Realtime TMT Quantification on the timsTOF series with PaSER

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Introduction

Proteomics experiments with the latest generation of mass spectrometers yield wide breadth and quantitation of a proteome. In hours, instruments like the timsTOF Pro 2 are capable of comprehensive identification of nearly all expressed proteins. The timsTOF Pro2 can use isobaric chemical tags such as TMT for multiplexed quantitation and improved parallelization. Isobaric chemical tags are a set of molecules with the same mass, but which generate distinct reporter ions upon fragmentation. The relative ratio of these reporter ions represents the relative abundance of the tagged peptides.

PaSER, Parallel Search Engine in Real-time is a proteomics data analysis platform that supports both dia-PASEF and dda-PASEF workflows in real time. As isobarically labelled peptides get measured with dda-PASEF workflows in the mass spectrometer, the data is streamed in real-time to the PaSER platform which can identify the labelled peptides and provide a list of all peptides immediately upon acquisition completion. Quantification of the reporter ions channels can be achieved in several minutes with only a few more clicks.

Methods

- 2 datasets were used to illustrate quantification of TMT data with PaSER
 - Single species dataset with TMT-labelling as a 9plex with three different dilution ratios
 - Three species mix labelled as a 6plex designed to provide a fixed ratio of Human, large changes in Yeast and small changes in Bacteria within a single sample

	Enzyme information														St	Static/Tixed modifications													
	Specificity					0	 none ○ one end ● both end 0 ○ 1 ● 2 ○ 3 ○ 4 ○ unlimited 									N-f	N-term static modification 🕢					229.1629							
	Max num internal missed cleavage Protease name, e.g., trypsin															0	C-t	C-term static modification				0.0							
							t	trypsin KR e cterm _ nterm									Am	Amino acid residue specific static modification 💋				57.02146 C 229.1629 K				_			
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Click	to show/hid	I 1 files uploade de spectral files J Q View S		rams																									
Display in exp page	ID	Search Results	TIMS Viz	DeltaMass	P SM Score	name	Misc quality check	Searched Scan/Total Scans	Searched Scan Ratio	Date	SQT File Count		Peptide IDs	Spectrum IDs		Peptide FDR (%)	Spectrum FDR (%)	Program	Search Params	DTA Select Filter Params	Search Status	Upload Files	DTASelect	mzident	Quantitative Analysis	Chro File		PTM-gene file download	Delete
0	156722	Result View		plot	plot		View	142937 / 144144	99.0 %	2021- 12-13		6592	19510	29426	0.99	0.46	0.48	ProLuCID	search xml	-p 1 -y 0 trypstatpfp 0.01modstat extrapl DBdm -t 1	complete path	N/A	run another	generate	run now path qid:	chro(right click save)		search results	×

port options: 🕢 CSV | 🕱 Excel | 🕢 XML | 🔂 PD Figure 1 – A: Search Parameters for TMT Data. B: Simple click to take real time search into quantification performance screen.

Isobaric tags used are completely customizable as well as allowing for the input of lot specific correction values

r parameters

sobaric purity filter : -1.0

mass tolernace: 50.0 ppm intensity threshold: 0.0 (sum of all reporter ions

um number of unique peptides per protein

0 156722

Tandem Scan Shift 0

MS3-based TMT 🗆 🔞

Data Set 1 continued:



2021-12-13

Data Set 2:











Figure 4 – TMT quantification and protein identification from a mixed species model. Human, Yeast and Bacteria were combined in 6 ratios to highlight large and small changes as indicated in the table (A), or graphically (B). C: Total protein identifications for each species are illustrated. D: For each species reference, the relative ratio quantification of each channel obtained either by PaSER or MaxQuant was normalized and plotted. Horizontal lines represent expected ratios.



Figure 3 – Quantitative analysis K562 lysate labelled as a TMT 9plex and run on the timsTOF Pro 2 using a dda-PASEF workflow. A: Experimental design indicating groups and expected ratios. B: Relative intensity of each channel as quantified by PaSER or MaxQuant. Colours represent grouping of samples, and error bars coefficient of variation. C: Individual peptide ratios were calculated relative to group 1 and then averaged. Box plots indicate observed ratios from PaSER and MaxQuant, dotted horizontal lines represent expected values. D: Violin plots indicating coefficient of variation for the PaSER quantification of replicates across the three groups.



93,419 PSMs encoding 63,496 peptides representing 4,388 protein groups were identified

Quantitative accuracy was validated by calculating ratio between groups for each peptide (relative to group 1) and processed the data in MaxQuant (reference) a known software for comparison

Quantitative ratios calculated from both software were in excellent agreement with each other as well as expected ratios



*Reference channels for each species are shown in



Data Set 2 continued:

- 5906 human, 3905 yeast, 1564 bacterial proteins quantified with at least 2 unique peptides
- Good quantitative accuracy in these complex samples across all channels was observed, and similar ratios and quantitative accuracy was observed by MaxQuant
- Both large (>2 fold change) and small (<1.5 fold change) were able to be quantified by PaSER from within the same sample

Summary

- The use of isobaric tags is a popular method to increase throughput of experiments
- Recently the bottleneck in large cohort studies has shifted from the acquisition to the analysis. PaSER alleviates this bottleneck by doing the identification of dia-PASEF, dda-PASEF, and TMT dda-PASEF in real time with identification results in seconds after run completion and quantitative analysis in minutes

Conclusion

- PaSER provides an accurate, fast and efficient workflow for the identification and quantification of isobarically labelled experiments (TMT, iTRAQ)
- Quantification of up to 10 isobaric tags can be done accurately, reproducibly, and quickly with the timsTOF platform coupled to PaSER
- Similar quantification results obtained with PaSER when compared to other leading software platforms

Download the Application Note





Questions about PaSER?



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Informatics: Peptide ID and Quantification

Innovation with Integrity