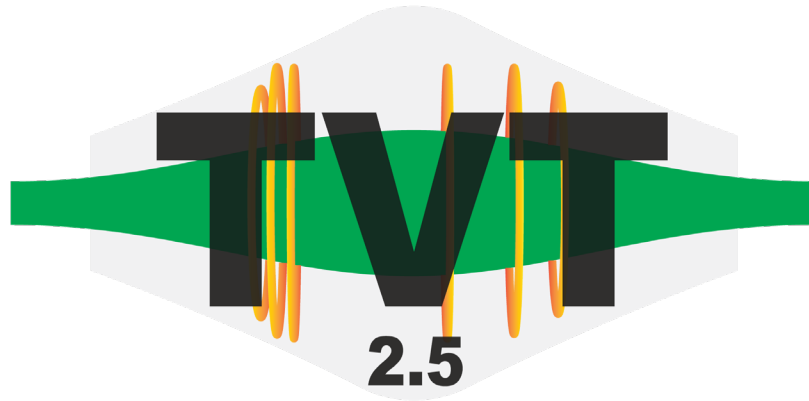


Easy Start Guide



1. **Select TKO standard and upload RAW file**
2. **Email notification**
3. **Viewer homepage**
4. **Settings/advanced configuration**
5. **Assess interference with TKO proteins**
6. **Check CVs of known unchanging proteins**
7. **Instrument performance statistics**
8. **Performance comparison across standards**

Xinyue Liu

Joao A. Paulo

<http://tko.hms.harvard.edu/>

1. Select TKO standard and upload RAW file

<http://tko.hms.harvard.edu/>



TKO Viewing Tool

Select Options and Upload a File to Begin

1. Select Plex and Enzyme

Plex

- TMT 6-plex
- TMT 9-plex
- TMT 11-plex
- TMT Pro 9-plex
- TMT Pro 16-plex
- TMT Pro 18-plex

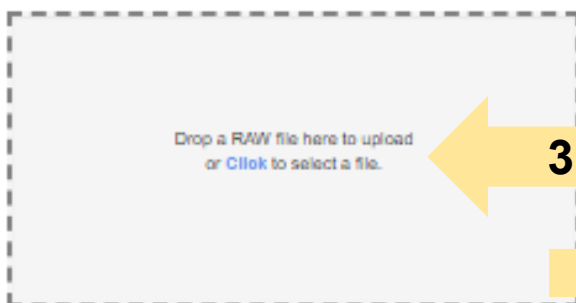
Enzyme

- LysC/Trypsin
- LysC

1. Select the enzyme

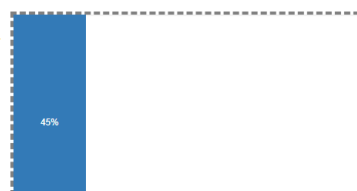
2. Select the TMT reagent

2. Upload File



3. Upload the file

2. Upload File



Analysis will begin when the upload finishes.

Database search powered by Comet MS/MS

NOTE: TMT uses reactive widgets to dynamically display data. As such it may be incompatible with the Microsoft Edge Browser or Internet Explorer. Please use another browser such as Google Chrome or Mozilla Firefox to ensure functionality.

Analysis will begin when the upload finishes.

[View Example Output](#)

[Download Example RAW File](#)

Pierce™ TMT11plex Yeast Digest Standard
Catalog Number A40939



GYGI LAB



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Lite Version - Upload a CSV File:

- [TKO 11-plex](#)
- [TKO 9-plex](#)

• **Example files**

2. Email notification

Please wait until the data is processed. This will take a few minutes.

Enter your e-mail to get a notification when the results are ready, or reload this page after a few minutes.

E-Mail Address: **1. Enter email address**

2. Click to start search

Notification Enabled

Please wait until the data is processed. This will take a few minutes.
You will get an e-mail to notify you when the data is ready.

- Receive email notification (~15 min)**

Your RAW file submitted on 2021-09-30 12:46 has been processed and the results are ready to view:

https://wren.hms.harvard.edu/tko_viewer/?id=6155e9d66e486 **3. Click on link**

RAW file processed: ea07033.raw
Search ID: 2252(for future comparison)

NOTE: TVT uses reactive widgets to dynamically display data. As such it is incompatible with the Microsoft Edge Browser (Internet Explorer). Please use another browser such as Google Chrome or Mozilla Firefox to ensure functionality.

Date	File	ID	SearchID
2021-09-30	ea07033.raw	6155e9d66e486	2252

3. Viewer homepage

The image shows a screenshot of a web application interface for protein analysis. At the top is a dark navigation bar with links: Home, TKO Proteins, Unchanging Proteins, Instrument Performance, Settings / Help, and Admin. A yellow callout box labeled "Drop-list options" points to the "Instrument Performance" dropdown menu, which is open and shows "Performance Stats" and "Performance Comparison". Below the navigation bar is a logo for "TVT 2.5" (TKO Viewing Tool) featuring a stylized chromatogram. To the right of the logo, a yellow callout box labeled "Overall Numbers" points to a statistics box that displays "Unfiltered Statistics: Total Proteins: 1439, Total Peptides: 5625" and "Data loaded successfully!". Below the logo and statistics is a welcome message: "Welcome! Please select an option to view below:". Underneath are four colored buttons: "TKO Proteins" (green), "Unchanging Prot." (red), "Instrument Stats" (blue), and "Settings / Help" (purple). A dashed yellow box highlights the "Instrument Stats" button, with a callout box labeled "Click for options" pointing to a modal window. The modal window has a title "Select an option for Instrument Performance:" and contains two buttons: "Instrument Statistics" and "Performance Comparison", along with a "Close" button at the bottom.

Drop-list options

Home TKO Proteins Unchanging Proteins Instrument Performance Settings / Help Admin

Performance Stats
Performance Comparison

Unfiltered Statistics:
Total Proteins: 1439
Total Peptides: 5625
Data loaded successfully!

Overall Numbers

Welcome! Please select an option to view below:

TKO Proteins Unchanging Prot.

Instrument Stats Settings / Help

TVT 2.5

TVT T.K.O. Viewing Tool

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thermo scientific

Protein Quant ID: 2131 | Search ID: 2252 | RAW: ea07033.raw

Click for options

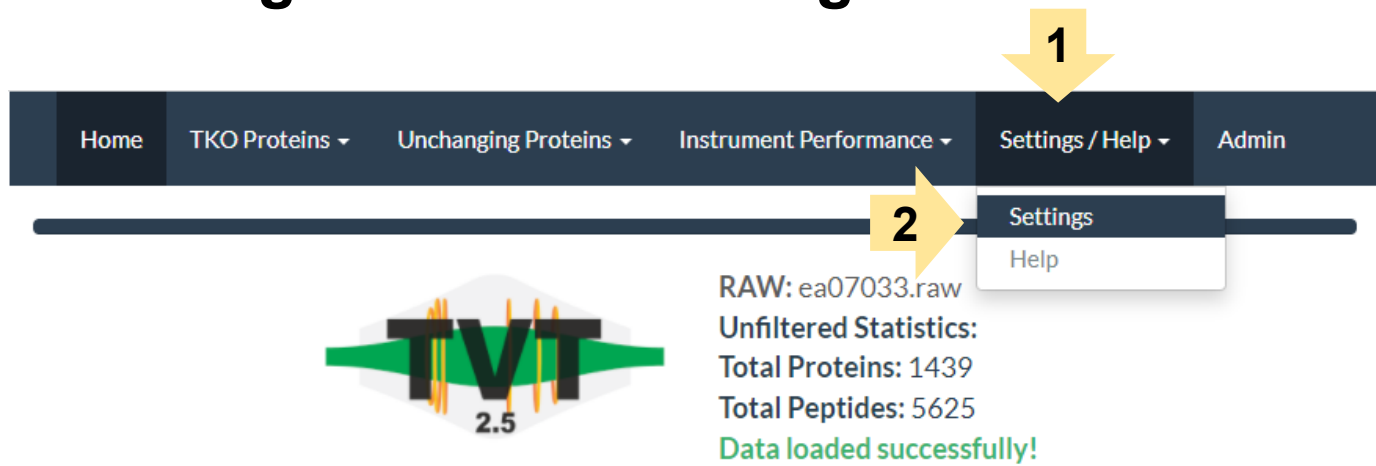
Select an option for Instrument Performance:

Instrument Statistics

Performance Comparison

Close

4. Settings/advanced configuration



Home TKO Proteins ▾ Unchanging Proteins ▾ Instrument Performance ▾ Settings / Help ▾ Admin

Settings
Help

RAW: ea07033.raw
Unfiltered Statistics:
Total Proteins: 1439
Total Peptides: 5625
Data loaded successfully!

Select TMT summed signal-to-noise threshold

Filter Settings:

Isolation Spec. Cut Off: 0.8 1

Sum Signal Cut Off: [Click To Confirm](#)

Select isolation specificity (isolation purity threshold)

Protein Choices:

Define your own lists TKO protein

TKO Proteins:	Unchanging Proteins:
KO Protein 1: <input type="text" value="MET6"/>	Unchanging Protein 1: <input type="text" value="GLN4"/>
KO Protein 2: <input type="text" value="PFK2"/>	Unchanging Protein 2: <input type="text" value="FRS2"/>
KO Protein 3: <input type="text" value="URA2"/>	Unchanging Protein 3: <input type="text" value="SCP160"/>

[Click To Update Proteins](#)


5. Assess interference with TKO proteins

Home TKO Proteins ▾ Unchanging Proteins ▾ Instrument Performance ▾ Settings / Help ▾ Admin

Select from TKO proteins menu

- KO Peptide Table
- KO Graphs
- Interactive Graph (IFI)

RAW: ea07033.raw
 Unfiltered Statistics:
 Total Proteins: 1439
 Total Peptides: 5625
 Data loaded successfully!



Only Show TKO Proteins?
 Show As Percentages?

KO peptide table

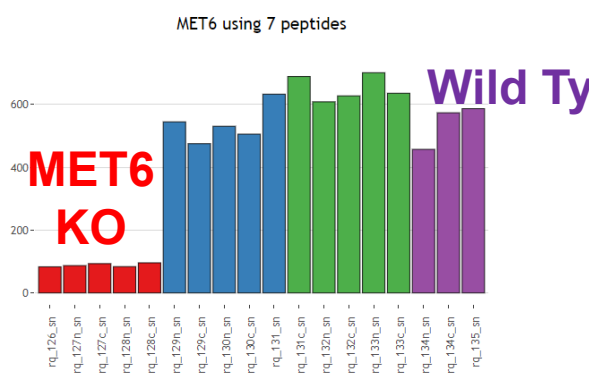
Gene Symbol:	Num. of Peptides:	IFI +/- Std.Dev.
MET6	7	0.842 +/- 0.066
PFK2	11	0.923 +/- 0.079
URA2	34	0.765 +/- 0.226
Total TKO	52	0.809 +/- 0.198

$$IFI = 1 - \frac{avg(TMT\ S: N\ for\ KO)}{avg(TMT\ S: N\ for\ nonKO)}$$

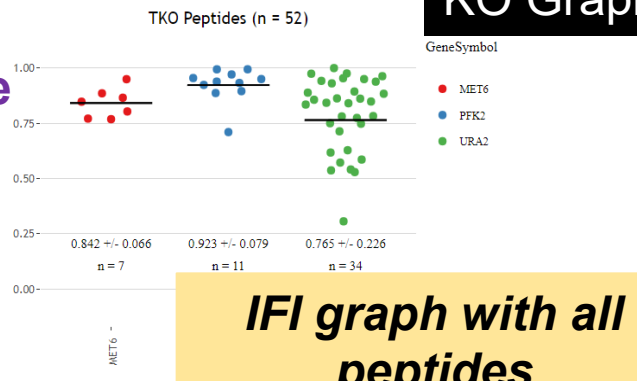
**Interference-Free Index (IFI):
 1 denotes no interference**

KO Graphs

MET6 using 7 peptides



TKO Peptides (n = 52)



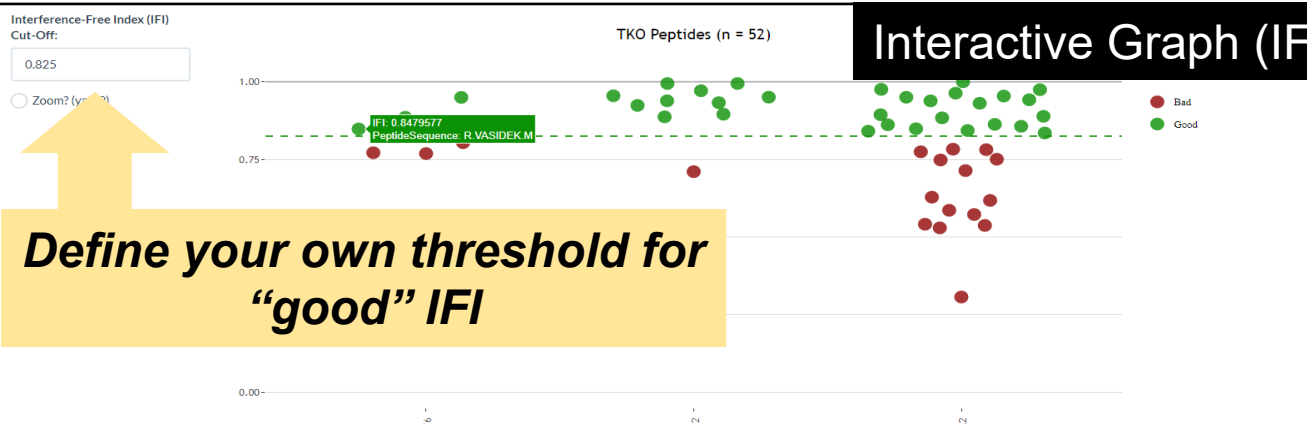
IFI graph with all peptides

Interactive Graph (IFI)

Interference-Free Index (IFI)
 Cut-Off:

Zoom? (yes/no)

TKO Peptides (n = 52)



Define your own threshold for "good" IFI


6. Check CVs of known unchanging proteins

Home TKO Proteins **Unchanging Proteins** Instrument Performance Settings / Help Admin

Unchanging Peptide Table
Unchanging Graphs

Select unchanging menu

RAW: ea07033.raw
Unfiltered Statistics:
Total Proteins: 1439
Total Peptides: 5625
Data loaded successfully!



Only Show Unchanging Proteins?
 Show As Percentages?

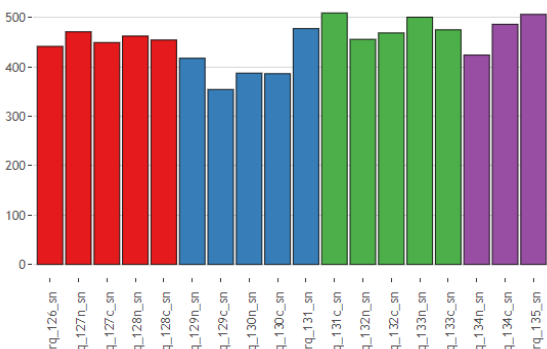
Gene Symbol:	Num. of Peptides:	%CV +/- Std.Dev.
GLN4	9	14.152% +/- 3.886
FRS2	7	15.523% +/- 3.774
SCP160	16	15.279% +/- 4.062
Total Unchanging	32	15.015% +/- 3.866

Unchanging Peptide Table

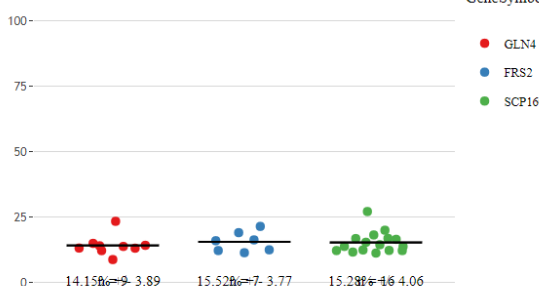
$$CV = \frac{SD}{Mean}$$

Calculates coefficient of variation (CV)

GLN4 using 9 peptides



Unchanging protein plots



CV graph of all peptides

7. Instrument performance statistics

Home TKO Proteins ▾ Unchanging Proteins ▾ Instrument Performance ▾ Settings / Help ▾ Admin

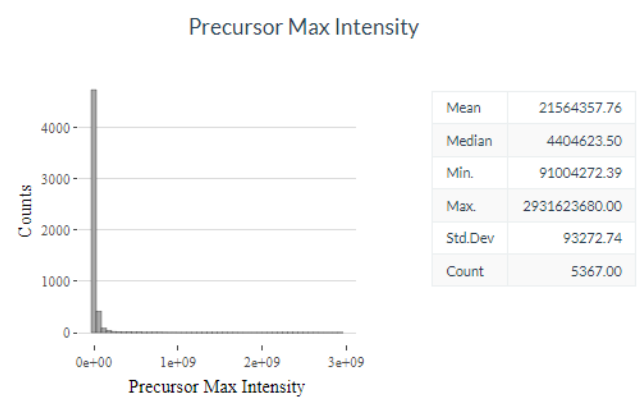
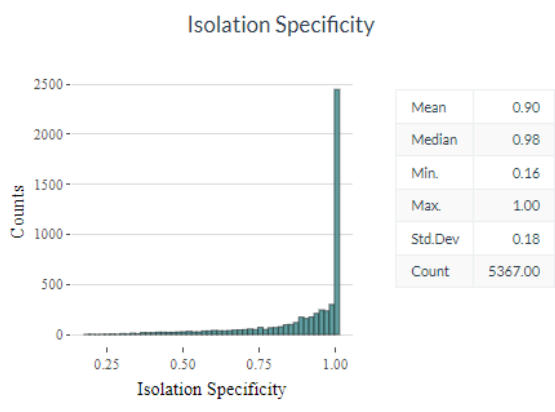
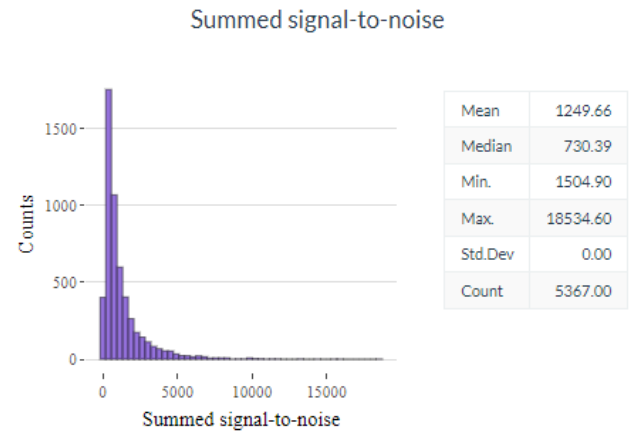
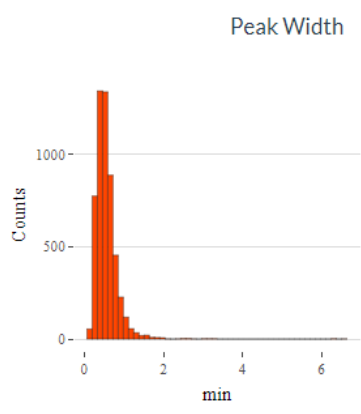
1. Check tables and plots

Performance Stats
Performance Comparison



RAW: Ca7033.raw
Unfiltered Statistics:
Total Proteins: 1439
Total Peptides: 5625
Data loaded successfully!

	Mean	Median	Minimum	Maximum	Std Dev		
Comet XCorr	9.81	9.56	4.18	21.00	2.49	Unique Peptides	4578
PPM	-0.52	-0.55	-31.02	20.09	1.54	Validated Reverse Hits	12
Missed Cleavages	0.03	0.00	0.00	2.00	0.17	Total Peptides	5364
Charge	2.47	2.00	2.00	5.00	0.56	Total Protein	1144
Peptide Length	9.59	9.00	6.00	30.00	3.01	Sensitivity	63.25%
						Success Rate	28.2%



8. Performance comparison across standards

Home TKO Proteins ▾ Unchanging Proteins ▾ Instrument Performance ▾ Settings / Help ▾ Admin

Performance Stats
Performance Comparison

1.

Unfiltered Statistics:
Total Proteins: 1439
Total Peptides: 5625
Data loaded successfully!

Performance Comparison:

To compare this run (Current) against others, put up to 9 other search ids into the box below, comma separated.
These search ids (SIDs) can be found at the bottom of the page of each instance of TVT.

Search ID(s) to compare:

2. Enter other SIDs to compare (Find SID in email - Section 2)

Confirm

WARNING: Once 'Confirm' is pressed, the application will take up to a minute per search id to fetch data and will be unresponsive.
Please wait for the graphs to load before manipulating other data.

Unfiltered Statistics:

	2252(Current)	2236
Total Proteins	1439	1253
Total Peptides	5625	4324

IFI Comparison:

Gene Symbol:	2252(Current)	2236
MET6	0.842 +/- 0.066	0.108 +/- 0.427
PFK2	0.923 +/- 0.079	-9.287 +/- 15.446
URA2	0.765 +/- 0.226	0.775 +/- 0.144
Total TKO	0.809 +/- 0.198	-0.712 +/- 5.776

Number of TKO Peptides:

Gene Symbol:	2252(Current)	2236
MET6	7	6
PFK2	11	3
URA2	34	14
Total TKO	52	23

Peak Width:

	2252(Current)	2236
Mean	0.56	1.32
Median	0.51	1.15
SD	0.30	0.84
XMax	6.55	17.11
XMin	0.10	0.06
Total:	5367.00	4134.00