



*Knowledge that will change your world*

## Evaluating XCMS analysis

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## 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**

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## Upload of data files to XCMS

- We uploaded last Wednesday files C4-C6 and G4-G6.
- Now go to XCMS – logon with your ID/Password

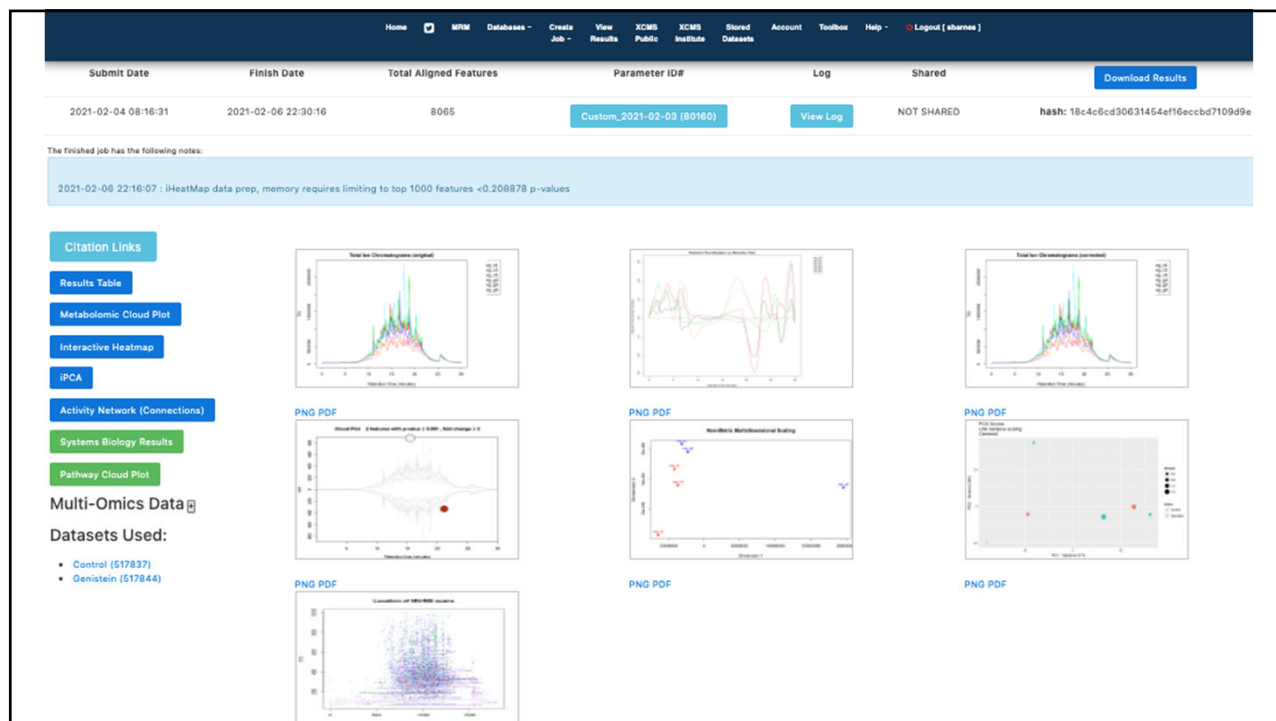


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## Results page

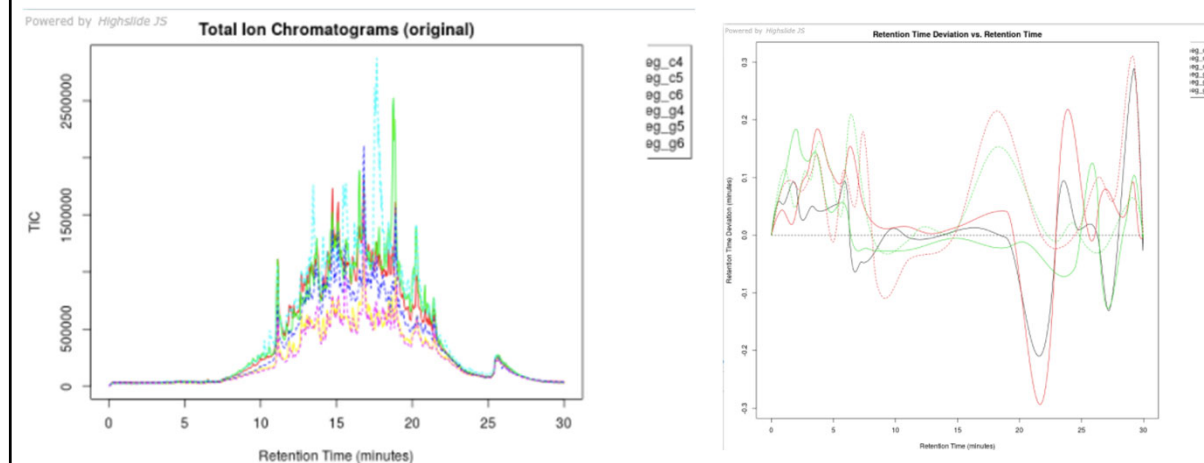
<input type="checkbox"/>	EXPTYPE	Status	JobID Filter	Progress	JobName	Datasets / Sources	Created	Parameters (ID#)	Group Filter	Share	Delete
<input type="checkbox"/>	PAIR	VIEW	1441291	job complete	P_2021-02-03_07.32	Control (#517837) Genistein (#517844)	2021-02-03 07.32.07	80160			

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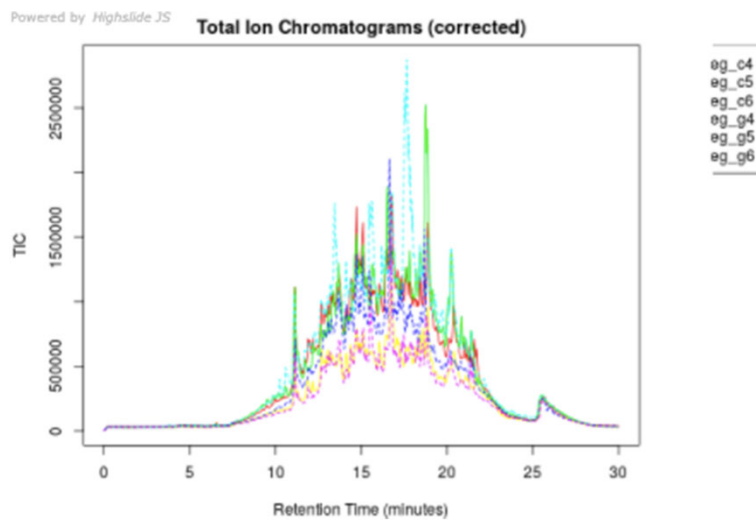
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## Overlay TICs and retention time variation



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## After retention time correction



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[Citation Links](#)[Results Table](#)[Metabolomic Cloud Plot](#)[Interactive Heatmap](#)[iPCA](#)[Activity Network \(Connections\)](#)[Systems Biology Results](#)[Pathway Cloud Plot](#)

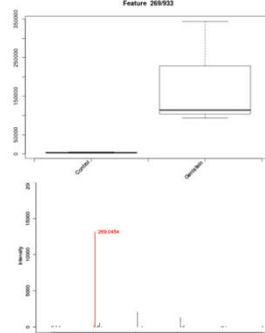
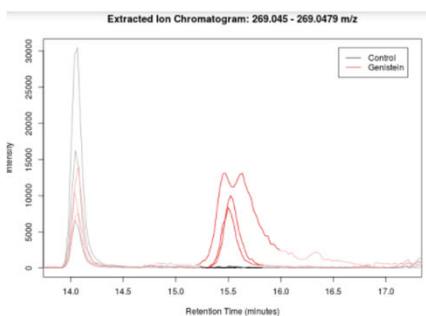
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## Results table

Job#1441291 : P\_2021-02-03\_07:32

Columns Hide isotopic peaks Page 12 of 81 100 View 1,101 - 1,200 of 8,066

featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean	isotopes	adducts	peakgroup	usernotes
2280	34.8	0.33622	UP	269.0463	20.54	60,880	6,931	241,418	[138][M]-		50	
594	54.1	0.15384	UP	269.0464	15.55	13,112	3,395	183,586	[137][M]-	[M-H-C6H8O6]	14	
2442	20.4	0.35095	UP	269.0464	18.04	139,434	72,941	1,491,219	[136][M]-	[M-H]- 270.05	1	
3881	1.9	0.47643	UP	269.0684	16.14	2,224	7,805	14,832	[132][M+1]-		17	
2588	1.2	0.36250	DOWN	269.0688	11.67	481	3,978	3,249		[M-H-C3H2O3]	69	
2966	1.7	0.39651	DOWN	269.0696	15.24	1,609	9,128	5,289			20	
107	5.1	0.04085	UP	269.0736	20.80	1,394	2,911	14,870		[M+Cl]- 234.1	35	
6802	1.2	0.81487	DOWN	269.0859	22.11	372	2,736	2,234		[M+Cl]- 234.1284		
5024	1.3	0.59098	DOWN	269.0895	17.68	2,339	12,031	9,542		[M+Cl]- 234.130		
8012	1.0	0.99098	DOWN	269.1038	19.27	2,176	13,151	13,096	[139][M]-	[M-H]- 270.10625		



ppn	NAME	ADDUCT	METLIN	TOXIN
2	SULFURETINE	M-H	990417	n
3	Apigenin	M-H	3397	n
3	Genistein	M-H	3398	n
3	Pelargonidin	M-H	3399	n
3	Aloe-emodin	M-H	41039	n
3	Emodin	M-H	41045	n
3	2-Hydroxychryso	M-H	41048	n
3	Baicalein	M-H	43859	n

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## 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

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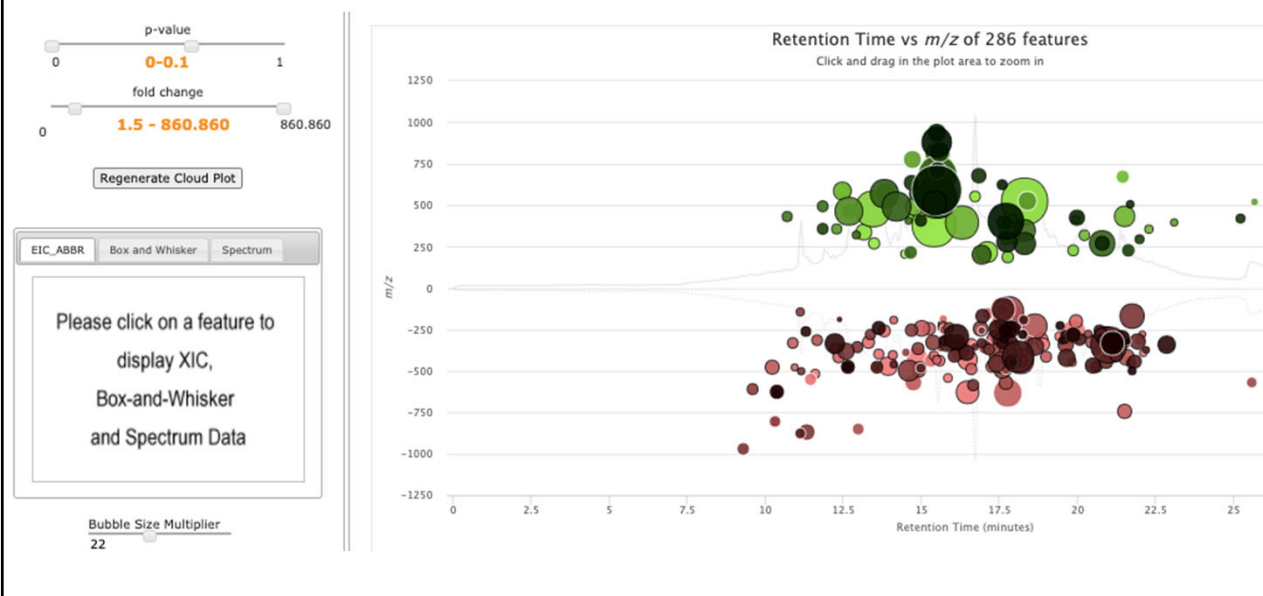
## Data ordered by peak group

Note that peak group members have similar retention times

featureidx	fold	pvalue	updown	mzmed	rtmed	maxint	dataset1_mean	dataset2_mean	Isotopes	adducts	peakgroup
0	0.0	0.00000e+0		0.00	0.00	0.00	0.00	0.00			
55	7.6	0.01922	DOWN	414.0482	18.12	7,363	47,012	6,194			1
152	1.7	0.05395	DOWN	234.1437	18.12	591	4,133	2,496	[77][M+1]-		1
315	1.6	0.09779	DOWN	354.1273	18.08	1,694	17,445	10,906	[324][M+1]-		1
372	1.6	0.10926	UP	387.0910	18.07	1,027	5,085	8,275		[M-H]- 388.10	1
388	1.7	0.11407	DOWN	371.1342	18.13	1,677	11,927	7,188			1
399	1.3	0.11680	DOWN	353.1245	18.13	6,500	62,096	46,501	[324][M]-		1
500	2.7	0.13633	DOWN	392.2094	18.16	2,912	23,182	8,437		[M-H+HCOOH]-	1
541	1.9	0.14504	UP	303.1027	18.10	1,508	6,250	12,148			1
570	1.6	0.14999	DOWN	233.1405	18.12	4,519	31,681	19,834	[77][M]-		1
699	1.6	0.16896	UP	511.2518	18.07	3,007	11,790	18,580		[M-2H+Na]- 49	1
738	10.9	0.17501	UP	463.0445	18.14	3,448	1,098	11,958			1
745	1.3	0.17637	DOWN	141.0936	18.10	440	3,291	2,603		[M-H]- 142.10	1
781	3.3	0.18118	DOWN	443.2280	18.04	7,439	92,328	27,603		[3M+2Cl]- 27	1
845	1.6	0.19085	DOWN	228.0161	18.17	1,103	4,382	2,705	[68][M+2]-		1
904	2.1	0.19740	DOWN	522.2368	18.14	2,166	15,145	7,082		[M-H+NH3]- 5	1
1055	1.4	0.21516	DOWN	307.1325	18.15	2,166	12,164	8,554		[M+Cl]- 272.1	1
1125	3.5	0.22418	UP	957.2084	18.13	1,697	1,720	6,009			1
1237	1.5	0.23839	UP	507.2077	18.17	1,631	11,347	16,863			1
1647	1.7	0.28099	DOWN	573.1803	18.11	1,734	10,791	6,501		[M-H+NaCOOH]-	1

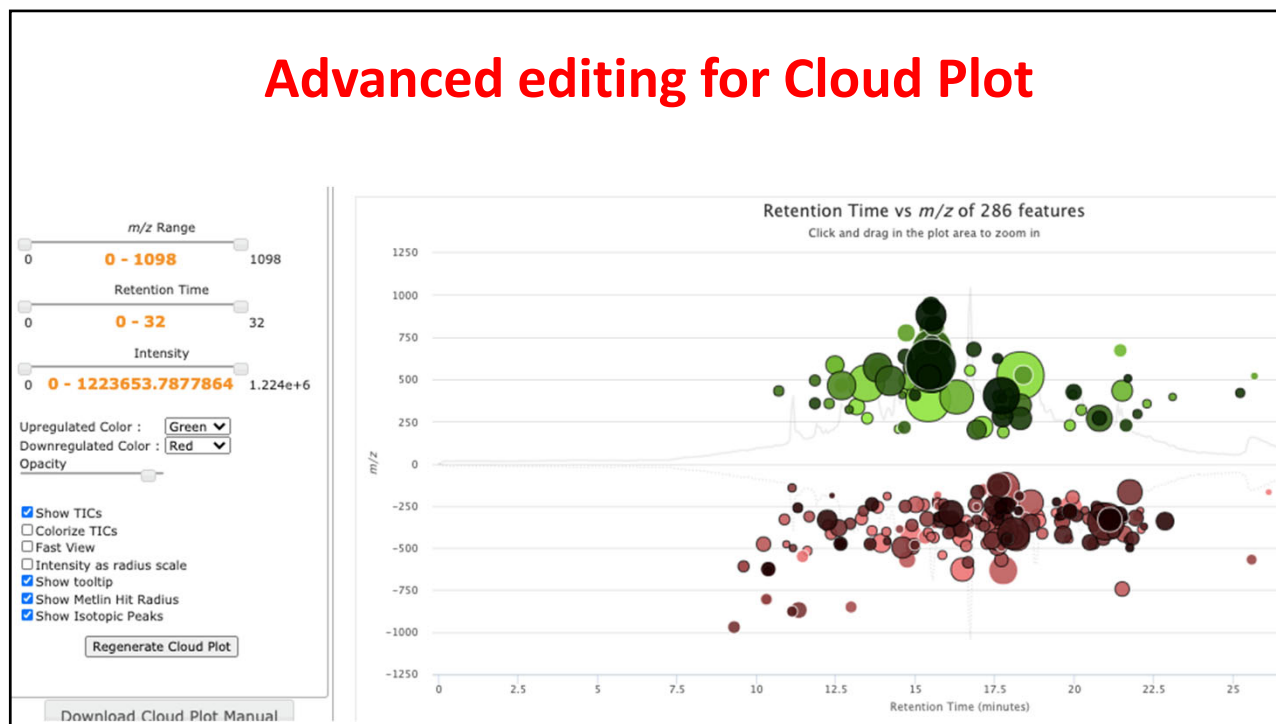
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## Cloud plot p<0.1, FC 1.5 and greater



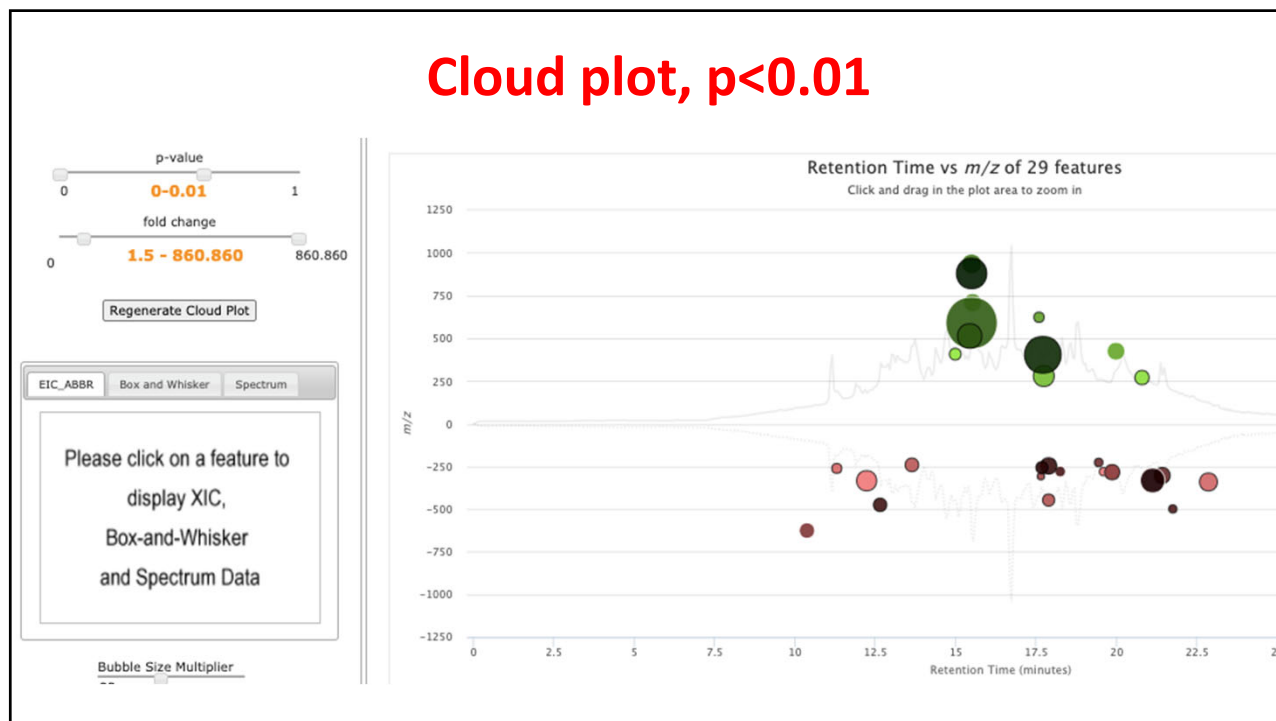
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## Advanced editing for Cloud Plot



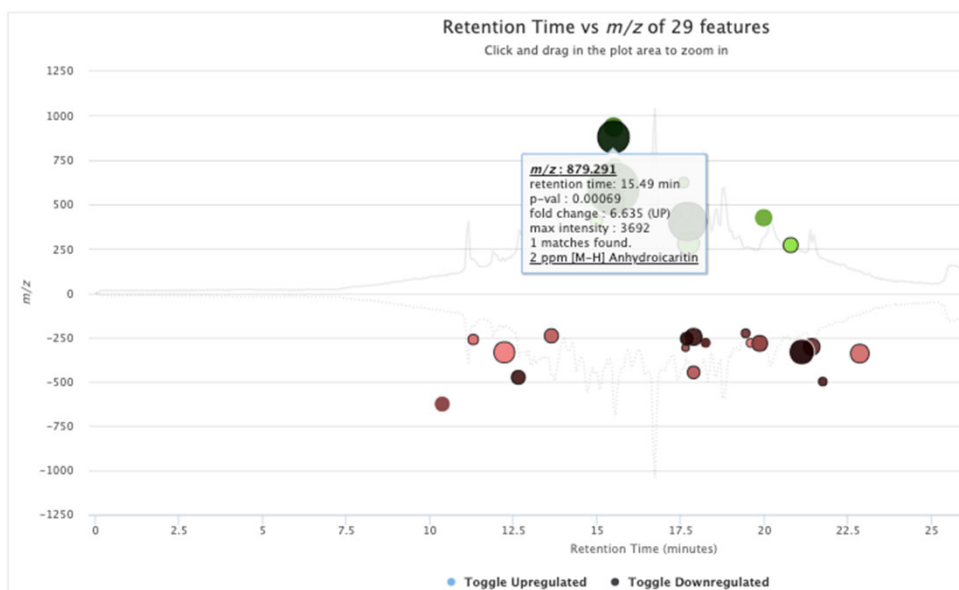
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## Cloud plot, $p < 0.01$



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## Mouse over to reveal spot parameters



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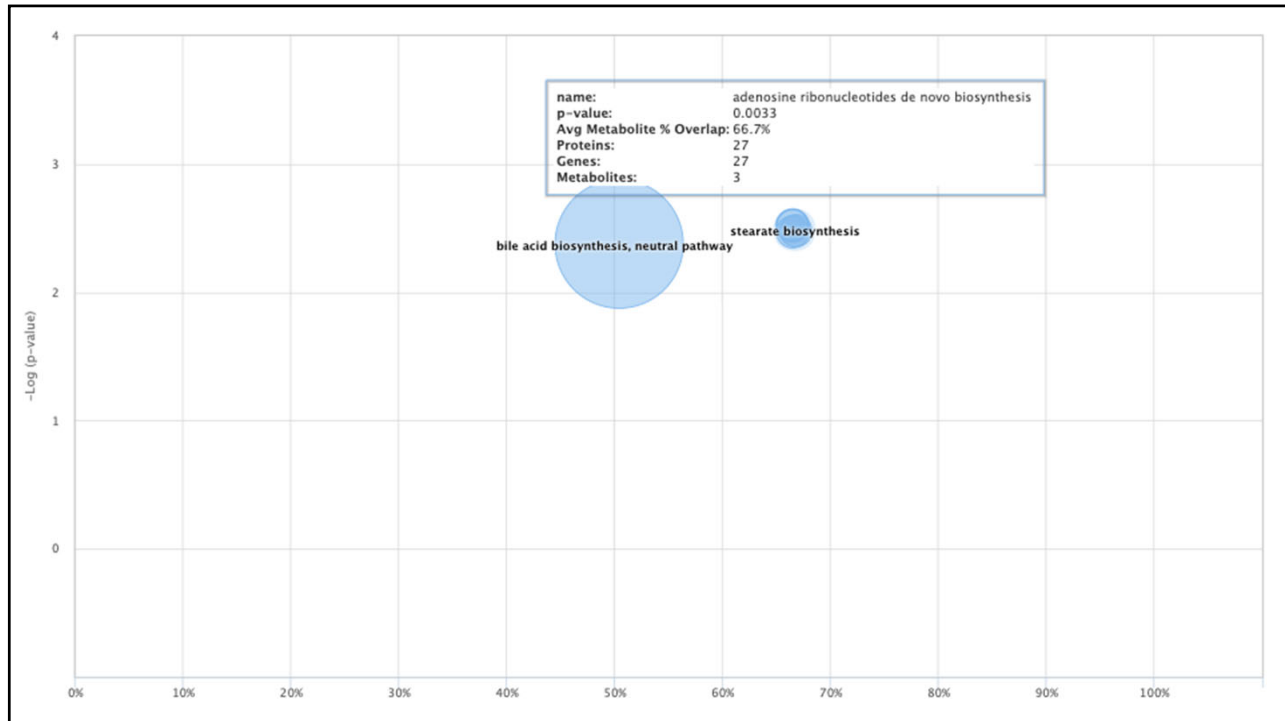
## Pathways identified as being affected by genistein

Pathway Cloud Plot	Pathway	Overlapping genes	All genes	Overlapping proteins	All proteins	Overlapping putative metabolites <sup>1</sup>	All metabolites <sup>2*</sup>	p-values
Predictive Metabolites Results	stearate biosynthesis	0	14	0	29	2	3	3.3e-3
	lipoate biosynthesis and incorporation	0	2	0	2	2	3	3.3e-3
	adenosine ribonucleotides <i>de novo</i> biosynthesis	0	27	0	27	2	3	3.3e-3
	bile acid biosynthesis, neutral pathway	0	24	0	13	2	4	4.3e-3

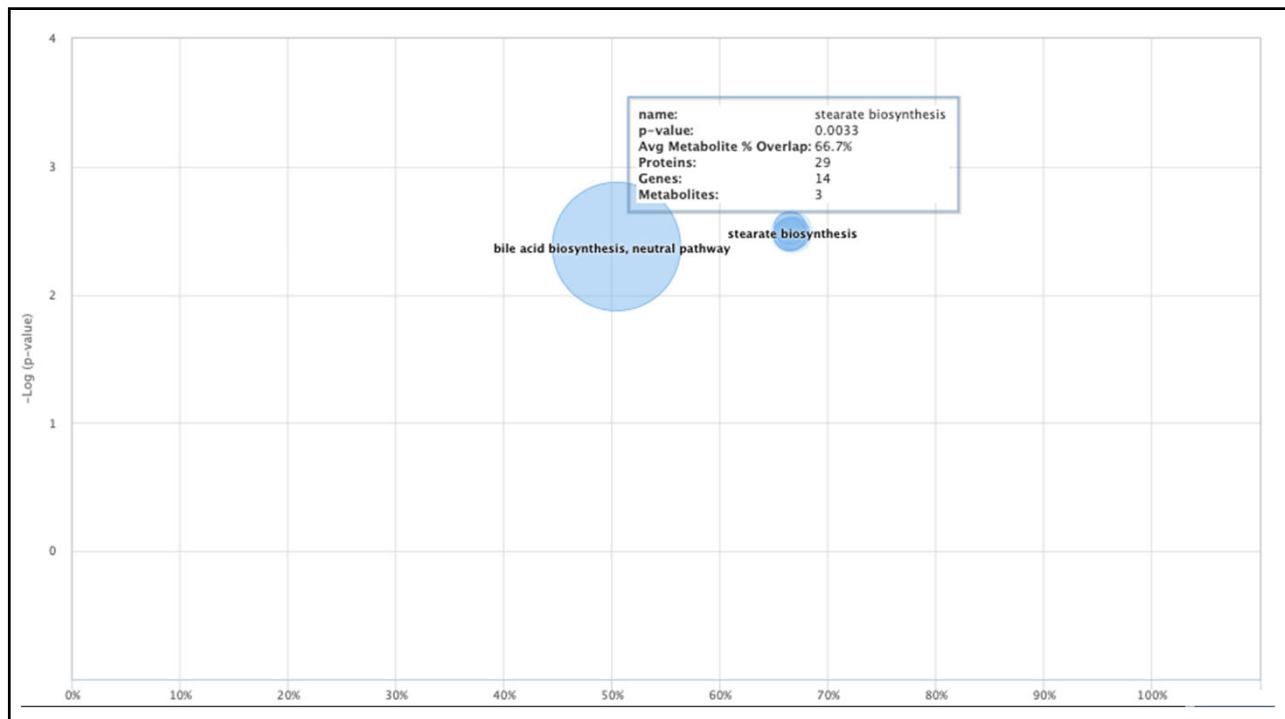
**Note: this analysis was done with the standard parameter file used in TMPL**

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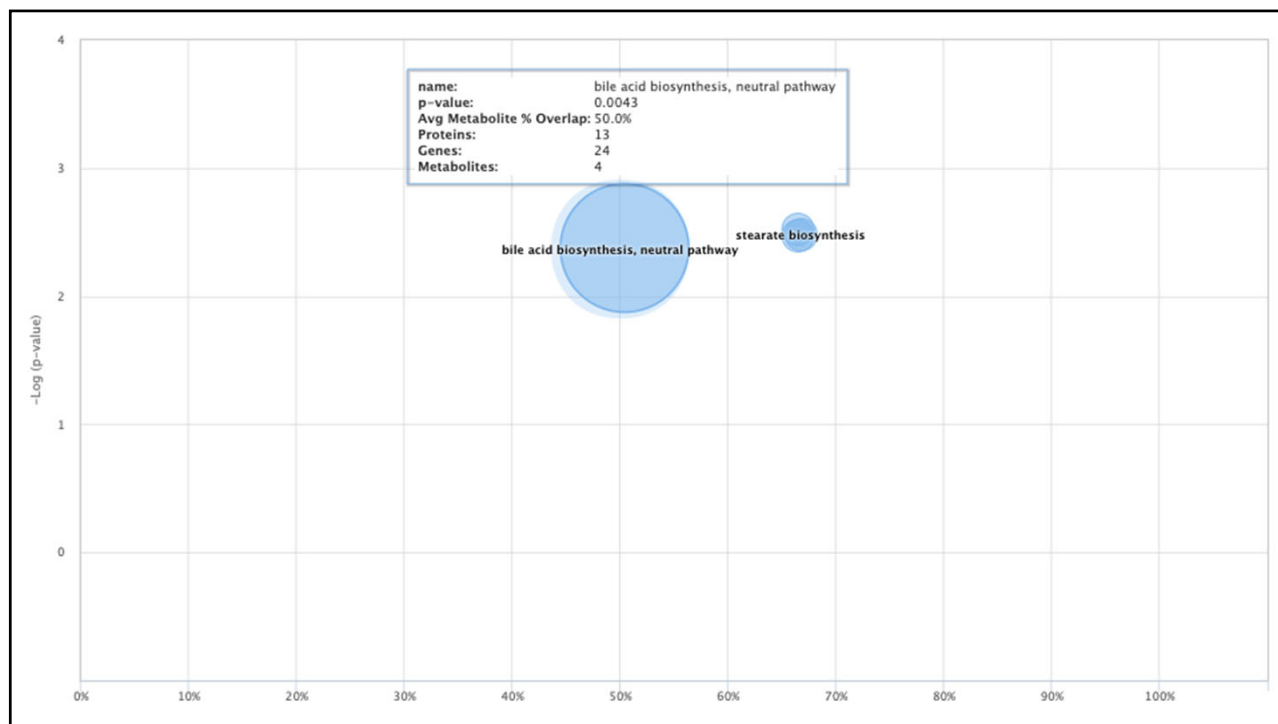




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