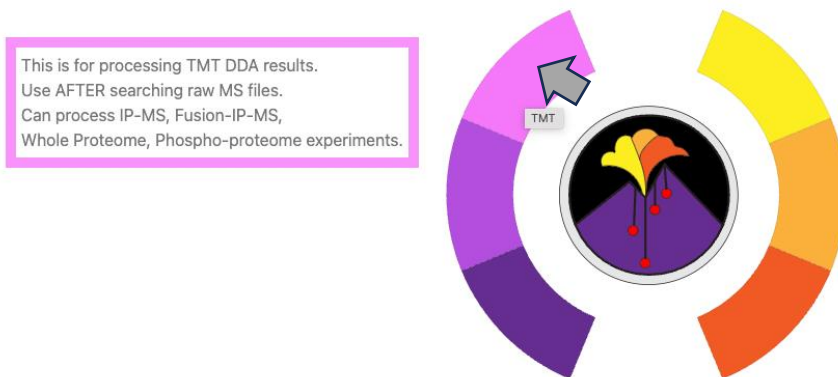# TMT (Whole Proteome)

**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 Whole proteome arm (highlighted with red arrow in the figure below) to access to workflow.
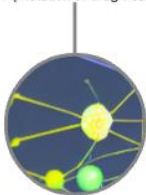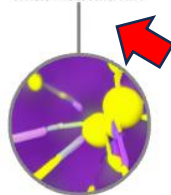
**Step 3**: Upload the annotation file. This is made using the experimental setup.

An example can be seen below taken from a benchmarking two whole proteome (yeast spiked into human) TMT10 experiment. Here each TMT label is associated with a separate condition. For example, ten implies Human:Yeast of 1:1, four implies Human: Yeast of 1:0.4 and one implied Human: Yeast of 1:0.1. 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 ('four','ten') and direction (Condition_2/Condition_1) means the following comparison will run – (ten/four).



**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 benchmarking whole-proteome 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". The second screenshot is of the down-sampled version of COMET search for the same benchmarking dataset.

As before hover over question mark for more information. Click on the "Sample File" in blue to download this example PSM files.

**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.

**For the example dataset choose "Protein-list Normalization" for type of column type normalization. Put "Human" in the text box.**



**Choose type of column-type normalization:**

For more information: ?

○ Global Normalization
● Protein-list Normalizati
○ No Column Normalizat

> Choose global normalization if you can assume that most proteins in your experiment are NOT going to be differentially up or down regulated for the conditions being compared.
> Choose protein list normalization and provide a list of uniprots in case you know of specific proteins that can be assumed to be unchanging between the conditions compared as a result of experimental setup.
> Choose no column normalization in case none of the situations discussed are applicable.

Input the uniprot ID's comma separated.
Example: P16140,P39107,P04806.
Enter - "human" or "yeast" or "ecoli" for whole proteome

[human]

[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.
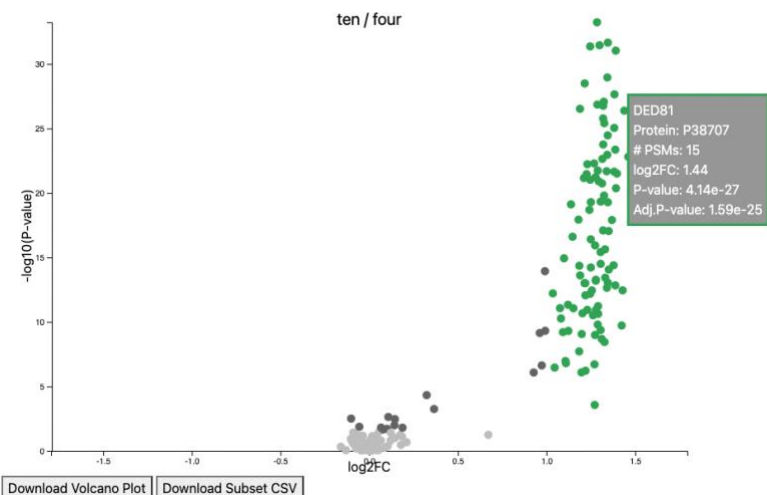
Value: 5

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



ten / four

DED81
Protein: P38707
# PSMs: 15
log2FC: 1.44
P-value: 4.14e-27
Adj.P-value: 1.59e-25

Download Volcano Plot    Download Subset CSV

## The output file (protein) looks like this –

| Protein | log2FC | pval | adjpval | Proteininoiso | Gene Symbol | # PSMs | log2_127C | log2_129N | log2_128N | log2_127N | log2_128C | log2_126 | PSM Cutoff |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P26784 | 1.38134962 | 2.23E-22 | 4.11E-21 | P26784 | RPL16A | 12 | -11.726424 | -11.768642 | -10.474821 | -10.422956 | -11.861789 | -10.440364 | 5 |
| Q96F63 | 0.34030288 | 0.06213941 | 0.10918158 | Q96F63 | CCDC97 | 2 | -11.408906 | -11.50002 | -11.205176 | -11.038491 | -11.745837 | -10.928838 | |
| P06280 | -0.0384584 | 0.90470072 | 0.91944126 | P06280 | GLA | 5 | -11.667265 | -11.250148 | -11.502813 | -11.65461 | -11.614298 | -11.353603 | |
| Q3E792 | 1.21820791 | 9.91E-14 | 7.75E-13 | Q3E792 | RPS25A | 5 | -11.50257 | -11.801045 | -10.503787 | -10.442304 | -11.89155 | -10.44524 | |
| Q12207 | 1.35761863 | 0.02973173 | 0.05577494 | Q12207 | NCE102 | 2 | -12.775087 | -12.264278 | -11.417469 | -10.79947 | -11.709065 | -10.554678 | |
| O43747 | 0.01812818 | 0.45050281 | 0.57056066 | O43747 | AP1G1 | 5 | -11.197248 | -11.623892 | -11.539199 | -11.481668 | -11.659592 | -11.425964 | |
| Q05506 | 1.28455969 | 5.85E-34 | 2.92E-31 | Q05506 | YDR341C | 28 | -11.761494 | -11.793693 | -10.552705 | -10.48513 | -11.818836 | -10.499439 | |
| P13861 | 0.02960514 | 0.48693274 | 0.60292664 | P13861 | PRKAR2A | 15 | -11.474795 | -11.56569 | -11.449339 | -11.573047 | -11.477439 | -11.490748 | |
| P53090 | 1.29083586 | 2.38E-11 | 1.47E-10 | P53090 | ARO8 | 8 | -11.587725 | -11.835221 | -10.628664 | -10.525632 | -11.852603 | -10.486275 | |
| P46109 | 0.02998512 | 0.12793197 | 0.2033059 | P46109 | CRKL | 8 | -11.602967 | -11.429439 | -11.372418 | -11.478999 | -11.412675 | -11.362643 | |
| P0DMV9 | -0.0457134 | 0.91229316 | 0.92339612 | P0DMV9 | HSPA1B | 76 | -11.520141 | -11.519697 | -11.498504 | -11.506837 | -11.440221 | -11.558125 | |
| O76095 | -0.1619452 | 0.30064374 | 0.41214623 | O76095 | JTB | 1 | -11.518074 | -11.376478 | -11.68002 | -11.377997 | -11.396209 | -11.588197 | |
| Q9BUJ2 | -0.1597208 | 0.47721427 | 0.5953248 | Q9BUJ2 | HNRNPUL1 | 6 | -11.439283 | -11.49517 | -11.732362 | -11.656188 | -11.525864 | -11.626989 | |
| Q8TEQ6 | 0.31017975 | 0.23309433 | 0.34009963 | Q8TEQ6 | GEMIN5 | 2 | -11.382307 | -11.885992 | -11.050817 | -11.340174 | -11.586252 | -11.575813 | |
| P36018 | 0.90554353 | 6.35E-07 | 2.60E-06 | P36018 | YPT52 | 4 | -11.648322 | -11.606236 | -10.749002 | -10.723762 | -11.868766 | -10.721091 | |
| P38791 | 0.99104217 | 1.16E-14 | 1.02E-13 | P38791 | DYS1 | 13 | -11.632256 | -11.647798 | -10.60166 | -10.764521 | -11.772198 | -10.84641 | |
| Q9UNH5 | 0.09600367 | 0.35627739 | 0.4715714 | Q9UNH5 | CDC14A | 1 | -11.497843 | | | -11.399855 | -11.381747 | -11.287731 | |
| P45973 | 0.06565392 | 0.6568898 | 0.74666973 | P45973 | CBX5 | 3 | -11.580473 | -11.565569 | -11.490778 | -11.477054 | -11.478546 | -11.498655 | |
| Q02878 | 0.00560888 | 0.68024662 | 0.76279341 | Q02878 | RPL6 | 17 | -11.570794 | -11.506728 | -11.481798 | -11.490027 | -11.482545 | -11.550941 | |
| Q969Q5 | -0.012839 | 0.81862909 | 0.86362772 | Q969Q5 | RAB24 | 1 | -11.398085 | -11.588733 | -11.53585 | -11.410924 | -11.465611 | -11.558748 | |
| P13674 | -0.1468863 | 0.58969541 | 0.68912883 | P13674 | P4HA1 | 1 | -11.296898 | -11.362917 | -11.510518 | -11.443784 | -11.710561 | -11.670512 | |
| P40926 | -0.0105211 | 0.53037069 | 0.63926322 | P40926 | MDH2 | 22 | -11.395903 | -11.465077 | -11.494215 | -11.462558 | -11.498413 | -11.482076 | |
| Q16204 | -0.0936251 | 0.47355475 | 0.59224016 | Q16204 | CCDC6 | 2 | -11.269886 | -11.574696 | -11.510263 | -11.732625 | -11.432712 | -11.363511 | |
| Q12287 | 1.32409542 | 0.00204293 | 0.00476366 | Q12287 | COX17 | 1 | -11.375748 | -11.838521 | -10.514425 | -10.407758 | -11.901406 | -10.541281 | |
| P15703 | 1.16134159 | 8.73E-12 | 5.75E-11 | P15703 | BGL2 | 4 | -11.648393 | -11.712628 | -10.556376 | -10.590355 | -11.843102 | -10.50546 | |
| Q8IY81 | 0.3241989 | 4.75E-05 | 0.00015283 | Q8IY81 | FTSJ3 | 6 | -11.666386 | -11.626663 | -11.280481 | -11.185315 | -11.59718 | -11.421914 | |
| P38986 | 1.38802544 | 3.15E-05 | 0.00010708 | P38986 | ASP1 | 1 | -11.866326 | -11.752365 | -10.547192 | -10.46865 | -11.873223 | -10.36434 | |
| P35178 | 0.80046002 | 0.00029336 | 0.00082704 | P35178 | RRP1 | 1 | -11.547308 | -11.632503 | -10.829477 | -10.92925 | -11.687103 | -10.746848 | |

ten-vs-four_FC_and_pval_YuLab

## The output file (peptide) looks like this -

| PeakID | AnnotatedSe | Charge | Fraction | Protein | 126 | 127C | 127N | 128C | 128N | 129C | 129N | 130C | 131 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [K].lkGDDLQ/ | [K].lkGDDLQ/ | 3 | HPH7.raw | P07910 | 0.00033327 | 0.0003802 | 0.00033692 | 0.00032673 | 0.00034862 | 0.00036727 | 0.00033562 | 0.00038573 | 0.00033549 |
| [K].dNQIYAIE | [K].dNQIYAIE | 3 | HPH5.raw | P38879 | 0.00074752 | 0.00026469 | 0.00075403 | 0.00024236 | 0.00075802 | 5.00E-05 | 0.00027379 | 3.55E-05 | 4.08E-05 |
| [R].dSGYGVS | [R].dSGYGVS | 2 | HPH3.raw | P13663 | 0.00070923 | 0.00026538 | 0.00030735 | 0.00022826 | 0.0006035 | 0.00010463 | 0.00032957 | 0.00010376 | 0.00010806 |
| [K].vEEQEQC | [K].vEEQEQC | 3 | HPH3.raw | P38701 | 0.00071763 | 0.00026167 | 0.00068688 | 0.00026264 | 0.00070311 | 7.07E-05 | 0.00031346 | 9.23E-05 | 4.98E-05 |
| [K].lYDLLGVS | [K].lYDLLGVS | 3 | HPH5.raw | P25294 | 0.00048221 | 0.00018885 | 0.00053643 | 0.00014272 | 0.00054058 | | 0.00022006 | | |
| [R].eAGEVkD | [R].eAGEVkD | 3 | HPH3.raw | P41805 | 0.00067574 | 0.00032078 | 0.00072417 | 0.00025971 | 0.00070877 | 4.73E-05 | 0.00032821 | 6.05E-05 | 4.64E-05 |
| [R].gkLEEEY/ | [R].gkLEEEY/ | 3 | HPH3.raw | P32589 | 0.00074303 | 0.00037201 | 0.0006442 | 0.0002381 | 0.00076626 | 5.02E-05 | 0.00032111 | 8.03E-05 | 5.33E-05 |
| [K].aGAHLQ( | [K].aGAHLQ( | 3 | HPH7.raw | P04406 | 0.00032877 | 0.00041784 | 0.00034462 | 0.00035303 | 0.00034255 | 0.0003306 | 0.00035312 | 0.00037582 | 0.00031547 |
| [R].gFLSDLP[ | [R].gFLSDLP[ | 3 | HPH6.raw | P05317 | 0.00069354 | 0.00037472 | 0.00071906 | 0.00025598 | 0.00069285 | 5.74E-05 | 0.00028641 | 5.68E-05 | 4.88E-05 |
| [R].ILNTFLER | [R].ILNTFLER | 2 | HPH8.raw | Q14204 | 0.00033171 | 0.00032232 | 0.00034876 | 0.00035939 | 0.0003956 | 0.00041236 | 0.00036113 | 0.00029225 | 0.00029544 |
| [R].gGYFDEF | [R].gGYFDEF | 2 | HPH5.raw | P11413 | 0.00028778 | 0.00044996 | 0.00036879 | 0.00027447 | 0.00034816 | 0.00037011 | 0.00033086 | 0.00040549 | 0.00032448 |
| [R].gWDDLFI | [R].gWDDLFI | 2 | HPH7.raw | Q14204 | 0.00035529 | 0.00041456 | 0.0003319 | 0.00031538 | 0.00033567 | 0.00033693 | 0.00033518 | 0.00041115 | 0.00032618 |
| [R].dNQLSEV | [R].dNQLSEV | 2 | HPH3.raw | Q14978 | 0.00034719 | 0.00029993 | 0.00036285 | 0.00038731 | 0.00035562 | 0.00036434 | 0.00036569 | 0.00031091 | 0.00033842 |
| [K].hLEDNTLI | [K].hLEDNTLI | 3 | HPH6.raw | P05739 | 0.00073257 | 0.00024626 | 0.00075546 | 0.0002609 | 0.00072149 | 4.87E-05 | 0.0003024 | 4.62E-05 | 4.75E-05 |
| [K].tLDVVER. | [K].tLDVVER. | 2 | HPH3.raw | P00942 | 0.00071685 | 0.00025749 | 0.00072301 | 0.00027892 | 0.00076609 | 6.17E-05 | 0.00029212 | 2.97E-05 | 3.49E-05 |
| [R].eLDLYDN | [R].eLDLYDN | 3 | HPH3.raw | Q15435 | 0.00033634 | 0.00042543 | 0.00037834 | 0.00030226 | 0.00041087 | 0.00029255 | 0.00037885 | 0.00037224 | 0.00026275 |
| [R].aEVSQPR | [R].aEVSQPR | 2 | HPH7.raw | P22314 | 0.00022849 | 0.00041725 | 0.00023279 | 0.00053172 | 0.00025502 | 0.00040786 | 0.00029357 | 0.00038406 | 0.00042454 |
| [-].mGSGAG | [-].mGSGAG | 2 | HPH1.raw | Q9Y5G0 | 0.00038931 | 0.000332 | 0.00061138 | 0.00032747 | 0.00052097 | 0.00022755 | 0.00029517 | 0.00028301 | 0.00017976 |
| [K].iEGVATP( | [K].iEGVATP( | 3 | HPH6.raw | P41056 | 0.00069567 | 0.00027261 | 0.00069091 | 0.00027398 | 0.00072077 | 7.28E-05 | 0.0003124 | 5.97E-05 | 6.19E-05 |
| [K].tHIGDGF( | [K].tHIGDGF( | 3 | HPH8.raw | Q12074 | 0.00071313 | 0.00091546 | 0.00077785 | 0.00028266 | 0.00073519 | 6.00E-05 | 0.00029518 | 3.66E-05 | 5.77E-05 |
| [K].sVDELITE | [K].sVDELITE | 3 | HPH2.raw | P05319 | 0.00075717 | 0.00032846 | 0.0007171 | 0.0002409 | 0.00070871 | 5.14E-05 | 0.0002749 | 4.88E-05 | 5.09E-05 |
| [K].INTAISNL | [K].INTAISNL | 2 | HPH5.raw | P07806 | 0.00038151 | 0.00031786 | 0.00046773 | 0.00036292 | 0.00041521 | | 0.0002059 | | |
| [K].aTGDETG | [K].aTGDETG | 2 | HPH2.raw | P61247 | 0.00033086 | 0.00039852 | 0.00031653 | 0.00032255 | 0.00031704 | 0.00035513 | 0.00032157 | 0.00044364 | 0.00035643 |
| [R].iHVLEAQ | [R].iHVLEAQ | 3 | HPH6.raw | Q9BSJ8 | 0.0003478 | 0.00034907 | 0.00032907 | 0.00036826 | 0.00035332 | 0.00035113 | 0.00034791 | 0.00034868 | 0.00035292 |
| [K].eIHFFGDI | [K].eIHFFGDI | 3 | HPH5.raw | P07283 | 0.00069107 | 0.00037161 | 0.00071913 | 0.00024916 | 0.00067682 | 5.42E-05 | 0.0003102 | 5.96E-05 | 4.97E-05 |
| [R].kQFDAAE | [R].kQFDAAE | 3 | HPH5.raw | P07263 | 0.0006758 | 0.00034609 | 0.00072885 | 0.0002552 | 0.00069311 | 6.18E-05 | 0.00030948 | 5.28E-05 | 5.30E-05 |
| [R].dMYEQF( | [R].dMYEQF( | 3 | HPH6.raw | P61011 | 0.00048423 | 0.00029595 | 0.00040941 | 0.00028214 | 0.00044429 | 0.00023958 | 0.00038542 | 0.000303 | 0.00030104 |
| [K].qLQNISL/ | [K].qLQNISL/ | 3 | HPH8.raw | Q14204 | 0.00056728 | 0.00040193 | 0.00025426 | 0.00025606 | 0.00040678 | 0.00029987 | 0.00036162 | 0.00030783 | 0.00029106 |

ten-vs-four_peptide_level_FC_an