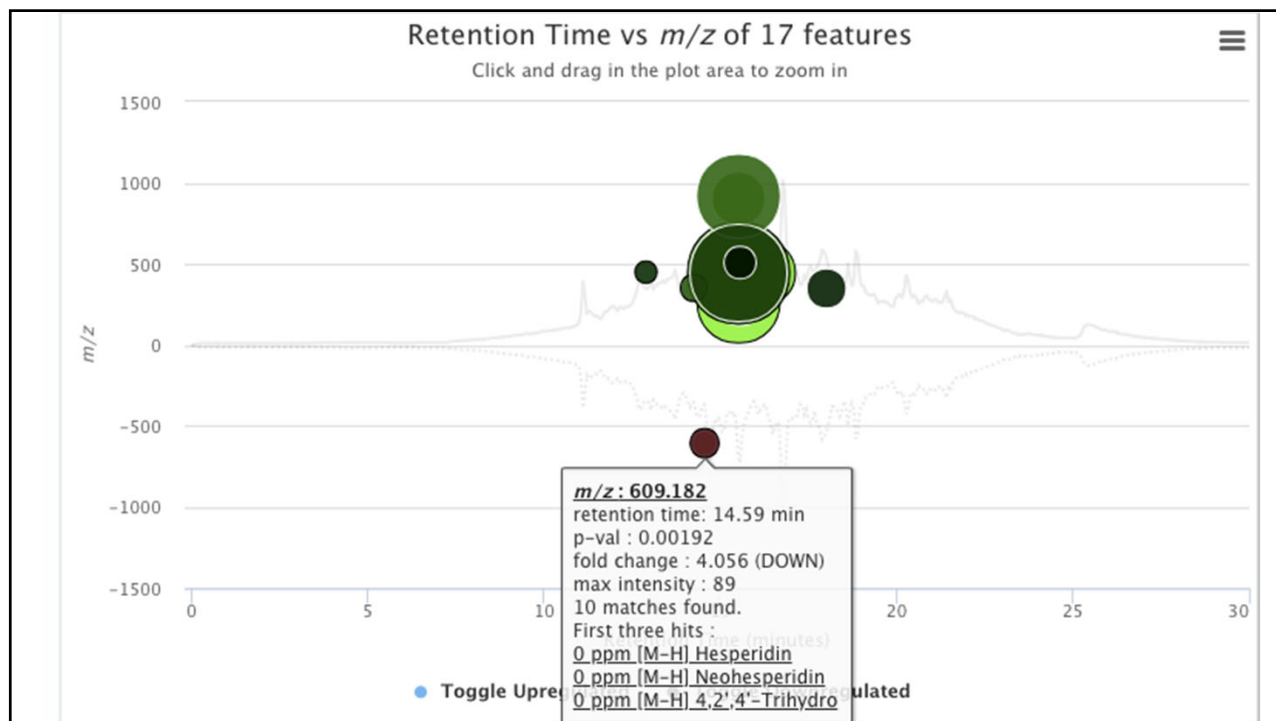
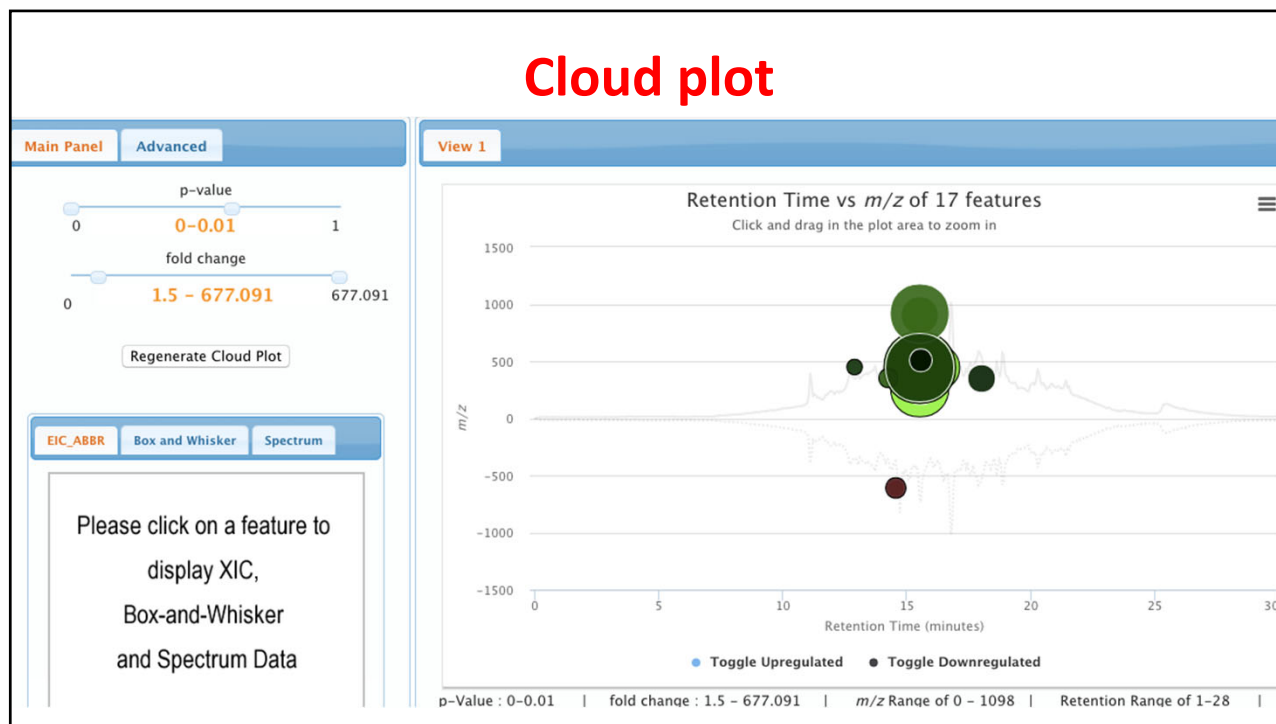
Job#1286073 : P_2019-01-27_20:05										
Columns Hide isotopic peaks Page 1 of 74 100										
featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean	isotopes	
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
1	4.8	0.00062	UP	508.0729	15.56	22	44	212		
2	6.7	0.00068	UP	348.9007	18.02	11	14	92		
2	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
3	2.9	0.00084	UP	449.0722	12.89	13	43	124		
4	109.5	0.00166	UP	443.6702	15.53	13	1	112		
5	4.0	0.00193	DOWN	609.1823	14.59	89	581	144	[728][M]-	
6	3.6	0.00202	UP	352.9973	14.25	20	61	223		
7	57.6	0.00296	UP	918.1215	15.53	10	1	36	[929][M+1]2-	
8	12.9	0.00376	UP	902.1550	15.54	9	3	38	[924][M]2-	
9	138.1	0.00548	UP	445.0773	15.53	1,734	128	17,617	[494][M]-	
10	64.1	0.00648	UP	446.0812	15.54	395	63	4,059	[494][M+1]-	
11	53.7	0.00684	UP	445.5776	15.53	13	2	81		
12	3.2	0.00692	UP	763.2073	15.60	7	15	49		
13	3.5	0.00732	UP	481.0435	14.97	5	11	39		
14	3.2	0.00754	DOWN	610.1852	14.59	33	200	63	[728][M+1]-	
15	19.3	0.00805	UP	445.0772	16.30	111	54	1,049		
16	10.4	0.00894	UP	270.0496	15.56	22	21	222	[99][M+1]-	
17	54.8	0.00909	UP	269.0459	15.53	122	23	1,255	[99][M]-	
18	615.5	0.01252	UP	668.6207	15.53	89	1	596	[771][M+1]2-	

Job#1286073 : P_2019-01-27_20:05										
Columns Hide isotopic peaks Page 1 of 74 100										
featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean		
2	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00		
5509	1.1	0.81689	DOWN	61.9936	19.16	29	707	657		
1607	1.1	0.37015	DOWN	61.9938	25.61	108	1,409	1,333		
1571	1.3	0.36494	UP	61.9938	25.98	165	3,221	4,076		
467	1.3	0.18131	UP	68.9999	25.73	29	544	699		
4042	1.3	0.64948	DOWN	69.0000	26.24	37	1,349	1,067		
1238	5.5	0.31731	UP	79.9594	17.39	14	9	47		
3121	1.1	0.55039	DOWN	88.9906	0.14	31	218	192		
5779	1.2	0.84934	UP	96.9625	26.01	27	395	455		
6212	1.1	0.89322	UP	96.9628	4.36	27	524	564		
6731	1.1	0.94195	DOWN	107.0523	15.13	26	108	101		
3407	1.1	0.58041	UP	112.9873	16.40	47	1,082	1,208		
2478	1.3	0.47656	UP	112.9874	26.81	82	599	784		
1594	1.1	0.36856	UP	112.9874	25.73	178	4,593	5,048		
3284	1.2	0.56753	UP	112.9875	14.97	37	1,031	1,191		
7131	1.0	0.98180	UP	112.9876	17.13	57	2,267	2,289		
6528	1.0	0.92176	DOWN	112.9882	20.58	63	1,486	1,462		
824	1.8	0.24933	DOWN	112.9885	26.21	207	7,867	4,360		
422	1.7	0.17115	UP	116.0376	16.51	11	41	68		

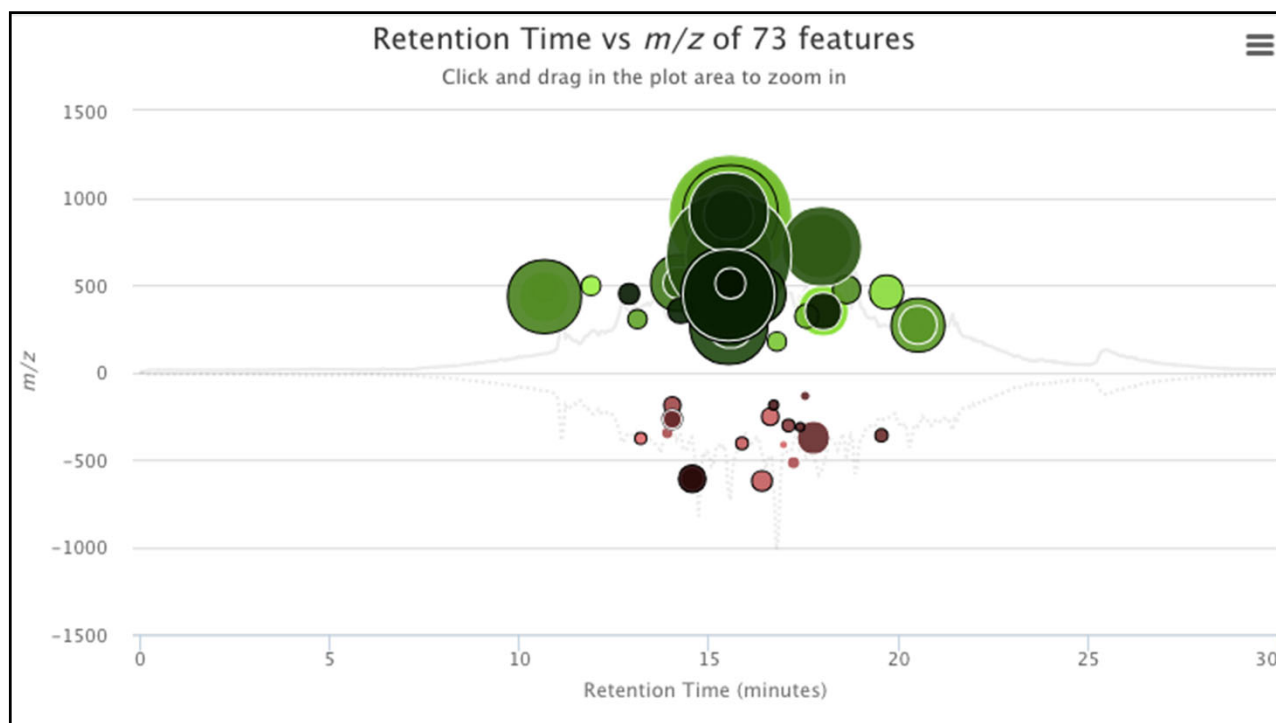
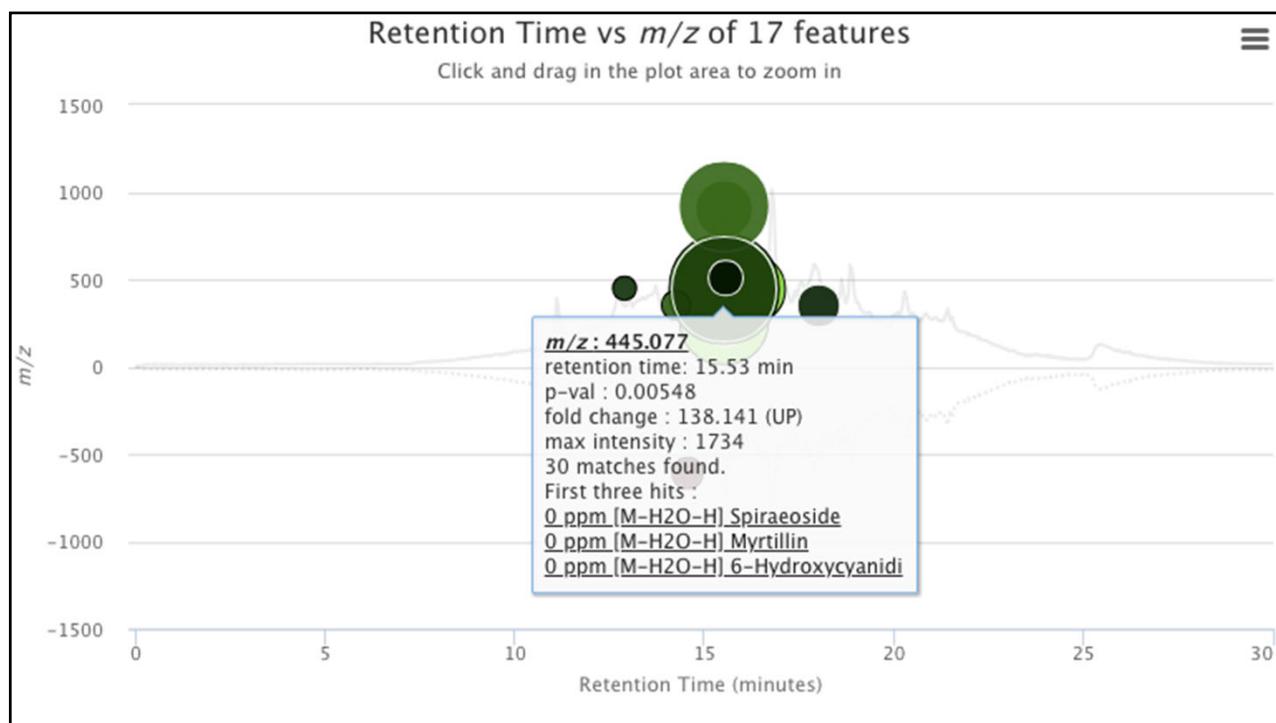
Job#1286073 : P_2019-01-27_20:05									
Columns Hide isotopic peaks Page 1 of 74 100									
featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean	
2	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00	
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00	
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00	
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00	
3121	1.1	0.55039	DOWN	88.9906	0.14	31	218	192	
6212	1.1	0.89322	UP	96.9628	4.36	27	524	564	
2675	1.4	0.49970	DOWN	191.0213	6.50	16	122	86	
3033	1.6	0.53904	DOWN	351.0566	6.50	10	25	16	
6463	1.1	0.91619	UP	333.0576	6.50	5	11	12	
5722	1.1	0.84272	DOWN	243.0631	6.50	7	19	16	
3352	1.4	0.57489	DOWN	129.0211	7.76	14	73	53	
3276	1.4	0.56665	DOWN	173.0105	7.77	42	210	148	
3678	1.6	0.60871	DOWN	330.4924	8.60	24	324	201	
2933	1.7	0.52789	DOWN	939.3695	8.60	10	40	23	
1705	1.8	0.38241	DOWN	661.9922	8.60	22	277	155	
4418	1.4	0.69351	UP	879.3847	8.77	15	36	51	
6765	1.0	0.94514	UP	283.0689	8.83	7	26	27	
1347	2.4	0.33466	DOWN	968.4482	8.85	7	28	12	
1182	2.9	0.30585	DOWN	967.4489	8.86	12	52	18	
6092	1.1	0.88116	DOWN	913.3536	8.91	10	36	32	
6888	1.0	0.95673	UP	296.1005	8.94	15	51	52	
5029	1.4	0.76488	UP	551.2454	9.00	13	36	49	

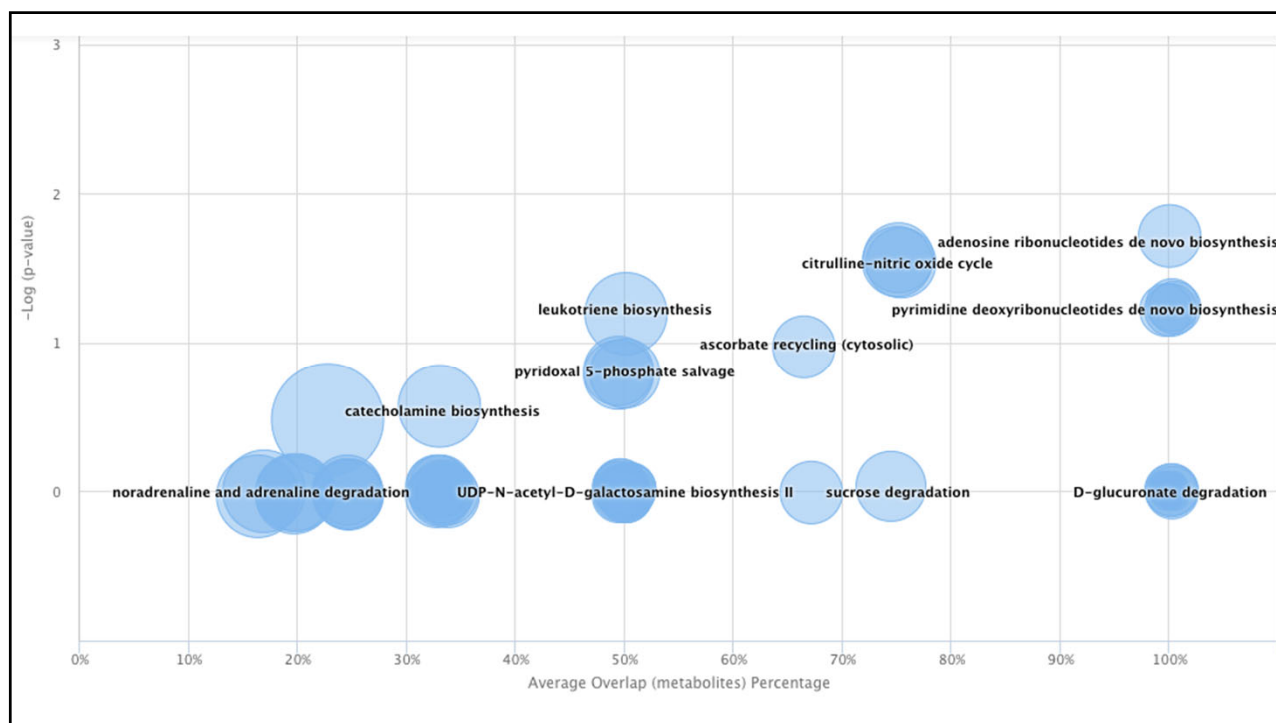
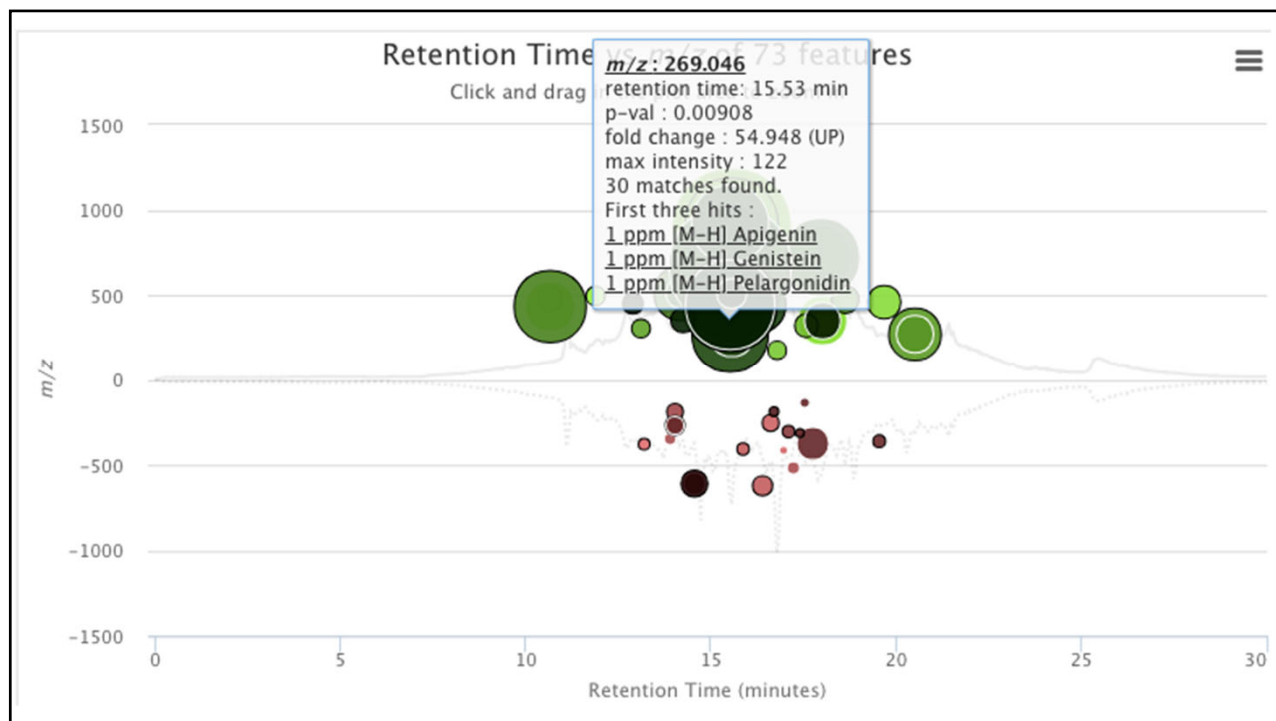
Job#1286073 : P_2019-01-27_20:05												
Columns Hide isotopic peaks Page 1 of 74 100 View												
featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean	isotopes	adducts	peakgroup	
				0.00	0.00	0.00	0.00	0.00				
				0.00	0.00	0.00	0.00	0.00				
				0.00	0.00	0.00	0.00	0.00				
				0.00	0.00	0.00	0.00	0.00				
108	1.8	0.07437	UP	470.2837	18.81	11	32	59				1
143	1.6	0.08868	DOWN	563.1533	18.81	5	31	19				1
181	2.2	0.10201	UP	622.3947	18.86	13	16	36				1
222	4.1	0.11451	DOWN	512.1313	18.78	12	44	11				1
322	2.9	0.14486	DOWN	511.1284	18.79	44	163	56				1
382	2.1	0.16211	DOWN	390.0652	18.85	12	72	35				1
509	3.3	0.19001	UP	424.2780	18.82	17	22	74	[440][M+1]-			1
1040	2.3	0.28342	DOWN	242.9104	18.82	13	51	23				1
1053	1.5	0.28573	DOWN	495.1675	18.85	42	430	282				1
1198	2.8	0.30904	UP	423.2748	18.83	62	79	221	[440][M]-			1
1218	1.8	0.31305	DOWN	425.1815	18.79	35	289	160				1
1320	1.8	0.33163	UP	469.2825	18.81	31	78	138				1
1407	1.3	0.34236	DOWN	343.1690	18.80	18	180	135				1
1548	1.5	0.36220	UP	342.1306	18.79	15	54	82	[252][M+1]-			1
2093	2.2	0.42854	UP	628.3083	18.85	24	53	116	[745][M+2]-			1
2228	1.7	0.44627	DOWN	240.9151	18.82	13	75	44				1
2303	1.6	0.45532	DOWN	800.3302	18.86	26	110	70				1
2530	2.0	0.48192	UP	672.2672	18.82	58	115	230	[772][M+1]-			1

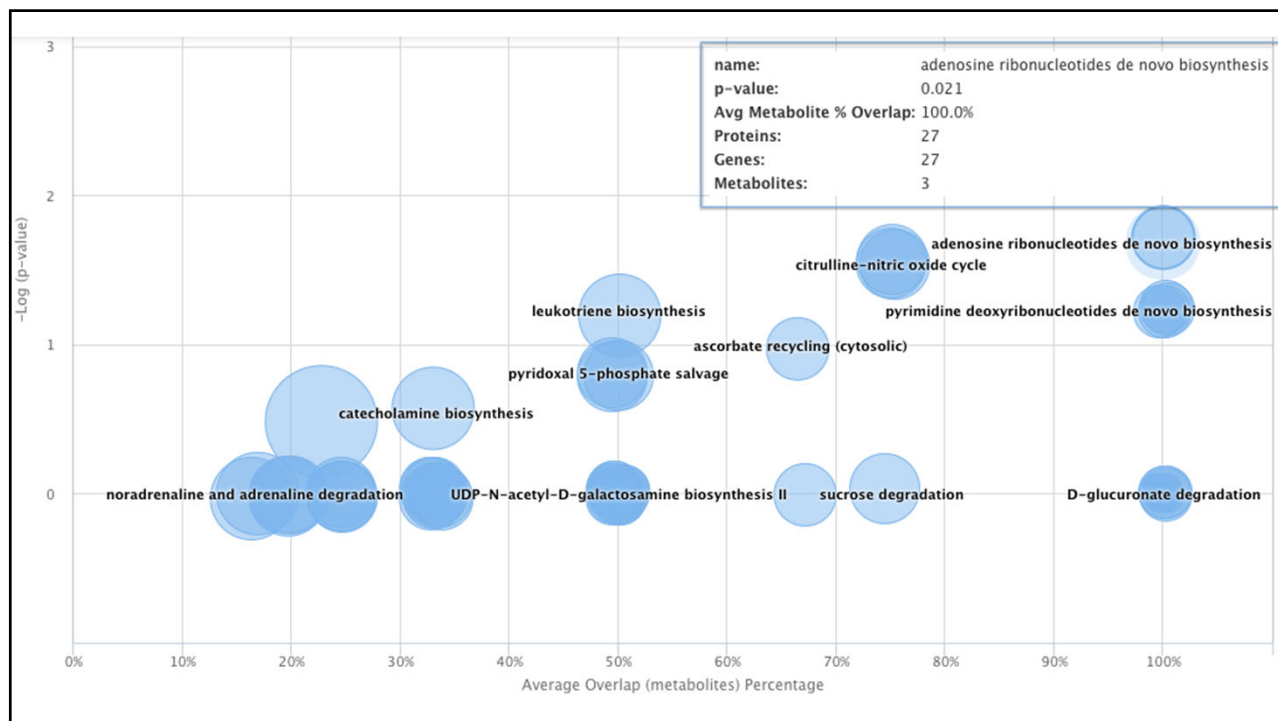
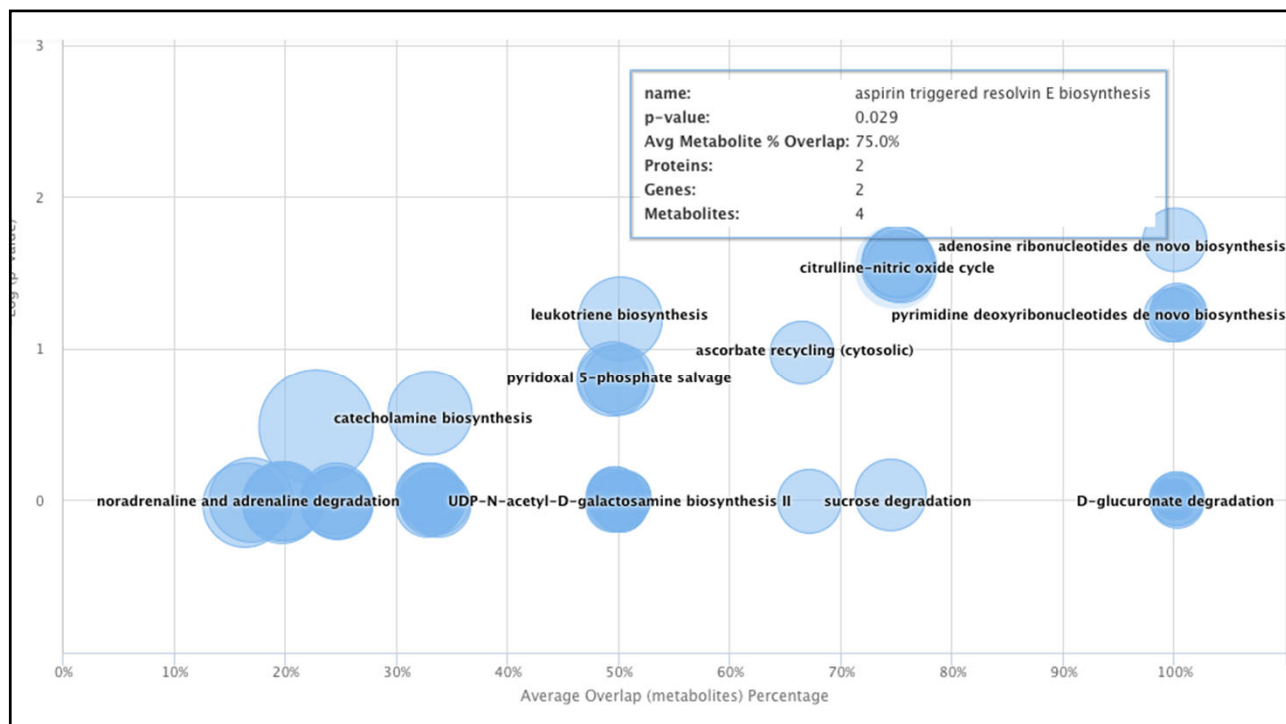
Note that peak group members have the same retention time

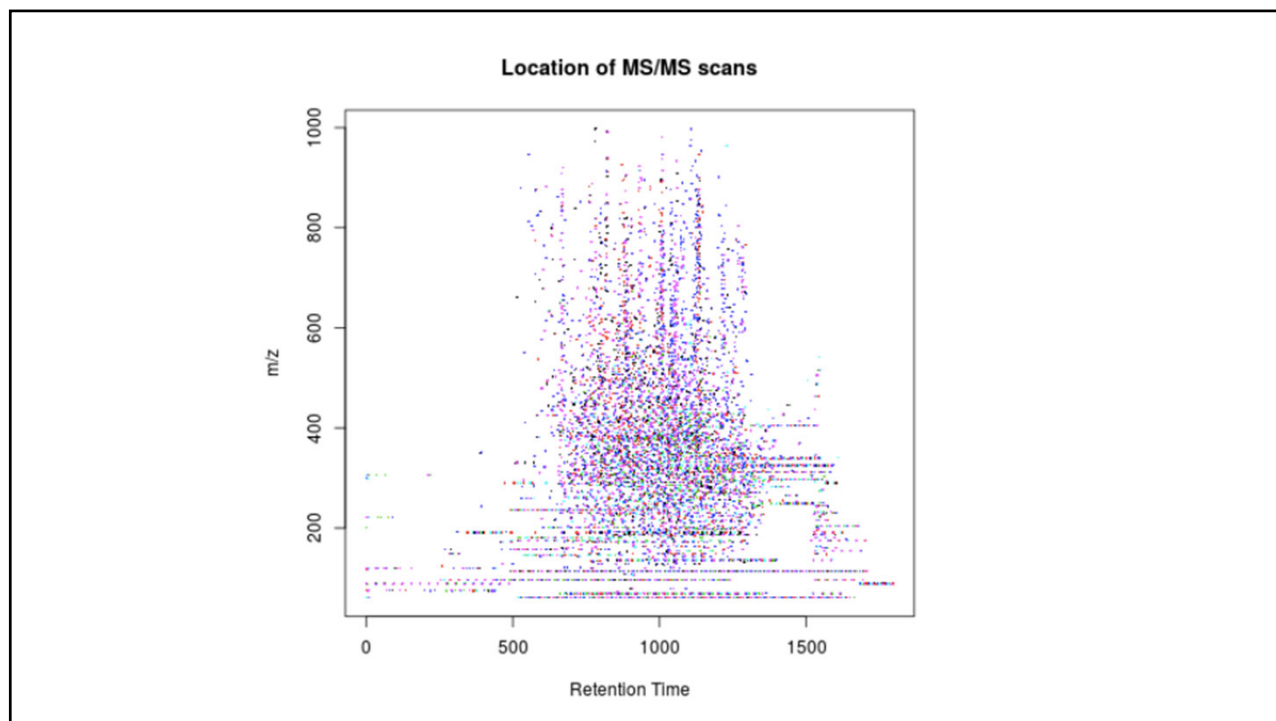












## Class exercise - MZmine

- Load all the C1-C6 and G1-G6 .mzxml files
- Locate the ions that have the ion (in negative) for p-ethylphenol glucuronide ( $C_{14}H_{18}O_7$ ) and p-ethylphenol sulfate ( $C_8H_{10}O_4S$ ) - what are their  $m/z$  values?
  - Make composite XICs for each of these ions (you may want to do C1-C3/G1-G3 and then separately C4-C6/G4-G6 – MZmine has only 6 colors to distinguish samples)
  - Get MSMS spectra of each one (hint choose the sample that has the most intense peak)
- Identify all the *masses* in each file -- from these generate chromatograms, and then deconvolute the chromatograms.
  - Look at the notes from the class on Jan 23
- Output the data into a .csv file (choose row ID,  $m/z$ , retention time, peak height, peak area and FWHM)
- Sort the file by retention time – identify ions that are co-eluting and are isotopes.

## Class exercise - XCMS

- Create a new job
- Upload the C1-C6 and G1-G6 .wiff and .wiffscan files
- Use the parameter settings from last time
- What are the most significant ions? Are their XICs convincing?
- Look at the Cloud plot – are there more significant ions at say  $p < 0.01$  than with just the group comparisons with  $n=3$ ?
- How different is the pathway plot?

## How to present your findings to me?

- Send the .csv file from MZmine
- Present captured pictures in either:
  - A Powerpoint file – make sure that you provide context, either on the slide or in the comment space underneath the slide
- OR
  - A Word file – provide in text the background to what you have done in the analysis
- All are due before class on Monday, February 11