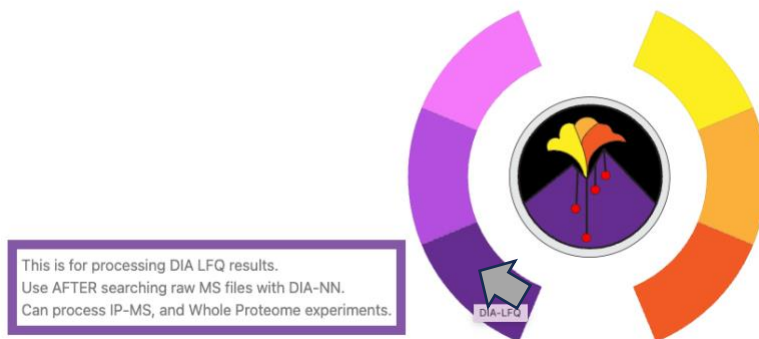


## DIA-LFQ (IP-MS)

**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 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.

### Analysis Type

For more information: ?

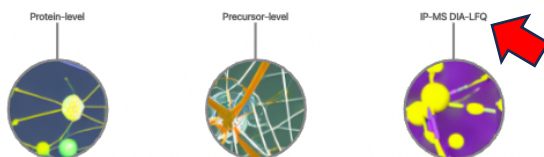
Magma (Linear Model)

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 Crosslinking (XL)-IP-MS DIA-LFQ experiment. Here each Channel is associated with a separate machine run. So, in the example below, MLH1\_Flag\_IP was run thrice and thus has three separate files generated which correspond to MLH1\_Flag\_IP\_OnBeadDigestion\_OnlyTrypsin\_RegularIPmethod\_DIA\_50min\_1pct.raw, MLH1\_Flag\_IP\_OnBeadDigestion\_OnlyTrypsin\_RegularIPmethod\_DIA\_50min\_1pct\_20230722164251.raw and MLH1\_Flag\_IP\_OnBeadDigestion\_OnlyTrypsin\_RegularIPmethod\_DIA\_50min\_1pct\_2023072224609.raw (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).** Control column here lets the underlying tool know whether the label is a control in your experimental setup or not.

	A	B	C
1	Channel	Label	Control
2	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct.raw	EGFP_Flag_IP	TRUE
3	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722142047.raw	EGFP_Flag_IP	TRUE
4	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722202358.raw	EGFP_Flag_IP	TRUE
5	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct.raw	EGFP_Flag_XLIP	TRUE
6	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722153152.raw	EGFP_Flag_XLIP	TRUE
7	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\EGFP_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722213505.raw	EGFP_Flag_XLIP	TRUE
8	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct.raw	MLH1_Flag_IP	FALSE
9	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722164251.raw	MLH1_Flag_IP	FALSE
10	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_2023072224609.raw	MLH1_Flag_IP	FALSE
11	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct.raw	MLH1_Flag_XLIP	FALSE
12	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722175352.raw	MLH1_Flag_XLIP	FALSE
13	E:\Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_XL-IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722235712.raw	MLH1_Flag_XLIP	FALSE

	A	B	C	D	E	F	G	H	I	J	K
1	Protein	Protein.Ids	Protein.Nam	Genes	First	Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct.raw	Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_20230722164251.raw	Raw_files\Yugandhar\MLH1_FLAG_DIA\MLH1_Flag_IP_OnBeadDigestion_OnlyTrypsin_RegularIPmethod_DIA_50min_1pct_2023072224609.raw			
2	ADA075B632	ADA075B632	KV229_HUM	IGKV2-29;IG	Immunoglob	1.40E+09	1.40E+09	1.55E+09	2.90E+09	2.60E+09	2.52E+09
3	ADA0C4D831	P23083	AA04	HV102_HUM	IGHV1-18;IG	215263	168652	159392	449150	580393	412736
4	A0FGR8-2	A0FGR8	A0F	ESY22_HUM	ESY22	1.62E+06	1.70E+06	1.71E+06	1.17E+06	1.29E+06	1.23E+06
5	A11L0T0	A11L0T0	ILVBL_HUM	ILVBL	Acetolactate	1.98E+06	2.07E+06	1.77E+06	1.09E+06	996161	1.11E+06
6	A11390	A11390	A113	PKHG3_HUM	PLEKHA7	2.78E+06	2.58E+06	2.64E+06	4.16E+06	4.04E+06	4.34E+06
7	A3KMH1	A3KMH1	A3K	VW48_HUM	VW48	148498	501907	228581	269880		409222
8	ASVXK5	ASVXK5	ASVX	CNOT1_HUM	CNOT1	836918	799941	815331	781413	833139	742079
9	A6NDG6	A6NDG6	PGP_HUM	PGP	Glycerol-3-ph	102522	257744	241364			
10	A6NHG4	P30	A6NHG4	P30	DOTL_HUM	DOTL	218858	234545		372187	
11	A6NHL2	A6NHL2	A6N	TBAL3_HUM	TBAL3	866238	944739	425061	365564	337135	375952
12	A6NHQ2	A6NHQ2	FBLL1_HUM	FBLL1	rRNA;tRNA;	6.44E+06	6.40E+06	7.62E+06	8.83E+06	8.37E+06	9.37E+06
13	A6NHR9	A6NHR9	A6N	SMHD1_HUM	SMCHD1		486888	528488	74614.9		
14	A6NKD2	P0C	P0C	TSYP1_TSPY1	Testis-specif	1.00E+06	1.49E+06	1.26E+06	1.10E+06	1.12E+06	
15	A6NKT7	J272	A6NKT7	J272	RGD33_HUM	RGD33	974959	957037	1.06E+06	617075	648722
16	AGZK13	C981	AGZK13	C981	RTL8A_HUM	RTL8A	709826	638522	493905	268735	260628
17	A8MV81	G91	A8MV81	G91	HIG1A_HUM	HIG1A	957636	873923	852198	547475	653799
18	A8MW9	P6	A8MW9	P6	RUXGL_HUM	SNRPB	363006	538340	530308	407288	619934
19	A9QM74	A9QM74	IMAB_HUM	KPNA7	Importin sub	1.84E+07	1.73E+07	1.92E+07	1.50E+06	1.64E+06	2.01E+06
20	B1ANS9	B1A	B1ANS9	B1A	WDR64_HUM	WDR64	1.84E+06	1.91E+06	1.94E+06	1.47E+06	1.88E+06
21	B2RKP0	P09	B2RKP0	P265	HGB1A_HUM	HMG13	229426	347944	349780	290033	409420
22	B5ME13	C99	B5ME13	C99	EIF3C_HUM	EIF3C	591627	588352	633462	976885	963948
23	E9PAV3	E9PV	Q13765	E9PV	NACAM_HUM	NACA	1.13E+06	1.28E+06	1.20E+06	3.24E+06	3.48E+06
24	E9PRG8	E9PRG8	CKO98_HUM	C11orf98	Uncharacteri	247512	331763	181969		387851	395922
25	O00116	O00116	ADAS_HUM	AGPS	Alkylidihydro	506470	409273	427843	364679	423011	451671
26	O00139	O00139	O00139	O00139	KIF2A_HUM	KIF2A	412936	534012	355273	341888	444609
27	O00148	O00148	O00148	O00148	DX39A_HUM	DX39A	1.93E+06	1.92E+06	1.93E+06	1.36E+06	1.73E+06
28	O00151	O00151	PDCL1_HUM	PDCL1	PDZ and LIM	4.28E+06	4.57E+06	4.49E+06	9.79E+06	1.06E+07	1.02E+07
29	O00154	O00154	BACH_HUM	ACOT7		188641		158165	355593	193799	388022
30	O00159	O00159	O00159	O00159	MYO1C_HUM	MYO1C	8.55E+06	8.15E+06	8.40E+06	8.94E+06	8.81E+06
31	O00161	O00161	SNP23_HUM	SNAP23	Synaptosomi	2.09E+06	1.89E+06	2.26E+06	2.26E+06	2.38E+06	2.22E+06
32	O00165	O00165	O00165	O00165	HAX1_HUM	HAX1	3.80E+06	3.98E+06	3.60E+06	2.90E+06	3.06E+06
33	O00170	O00170	AIP_HUMAN	AIP	AH receptor-	127367		99057.5	303901	169820	
34	O00178	O00178	GTPB1_HUM	GTPBP1	GTP-binding protein 1						
35	O00203	O00203	O00203	O00203	AP3B1_HUM	AP3B1	108135	152141	103066	293063	417799
36	O00217	O00217	NDUS8_HUM	NDUS8	NADH dehyd	1.04E+06	933267	1.01E+06		837333	799983
37	O00231	O00231	O00231	O00231	PSMD11_HUM	PSMD11	950796	694329	763192	816313	793852

(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, Label and Control. Channel are the column header(s) in the DIANN output specifying quantification per row. Also need to specify which channels should be considered as "Controls". 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 "-" character.

**Step 4:** Choose the conditions you want to compare in a pairwise fashion. And choose the direction of comparison. So, choosing comparison ('MLH1\_Flag\_IP', 'EGFP\_Flag\_IP') and direction (Condition\_1/Condition\_2) means the following comparison will run – (MLH1\_Flag\_IP/EGFP\_Flag\_IP).

2 **Select the Comparison You Would Like to Perform**

Choose a comparison with control

('MLH1\_Flag\_IP', 'EGFP\_Flag\_IP')

('MLH1\_Flag\_IP', 'EGFP\_Flag\_XLIP')

('MLH1\_Flag\_XLIP', 'EGFP\_Flag\_IP')

('MLH1\_Flag\_XLIP', 'EGFP\_Flag\_XLIP')

OR Choose a comparison NOT with control

('MLH1\_Flag\_IP', 'MLH1\_Flag\_XLIP')

Choose the direction for comparison:

(Condition\_1/Condition\_2)

(Condition\_2/Condition\_1)

**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 real XL-IP-DIA-LFQ experiment and looks like the screenshot below.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	
1	Protein	Protein.Ids	Protein.Nam	Genes	First.Protein.E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	Raw_files E	
2	A0A075B652	A0A075B652	KV229_HUM	IKV2V_29	IGH	Immunoglob	2.90E+09	2.60E+09	2.52E+09	2.68E+09	2.82E+09	2.86E+09	1.40E+09	1.40E+09	1.55E+09	1.99E+09	1.91E+09	1.79E+09
3	A0A0C4D31	P23083	A0A4 HV102_HUM	IGHV1-18	IGH	Immunoglob	449150	580393	412736	778222	650374	862940	215263	168652	159392	424059	373572	424897
4	A0FGR8-2	A0FGR8	ADAF ESYT2_HUM	ESYT2	IGH	Immunoglob	1.17E+06	1.29E+06	1.23E+06	2.14E+06	1.98E+06	2.09E+06	1.62E+06	1.70E+06	1.71E+06	2.00E+06	2.06E+06	2.12E+06
5	A1L0T0	A1L0T0	ILVB1_HUM	ILVB1	IGH	Immunoglob	1.09E+06	996161	1.11E+06	1.31E+06	1.12E+06	1.32E+06	1.98E+06	2.07E+06	1.77E+06	1.25E+06	1.30E+06	1.45E+06
6	A1L390	A1L390	A1L3 PKH63_HUM	PLEKH63	IGH	Immunoglob	4.16E+06	4.04E+06	4.34E+06	401131	391300	148498	501907	228581	410644	333703	411825	
7	A3MHH1A39	A3MHH1A39	VWVAB_HUM	VWVAB	IGH	Immunoglob	269880		409222	440298	138E+06	1.27E+06	1.16E+06	836918	799941	815231	1.56E+06	1.53E+06
8	AS7K6AS7A	AS7K6AS7A	ASHY CNO11_HUM	CNO11	IGH	Immunoglob	781413	833139	742079	1.06E+06	883094	883600	102522	257744	241364	906553	886404	887211
9	AMNDG6	AMNDG6	PGP_HUM	PGP	IGH	Immunoglob												
10	AMNHG4-P30	AMNHG4-P30	DDTL_HUM	DDTL	IGH	Immunoglob	372187			1.90E+06	1.93E+06	1.59E+06	218858	234545		1.44E+06	1.35E+06	1.42E+06
11	AMNHL2	AMNHL2	AMNHL2A3_HUM	TUBAL3	IGH	Immunoglob	365564	337135	375952	754585	762735	955978	866238	944739	425061	681160	1.74E+06	1.53E+06
12	AMNHQ2	AMNHQ2	FBLL1_HUM	FBLL1	IGH	Immunoglob	8.83E+06	8.37E+06	9.37E+06	9.07E+06	8.40E+06	9.92E+06	6.44E+06	6.40E+06	7.62E+06	8.25E+06	7.10E+06	1.12E+07
13	AMNHR9	AMNHR9	AMNHR9A6	SMMD1_HUM	SMMD1	IGH	Immunoglob	74614.9		363720	478671	431217		486888	528488	328413	379881	155739
14	AMND2-POC	POCV98	QD11 TSPY1_HUM	TSPY1	IGH	Immunoglob	1.10E+06	1.12E+06		889802	999624	902117	1.00E+06	1.49E+06	1.26E+06	980294	1.06E+06	1.05E+06
15	AMNKT7-Q7Z	AMNKT7-Q7Z	RGPD3_HUM	RGPD3	IGH	Immunoglob	617075	648722	455533	432809	468925	974959	957037	1.06E+06	655554	774521	639240	
16	ASZK13	ASZK13	Q9V1 RTL8A_HUM	RTL8A	IGH	Immunoglob	268735	250628	201644	340574	336303	267203	709816	638522	493905	485821	361225	379762
17	AMV81-Q91	AMV81-Q91	HG1A_HUM	HG1A	IGH	Immunoglob	547475	653799	618510	411819	431490	318022	957636	879233	852198	352153	360802	534953
18	AMW09-P6	AMW09-P6	RUXGL_HUM	RNRPG	IGH	Immunoglob	407288	619934	336882	882101	1.07E+06	397583	363006	538340	530308	1.03E+06	917596	292315
19	A9QM74	A9QM74	IMAB_HUM	PKNA7	IGH	Immunoglob	1.50E+06	1.64E+06	2.01E+06	2.34E+06	2.84E+06	2.02E+06	1.84E+07	1.73E+07	1.92E+07	1.88E+07	2.28E+07	1.78E+07
20	B1ANS9-B1A	B1ANS9-B1A	WDR64_HUM	WDR64	IGH	Immunoglob	1.47E+06	1.88E+06	1.85E+06	7.39E+06	7.80E+06	8.23E+06	1.84E+06	1.91E+06	1.94E+06	8.86E+06	8.10E+06	8.91E+06
21	B2RKP0-PO9	B2RKP0-P261	HGB1A_HUM	HMG1A	IGH	Immunoglob	290033	409420	446437	1.37E+06	1.14E+06	1.21E+06	229426	347944	349780	1.72E+06	1.35E+06	1.02E+06
22	BSME19-Q96	Q99613-Q99	E1F3C_HUM	E1F3C	IGH	Immunoglob	976885	963848	879819	1.75E+06	1.91E+06	1.90E+06	591627	588352	633462	2.01E+06	2.05E+06	2.09E+06
23	EPAV3-E99	Q13765-E99	NACAM_HUM	NACA	IGH	Immunoglob	3.24E+06	3.48E+06	3.29E+06	2.47E+06	2.23E+06	2.28E+06	1.13E+06	1.28E+06	1.20E+06	2.01E+06	2.01E+06	2.08E+06
24	E9RIG8	E9RIG8	CK98_HUM	C11orf98	IGH	Immunoglob	387851	395922	2.07E+06	2.56E+06	2.44E+06	2.47E+06	247512	331763	181969	1.41E+06	1.40E+06	1.75E+06
25	O00116	O00116	ADAS_HUM	AGPS	IGH	Immunoglob	364679	423011	451671	638223	731907	616340	506470	409273	427843	658818	461308	644474
26	O00139-O00	O00139-O00	KIF2A_HUM	KIF2A	IGH	Immunoglob	355273	341888	444609	403073	353022	405481	412936	534012		464241	940451	517530
27	O00148	O00148	O0X39A_HUM	DX39A	IGH	Immunoglob	1.36E+06	1.73E+06	1.78E+06	5.49E+06	5.56E+06	5.15E+06	1.93E+06	1.92E+06	1.93E+06	4.54E+06	4.59E+06	4.53E+06
28	O00151	O00151	PD11_HUM	PD1M1	IGH	Immunoglob	9.79E+06	1.06E+07	1.02E+07	796953	672377	810698	4.28E+06	4.57E+06	4.49E+06	888360	777331	669602
29	O00154-O00	O00154-O00	BACH2_HUM	ACOT7	IGH	Immunoglob	355593	193799	388022	940390	1.06E+06	952322	188641		158165	453036	955565	834410
30	O00159-O00	O00159-O00	MYO1C_HUM	MYO1C	IGH	Immunoglob	8.94E+06	8.81E+06	8.26E+06	658439	517769		8.59E+06	8.15E+06	8.40E+06	645515	506079	487860
31	O00161	O00161	SNP23_HUM	SNAP23	IGH	Immunoglob	2.26E+06	2.38E+06	2.22E+06	152755	207643	2.09E+06	1.89E+06	2.26E+06	2.16828	203045	197793	
32	O00165-O00	O00165-O00	HAK1_HUM	HAK1	IGH	Immunoglob	2.90E+06	3.06E+06	3.03E+06	3.41E+06	3.61E+06	3.61E+06	3.80E+06	3.98E+06	3.60E+06	3.96E+06	3.62E+06	3.76E+06
33	O00170	O00170	AIP_HUMAN	AIP	IGH	Immunoglob	303901	169820		288790	276414		127367		99057.5	193370		
34	O00178	O00178	GTPB1_HUM	GTPB1	IGH	Immunoglob				305543	196713	277568				185381	216475	140230
35	O00203-O00	O00203-O00	AP3B1_HUM	AP3B1	IGH	Immunoglob	293063		417799	692063	523795	571894	108135	152141	103066	419018	351206	346268

**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.Nam	Genes
2	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
3	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
4	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
5	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
6	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
7	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	Q96JP5;Q96JP5-2		Q96JP5;Q96J ZFP91_HUM ZFP91		
8	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
9	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
10	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
11	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
12	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
13	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
14	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
15	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
16	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_IP_OnBeadDig	P36578		P36578	RL4_HUMAN	RPL4
17	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
18	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
19	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_MLH1_Flag_XL-IP_OnBead	P36578		P36578	RL4_HUMAN	RPL4
20	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	O76031		O76031	CLPX_HUMA	CLPX
21	E:\Raw_files\Yugandhar\MLH1_FLAG_DI_EGFP_Flag_XL-IP_OnBead	O76031		O76031	CLPX_HUMA	CLPX

**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 ('MLH1\_Flag\_XLIP', 'MLH1\_Flag\_IP') 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 'Protein' column of your file used for quantification (input in step 5). The example below of "P40692" corresponds to MLH1 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.

**Step 8:** 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 MLH1\_Flag\_IP vs EGFP\_Flag\_IP then #PSMs is #Precursors for MLH1\_Flag\_IP). 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!**

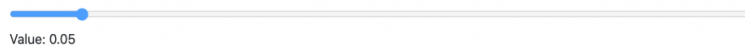
[Click Here to Download the Result File](#)

[Click Here to Download the baseline Volcano plot \(PDF\)](#)

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



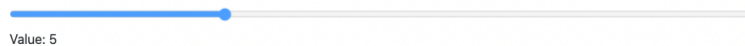
Adjusted P-value cutoff choice: Drag the slider to display the current value.



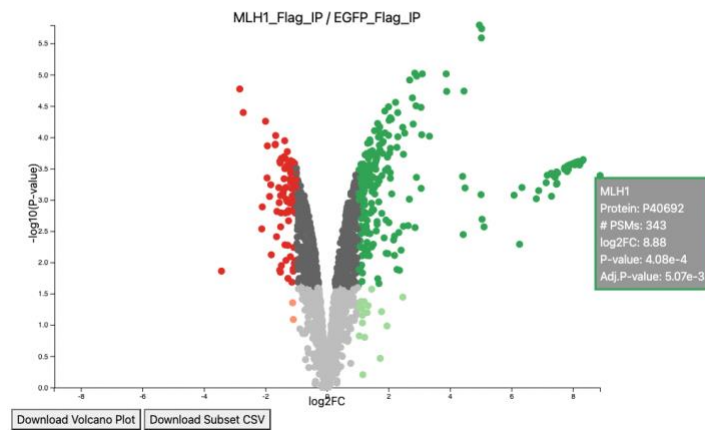
PSM cutoff choice: Drag the slider to display the current value.



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



Enter Uniprot IDs separated by @  
Example: Q08945@Q9UQE7



**The output file looks like this –**

	A	B	C	D	E	F	G	H	I	J	K
1	Protein	log2FC	pval	adjpval	#Precursors-IN-EGFP_Flag_IP	#Precursors-IN-EGFP_Flag_IP	#Precursors-IN-MLH1_Flag_IP	#Precursors-IN-MLH1_Flag_IP	# PSMs	PSM Cutoff	Gene Symbol
2	Q9P2E9-Q9P	-0.1310916	0.6622473	0.70318742	24	15	23	14	14	5	RRBP1
3	PC254	0.03807911	0.47193729	0.53049704	9	9	9	9	9	5	RF526
4	Q9H9P2	6.66294802	0.00310121	0.01193245	2	0	3	2	2	5	NLEFA
5	P29992	-0.6696114	0.00943703	0.02501395	7	12	9	12	12	5	GNA11
6	Q9H000	1.80986422	0.00018111	0.00504108	20	32	36	45	45	5	MKRN2
7	P27708	0.95637541	0.00042942	0.00510087	181	182	182	183	183	5	CAD
8	Q92499	-0.7424515	0.00085937	0.0063806	57	71	56	68	68	5	DDX1
9	Q92522	0.40670387	0.05422999	0.09103346	14	12	15	12	12	5	H5FX
10	P09493-5	0.11887802	0.1928363	0.25092093	3	6	3	6	6	5	TPM1
11	P42224-P422	-0.3858135	0.87227389	0.89137175	3	5	4	5	5	5	STAT1
12	Q02543	0.08212855	0.3511961	0.41697412	27	28	27	30	30	5	RH18A
13	PC1006	-0.2050463	0.15498602	0.21113895	10	11	11	10	10	5	RAB8A
14	Q9NHQ2-Q9H	-0.6133268	0.16928195	0.22665583	4	5	5	2	2	5	INTS7
15	P38646	0.88176842	0.00057634	0.00552413	120	120	123	123	123	5	HSPA9
16	P27348	0.43010554	0.03000871	0.05716832	15	15	15	15	15	5	YWHAQ
17	Q15404-Q15	6.71552958	0.0036168	0.01302898	6	0	6	3	3	5	RSU1
18	Q555J5-Q55	-0.7008213	0.02678653	0.05226437	11	11	12	12	12	5	HP1BP3
19	Q9BX63	5.01086839	0.00082581	0.00631926	0	3	109	138	138	5	BRIP1