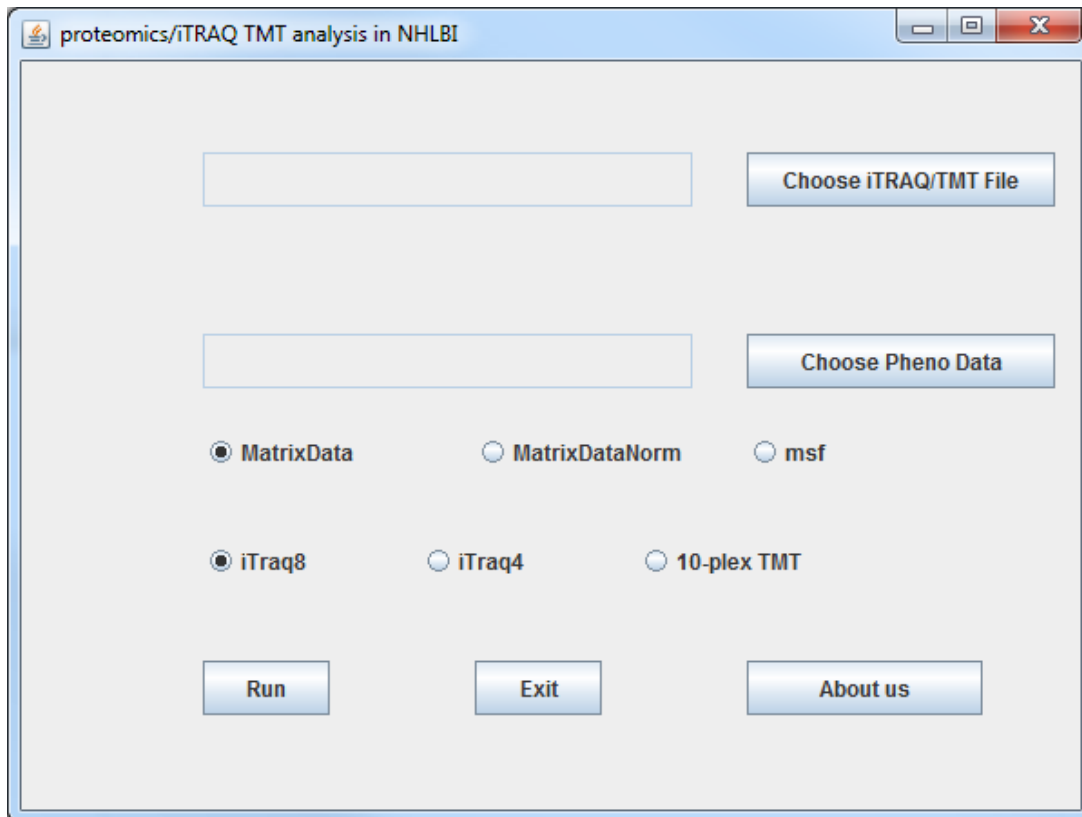


Manual

We developed software to conduct iTRAQ data analysis with Java.

Here is the step by step instruction of software

After downloading the software, double click iTRAQGU to start, the following interface should come out, otherwise please check whether java is installed on your computer.



Three type of iTRAQ data can be accepted. The first column of MatrixData type file is peptide sequence, the second column is the corresponding protein accession (group) ID. The following columns are the intensity of all tracks.

Pheno Data is tab-delimited text file, so far, only two classes are allowed.

We can use msf file directly, it takes around two hours to extract data from a typical msf file.

You also need tell it is a 4-iTRAQ or 8 iTRAQ.

After CLICK Run button, the following frame should come out

Proteome Data										
peptide	113	114	115	116	117	118	119	121	protein	
cQYVTEK	44214.39108	45833.19512	45953.43052	49910.50154	45431.68932	45753.53539	49531.11734	44747.05274	protein41	▲
iAIPGLAGA...	1963.981922	2051.223948	1874.099182	2551.566164	2185.939208	2404.381157	2586.187544	2414.199692	protein310	≡
aSITALEAK	45198.05374	46780.15267	47441.15356	47086.81628	47327.76126	45810.68225	50261.84934	50612.29296	protein54	
iTVTSEVPF...	53835.57525	53217.42344	55887.88276	57510.07478	53941.47123	53912.50151	56893.15068	52993.49218	protein391	
IVDQNIQSF...	1976.482576	2104.619185	2427.32521	2451.569404	2217.722272	2066.19849	2160.479874	2173.016572	protein562	
tDTVLILcR	14566.88952	14585.68626	16642.77721	12506.23399	14128.71005	14160.0012	16345.15779	14581.81348	protein795	
eQmEAEIA...	44539.741	44342.26795	45536.65426	47039.3231	44243.87458	46084.32161	45812.01566	45200.7085	protein109	
aYHEQLSV...	6427.357193	6137.292108	5972.404974	4994.851742	6248.590029	5962.490018	6669.994406	5591.057846	protein30	
wGDAGAEY...	507.592869	390.6696596	444.395126	490.2094214	491.5580838	458.5277646	511.3729526	545.2014008	protein1013	
IYDSmk	108528.0484	110033.9042	116394.2768	119875.9528	112569.2311	113123.9426	117052.0773	110423.7344	protein575	
eAVELPLT...	13532.16212	13449.39107	13837.89567	17431.5191	14270.13978	13436.53859	14457.92149	13864.28784	protein65	
sLGTIQQcc...	1515.29395	1715.83705	1847.415438	1471.247153	1553.301601	1466.29849	1487.60388	1721.056519	protein756	
rAPFDLFE...	62777.39266	63642.58739	66554.16858	73013.15992	66747.1611	63212.85664	77054.83204	73727.86866	protein707	
iFGVTTLDI...	76081.22015	75611.25405	81004.934	85603.47306	79995.15136	80929.51768	87368.63912	80564.75074	protein328	
ITFDITTFSP...	7968.062316	8193.085711	8822.30399	9541.66087	8080.147277	8348.023211	9507.247672	8821.789062	protein552	
aTIGADFLTk	10598.51691	10864.58613	11804.51975	12695.33611	10917.73675	11178.07852	11346.65109	10966.86914	protein59	
qEVESELLV...	4421.897256	4668.511255	4628.219966	4071.895188	4379.709297	4868.859111	5230.143602	4969.466674	protein674	
vGTFSEVvk...	5946.644994	5863.524202	5006.494484	5075.939658	6079.253228	5597.96957	4879.469724	5705.255982	protein916	
sNTPILVDGk	198223.728	202140.3225	204950.095	211501.016	204519.4449	205487.972	213967.1294	191982.2246	protein764	
dIELHLESS...	2450.308212	2693.541954	2719.387756	2375.834334	2443.890369	2467.773125	2142.685544	2290.046386	protein24	
IELQGPR	4704.459357	4833.528847	5265.02019	5016.789868	4716.581603	4896.174917	5334.094892	4551.638184	protein446	
gHELVPLE...	4504.625376	4166.929953	4591.101852	3273.555242	4340.841427	3903.136818	4996.168884	4344.072754	protein218	
sIPAYLAET...	7935.305697	8211.995269	8524.9929	9549.088442	8549.250029	8597.083369	9250.928604	9282.094758	protein745	
eALNScNk	12649.98894	12444.89486	13299.90544	13243.72793	12826.51567	13046.64146	13132.99563	10923.20264	protein60	
gNDIIAAK	10580.75973	9984.133942	9272.262088	10281.14217	9989.967935	10235.92697	11971.30045	10375.77148	protein239	
ITVADIP	6277.212221	6616.221067	7266.506106	6822.402042	6621.001823	6400.207567	6852.252569	6052.4202	protein560	▼

NormaliZation with Mean (20%-80%)

Save the Result

This is the original data, or the data extracted from msf file. We use the **mean and standard deviation of peptides ranking 20-80%** to normalize the data.

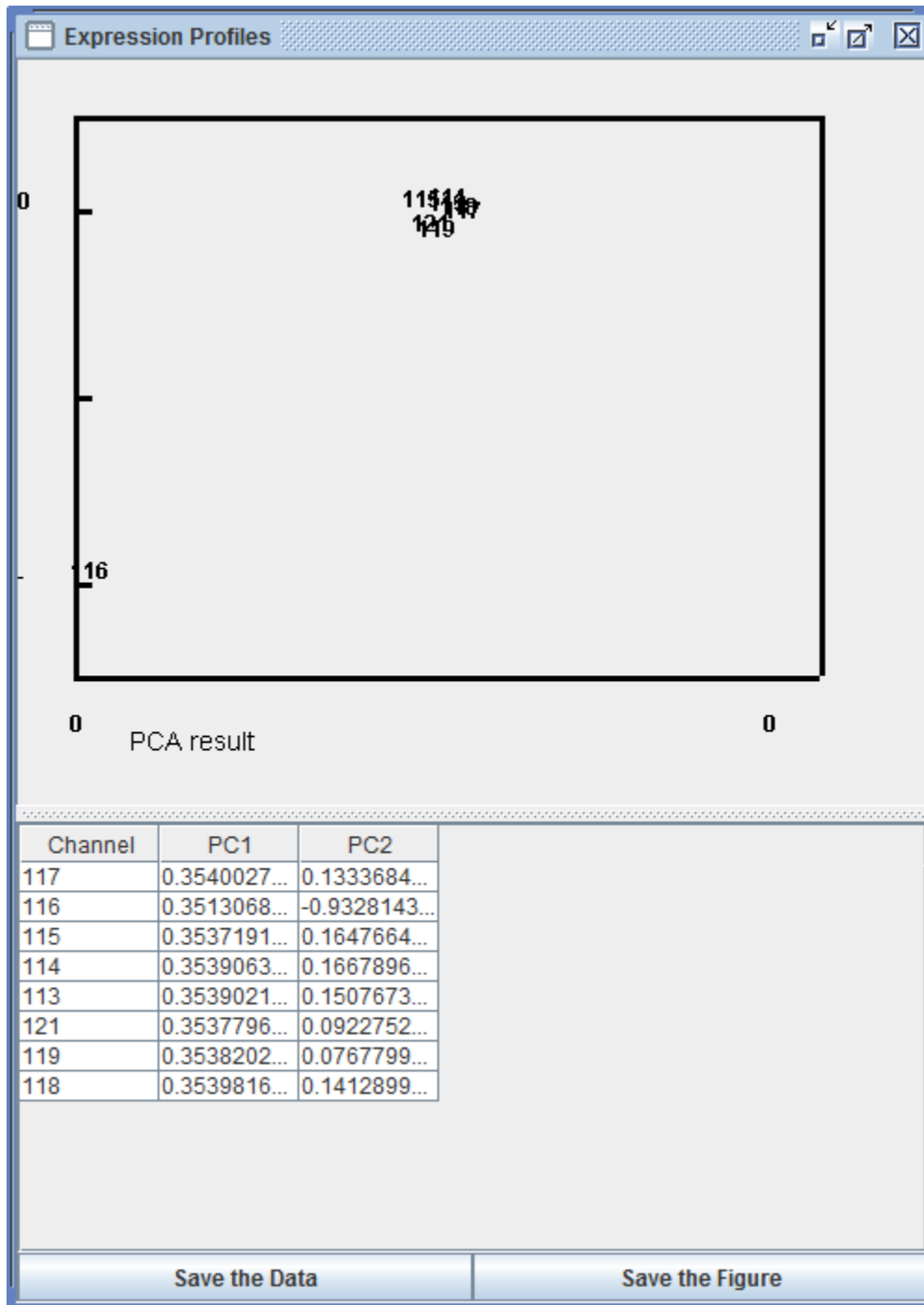
After CLICK Normalization with Mean (20-80%). The following frame should come out.

peptide	113	114	115	116	117	118	119	121	protein
QVYITEK	12.233479635071303	12.2165026483930516	12.209320906021032	12.34644811237365	12.23358901050553	12.2444310067396803	12.329195697491002	12.28033394291993398	protein41
IAUPLGAGAGNSVLVSNLN	8.661220917401855	8.70809894880295	8.56100705031553	8.96826391399102	8.778696933580541	8.885913450789092	8.947565880867873	8.930821741218345	protein319
ISTALEAK	12.25857948182505	12.273840259698307	12.245883384630361	12.28028472580212	12.281949733829998	12.24785488723852	12.3458799515497057	12.4211642439734963	protein54
IVTISEVPSK	12.458067424855473	12.432527786374217	12.420816704325053	12.50815141328618	12.439686572360684	12.433014610445445	12.487820782719478	12.474385883405055	protein391
IQDQHFSTYLR	8.6884584792861	8.730265421465523	8.85565448448265	8.91269539127382	8.785142107185919	8.713205163758478	8.741570227315187	8.810974092245085	protein452
IQVLYLR	10.96694996288193	10.945246435186757	11.051218274589615	10.769547194031048	10.90472753133547	10.90854635154229	11.059295019350395	10.994007721410345	protein795
KEMEAHILK	12.241842775715582	12.212825917420767	12.19806304437811	12.29134837687076	12.205168693658734	12.254648406857497	12.239706019126114	12.20191735562095	protein109
KVIEGLSDVAETNACFEPAK	10.033534816871987	9.958384675913374	9.862622964117655	9.72307287134405	9.97525344898337	9.921905198852163	10.032706397222556	9.89425899161222	protein30
IQDQHFSTYLR	7.13778340037011	8.184697017484415	8.19842606402489	7.07846837720356	7.078642463445138	6.8697189564118555	7.09114133735651	7.237161027184456	protein1913
YDNEK	12.25778881787554	12.248922629376375	12.20902229281014	13.345125325929472	12.26907379583238	13.279022697541244	13.314214152929598	13.3165313264742	protein575
6AVELPLTHPELYK	10.88290028781732	10.852783481744764	10.840760926322043	10.917928197597725	10.916071085740197	10.848723679816835	10.91873334428064	10.93611909859541	protein65
ITGCDPLK	8.38530111201845	8.504545268870429	8.44654774230768	8.33083802114571	8.38454748085176	8.321771633700749	8.31416850817298	8.54255643061638	protein756
HPPELFEHR	12.633349552637422	12.624670389357277	12.631699919657281	12.780131701488028	12.673640795004197	12.619153952132022	12.635250820047727	12.853214510311534	protein707
6FVTLTLVLR	12.852601482387146	12.821213781196928	12.855758117282437	12.951413670134966	12.87990679415611	12.886991280512518	12.979130263548878	12.94581294128149	protein328
ITFDITTSPTNGK	10.278748324835514	10.28774300943722	10.327486526956	10.451238847405774	10.28811242238357	10.30579729747556	10.43865757429624	10.41746703086549	protein552
RVSESLIITEAHR	10.604161863413054	10.60947563055387	10.69537115648389	10.789448495435995	10.6170012044190188	10.638059815203888	10.641233163758125	10.687196289534072	protein619
GVTFSEVPAAGTVEQDS	9.60701545698927	9.64654145029822	9.59187686728228	8.480889715478363	9.570037146299436	9.600793920648868	9.76418689770058	9.75905037787149	protein674
6NTPLEVDK	13.944839673339654	13.94225499146949	13.914236889034671	13.992114613474252	13.943917515652456	13.959918000107854	14.00500115244138	13.951138416693329	protein764
SEDLSSSSSHQTDHQR	9.933591424070558	9.91955524269546	9.865511127145959	8.875941700801146	8.905775277579702	8.91559896543075	8.742097752959709	8.87025874641079	protein624
ELQDPR	9.67167324207342	9.695141953827391	9.7388737919611957	9.72894695244072	9.654824401765098	9.697145795393258	9.77821668691273	9.858302277642515	protein445
QHELVLQER	9.62815852810048	9.51686617199849	9.582693887290626	9.242188932519402	9.560248305236728	9.43887529536571	9.70176123357056	9.604755427824406	protein218
8IPAYLAETLYYAmk	10.27448221638217	10.250371119546591	10.28836553795823	10.462125546223213	10.33240397323505	10.3393228244447	10.407354881509184	10.475818064172884	protein745
6ANSQW	10.808007113793385	10.742971451050225	10.789544609581798	10.834838752689298	10.794544717614826	10.8191330479576	10.808888751703258	10.662589280011375	protein610
QEDIAHK	10.6022496891332	10.51313143292598	10.28402262309207	10.54526984687117	10.509286238735966	10.538364088806513	10.5281172823379	10.603652515536217	protein238
ITVADIR	10.004871810492372	10.043891489470509	10.0470637595228	10.080830835241187	10.042959077148926	10.020259077148926	10.06399844727074	10.144438183255583	protein560
IAVTEGcQFSR	10.004784522577711	9.985728063237932	10.000574811250444	9.97842408911128	9.958887422511486	9.822801216882387	9.986475295296843	9.99715180024005	protein587
ITNIPQLLQK	8.530253744083954	8.69428115527481	8.50873718757118	9.132519685092829	8.899337015729166	9.01833489575058	9.025897878339449	8.911470396321248	protein389
HEVLEVDGHR	9.8956867827098	9.87369756895335	9.821927163478967	10.08673840889917	9.91271778972078	9.937305438677164	10.04547048120481	10.04774763121869	protein492
IEASIAHALETLEQHR	10.609327160914876	10.66302633891129	10.511350854533683	10.70219702563307	10.609609413756615	10.611871769019002	10.6158300800975728	10.627257040015246	protein796
IFDQAFCLR	12.015436338840095	12.03145480433797	12.03145480433797	12.03830333142034	12.034329063445149	12.050907083648355	12.09819188367787	12.142988445188824	protein452
QTNLCVGGDSLTGANLRR	10.053343113229637	10.136141886829528	10.13593257706822	9.988893487884458	10.092033118953026	10.17217384513056	10.111418614608835	10.038762317738195	protein229
IEVAHLVR	13.113970615480138	13.62712638575682	13.57712655493484	13.875548192776	13.62398471785993	13.8476880269123	13.874378840117678	13.687236011663063	protein425
6ALSALAHFLNK	9.0242791141238	8.988753928968952	8.90442654741558	9.171889575757586	8.97117495056187	8.942051328288194	8.938813971095549	9.050248379551672	protein502
IVEMTSK	14.01971286582484	14.03562653580803	14.03562653580804	14.25238276785545	14.037470210077313	14.071454827332200	14.126093404129052	14.136117266103403	protein393
QTVYVNTILITTR	13.851888345099881	13.841305059315053	13.837164701345658	13.889587196914657	13.829154291438536	13.8047288728480136	13.858621155998024	13.894942091479125	protein247
ILVQVYTEDAK	12.53988278117134	12.54961928835126	12.541735619787653	12.5698059115235	12.559025687455531	12.5344954486330911	12.62494848491784	12.65052084154859	protein488
ILEAVQK	9.426959708404758	9.51333064635676	9.415555078830956	9.46178099435397	9.452725548350742	9.542683575665555	9.537033140407537	9.454527937502071	protein20
6EGAGHQK	12.250614320245845	12.2648810836482	12.18143949992138	12.275320425148886	12.248040406498786	12.286456470653425	12.424913419901532	12.164318956788818	protein11
ILKIDQK	11.244130560273452	11.27980815731568	11.248399703022895	11.309670873741372	11.22796732908333	11.228746107907827	11.347085843505966	11.385448278276706	protein484
ITVYFSLQLR	10.112225292695954	10.259197122144525	10.21046232760996	10.19172141469345	10.211524591079594	10.13008948772172	10.17530250295399	10.16167891960579	protein716
QADLVSEQLHRR	9.424666178780232	9.3287245859951	9.52087674398273	9.326300463611474	9.30518043454201	9.480036196330486	9.31430428020421	9.403973350170075	protein559
BLVLYK	12.659216345777469	12.856428978012883	12.851136217800591	12.878196132520993	12.861680101915942	12.850002449669228	12.995155544154527	12.94562875644925	protein222
SYGPAPGAGHYQEESNL	10.059524919717335	10.074175342987	9.95121500213168	10.075795240898195	9.882127875909142	9.914925141324487	9.89515826271587	9.833061978822228	protein791
HALAYKQAPLQLTQK	8.1735049245938	8.158781151388305	8.114511073365744	8.328897842861829	8.13610778323251	8.25676937406921	8.262921312777144	8.2781158820834	protein763
QVYSTERIKLL	11.201038074711584	11.20794860788673	11.2550416314514	11.350051448195453	11.15788179565217	11.183744113192126	11.245988701736352	11.279533331641053	protein702
IVADIR	13.688271202244584	13.831647701032974	13.832324768659794	13.91260156805276	13.82765081752198	13.846361088238481	13.916823403819026	13.90135146147722	protein433
ILILPDMLK	11.706252975219599	11.74458028774517	11.850502068919432	12.00759099709191	11.68142623117187	11.59547452031163	11.832751162449287	12.020459687057988	protein160
QVYEVLTQTK	9.78034674809967	9.871715705139044	9.842637917102252	9.8624239383539	9.8457918868879	9.8299273358011	9.74561739817592	9.97154992056798	protein119
6QLNCGQVTR	13.405049451533893	13.383796482714619	13.354118057969142	13.349784788863917	13.36078901493438	13.401619576822428	13.459404167572987	13.486190906550948	protein15
IFLEELSFQVGEHGR	5.9817659107438725	6.21550177193426	5.656161032026051	6.433655017004285	5.545487452614199	6.368487655316707	6.052379652880771	6.0263568138767915	protein329
ILALPYAK	12.637168456570383	12.684343659415532	12.638931048173745	12.675890971420888	12.668326275918892	12.72126266299945	12.72419020078841	12.72419020078841	protein20
QEVLYNFWGR	6.343370815303813	6.31291544384381	6.51635364219594	6.547774998195254	6.289972508958999	6.30955969786974	6.330381323390009	6.526031766502185	protein672
PCA	Weighted method	Cauchy fitting	Rank Sum	Ttest	Save the Result				

This is the normalized data. Note that the intensities are log 10 normalized. We can conduct PCA analysis. Two pairwise statistical methods, T test, ranksum are included. We also fit the ratios of all peptides with Cauchy distribution and use the fitted distribution to estimate the statistical significance (It is assumed that ratio of two normal distributions, which is the case is iTRAQ follow Cauchy distribution).

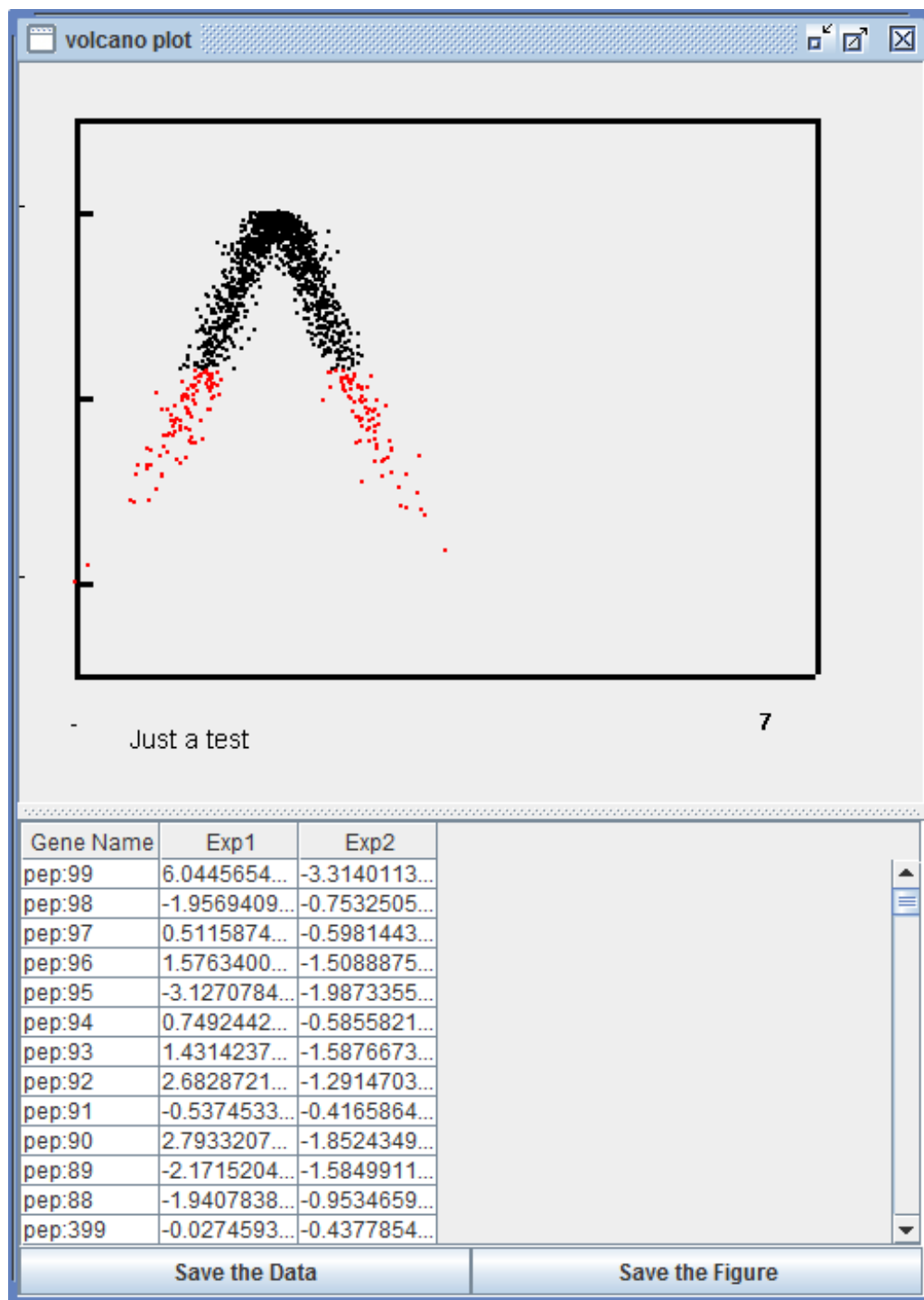
The weighted method is a kind of linear model. It assumes the statistical variance is dependent on the number of peptides and their intensities (the detailed can be found in “**Defining, Comparing, and Improving iTRAQ Quantification in Mass Spectrometry Proteomics Data**”). It fits the relationship between variance and intensity with LOWESS for different number of peptides for certain protein. We modified the method and calculate the weighted ratio with error estimations. The basic idea behind this method is that the proteins with low intensity and small number of unique peptides tend to have higher variance.

Below figure shows the result of PCA analysis. We can save the PC1 and PC2, and save the Figure (resolution =200DPI, can change)



Below figure shows the result of t test. We got two tables, one is the result at peptide level, another one is the result at protein level. We can save the results and draw volcano plots

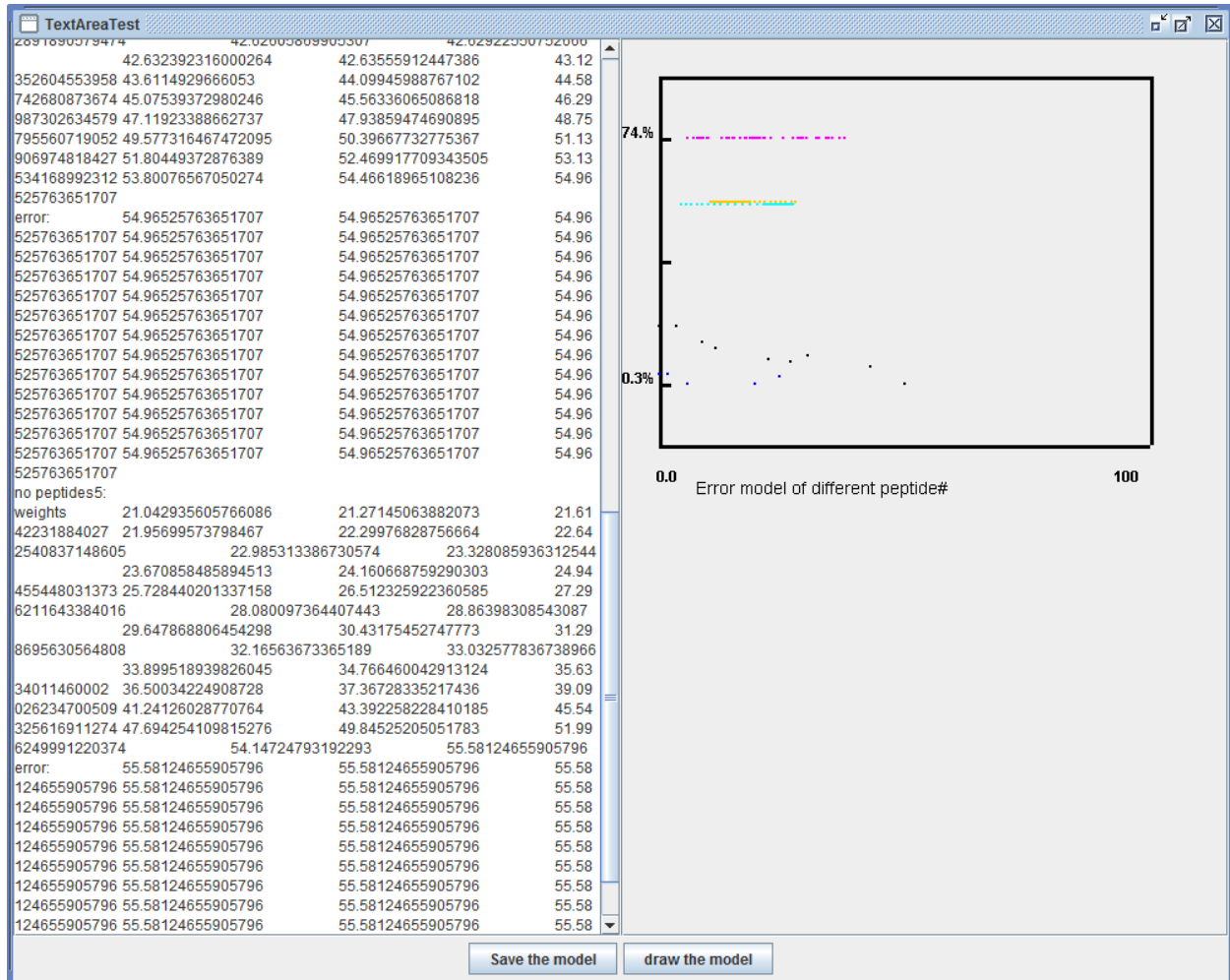
iTRAQ Result			
protein	peptides #	t value	pvalue
protein131	1	-0.7693321495350608	0.4708874412737347
protein373	1	1.7120842855234826	0.1377222111206564
protein132	1	-0.9777555864192271	0.3659556619376123
protein374	1	0.13329613586727407	0.8983175089135136
protein133	1	0.6521682007335402	0.5384590338874942
protein375	1	-0.2668188856954916	0.7985420347148116
protein134	1	1.0307786450148488	0.34239238746862055
protein376	1	-2.4679893495915577	0.048590009926500866
protein370	1	-3.451913183465316	0.013601115084972637
protein371	1	-1.6259157809268818	0.15509256483495304
protein130	1	1.3165298679656021	0.23604825178902544
protein372	1	-1.2997208745350806	0.2413948315844423
peptides #	t value		pvalue
cQYVTEk	0.32236638416123875		0.7581118486455285
iAIPGLAGAGNSVLLVSNLNPER	1.7202030599434581		0.13618621264108155
aSITALEAk	1.525274740466925		0.17803521111765463
iTVTSEVPFSk	0.0321301240318876		0.9754104329089262
IVDQNIJSFYLSR	-0.6188549885698093		0.5587597839133152
tDTVLILcR	0.4656406246808643		0.6578952802349662
eQmEAEIAHLk	0.5469515759623645		0.6041426892306712
aYHEQLSVAEITNAcFEPANQmVk	0.7598568060106468		0.47612580511397673
wGDAGAEYVVESTGVFTTMEk	1.2420691424015222		0.2605567145898662
IYDSmk	0.5400932512861065		0.6085796629050413
eAVELPLTHFELYk	-0.36141890606418586		0.7301722304680158
sLGTIQQccDAIDHLcR	-0.6968678015094285		0.5119671121496698
ProteinVolcano		Save the Protein Result	PeptideVolcano
		Save the Peptide Result	



This is the volcano plots. Can save the result and save the figure.

Ranksum and Cauchy fitting' results can be conducted with same way.

After clicking Weighted method, we can get two frames. One is the summary of the model. And one is used to show the result.



We can see the weigh errors relationships for different number of peptides. The right part is the figure (working on it to beautify)

iTRAQ Result										
protein	protein_wet...	protein_rati...	protein_pe...	error_matri...	protein_wet...	protein_rati...	protein_pe...	error_matri...	protein_wet...	pro...
protein131	60.818882...	1.0001742...	1.0	7.6631443...	60.818882...	0.9998257...	1.0	7.6631443...	60.818882...	0.98
protein373	7.0577140...	0.9668061...	1.0	16.528579...	7.0577140...	1.0331938...	1.0	16.528579...	7.0577140...	1.15
protein132	23.278991...	1.0104028...	1.0	12.122116...	23.278991...	0.9895971...	1.0	12.122116...	23.278991...	1.14
protein374	53.681998...	1.0000446...	1.0	8.0711625...	53.681998...	0.9999553...	1.0	8.0711625...	53.681998...	1.05
protein133	17.739082...	1.0037580...	1.0	13.594926...	17.739082...	0.9962419...	1.0	13.594926...	17.739082...	1.02
protein375	23.278991...	0.9862230...	1.0	12.122116...	23.278991...	1.0137769...	1.0	12.122116...	23.278991...	1.12
protein134	86.444362...	0.9860752...	1.0	3.8621523...	86.444362...	1.0139247...	1.0	3.8621523...	86.444362...	1.05
protein376	23.278991...	0.9534090...	1.0	12.122116...	23.278991...	1.0465909...	1.0	12.122116...	23.278991...	1.11
protein370	44.541267...	0.9684535...	1.0	8.9442920...	44.541267...	1.0315464...	1.0	8.9442920...	44.541267...	1.02
protein371	7.0577140...	1.0563859...	1.0	16.528579...	7.0577140...	0.9436140...	1.0	16.528579...	7.0577140...	1.06
protein130	23.278991...	0.9828964...	1.0	12.122116...	23.278991...	1.0171035...	1.0	12.122116...	23.278991...	1.09
protein372	7.0577140...	1.0564656...	1.0	16.528579...	7.0577140...	0.9435343...	1.0	16.528579...	7.0577140...	1.06
protein139	7.0577140...	0.9986229...	1.0	16.528579...	7.0577140...	1.0013770...	1.0	16.528579...	7.0577140...	1.25
protein135	53.681998...	0.9893747...	1.0	8.0711625...	53.681998...	1.0106252...	1.0	8.0711625...	53.681998...	1.06
protein377	86.444362...	0.9834702...	1.0	3.8621523...	86.444362...	1.0165297...	1.0	3.8621523...	86.444362...	1.09
protein136	53.681998...	1.0170769...	1.0	8.0711625...	53.681998...	0.9829230...	1.0	8.0711625...	53.681998...	1.07
protein378	23.278991...	0.9470086...	1.0	12.122116...	44.541267...	1.0529913...	1.0	8.9442920...	44.541267...	1.06
protein137	7.0577140...	0.9628173...	1.0	16.528579...	7.0577140...	1.0371826...	1.0	16.528579...	7.0577140...	1.04
protein379	23.278991...	1.0814543...	1.0	12.122116...	23.278991...	0.9185456...	1.0	12.122116...	23.278991...	1.10
protein138	44.541267...	0.9191140...	1.0	8.9442920...	44.541267...	1.0808859...	1.0	8.9442920...	44.541267...	1.18
protein120	60.818882...	0.9772401...	1.0	7.6631443...	60.818882...	1.0227598...	1.0	7.6631443...	60.818882...	1.08
protein362	17.739082...	1.0081025...	1.0	13.594926...	17.739082...	0.9918974...	1.0	13.594926...	17.739082...	1.12
protein121	53.681998...	1.0641156...	1.0	8.0711625...	53.681998...	0.9358843...	1.0	8.0711625...	53.681998...	1.22
protein363	60.818882...	1.0023507...	1.0	7.6631443...	60.818882...	0.9976492...	1.0	7.6631443...	60.818882...	1.06
protein122	53.681998...	0.9864244...	1.0	8.0711625...	53.681998...	1.0135755...	1.0	8.0711625...	53.681998...	1.10

Close
Save the Result
Average the Result

The Table shows the result of weighted method. Each channel has four columns. The weight (intensity), the estimated ratio, the number of peptides, the error estimation of ratio (percentage)

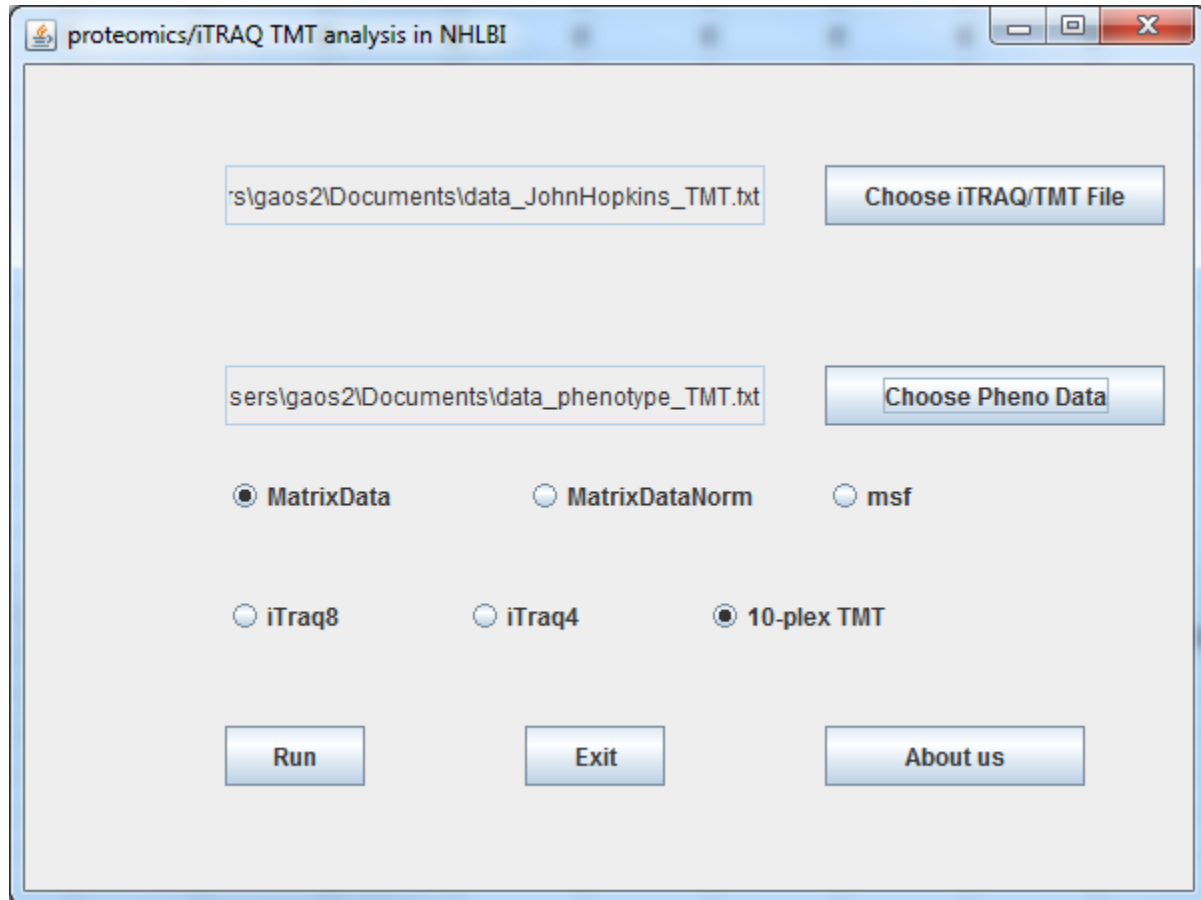
After clicking “Average the Result”. The program uses the variation as the weight to generate a ratio of proteins with phenotypes. And the following frame will come out.

Pathway Result			
protein	peptides #	Value	pvalue
protein1049	1.0	0.9868700366720126	0.0010047023913679635
protein35	3.0	1.0192992848127482	0.0010129159687056744
protein968	1.0	0.9870432539168431	0.00101553883102021
protein703	1.0	0.987045281991911	0.0010156670896269045
protein85	1.0	0.9870548166913977	0.0010162705125906402
protein935	1.0	0.9870732042200838	0.0010174362289695723
protein245	1.0	0.9870817224660976	0.0010179771668068541
protein330	1.0	1.0192143982930806	0.0010182527505702144
protein914	1.0	1.019197786630753	0.0010193037149541784
protein493	1.0	0.9871323522981162	0.0010212042244971975
protein609	1.0	1.019157040074115	0.001021890837414774
protein340	1.0	0.9871927335203788	0.001025079631428515
protein332	1.0	0.9872245801011268	0.0010271354732362533
protein925	1.0	1.0190302046290278	0.0010300288636728716
protein495	1.0	0.9873335102278492	0.0010342300889844458
protein452	1.0	1.0188804581628965	0.0010398055920608495
protein640	1.0	0.9874426409973572	0.0010414365916469246
protein852	1.0	1.0188020753296498	0.00104499756930021
protein468	1.0	0.9874981288248366	0.0010451393516472496
protein938	1.0	1.0187630890356385	0.0010475993546497087
protein212	1.0	0.9875940227627301	0.001051600817884074
protein587	1.0	1.018610631764765	0.0010578995228976984
protein347	1.0	0.9878132088597193	0.0010666737537824223
protein838	1.0	1.0184136085898505	0.0010715149173735616
protein892	1.0	1.0183793300526875	0.0010739196769689041
protein1020	1.0	1.0182574160675247	0.00107549237827020074

The pvalues need to be adjusted.

The following part is the result from TMT proteomics data. The sample data was downloaded from http://www.biostat.jhsph.edu/~kkammers/software/CVproteomics/TMT_experiment.csv

The sample data is reformatted and renamed to data_JohnHopkins_TMT and data_phenotype_TMT



After CLICK Run button, the following frame should come out

Proteome Data											
peptide	X126	X127_N	X127_C	X128_N	X128_C	X129_N	X129_C	X130_N	X130_C	X131	protein
aQEDL	2199727...	313912.9...	677040.4...	2093226...	2.215218...	2318118...	880272.4...	666260.7...	628410.0...	585675.3...	21361657
dDEPAV/k	1965852...	1537493...	982955.0...	2278005...	4262563...	3746353...	447908.8...	2588878...	479377.6...	2804112...	90669511
aLASLAT	497381.2...	2571366...	1.202949...	9236156...	391651.3...	1759883...	374388.0...	1275037...	2082623...	192712.2...	21359969
aDVFHA	4976873...	2901622...	2881726...	1.300327...	5721048...	994521.3...	1097624...	961874.2...	768550.8...	2197554...	21361794
dTDSINL	1651345...	2.566566...	6011535...	1235615...	1622253...	3678017...	44721.00...	407384.1...	161845.6...	605040.4...	4507943
dLNDRP	605361.1...	1165664...	2195390...	1351133...	1801050...	718412.4...	250125.2...	2991126...	36151.79...	1198173...	1187223...
dQMQQD	386068.6...	1.159327...	679053.7...	229760.8...	641888.6...	1097443...	770031.4...	2308532...	4680090...	307038.6...	62422577
aLIEAEk	879527.2...	8470118...	324314.8...	1571827...	5669931...	294187.5...	1119431...	564157.8...	468441.8...	1468794...	6005721
aPmVNP	7141736...	5412024...	3119707...	350920.3...	1872752...	2722785...	8220236...	150563.8...	496310.6...	163703.9...	1229391
aELGMG	279011.3...	232842.7...	3971301...	816337.3...	5531563...	5687866...	1131646...	857110.2...	7171599...	951646.0...	3347244...
aInSTIT	389647.8...	1555541...	1.298507...	1575647...	802991.0...	368210.5...	566753.0...	1932792...	1981822...	919643.6...	4759198
aTPDEA	4719379...	3545649...	1080739...	1309021...	798655.8...	350229.2...	1038276...	1718424...	3804599...	333972.2...	24371272
aQLLQP	2747981...	2223405...	1076618...	2264293...	1.137682...	91212.50...	499123.0...	2887447...	354922.7...	992169.6...	1557229...
eEAYNS	151964.2...	755420.7...	651227.4...	2997910...	1007329...	3913581...	1919697...	206896.2...	361134.8...	267709.1...	31621305
aALcAVH	907753.1...	720697.8...	354109.3...	1051171...	297919.1...	453448.5...	589306.9...	652810.0...	664002.5...	51888.65...	71772942
aEDGSVI	294877.7...	952038.3...	3242258...	7.051366...	713223.1...	252283.8...	556561.6...	2086240...	5359828...	2683339...	2098628...
aTDGSL	498452.0...	1105501...	1275795...	846654.4...	569943.6...	180809.4...	606614.5...	243690.6...	331123.2...	946706.5...	4885637
eDLVEEI	3042367...	313258.0...	1060590...	279876.9...	1169470...	159809.3...	132035.7...	1286052...	1035676...	1509888...	3844755...
aSQAqP	2528074...	1549644...	266318.0...	7131482...	4783689...	2666240...	1006477...	731430.3...	315235.7...	1270497...	2958423...
dVDAVDk	1182159...	2489566...	2456134...	1.292631...	1.359620...	88323.97...	501545.5...	1002936...	1675877...	262652.0...	5454032
aLGDSS	3280309...	2029175...	968031.2...	4223551...	1062121...	1620945...	1248820...	2572390...	1010870...	476921.1...	14150169
eAGVEm	439203.2...	1081238...	1.072643...	1986520...	1709985...	899845.7...	1189939...	352804.1...	1911769...	3011350...	45439306
eALNScNk	977561.6...	3.378622...	216164.1...	233160.8...	2470253...	118978.9...	197367.6...	210625.3...	342011.5...	240898.9...	55956788
dLAIGLR	265528.4...	5281291...	1010501...	3741773...	500921.8...	2784941...	1283593...	350137.9...	313990.0...	626177.0...	56243599
dVmPEV	2062906...	479696.1...	143435.6...	334715.9...	2140515...	568105.3...	555164.5...	102668.7...	165591.8...	746664.4...	18201905
dCICRM	2674247...	274667.7...	2.062628...	422168.0...	060708.0...	522176.1...	420220.2...	820058.4...	4950825...	250278.6...	4502525

NormaliZation with Mean (20%-80%)

Save the Result

This is the original data, or the data extracted from msf file. We use the **mean and standard deviation of peptides ranking 20-80%** to normalize the data.

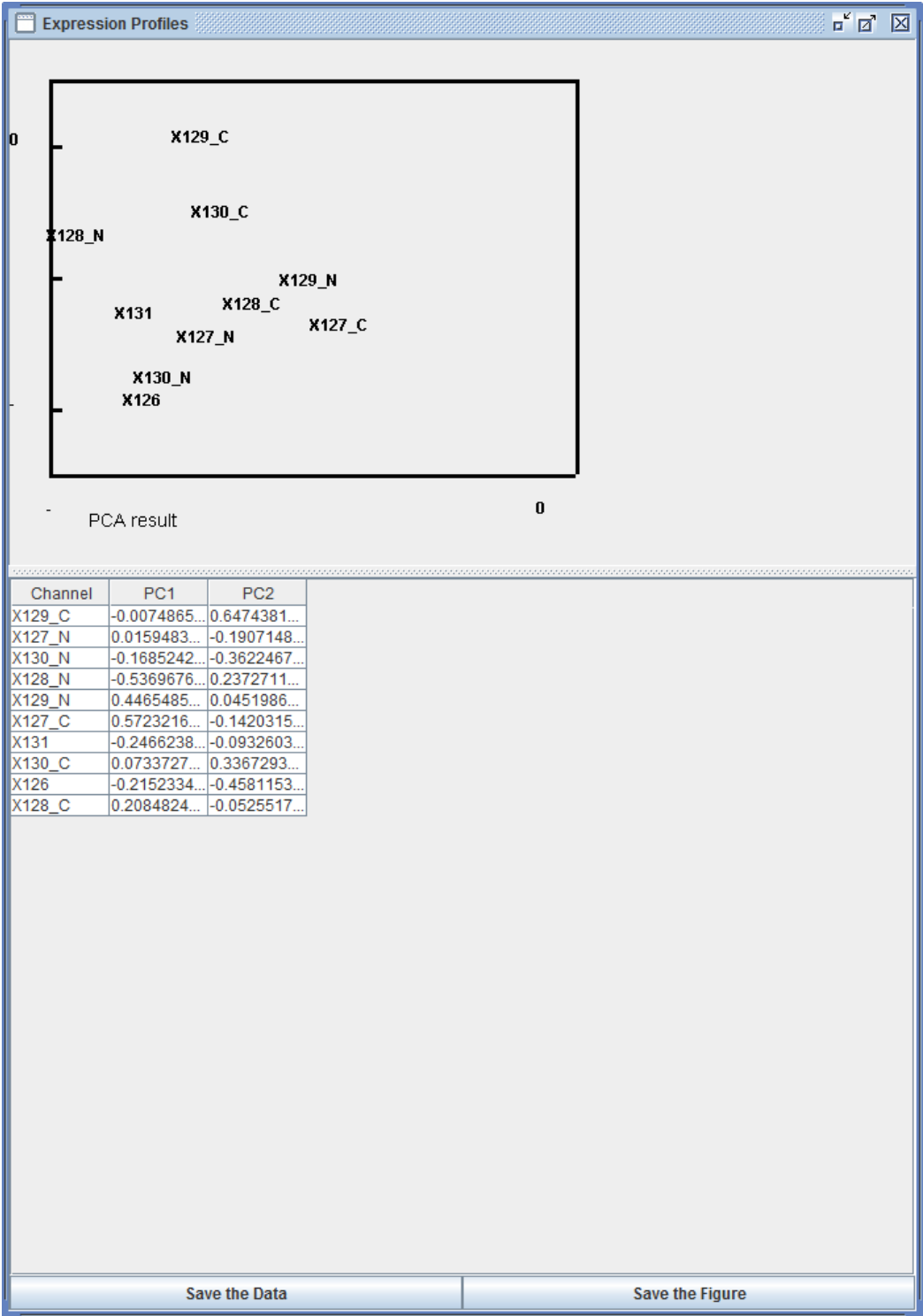
After CLICK Normalization with Mean (20-80%). The following frame should come out.

LOG10 Normalized iTRAQ Data													
peptide	X126	X127_N	X127_C	X128_N	X128_C	X129_N	X129_C	X130_N	X130_C	X131	protein		
aQEDL	26.0831356831	20.7290278362	24.5314088777	20.8777438055	27.4822777517	27.9668066158	23.2381145319	22.6732571583	22.5801912507	24.161274261	21361657		
dDEPAVK	25.8790764149	23.3883708446	25.2250811939	21.0019220085	24.7606322576	28.8649568142	22.1131174491	24.9246168351	22.1312067950	26.9536369722	90689511		
aLASLATLES	23.3841931078	24.2491861852	29.8849214455	23.0567795789	20.8182774624	27.4513199590	21.8145736923	23.7498394056	24.5674869490	22.1793206295	21359969		
aDVFHAYLSLK	27.5653136527	24.4514375177	27.2262517356	23.5589253355	25.2466136339	26.3834397274	23.6055511348	23.2823383233	22.9140863868	26.5190382210	21361794		
dTDSINLYK	25.5625953860	28.1001562183	28.5942945324	20.1039383755	23.165275847	28.8305128652	18.2765308039	21.8572990687	20.3302453125	24.2192787927	4507943		
dLNDRPVGP	23.7408525647	22.9249549146	26.7201276908	20.2351347343	23.3379339723	25.7749441630	21.1429956700	25.1641760339	17.8441723252	25.4375479631	118722349		
dQMDDQVIMVLK	22.9242887922	26.7699290849	24.5369333316	17.6344471038	21.6341488697	25.5676935811	23.0153266967	24.7345080052	25.9104206253	23.0098116103	62422577		
lALIEAK	24.4189943938	26.2445507170	23.1620159617	20.4572268113	25.2317921149	24.1044302798	23.6383077074	22.3973363992	22.0929323293	25.8006501524	6005721		
pPmVNPTLGV	28.2209357477	25.4948143287	27.3738854318	18.2561486196	23.4024045643	28.2678561501	26.9581062347	20.2062734900	22.1887815269	21.8884392831	12239155		
eELMGDSSTsq	22.3347296530	20.2289720941	27.8229370511	19.4954825824	25.1909914222	29.6462138233	23.6563785015	23.0910610403	26.6183213087	25.0268086236	334724455		
allnSTITPNMTf	22.9410411595	23.4079049035	30.0271399242	20.4607896578	22.0039486882	24.5243613910	22.5049671775	24.4398451847	24.4852020543	24.9658166344	4759198		
aTPDEAADLER	27.4688737847	24.7869554671	25.4015303733	20.1886541372	21.9950087669	24.4306840711	23.5129957832	24.2448514937	25.5669174926	23.1597362124	24371272		
aQLLOLPTLEIN	26.4871117280	24.0058177414	25.3944232890	20.9930509323	26.3818418367	21.9133942541	22.2933835936	25.1056619168	21.6326586425	25.1011625957	155722983		
eEAYNSLMK	21.2317083982	22.1989022161	24.4590854852	21.4050402553	22.3783499377	28.9466649566	24.536328166	20.7334602454	21.6614349573	22.7654076847	31621305		
aALCAVHVR	24.4763375387	22.1201411453	23.3255410760	19.8666250387	20.3665322296	24.9139624614	22.5699442610	22.6394279055	22.6715676697	19.8398121241	71772942		
eEDGSLVIDELI	22.4351339363	22.5861061017	27.4455736460	26.0406296137	21.8081744050	23.8169209791	22.4747528452	24.5665671013	26.1353482507	26.8751392292	209862831		
aTDSLOSED	23.3880971950	22.8362566538	25.7102414423	19.5490109197	21.4378324643	23.1936567817	22.6181423233	21.0049610492	21.5175352187	25.0175295921	4885637		
eDLVEIK	26.6718509867	20.7255320772	25.3665150148	17.9240871745	22.6248207702	22.9626545603	20.0791943620	23.7641073417	23.4088460267	25.8498507470	384475554		
aSOAQPADQEL	26.3356959632	23.4015475707	22.7954444252	22.6771607368	24.9511091501	28.2285909526	23.4612028243	22.8280490454	21.4359833699	25.5420511394	295842359		
dVDADVK	24.9558193426	24.1950734225	26.9289345683	23.5502113228	26.6761452664	27.2974490661	23.8204314958	23.3516776305	24.2070917703	22.7314035468	5454032		
aLGDSSPSQDA	26.8085583274	23.8528134559	25.1966164988	21.9081919578	22.4658173375	27.2974490661	23.8204314958	24.1910419239	23.3683085086	23.7950173128	14513061		
eAGVEmGDDE	23.1583730558	22.7991106477	29.6716097010	20.8009379121	23.2522504888	26.1962640845	23.7400128138	21.6187046594	24.4255139740	27.0807698524	45439306		
eALNSGNK	24.1048350921	28.5602862334	22.4072351000	17.6560110687	23.8597007638	22.4106320346	20.7485492351	20.7630912436	21.5711967102	22.5772555195	55956788		
dLAIGLR	22.2448141824	25.4538852809	25.2765045552	21.7304001969	21.2246552768	28.3100884683	23.8661619831	21.6061222017	21.4294159611	24.2805041222	56243599		
dVmpPvNkVLDK	25.9655584491	21.4387867680	21.6441285771	18.1867490055	23.6230953324	25.3357419134	22.4705678580	19.5711893094	20.3681977394	24.5942863654	18201905		
dGIEPMWDEK	26.4377385528	20.5054837749	30.8881375328	18.5309645363	22.8215829562	31.3644753618	25.9394648849	19.6042732890	24.3628119194	24.5881089683	34878777		
dEVFYEIFR	26.7842204995	22.0133293202	25.8968031217	21.8239690953	23.1758053941	28.0881094097	23.3074471859	21.4406131287	20.2818901958	19.1282389910	166197710		
eAQSDLNK	25.7108756858	23.5336541018	29.5174997873	19.1037069430	22.8215829562	31.3644753618	25.9394648849	19.6042732890	24.3628119194	24.5881089683	34878777		
dALVSGALESTK	26.9560660694	27.0115575229	29.0054739001	19.7297656557	20.9084814896	24.8951106996	24.5429619012	23.6620008267	24.1398767393	25.0012014015	82546843		
dAEDALHNLDRK	23.1684454086	24.8781890169	24.3784150595	19.5860148935	20.3060260696	28.7023033954	24.3356163760	24.5158418841	20.4635384374	26.3750545041	300360554		
dQVVIDGR	22.4857205876	25.6624974738	26.0697210740	24.7382710819	22.7505966722	26.0445907998	21.6613352791	25.2613158640	24.8128018255	25.5506929053	5902018		
dTICNQDER	25.4524393299	26.3225006430	28.1912719707	15.7424025013	21.8785205016	26.6811440108	22.9378471326	21.8389053138	24.3285349885	29.8873295813	7656991		
aATVYIGILR	24.6703917681	25.7980160172	30.499990432	15.3414467861	22.9403912261	28.0204140375	22.5991326336	24.7386207698	23.6961828348	22.6915984231	145275200		
aAGDSNLK	27.2239032082	22.3907795661	25.5421151405	22.5098577262	21.8519308236	23.0336691958	22.6045110340	24.1133909599	25.6543774738	23.6759637848	25777730		
eEVGVIAK	29.4022476401	20.4360911944	22.2641066506	21.9281558216	24.4325660396	25.5824421410	25.6565327763	23.9982628112	23.8986278360	24.9015068272	34482047		
dSEQVAELK	26.4542324783	22.8364180808	25.5758869867	19.5074844192	25.3069220985	29.3329616303	21.6593200647	23.9052296585	19.6946927393	25.1150328961	4505541		
aADMLSGPR	22.5989713003	22.6796089114	26.1479019066	17.1911012841	21.9720704038	23.9573210290	21.8752533696	21.8959105169	20.5833003716	26.8132270594	25777734		
dGSPADDDLEK	26.8284684179	23.1934220311	21.7578563450	21.4900104200	23.5758170895	26.4058770396	22.3613213127	20.2110243616	26.3058206336	24.2037605852	14149738		
eEGLAEETLK	23.9477675463	23.7558289587	25.3032831575	21.9707340745	22.6014926404	29.5856665450	23.9671007955	22.3829622127	22.3793040055	24.0545472089	40018629		
dFDPVHGDVVK	23.7428773368	22.7072570837	24.4791933365	21.3422012474	23.6106079068	27.0244593734	21.1271477984	25.6545676404	26.6297601829	27.1486887266	162287326		
												PCA Weighted method Cauchy Fitting Rank Sum Ttest Save the Result	

This is the normalized data. Note that the intensities are log 10 normalized. We can conduct PCA analysis. Two pairwise statistical methods, T test, ranksum are included. We also fit the ratios of all peptides with Cauchy distribution and use the fitted distribution to estimate the statistical significance (It is assumed that ratio of two normal distributions, which is the case is iTRAQ follow Cauchy distribution).

The weighted method is a kind of linear model. It assumes the statistical variance is dependent on the number of peptides and their intensities (the detailed can be found in “**Defining, Comparing, and Improving iTRAQ Quantification in Mass Spectrometry Proteomics Data**”). It fits the relationship between variance and intensity with LOWESS for different number of peptides for certain protein. We modified the method and calculate the weighted ratio with error estimations. The basic idea behind this method is that the proteins with low intensity and small number of unique peptides tend to have higher variation.

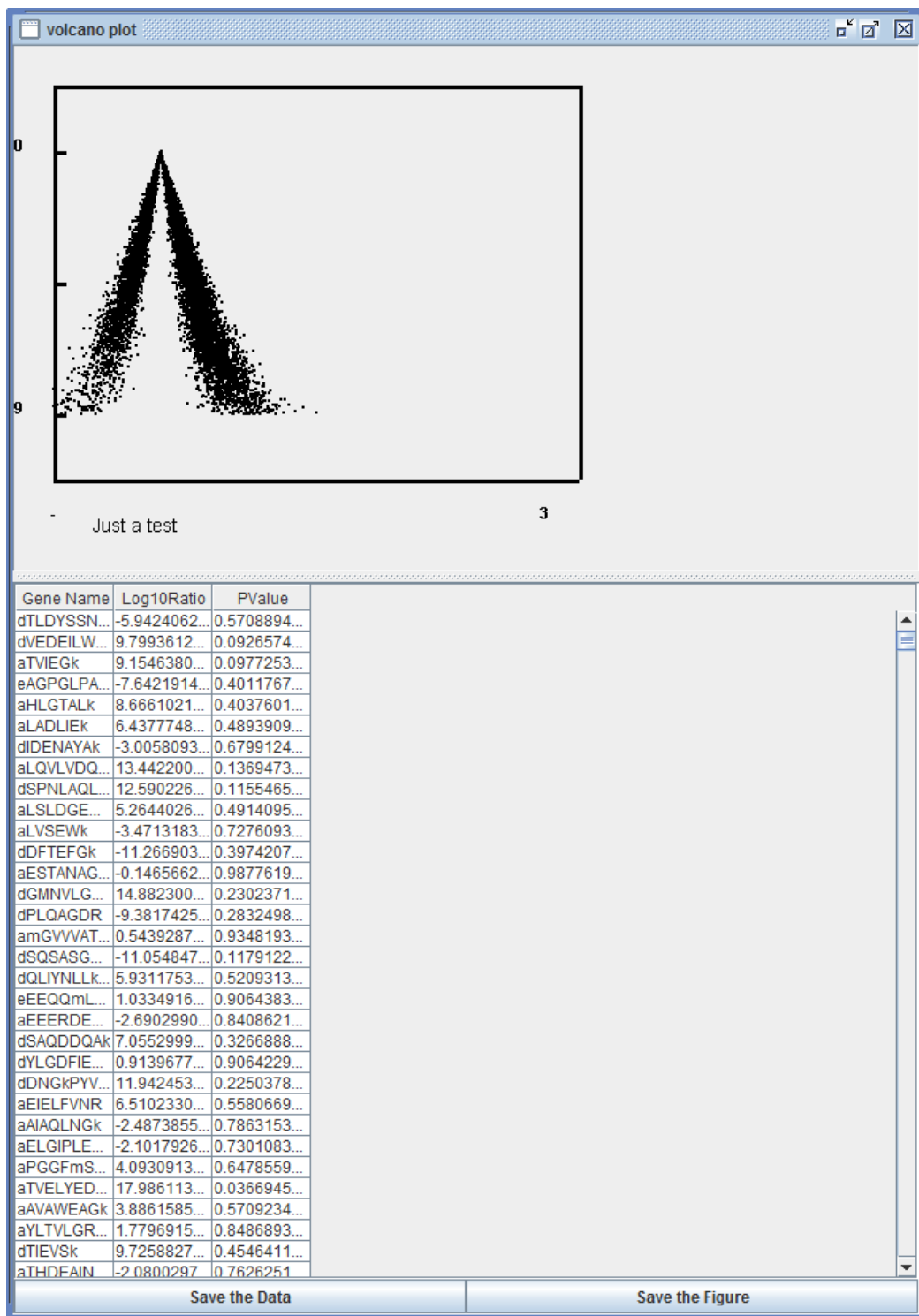
Below figure shows the result of PCA analysis. We can save the PC1 and PC2, and save the Figure (resolution =200DPI, can change)



Below figure shows the result of t test. We got two tables, one is the result at peptide level, another one is the result at protein level. We can save the results and draw volcano plots

iTRAQ Result			
protein	peptides #	tvalue	pvalue
34787409	1	1.944503618520127	0.08773396683845402
203098013	2	1.2048593359489113	0.26268758394777425
126012573	1	0.49776990317288095	0.6320387079957914
5031653	1	0.210670502128914	0.8384114027093235
41152506	2	0.10059670832237433	0.9223460187956352
126012571	4	0.059650503211308104	0.9538971734897348
118600971	1	0.4230279342925981	0.6834171341454418
118600975	3	-0.42055425280098346	0.6851499873333045
4759302	3	0.6309861122175754	0.5456501797692233
148746189	1	-0.3125353054148328	0.7626251962990607
34878787	1	0.4242775076697155	0.682542545702626
7661728	1	1.3435112257277746	0.21596754342412217
7661734	1	-2.53703256115883	0.03487150420827301
13375779	2	0.4422130828206394	0.6700460129770258
peptides #	tvalue	pvalue	
aQEDL	0.10831612258110264	0.9164123602833589	
dDEPAVh	0.596919261175894	0.5670664961484595	
aLASLATLESVVQER	-0.17944613260375092	0.8620491097271521	
aDVFHAYLSLLk	-0.9650923909690916	0.3627619919642999	
dTDSINLYk	-0.9979489726190541	0.3475281234699794	
dLNDRPVGP	-0.17087863856427768	0.8685620110447672	
dQMQQDVMVLk	1.1494281450437296	0.2835806539566037	
aLIEAEK	-0.24702039211007776	0.8111134868000769	
aPmVNPTLGVHEADLLk	-0.2733317292020404	0.7915220426562966	
aELGMGDSTSpSPiik	1.3278719047284826	0.22085622360806623	
aInSTITPNmTFTk	0.24475375288889017	0.8128081015493025	
aTPDEADLER	0.158734310355881	0.8778121807554218	
aQLLQPTLEINPR	-1.1253036769074953	0.2930854548620083	
eEAYNSLMk	0.8936699764869517	0.3975853502291171	

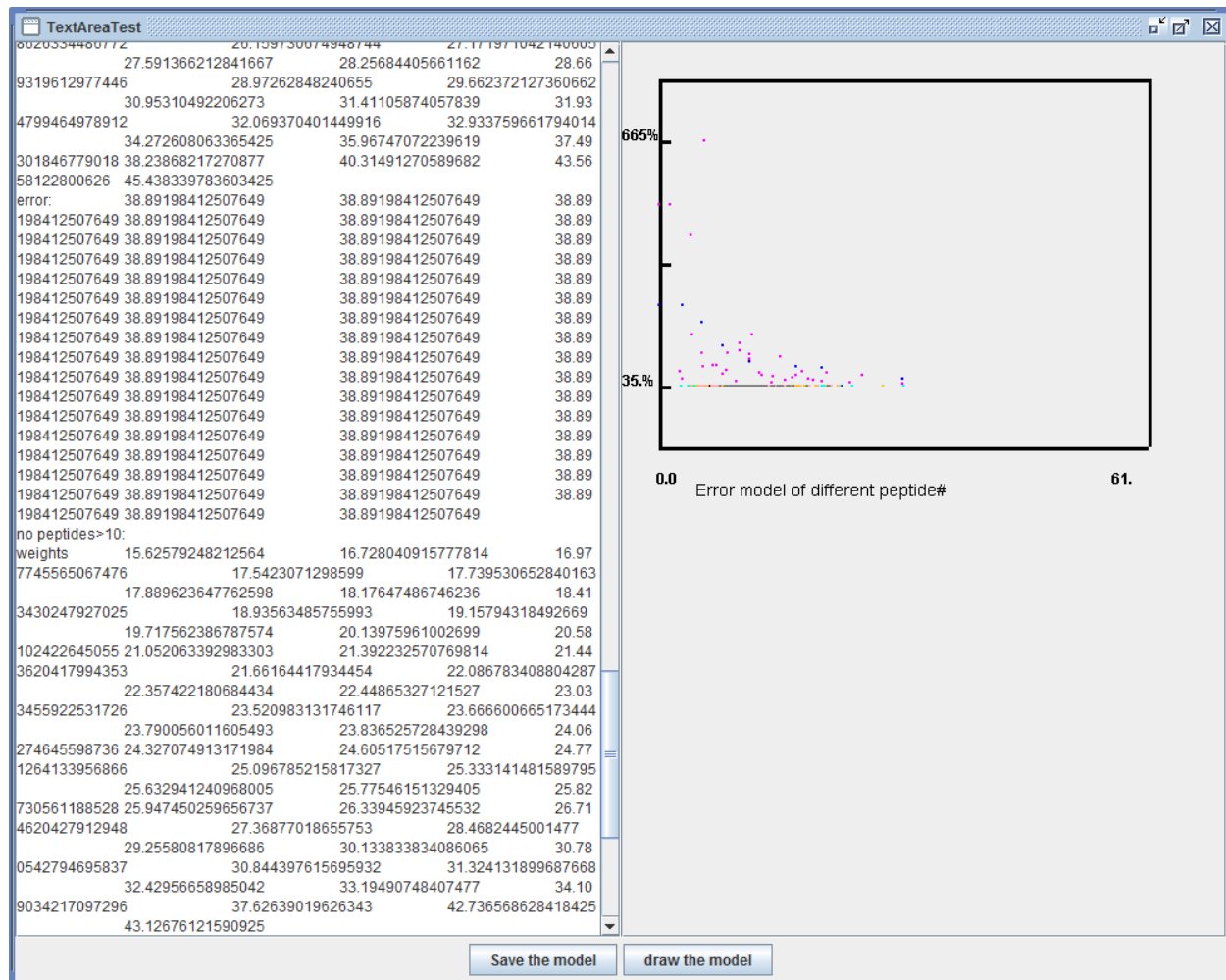
ProteinVolcano
Save the Protein Result
PeptideVolcano
Save the Peptide Result



This is the volcano plots. Can save the result and save the figure.

Ranksum and Cauchy fitting' results can be conducted with same way.

After clicking Weighted method, we can get two frames. One is the summary of the model. And one is used to show the result.



We can see the weigh errors relationships for different number of peptides. The right part is the figure (working on it to beautify)

iTRAQ Result										
protein	protein_wet	protein_rati	protein_pe...	error_matri...	protein_wet	protein_rati	protein_pe...	error_matri...	protein_wet	pro
34787409	16.117885...	1.8558608...	1.0	1263.6024...	6.1656495...	0.1441391...	1.0	1997.7494...	6.1656495...	0.25
203098013	22.843284...	1.3857906...	2.0	632.48720...	14.537663...	0.6095066...	2.0	796.17092...	8.6245165...	2.05
126012573	11.083383...	0.5369139...	1.0	1647.2416...	16.117885...	1.4630860...	1.0	1263.6024...	16.117885...	11.9
5031653	11.083383...	1.1249686...	1.0	1647.2416...	11.083383...	0.8750313...	1.0	1647.2416...	11.083383...	4.47
41152506	61.691070...	1.9124562...	2.0	86.345713...	11.141767...	0.0952133...	2.0	840.58396...	37.888229...	0.38
126012571	42.680265...	1.3625112...	4.0	41.078402...	31.342396...	0.7968978...	4.0	41.078402...	41.037744...	1.39
118600971	11.083383...	0.5893599...	1.0	1647.2416...	22.909678...	1.4106400...	1.0	893.73329...	22.909678...	1.02
118600975	52.661775...	1.1477870...	3.0	44.821841...	41.924673...	0.7879464...	3.0	44.821841...	37.982575...	0.85
4759302	19.474101...	0.9563810...	3.0	44.821841...	36.343330...	1.5606584...	3.0	44.821841...	22.279652...	0.43
148746189	61.691070...	0.8066700...	1.0	255.01751...	61.691070...	1.1933299...	1.0	255.01751...	41.173272...	0.55
34878787	61.691070...	0.3094133...	1.0	255.01751...	61.691070...	1.6905866...	1.0	255.01751...	16.117885...	0.06
7661728	34.603185...	1.6201656...	1.0	624.27452...	16.117885...	0.3798343...	1.0	1263.6024...	11.083383...	0.32
7661734	61.691070...	0.9071656...	1.0	255.01751...	61.691070...	1.0928343...	1.0	255.01751...	41.173272...	0.35
13375779	51.432171...	1.2616725...	2.0	188.01861...	48.147128...	0.7398308...	2.0	223.15459...	51.432171...	1.53
56699407	11.083383...	0.3178348...	2.0	841.33275...	32.041475...	1.6865088...	2.0	439.09549...	28.645579...	1.45
56237023	30.631448...	1.0719964...	3.0	44.821841...	25.055444...	0.9717234...	3.0	44.821841...	22.909678...	1.00
7661730	41.173272...	1.6758878...	1.0	527.31396...	16.117885...	0.3241121...	1.0	1263.6024...	41.173272...	2.65
153791497	6.1656495...	0.1145719...	1.0	1997.7494...	41.173272...	1.8854280...	1.0	527.31396...	22.909678...	0.56
21361361	42.378867...	1.0753837...	5.0	38.891984...	42.378867...	1.0002945...	5.0	38.891984...	18.854681...	0.87
4507645	28.756432...	1.2774932...	2.0	514.61743...	22.843284...	0.7481271...	2.0	632.48720...	16.996530...	2.52
61743967	8.6245165...	0.2713169...	2.0	874.42424...	23.669461...	1.7474683...	2.0	621.49664...	23.669461...	1.34
4507635	6.1656495...	1.2723211...	1.0	1997.7494...	6.1656495...	0.7276788...	1.0	1997.7494...	6.1656495...	3.80
118600961	61.691070...	0.7974764...	1.0	255.01751...	61.691070...	1.2025235...	1.0	255.01751...	41.173272...	0.30
45446740	11.083383...	1.7243469...	2.0	841.33275...	6.1656495...	0.2565855...	2.0	912.09810...	11.083383...	4.04
14110680	38.275307...	1.1788842...	5.0	38.891984...	40.734370...	1.0285797...	5.0	38.891984...	26.230351...	0.76

Close
Save the Result
Average the Result

The Table shows the result of weighted method. Each channel has four columns. The weight (intensity), the estimated ratio, the number of peptides, the error estimation of ratio (percentage)

After clicking “Average the Result”. The program uses the variation as the weight to generate a ratio of proteins with phenotypes. And the following frame will come out.

The pvalues need to be adjusted.