Run & Done isobaric quantitation with PaSER on the timsTOF platform

In this application note we demonstrate, how the timsTOF platform with PaSER provides an easy, robust and quantitatively accurate workflow for the analysis of isobaric labeled experiments (such as TMT and iTRAQ).

Abstract

PaSER's real-time GPU powered search capability provides identifications for isobarically labeled experiments at the end of the acquisition, with quantitation only a few clicks and minutes away. We utilize two datasets with known ground truths, with regards to expected ratios between labeled channels, to illustrate this workflow. In both datasets, PaSER® is able to precisely and accurately reproduce the expected ratios.

Introduction

Proteomics experiments with the latest generation of mass spectrometers provide wide breadth, depth, and quantitation of a proteome. In just hours instruments like the timsTOF Pro 2 are capable of near-comprehensive identification of the majority of expressed proteins. State-of-the-art mass spectrometers efficiently transfer ions into the vacuum, but only use a small fraction of the ion beam for mass analysis. The recently launched timsTOF Pro 2 achieves a nearly 100% duty cycle by parallel ion storage and sequential release

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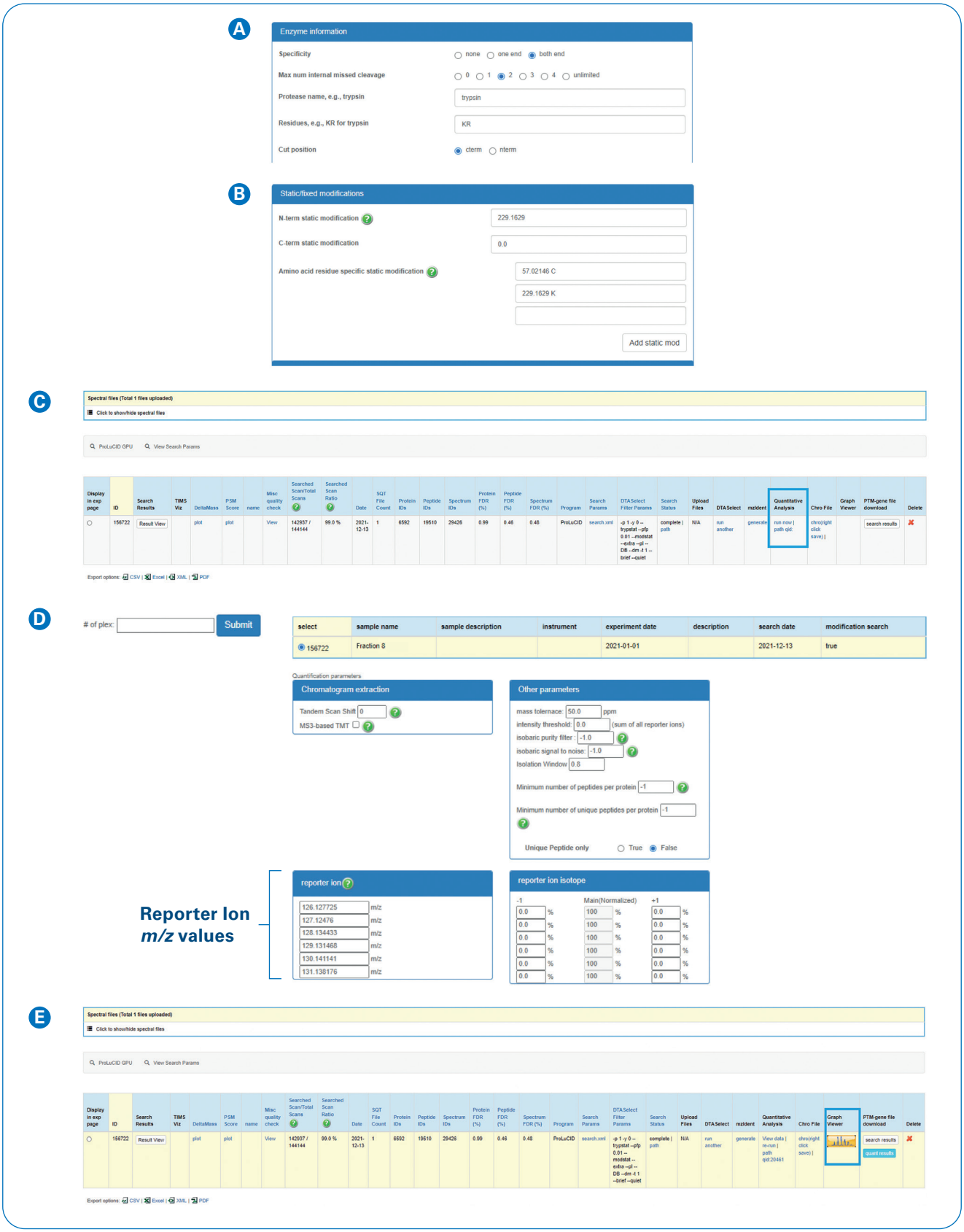


Figure 1: Screenshots depicting the simplicity of TMT analysis in PaSER. General search parameters, such as (A) enzymes and (B) static modifications, are set prior to data acquisition to allow for real-time identification to take place. Parameters are fully customizable for any set of reporter ions being used. (C) Once the database search is completed, user will select quantitative analysis and will be presented with options (D) that allow the user to specify the reporter ions used and desired tolerances. After clicking submit, users can access the results with the press of a button as shown in (E).

from a trapped ion mobility (TIMS) device into a quadrupole time of flight mass analyzer. Synchronizing the release of ions from the TIMS device with the quadrupole (PASEF; Parallel Accumulation Serial Fragmentation) increases the MS/MS sequencing speed more than 10-fold while simultaneously boosting sensitivity in online DDA experiments [1,2].

Data-independent acquisition (DIA) facilitates reproducible and accurate protein identification and quantification across large sample cohorts. This is achieved by using isolation of wide quadrupole windows, rather than selecting individual precursors, to ensure that all precursor ions are fragmented in every sample. Ion mobility provides an additional dimension of separation for complex samples, which can also be used for aligning precursor and fragment ions. By making use of reproducible mobility values from the timsTOF Pro, we extend the PASEF principle to DIA resulting in the dia-PASEF® acquisition method [3]. dia-PASEF shows excellent results, identifying more than 4000 yeast protein groups in a single shot 30 min gradient or more than 6400 protein groups from a HeLa digest [4].

Additional to robust, reproducible and accurate quantitation in dia-PASEF, the timsTOF platform is capable of utilizing mass-differential quantitation techniques, including isobaric chemical tags (e.g. TMT and iTRAQ), for multiplexed quantitation and improved parallelization. Isobaric chemical tags are a set of molecules with the same mass but which

TMT channel	Group	Expected ratio
126	1	1.0
127C	2	0.5
128C	3	0.2
129C	1	1.0
130C	2	0.5
131C	3	0.2
132C	1	1.0
133C	2	0.5
134N	3	0.2

Figure 2: K562 lysate was labelled with TMT and grouped together as indicated in (A). Quantitative relative intensities obtained by PaSER (B) and MaxQuant (C) were plotted with error bars representing CVs. Box-plots illustrate the average ratio of group 2 and 3 normalized to group 1 for data generated on (D) PaSER and (E) MaxQuant. Expected ratios for each group are indicated by horizonal lines. (F) Coefficient of variation for the quantification across replicates from the three groups.

generate distinct reporter ions upon fragmentation. The relative ratio of these reporter ions represents the relative abundance of the tagged molecules (peptides).

PaSER, Parallel Search Engine in Real-time, is a proteomics data analysis platform that supports DDA and DIA workflows. PaSER is able to identify isobarically labeled peptides in real-time and provide the list of identified peptides immediately at the end of the run. Quantitation of the reporter ion channels can then be achieved with a few more clicks [5,6]. This allows PaSER to provide a fast and efficient workflow for the analysis of isobaric labeled experiments (such as TMT and iTRAQ).

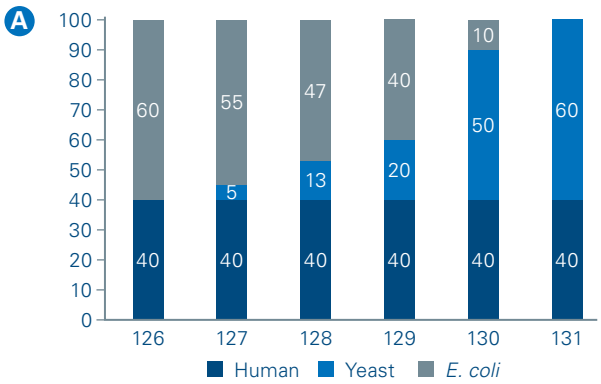
In this application note, we demonstrate the performance of PaSER for the analysis of isobaric chemical tags on the timsTOF Pro mass spectrometer (Bruker Daltonics GmbH & Co. KG) on two data sets.

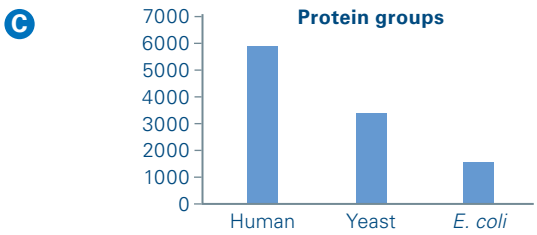
It's trivial to include isobaric labeled tags in PaSER search parameters. There are several recommendations that can be noted, such as enzyme specificity be set for both termini (Figure 1A). The isobaric labeled tags can be specified as either variable modifications or static modifications. For estimating labeling efficiency, it's preferable to have the isobaric labeled tags as variable modification. For final experiments, it's preferrable to consider the isobaric labeled tags as static modifications (Figure 1B). With the search parameter saved, generate a PaSER key with the project of your choice and the saved search parameter. Utilize this PaSER key in Compass® HyStar software to acquire data and have it processed by PaSER.

Once acquisition has been completed, look at the search results in PaSER Data Viewer (PDV). Along with the number of identifications and the reported false discovery rate (FDR), quantitative analysis can be started by choosing "Run Now" in the Quantitative Analysis column (Figure 1C). Ensure the number and mass of the reporter ions are correctly specified. Specify other parameters, such as the reporter ion mass tolerance, intensity threshold and purity correction values as desired and then click submit (Figure 1D). Quantitative analyses are accessible via the "View Data" link under the Quantitative Analysis column or the Graph Viewer icon (Figure 1E).

To illustrate the use of the isobaric labeled quantitative workflow in PaSER, we utilized two datasets. First a single species dataset with TMT labeled as a 9-plex, consisting of 3 different ratios (5:2.5:1) in triplicate (Figure 2A), which was kindly provided by the laboratory of Dr. Anne-Claude Gingras at the Lunenfeld-Tanenbaum Research Institute (Toronto, Canada). As described above, following acquisition and database searching, quantitation was triggered for the 9 channels (see Figure 2A for experimental setup). 93,419 PSMs encoding 63,496 peptides representing 4388 protein groups were identified. To validate the quantitative accuracy, we calculated the ratio between groups for each peptide, where group 1 served as the reference. We also processed the data with MaxQuant (v2.0.3) [7] as a comparison (Figure 2B&C). The quantitative ratios calculated from both software were in excellent agreement with the expected ratios of 1:0.5:0.2 (Figure 2D & E). Furthermore, the average relative intensity reporter ion signal from each group is in line with the anticipated results of 100%:49%:23%. To evaluate the quantitative precision in PaSER, we visualized the coefficient of variation in each group (Figure 2F). The median CV were 5.9%, 7.7% and 7.4% for Group 1, 2 and 3, respectively. Taken together, PaSER is able to produce great quantitative accuracy and precision for isobarically tagged datasets and fully supports N-plex workflows.

Next we utilized a more complex and challenging sample provided by the laboratory of Dr. Steven Carr at the Broad Institute of MIT and Harvard. This dataset consisted of three species mixed across 6 channels to provide a fixed ratio of 1:1 (Human proteome), large changes (1:1 to 1:12, Yeast proteome) or small changes (1:1 to 1:6, *E. coli* proteome) (see Figure 3A and B) within a single sample. For each species a reference channel was selected based on the specific channel in which it had the least interference (Figure 3B). Overall 5906 human, 3905 yeast and 1564 *E. coli* protein groups were quantified with at least 2 unique peptides (Figure 3C). PaSER showed good quantitative accuracy in complex samples across all channels and species (Figure 3D upper panels). PaSER was able to quantify both large (>2 fold change) and small changes (<1.5 fold change) in protein levels without issue. Similar quantitative accuracy was observed with MaxQuant (Figure 3D lower panels). It should be noted that the isotopic purity for each channel was unknown, and purity correction factors were not applied. We speculate that the quantitation accuracy could be further improved by applying purity correction (a supported feature).





B

Channel	Species	Expected ratio vs. reference channel	Expected ratio	
126	Human	1	(1:1)	
127	Human	1	(1:1)	
128	Human	1	(1:1)	
129	Human	1	(1:1)	
130	Human	1	(1:1)	
131	Human	1	(1:1)	
126	Yeast	0	0	Large changes
127	Yeast	0,08	(1:12)	Large changes
128	Yeast	0,22	(1:4.6)	Large changes
129	Yeast	0,33	(1:3)	Large changes
130	Yeast	0,83	(1:1.2)	Large changes
131	Yeast	1	(1:1)	Large changes
126	*E. coli.*	1	(1:1)	Small changes
127	*E. coli.*	0,92	(1:1.1)	Small changes
128	*E. coli.*	0,78	(1:1.3)	Small changes
129	*E. coli.*	0,67	(1:1.5)	Small changes
130	*E. coli.*	0,17	(1:6)	Small changes
131	*E. coli.*	0	0	Small changes

**Reference channels for each species are shown in bold*

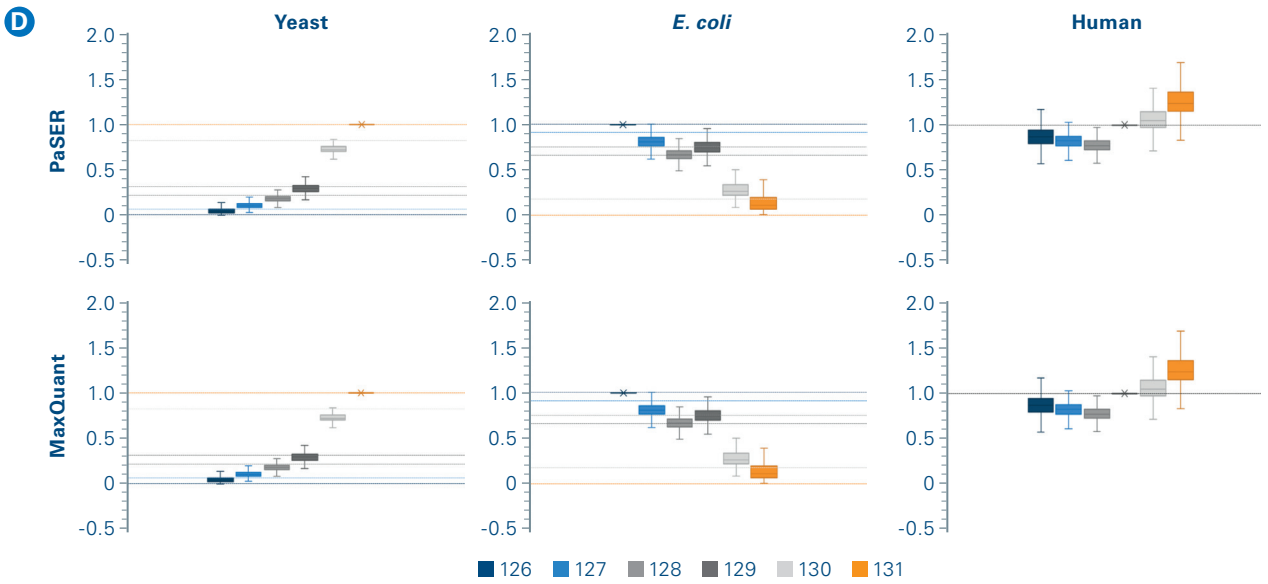


Figure 3: TMT quantification and protein identification from a mixed-species model. Human, Yeast, and E. coli were combined in 6 ratios as indicated graphically (A) or by tabular view (B). (C) Total proteins identified for each species are shown. The species-specific relative quantification of each channel obtained from PaSER or MaxQuant was normalized and plotted (D), with expected values represented by horizontal lines.

Conclusion

- Quantification with up to 10 isobaric tags can be done accurately, reproducibly, and quickly with the timsTOF platform coupled to PaSER
- Quantification by PaSER platform is comparably accurate to other software platforms
- Run & Done technology allows for identification in real-time and accurate quantification minutes afterwards
- Available to all PaSER customers without additional modules or updates

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www.bruker.com/timstof

References

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