



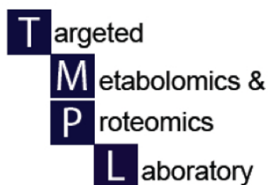
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GBS 724 02-05-16

## Working with the data

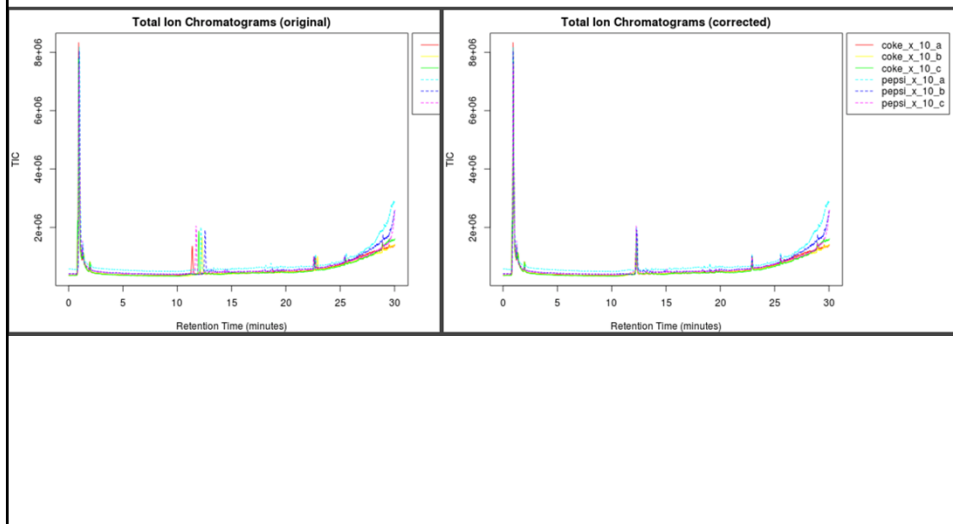
Steve Barnes, PhD



## Examining output on XCMS

- **First, we'll logon to XCMS and inspect two datasets**
  - Coke via Pepsi
- **Then we'll look at the second dataset**
  - Non-irradiated diet vs irradiated diet
- **We'll download the XCMS output file**
  - Make a Volcano plot
  - Prepare files for MetaboAnalyst

## Rt correction - Coke vs Pepsi



## Let's go to XCMSonline

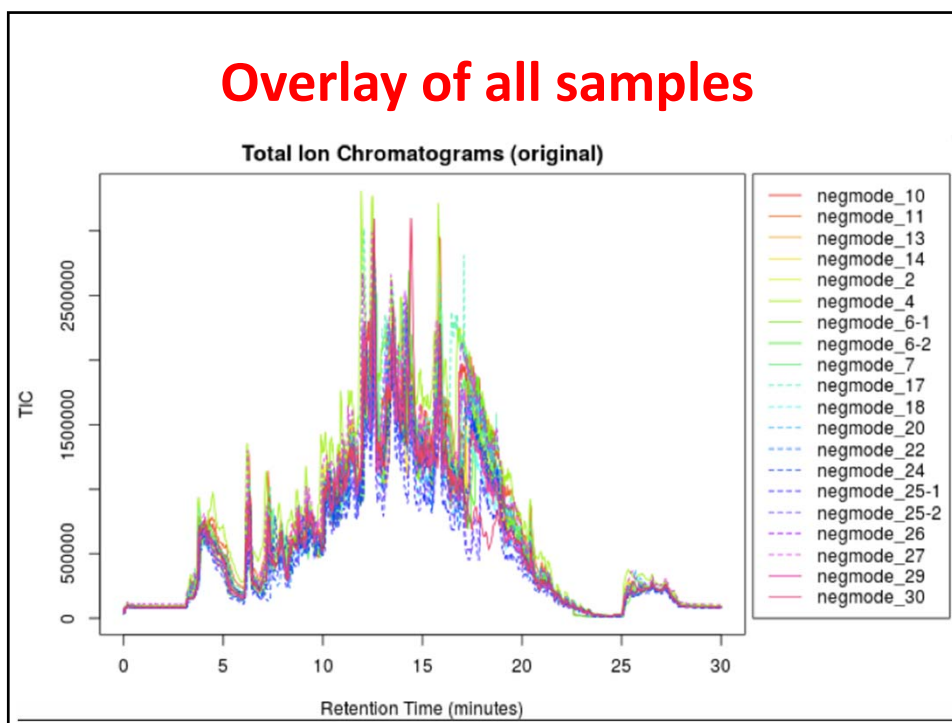
View Jobs

Job Count: 115

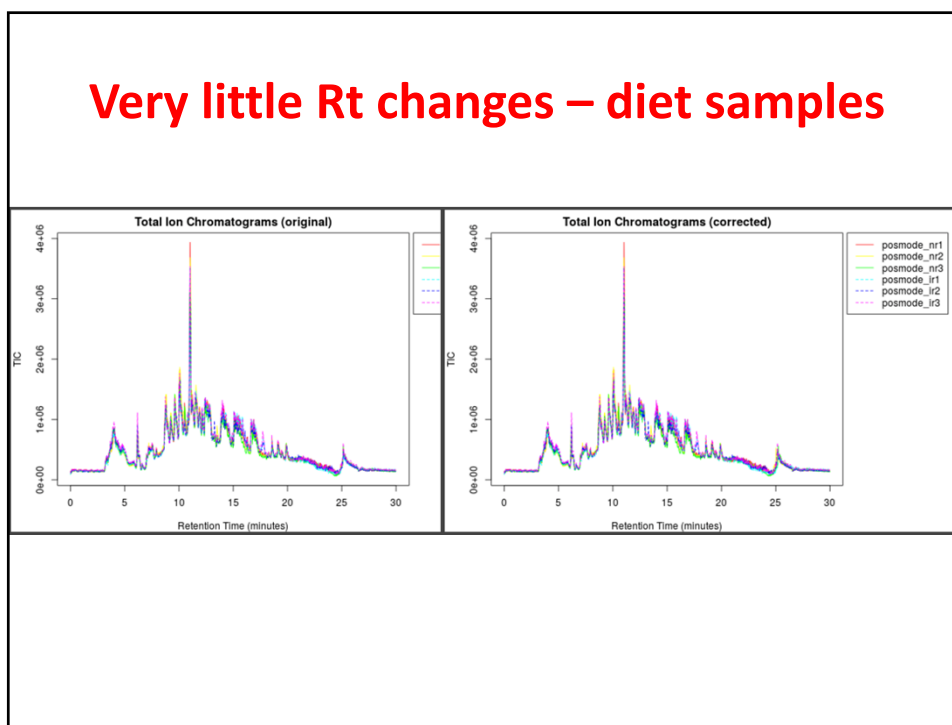
Search Jobs:  Search Clear View Public Shares

Exp Type	Status	ID	Progress	Job Name	Datasets (ID#) [control]	Created	Parameters (ID#)	Group	Shared
PAIR	<span>VIEW</span>	1080273	<span>job complete</span> 100%	pair_2015-10-26_10:27	Miller_negmo (150494)* Miller_negmo (150695)	2015-10-26 10:27:57	NanoLC Neg (10374)		<span>✖</span>
MULTI	<span>VIEW</span>	1078919	<span>job complete</span> 100%	15_1016_Ser2_Pos_AshleeData	Ser2_41B_Po (#148944) Ser2_41B_P (#148945)	2015-10-16 05:39:28	5600 Trpl (15851)	Public	NA
MULTI	<span>VIEW</span>	1078821	<span>job complete</span> 100%	15_1015_Ser2_Neg_AshleeData	Ser2_41B_Neg (#148813) Ser2_41B_N (#148814)	2015-10-15 13:58:44	5600 Trpl (15859)	Public	NA
MULTI	<span>VIEW</span>	1078638	<span>job complete</span> 100%	15_1014_Ser1_Pos_AshleeData	Ser1_41B_P (#148625) Ser1_41_Po (#148626)	2015-10-14 12:04:08	5600 Trpl (15851)	Public	NA
MULTI	<span>VIEW</span>	1078579	<span>job complete</span> 100%	15_1014_Ser1_Neg_AshleeData	Ser1_41B_N (#148595) Ser1_41_Neg (#148596)	2015-10-14 06:14:27	5600 Trpl (15859)	Public	NA
PAIR	<span>VIEW</span>	1077952	<span>job complete</span> 100%	pair_2015-10-09_09:04	McLean_Ser1A (147900)* McLean_NewSe (147694)	2015-10-09 09:04:32	nanoLC_560 (9920)		<span>✖</span>
PAIR	<span>VIEW</span>	1077790	<span>job complete</span> 100%	McLean_newFF_100815	McLeanNew_No (147860)* McLean_NewCb (147861)	2015-10-08 12:03:32	nanoLC_560 (9920)		<span>✖</span>
PAIR	<span>VIEW</span>	1077057	<span>job complete</span> 100%	pair_2015-10-02_12:32	FruitFly_Tes (147174)* WaterBlankPo (147166)	2015-10-02 12:32:16	nanoLC_560 (9920)		<span>✖</span>
PAIR	<span>VIEW</span>	1076715	<span>job complete</span> 100%	Repeat_GrubbsUrline_Negmode_093015	Grubbs_Urine (107534)* Grubbs_Urine (107628)	2015-09-30 10:38:05	NanoLC600 (10377)		<span>✖</span>

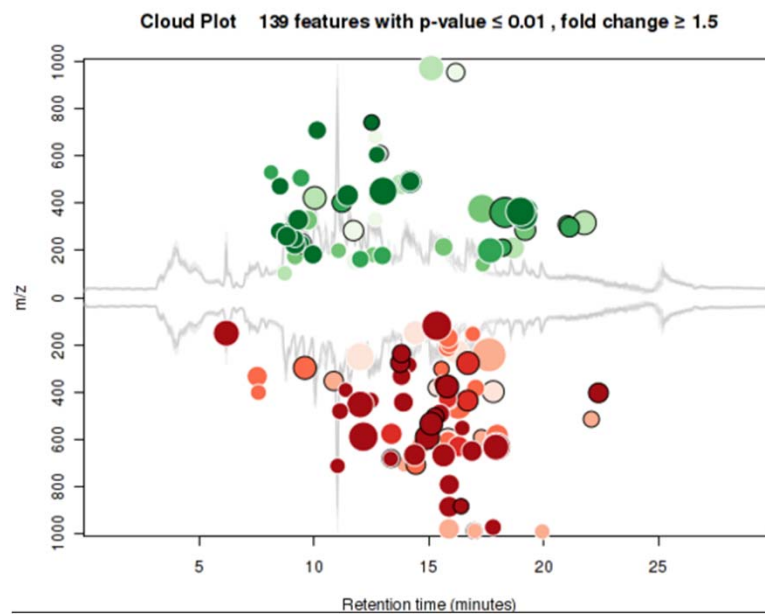
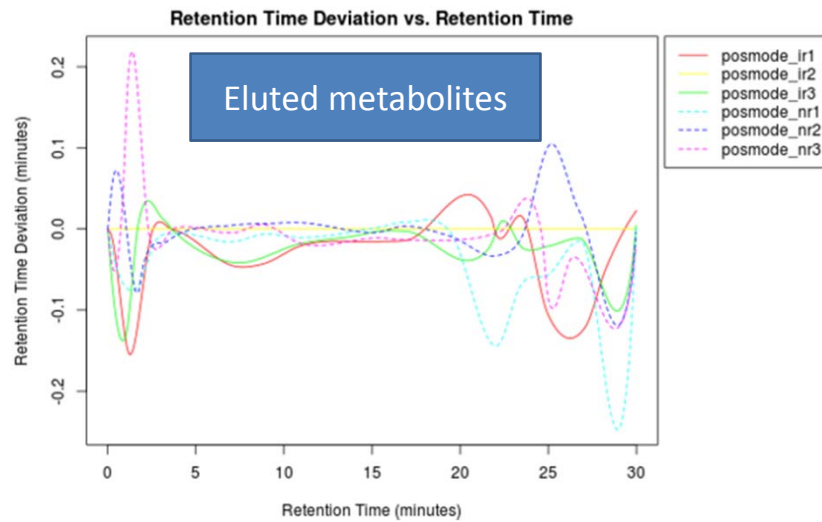
## Overlay of all samples



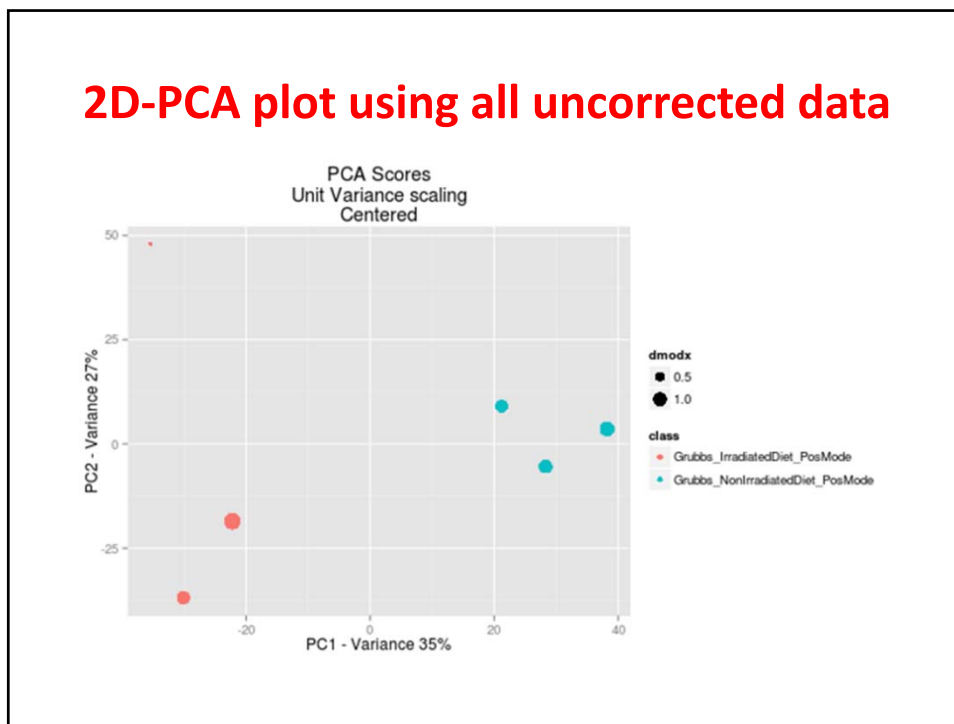
## Very little Rt changes – diet samples



## Rt variation for all samples



## 2D-PCA plot using all uncorrected data



## Download the processed Excel file

**Pairwise Results Summary: Grubbs\_Diet\_PosMode (#1047227)** [Download Results](#) hash: c6b73a0e273acf3290623276d9d88b2

Submit Date	Finish Date	Paired Samples	Total Aligned Features	Parameter ID#	Log	Shared
2015-02-05 17:51:50	2015-02-06 10:37:58	False	3099	nanol_C_5609_TripleTO (9920)	<a href="#">View Log</a>	NOT SHARED

**WARNINGS:**

2015-02-06 10:36:05 : HeatMap data prep, memory requires limiting to top 1000 features <0.16336 p-values

[View Results Table](#)

[View Interactive Cloud Plot](#)

[View Interactive Heatmap](#)

[View PCA](#)

**Datasets Used**

- Grubbs\_Irradiat (108909)
- Grubbs\_NonIrrad (109003)

## Synopsis

- Downloading data files from XCMSOnline
- Opening the .zip file
- Creating the Excel file from result.tsv file
- Why a Volcano plot?
  - Making a volcano plot
  - Using a macro?
- MetaboAnalyst

▶	boxplot	Feb 6, 2015, 10:17 AM	--	Folder
	CloudPlot-svg.svg	Feb 6, 2015, 10:37 AM	575 KB	SVG document
	CloudPlot.pdf	Feb 6, 2015, 10:37 AM	234 KB	PDF Document
	CloudPlot.png	Feb 6, 2015, 10:37 AM	64 KB	PNG image
▶	EIC	Feb 6, 2015, 10:18 AM	--	Folder
	Heatmap_1047227.png	Feb 6, 2015, 10:18 AM	46 KB	PNG image
	Heatmap_Cor_1047227.png	Feb 6, 2015, 10:36 AM	376 KB	PNG image
	MDS.pdf	Feb 6, 2015, 10:36 AM	5 KB	PDF Document
	MDS.png	Feb 6, 2015, 10:36 AM	16 KB	PNG image
	MVstats_ScalingPlot_1047227.pdf	Feb 6, 2015, 10:36 AM	82 KB	PDF Document
	PCA-diagnostics.pdf	Feb 6, 2015, 10:36 AM	5 KB	PDF Document
	PCA-diagnostics.png	Feb 6, 2015, 10:36 AM	5 KB	PNG image
	PCA-loadings-all.pdf	Feb 6, 2015, 10:36 AM	29 KB	PDF Document
	PCA-loadings-all.png	Feb 6, 2015, 10:36 AM	18 KB	PNG image
	PCA.pdf	Feb 6, 2015, 10:36 AM	5 KB	PDF Document
	PCA.png	Feb 6, 2015, 10:36 AM	9 KB	PNG image
	result.tsv	Feb 6, 2015, 10:37 AM	1 MB	Plain Text
	rtcor.pdf	Feb 6, 2015, 10:15 AM	51 KB	PDF Document
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	XCMS.diffreport..Grubbs_Irra...nIrradiatedDiet_PosMode.tsv	Feb 6, 2015, 10:18 AM	1.2 MB	Plain Text
	XCMS.diffreport..Grubbs_Irra...IrradiatedDiet_PosMode.xlsx	Feb 6, 2015, 10:18 AM	919 KB	Micros...(xlsx)
	XCMSOnline_log.txt	Feb 6, 2015, 10:37 AM	2 KB	Plain Text

Open this Excel file



# The Excel DiffReport from XCMS

	name	fold	log2fold	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	npeaks	Grubbs_lrr	Grubbs_No	maxint
1	M260T9	2.066231	1.047001	52.9784	4.48E-06	0.008947	UP	260.1494	260.147	260.1507	8.820283	8.79305	8.82015	3	0	3	812
2	M554T16	1.603843	-0.68153	-21.311	5.35E-05	0.034248	DOWN	553.7874	553.7773	553.7881	16.4607	16.45587	16.46268	3	3	0	1036
3	M365T19	4.732957	2.242742	28.44062	6.61E-05	0.034248	UP	365.3147	365.3146	365.3152	18.99483	18.9895	19.00633	3	0	3	1405
4	M250T9	1.72652	0.787867	18.03406	8.97E-05	0.034248	UP	250.0274	250.0261	250.0306	9.184317	9.157483	9.206233	3	0	3	1002
5	M347T19	4.266528	2.093062	16.25312	9.19E-05	0.034248	UP	347.2915	347.29	347.2931	19.12692	19.08167	19.17217	2	0	2	978
6	M590T12	5.036719	-2.33248	-20.0766	0.000103	0.034248	DOWN	590.3727	590.3708	590.3746	12.15744	12.14798	12.1669	2	2	0	570
7	M482T11	1.712481	-0.77609	-17.9095	0.00015	0.040827	DOWN	481.9515	481.9483	481.9547	11.14359	11.11702	11.17017	2	2	0	406
8	M330T9	2.258089	1.175103	15.09507	0.000163	0.040827	UP	330.1959	330.1949	330.1965	9.323967	9.31655	9.328883	3	0	3	2591
9	M535T15	2.503811	-1.32413	-20.1752	0.000199	0.044224	DOWN	535.1078	535.0998	535.1093	15.11455	15.11107	15.14075	3	3	0	5038
10	M669T16	2.802279	-1.4866	-11.4148	0.000354	0.0561	DOWN	669.1933	669.1884	669.1981	15.64988	15.64847	15.65128	2	2	0	1290
11	M454T12	3.772682	-1.91559	-16.3974	0.000366	0.0561	DOWN	453.794	453.7929	453.7981	12.02213	12.00212	12.02257	3	3	0	539
12	M238T14	1.791233	-0.84095	-19.4555	0.000393	0.0561	DOWN	238.0703	238.0672	238.0735	13.81681	13.81423	13.81938	2	2	0	2098
13	M634T18	3.466381	-1.79343	-14.2648	0.000412	0.0561	DOWN	634.2947	634.2865	634.296	17.92467	17.91633	17.95633	3	3	0	1630
14	M434T11	2.382244	1.252321	10.7818	0.00051	0.0561	UP	433.9209	433.9164	433.9215	11.47543	11.45125	11.47647	3	0	3	614
15	M507T15	1.82248	-0.8659	-10.9623	0.000524	0.0561	DOWN	507.113	507.1056	507.1147	15.27278	15.24695	15.27437	6	3	3	14195
16	M431T14	1.366759	0.450759	13.74105	0.000552	0.0561	UP	431.1934	431.1906	431.203	13.74835	13.69858	13.75413	3	0	3	2065
17	M667T14	2.485365	-1.31346	-13.247	0.000602	0.0561	DOWN	667.2951	667.2859	667.2966	14.38968	14.35893	14.41728	6	3	3	21387
18	M120T15	5.182828	-2.37375	-10.0467	0.000623	0.0561	DOWN	119.5384	119.5375	119.5398	15.35508	15.32717	15.35515	3	3	0	812
19	M684T13	1.620399	-0.69635	-10.3018	0.000635	0.0561	DOWN	684.1574	684.1572	684.1576	13.35001	13.3386	13.36142	2	2	0	726
20	M281T9_1	1.82361	0.866797	12.53908	0.000655	0.0561	UP	281.1265	281.1228	281.137	8.502767	8.471933	8.5337	4	1	3	14440
21	M404T22	2.007163	-1.00516	-9.56783	0.00067	0.0561	DOWN	404.2992	404.2916	404.2996	22.38883	22.38433	22.4315	3	3	0	695
22	M793T16	2.165377	-1.1462	-10.177	0.000678	0.0561	DOWN	793.3024	793.2985	793.3063	15.89646	15.89375	15.89917	2	2	0	992
23	M183T15	1.325866	-0.40694	-9.70303	0.000749	0.0561	DOWN	183.0737	183.0701	183.0784	14.72995	14.69763	14.75793	3	3	0	4434
24	M668T14	2.140483	-1.09794	-9.35323	0.000761	0.0561	DOWN	668.2976	668.2889	668.2999	14.3624	14.33207	14.3897	6	3	3	7588
25	M885T16	1.528875	-0.61247	-9.64172	0.000805	0.0561	DOWN	885.4805	885.4799	885.4845	16.41087	16.3983	16.4331	5	2	3	729
26	M633T18	4.3521	-2.12171	-15.0552	0.000825	0.0561	DOWN	633.2917	633.286	633.2957	17.95517	17.91633	17.98117	4	3	1	4500
27	M651T17	2.229124	-1.15648	-9.09566	0.000834	0.0561	DOWN	651.2968	651.2948	651.3025	16.88233	16.85467	16.91183	5	2	3	2671
28	M593T15	2.76298	-1.46623	-10.994	0.000838	0.0561	DOWN	593.1483	593.1424	593.1494	14.9416	14.93808	14.96662	5	3	2	11724

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
	name	fold	log2fold	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	
1	M260T9	2.0662	1.04700	52.9784	0.000004	0.008947	UP	260.1494	260.1470	260.1507	8.820	8.793	8.820	
2	M554T16	1.6038	-0.68153	-21.3110	0.000056	0.034248	DOWN	553.7874	553.7773	553.7881	16.461	16.456	16.463	
3	M365T19	4.7330	2.24274	28.4406	0.000064	0.034248	UP	365.3147	365.3146	365.3152	18.995	18.990	19.006	
4	M250T9	1.7265	0.78787	18.0341	0.000090	0.034248	UP	250.0274	250.0261	250.0306	9.184	9.157	9.206	
5	M347T19	4.2665	2.09306	16.2531	0.000092	0.034248	UP	347.2915	347.2900	347.2931	19.127	19.082	19.172	
6	M590T12	5.0367	-2.33248	-20.0766	0.000103	0.034248	DOWN	590.3727	590.3708	590.3746	12.157	12.148	12.167	
7	M482T11	1.7125	-0.77609	-17.9095	0.000150	0.040827	DOWN	481.9515	481.9483	481.9547	11.144	11.117	11.170	
8	M330T9	2.2581	1.17510	15.0951	0.000163	0.040827	UP	330.1959	330.1949	330.1965	9.324	9.317	9.329	
9	M535T15	2.5038	-1.32413	-20.1752	0.000199	0.044224	DOWN	535.1078	535.0998	535.1093	15.115	15.111	15.141	
10	M669T16	2.8023	-1.48660	-11.4148	0.000354	0.056100	DOWN	669.1933	669.1884	669.1981	15.650	15.648	15.651	
11	M454T12	3.7727	-1.91559	-16.3974	0.000366	0.056100	DOWN	453.7940	453.7929	453.7981	12.022	12.002	12.023	
12	M238T14	1.7912	-0.84095	-19.4555	0.000393	0.056100	DOWN	238.0703	238.0672	238.0735	13.817	13.814	13.819	
13	M634T18	3.4664	-1.79343	-14.2648	0.000412	0.056100	DOWN	634.2947	634.2865	634.2960	17.925	17.916	17.956	
14	M434T11	2.3822	1.25232	10.7818	0.000510	0.056100	UP	433.9209	433.9164	433.9215	11.475	11.451	11.476	
15	M507T15	1.8225	-0.86590	-10.9623	0.000524	0.056100	DOWN	507.1130	507.1056	507.1147	15.273	15.247	15.274	
16	M431T14	1.3668	0.45076	13.7411	0.000552	0.056100	UP	431.1934	431.1906	431.2030	13.748	13.699	13.754	
17	M667T14	2.4854	-1.31346	-13.2470	0.000602	0.056100	DOWN	667.2951	667.2859	667.2966	14.390	14.359	14.417	
18	M120T15	5.1828	-2.37375	-10.0467	0.000623	0.056100	DOWN	119.5384	119.5375	119.5398	15.355	15.327	15.355	
19	M684T13	1.6204	-0.69635	-10.3018	0.000635	0.056100	DOWN	684.1574	684.1572	684.1576	13.350	13.339	13.361	
20	M281T9_1	1.8236	0.86680	12.5391	0.000655	0.056100	UP	281.1265	281.1228	281.1370	8.503	8.472	8.654	
21	M404T22	2.0072	-1.00516	-9.5678	0.000670	0.056100	DOWN	404.2992	404.2916	404.2996	22.389	22.384	22.432	
22	M793T16	2.1654	-1.1462	-10.1770	0.000678	0.056100	DOWN	793.3024	793.2985	793.3063	15.896	15.894	15.899	
23	M183T15	1.3259	-0.40694	-9.7030	0.000749	0.056100	DOWN	183.0737	183.0701	183.0784	14.730	14.698	14.758	
24	M668T14	2.1405	-1.09794	-9.3532	0.000761	0.056100	DOWN	668.2976	668.2889	668.2999	14.362	14.332	14.390	
25	M885T16	1.5289	-0.61247	-9.6417	0.000805	0.056100	DOWN	885.4805	885.4799	885.4845	16.411	16.398	16.433	
26	M633T18	4.3521	-2.12171	-15.0552	0.000825	0.056100	DOWN	633.2917	633.2860	633.2957	17.955	17.916	17.981	
27	M651T17	2.2291	-1.15648	-9.0957	0.000834	0.056100	DOWN	651.2968	651.2948	651.3025	16.882	16.855	16.912	
28	M593T15	2.7630	-1.46623	-10.9940	0.000838	0.056100	DOWN	593.1483	593.1424	593.1494	14.942	14.938	14.967	
29	M888T16	2.2822	-1.19042	-10.1014	0.000905	0.056100	DOWN	887.8479	887.8433	887.8524	15.891	15.887	15.894	
30	M452T13	4.3433	2.11878	14.8409	0.000928	0.056100	UP	451.7763	451.7747	451.7793	13.011	12.983	13.024	
31	M493T14	2.0469	1.03347	10.0698	0.000932	0.056100	UP	493.1546	493.1534	493.1588	14.198	14.197	14.202	

## Areas of aligned metabolites by sample

posmode_ir1	posmode_ir2	posmode_ir3	posmode_nr1	posmode_nr2	posmode_nr3
7,303	7,394	6,989	14,789	15,056	14,964
8,508	8,151	8,508	5,080	5,225	5,386
2,988	3,739	3,422	15,238	16,371	16,425
4,370	4,355	3,957	7,263	7,493	7,140
3,247	3,907	5,368	18,064	16,741	18,621
3,469	3,554	3,737	549	642	945
3,766	3,661	3,826	2,046	2,274	2,252
17,714	20,889	20,577	47,107	43,613	42,913
54,982	55,692	59,722	24,224	21,401	22,430
14,434	12,701	12,837	5,257	5,260	3,746
4,918	4,559	4,780	890	1,421	1,468
20,938	21,621	21,088	10,988	12,403	12,142
20,434	19,642	22,604	6,593	6,562	4,928
3,707	4,081	4,871	10,632	10,286	9,241
132,737	142,214	148,453	84,295	75,865	72,163
13,723	12,724	13,535	17,860	18,335	18,451
196,652	198,218	211,370	92,115	85,725	66,085
5,351	4,463	4,952	1,491	453	905
7,646	8,392	8,305	5,128	5,257	4,638
52,549	60,726	63,555	110,561	108,240	103,668

irradiated diet

non-irradiated diet

## Sort the data by retention time

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
	name	fold	log2fold	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax	
1	1136	M193T3	1.3875	-0.47254	-1.7482	0.206438	0.363095	DOWN	193.0538	193.0537	193.0538	3.313	3.312	3.313
2	2681	M73T3_1	1.0343	-0.04860	-0.2801	0.804100	0.598244	DOWN	72.5282	72.5268	72.5291	3.324	3.305	3.405
3	1877	M84T3	1.0647	-0.09038	-0.8206	0.459843	0.489536	DOWN	84.0368	84.0364	84.0370	3.338	3.313	3.365
4	1361	M131T3_2	1.1376	-0.18596	-1.2585	0.277228	0.406430	DOWN	131.0178	131.0169	131.0179	3.345	3.324	3.379
5	2590	M179T3	1.0448	0.06323	0.3372	0.757224	0.584204	UP	179.0112	179.0109	179.0119	3.345	3.334	3.365
6	2856	M64T3	1.0071	-0.01023	-0.1709	0.878055	0.614022	DOWN	63.5244	63.5241	63.5248	3.345	3.318	3.379
7	813	M51T3	1.5063	0.59105	2.1012	0.109525	0.269192	UP	50.5172	50.5165	50.5178	3.350	3.348	3.353
8	2800	M78T3_1	1.0330	0.04677	0.2066	0.853260	0.608924	UP	77.5253	77.5229	77.5279	3.351	3.324	3.368

Scroll down to Rt 5.00 min

886	3085	M184T5	1.0027	0.00384	0.0098	0.992751	0.642738	UP	183.5468	183.5439	183.5494	4.964	4.901	4.980
887	2549	M192T5	1.0376	-0.05329	-0.3797	0.732150	0.573820	DOWN	191.5392	191.5388	191.5396	4.971	4.936	4.983
888	2345	M314T5	1.0993	0.13654	0.5069	0.639000	0.544471	UP	313.5067	313.5063	313.5070	4.972	4.964	4.980
889	812	M397T5	1.2059	-0.27011	-2.4796	0.109518	0.269192	DOWN	397.0391	397.0375	397.0404	4.978	4.964	5.034
890	996	M220T5	1.0758	-0.10539	-1.7194	0.161833	0.324674	DOWN	219.5372	219.5367	219.5376	4.986	4.965	5.011
891	1300	M443T5	1.2790	-0.35499	-1.3335	0.257810	0.396275	DOWN	443.0460	443.0435	443.0472	5.000	4.924	5.038
892	2179	M276T5	1.0704	-0.09815	-0.6349	0.578423	0.529925	DOWN	275.9916	275.9913	275.9917	5.006	4.983	5.034
893	2456	M396T5	1.0426	-0.06023	-0.4453	0.690727	0.561856	DOWN	396.0343	396.0323	396.0348	5.006	4.991	5.062
894	2187	M259T5	1.1427	-0.19248	-0.6451	0.581173	0.530554	DOWN	258.5479	258.5443	258.5516	5.014	4.965	5.062

Keep metabolites eluting between 5.00 and 25.00 minutes



## Creating .csv files for each sample

A	B	C
mzmed	rtmed	posmode_ir1
260.1494	8.820	7,303
553.7874	16.461	8,508
365.3147	18.995	2,988
250.0274	9.184	4,370
347.2915	19.127	3,247
590.3727	12.157	3,469
481.9515	11.144	3,766
330.1959	9.324	17,714
535.1078	15.115	54,982
669.1933	15.650	14,434
453.7940	12.022	4,918
238.0703	13.817	20,938
634.2947	17.925	20,434
433.9209	11.475	3,707
507.1130	15.273	132,737
431.1934	13.748	13,723
667.2951	14.390	196,652
119.5384	15.355	5,351
684.1574	13.350	7,646

- Copy the median  $m/z$  and median Rt values into a new Excel file. Then copy the column of areas from the first sample in Group\_1. Save as an Excel .csv file.
  - Note that the file name must not have spaces – use an underscore instead of a space.
- Leave the file open and replace the yellow column with the areas from the next Group\_1 sample. Save as a second .csv file.
- Continue until all Group\_1 and Group\_2 samples have a corresponding .csv file.

## Preparing a .zip file

- Put each of the .csv files for group\_1 samples into a folder named “Group\_1”.
- Put each of the .csv files for group\_2 samples into a folder named “Group\_2”.
- Click on Group\_1 and Group\_2 folders and combine to form a .zip file.
  - Rename the .zip file as [your\_name].zip
- You’re now ready to submit it to MetaboAnalyst