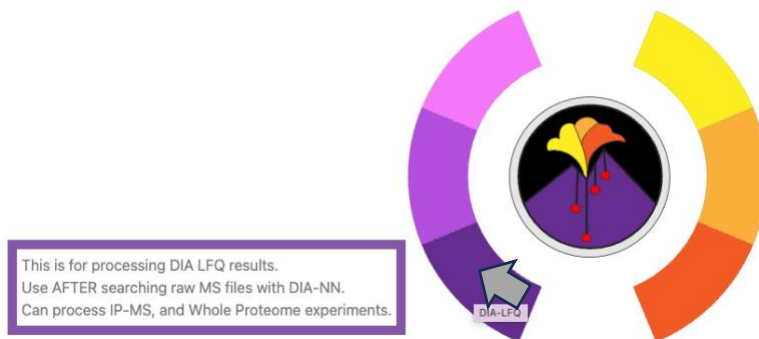


DIA-LFQ (Protein level)

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 analysis type (MAGMa LM for a two-sample t-test and MAGMa Limma for a one sample t-test)

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

Analysis Type

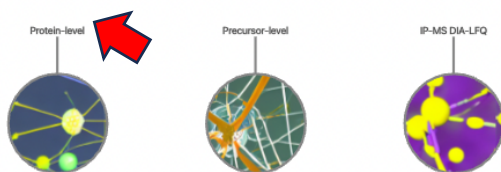
For more information: ?

- Magma (Linear Model)
 Magma (Limma)

Choose the strategy for analysis with two choices:

1. Choose the model type to run the differential expression analysis with (between linear model (two sample) vs limma (one sample))
2. Choose the type of analysis (Whole Proteome - Precursor or Protein level) or IP-DIA-LFQ. Output files from DIA-NN should be used as input after.

NOTE: No default set but recommend setting of Protein level/IP-DIA + Magma (limma model). Check "About" page or manuscript for more details.



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

An example can be seen below taken from a benchmarking whole proteome (human with yeast spike in) DIA-LFQ experiment. Here each Channel is associated with a separate machine run. So, in the example below, condition “Sample A” (Human: Yeast:: 1:1) was run thrice and thus has three separate files generated which correspond to two columns in the protein level quantification file (highlighted in green). **These are headers of the files that would be input in step 5 below (see second screen shot for corresponding color in column header).**

| | A | | | | | | | | | | B | | | | | | | | | | |
|----|--|--|--|--|--|--|--|--|--|--|---|--|--|--|--|--|--|--|--|--|----------|
| 1 | Channel | | | | | | | | | | | | | | | | | | | | Label |
| 2 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2524.d | | | | | | | | | | | | | | | | | | | | Sample A |
| 3 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2525.d | | | | | | | | | | | | | | | | | | | | Sample A |
| 4 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2526.d | | | | | | | | | | | | | | | | | | | | Sample A |
| 5 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2531.d | | | | | | | | | | | | | | | | | | | | Sample B |
| 6 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2532.d | | | | | | | | | | | | | | | | | | | | Sample B |
| 7 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2533.d | | | | | | | | | | | | | | | | | | | | Sample B |
| 8 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2535.d | | | | | | | | | | | | | | | | | | | | Sample C |
| 9 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2536.d | | | | | | | | | | | | | | | | | | | | Sample C |
| 10 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2537.d | | | | | | | | | | | | | | | | | | | | Sample C |

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O |
|----|---------|-------------|-------------|---------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------|---|
| 1 | Protein | Protein.Ids | Protein.Nam | Genes | First.Protein. | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\2023_May_C | |
| 2 | A2RTX5 | A2RTX5 | SYTC2_HUM | TARS3 | Threonine-t | 15944.2 | 16235.8 | 13334.3 | 18015.2 | 14564.8 | 16957.2 | 13874.6 | 13973.4 | 15230.1 | |
| 3 | LOR8F8 | LOR8F8 | MIDUO_HUN | MIEF1 | MIEF1 upstre | 20306.6 | 31025.5 | 26134.2 | 40500 | 38305.6 | 33994.6 | 27231.9 | 23019.6 | 25284 | |
| 4 | O00257 | O00257 | CBX4_HUMA | CBX4 | E3 SUMO-pr | 31290.3 | 25705.3 | 31914.9 | 32556.9 | 30615.1 | 32384.1 | 25321.9 | 26297.9 | 27565.1 | |
| 5 | O00258 | O00258 | GET1_HUMA | GET1 | Guided entry | 4101.55 | 5184.99 | 4891.59 | 5610.92 | 4383.75 | 4045.87 | 4969.71 | 4638.22 | 4514.36 | |
| 6 | O00264 | O00264 | PGRC1_HUM | PGRMC1 | Membrane-a | 343183 | 340696 | 326223 | 442980 | 467482 | 451763 | 438401 | 425337 | 441216 | |
| 7 | O00469 | O00469 | PLOD2_HUM | PLOD2 | Procollagen- | 50505.6 | 52641.1 | 51054.5 | 60625.4 | 60709.3 | 60564.4 | 56156.1 | 54538.3 | 56712.9 | |
| 8 | O14639 | O14639 | ABL1M1_HUN | ABLIM1 | Actin-binding | 4485.57 | 15124.2 | 14480.6 | 16178.3 | 19332.8 | 18881.9 | 15269.6 | 13856 | 15082.2 | |
| 9 | O14657 | O14657 | TOR1B_HUM | TOR1B | Torsin-1B | 19376.3 | 18249.9 | 17701.6 | 20595.4 | 23204.8 | 13031.6 | 18856.8 | 17889.5 | 19488.8 | |
| 10 | O15126 | O15126 | SCAM1_HUN | SCAMP1 | Secretory car | 29889.3 | 24671.2 | 30634.5 | 37534.6 | 37673.6 | 42550.1 | 35807.3 | 35401.7 | 32844.4 | |
| 11 | O15304 | O15304 | SIVA_HUMA | SIVA1 | Apoptosis rei | 10081.2 | 7646.25 | 6616.17 | 1996.03 | 5434.4 | 7202.26 | 7641.77 | 7319.09 | 4708.16 | |
| 12 | O15321 | O15321 | TM9S1_HUM | TM9SF1 | Transmembr | 20380.2 | 20410.9 | 18020.2 | 16311.2 | 15967 | 20695.8 | 19330.4 | 16006.9 | 16209.2 | |
| 13 | O43292 | O43292 | GPA1_HUM | GPA1 | Glycosylphos | 17202.3 | 23281.9 | 15531.9 | 15748.9 | 20459.9 | 17869.3 | 19818.7 | 17386.3 | 18820.7 | |
| 14 | O43447 | O43447 | PIIH_HUMAI | PIIH | Peptidyl-prol | 419765 | 468936 | 396211 | 450160 | 431498 | 413610 | 442408 | 406987 | 415182 | |
| 15 | O43567 | O43567 | RNF13_HUM | RNF13 | E3 ubiquitin- | 7219.94 | 5406.75 | 8014.85 | 8639.45 | 9969.08 | 5437.31 | 10265.9 | 8413.95 | 8364.91 | |
| 16 | O43663 | O43663 | PRC1_HUMA | PRC1 | Protein regul | 170001 | 189163 | 151910 | 246849 | 230180 | 207287 | 172238 | 155032 | 174000 | |
| 17 | O43929 | O43929 | ORC4_HUM | ORC4 | Origin recogni | 46247.6 | 50641.1 | 52583.5 | 65225.3 | 63211.1 | 62048 | 53586.3 | 52464.5 | 56440.2 | |
| 18 | O60826 | O60826 | CCD22_HUM | CCDC22 | Coiled-coil d | 70250.5 | 63085 | 70690.2 | 72434.8 | 66947.7 | 70907.2 | 69190.9 | 69623.2 | 66484.8 | |
| 19 | O75113 | O75113 | N4BP1_HUM | N4BP1 | NEDD4-bindi | 41987.7 | 41924.5 | 33715.9 | 41820.6 | 44669.1 | 42690 | 35225.5 | 37276.3 | 40062.3 | |
| 20 | O75121 | O75121 | MFA3L_HUM | MFAP3L | Microfibrilla | 6653.58 | 8527.04 | 9984.36 | 9157.86 | 10465 | 10372.1 | 6270.57 | 11724.9 | 9080.72 | |
| 21 | O75150 | O75150 | BRE1B_HUM | RNF40 | E3 ubiquitin- | 60382.3 | 65078.3 | 65609.7 | 79460.4 | 73603.8 | 74264.1 | 72510.9 | 69577.8 | 69485.5 | |
| 22 | O75417 | O75417 | DPOLQ_HUM | POLQ | DNA polyme | 13776.2 | 9876.68 | 13463.9 | 10645.6 | 14391.4 | 11181.3 | 13195.2 | 11602.1 | 10996.7 | |
| 23 | O75525 | O75525 | KHDR3_HUM | KHDRB53 | KH domain-c | 334471 | 322770 | 321972 | 367780 | 360167 | 356982 | 365355 | 364374 | 351086 | |
| 24 | O94788 | O94788 | AL1A2_HUM | ALDH1A2 | Retinal dehy | 99285 | 98671.8 | 97095.1 | 119611 | 115296 | 105823 | 108459 | 105469 | 93010.6 | |
| 25 | O94905 | O94905 | ERLN2_HUM | ERLN2 | Erlin-2 | 47687 | 58778.7 | 54064.7 | 67397.6 | 66855 | 60591.2 | 57872.3 | 59537.2 | 53766.4 | |
| 26 | O95201 | O95201 | ELAVL1_HUM | ELAVL1 | RNA-bindin | 24406.6 | 27770.7 | 15361 | 23070.0 | 22676.4 | 18148.5 | 18560.7 | 21103.6 | 17053.7 | |

(the pg_matrix file is inputted in step 5)

You can also hover over question mark for more details. [Click on the “Sample File” in blue to download this example annotation file.](#)

1 Upload the 'Annotation file'

For more information: ?

Select a file:

No file chosen

[Sample file](#)

This file is to assign labels to channels according to experimental setup. Columns needed in this file are - Channel and Label. Channel are the column header(s) in the DIANN output specifying quantification per row. Important to choose the conditions to be compared.

NOTE: File name should not have spaces and should be ".csv" format.

NOTE(2): The "Label" column should not have spaces and "-" character.

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

2

Select the Comparison You Would Like to Perform

Choose a comparison

- ('Sample A', 'Sample B')
- ('Sample A', 'Sample C')
- ('Sample B', 'Sample C')

Choose the direction for comparison:

- (Condition_1/Condition_2)
- (Condition_2/Condition_1)

Reset

Step 5: Upload the input files (protein level aggregated) for quantification.

For DIA-NN search, upload a tab separated file of quantification file (aggregated to protein level). The file usually has “pg_matrix.tsv” in the name. **Set the column name as “Protein” that you want to use as the unique identifier** (every point on your volcano plot would be associated with a unique value in that column). Make sure the files have the following columns – 'Protein', 'Genes'.

As before hover over question mark for more information. [Click on the “Sample File” in blue to download this example protein level aggregated file.](#)

3

Upload a tab separated Bruker+DIANN text file with protein level information.

For more information: ?

Select a file:

- This field is required

Choose File No file chosen

Reset

[Sample file](#)

This file is the output from DIA-NN search in the form of tab delimited ".tsv" file of all information aggregated to protein level.

NOTE: After running DIA-NN the output file should probably have "pg_matrix.tsv" in the name.

NOTE (2): IMPORTANT!! Set column name as "Protein" for the column to be used as unique protein ID's.

The example is taken from a down-sampling of real benchmarking DIA-LFQ experiment run on Bruker timsTOF HT and looks like the screenshot below.

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O |
|----|---------|-------------|-------------|---------|----------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|-------|
| 1 | Protein | Protein.Ids | Protein.Nam | Genes | First.Protein | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\202 | D:\Data\2023 | May_1 |
| 2 | A2RTX5 | A2RTX5 | SYTC2_HUM | TARS3 | Threonine--t | 15944.2 | 16235.8 | 13334.3 | 18015.2 | 14564.8 | 16957.2 | 13874.6 | 13973.4 | 15230.1 | |
| 3 | LOR8F8 | LOR8F8 | MIDUO_HUM | MIEF1 | MIEF1 upstre | 20306.6 | 31025.5 | 26134.2 | 40500 | 38305.6 | 33994.6 | 27231.9 | 23019.6 | 25284 | |
| 4 | O00257 | O00257 | CBX4_HUMA | CBX4 | E3 SUMO-pr | 31290.3 | 25705.3 | 31914.9 | 32556.9 | 30615.1 | 32384.1 | 25321.9 | 26297.9 | 27565.1 | |
| 5 | O00258 | O00258 | GET1_HUMA | GET1 | Guided entry | 4101.55 | 5184.99 | 4891.59 | 5610.92 | 4383.75 | 4045.87 | 4969.71 | 4638.22 | 4514.36 | |
| 6 | O00264 | O00264 | PGRC1_HUM | PGRMC1 | Membrane-a | 343183 | 340696 | 326223 | 442980 | 467482 | 451763 | 438401 | 425337 | 441216 | |
| 7 | O00469 | O00469 | PLOD2_HUM | PLOD2 | Procollagen- | 50505.6 | 52641.1 | 51054.5 | 60625.4 | 60709.3 | 60564.4 | 56156.1 | 54538.3 | 56712.9 | |
| 8 | O14639 | O14639 | ABL1M1_HUN | ABLIM1 | Actin-binding | 4485.57 | 15124.2 | 14480.6 | 16178.3 | 19332.8 | 18881.9 | 15269.6 | 13856 | 15082.2 | |
| 9 | O14657 | O14657 | TOR1B_HUN | TOR1B | Torsin-1B | 19376.3 | 18249.9 | 17701.6 | 20595.4 | 23204.8 | 13031.6 | 18856.8 | 17889.5 | 19488.8 | |
| 10 | O15126 | O15126 | SCAM1_HUN | SCAMP1 | Secretory cai | 29889.3 | 24671.2 | 30634.5 | 37534.6 | 37673.6 | 42550.1 | 35807.3 | 35401.7 | 32844.4 | |
| 11 | O15304 | O15304 | SIVA_HUMA | SIVA1 | Apoptosis re | 10081.2 | 7646.25 | 6616.17 | 1996.03 | 5434.4 | 7202.26 | 7641.77 | 7319.09 | 4708.16 | |
| 12 | O15321 | O15321 | TM9S1_HUM | TM9SF1 | Transmembr | 20380.2 | 20410.9 | 18020.2 | 16311.2 | 15967 | 20695.8 | 19330.4 | 16006.9 | 16209.2 | |
| 13 | O43292 | O43292 | GPAA1_HUN | GPAA1 | Glycosylphos | 17202.3 | 23281.9 | 15531.9 | 15748.9 | 20459.9 | 17869.3 | 19818.7 | 17386.3 | 18820.7 | |
| 14 | O43447 | O43447 | PIIH_HUMAI | PIIH | Peptidyl-prol | 419765 | 468936 | 396211 | 450160 | 431498 | 413610 | 442408 | 406987 | 415182 | |
| 15 | O43567 | O43567 | RNF13_HUM | RNF13 | E3 ubiquitin- | 7219.94 | 5406.75 | 8014.85 | 8639.45 | 9969.08 | 5437.31 | 10265.9 | 8413.95 | 8364.91 | |
| 16 | O43663 | O43663 | PRC1_HUMA | PRC1 | Protein regul | 170001 | 189163 | 151910 | 246849 | 230180 | 207287 | 172238 | 155032 | 174000 | |
| 17 | O43929 | O43929 | ORC4_HUM | ORC4 | Origin recogni | 46247.6 | 50641.1 | 52583.5 | 65225.3 | 63211.1 | 62048 | 53586.3 | 52464.5 | 56440.2 | |
| 18 | O60826 | O60826 | CCD22_HUM | CCDC22 | Coiled-coil d | 70250.5 | 63085 | 70690.2 | 72434.8 | 66947.7 | 70907.2 | 69190.9 | 69623.2 | 66484.8 | |
| 19 | O75113 | O75113 | N4BP1_HUM | N4BP1 | NEDD4-bindin | 41987.7 | 41924.5 | 33715.9 | 41820.6 | 44669.1 | 42690 | 35225.5 | 37276.3 | 40062.3 | |
| 20 | O75121 | O75121 | MFA3L_HUM | MFAP3L | Microfibrillar | 6653.58 | 8527.04 | 9984.36 | 9157.86 | 10465 | 10372.1 | 6270.57 | 11724.9 | 9080.72 | |
| 21 | O75150 | O75150 | BRE1B_HUM | RNF40 | E3 ubiquitin- | 60382.3 | 65078.3 | 65609.7 | 79460.4 | 73603.8 | 74264.1 | 72510.9 | 69577.8 | 69485.5 | |
| 22 | O75417 | O75417 | DPOLQ_HUM | POLQ | DNA polyme | 13776.2 | 9876.68 | 13463.9 | 10645.6 | 14391.4 | 11181.3 | 13195.2 | 11602.1 | 10996.7 | |
| 23 | O75525 | O75525 | KHDR3_HUM | KHDRB53 | KH domain-c | 334471 | 322770 | 321972 | 367780 | 360167 | 356982 | 365355 | 364374 | 351086 | |
| 24 | O94788 | O94788 | AL1A2_HUM | ALDH1A2 | Retinal dehy | 99285 | 98671.8 | 97095.1 | 119611 | 115296 | 105823 | 108459 | 105469 | 93010.6 | |
| 25 | O94905 | O94905 | ERLN2_HUM | ERLIN2 | Erlin-2 | 47687 | 58778.7 | 54064.7 | 67397.6 | 66855 | 60591.2 | 57872.3 | 59537.2 | 53766.4 | |

Step 6: Enter the full file generated after DIA-NN search. This is used to compute the number of precursors identified per label type (specified in the annotation file in step 3). **Here also, set the column name as 'Protein' for the column to be used for unique identifiers (like step 5).**

Usually, this file is very large so you can remove extra columns to reduce size. Just make sure the file have the following columns – 'File.Name', 'Run', 'Protein', 'Protein.Ids', 'Protein.Names', 'Genes'.

As before hover over question mark for more information. [Click on the "Sample File" in blue to download this example file for this example \(screenshot of what the file looks like where Protein.Groups set to Protein\).](#)

4

Upload a tab separated Bruker+DIANN text file with ALL information.

For more information: ?

- This field is required

Choose File No file chosen

Reset

Sample file

This file is the output from DIA-NN search in the form of tab delimited ".tsv" file of all information NOT aggregated. Used for finding # Precursors etc per protein. Potential way to filter out noise.

NOTE: After running DIA-NN the output file that is the name of the run in ".tsv" format.

NOTE (2): IMPORTANT!! Set column name as "Protein" for the column to be used as unique protein ID's.

| | A | B | C | D | E | F |
|----|--|-------------|---------|-------------|---------------|-------|
| 1 | File.Name | Run | Protein | Protein.Ids | Protein.Names | Genes |
| 2 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2524.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 3 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2525.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 4 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2526.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 5 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2531.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 6 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2532.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 7 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2533.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 8 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2536.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 9 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2537.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 10 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2524.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 11 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2525.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 12 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2526.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 13 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2531.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 14 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2532.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 15 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2533.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 16 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2535.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 17 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2536.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 18 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2537.d | 20230509_Si | Q12118 | Q12118 | SGT2_YEAST | SGT2 |
| 19 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2524.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 20 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2525.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 21 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2526.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 22 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2531.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 23 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2532.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 24 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2533.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 25 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2535.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 26 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2536.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |
| 27 | D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2537.d | 20230509_Si | P26641 | P26641 | EF1G_HUMAN | EEF1G |

Step 7: Choose whether to do imputation on your data or not. Recommended to not penalize proteins that are not going to be identified/quantified in the Control. In certain situations, this is a given (for example, viral bait would never be endogenously expressed and hence might be completely missing in Control runs depending on type of control being compared to).

A successful start of run with look like this –

Run the Analysis

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

Loading...

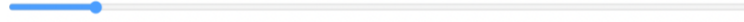
The output page (given a successful run) looks like the screenshot below. You can download the protein level differential expression analysis results as well as the pdf of resulting volcano plot (set at 5 PSM cutoff – **where # PSMs is set as the # of Precursors of the numerator condition. So, if comparison is Sample A vs Sample B then #PSMs is #Precursors for Sample A**). 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](#)

[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

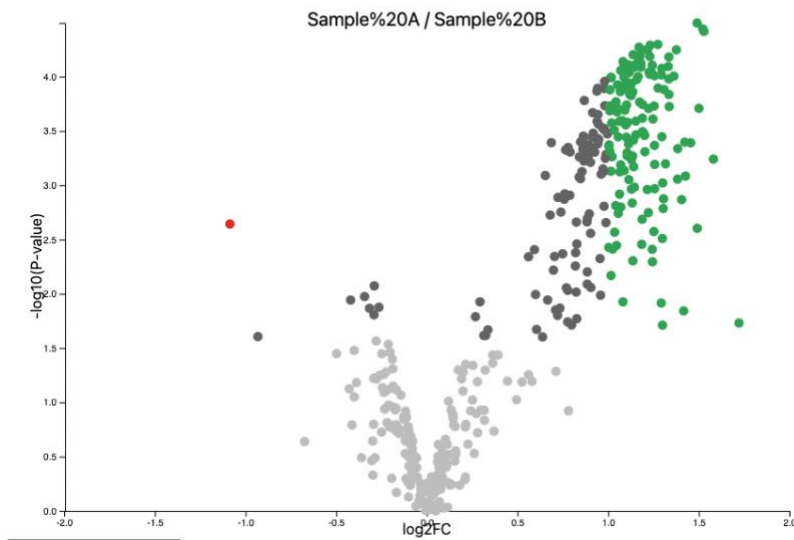
Size of scatter point: Drag the slider to display the current value.



Value: 5

Enter Uniprot IDs separated by @
Example: Q08945@Q9UQE7

Update Volcano Plot



The output file looks like this –

| | A | B | C | D | E | F | G | H | I | J |
|----|---------|------------|------------|------------|--------------|--------------|--------------|--------|------------|-------------|
| 1 | Protein | log2FC | pval | adjpval | #Precursors- | #Precursors- | #Precursors- | # PSMs | PSM Cutoff | Gene Symbol |
| 2 | P39966 | 1.10159864 | 0.00018238 | 0.00131265 | 37 | 29 | 38 | 37 | 5 | PTC2 |
| 3 | P00445 | 1.52273032 | 3.64E-05 | 0.00117586 | 47 | 45 | 44 | 47 | 5 | SOD1 |
| 4 | P25635 | 0.97145326 | 0.00070753 | 0.00233014 | 33 | 16 | 31 | 33 | 5 | PWP2 |
| 5 | P13186 | 0.82488338 | 0.00218761 | 0.00559936 | 23 | 7 | 18 | 23 | 5 | KIN2 |
| 6 | P53110 | 1.05599636 | 0.00181425 | 0.00481849 | 8 | 1 | 4 | 8 | 5 | YGL159W |
| 7 | P13861 | 0.03717888 | 0.59514466 | 0.65479168 | 77 | 81 | 81 | 77 | 5 | PRKAR2A |
| 8 | Q12502 | 1.13465163 | 0.00053816 | 0.00198398 | 14 | 6 | 13 | 14 | 5 | LDB19 |
| 9 | P36104 | 0.77748475 | 0.00929662 | 0.02005472 | 17 | 3 | 16 | 17 | 5 | SWD2 |
| 10 | Q9Y5X4 | -1.1916566 | 0.00150702 | 0.00415904 | 3 | 3 | 3 | 3 | 5 | NR2E3 |
| 11 | Q9NYL9 | 0.14576137 | 0.14016324 | 0.20855615 | 47 | 59 | 52 | 47 | 5 | TMOD3 |
| 12 | Q9NQL2 | -0.6744407 | 0.22962786 | 0.31163781 | 8 | 12 | 12 | 8 | 5 | RRAGD |
| 13 | P28791 | 0.81897296 | 0.0055468 | 0.01239873 | 6 | 0 | 4 | 6 | 5 | SEC20 |
| 14 | P40357 | 0.84109681 | 0.00054833 | 0.00198547 | 33 | 17 | 34 | 33 | 5 | SEC9 |
| 15 | Q9Y597 | -0.1153102 | 0.50408832 | 0.58592854 | 12 | 18 | 12 | 12 | 5 | KCTD3 |
| 16 | Q96MY7 | 0.26524255 | 0.0217614 | 0.04103104 | 3 | 3 | 3 | 3 | 5 | FAM161B |
| 17 | P37838 | 1.20147305 | 0.00034735 | 0.00170026 | 36 | 23 | 36 | 36 | 5 | NOP4 |
| 18 | Q2V2M9 | -0.2856924 | 0.32382714 | 0.41123549 | 5 | 6 | 4 | 5 | 5 | FHOD3 |
| 19 | Q96KQ4 | -0.2925342 | 0.06000278 | 0.10221164 | 19 | 21 | 20 | 19 | 5 | PPP1R13B |
| 20 | P03872 | 1.08172197 | 0.01186634 | 0.02452709 | 7 | 2 | 7 | 7 | 5 | REP2 |
| 21 | Q9BXW7 | -0.0346588 | 0.56008565 | 0.63025584 | 77 | 87 | 91 | 77 | 5 | HDHD5 |
| 22 | Q8TF01 | 0.13667344 | 0.57201054 | 0.64075557 | 9 | 12 | 12 | 9 | 5 | PNISR |
| 23 | P40040 | 1.13585458 | 0.00013806 | 0.00117586 | 15 | 13 | 15 | 15 | 5 | THO1 |
| 24 | P19880 | 1.37645951 | 5.67E-05 | 0.00117586 | 25 | 14 | 22 | 25 | 5 | YAP1 |
| 25 | P35180 | 1.12828748 | 0.0005825 | 0.00203529 | 17 | 10 | 18 | 17 | 5 | TOM20 |
| 26 | P00100 | 1.13853731 | 0.00108764 | 0.00211733 | 51 | 30 | 46 | 51 | 5 | PPA4 |