# High speed, high sensitivity and high reproducible and accurate label-free quantification using the PASEF method on a TIMS-QTOF

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**Gary Kruppa[1], Markus Lubeck[2], Heiner Koch[2], Verena Tellström[2], Paul Shan[3], Juergen Cox[4], Scarlet Koch[2]**

[1]Bruker Daltonics Inc., Billerica, USA [2]Bruker Daltonik GmbH, Bremen, Germany
[3]Bioinformatics Solutions Inc., Waterloo, Canada
[4]Max-Planck-Institute of Biochemistry, Martinsried, Germany

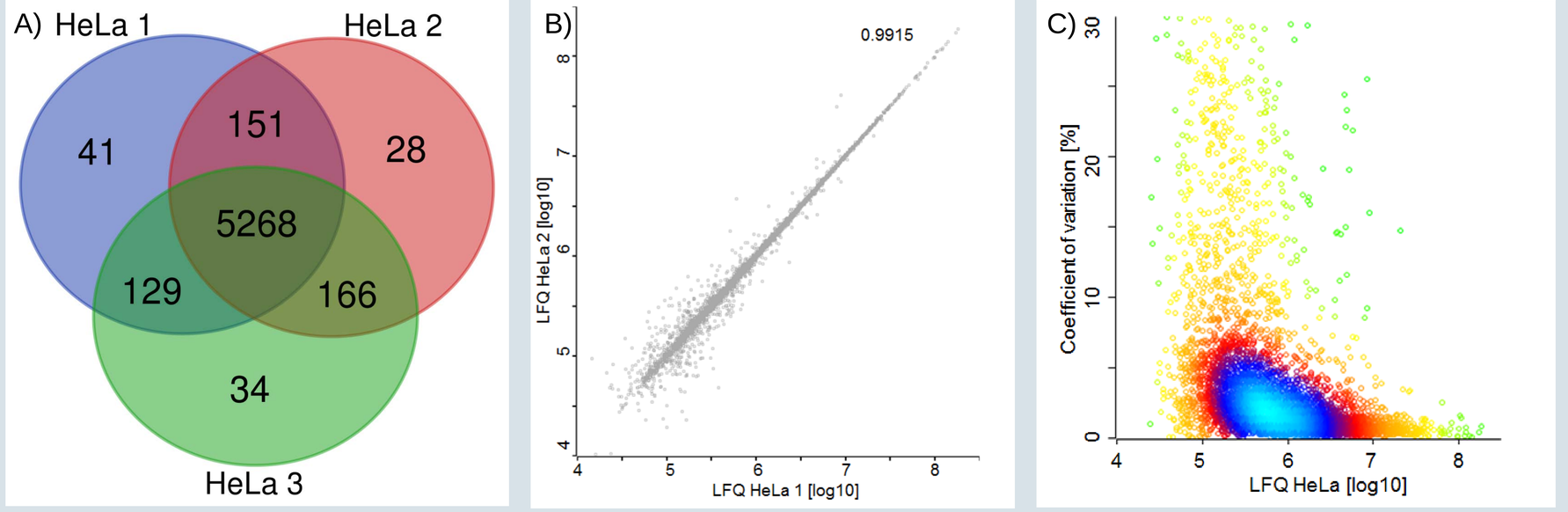


Fig. 2 High reproducibility with low sample amounts: A) Overlap (96%) of protein identifications of HeLa triplicate analysis using a 90 min gradient. Proteins that were identified with at least 2 peptides are considered here. B) LFQ intensities of two HeLa replicates. C) CVs of all three HeLa replicates.

## Introduction

Mass spectrometry (MS)-based proteomics has become a powerful technology for the identification and quantification of thousands of proteins. The quality of quantification is mainly depending on three different parameters: performance of the instrument, optimized acquisition methods and powerful data analysis software. We recently introduced the Parallel Accumulation Serial Fragmentation (PASEF) method (Meier et al., JPR 2015) on a TIMS-QTOF instrument, delivering high robustness, speed and sensitivity, which are very important when performing shotgun proteomics experiments. Here we show highly reproducible and accurate quantification using this instrument with the PASEF acquisition method and the software packages PEAKS and MaxQuant which have optimized several parameters of their algorithms for the processing of 4-dimensional PASEF data.

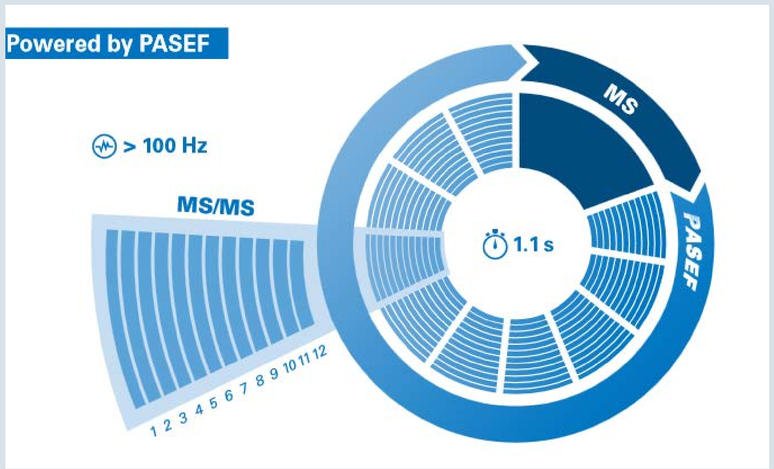


Fig. 1 The PASEF acquisition method: A PASEF cycle (total: 1.1s) consists of 1 TIMS MS scan (100 ms) and 10 PASEF MS/MS scans (each 100 ms) for shotgun proteomics experiments using a 90 min gradient. Per PASEF scan on average 12 different precursors are selected for MS/MS resulting in a sequencing speed of > 120 Hz.

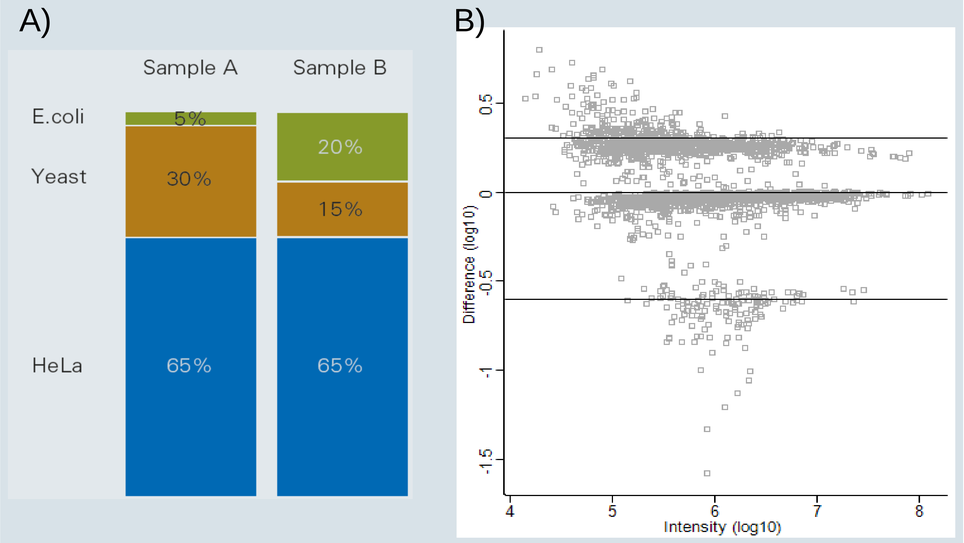


Fig. 3 Accurate label-free quantification: A) Overview of the experimental design. B) Protein level quantitative results of the spiked in experiment. Log-transformed ratios (sample A/sample B) of yeast, human and E.coli proteins are plotted over log-transformed LFQ intensities.
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## Methods

A nanoElute (Bruker Daltonics) nano-flow LC was coupled to a high-resolution TIMS-QTOF (timsTOF Pro, Bruker Daltonics) with a CaptiveSpray ion source (Bruker Daltonics). The peptide mixtures (≤ 200 ng) were loaded onto a 250 mm pulled emitter column (IonOpticks, Australia). Chromatographic separation was carried out using a linear gradient of 5-30% buffer B (100% ACN and 0.1% FA) at a flow rate of 400 nl/min over 90 min. LC-MS/MS data were acquired using the PASEF method with a total cycle time of 1.1 s, including 1 TIMS MS scan and 10 PASEF MS/MS scans. Data analysis was performed using PEAKS studio (Bioinformatics solutions Inc.) and MaxQuant (Jürgen Cox, Max Planck Institute of Biochemistry).

## Results

To investigate the quantitative performance of the PASEF method for shotgun proteomics, we first analyzed 200 ng of a complex peptide mixture derived from a mammalian cell line in a single-acquisition using a 90 min gradient and optimized the PASEF parameters accordingly. Best results could be achieved by using a cycle time of 1.1s including 1 TIMS MS scan (100 ms) and 10 PASEF scans (100 ms each) containing on average 12 MS/MS scans per PASEF scan (Figure 1). Deep proteome coverage could be achieved with more than 5300 protein families identified in each run with at least 2 peptides with good reproducibility when comparing the overlap (96%) of protein identifications between replicates (Figure 2A). A comparison of label free intensities between technical replicates showed excellent reproducibility with a R2 = 0.99 using a 90 min gradient (Figure 2B).

To evaluate the reproducibility of PASEF, we determined the coefficient of variation (CV) of the label free quantification (LFQ) intensities. For more than 98% of the quantified proteins the CV was smaller than 10% (Figure 2C). Accurate quantification of differentially expressed proteins remains challenging over a wide concentration range and profits from a robust analytical platform. For the evaluation of the accuracy we used a high-complexity protein mixture.

Background human proteins were spiked in equal amounts while E.coli and Yeast were spiked in 1:4 and 2:1 and measured in quaduplicates (150 ng injected) (Figure 3A). The results show that good accurate quantitation can be obtained with high sample complexity, dynamic range and even for low-fold changes in concentration of factor 2 and 4 (Figure 3B).

## Conclusions

- Extremely high sequencing speed (> 120 Hz) provided by PASEF
- High depth of proteome coverage even with low sample amounts (< 200 ng)
- High reproducibility in identification and label free quantification
- PASEF provides accurate label free quantification
- TIMS PASEF is therefore very well suited for large cohort and high complexity shotgun proteomics studies

timsTOF Pro