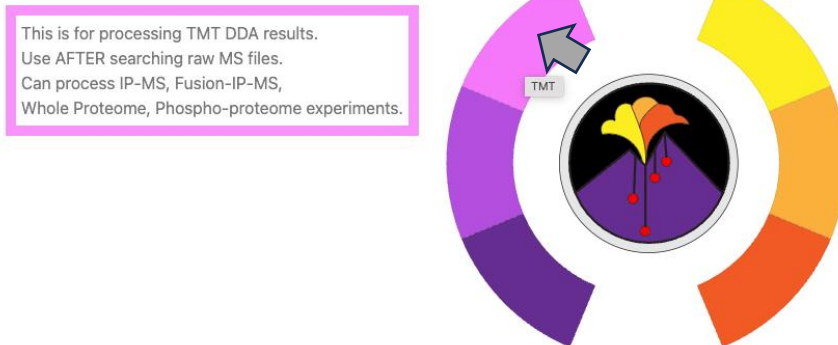


## TMT (IP-MS)

**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.

### Analysis Type

For more information: ?

Use COMET Search results:

Use SEQUEST Search results:

Choose the strategy for analysis with two choices:

Magma (Linear Model)

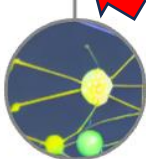
1. Choose the search engine that was used for generating the PSM list.

Magma (Limma)

2. Choose the model type to run the differential expression analysis with (between linear model (two sample) vs limma (one sample))

NOTE: No default set but recommend setting of COMET search + Magma (linea model). Check "About" page or manuscript for more details.

IP-TMT (Mutation or drug treatment)



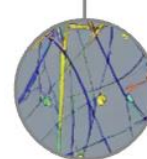
IP-TMT (Fusions)



Whole Proteome TMT



PhosphoProteome TMT



**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.](#)

	A	B	C
1	Channel	Label	Control
2	126	Vector	TRUE
3	127N	N	FALSE
4	127C	M	FALSE
5	128N	orf3a	FALSE
6	128C	orf6	FALSE
7	129N	Vector	TRUE
8	129C	N	FALSE
9	130N	M	FALSE
10	130C	orf3a	FALSE
11	131	orf6	FALSE

1 Upload the 'Annotation file'

For more information: ?

Select a file:

Choose File No file chosen

Submit Reset

[Sample file](#)

This file is to assign labels to channels according to experimental setup. Columns necessary for the file are Channel, Label and Control. Channel is to specify the TMT tags used in the experiment. Label is to specify the biological condition encoded by the corresponding TMT tag. Control column is to specify which conditions 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.

**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).

2 Select the Comparison You Would Like to Perform

Choose a comparison with control

- ('N', 'Vector')
- ('M', 'Vector')
- ('orf3a', 'Vector')
- ('orf6', 'Vector')

OR Choose a comparison NOT with control

- ('N', 'M')
- ('N', 'orf3a')
- ('N', 'orf6')
- ('M', 'orf3a')
- ('M', 'orf6')
- ('orf3a', 'orf6')

Choose the direction for comparison:

- (Condition\_1/Condition\_2)
- (Condition\_2/Condition\_1)

Reset

**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.](#)

**3 Upload a tab separated PD text file with "S/N" values**

For more information: ?

Select a file:

- This field is required.

[Choose File](#) No file chosen

[Reset](#)

[Sample file](#)

This file is the output from Proteome Discoverer(PD) search in the form of tab delimited ".txt" file of all PSMs passing 1% FDR threshold. The reporter ion intensities need to be exported as signal-to-noise ratios.

NOTE: File name should not have spaces.

For PD search ensure the following columns are present - 'Intensity', 'Annotated Sequence', 'Master Protein Accessions', 'Protein Accessions', 'Isolation Interference [%]', 'Spectrum File', 'Charge', 'RT [min]', '# Protein Groups' Ensure the column "Intensity" corresponding to precursor ion intensities associated with each PSM is uploaded.

**4 Upload a tab separated PD text file with "Intensity" values**

For more information: ?

Select a file:

- This field is required.

[Choose File](#) No file chosen

[Reset](#)

[Sample file](#)

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

#	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Checked	Confidence	Identifying N-PSM	Ambiguous Sequence	Annotated S1-Modification	# Proteins	Master Prot	Protein Acce	# Missed Cle	Charge	DeltaScore	DeltaCn	Rank	Search Engr	m/z [Da]	MH+ [Da]	Theo. MH+	[Delta]m		
2	FALSE	High	Sequest HT ( Unambiguous )	MVYSTCLNI [R].mVYSTCS N-Term(TMT)		1	Q08123	Q08123	0	3	0.6418	0	1	1	1014.20433	3040.59843	3040.59868	-		
3	FALSE	High	Sequest HT ( Unambiguous )	LSSQEESGTF [K].SSQEESI N-Term(TMT)		1	Q593C4	Q593C4	0	3	0.4362	0	1	1	745.40162	2234.19031	2234.18996	-		
4	FALSE	High	Sequest HT ( Unambiguous )	NINCSEESFC [K].mNCSIEE N-Term(TMT)		1	P35914	P35914	0	2	0.6783	0	1	1	863.42631	1725.84534	1725.84275	-		
5	FALSE	High	Sequest HT ( Unambiguous )	DFTPAVCTEL [R].#FTPACT N-Term(TMT)		1	P30041	P30041	0	2	0.6601	0	1	1	812.91383	1624.82038	1624.82022	-		
6	FALSE	High	Sequest HT ( Unambiguous )	VQEAARPEE [R].vQEAARF N-Term(TMT)		1	Q591Y6	Q591Y6	0	3	0.6125	0	1	1	751.09167	2251.26047	2251.26076	-		
7	FALSE	High	Sequest HT ( Unambiguous )	KQDEPIDFM [K].KQDEPID N-Term(TMT)		1	P40227	P40227	1	3	0.4389	0	1	1	933.17702	2797.51551	2797.51569	-		
8	FALSE	High	Sequest HT ( Unambiguous )	DLPTSPVLV [K].#LPTSPV N-Term(TMT)		1	Q56F86	Q56F86	0	3	0.716	0	1	1	972.50092	2915.48882	2915.48443	-		
9	FALSE	High	Sequest HT ( Unambiguous )	NAGVEGSLV [K].#AGVEG N-Term(TMT)		1	P10809	P10809	0	2	0.4597	0	1	1	837.4959	1673.98453	1673.98381	-		
10	FALSE	High	Sequest HT ( Unambiguous )	MLDAEDVW [K].mLDAEDI N-Term(TMT)		1	Q43707	Q43707	0	3	0.25	0	1	1	764.4049	2291.20014	2291.19533	-		
11	FALSE	High	Sequest HT ( Unambiguous )	INPDGGSVY [R].#NPDGSC N-Term(TMT)		1	Q59280	Q59280	0	2	0.6529	0	1	1	980.52038	1960.03349	1960.03372	-		
12	FALSE	High	Sequest HT ( Unambiguous )	LFQVSLDAP [R].#FQVSTL N-Term(TMT)		1	P33992	P33992	0	4	0.7153	0	1	1	916.2084	3661.81177	3661.81045	-		
13	FALSE	High	Sequest HT ( Unambiguous )	VFMDSGDEL [R].#FMDSG N-Term(TMT)		1	P08238	P08238	0	3	0.3068	0	1	1	808.44279	1603.11183	1603.1087	-		
14	FALSE	High	Sequest HT ( Unambiguous )	QAAPVTLQLL [K].#AAPVTL N-Term(TMT)		1	Q59K52	Q59K52	0	3	0.6465	0	1	1	838.8252	2514.46103	2514.45835	-		
15	FALSE	High	Sequest HT ( Unambiguous )	YMEEAIMQP [K].#MEEAIM N-Term(TMT)		1	Q03393	Q03393	0	3	0.1608	0	1	1	693.69435	2079.06849	2079.0655	-		
16	FALSE	High	Sequest HT ( Unambiguous )	YMEEAIMQP [K].#MEEAIM N-Term(TMT)		1	Q03393	Q03393	0	3	0.2507	0	1	1	693.69372	2079.06661	2079.0655	-		
17	FALSE	High	Sequest HT ( Unambiguous )	LGRPTLSSEV [K].#GRPTLS N-Term(TMT)		1	Q8N8X0	Q8N8X0	0	4	0.3006	0	1	1	857.96936	3428.85562	3428.85333	-		
18	FALSE	High	Sequest HT ( Unambiguous )	GVMLAVDAN [R].#VMLAVI N-Term(TMT)		1	P10809	P10809	0	3	0.4805	0	1	1	635.04914	1903.13286	1903.13384	-		
19	FALSE	High	Sequest HT ( Unambiguous )	MARKVEVLP [R].#ARVEE N-Term(TMT)		1	Q14980	Q14980	0	3	0.5425	0	1	1	872.475	2615.41045	2615.41147	-		
20	FALSE	High	Sequest HT ( Unambiguous )	SEEPGAPLV [R].#EPPGAP N-Term(TMT)		1	P53814	P53814	0	3	0.5139	0	1	1	852.45243	2555.34274	2555.34272	-		
21	FALSE	High	Sequest HT ( Unambiguous )	KAGTQIEID [R].#AGTQIE N-Term(TMT)		1	Q43707	Q43707	1	3	0.3398	0	1	1	698.71287	2094.12406	2094.12315	-		
22	FALSE	High	Sequest HT ( Unambiguous )	LLVDADNCL [R].#LVDADN N-Term(TMT)		1	Q59Z82	Q59Z82	0	3	0.6821	0	1	1	518.94678	1554.82579	1554.82598	-		
23	FALSE	High	Sequest HT ( Unambiguous )	LFQVSLDAP [R].#FQVSTL N-Term(TMT)		1	P33992	P33992	0	4	0.5797	0	1	1	912.20978	3645.81728	3645.81554	-		
24	FALSE	High	Sequest HT ( Unambiguous )	KLDAIEEICT [R].#LDAIEE N-Term(TMT)		1	Q59615	Q59615	1	3	0.6754	0	1	1	760.42205	2279.25161	2279.25201	-		
25	FALSE	High	Sequest HT ( Unambiguous )	TALINSTGEE [R].#ALINST N-Term(TMT)		1	P02545	P02545	0	2	0.6814	0	1	1	860.95866	1720.91005	1720.91011	-		
26	FALSE	High	Sequest HT ( Unambiguous )	NSPPKPF [R].#NSPKF N-Term(TMT)		1	Q9K876	Q9K876	0	4	0.6564	0	1	1	829.19827	3313.72125	3313.72601	-		

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 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 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

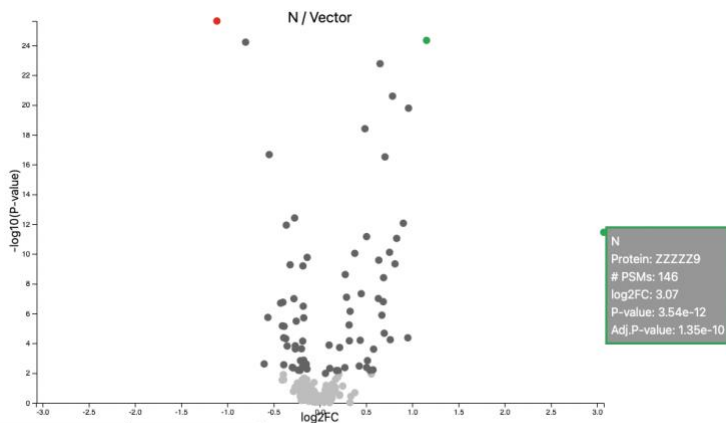
Size of scatter point: Drag the slider to display the current value.



Value: 5

Enter Uniprot IDs separated by @  
Example: Q08945@Q9UQE7

Update Volcano Plot



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	ZZZZZ29	3.0718912	3.54E-12	1.35E-10	N	146	-10.672234	-15.08168	-13.583132	-10.812606	5
3	Q9Y6V0	-0.3992096	1.76E-07	2.64E-06	IVNS1ABP	17	-12.251674	-11.739679	-11.831093	-12.245438	
4	Q9Y6W5	-0.097623	0.4514805	0.65563717	WASF2	2	-12.30738	-12.097758	-12.174227	-12.207526	
5	Q9Y6W8	-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	Q9Y411	-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	Q9Y389	-0.5734296	0.03109339	0.10937817	RRP15	1	-12.168614	-11.457073	-11.646169	-12.083355	
18	Q9Y3A5	0.03156629	0.64335394	0.78596395	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	Q9Y211	-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	

### 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].gDPGDDQ	[R].gDPGDDQ		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].gAEEPPP	[R].gAEEPPP		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].kVQSDGc	[K].kVQSDGc		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].sEPEGAP	[R].sEPEGAP		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].gLFDEEM	[R].gLFDEEM		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