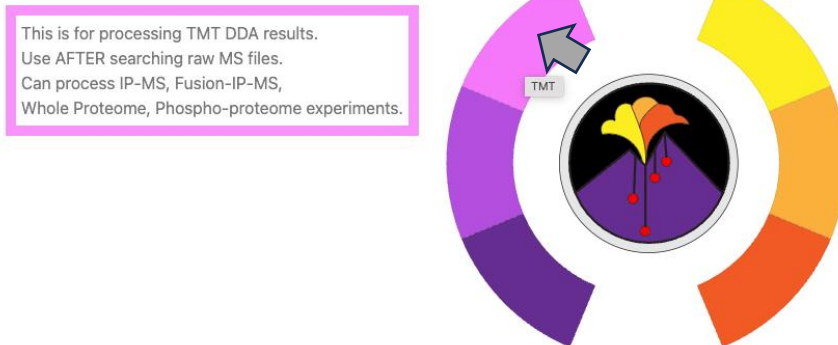# TMT (IP-MS)

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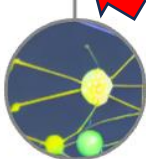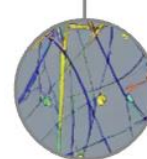
Step 3: Upload the annotation file. This is made using the experimental setup.

An example can be seen below taken from a SARS-CoV2 IP-MS TMT10 experiment. Here each TMT label is associated with a separate IP with Vector coding for non-viral transfected samples and N, M, orf3a and orf6 coding for IPs of specific viral 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.

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

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 down sampled to 500 proteins from a real IP experiment and looks like the screenshot below.

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.

3

Upload a tab separated COMET search output text file after libra quantification

For more information: **?**

Select a COMET output file:

- This field is required.

Choose File | No file chosen

Reset

Sample file

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 ('N','M) 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 "**ZZZZZ9**" corresponds to N viral bait. As before hover over question mark to get more information.

6 ## Enter the Uniprot ID of your bait below:

For more information: **?**

- This field is required.

| ZZZZZ9 | annotation_1220.csv |

Reset

Step 8: 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 –

Run the Analysis

Your job has been succesfully submitted, you will be redirected to the result page once the analysis is complete

↻ Loading...

The output page (given a successful run) looks like the screenshots 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.
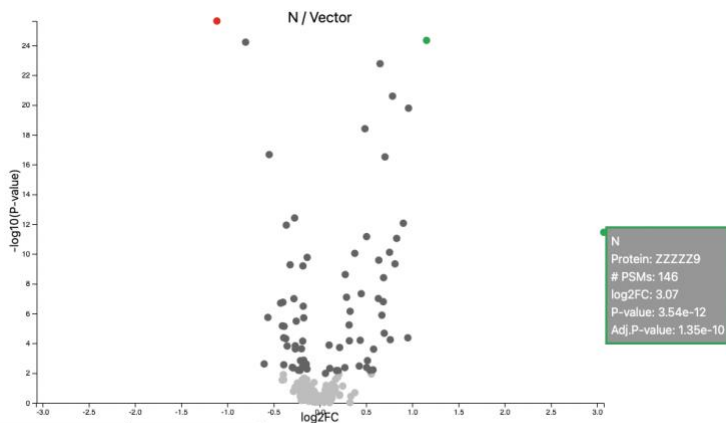
Value: 5

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]

N / Vector

N
Protein: ZZZZZ9
PSMs: 146
log2FC: 3.07
P-value: 3.54e-12
Adj.P-value: 1.35e-10

[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_127N	log2_126	log2_129N	log2_129C	PSM Cutoff
2	ZZZZZ9	3.0718912	3.54E-12	1.35E-10	N	146	-10.672234	-15.08168	-13.583132	-10.812606	5
3	Q9Y6Y0	-0.3992096	1.76E-07	2.64E-06	IVNS1ABP	17	-12.251674	-11.739879	-11.831093	-12.245438	
4	Q9Y6W5	-0.097623	0.45143805	0.65663717	WASF2	2	-12.30738	-12.097758	-12.174227	-12.207526	
5	Q9Y6N8	-0.3119672	0.13224471	0.29282757	CDH10	1	-11.943675	-11.510728	-11.766183	-11.966535	
6	Q9Y6D6	-0.4712345	0.05440267	0.16429101	ARFGEF1	1	-12.630295	-11.998228	-12.136671	-12.447415	
7	Q9Y6D5	-0.0912389	0.40734843	0.62182093	ARFGEF2	2	-12.145137	-12.142974	-12.182493	-12.385237	
8	Q9Y617	0.01986107	0.63261716	0.78054257	PSAT1	12	-12.880707	-13.286997	-12.62676	-12.878715	
9	Q9Y613	-0.1253561	0.7079511	0.83407065	FHOD1	7	-11.963397	-11.91926	-11.882098	-11.931049	
10	Q9Y5Q8	-0.119137	0.2240163	0.4177146	GTF3C5	1	-12.103456	-11.895853	-11.941819	-11.973707	
11	Q9Y4P3	0.31967334	6.79E-05	0.00066084	TBL2	12	-11.879102	-12.306116	-12.202053	-12.015355	
12	Q9Y4I1	-0.1851581	7.16E-05	0.00068326	MYO5A	21	-11.763877	-11.566686	-11.617389	-11.775686	
13	Q9Y4F5	-0.0906183	0.70532161	0.83389643	CEP170B	4	-11.897222	-11.99269	-11.901107	-12.005734	
14	Q9Y490	-0.2553572	3.28E-06	4.07E-05	TLN1	44	-12.001374	-11.774391	-11.779223	-12.068989	
15	Q9Y3D6	0.02594121	0.45331078	0.65743317	FIS1	2	-12.323645	-12.281948	-12.308564	-12.153915	
16	Q9Y3C4	-0.1336602	0.43562032	0.64653463	TPRKB	3	-12.187928	-12.144334	-12.068884	-12.202555	
17	Q9Y3B9	-0.5734296	0.03109339	0.10937817	RRP15	1	-12.168614	-11.457073	-11.646169	-12.083355	
18	Q9Y3A5	0.03156629	0.64335394	0.78596935	SBDS	6	-12.377827	-12.561993	-12.418118	-12.471679	
19	Q9Y333	0.07921899	0.37546032	0.59120102	LSM2	2	-11.950093	-12.088086	-12.112609	-12.12578	
20	Q9Y312	0.35838836	0.12696953	0.28423424	AAR2	1	-11.496078	-11.991519	-11.982878	-11.774099	
21	Q9Y305	-0.1149162	0.2159682	0.40730124	ACOT9	9	-12.143957	-12.088168	-12.002298	-12.200503	
22	Q9Y2Z0	-0.228984	0.05924786	0.17108135	SUGT1	4	-12.306539	-11.984594	-11.943304	-12.11874	
23	Q9Y2R4	0.7618811	5.76E-05	0.00058263	DDX52	5	-11.685664	-12.381097	-12.167263	-11.682783	
24	Q9Y2L1	-0.0629616	0.04265283	0.13827321	DIS3	11	-12.120913	-11.928606	-12.01892	-12.050285	
25	Q9Y2B0	0.03537214	0.75396816	0.86167789	CNPY2	3	-12.282849	-12.150666	-12.098583	-12.074646	
26	Q9Y2A7	-0.2114628	0.35630419	0.57193164	NCKAP1	9	-12.364685	-11.916955	-12.074081	-12.139341	
27	Q9Y277	-0.0850129	0.45740804	0.66144136	VDAC3	5	-12.205529	-12.120516	-12.086573	-12.108303	

N-vs-Vector_FC_and_pval_YuLab +

The output file (peptide) looks like this -

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	PeakID	AnnotatedSe	Charge	Fraction	Protein	126	127C	127N	128C	128N	129C	129N	130C	130N	131
2	[R].gDPGDQ	[R].gDPGDQ	2	CACO2-M-N-	Q9NZB2	0.00021616	0.00021216	0.00034582	0.00022341	0.00024994	0.00031672	0.0002019	0.00023626	0.00020781	0.00021444
3	[K].qQSPQEP	[K].qQSPQEP	2	CACO2-M-N-	Q9UHB6	0.00042107	0.00021156	0.00028114	0.00020295	0.00013135	0.0002725	0.00040354	0.00012127	0.00021134	0.00021761
4	[K].sSSSSQk.	[K].sSSSSQk.	2	CACO2-M-N-	Q7Z6E9	0.00025232	0.00023308	0.00027358	0.00030263	0.00016141	0.00029682	0.00026452	0.00012289	0.00025645	0.00028286
5	[K].sSGNSSS	[K].sSGNSSS	3	CACO2-M-N-	Q12797	0.00015738	0.00028482	0.00025586	8.91E-05	0.00039362	0.00027115	0.00019116	0.00039067	0.00029963	0.0001775
6	[K].aDPPATE	[K].aDPPATE	2	CACO2-M-N-	Q9HAV7	0.00018737	0.00017152	0.00021111	0.00017592	0.00052175	0.00020538	0.00017681	0.00052905	0.00017043	0.00015815
7	[R].gAEEEPP	[R].gAEEEPP	3	CACO2-M-N-	Q13144	0.00025955	0.0002156	0.00021335	0.00030073	0.00034176	0.00022475	0.0002122	0.00027332	0.00022124	0.00023671
8	[K].dVLGPST	[K].dVLGPST	3	CACO2-M-N-	Q9BZF1	0.00020167	0.00021381	0.0001735	0.0001951	0.00047064	0.00017209	0.00020436	0.00048836	0.00023541	0.00018928
9	[K].kVQSDG(	[K].kVQSDG(	3	CACO2-M-N-	Q08211	0.00015867	0.00018885	0.00040189	0.00019502	0.00025694	0.00039308	0.00017012	0.00023863	0.00016536	0.00019638
10	[K].aQNTWG	[K].aQNTWG	2	CACO2-M-N-	P02545	0.00014872	0.00037766	0.00026979	0.000168	0.00035855	0.00027262	0.00017317	0.0002941	0.000315	0.00015332
11	[R].aEAGDNI	[R].aEAGDNI	2	CACO2-M-N-	P49411	0.00020724	0.00029041	0.00020505	0.00020192	0.00033155	0.0002135	0.00022007	0.00038548	0.00028829	0.00019794
12	[R].gPPPSYG	[R].gPPPSYG	2	CACO2-M-N-	P38159	0.00038289	0.00020746	0.00017635	0.00016307	0.00022172	0.00026364	0.00034138	0.0002551	0.0002493	0.00024859
13	[K].gATYPSEI	[K].gATYPSEI	2	CACO2-M-N-	Q8WWI1	0.00034318	0.00029303	0.00031769	0.00019894	5.92E-05	0.00033372	0.00035512	4.88E-05	0.00031538	0.00020116
14	[K].yTSQLQV	[K].yTSQLQV	2	CACO2-M-N-	Q9Y4F5	0.00022376	0.00018933	0.00018429	0.00018982	0.00043128	0.00020701	0.00021911	0.00040733	0.00023977	0.00022461
15	[R].sEEPGAP	[R].sEEPGAP	3	CACO2-M-N-	P53814	0.00028476	0.00043613	0.00034073	0.00019219	3.63E-05	0.00030193	0.00027755	2.44E-05	0.00044454	0.00018938
16	[R].tEmENEF	[R].tEmENEF	3	CACO2-M-N-	P05787	0.00015761	9.63E-05	0.00018939	0.00030163	0.00041983	0.00021147	0.00016285	0.00047384	9.70E-05	0.00033317
17	[R].gLFDEEN	[R].gLFDEEN	3	CACO2-M-N-	Q15149	0.00034058	0.00020768	0.00030982	0.00033825	3.36E-05	0.00031731	0.00032032	2.32E-05	0.00019888	0.00032301
18	[R].aAcQEAC	[R].aAcQEAC	3	CACO2-M-N-	Q53H12	0.00027176	0.00027374	0.00019873	0.00026845	0.00024433	0.00019694	0.00027315	0.00026036	0.00026113	0.00028086
19	[K].sTAMNES	[K].sTAMNES	2	CACO2-M-N-	Q9BY42	0.00023297	0.00034122	0.00034547	0.00029125	0.00015363	0.00020905	0.00026482	0.00014346	0.00026114	0.00025626

N-vs-Vector_peptide_level_FC_an +