THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Knowledge that will change your world

Working with metabolomics data

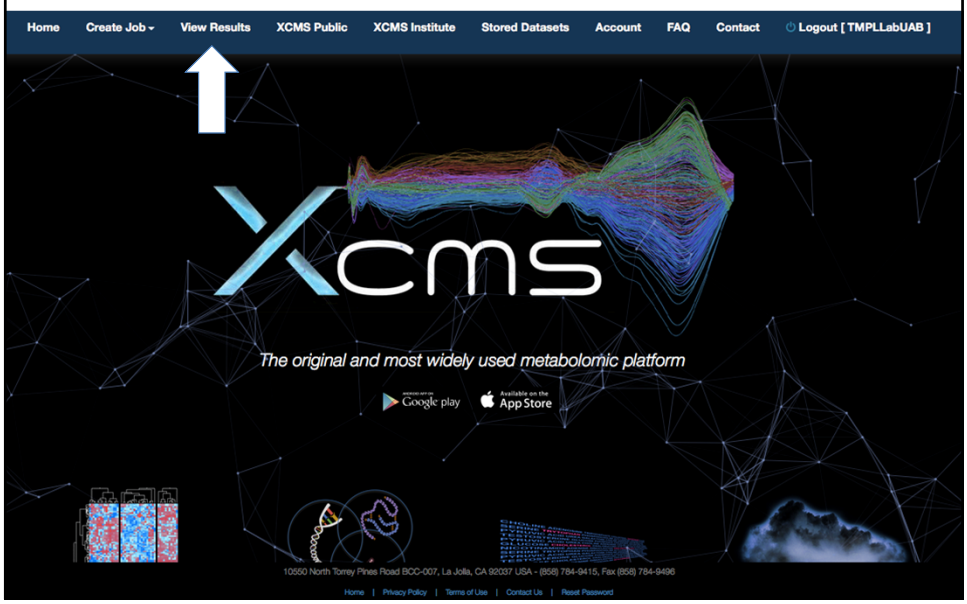
Steve Barnes, PhD

Targeted Metabolomics & Proteomics Laboratory

Examining output on XCMS

- First, we'll logon to XCMS and inspect the non-irradiated diet vs irradiated diet
- We'll download the XCMS output file
  - Make a Volcano plot
  - Prepare files for MetaboAnalyst

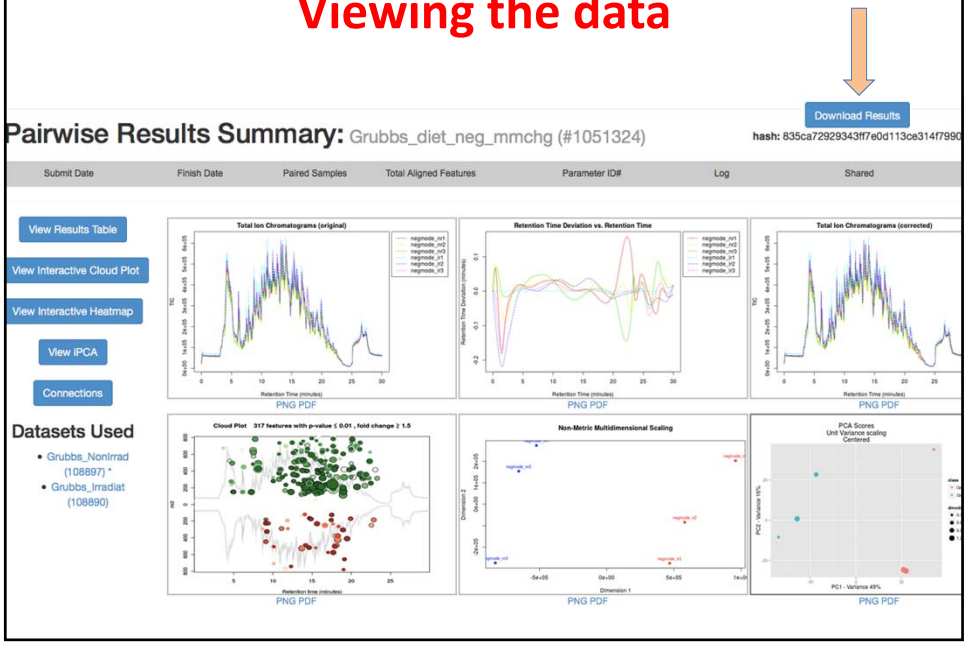
Logon to XCMS with your name and password



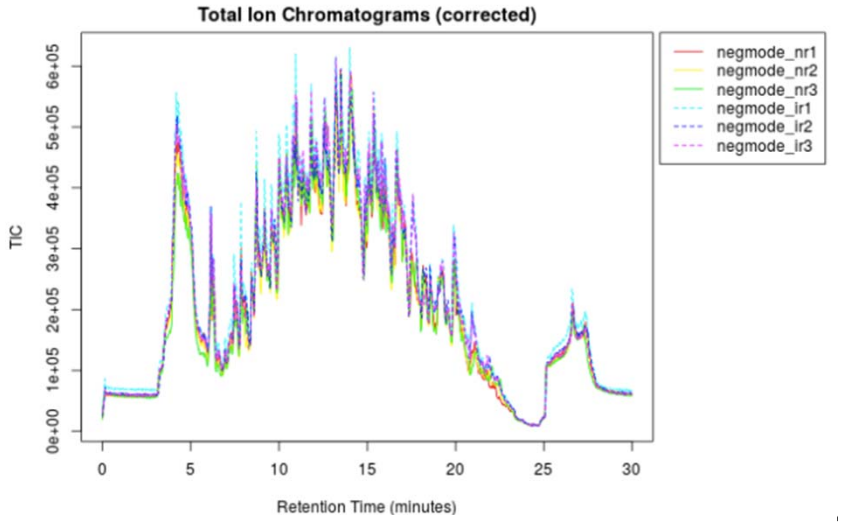
Viewing the datasets

	PAIR	VIEW	1052098	job complete 100%	MCF10AT_NTVec vs KD1_NegMode	MCF10AT_NT (#89883) MCF10AT_KD (#89884)	2015-03-19 16:22:46	NanoLc Neg (10374)		Shared [Stop sharing]	✖
	PAIR	VIEW	1052085	job complete 100%	MCF10AT_NTVec vs KD1_PosMode	MCF10AT_NT (#89683) MCF10AT_KD (#89684)	2015-03-19 14:10:30	nanoLC_560 (9920)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051960	job complete 100%	PAIR_2015-03-18_17:24	Sham_Cutts (#115973) GSE_Cutts_ (#115883)	2015-03-18 17:24:58	nanoLC_560 (9920)			✖
	PAIR	VIEW	1051941	job complete 100%	SUM159VMP_VS_NF2c16_NegMode	SUM159_VMP (#89938) SUM159_NF2 (#89955)	2015-03-18 13:24:52	NanoLc Neg (10374)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051935	job complete 100%	SUM159VMP_VS_NF2c16_PosMode	SUM159_VMP (#89688) SUM159_NF2 (#89689)	2015-03-18 12:07:33	nanoLC_560 (9920)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051415	job complete 100%	Grubbs_urine_pos_mmchg	Grubbs_Uri (#107301) Grubbs_Uri (#107315)	2015-03-12 19:11:09	nanoLC_560 (9920)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051379	job complete 100%	Aman Set 3 Comparison	Aman_Set3_ (#115309) Aman_Set3- (#115234)	2015-03-12 13:21:25	nanoLC_560 (9920)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051373	job complete 100%	Grubbs_urine_neg_mmchg	Grubbs_Uri (#107534) Grubbs_Uri (#107626)	2015-03-12 12:19:17	NanoLC5600 (10377)			✖
	PAIR	VIEW	1051324	job complete 100%	Grubbs_diet_neg_mmchg	Grubbs_Non (#108897) Grubbs_Irr (#108890)	2015-03-12 05:55:12	NanoLc Neg (10374)		Shared [Stop sharing]	✖
	PAIR	VIEW	1051271	job complete 100%	Grubbs_diet_pos_mmchg	Grubbs_Non (#109003) Grubbs_Irr (#108990)	2015-03-11 22:05:58	nanoLC_560 (9920)		Shared [Stop sharing]	✖
	PAIR	VIEW	1050983	job complete 100%	Sera Positive Mode	McLean_Har (#114812) McLean_Har (#114794)	2015-03-09 22:30:49	nanoLC_560 (9920)			✖
	PAIR	VIEW	1047227	job complete 100%	Grubbs_Diet_PosMode	Grubbs_Irr (#108990) Grubbs_Non (#109003)	2015-02-05 17:51:27	nanoLC_560 (9920)		Shared [Stop sharing]	✖

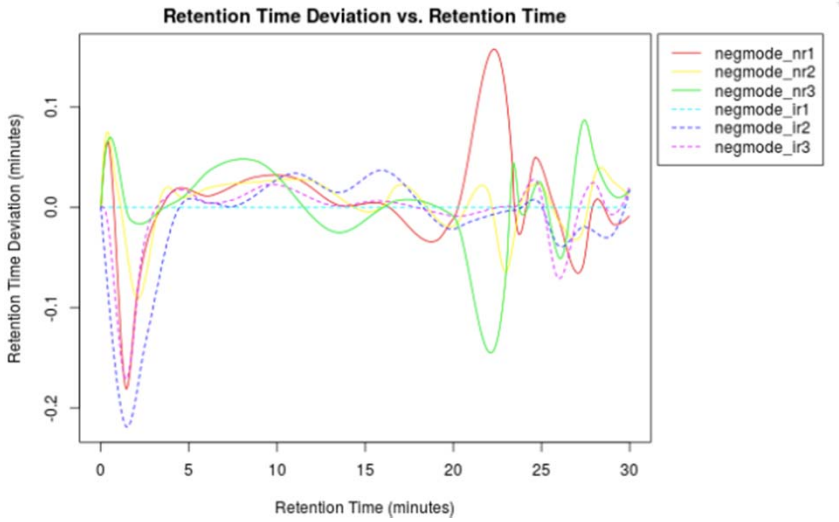
Viewing the data



Total ion chromatograms



High reproducibility of retention times



Synopsis

- Opening the .zip file
- Creating the Excel file from result.tsv file
- Why a Volcano plot?
  - Making a volcano plot
  - Selecting ions that reach significance and fold-change cutoffs
- Preparing for MetaboAnalyst

boxplot	Mar 12, 2015, 6:19 AM	--	Folder
CloudPlot-svg.svg	Mar 12, 2015, 6:52 AM	968 KB	SVG document
CloudPlot.pdf	Mar 12, 2015, 6:52 AM	366 KB	PDF Document
CloudPlot.png	Mar 12, 2015, 6:52 AM	99 KB	PNG image
EIC	Mar 12, 2015, 6:21 AM	--	Folder
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Heatmap_Cor_1051324.png	Mar 12, 2015, 6:51 AM	356 KB	PNG image
MDS.pdf	Mar 12, 2015, 6:51 AM	5 KB	PDF Document
MDS.png	Mar 12, 2015, 6:51 AM	15 KB	PNG image
mummichog	Mar 12, 2015, 6:24 AM	--	Folder
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PCA-diagnostics.pdf	Mar 12, 2015, 6:51 AM	5 KB	PDF Document
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result.tsv	Mar 12, 2015, 6:52 AM	1.4 MB	Plain Text
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TICs.png	Mar 12, 2015, 6:13 AM	62 KB	PNG image
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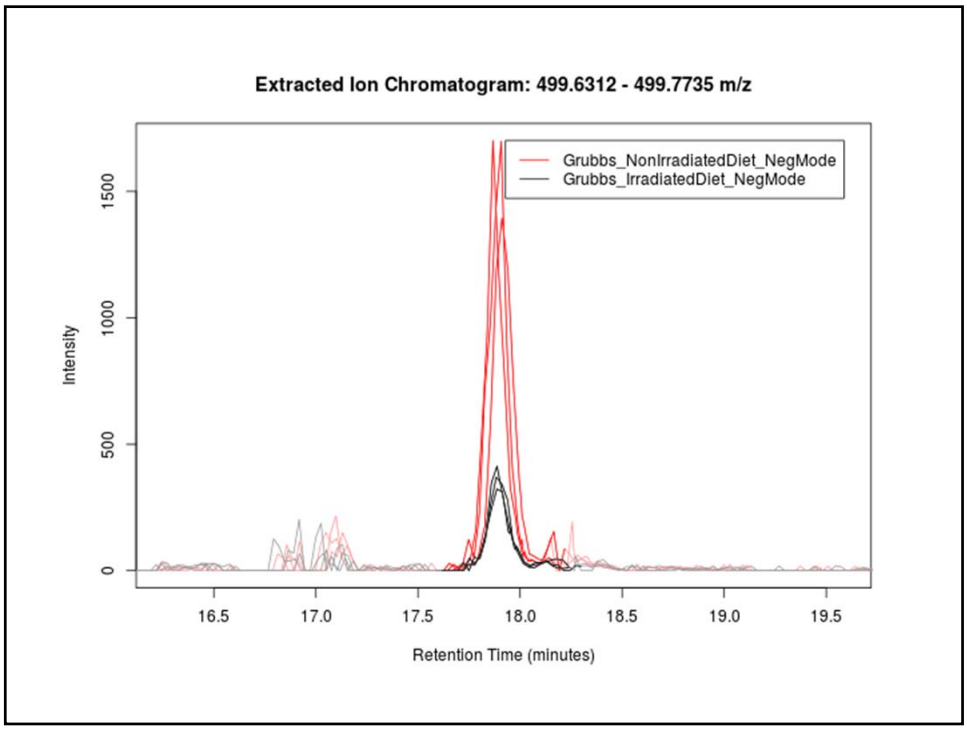
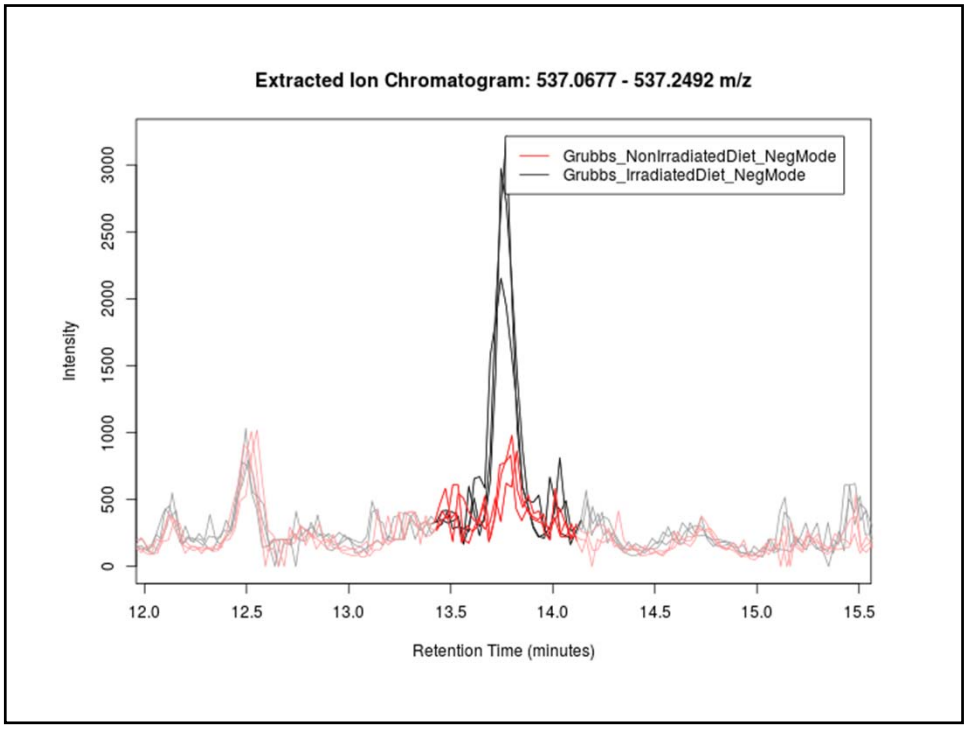
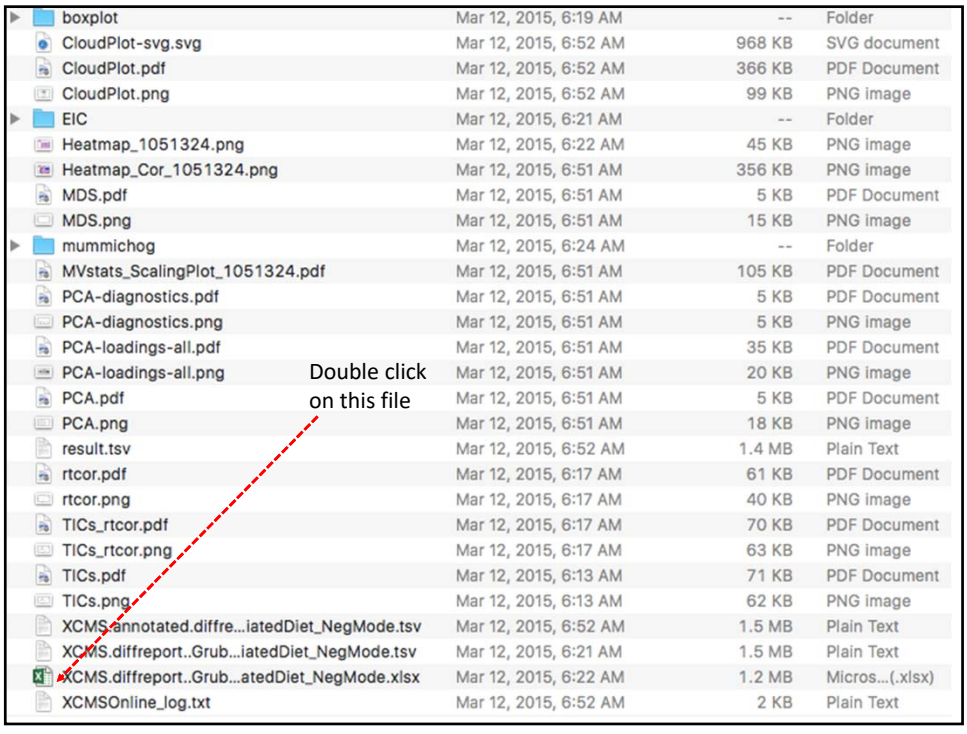
XCMS.diffreport..Grub...atedDiet_NegMode.xlsx	Mar 12, 2015, 6:22 AM	1.2 MB	Micros...(.xlsx)
XCMSOnline_log.txt	Mar 12, 2015, 6:52 AM	2 KB	Plain Text

Ion chromatograms




Extracted Ion Chromatogram: 537.0677 - 537.2492 m/z
Grubbs_NonIrradiatedDiet_NegMode
Grubbs_IrradiatedDiet_NegMode
Intensity
0
500
1000
1500
2000
2500
3000
12.0
12.5
13.0
13.5
14.0
14.5
15.0
15.5
Retention Time (minutes)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CloudPlot.pdf Mar 12, 2015, 6:52 AM 366 KB PDF Document
CloudPlot.png Mar 12, 2015, 6:52 AM 99 KB PNG image
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Heatmap_Cor_1051324.png Mar 12, 2015, 6:51 AM 356 KB PNG image
MDS.pdf Mar 12, 2015, 6:51 AM 5 KB PDF Document
MDS.png Mar 12, 2015, 6:51 AM 15 KB PNG image
mummichog Mar 12, 2015, 6:24 AM -- Folder
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PCA-diagnostics.pdf Mar 12, 2015, 6:51 AM 5 KB PDF Document
PCA-diagnostics.png Mar 12, 2015, 6:51 AM 5 KB PNG image
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rtcor.pdf Mar 12, 2015, 6:17 AM 61 KB PDF Document
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TICs_rtcor.pdf Mar 12, 2015, 6:17 AM 70 KB PDF Document
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TICs.pdf Mar 12, 2015, 6:13 AM 71 KB PDF Document
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XCMS.annotated.diffre...iatedDiet_NegMode.tsv Mar 12, 2015, 6:52 AM 1.5 MB Plain Text
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XCMS.diffreport..Grub...atedDiet_NegMode.xlsx Mar 12, 2015, 6:22 AM 1.2 MB Micros...(.xlsx)
XCMSOnline_log.txt Mar 12, 2015, 6:52 AM 2 KB Plain Text
Double click on this file

The Excel DiffReport from XCMS

	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		M500T18_	3.177186	-1.66775	-69.9353	4.25E-07	0.000591	DOWN	499.7199	499.7144	499.721	17.9395	17.8955	17.94383
2		M251T15	2.538102	1.34375	57.295	7.07E-07	0.000591	UP	251.0044	251.0023	251.0067	14.64618	14.62393	14.66083
3		M417T18	2.457688	1.297302	55.09394	1.17E-06	0.000651	UP	417.2121	417.2091	417.2133	17.85967	17.82967	17.8865
4		M199T19	2.493736	1.318309	40.96574	2.92E-06	0.001223	UP	199.1334	199.1321	199.1345	18.99725	18.97583	19.02717
5		M537T14_	1.778737	0.830853	31.72212	1.16E-05	0.003885	UP	537.1257	537.1252	537.1263	13.75913	13.74583	13.77243
6		M325T11	1.695199	0.761455	49.07387	1.61E-05	0.003885	UP	325.0931	325.0922	325.0951	11.21367	11.20732	11.25245
7		M144T16	2.012298	1.008844	33.20396	1.69E-05	0.003885	UP	144.0457	144.0453	144.0465	16.20212	16.19118	16.21943
8		M357T13_	2.594144	1.375258	32.88002	1.92E-05	0.003885	UP	357.0822	357.082	357.0837	12.66457	12.65622	12.68867
9		M426T8	6.841469	2.774306	22.7141	2.37E-05	0.003885	UP	426.0323	426.0321	426.0345	8.13925	8.13335	8.157967
10		M347T13	1.995841	0.996997	22.19083	2.44E-05	0.003885	UP	347.168	347.1634	347.172	13.05983	13.00283	13.11525
11		M591T15_	2.88852	1.530331	31.66855	2.55E-05	0.003885	UP	591.1369	591.135	591.1397	14.92045	14.89628	14.96255
12		M630T16	2.300034	1.201655	23.19857	3E-05	0.004174	UP	629.784	629.7792	629.7876	16.32718	16.27417	16.34668
13		M438T12_	1.608298	0.685535	24.90868	3.44E-05	0.004174	UP	438.1711	438.1709	438.1714	12.30179	12.27012	12.33347
14		M425T8	4.383821	2.132189	20.21984	3.77E-05	0.004174	UP	425.045	425.0435	425.0457	8.13335	8.112117	8.157967
15		M151T15	2.067456	1.047856	20.92318	4.47E-05	0.004174	UP	151.0399	151.0366	151.0405	14.53876	14.51367	14.5699
16		M506T15	1.435551	0.521604	20.18947	4.83E-05	0.004174	UP	506.1093	506.1066	506.1145	15.21824	15.18848	15.25067
17		M789T15	2.016565	1.0119	18.58276	5.06E-05	0.004174	UP	789.2271	789.2264	789.2278	14.96682	14.95903	14.9746
18		M315T16	3.633561	1.861384	25.13321	5.14E-05	0.004174	UP	315.143	315.1425	315.1436	16.35723	16.35473	16.38143
19		M641T16_	3.070745	1.618589	24.93903	5.26E-05	0.004174	UP	640.7743	640.774	640.7759	16.35723	16.35473	16.38143
20		M582T16_	1.484257	0.569741	18.25259	5.37E-05	0.004174	UP	582.278	582.2752	582.2823	15.50497	15.47808	15.51025

This file has 3,906 lines – we need to apply filters

Content of the Excel file

	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		M500T18_	3.177186	-1.66775	-69.9353	4.25E-07	0.000591	DOWN	499.7199	499.7144	499.721	17.9395	17.8955	17.94383
2		M251T15	2.538102	1.34375	57.295	7.07E-07	0.000591	UP	251.0044	251.0023	251.0067	14.64618	14.62393	14.66083
3		M417T18	2.457688	1.297302	55.09394	1.17E-06	0.000651	UP	417.2121	417.2091	417.2133	17.85967	17.82967	17.8865
4		M199T19	2.493736	1.318309	40.96574	2.92E-06	0.001223	UP	199.1334	199.1321	199.1345	18.99725	18.97583	19.02717
5		M537T14_	1.778737	0.830853	31.72212	1.16E-05	0.003885	UP	537.1257	537.1252	537.1263	13.75913	13.74583	13.77243
6		M325T11	1.695199	0.761455	49.07387	1.61E-05	0.003885	UP	325.0931	325.0922	325.0951	11.21367	11.20732	11.25245
7		M144T16	2.012298	1.008844	33.20396	1.69E-05	0.003885	UP	144.0457	144.0453	144.0465	16.20212	16.19118	16.21943
8		M357T13_	2.594144	1.375258	32.88002	1.92E-05	0.003885	UP	357.0822	357.082	357.0837	12.66457	12.65622	12.68867
9		M426T8	6.841469	2.774306	22.7141	2.37E-05	0.003885	UP	426.0323	426.0321	426.0345	8.13925	8.13335	8.157967
10		M347T13	1.995841	0.996997	22.19083	2.44E-05	0.003885	UP	347.168	347.1634	347.172	13.05983	13.00283	13.11525
11		M591T15_	2.88852	1.530331	31.66855	2.55E-05	0.003885	UP	591.1369	591.135	591.1397	14.92045	14.89628	14.96255
12		M630T16	2.300034	1.201655	23.19857	3E-05	0.004174	UP	629.784	629.7792	629.7876	16.32718	16.27417	16.34668
13		M438T12_	1.608298	0.685535	24.90868	3.44E-05	0.004174	UP	438.1711	438.1709	438.1714	12.30179	12.27012	12.33347
14		M425T8	4.383821	2.132189	20.21984	3.77E-05	0.004174	UP	425.045	425.0435	425.0457	8.13335	8.112117	8.157967
15		M151T15	2.067456	1.047856	20.92318	4.47E-05	0.004174	UP	151.0399	151.0366	151.0405	14.53876	14.51367	14.5699
16		M506T15	1.435551	0.521604	20.18947	4.83E-05	0.004174	UP	506.1093	506.1066	506.1145	15.21824	15.18848	15.25067
17		M789T15	2.016565	1.0119	18.58276	5.06E-05	0.004174	UP	789.2271	789.2264	789.2278	14.96682	14.95903	14.9746
18		M315T16	3.633561	1.861384	25.13321	5.14E-05	0.004174	UP	315.143	315.1425	315.1436	16.35723	16.35473	16.38143
19		M641T16_	3.070745	1.618589	24.93903	5.26E-05	0.004174	UP	640.7743	640.774	640.7759	16.35723	16.35473	16.38143
20		M582T16_	1.484257	0.569741	18.25259	5.37E-05	0.004174	UP	582.278	582.2752	582.2823	15.50497	15.47808	15.51025

Sort the data by retention time

	name	fold	log2fold	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
1208	M209T3	1.369143	0.453273	2.709012	0.055919	0.077433	UP	208.9602	208.9598	208.9611	3.28215	3.265233	3.3416
378	M323T3	1.434411	0.520459	5.353103	0.006801	0.030027	UP	322.938	322.9359	322.9407	3.32005	3.295083	3.361817
549	M271T3	1.186928	0.247232	5.44988	0.01248	0.038025	UP	270.9417	270.9411	270.9425	3.32005	3.295083	3.361817
895	M387T3	1.394198	0.479436	4.649027	0.031911	0.059621	UP	386.9358	386.9344	386.9368	3.32005	3.295083	3.361817
1689	M272T3	1.088879	0.122844	2.524051	0.111581	0.110419	UP	271.941	271.9398	271.9434	3.326167	3.29085	3.361817
892	M325T3	1.375868	0.460342	3.79855	0.031801	0.059554	UP	324.9303	324.929	324.9401	3.32705	3.30445	3.361817
1378	M391T3	1.312616	0.392445	3.507716	0.071514	0.086812	UP	390.9101	390.9064	390.9131	3.32705	3.30445	3.367883
1623	M291T3	1.256324	-0.32921	-2.50694	0.102207	0.105294	DOWN	290.9445	290.9436	290.9464	3.327583	3.295083	3.361817
749	M324T3	1.275929	0.351548	3.759227	0.022331	0.049864	UP	323.9378	323.9374	323.9399	3.331108	3.298017	3.361817
2099	M154T3	1.037093	0.052546	1.655429	0.189385	0.150928	UP	154.0059	154.0023	154.0117	3.331492	3.30095	3.361817

Scroll down to Rt 5.00 min

1845	M387T5	1.154483	0.207247	2.022386	0.135817	0.123138	UP	387.114	387.113	387.1145	4.978417	4.97	4.99685
2204	M388T5	1.139738	0.188702	1.768345	0.215874	0.163842	UP	388.1168	388.115	388.1176	4.978417	4.970667	4.99615
1315	M404T5	1.345221	-0.42784	-2.9703	0.065723	0.083602	DOWN	404.1036	404.1014	404.1047	4.983425	4.945983	5.010417
1128	M564T5	1.522219	0.606175	3.224519	0.049554	0.073486	UP	564.0012	563.9964	564.0092	4.984925	4.917883	5.0261
2862	M772T5_2	1.106141	-0.14554	-0.84381	0.44698	0.26116	DOWN	772.2342	772.227	772.2367	4.992317	4.97	5.037
3327	M643T5_1	1.122221	-0.16636	-0.46213	0.67351	0.338631	DOWN	642.6763	642.6715	642.6794	4.992317	4.930767	4.99685
1060	M419T5	1.160736	-0.21504	-3.76575	0.042847	0.067616	DOWN	419.1222	419.1178	419.1251	4.998867	4.97	5.037
3479	M418T5	1.009913	0.01423	0.343979	0.752961	0.362038	UP	418.1259	418.1251	418.1274	4.999242	4.99615	5.037
805	M534T5	2.270077	1.182742	4.124107	0.02608	0.054177	UP	534.0049	533.9957	534.0074	4.999617	4.99685	5.09565
2042	M768T5	1.190096	-0.25108	-1.66652	0.174057	0.142584	DOWN	768.2396	768.2371	768.241	4.999617	4.992317	5.022167
1207	M446T5	1.377175	0.461712	2.687478	0.055893	0.077433	UP	446.1187	446.1181	446.1194	5.003833	4.970667	5.037

Delete all these records

Keep metabolites eluting between 5.00 and 25.00 minutes

Adding –log p-value

	name	fold	log2fold	-log P-value	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
1	M500T18_1	3.17718625	-1.6677497	6.37194281	-69.935309	4.2468E-07	0.00059116	DOWN	499.71989	499.714381	499.721035	17.9395	17.8955	17.9438333
96	M165T15	1.66018323	-0.7313425	3.24367484	-36.133032	0.00057059	0.00994236	DOWN	165.055502	165.054308	165.056684	14.6864667	14.6478333	14.71805
47	M574T18	2.13905717	-1.096975	3.78771429	-29.64074	0.00016304	0.00569183	DOWN	574.266065	574.263028	574.272462	17.7115	17.6941667	17.7368333
38	M517T15_2	1.75943722	-0.815114	3.93600908	-27.522969	0.00011588	0.00501453	DOWN	517.194812	517.191996	517.198037	14.6337667	14.59155	14.6643167
42	M574T17_2	1.59552933	-0.6740351	3.89904915	-23.534148	0.00012617	0.00502501	DOWN	573.751404	573.745446	573.752077	17.0508333	17.0495	17.1253333
69	M567T12_1	1.21578562	-0.2818889	3.50811164	-22.910965	0.00031038	0.00742882	DOWN	567.214555	567.212376	567.21642	12.4962667	12.4584667	12.5235833
206	M504T10	2.04744309	-1.0338234	2.65836289	-18.693823	0.00219602	0.01783223	DOWN	504.138121	504.136349	504.14049	10.1241	10.1203667	10.1441333
21	M175T15_2	1.75024008	-0.8075528	4.26057989	-18.65345	5.4881E-05	0.00417379	DOWN	175.097496	175.096898	175.098239	14.8694333	14.8666333	14.8797
30	M529T12	1.20815162	-0.2728015	4.06091097	-18.56302	8.6914E-05	0.0046548	DOWN	529.193978	529.191284	529.195264	11.8747	11.8619333	11.9029833
177	M231T16_2	3.00517465	-1.5874488	2.79422291	-16.956079	0.00160612	0.01517888	DOWN	231.15918	231.158231	231.160844	15.6376667	15.5836667	15.63795
46	M493T18_2	2.09585981	-1.0675422	3.80434853	-16.138394	0.00015691	0.00569183	DOWN	493.237441	493.235949	493.237575	18.1235	18.1161667	18.1653333
56	M287T12	1.49870741	-0.5837188	3.63982195	-14.759869	0.00022918	0.00684582	DOWN	287.077916	287.073836	287.083574	12.4962667	12.40445	12.55065
124	M565T12_1	1.40852493	-0.4941851	3.08759847	-14.151015	0.00081734	0.01094051	DOWN	565.215654	565.215406	565.217814	12.4962667	12.4853	12.5235833
67	M175T15_1	1.65329893	-0.7253476	3.54719875	-14.110635	0.00028366	0.0070821	DOWN	175.076333	175.075715	175.077592	14.8742	14.8672667	14.8829167
57	M490T14_1	2.28133744	-1.1898799	3.62228199	-13.866683	0.00023863	0.00700292	DOWN	490.13636	490.134832	490.13757	14.1682333	14.1636833	14.1689833
170	M490T14_2	2.64201512	-1.4016387	2.82058063	-13.640201	0.00151154	0.01487326	DOWN	490.150671	490.14958	490.151411	14.2480333	14.2477833	14.26975
55	M187T17	1.65717691	-0.7287276	3.64858184	-13.139663	0.0002246	0.00683111	DOWN	187.097276	187.096754	187.098391	17.4558333	17.4535	17.4841667
327	M587T12	1.21922912	-0.2859693	2.27459764	-12.965168	0.00531377	0.02715163	DOWN	587.234503	587.233346	587.23698	12.2213833	12.1999667	12.2525333
324	M289T11	1.803374	-0.8506986	2.28411244	-12.840128	0.00519861	0.02682321	DOWN	289.082598	289.076863	289.086635	11.1201167	11.10205	11.1440333
74	M317T11	1.18992599	-0.2508718	3.44300584	-12.785979	0.00035892	0.00808092	DOWN	317.082663	317.080868	317.083426	11.208525	11.1920667	11.2338

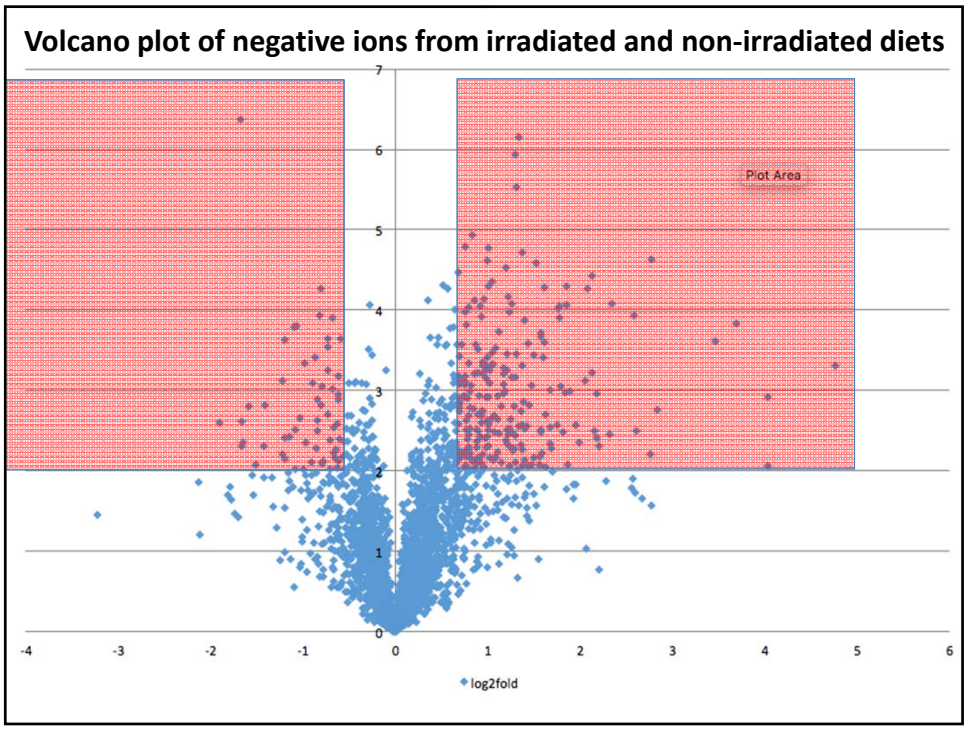
Insert an extra column next to the "log2fold" column

In the first of this column enter "=-log(g2) and <return>
Copy this value and paste to all the others in the same column

Making a volcano plot

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
		name	fold	log2fold	-log P-value	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
1		M500T18_1	3.17718625	-1.6677497	6.37194281	-69.935309	4.2468E-07	0.00059116	DOWN	499.71989	499.714381	499.721035	17.9395	17.8955	17.9438333
96		M165T15	1.66018323	-0.7313425	3.24367484	-36.133032	0.00057059	0.00994236	DOWN	165.055502	165.054308	165.056684	14.6864667	14.6478333	14.71805
47		M574T18	2.13905717	-1.096975	3.78771429	-29.64074	0.00016304	0.00569183	DOWN	574.266065	574.263028	574.272462	17.7115	17.6941667	17.7368333
38		M517T15_2	1.75943722	-0.815114	3.93600908	-27.522969	0.00011588	0.00501453	DOWN	517.194812	517.191996	517.198037	14.6337667	14.59155	14.6643167
42		M574T17_2	1.59552933	-0.6740351	3.89904915	-23.534148	0.00012617	0.00502501	DOWN	573.751404	573.745446	573.752077	17.0508333	17.0495	17.1253333
69		M567T12_1	1.21578562	-0.2818889	3.50811164	-22.910965	0.00031038	0.00742882	DOWN	567.214555	567.212376	567.21642	12.4962667	12.4584667	12.5235833
206		M504T10	2.04744309	-1.0338234	2.65836289	-18.693823	0.00219602	0.01783223	DOWN	504.138121	504.136349	504.14049	10.1241	10.1203667	10.1441333
21		M175T15_2	1.75024008	-0.8075528	4.26057989	-18.65345	5.4881E-05	0.00417379	DOWN	175.097496	175.096898	175.098239	14.8694333	14.8666333	14.8797
30		M529T12	1.20815162	-0.2728015	4.06091097	-18.56302	8.6914E-05	0.0046548	DOWN	529.193978	529.191284	529.195264	11.8747	11.8619333	11.9029833
177		M231T16_2	3.00517465	-1.5874488	2.79422291	-16.956079	0.00160612	0.01517888	DOWN	231.15918	231.158231	231.160844	15.6376667	15.5836667	15.63795
46		M493T18_2	2.09585981	-1.0675422	3.80434853	-16.138394	0.00015691	0.00569183	DOWN	493.237441	493.235949	493.237575	18.1235	18.1161667	18.1653333
56		M287T12	1.49870741	-0.5837188	3.63982195	-14.759869	0.00022918	0.00684582	DOWN	287.077916	287.073836	287.083574	12.4962667	12.40445	12.55065
124		M565T12_1	1.40852493	-0.4941851	3.08759847	-14.151015	0.00081734	0.01094051	DOWN	565.215654	565.215406	565.217814	12.4962667	12.4853	12.5235833
67		M175T15_1	1.65329893	-0.7253476	3.54719875	-14.110635	0.00028366	0.0070821	DOWN	175.076333	175.075715	175.077592	14.8742	14.8672667	14.8829167
57		M490T14_1	2.28133744	-1.1898799	3.62228199	-13.866683	0.00023863	0.00700292	DOWN	490.13636	490.134832	490.13757	14.1682333	14.1636833	14.1689833
170		M490T14_2	2.64201512	-1.4016387	2.82058063	-13.640201	0.00151154	0.01487326	DOWN	490.150671	490.14958	490.151411	14.2480333	14.2477833	14.26975
55		M187T17	1.65717691	-0.7287276	3.64858184	-13.139663	0.0002246	0.00683111	DOWN	187.097276	187.096754	187.098391	17.4558333	17.4535	17.4841667
327		M587T12	1.21922912	-0.2859693	2.27459764	-12.965168	0.00531377	0.02715163	DOWN	587.234503	587.233346	587.23698	12.2213833	12.1999667	12.2525333
324		M289T11	1.803374	-0.8506986	2.28411244	-12.840128	0.00519861	0.02682321	DOWN	289.082598	289.076863	289.086635	11.1201167	11.10205	11.1440333
74		M317T11	1.18992599	-0.2508718	3.44500584	-12.785979	0.00035892	0.00808092	DOWN	317.082663	317.080868	317.083426	11.208525	11.1920667	11.2338

Highlight columns D and E and create a chart



Selecting cut-offs

- We should now sort the data in the Excel file by p-values (from smallest to largest)
- Copy the metabolite ions that have p-values <0.01 into a new sheet
- Now resort these data by fold-change
  - these can be increased (>1.5) or decreased (<0.667)

Selecting all ions with p < 0.01 and making a new sheet

1	M500T18_1	3.17718625	-1.6677497	6.37194281	-69.935309	4.2468E-07	0.00059116	DOWN	499.71989	499.714381	499.721035	17.9395	17.8955	17.9438333
2	M251T15	2.5381022	1.34375016	6.15069974	57.2949952	7.0681E-07	0.00059116	UP	251.004448	251.00233	251.006679	14.646175	14.6239333	14.6608333
3	M417T18	2.45768759	1.29730154	5.93242838	55.093938	1.1683E-06	0.00065146	UP	417.212131	417.209144	417.213252	17.8596667	17.8296667	17.8865
4	M199T19	2.49373586	1.31830866	5.53403205	40.9657391	2.9239E-06	0.00122277	UP	199.133402	199.132069	199.134536	18.99725	18.9758333	19.0271667
5	M537T14_2	1.77873662	0.83085291	4.93437072	31.7221237	1.1631E-05	0.00388536	UP	537.125737	537.125207	537.126266	13.7591333	13.7458333	13.7724333
6	M325T11	1.69519907	0.7614547	4.79236518	49.0738693	1.613E-05	0.00388536	UP	325.093116	325.092202	325.095085	11.2136667	11.2073167	11.25245
7	M144T16	2.01229805	1.00884401	4.77150826	33.2039585	1.6924E-05	0.00388536	UP	144.045677	144.045279	144.046454	16.2021167	16.1911833	16.2194333
8	M357T13_2	2.59414359	1.37525834	4.71777294	32.8800228	1.9153E-05	0.00388536	UP	357.082227	357.081993	357.083674	12.6645667	12.6562167	12.6886667
9	M426T8	6.84146926	2.77430619	4.6257974	22.7140999	2.367E-05	0.00388536	UP	426.03226	426.032122	426.034541	8.13925	8.13335	8.15796667
10	M347T13	1.99584111	0.99699687	4.61237735	22.1908286	2.4413E-05	0.00388536	UP	347.167955	347.163411	347.172006	13.059825	13.0028333	13.11525

480	M611T16_2	1.17035124	-0.2269416	2.00908774	-5.3602012	0.00979292	0.03401502	DOWN	611.278173	611.27616	611.284794	15.8765083	15.8224333	15.9544333
482	M711T18	1.26827471	-0.3428673	2.00794425	-7.6797212	0.00981874	0.03401502	DOWN	711.31911	711.31715	711.322138	18.05925	17.9893333	18.1333333
483	M576T17_2	1.13201205	0.17888932	2.00693443	5.45812005	0.0098416	0.03401502	UP	576.245602	576.244742	576.249542	17.081	16.9721667	17.2375
484	M115T16	1.41760411	0.5034547	2.00604191	4.72201644	0.00986184	0.03401502	UP	115.004417	115.001493	115.006234	15.5579167	15.5327833	15.56165
485	M342T9	1.47951495	0.56512427	2.00572431	6.37522916	0.00986906	0.03401502	UP	342.090705	342.087875	342.09202	9.3506	9.30873333	9.37555
486	M197T10	1.26666403	0.34103392	2.0030799	6.82066584	0.00992933	0.03401502	UP	197.025861	197.023886	197.027836	9.56196667	9.55711667	9.56681667
487	M201T18	1.31936278	0.39984131	2.00283676	4.70021934	0.00993489	0.03401502	UP	201.105998	201.103323	201.107267	17.6155833	17.5908333	17.6258333
488	M162T11	1.09119523	0.12590925	2.00277294	6.75086139	0.00993635	0.03401502	UP	162.053951	162.052952	162.054782	11.4542	11.4454167	11.4963667
489	M131T14_1	1.31291812	-0.3927769	2.00218947	-5.5314033	0.00994971	0.03401502	DOWN	131.050967	131.048953	131.056393	13.7197	13.6934667	13.7197667
490	M687T15_2	1.30430893	0.38328562	2.001569	5.3380585	0.00996394	0.03401502	UP	686.755271	686.753349	686.757192	15.1120417	15.1079667	15.1161167

Selecting those with fold-change >1.5 and new page

name	fold	log2fold	-log P-value	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
M241T18_1	27.4045917	4.77634573	3.30532767	40.46059	0.00049508	0.00920165	UP	241.108312	241.107471	241.109437	17.59125	17.5871667	17.6198333
M240T18	16.4294376	4.03821119	2.05479101	10.0229195	0.00881473	0.03293413	UP	240.359872	240.356187	240.361536	17.5906667	17.5871667	17.5916667
M242T18	16.3870912	4.03448789	2.91477253	27.6130715	0.00121682	0.013362	UP	242.110341	242.109733	242.111274	17.5906667	17.5871667	17.5916667
M424T8_1	13.0179292	3.70242807	3.83389494	30.5724419	0.00014659	0.00557299	UP	424.028175	424.026455	424.029895	8.12178333	8.11845	8.12511667
M424T8_2	11.1363641	3.47720638	3.61174395	29.8423152	0.00024449	0.00703395	UP	424.0396	424.036272	424.044862	8.1363	8.12225	8.15796667
M227T16	7.16213011	2.84038873	2.75564323	19.5959381	0.00175532	0.01604506	UP	227.092249	227.090325	227.093361	15.8502167	15.8489333	15.8756833
M426T8	6.84146926	2.77430619	4.6257974	22.7140999	2.367E-05	0.00388536	UP	426.03226	426.032122	426.034541	8.13925	8.13335	8.15796667
M171T18_2	6.77813538	2.76088845	2.21189773	12.4050347	0.00613907	0.0288409	UP	171.102926	171.102581	171.103387	17.6959167	17.6403333	17.698
M278T15	6.10860512	2.61084298	2.49625447	16.1413878	0.00318967	0.02084209	UP	278.101274	278.100858	278.105129	14.64535	14.6205333	14.6608333
M184T9	1.53161283	-0.6150517	2.95136904	-8.3891049	0.00111849	0.01290324	DOWN	184.01267	184.009146	184.016193	9.12173333	9.12105	9.12241667
M655T19	1.52860376	-0.6122145	3.17714123	-10.355352	0.00066506	0.01049515	DOWN	655.441309	655.431657	655.442502	19.139	19.1328333	19.1483333
M605T13	1.52721469	0.61090289	2.83316714	7.78489386	0.00146836	0.01470795	UP	605.241635	605.240209	605.245899	12.844175	12.83055	12.8973
M505T10_2	1.52573259	-0.6095021	2.88092563	-8.1460738	0.00131545	0.01358432	DOWN	505.142079	505.139106	505.14635	10.1496833	10.14745	10.1714167
M448T10_2	1.52445007	0.6082889	2.03801647	9.29372235	0.00916186	0.03356554	UP	448.137373	448.128591	448.139353	9.63695	9.6166	9.68833333
M519T15	1.52108123	-0.6050972	2.40027776	-6.5189034	0.00397853	0.02302821	DOWN	518.614618	518.611173	518.618064	15.481275	15.4780833	15.4844667
M243T17	1.5163421	0.60059527	2.76595921	7.7550418	0.00171412	0.01584156	UP	243.123357	243.122711	243.124292	16.7584167	16.7355	16.7886667
M761T16	1.51582446	0.60010269	3.7779874	15.3680847	0.00016673	0.00569183	UP	761.225784	761.219871	761.226168	15.56165	15.4844667	15.6112333
M290T18	1.51566902	0.59995474	2.3382065	6.0786388	0.0045898	0.02509041	UP	290.172463	290.168635	290.173506	17.9403333	17.8955	17.9438333
M232T15	1.51137331	0.59586005	2.1964941	9.93687023	0.00636071	0.02907104	UP	232.114907	232.111812	232.115896	15.0644167	15.0585833	15.0818333

Selecting those with fold-change increase >1.5 and new page

Sort by UP/DOWN

	name	fold	log2fold	-log P-value	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
1														
2	M241T18_1	27.4045917	4.77634573	3.30532767	40.46059	0.00049508	0.00920165	UP	241.108312	241.107471	241.109437	17.59125	17.5871667	17.6198333
3	M240T18	16.4294376	4.03821119	2.05479101	10.0229195	0.00881473	0.03293413	UP	240.359872	240.356187	240.361536	17.5906667	17.5871667	17.5916667
4	M242T18	16.3870912	4.03448789	2.91477253	27.6130715	0.00121682	0.013362	UP	242.110341	242.109733	242.111274	17.5906667	17.5871667	17.5916667
5	M424T8_1	13.0179292	3.70242807	3.83389494	30.5724419	0.00014659	0.00557299	UP	424.028175	424.026455	424.029895	8.12178333	8.11845	8.12511667
6	M424T8_2	11.1363641	3.47720638	3.61174395	29.8423152	0.00024449	0.00703395	UP	424.0396	424.036272	424.044862	8.1363	8.12225	8.15796667
7	M227T16	7.16213011	2.84038873	2.75564323	19.5959381	0.00175532	0.01604506	UP	227.092249	227.090325	227.093361	15.8502167	15.8489333	15.8756833
8	M426T8	6.84146926	2.77430619	4.6257974	22.7140999	2.367E-05	0.00388536	UP	426.03226	426.032122	426.034541	8.13925	8.13335	8.15796667
9	M171T18_2	6.77813538	2.76088845	2.21189773	12.4050347	0.00613907	0.0288409	UP	171.102926	171.102581	171.103387	17.6959167	17.6403333	17.698
10	M278T15	6.10860512	2.61084298	2.49625447	16.1413878	0.00318967	0.02084209	UP	278.101274	278.100858	278.105129	14.64535	14.6205333	14.6608333
338	M587T8	1.17946394	0.23813131	2.24949465	5.44286982	0.00562996	0.02794546	UP	587.123047	587.120063	587.129497	7.51885	7.49283333	7.5402
339	M431T12_3	1.17183289	0.22876684	2.10375786	6.61525602	0.00787485	0.03165926	UP	431.191141	431.190612	431.191451	12.1466167	12.1357167	12.15485
340	M403T19	1.14660846	0.19737283	2.04169228	4.81730337	0.00908464	0.03347246	UP	403.269495	403.268237	403.270058	18.9244167	18.9113333	18.973
341	M545T10	1.14353987	0.19350666	2.73436639	7.36957773	0.00184346	0.01631577	UP	545.172989	545.170135	545.175188	9.96228333	9.93963333	9.98838333
342	M295T14	1.13866277	0.18734054	2.2077779	7.17433484	0.00619758	0.0288409	UP	295.058287	295.057779	295.059344	14.0641	14.0602	14.0875333
343	M383T12_1	1.1348121	0.18245344	2.17530962	5.61475903	0.00667868	0.03002729	UP	383.077906	383.077749	383.080144	12.4962667	12.4313833	12.49645
344	M635T11_2	1.13366549	0.180995	2.12820167	7.22587127	0.00744386	0.03105201	UP	635.23033	635.226956	635.231231	11.0496833	10.9833667	11.0760167
345	M576T17_2	1.13201205	0.17888932	2.00693443	5.45812005	0.0098416	0.03401502	UP	576.245602	576.244742	576.249542	17.081	16.9721667	17.2375
346	M231T16_1	1.13052077	0.1769875	2.66711209	13.5258148	0.00215223	0.01773485	UP	231.138481	231.13648	231.139489	15.6399167	15.6359833	15.64
347	M162T11	1.09119523	0.12590925	2.00277294	6.75086139	0.00993635	0.03401502	UP	162.053951	162.052952	162.054782	11.4542	11.4454167	11.4963667

Selecting those with fold-change decrease >1.5 and new page

	name	fold	log2fold	-log P-value	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
2	M377T18	3.72924023	-1.8988817	2.59661412	-9.9493137	0.00253155	0.01916149	DOWN	377.280273	377.279809	377.280485	18.2675	18.2525	18.299
3	M500T18_1	3.17718625	-1.6677497	6.37194281	-69.935309	4.2468E-07	0.00059116	DOWN	499.71989	499.714381	499.721035	17.9395	17.8955	17.9438333
4	M424T18	3.15396982	-1.6571689	2.30821987	-8.465829	0.0049179	0.02644019	DOWN	424.286919	424.285903	424.288206	18.2798333	18.2675	18.299
5	M493T18_1	3.14854207	-1.6546839	2.61484641	-12.339822	0.00242747	0.01871241	DOWN	492.732075	492.731096	492.733053	17.9241667	17.908	17.9403333
6	M251T17	3.12450783	-1.643629	2.34919635	-11.996006	0.00447511	0.02464783	DOWN	251.059425	251.059206	251.060194	16.6583333	16.6248	16.6707833
7	M231T16_2	3.00517465	-1.5874488	2.79422291	-16.956079	0.00160612	0.01517888	DOWN	231.15918	231.158231	231.160844	15.6376667	15.5836667	15.63795
8	M768T12	2.84825797	-1.5100798	2.07827637	-5.0543868	0.00835071	0.03256133	DOWN	767.829662	767.826195	767.833128	11.6209083	11.60575	11.6360667
9	M525T14_2	2.6848561	-1.4248448	2.30517791	-6.0601844	0.00495247	0.02646754	DOWN	525.284116	525.27453	525.287438	13.6901417	13.6671667	13.7188
10	M490T14_2	2.64201512	-1.4016387	2.82058063	-13.640201	0.00151154	0.01487326	DOWN	490.150671	490.14958	490.151411	14.2480333	14.2477833	14.26975

117	M419T11_1	1.17810989	-0.2364741	2.41792855	-7.9513274	0.00382007	0.02265429	DOWN	419.035272	419.033926	419.03625	11.3799333	11.3681333	11.3881667
118	M611T16_2	1.17035124	-0.2269416	2.00908774	-5.3602012	0.00979292	0.03401502	DOWN	611.278173	611.27616	611.284794	15.8765083	15.8224333	15.9544333
119	M565T15	1.16633313	-0.2219799	2.23155622	-6.7354962	0.00586737	0.02853127	DOWN	565.120094	565.119191	565.121328	14.6141	14.59155	14.6643167
120	M535T14	1.16407717	-0.2191867	2.26682318	-6.1061829	0.00540975	0.02737525	DOWN	535.110262	535.106754	535.111276	13.5184917	13.4834667	13.5622667
121	M221T19	1.15855517	-0.2123267	2.15628902	-5.6641662	0.00697768	0.03031699	DOWN	221.084846	221.08127	221.085397	19.0063333	18.9436667	19.0306667
122	M119T14	1.1541478	-0.206828	2.25839361	-9.9584077	0.00551577	0.02758935	DOWN	119.039425	119.033334	119.042616	14.0117333	14.0084	14.0381833
123	M621T15	1.14909666	-0.2005002	2.13061062	-5.1113482	0.00740269	0.03095745	DOWN	621.206427	621.205274	621.20758	15.1936583	15.1884833	15.1988333
124	M607T16	1.11674864	-0.1593045	2.01321577	-5.863233	0.00970028	0.03401502	DOWN	607.130977	607.129251	607.131787	15.9016833	15.87635	15.9026667
125	M517T12_1	1.08641081	-0.1195697	2.06759187	-5.3916682	0.00855871	0.03280741	DOWN	517.213031	517.208377	517.213509	12.1466167	12.1357167	12.1986333
126	M451T16	1.06454484	-0.0902367	3.25022678	-10.111341	0.00056205	0.00994236	DOWN	451.211411	451.208912	451.217074	16.034225	16.0070167	16.05845

Preparing for analysis using MetaboAnalyst

http://www.metaboanalyst.ca

Reducing the number of ions

Metaboanalyst will filter ions if you don't

	A	name	fold	log2fold	tstat	pvalue	qvalue	updown	mzmed	mzmin	mzmax	rtmed	rtmin	rtmax
1														
2	1207	M446T5	1.377175	0.461712	2.687478	0.055893	0.077433	UP	446.1187	446.1181	446.1194	5.003833	4.970667	5.037
3	40	M149T5	1.90913	0.932916	18.0073	0.00012	0.005015	UP	149.0452	149.0443	149.0455	5.004642	4.99615	5.09565
4	1904	M428T5	1.113881	-0.1556	-1.82025	0.145891	0.128174	DOWN	428.1189	428.1158	428.122	5.006933	4.97	5.043867
5	2305	M415T5	1.10218	-0.14036	-1.37875	0.24297	0.176326	DOWN	415.1069	415.106	415.1077	5.007133	4.99615	5.018117
6	3204	M431T5	1.088047	0.121741	0.55343	0.614273	0.320704	UP	431.0058	430.9997	431.0088	5.008492	4.97	5.0903
7	2951	M417T5	1.026591	-0.03786	-0.79944	0.484807	0.274812	DOWN	417.1238	417.122	417.1243	5.022617	4.999617	5.043867
8	2828	M461T5	1.211062	0.276273	0.875784	0.436068	0.257935	UP	461.0385	461.0379	461.0397	5.0238	5.010417	5.079233
9	1603	M737T5	1.461053	0.547009	2.301815	0.100029	0.10421	UP	737.1304	737.1269	737.1334	5.0261	4.970667	5.043867
10	2473	M430T5	1.09405	-0.12968	-1.19016	0.304376	0.205884	DOWN	430.118	430.1169	430.119	5.03155	5.022167	5.049267
11	620	M445T5	1.637928	0.711872	4.193747	0.015944	0.04293	UP	445.1174	445.1162	445.1178	5.03155	4.99685	5.0698
12	934	M134T5	1.160373	0.214588	3.212711	0.033865	0.060651	UP	134.0173	134.0161	134.0181	5.052367	4.992317	5.204667
13	647	M59T5	1.557694	0.639411	4.258795	0.017423	0.045047	UP	59.01358	59.01234	59.01613	5.063733	4.972833	5.09565
14	1919	M528T5	1.266863	0.341261	2.016901	0.14872	0.129638	UP	528.0399	528.0329	528.0433	5.078825	5.018117	5.116783
15	1091	M498T5	1.984819	0.989007	4.038585	0.046207	0.07082	UP	498.0279	498.0252	498.0327	5.087442	5.023067	5.131433

3087 ions – too many – need to reduce to 2000

3077	1706	M253T23	1.416906	-0.50274	-2.06326	0.114028	0.11168	DOWN	253.1829	253.1801	253.1839	23.30983	23.2595	23.36067
3078	2067	M515T24	1.091546	0.126373	1.903596	0.18148	0.146867	UP	514.9764	514.9749	514.9768	23.57683	23.5595	23.59583
3079	3049	M316T24_	1.126937	0.172406	0.704824	0.528703	0.289953	UP	316.257	316.2568	316.2576	23.67358	23.66283	23.695
3080	3307	M315T24_	1.069737	0.097255	0.469644	0.665489	0.336622	UP	315.2532	315.253	315.2548	23.67517	23.66217	23.69017
3081	606	M239T24	1.528422	-0.61204	-4.80953	0.015381	0.042457	DOWN	239.1648	239.1641	239.1658	23.74325	23.731	23.77283
3082	497	M415T24	1.750608	0.807856	4.712873	0.010264	0.034546	UP	415.2702	415.2695	415.271	23.82217	23.8215	23.82283
3083	3454	M293T24	1.035401	-0.05019	-0.38613	0.735035	0.355976	DOWN	293.2115	293.2107	293.2126	23.82742	23.79517	23.85517
3084	3810	M315T24_	1.010898	0.015637	0.076505	0.944836	0.414735	UP	315.2533	315.253	315.2539	24.01042	23.99983	24.0295
3085	3630	M316T24_	1.029368	-0.04176	-0.22468	0.84074	0.387428	DOWN	316.2574	316.2568	316.2581	24.01042	23.99983	24.0245
3086	1617	M661T24	1.103245	-0.14175	-2.1225	0.101419	0.104917	DOWN	660.9736	660.973	660.9755	24.471	24.44683	24.48183
3087	3726	M295T25	1.048568	-0.06842	-0.14967	0.892584	0.400688	DOWN	295.2277	295.2274	295.2282	24.54858	24.508	24.58
3088	3905	M296T25	1.00045	0.000649	0.001523	0.99891	0.427899	UP	296.2314	296.231	296.2319	24.56108	24.55383	24.56833

Order intensity according to treatment group

	A	fold	log2fold	tstat	pvalue	qvalue	mzmed	Rt_med	Mean_NR	Mean_IR
1										
2	2642	1.02133818	-0.0304606	-1.0167497	0.3679932	0.23299293	253.032519	13.693675	3306740.23	3237655
3	2050	1.06546213	0.09147932	1.64240724	0.1769922	0.14436164	305.070478	13.2466667	1696194.92	1807231
4	2363	1.03350529	0.04754577	1.31253109	0.26364298	0.18663292	293.114861	10.9970917	1498747.05	1548963
5	2062	1.06204886	0.08685014	1.83471727	0.17924862	0.14541289	387.165616	11.766275	1381508.16	1467229
6	219	1.56524555	0.646389	8.04462309	0.00249317	0.01904335	329.231644	19.98	908081.785	1421371
7	906	1.05982475	-0.0838257	-3.2232179	0.03249886	0.06000338	269.045847	15.4403833	1446007.76	1364384
8	3584	1.00772798	-0.0111063	-0.2483095	0.81740307	0.38137667	461.109237	12.6152917	1342598.38	1332302
9	3514	1.02524051	-0.0359624	-0.3227556	0.77687837	0.36981714	191.019737	6.29196667	1330544.62	1297788
10	262	2.10952508	1.07691824	17.1166071	0.00332954	0.0212578	187.097625	15.8236583	466760.497	984643
11	1204	1.04541773	-0.0640795	-3.3960413	0.05562863	0.07728721	477.103942	14.181775	949647.413	908390
12	3603	1.01149995	0.01649625	0.24489561	0.82505665	0.38304982	503.119673	15.1001833	837553.526	847185
13	2744	1.04975796	0.07005672	0.9921432	0.40313583	0.24569897	309.109607	8.73341667	730056.217	766382
14	3686	1.00585021	-0.0084155	-0.1880312	0.86797629	0.39388838	519.115262	16.6849167	750027.76	745665
15	3722	1.00579783	0.00834035	0.15323255	0.89011985	0.4000441	467.213875	16.0323333	696267.638	700304

Select the top 2000 ions

1990	2401	1.36338159	-0.4471894	-1.4532346	0.27812273	0.19376712	630.313619	17.0609167	7976.77407	5851
1991	1663	1.17585328	0.23370805	2.17623743	0.10786161	0.10849518	285.038382	14.1529083	4974.98016	5850
1992	3226	1.05094952	0.07169337	0.53776289	0.6215183	0.32227403	253.240184	13.6925833	5551.67508	5835
1993	331	1.71149617	0.77525806	6.8723704	0.00541689	0.02737525	165.05593	11.56685	3408.55073	5834
1994	3445	1.04068237	-0.0575298	-0.3728769	0.73078619	0.35479787	555.083177	15.2506667	6069.97147	5833
1995	711	1.18215249	-0.2414161	-4.2605693	0.02073158	0.04877515	582.082969	15.4834167	6872.78357	5814
1996	296	1.46569412	-0.5515841	-7.7272252	0.00427027	0.02413235	339.070163	14.5664833	8516.5468	5811
1997	349	1.28161543	-0.3579634	-6.5367788	0.00601293	0.02874864	624.267431	11.6629	7446.25062	5810
1998	2974	1.11100076	-0.1518598	-0.8069474	0.49568244	0.27880357	603.285239	13.2274	6453.07992	5808
1999	1022	1.58584765	0.66525418	4.24548217	0.04075359	0.06657426	517.298822	20.1105	3662.08997	5808
2000	3137	1.07937018	0.11018974	0.61520781	0.57876407	0.30857698	337.002039	15.6117667	5366.47259	5792

Areas of aligned metabolites by sample

negmode_nr1	negmode_nr2	negmode_nr3	negmode_ir1	negmode_ir2	negmode_ir3
3320701	3211285	3388235	3247804	3308733	3156426
1650544	1652507	1785534	1908024	1746573	1767097
1502083	1537549	1456609	1602389	1497667	1546834
1348351	1396739	1399435	1538662	1387977	1475049
942173	839275	942798	1519979	1335058	1409076
1476742	1443824	1417457	1401542	1343338	1348272
1389740	1326330	1311725	1399942	1301020	1295945
1395260	1463727	1132647	1307379	1276028	1309956
469492	464640	466149	1044107	964298	945524
963917	957988	927037	903994	904583	916594
846739	810984	854938	919809	799096	822651
751388	734458	704322	833628	740626	724893
757382	706826	785876	744625	749518	742854
685156	711925	691722	750467	674733	675713
804585	760221	750478	726812	609044	647972
717398	709880	739525	670560	613870	640035
513949	585959	582984	543410	498409	586219
22783	17915	17732	557699	528582	514971
501645	507853	464691	515378	475918	482951
477094	482742	490705	495190	477892	475722

non-irradiated diet irradiated diet

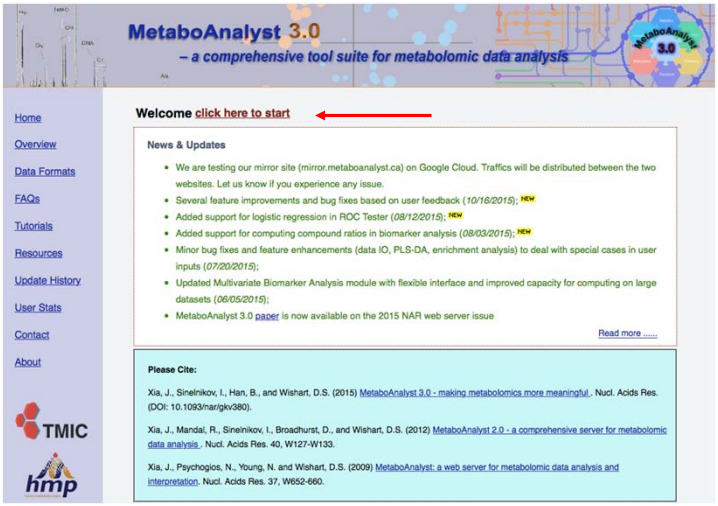
Creating .csv files for each sample

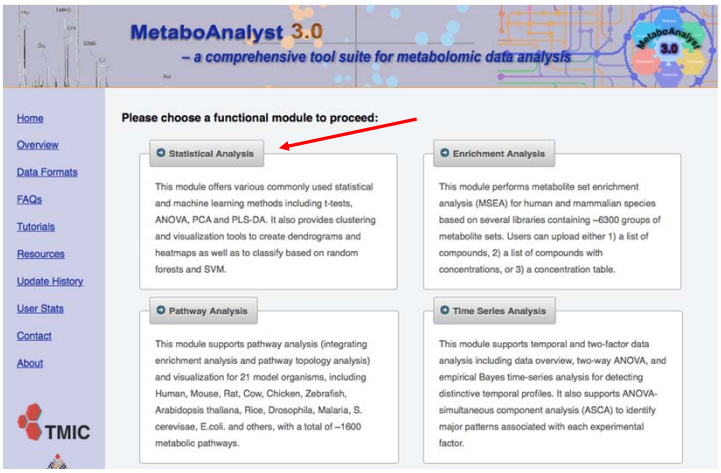
	mzmed	rtmed	negmode_nr1
2	499.71989	17.9395	9846
3	251.004448	14.646175	28534
4	417.212131	17.8596667	6340
5	199.133402	18.99725	18534
6	537.125737	13.7591333	17847
7	325.093116	11.2136667	23162
8	144.045677	16.2021167	26142
9	357.082227	12.6645667	12999
10	426.03226	8.13925	893
11	347.167955	13.059825	10415
12	591.136922	14.92045	26263
13	629.783983	16.3271833	3051
14	438.171148	12.3017917	15492
15	425.045022	8.13335	3088
16	151.039947	14.5387583	7302
17	506.109327	15.2182417	23595
18	789.227085	14.9668167	10301
19	315.142993	16.3572333	6312
20	640.774334	16.3572333	3677
21	582.278039	15.5049667	13805

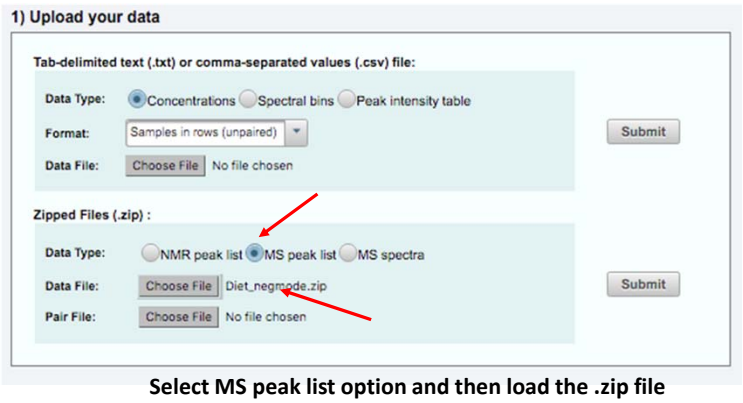
- **Copy the median *m/z* and median Rt values into a new Excel file. Then copy the column of areas from the first sample in Group_1. Save as an Excel .csv file.**
  - **Note that the file name must not have spaces – use an underscore instead of a space.**
- **Leave the file open and replace the yellow 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

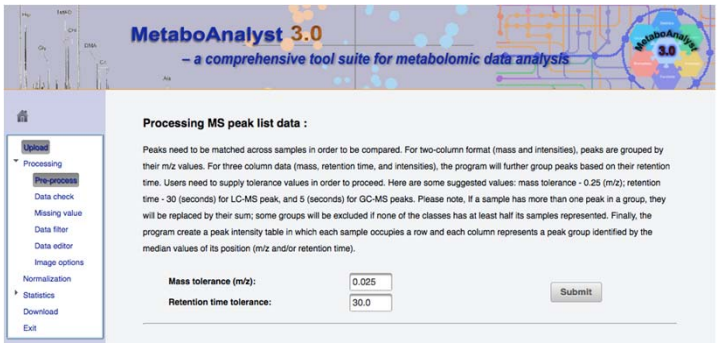
- Put each of the .csv files for group_1 samples into a folder named “Group_1”.
- Put each of the .csv files for group_2 samples into a folder named “Group_2”.
- Click on Group_1 and Group_2 folders and combine to form a .zip file.
  - Rename the .zip file as [your_name].zip
- You’re now ready to submit it to MetaboAnalyst
- http://www.metaboanalyst.ca

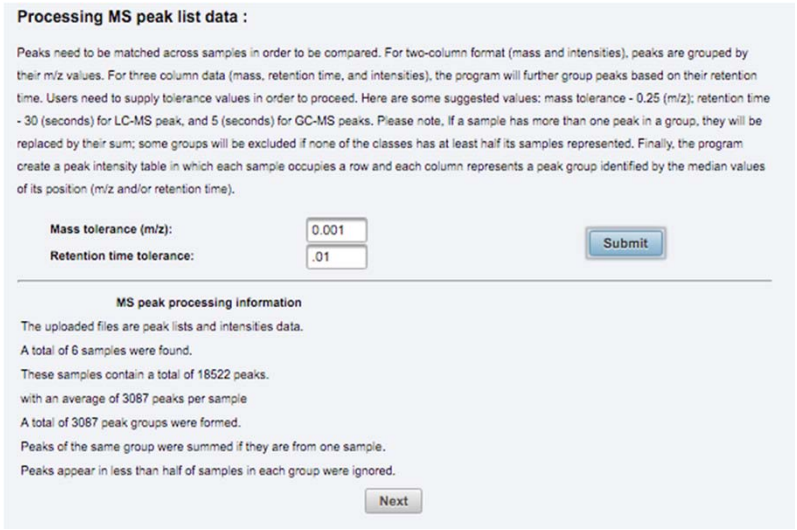


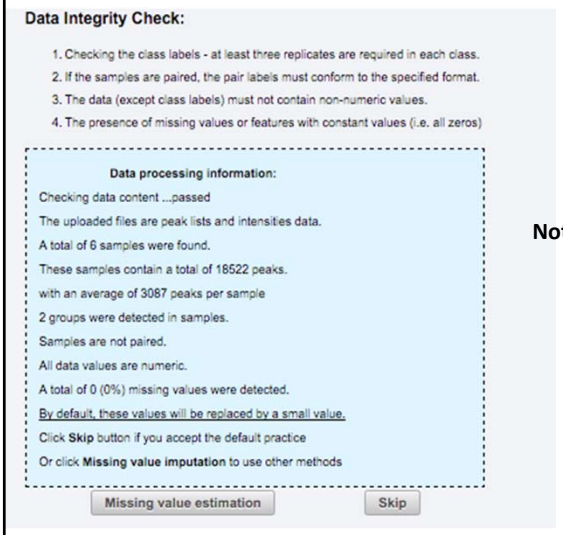




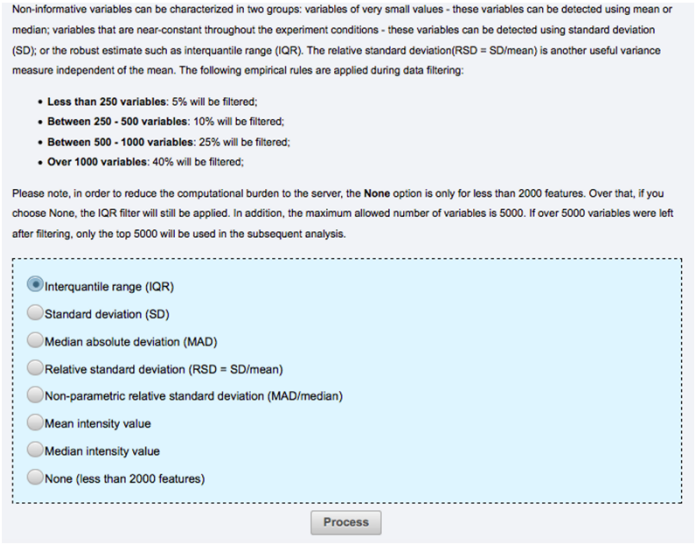
Select MS peak list option and then load the .zip file

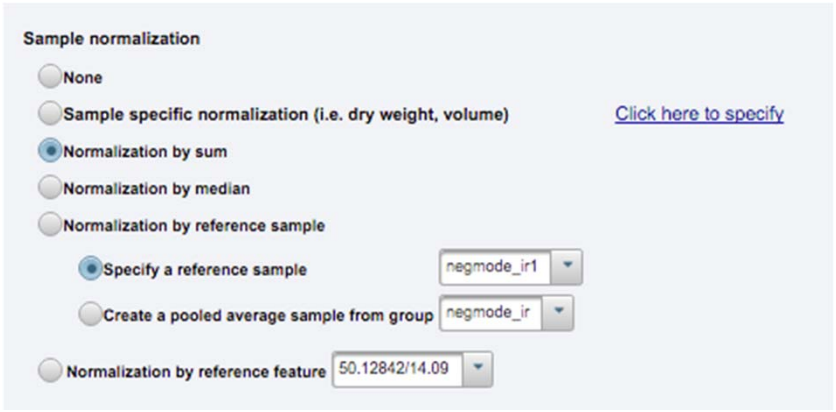
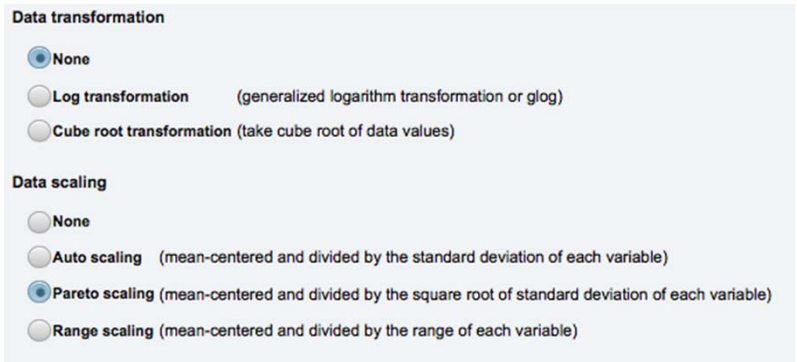

MetaboAnalyst 3.0
– a comprehensive tool suite for metabolomic data analysis
Upload
Processing
Pre-process
Data check
Missing value
Data filter
Data editor
Image options
Normalization
Statistics
Download
Exit
Processing MS peak list data :
Peaks need to be matched across samples in order to be compared. For two-column format (mass and intensities), peaks are grouped by their m/z values. For three column data (mass, retention time, and intensities), the program will further group peaks based on their retention time. Users need to supply tolerance values in order to proceed. Here are some suggested values: mass tolerance - 0.25 (m/z); retention time - 30 (seconds) for LC-MS peak, and 5 (seconds) for GC-MS peaks. Please note, If a sample has more than one peak in a group, they will be replaced by their sum; some groups will be excluded if none of the classes has at least half its samples represented. Finally, the program create a peak intensity table in which each sample occupies a row and each column represents a peak group identified by the median values of its position (m/z and/or retention time).
Mass tolerance (m/z):
0.025
Retention time tolerance:
30.0
Submit


Processing MS peak list data :
Peaks need to be matched across samples in order to be compared. For two-column format (mass and intensities), peaks are grouped by their m/z values. For three column data (mass, retention time, and intensities), the program will further group peaks based on their retention time. Users need to supply tolerance values in order to proceed. Here are some suggested values: mass tolerance - 0.25 (m/z); retention time - 30 (seconds) for LC-MS peak, and 5 (seconds) for GC-MS peaks. Please note, If a sample has more than one peak in a group, they will be replaced by their sum; some groups will be excluded if none of the classes has at least half its samples represented. Finally, the program create a peak intensity table in which each sample occupies a row and each column represents a peak group identified by the median values of its position (m/z and/or retention time).
Mass tolerance (m/z):
0.001
Retention time tolerance:
.01
Submit
MS peak processing information
The uploaded files are peak lists and intensities data.
A total of 6 samples were found.
These samples contain a total of 18522 peaks.
with an average of 3087 peaks per sample
A total of 3087 peak groups were formed.
Peaks of the same group were summed if they are from one sample.
Peaks appear in less than half of samples in each group were ignored.
Next



Note that XCMSonline filled in peaks





Data options before stats analysis