

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

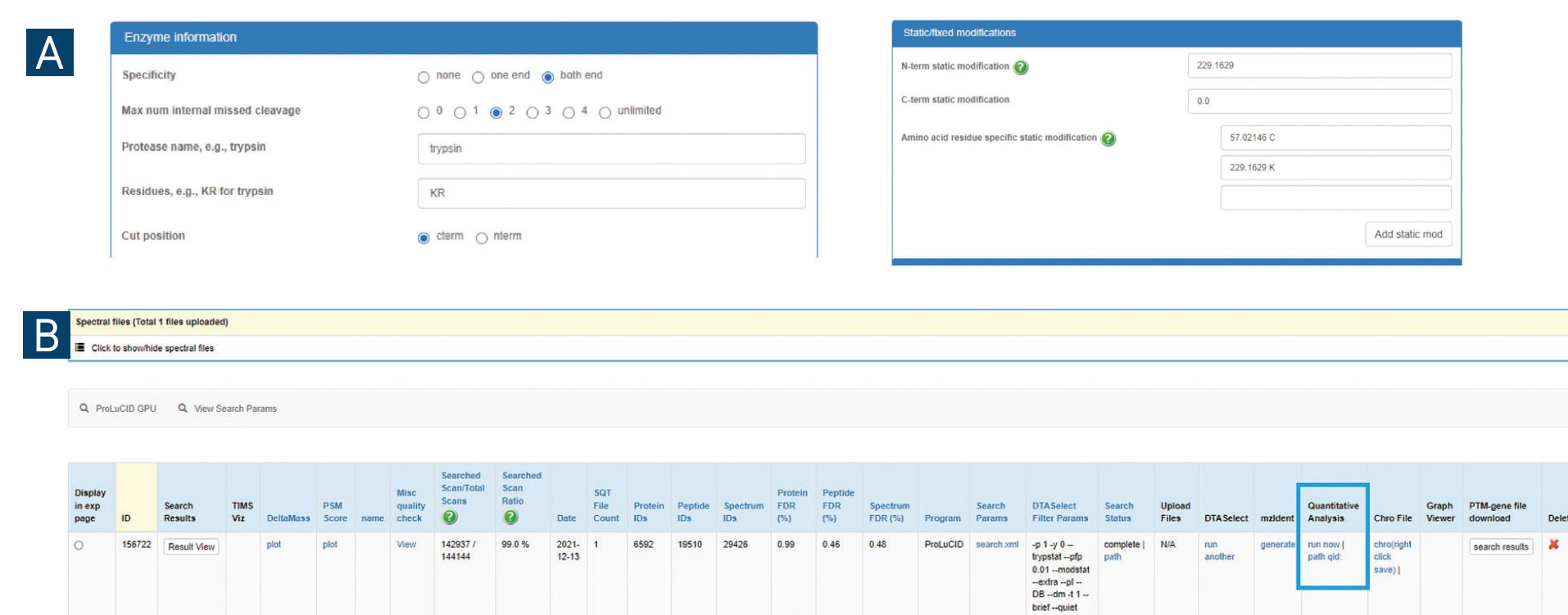


Figure 1 – A: Search Parameters for TMT Data. B: Simple click to take real time search into quantification performance screen.

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

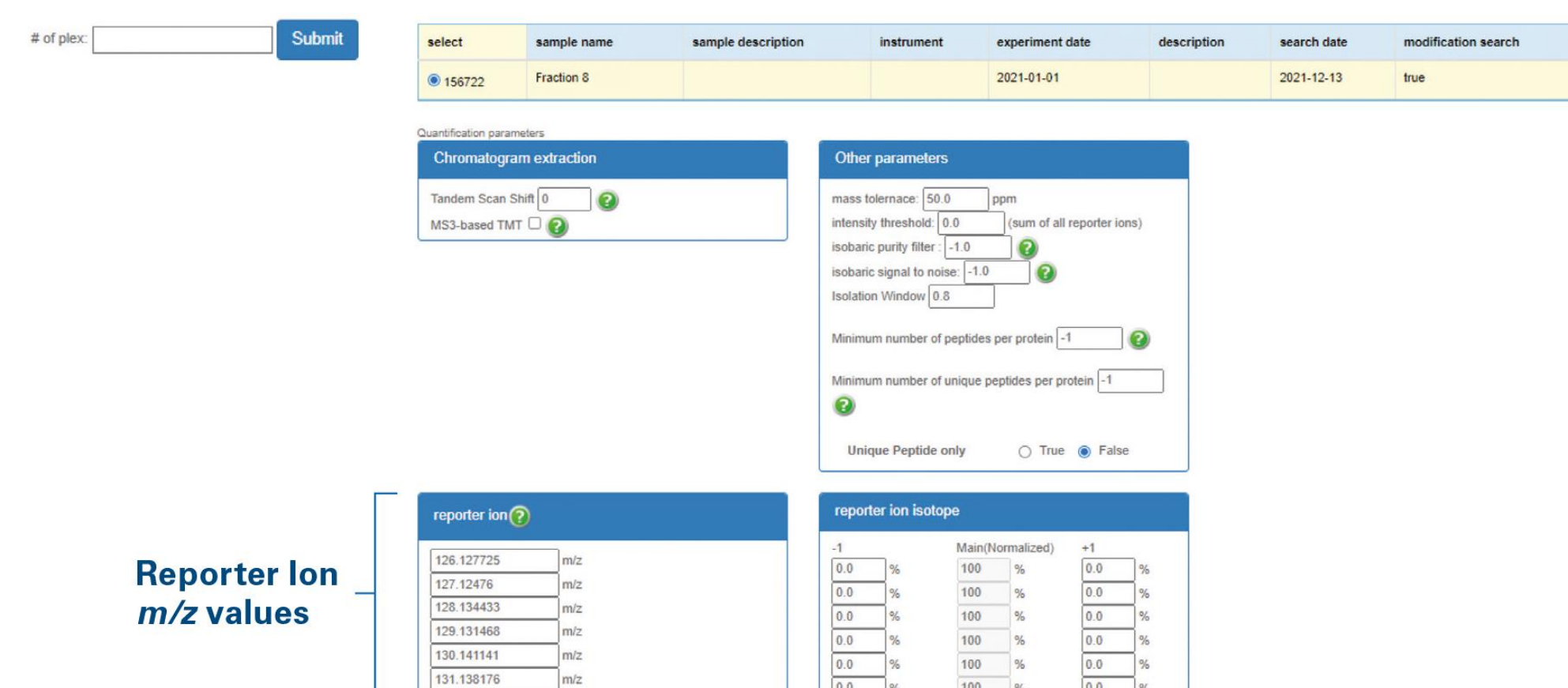


Figure 2 – Overview of Isobaric Tag Quantification Parameters in PaSER

Results

Data Set 1:

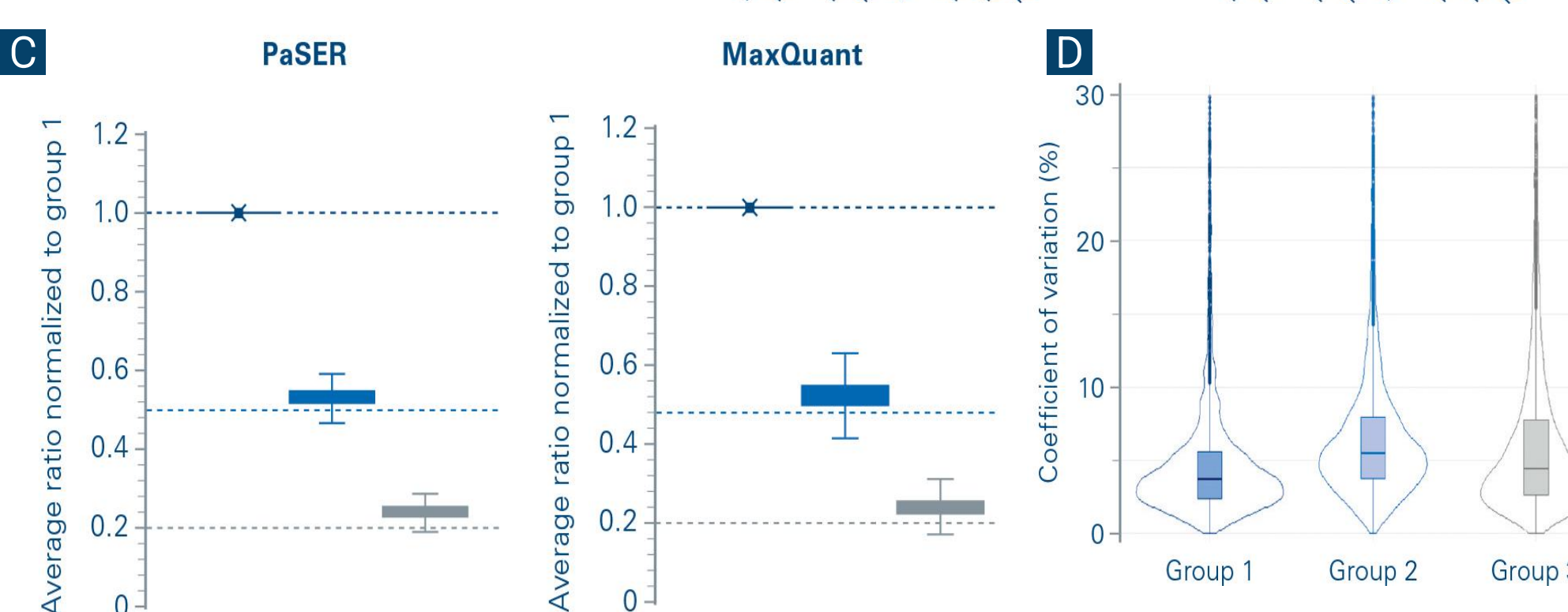
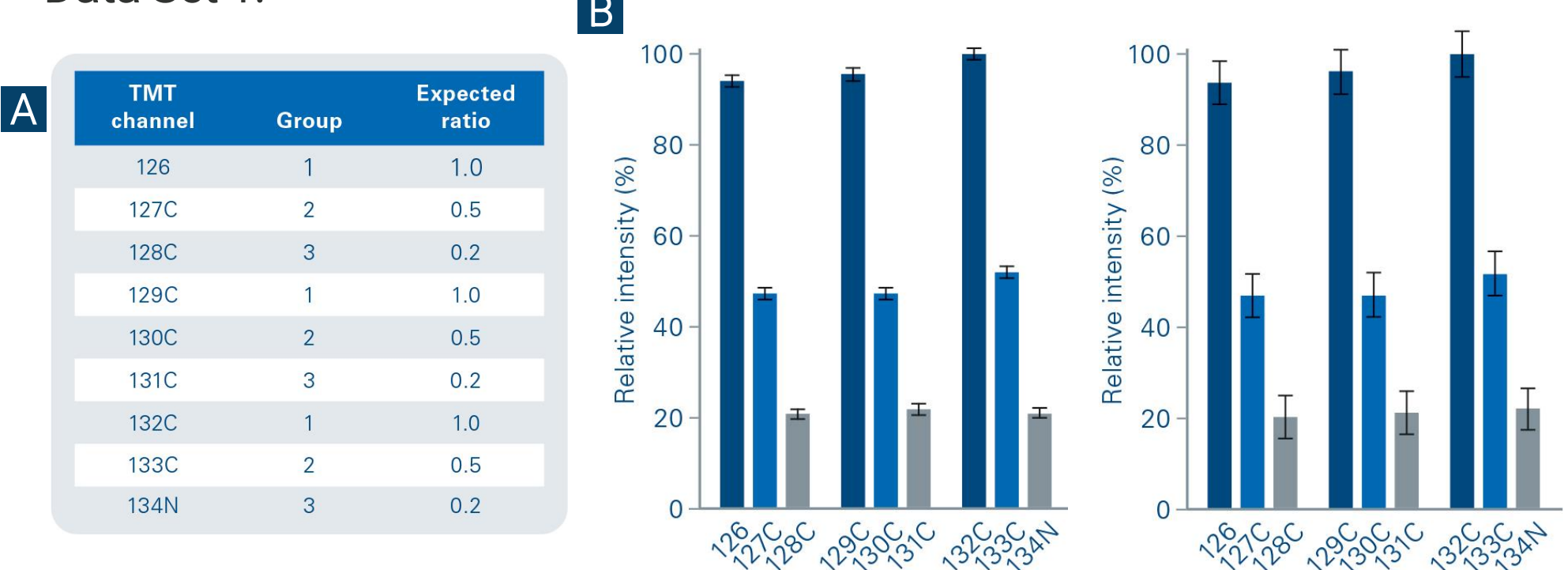
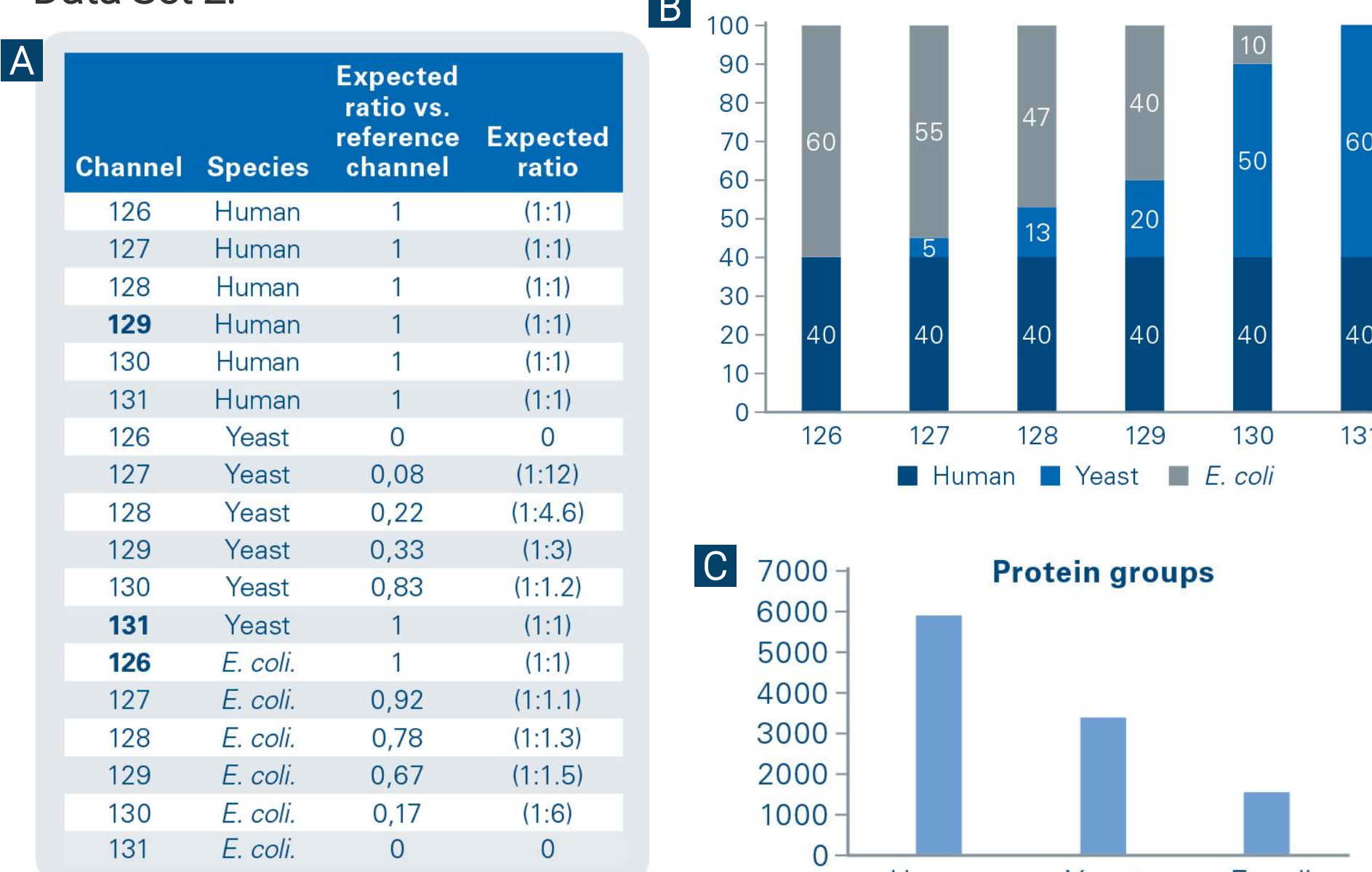


Figure 3 – Quantitative analysis K562 lysate labelled as a TMT 9plex and run on the timsTOF Pro 2 using a dda-PASEF workflow. A: Experimental design indicating groups and expected ratios. B: Relative intensity of each channel as quantified by PaSER or MaxQuant. Colours represent grouping of samples, 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.

Data Set 1 continued:

- 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

Data Set 2:



*Reference channels for each species are shown in

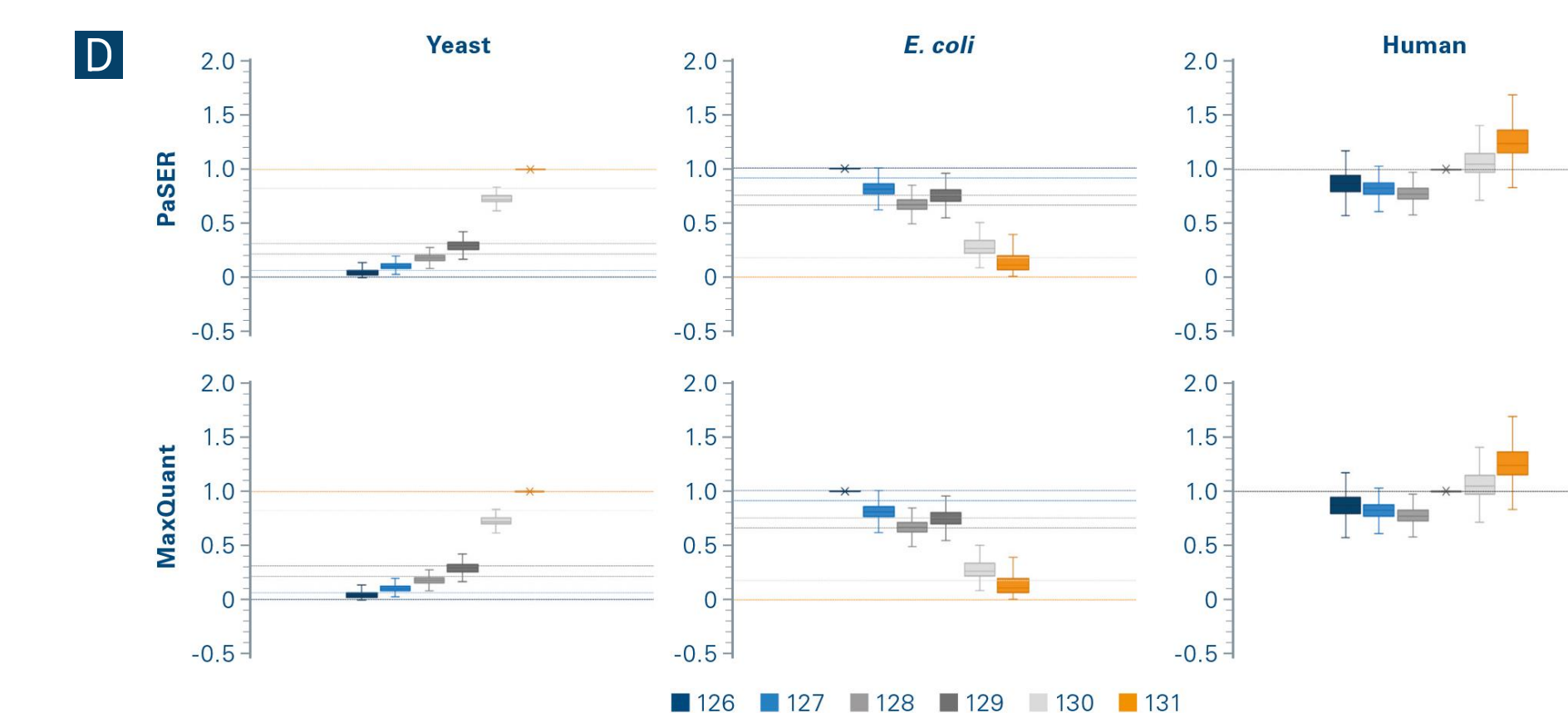


Figure 4 – TMT quantification and protein identification from a mixed species model. Human, Yeast and Bacteria were combined in 6 ratios to highlight large and small changes as indicated in the table (A), or graphically (B). C: Total protein identifications for each species are illustrated. D: For each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

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

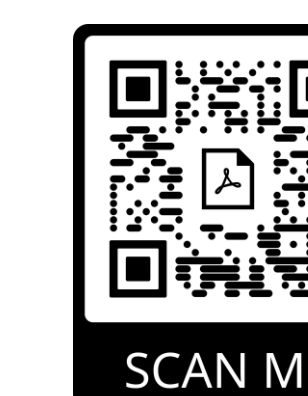
Summary

- 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

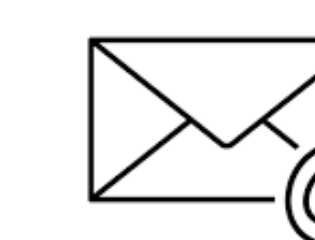
Conclusion

- 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