Single-cell equivalent sensitivity on timsTOF Pro with 35 minutes gradient time enabled by label-free reference run approach

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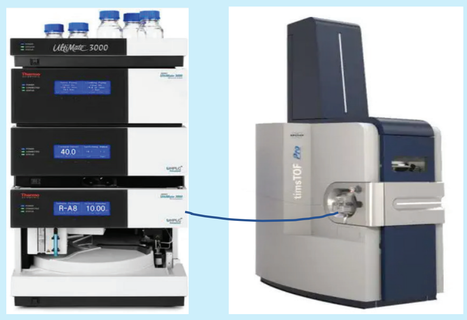
Introduction

Single-cell proteomics requires both high sensitivity and high sample throughput, i.e. rapid LC gradients with high scanning speed mass spectrometry. Besides the dedicated timsTOF SCP (Single Cell Proteomics) instrument, which was described in a recent preprint,[1)] the versatile timsTOF Pro instrument, which was designed for shotgun proteomics experiments using standard sample amounts,[2-3)] also offers a "high sensitivity" mode. We present a method that enables single-cell proteomics on this instrument, reasoning that concomitant search of reference samples could increase the sensitivity of peptide and protein detection in DIA (Data Independent Acquisition) measurements by the machine learning algorithms implemented in current DIA analysis software.

Methods

LC - MS



U3000 Separation

timsTOF Pro Identification & Quantification

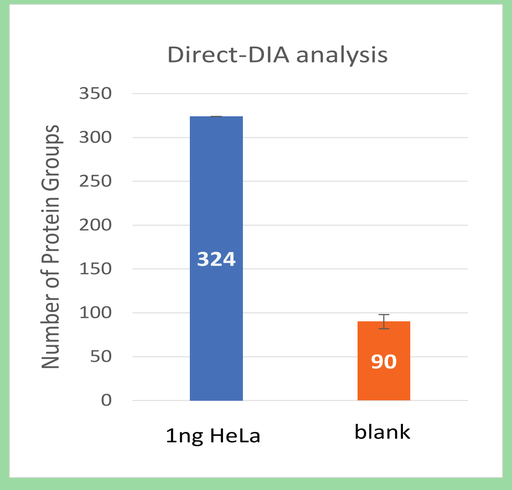
U3000 RSLC system
- Bruker Ten reversed-phase column
- 35 min net LC-gradient time
- direct injection (no trap column)
- UDP method for TFE rinse between LC-runs[4)]

timsTOF Pro mass spectrometer
- "high sensitivity" mode enabled
- data-dependent (DDA) vs. data-independent (DIA) analysis
- 1 MS + 8 DIA-PASEF scans (x 3 mobility windows), each 160ms

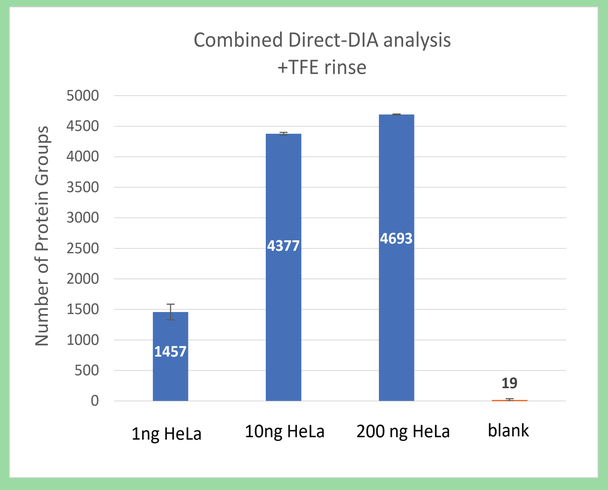
Data analysis software
- Spectromine 3.2 (DDA)
- Spectronaut 16 (DIA)

Samples
- commercial HeLa protein digest (Pierce)
- injected amounts: 200ng, 10ng, 1ng
- quintuplicate replicates (HeLa), triplicates (blank runs)

Results

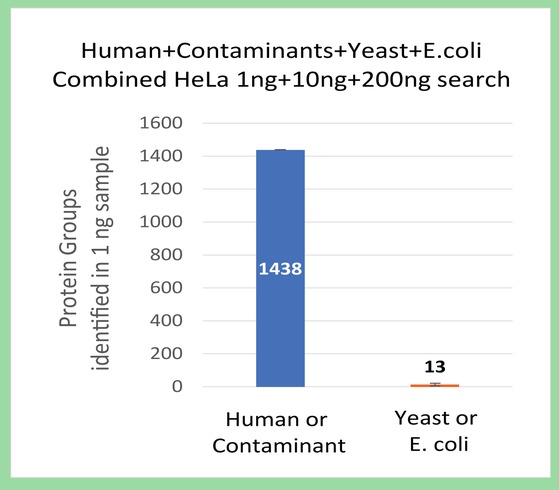


"Direct DIA" searches with Spectronaut identified on average 324 protein groups in 1ng of HeLa samples. However, a considerable number of spurious protein groups was also identified in blank runs. LC-gradient time was 35 min in all cases.

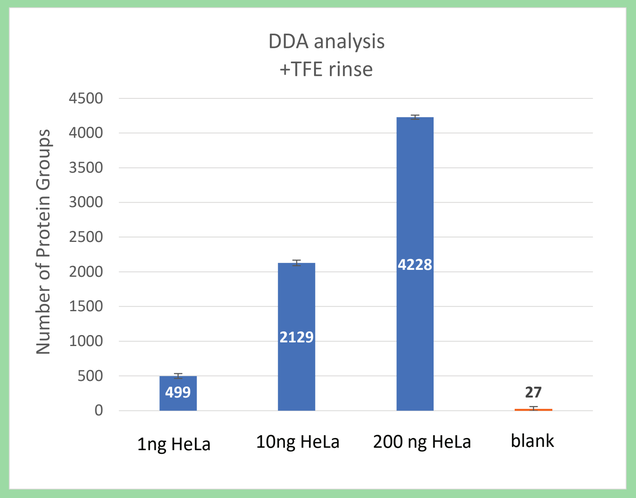


A combined Direct DIA analysis of 1ng together with 10ng and 200ng "reference" DIA runs acquired with the same 35 min gradient increased identifications to 1457 protein groups for the 1ng samples (ca 4.5-fold increase).

In addition, protein identifications in blank runs were strongly reduced by injecting a small amount of Trifluoroethanol (TFE) to wash the autosampler and the analytical column.[4)]



We also evaluated our "reference run" search strategy using a fasta database that comprised 10498 sequences from Yeast and E.coli in addition to 20715 human and contaminant sequences. The small number of spurious identification from the two organisms not present in the sample provides further support that results obtained by our reference run approach are valid.



Finally, we compared figures of merit of our DIA method to the alternative approach of DDA (data dependent acquisition). While the number of identified protein groups with 200ng HeLa in DDA analyses were only moderately smaller than with DIA, results indicate that for low sample amounts, our "DIA reference run" approach increases the number of identified protein groups more than twice. Data completeness was also superior with DIA (data not shown).

References

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Acknowledgements

This work was supported by WWTF grant LS20-079 "Closing the Loop in Hemodialysis" (Life Sciences 2020 - Precision Medicine) https://hd-research.net/