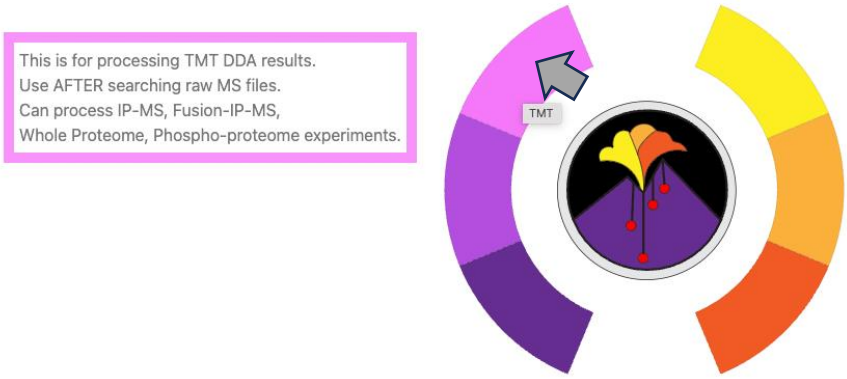
TMT (IP-MS Fusion)

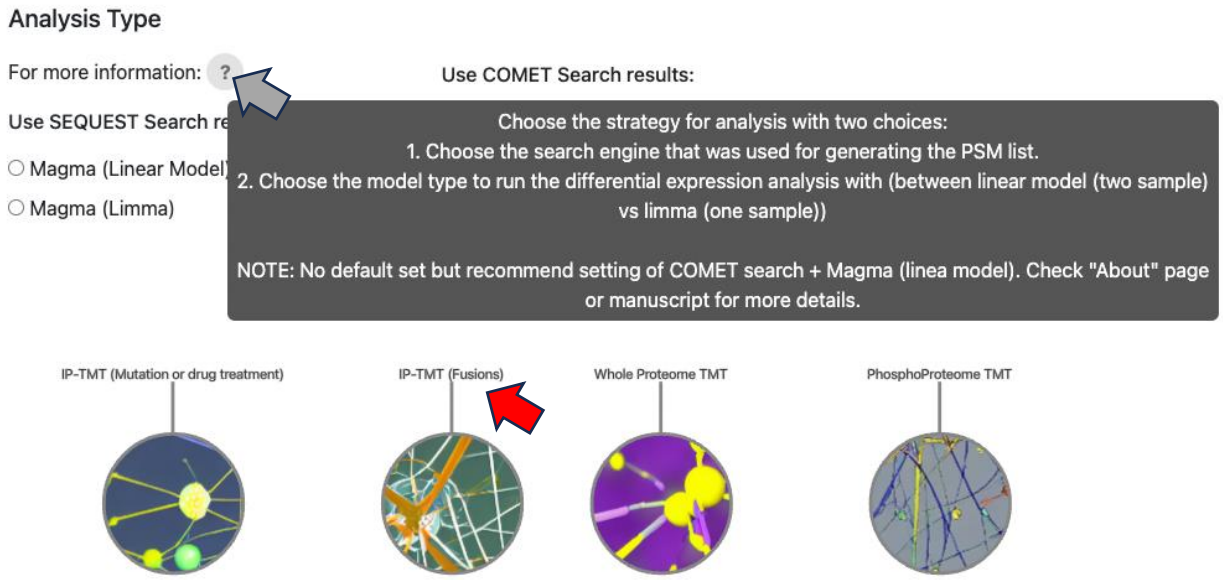
Step 1: Click on the TMT tab on the home page to access all the TMT workflows (as shown in figure below with the grey arrow).



Step 2: Choose the type of analysis to run. You can hover over the question mark to get more details on the different choices and recommended settings (as shown by the grey arrow below). The choices to be made here include:

1. The choice of search engine (SEQUEST in Proteome Discoverer vs COMET)
2. The choice of analysis type (MAGMa LM for a two-sample t-test and MAGMa Limma for a one sample t-test)

Click on the IP-TMT arm (highlighted with red arrow in the figure below) to access to workflow.

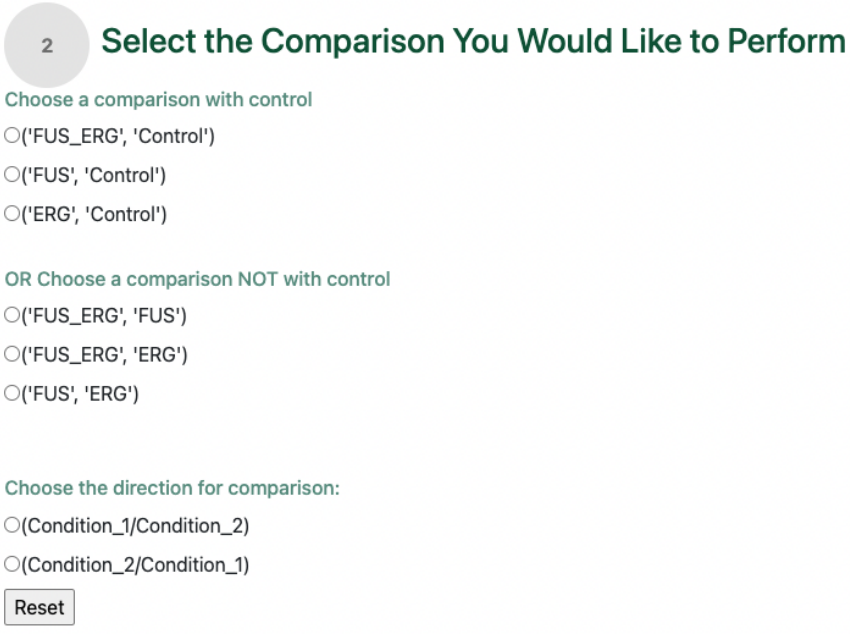


Step 3: Upload the annotation file. This is made using the experimental setup.

An example can be seen below taken from a Fusion IP-MS TMT10 experiment. Here each TMT label is associated with a separate IP with FUS_ERG coding for IP of overexpression of the fusion of FUS and ERG proteins and FUS and ERG coding for IPs of endogenous baits which are overexpressed. Control column here lets the underlying tool know whether the label is a control in your experimental setup or not. You can also hover over question mark for more details. Click on the "Sample File" in blue to download this example annotation file.

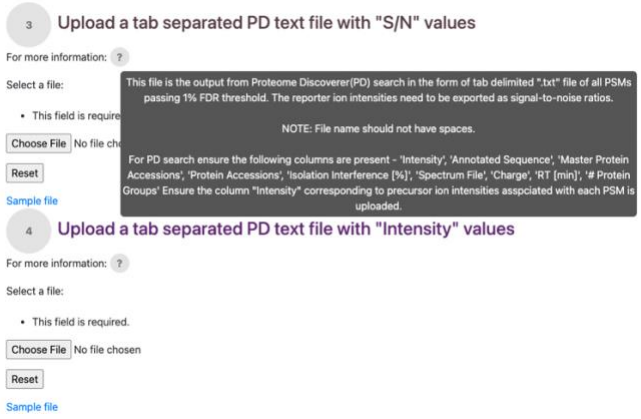
	A	B	C
1	Channel	Label	Control
2	126	none	FALSE
3	127N	FUS_ERG	FALSE
4	127C	Control	TRUE
5	128N	FUS_ERG	FALSE
6	128C	Control	TRUE
7	129N	FUS	FALSE
8	129C	ERG	FALSE
9	130N	FUS	FALSE
10	130C	ERG	FALSE
11	131	none	FALSE

Step 4: Choose the conditions you want to compare in a pairwise fashion. And choose the direction of comparison. So, choosing comparison ('FUS','Control') and direction (Condition_1/Condition_2) means the following comparison will run – (Fus/Control).



Step 5: Upload the input files (PSM list) for quantification.

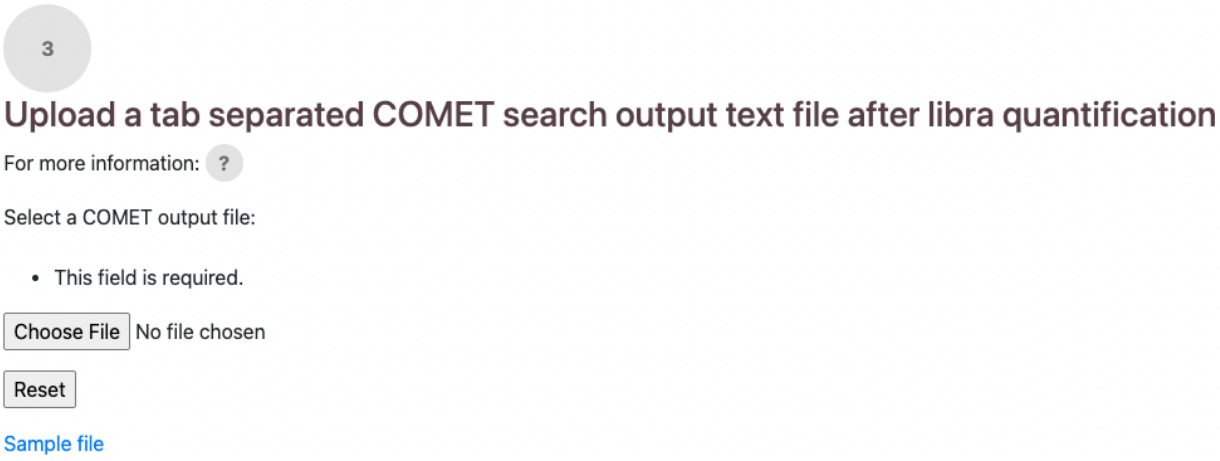
For SEQUEST in Proteome Discoverer search, upload a tab separated file of PSM (filtered at 1% FDR or filter cutoff of choice) and associated reporter ion abundance (per TMT channel). The abundances must be exported as signal-to-noise as well raw reporter intensities. Make sure the files have the following columns – 'Intensity', 'Annotated Sequence', 'Master Protein Accessions', 'Protein Accessions', 'Isolation Interference [%]', 'Spectrum File', 'Charge', 'RT [min]', '# Protein Groups'.
As before hover over question mark for more information. Click on the "Sample File" in blue to download this example PSM files.



The example has been taken from a real IP experiment and looks like the screenshot below.

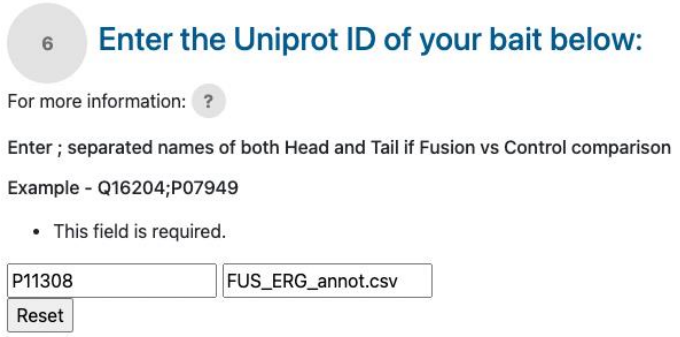
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	Checked	Confidence	Identifying N	PSM Ambigu	Sequence	Annotated Se	Modification	# Proteins	Master Prote	Protein Acce	# Missed Cle	Charge	DeltaScore	DeltaCn	Rank	Search Engin	m/z [Da]	MH+ [Da]	Theo. MH+ [I	DeltaM [ppm
2	FALSE	High	Sequest HT (	Unambiguou	LCYVALDFEQ	[K].LcYVALDF	N-Term(TMT	3	P60709	P60709; Q6S	0	4	0.4027	0	1	1	752.87937	3008.49563	3008.4997	-1.35
3	FALSE	High	Sequest HT (	Unambiguou	MTSTPETLLE	[K].mTSTPET	N-Term(TMT	2	Q9BZI7	Q9BZI7-2; Q	0	3	0.3043	0	1	1	717.3936	2150.16624	2150.16666	-0.19
4	FALSE	High	Sequest HT (	Unambiguou	DHLYGTLDPK	[K].dHLYGTLI	N-Term(TMT	1	Q14204	Q14204	0	3	0.5974	0	1	1	544.28443	1630.83875	1630.83865	0.06
5	FALSE	High	Sequest HT (	Unambiguou	GHYTEGAELV	[K].gHYTEGA	N-Term(TMT	6	Q13885; P07	Q13885; Q13	0	3	0.5666	0	1	1	730.05321	2188.14509	2188.14473	0.16
6	FALSE	High	Sequest HT (	Unambiguou	AETECQNTEY	[R].aETEcQN	N-Term(TMT	1	P13645	P13645	0	3	0.544	0	1	1	847.76806	2541.28964	2541.29069	-0.41
7	FALSE	High	Sequest HT (	Unambiguou	IIDEDGLLNLI	[K].IIDEDGLL	N-Term(TMT	2	P35251	P35251; P35	0	2	0.4643	0	1	1	806.97765	1612.94802	1612.94714	0.55
8	FALSE	High	Sequest HT (	Unambiguou	TVVTGIEMFI	[R].tVVTGIEr	N-Term(TMT	1	P49411	P49411	0	3	0.0508	0	1	1	579.33211	1735.98177	1735.9817	0.04
9	FALSE	High	Sequest HT (	Unambiguou	YRPEEVDDA	[K].yRPEEVD	N-Term(TMT	6	Q9HAU0	Q9HAU0; Q9	0	3	0.4404	0	1	1	598.33243	1792.98272	1792.98453	-1.01
10	FALSE	High	Sequest HT (	Unambiguou	IEGDMIVCAA	[R].iEGDMIV	N-Term(TMT	1	P46777	P46777	0	3	0.4209	0	1	1	792.42121	2375.24909	2375.25034	-0.53
11	FALSE	High	Sequest HT (	Unambiguou	NDVLDSLGIS	[R].nDVLDSL	N-Term(TMT	1	Q9UBE0	Q9UBE0	0	3	0.1287	0	1	1	815.10198	2443.29139	2443.29179	-0.16
12	FALSE	High	Sequest HT (	Unambiguou	VIMGEEVEPI	[R].vImGEEV	N-Term(TMT	1	P27708	P27708	0	3	0.3392	0	1	1	887.82493	2661.46022	2661.46073	-0.19
13	FALSE	High	Sequest HT (	Unambiguou	HSDGNLCVK	[R].hSDGNLc	N-Term(TMT	2	P49458	P49458-2; P4	0	3	0.2636	0	1	1	496.6062	1487.80404	1487.80407	-0.02
14	FALSE	High	Sequest HT (	Unambiguou	DLYANTVLSC	[K].dLYANTV	N-Term(TMT	2	P60709	P60709; P63	0	3	0.5789	0	1	1	815.41668	2444.23549	2444.23289	1.06
15	FALSE	High	Sequest HT (	Unambiguou	EQSLPSVMG	[K].eQSLPSV	N-Term(TMT	1	Q9NZ32	Q9NZ32	0	3	0.5396	0	1	1	858.13337	2572.38555	2572.39443	-3.45
16	FALSE	High	Sequest HT (	Unambiguou	SKPNMNYDK	[K].skPNmNY	N-Term(TMT	9	P11308	P11308-4; Q	0	4	0.3122	0	1	1	450.75345	1799.99195	1799.99288	-0.52
17	FALSE	High	Sequest HT (	Unambiguou	LPEDPLLSGLI	[K].lPEDPLLS	N-Term(TMT	1	P49327	P49327	0	3	0.5943	0	1	1	746.11145	2236.31979	2236.32046	-0.3
18	FALSE	High	Sequest HT (	Unambiguou	GWFDAEGQ	[R].gWFDAE	N-Term(TMT	1	P27694	P27694	0	3	0.4399	0	1	1	822.76835	2466.2905	2466.29168	-0.48
19	FALSE	High	Sequest HT (	Unambiguou	DLYANTVLSC	[K].dLYANTV	N-Term(TMT	2	P60709	P60709; P63	0	3	0.3366	0	1	1	815.41622	2444.23412	2444.23289	0.5
20	FALSE	High	Sequest HT (	Unambiguou	MELQEIQLK	[K].mELQEIQ	N-Term(TMT	7	P06753-5; P(	P06753-5; P(	0	2	0	0	1	1	795.46998	1589.93269	1589.93369	-0.63
21	FALSE	High	Sequest HT (	Unambiguou	IAQLEEQLDN	[K].iAQLEEQI	N-Term(TMT	2	P35579	P35579; P35	0	3	0.5915	0	1	1	663.70157	1989.09015	1989.09046	-0.15
22	FALSE	High	Sequest HT (	Unambiguou	STASLLATQE	[R].sTASLLAT	N-Term(TMT	5	Q9Y2D5-6	Q9Y2D5-7; Q	0	3	0.3711	0	1	1	813.77922	2439.32311	2439.32053	1.06
23	FALSE	High	Sequest HT (	Unambiguou	GVSFYEVPPF	[R].gVSFYEV	N-Term(TMT	3	O00159-3	O00159; O0C	0	3	0.6437	0	1	1	832.77652	2496.31502	2496.31246	1.02
24	FALSE	High	Sequest HT (	Unambiguou	AMQDAEVSK	[K].amQDAE	N-Term(TMT	1	P38646	P38646	0	2	0.5155	0	1	1	726.89192	1452.77656	1452.77685	-0.2
25	FALSE	High	Sequest HT (	Unambiguou	ENSASQISQL	[R].eNSASQI	N-Term(TMT	11	P39880	P39880-3; P3	0	3	0.4873	0	1	1	773.75648	2319.2549	2319.25562	-0.31
26	FALSE	High	Sequest HT (	Unambiguou	HNPEQK	[K].hNPEQk	N-Term(TMT	3	Q8ND76	Q8ND76; Q8	0	3	0.199	0	1	1	404.23582	1210.69289	1210.69444	-1.28
27	FALSE	High	Sequest HT (	Unambiguou	IEEGLDQINK	[R].iEEGLDQ	N-Term(TMT	2	O00161	O00161-2; O	0	3	0.0866	0	1	1	539.64673	1616.92565	1616.92596	-0.19
28	FALSE	High	Sequest HT (	Unambiguou	NKDQGTYED	[K].nkDQGTY	N-Term(TMT	2	P60660	P60660; P60	1	3	0.6396	0	1	1	749.05411	2245.14776	2245.1501	-1.04
29	FALSE	High	Sequest HT (	Unambiguou	DASVAEAWL	[R].dASVAEA	N-Term(TMT	3	Q01082-2; Q	Q01082-2; Q	0	3	0.726	0	1	1	774.4037	2321.19653	2321.19749	-0.41
30	FALSE	High	Sequest HT (	Unambiguou	KEPPIIMTVPF	[R].kEPPIIMT	N-Term(TMT	8	Q9UJV4	O60FE8; O6(	1	3	0.3257	0	1	1	601.35274	1802.04367	1802.04325	0.23

For COMET search after Peptide-Prophet and Libra, upload a tab separated file of PSM (filtered at 0.9 probability or the probability associated with FDR cutoff choice) and associated reporter ion abundance (per TMT channel). Make sure the files have the following columns – "spectrum","precursor_intensity","peptide","assumed_charge","retention_time_sec".
As before hover over question mark for more information. Click on the "Sample File" in blue to download this example PSM files.



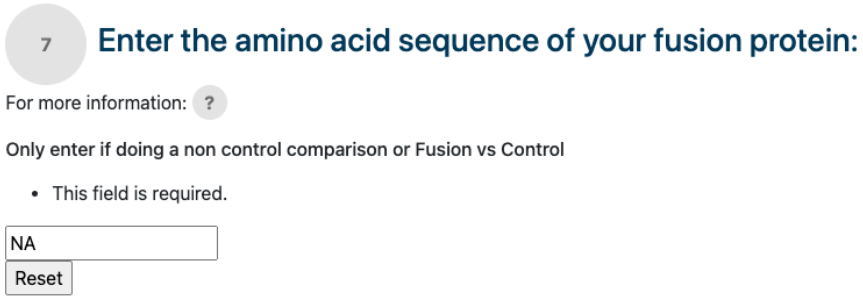
Step 6: You can modify settings (using fractions, doing row normalization and type of column normalization) for your specific analysis. Recommendation is to use the default settings. Hover over question mark for more information.

Step 7: Enter the Uniprot-ID of the bait that is pulled down in your IP-MS experiment. This is especially important for the non-control comparisons (if chosen for example ('FUS_ERG','FUS') in step 4) to normalize by bait for Condition 2-vs-Condition 1 type of comparisons (for example, drug treatments vs non-drug treatment). If you want to switch off bait normalization for non-control comparison type in "NA" in this field. Make sure the Uniprot-ID is in the 'Master Protein Accessions' column of your PSM files if SEQUEST search is run or column 'protein' if COMET search is run (input in step 5). The example below of "**P11308**" corresponds to ERG bait. As before hover over question mark to get more information.



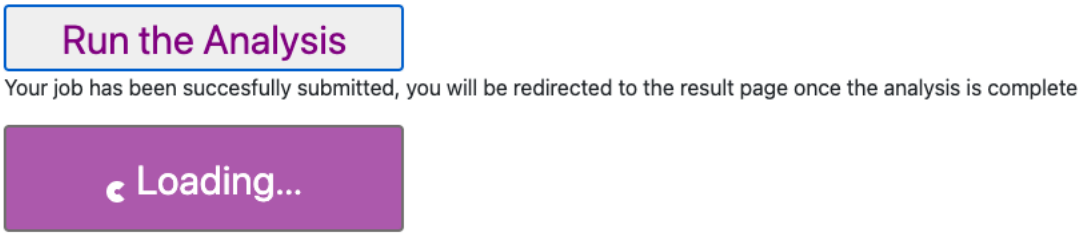
Step 8: Enter the amino acid sequence of the fusion protein. This is important to remove peptides that would not belong to fusion protein for the analyses that involve fusion condition (in the example here, these would be FUS_ERG/Control, FUS_ERG/FUS, FUS_ERG/ERG)

For all other possible comparisons, enter "NA" here.



Step 9: Choose whether to do imputation on your data or not. Recommendation is to not to not introduce biases.

A successful start of run with look like this –



The output page (given a successful run) looks like the screenshot below. You can download the protein level and peptide level differential expression analysis results as well as the pdf of resulting volcano plot (set at 5 PSM cutoff). You can highlight specific proteins on the volcano plot by entering the associated Uniprot IDs. You can dynamically set the thresholds and download the resulting file, as well as subset the result file with user-defined thresholds. –
–

Success!

Click Here to Download the Result File (Protein level)
Click Here to Download the Result File (Peptide level)
Click Here to Download the baseline Volcano plot (PDF)
FC cutoff choice: Drag the slider to display the current value.

Value: 2

Adjusted P-value cutoff choice: Drag the slider to display the current value.

Value: 0.05

PSM cutoff choice: Drag the slider to display the current value.

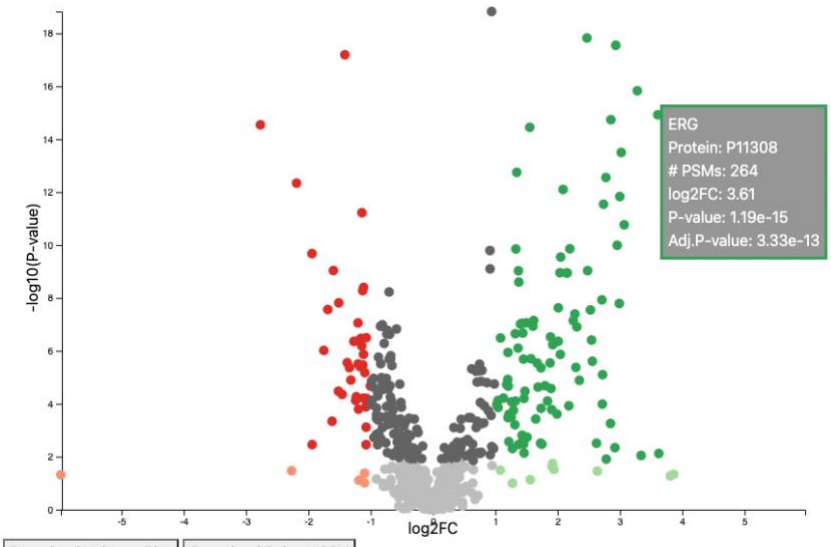
Value: 5

Size of scatter point: Drag the slider to display the current value.

Value: 5

Enter Uniprot IDs separated by @
Example: Q08945@Q9UQE7

Update Volcano Plot



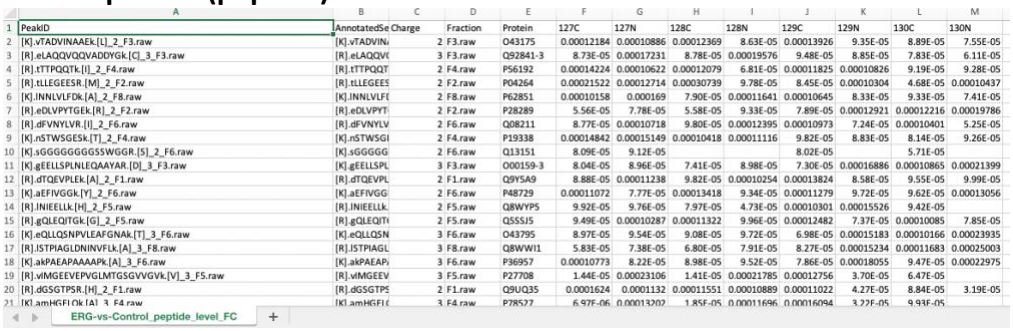
Download Volcano Plot | Download Subset CSV

The output file (protein) looks like this –

	A	B	C	D	E	F	G	H	I	J	K
1	Protein	log2FC	pval	adjpval	Gene Symbo	# PSMs	log2_129C	log2_130C	log2_128C	log2_127C	PSM Cutoff
2	Q8NI27	0.30702633	0.39924374	0.52091505	THOC2	2	-12.990649	-13.324952	-13.41241	-13.221622	5
3	P49257	-0.6093058	0.00834517	0.03287657	LMAN1	3	-13.402203	-13.710232	-12.931258	-12.583512	
4	P55769	1.14329422	0.01046787	0.03928972	SNU13	3	-13.322712	-12.879798	-14.079682	-14.382377	
5	Q12857	2.22252839	0.09058921	0.18675657	NFIA	1	-12.864906	-13.32071	-14.591746	-15.911478	
6	Q9P0M6	2.01061233	2.36E-08	1.01E-06	H2AFY2	10	-12.829975	-13.182418	-14.655948	-16.07195	
7	Q5SY16	0.38778965	0.15219025	0.26455501	NOL9	2	-12.903889	-13.233592	-13.711621	-13.298867	
8	P19623	0.05417775	0.91492509	0.94686273	SRM	1	-12.706159	-13.361297	-13.230607	-12.924184	
9	P32322	0.79034712	0.10893608	0.21278764	PYCR1	1	-12.583368	-13.134966	-13.648536	-13.605844	
10	P49411	0.9142341	1.61E-10	1.23E-08	TUFM	27	-12.491338	-13.118713	-13.832979	-13.74906	
11	P04181	-0.321085	0.2886737	0.40658331	OAT	3	-12.800733	-13.713797	-13.100591	-12.658733	
12	P30044	-0.839435	0.00357765	0.0173092	PRDX5	3	-13.033574	-14.110908	-12.667489	-12.183243	
13	Q8IY81	0.3935487	0.01534274	0.05273869	FTSJ3	7	-13.29844	-13.066811	-13.298402	-13.740591	
14	P06396	-0.1491613	0.07946749	0.17203614	GSN	13	-13.602259	-13.327337	-13.312501	-13.289177	
15	Q9UHI6	-0.7585537	0.13665519	0.24650946	DDX20	1	-13.10758	-13.632751	-12.435501	-12.782207	
16	P26583	0.47364799	0.21551073	0.33243768	HMGB2	2	-12.782814	-13.349486	-13.256262	-13.519597	
17	Q96HS1	0.11666649	0.72599822	0.80623185	PGAM5	4	-13.021722	-13.238948	-13.185093	-13.307347	
18	P35221	-0.1724584	0.8357654	0.88325207	CTNNA1	19	-13.812784	-13.32509	-13.286529	-13.164871	
19	O60306	-0.4419448	0.03044651	0.08857518	AQR	1	-13.304495	-13.365179	-12.965247	-12.819277	
20	O15213	1.22489396	0.02555231	0.0779136	WDR46	1	-13.213743	-12.970104	-14.158282	-14.474442	
21	O96IZ0	-0.761309	0.00806554	0.03207058	PAWR	1	-13.425547	-13.553341	-12.702026	-12.753227	

ERG-vs-Control_FC_and_pval_YuLa

The output file (peptide) looks like this -



	A	B	C	D	E	F	G	H	I	J	K	L	M
1	PeakID	AnnotatedSe	Charge	Fraction	Protein	127C	127N	128C	128N	129C	129N	130C	130N
2	[K].vTADVINAAEK.[L]_2_F3.raw	[K].vTADVIN/	2	F3.raw	O43175	0.00012184	0.00010886	0.00012369	8.63E-05	0.00013926	9.35E-05	8.89E-05	7.55E-05
3	[R].eLAQQVQQVADDYGk.[C]_3_F3.raw	[R].eLAQQV(	3	F3.raw	Q92841-3	8.73E-05	0.00017231	8.78E-05	0.00019576	9.48E-05	8.85E-05	7.83E-05	6.11E-05
4	[R].tTTPQQTk.[I]_2_F4.raw	[R].tTTPQQT	2	F4.raw	P56192	0.00014224	0.00010622	0.00012079	6.81E-05	0.00011825	0.00010826	9.19E-05	9.28E-05
5	[R].tLLEGEESR.[M]_2_F2.raw	[R].tLLEGEES	2	F2.raw	P04264	0.00021522	0.00012714	0.00030739	9.78E-05	8.45E-05	0.00010304	4.68E-05	0.00010437
6	[K].INNLVLFDk.[A]_2_F8.raw	[K].INNLVLF(	2	F8.raw	P62851	0.00010158	0.000169	7.90E-05	0.00011641	0.00010645	8.33E-05	9.33E-05	7.41E-05
7	[R].eDLVPYTGEk.[R]_2_F2.raw	[R].eDLVPYT	2	F2.raw	P28289	5.56E-05	7.78E-05	5.58E-05	9.33E-05	7.89E-05	0.00012921	0.00012216	0.00019786
8	[R].dFVNYLVR.[I]_2_F6.raw	[R].dFVNYLV	2	F6.raw	Q08211	8.77E-05	0.00010718	9.80E-05	0.00012395	0.00010973	7.24E-05	0.00010401	5.25E-05
9	[K].nSTWSGESk.[T]_2_F4.raw	[K].nSTWSGI	2	F4.raw	P19338	0.00014842	0.00015149	0.00010418	0.00011116	9.82E-05	8.83E-05	8.14E-05	9.26E-05
10	[K].sGGGGGGGGSSWGGR.[S]_2_F6.raw	[K].sGGGGG	2	F6.raw	Q13151	8.09E-05	9.12E-05			8.02E-05		5.71E-05	
11	[K].gEELLSPLNLEQAAYAR.[D]_3_F3.raw	[K].gEELLSPL	3	F3.raw	O00159-3	8.04E-05	8.96E-05	7.41E-05	8.98E-05	7.30E-05	0.00016886	0.00010865	0.00021399
12	[R].dTQEVPLEk.[A]_2_F1.raw	[R].dTQEVPL	2	F1.raw	Q9Y5A9	8.88E-05	0.00011238	9.82E-05	0.00010254	0.00013824	8.58E-05	9.55E-05	9.99E-05
13	[K].aEFIVGGk.[Y]_2_F6.raw	[K].aEFIVGG	2	F6.raw	P48729	0.00011072	7.77E-05	0.00013418	9.34E-05	0.00011279	9.72E-05	9.62E-05	0.00013056
14	[R].INIEELLk.[H]_2_F5.raw	[R].INIEELLk.	2	F5.raw	Q8WYP5	9.92E-05	9.76E-05	7.97E-05	4.73E-05	0.00010301	0.00015526	9.42E-05	
15	[R].gQLEQITGk.[G]_2_F5.raw	[R].gQLEQIT(	2	F5.raw	Q5SSJ5	9.49E-05	0.00010287	0.00011322	9.96E-05	0.00012482	7.37E-05	0.00010085	7.85E-05
16	[K].eQLLQSNPVLEAFGNAk.[T]_3_F6.raw	[K].eQLLQSN	3	F6.raw	O43795	8.97E-05	9.54E-05	9.08E-05	9.72E-05	6.98E-05	0.00015183	0.00010166	0.00023935
17	[R].ISTPIAGLDNINVFLk.[A]_3_F8.raw	[R].ISTPIAGL	3	F8.raw	Q8WWI1	5.83E-05	7.38E-05	6.80E-05	7.91E-05	8.27E-05	0.00015234	0.00011683	0.00025003
18	[K].akPAEAPAAAAPk.[A]_3_F6.raw	[K].akPAEAP/	3	F6.raw	P36957	0.00010773	8.22E-05	8.98E-05	9.52E-05	7.86E-05	0.00018055	9.47E-05	0.00022975
19	[R].vIMGEEVEPVGLMTGSGVVGVk.[V]_3_F5.raw	[R].vIMGEEV	3	F5.raw	P27708	1.44E-05	0.00023106	1.41E-05	0.00021785	0.00012756	3.70E-05	6.47E-05	
20	[R].dGSGTPSR.[H]_2_F1.raw	[R].dGSGTPS	2	F1.raw	Q9UQ35	0.0001624	0.0001132	0.00011551	0.00010889	0.00011022	4.27E-05	8.84E-05	3.19E-05
21	[K].amHGFLQk.[A]_3_F4.raw	[K].amHGFLC	3	F4.raw	P78527	6.97E-06	0.00013202	1.85E-05	0.00011696	0.00016094	3.22E-05	9.93E-05	

ERG-vs-Control_peptide_level_FC +