Streamlined sample processing coupled to PASEF strategy for in-depth proteome quantification

[1]Katrin Hartinger, [1]Nils Kulak, [2]Nagarjuna Nagaraj, [1]Fabian Hosp
[1]PreOmics GmbH, Martinsried, Germany | [2]Bruker Daltonic GmbH, Bremen, Germany

1 Introduction

Sample preparation is a critical component of the overall MS-based proteomics workflow. Here, we present automated sample processing for either label-free or chemical labeling samples coupled to a timsTOF Pro with parallel accumulation-serial fragementation (PASF) acquisition mode. We show deep proteome coverage for human plasma and accurate quantification of TMT ratios for mixed HeLa and yeast samples by reduced TMT ratio compression due to ion mobility separation.

2 Material & Methods

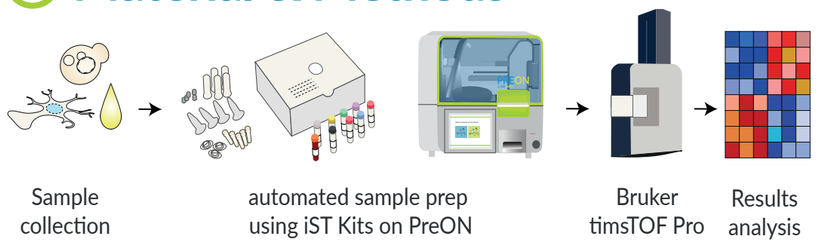

Fig. 1 | Non-depleted human plasma, HeLa and yeast samples were processed fully automated on the PreON platform using iST (LFQ) or iST-NHS (TMT) kits. 100 µg protein equivalents were used in quadruplicates. TMTsixplex:peptide ratio was 4:1. Data acquisition on timsTOF Pro with a 60 min method; data analysis using MaxQuant.

3 Results

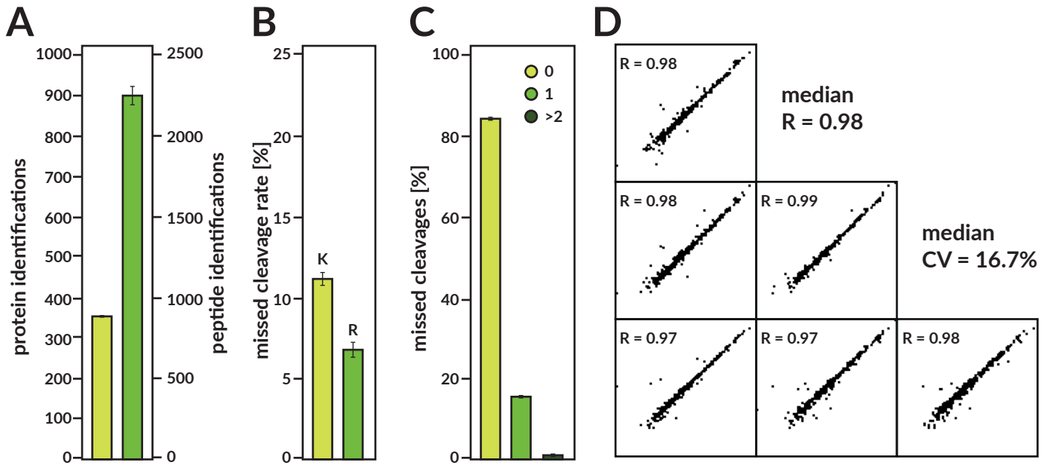

Fig. 2 | (A) Protein & peptide IDs for human plasma. (B+C) Percentage & number of total missed cleavages. (D) Technical variability of quadruplicates.

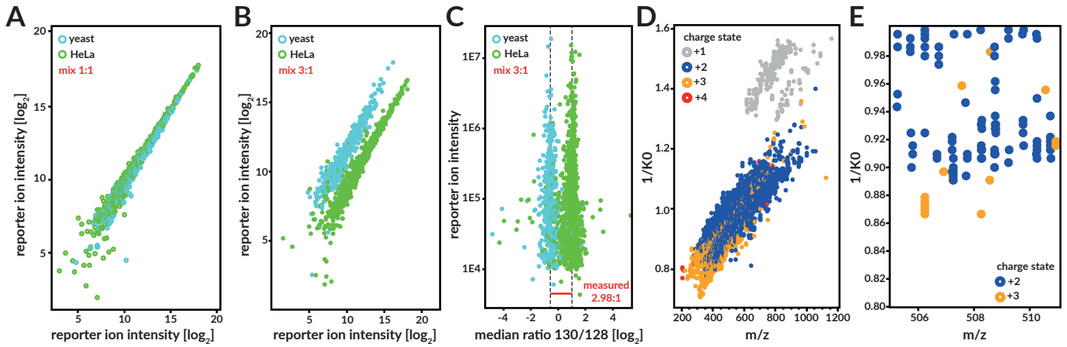

Fig. 3 | (A+B) HeLa & yeast mix experiments & ratio distribution close to expected ratio. (D+E) Benefits of PASEF for cofragmentation. Reduced ratio compression as ion mobility separates ions with the same m/z ratio.

4 Discussion & Outlook

We demonstrate identification of >350 proteins from non-depleted human plasma in single-shot MS runs using the timsTOF Pro with PASEF acquisition mode and excellent quantitative reproducibility and technical variability. Furthermore, PASEF enabled highly accurate quantification of TMT-sixplex samples close to the theoretical mixing ratio by reducing the TMT ratio compression.

We aim to automate sample processing for both label-free or chemical labeling applications coupled to PASEF acquisition for a wide range of sample types and biological questions.

5 Contact

www.preomics.com | hosp@preomics.com

PREOMICS