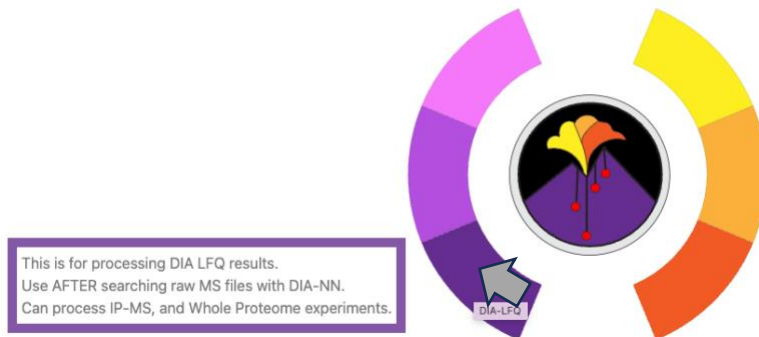


DIA-LFQ (Precursor level)

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 Precursor-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 precursor 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	P	Q	R	S
1	Protein	Protein.Ids	Protein.Nam	Genes	First.Protein	Proteotypic	Stripped.Seq	Modified.Seq	Precursor.Ch	Precursor.Id	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2524.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2525.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleA_500ng_dia-PASEF_30min_Slot1-2_1_2526.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2531.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2532.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleB_500ng_dia-PASEF_30min_Slot1-3_1_2533.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2535.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2536.d	D:\Data\2023_May_Cornell_YU\Data_Cornell_HT\500_ng\20230509_SampleC_500ng_dia-PASEF_30min_Slot1-4_1_2537.d
2	Q12118	Q12118	SGT2_YEAST	SGT2	Small glutan	1	AAHSSLSKEY	AAHSSLSKEY	2	AAHSSLSKEY	4118.52	3632.14	6831.14	1987.36	2652.5	4124.63	791.477	430.808	
3	Q12118	Q12118	SGT2_YEAST	SGT2	Small glutan	1	AAHSSLSKEY	AAHSSLSKEY	3	AAHSSLSKEY	25385.4	23775.1	27311.9	14640.8	14555.8	15606.3	4497.9	3590.88	4104.11
4	P26641	P26641	EF1G_HUMA	EEF1G	Elongation fa	1	AAAPAPEEEF	AAAPAPEEEF	3	AAAPAPEEEF	61254.8	58418.5	57521.4	31769.7	29493.5	20863.5	45257.9	47508.3	40846.8
5	P26641	P26641	EF1G_HUMA	EEF1G	Elongation fa	1	AAAPAPEEEF	AAAPAPEEEF	3	AAAPAPEEEF	40099.3	38782.8	36340.7	21958	16672.7		31053.9	31355.1	28188.7
6	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	140.586	1274.24		1730.57	2440.82	1289.6	522.119	480.756	
7	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	1878.96	1420.91	831.661	1727.11	5240.02	1621.68	3473.97	963.296	2353.84
8	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	1072.74	1766.49	809.352	2245.31	1209.04	1314.57	903.286	723.938	1322.2
9	P32263	P32263	P5CR_YEAST	PRO3	Pyroline-5-c	1	AAETAFAAF	AAETAFAAF	2	AAETAFAAF	172899	262555	251928	120590	121845	122748	40707	36592.1	37657.9
10	P32843	P32843	YME2_YEAST	YME2	Mitochondria	1	AAFTYLINDP	AAFTYLINDP	2	AAFTYLINDP	27741.3	27814.2	27207.4		11269.7	12848.9		6191.31	
11	Q12802	Q12802	AKP13_HUM	AKAP13	A-kinase anc	1	AAGALLTEGI	AAGALLTEGI	3	AAGALLTEGEAC	(UniMod-4)	HMSLSPELPLTK3		1425.04	995.146	1544.13	1451.19	1526.91	
12	Q86UP2	Q86UP2	KTN1_HUMA	KTN1	Kinectin	1	AAGDTTVIEN	AAGDTTVIEN	2	AAGDTTVIEN	598.69	616.493	291.581	1543.25	1421.9	1083.61	757.12		
13	Q86UP2	Q86UP2	KTN1_HUMA	KTN1	Kinectin	1	AAGDTTVIEN	AAGDTTVIEN	2	AAGDTTVIENS	OVSPETSESKE2			1260.41	1464.05	480.705	1330.64	1546.42	
14	P00560	P00560	PGK_YEAST	PGK1	Phosphoglyc	1	AAGFLEEK	AAGFLEEK	2	AAGFLEEK	4680000	5250000	5210000	2790000	2680000	2770000	1200000	1160000	1150000
15	P25367	P25367	RNQ1_YEAS	RNQ1	[PIN+] prion	1	AAGGSSSFA	AAGGSSSFA	2	AAGGSSSFA	94090.5	101345	102524	25657.6	35108.1	37521.1	11477.9	11095.2	13728.2
16	P14907	P14907	NSP1_YEAS	NSP1	Nucleoporin	1	AAISFGAKPE	AAISFGAKPE	6	AAISFGAKPE	64364.1	57439.6	50843.2	47193.6	44591.5	45465.6	9779.06	9707.16	10626.1
17	P14907	P14907	NSP1_YEAS	NSP1	Nucleoporin	1	AAISFGAKPE	AAISFGAKPE	3	AAISFGAKPE	52668.9	39880.8	44429.4	42132.4	41837.3	36861.1	30650.2	30293.2	29447
18	O43567	O43567	RNF13_HUM	RNF13	E3 ubiquitin-	1	AAIVHNVDSI	AAIVHNVDSI	4	AAIVHNVDSI	4870.14	4173.55	6358.48	7134.3	7440.29	4282.56	10013.7	7048.88	6290.13
19	O43567	O43567	RNF13_HUM	RNF13	E3 ubiquitin-	1	AAIVHNVDSI	AAIVHNVDSI	3	AAIVHNVDSI	8665.31	5538.55		8174.12	10624.7	5439.48	7984.25	7827.64	8835.46
20	P24941	P24941	CDK2_HUMA	CDK2	Cyclin-depen	1	AALAHFFPQI	AALAHFFPQI	2	AALAHFFPQD	VTKPVPHLR2			0				223.463	
21	P24941	P24941	CDK2_HUMA	CDK2	Cyclin-depen	1	AALAHFFPQI	AALAHFFPQI	3	AALAHFFPQI	4711.45	4731.23	7157.64	27229.3	28058.8	31845.6	12664.6	15826.5	16076.7
22	P24941	P24941	CDK2_HUMA	CDK2	Cyclin-depen	1	AALAHFFPQI	AALAHFFPQI	4	AALAHFFPQI	11087.6	14199.8	11122.8	20675.4	9654.48	17851.6	24926.5	19641.6	20203
23	P32366	P32366	VAOD_YEAS	VMA6	V-type proto	1	AALANVVEYI	AALANVVEYI	2	AALANVVEYR2				38276.6	25925.6	13529.6			
24	Q12929	Q12929	EPS8_HUMA	EPS8	Epidermal gr	1	AALDSSGSS	AALDSSGSS	2	AALDSSGSS	6192.74	2296.98	8085.11	6066.26	5160.03	8153.7	7710.75	6444.89	4114.16
25	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	2	AALLELWELR	9823.82	8137.45	7059.11	16652	11641.7	11206.8	4844.32	5813.77	4793.56
26	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	2	AALLELWELRR2				6623.41	4531.36	3189.69			
27	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	3	AALLELWELR	19708.7	19926.8	11471.5	48999.3	35518	31286.8	3783.79	3046.12	3228.35

(the pr_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 (precursor level aggregated) for quantification.

For DIA-NN search, upload a tab separated file of quantification file (aggregated to precursor level). The file usually has “pr_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 precursor level aggregated file.](#)

3

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

NOTE: After running DIA-NN the output file should probably have "pr_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	P	Q	R	S
1	Protein	Protein.Ids	Protein.Nam	Genes	First.Protein	Proteotypic	Stripped.Seq	Modified.Set	Precursor.Ch	Precursor.Id	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\202
2	Q12118	Q12118	SGT2_YEAST	SGT2	Small glutan	1	AAHSLSKEY	AAHSLSKEY	2	AAHSLSKEY	4118.52	3632.14	6831.14	1987.36	2652.5	4124.63		791.477	430.808
3	Q12118	Q12118	SGT2_YEAST	SGT2	Small glutan	1	AAHSLSKEY	AAHSLSKEY	3	AAHSLSKEY	25385.4	23775.1	27311.9	14640.8	14555.8	15606.3	4497.9	3590.88	4104.11
4	P26641	P26641	EF1G_HUM	EEF1G	Elongation fa	1	AAAPAEPEE	AAAPAEPEE	3	AAAPAEPEE	61254.8	58418.5	57521.4	31769.7	29493.5	20863.5	45257.9	47508.3	40846.8
5	P26641	P26641	EF1G_HUM	EEF1G	Elongation fa	1	AAAPAEPEE	AAAPAEPEE	3	AAAPAEPEE	40099.3	38782.8	36340.7	21958	16672.7		31053.9	31355.1	28188.7
6	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	140.586	1274.24		1730.57	2440.82	1289.6	522.119	480.756	
7	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	1878.96	1420.91	831.661	1727.11	5240.02	1621.68	3473.97	963.296	2353.84
8	Q13586	Q13586	STIM1_HUM	STIM1	Stromal inte	1	AADALNAM	AADALNAM	2	AADALNAM	1072.74	1766.49	809.352	2245.31	1209.04	1314.57	903.286	723.938	1322.2
9	P32263	P32263	PSCR_YEAST	PRO3	Proline-5-c	1	AAETAFAA	AAETAFAA	2	AAETAFAA	172899	262555	251928	120590	121845	122748	40707	36592.1	37657.9
10	P32843	P32843	YME2_YEAST	YME2	Mitochondri	1	AAFTYLNDP	AAFTYLNDP	2	AAFTYLNDP	27741.3	27814.2	27207.4		11269.7	12848.9		6191.31	
11	Q12802	Q12802	AKP13_HUM	AKAP13	A-kinase anc	1	AAGALLTEG	AAGALLTEG	3	AAGALLTEG	(UniMod:4)	HMSLSSPELPLTK3			1425.04	995.146	1544.13	1451.19	1526.91
12	Q86UP2	Q86UP2	KTN1_HUM	KTN1	Kinectin	1	AAGDVTVIEN	AAGDVTVIEN	2	AAGDVTVIEN	598.69	616.493	291.581	1543.25	1421.9	1083.61	757.12		
13	Q86UP2	Q86UP2	KTN1_HUM	KTN1	Kinectin	1	AAGDVTVIEN	AAGDVTVIEN	2	AAGDVTVIEN	598.69	616.493	291.581	1543.25	1421.9	1083.61	757.12		
14	P00560	P00560	PGK_YEAST	PGK1	Phosphoglyc	1	AAGFLEK	AAGFLEK	2	AAGFLEK	4680000	5250000	5210000	2790000	2680000	2770000	1200000	1160000	1150000
15	P25367	P25367	RNQL_YEAST	RNQL	[PIN+] prion	1	AAGGSSFA	AAGGSSFA	2	AAGGSSFA	94090.5	101345	102524	25657.6	35108.1	37521.1	11477.9	11095.2	13728.2
16	P14907	P14907	NSP1_YEAST	NSP1	Nucleoporin	1	AAISFGAKPE	AAISFGAKPE	2	AAISFGAKPE	64364.1	57439.6	50843.2	47193.6	44591.5	45465.6	9779.06	9707.16	10626.1
17	P14907	P14907	NSP1_YEAST	NSP1	Nucleoporin	1	AAISFGAKPE	AAISFGAKPE	3	AAISFGAKPE	52668.9	39880.8	44429.4	42132.4	41837.3	36861.1	30650.2	30293.2	29447
18	O43567	O43567	RNF13_HUM	RNF13	E3 ubiquitin-	1	AAIVHNVDI	AAIVHNVDI	3	AAIVHNVDI	4870.14	4173.55	6358.48	7134.3	7440.29	4282.56	10013.7	7048.88	6290.13
19	O43567	O43567	RNF13_HUM	RNF13	E3 ubiquitin-	1	AAIVHNVDI	AAIVHNVDI	3	AAIVHNVDI	8665.31	5538.55		8174.12	10624.7	5439.48	7984.25	7827.64	8835.46
20	P24941	P24941	CDK2_HUM	CDK2	Cyclin-depen	1	AALAHFFFQI	AALAHFFFQI	2	AALAHFFFQI					0			223.463	
21	P24941	P24941	CDK2_HUM	CDK2	Cyclin-depen	1	AALAHFFFQI	AALAHFFFQI	3	AALAHFFFQI	4711.45	4731.23	7157.64	27229.3	28058.8	31845.6	12664.6	15826.5	16076.7
22	P24941	P24941	CDK2_HUM	CDK2	Cyclin-depen	1	AALAHFFFQI	AALAHFFFQI	4	AALAHFFFQI	11087.6	14199.8	11122.8	20675.4	9654.48	17851.6	24926.5	19641.6	20203
23	P32366	P32366	VADL_YEAST	VMA6	V-type proto	1	AALANVVEYI	AALANVVEYI	2	AALANVVEYI				38276.6	25925.6	13529.6			
24	Q12929	Q12929	EP5B_HUM	EP5B	Epidermal gr	1	AALDSSGSS	AALDSSGSS	2	AALDSSGSS	6192.74	2296.98	8085.11	6066.26	5160.03	8153.7	7710.75	6444.89	4114.16
25	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	2	AALLELWELR	9823.82	8137.45	7059.11	16652	11641.7	11206.8	4844.32	5813.77	4793.56
26	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	2	AALLELWELR				6623.41	4531.36	3189.69			
27	Q13813	Q13813	SPTN1_HUM	SPTAN1	Spectrin alp	1	AALLELWELR	AALLELWELR	3	AALLELWELR	19708.7	19926.8	11471.5	48999.3	35518	31286.8	3783.79	3046.12	3228.35

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

[Sample file](#)

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...

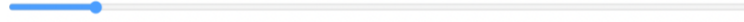
The output page (given a successful run) looks like the screenshot below. You can download the protein level differential expression analysis results as well as the pdf of resulting volcano plot (set at 5 PSM cutoff – **where # PSMs is set as the # of Precursors of the numerator condition. So, if comparison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



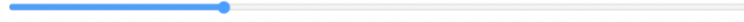
Value: 0.05

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



Value: 5

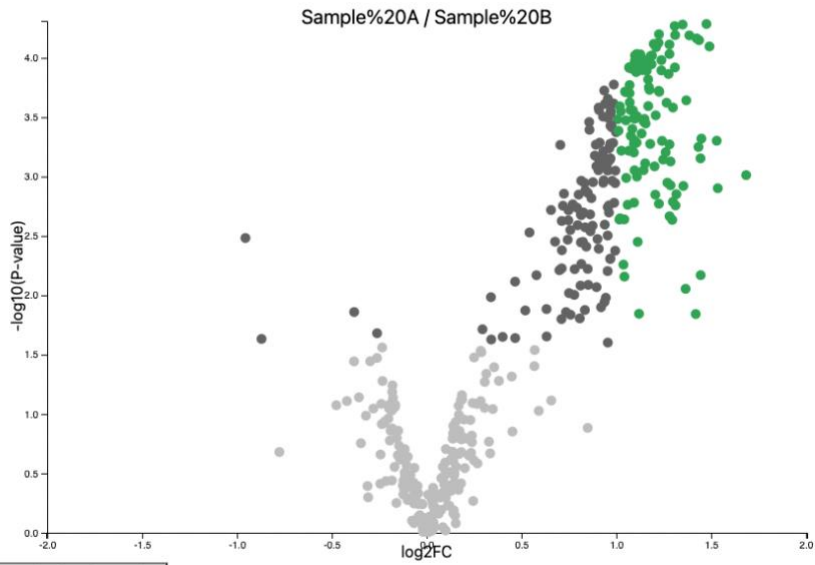
Size of scatter point: Drag the slider to display the current value.



Value: 5

Enter Uniprot IDs separated by @
Example: Q08945@Q9UQE7

Update Volcano Plot



Download 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	1.10838495	9.26E-05	0.0017081	37	29	38	37	5	PTC2
3	P00445	1.4753897	5.18E-05	0.0017081	47	45	44	47	5	SOD1
4	P25635	0.71286771	0.00417077	0.00995344	33	16	31	33	5	PWP2
5	P13186	0.79626951	0.00256663	0.00674423	23	7	18	23	5	KIN2
6	P53110	0.93767899	0.01137212	0.02390565	8	1	4	8	5	YGL159W
7	P13861	0.0874769	0.34870746	0.45094629	77	81	81	77	5	PRKAR2A
8	Q12502	1.09255841	0.00062612	0.00273721	14	6	13	14	5	LDB19
9	P36104	0.851709	0.00816012	0.01807667	17	3	16	17	5	SWD2
10	Q9Y5X4	-1.1908191	0.0011056	0.00381347	3	3	3	3	5	NR2E3
11	Q9NYL9	0.25020694	0.03340643	0.06251052	47	59	52	47	5	TMOD3
12	Q9NQL2	-0.7764938	0.20896162	0.30127775	8	12	12	8	5	RRAGD
13	P28791	0.83571136	0.00267473	0.00691788	6	0	4	6	5	SEC20
14	P40357	0.81309763	0.0020769	0.00580292	33	17	34	33	5	SEC9
15	Q9Y597	-0.1003788	0.51821962	0.59770831	12	18	12	12	5	KCTD3
16	Q96MY7	0.28483468	0.02483587	0.04755395	3	3	3	3	5	FAM161B
17	P37838	1.17485314	0.00018567	0.001917	36	23	36	36	5	NOP4
18	Q2V2M9	-0.1007145	0.53065307	0.60681162	5	6	4	5	5	FHOD3
19	Q96KQ4	-0.3829571	0.03603618	0.06642491	19	21	20	19	5	PPP1R13B
20	P03872	1.11983113	0.01429704	0.02871031	7	2	7	7	5	REP2
21	Q9BXW7	-0.0062404	0.75261638	0.79783797	77	87	91	77	5	HDHD5
22	Q8TF01	0.12850717	0.44433427	0.53799297	9	12	12	9	5	PNISR
23	P40040	1.16045282	0.00012793	0.0017081	15	13	15	15	5	THO1
24	P19880	1.36893148	0.00022766	0.00207905	25	14	22	25	5	YAP1

Sample A-vs-Sample B_FC_and_pva