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Pipetting-free single cell analysis with the label-free proteoCHIP and the proteoCHIP Evo96 for high sensitivity proteomics on the timsTOF SCP and the timsTOF Ultra

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Introduction

Throughout the single cell proteomics sample preparation, it is critical to minimize protein losses due to limited amount of starting material. Improved sample preparation steps such as protein extraction, minimized exposure of samples to surfaces as well as optimized sample storage and transfer conditions are crucial for high-performance single cell proteomics with high protein identification and reproducible quantification. Recent enhancements in trapped ion mobility spectrometry (TIMS) coupled to fast and sensitive mass spectrometry established in the timsTOF SCP and further optimized in the timsTOF Ultra, as well as automated single cell isolation and sample preparation in the cellenONE® platform and fast and robust liquid chromatography with the Evosep One in Whisper mode or the nanoElute 2 with 150 – 250 nL/min flow rates for ultra-sensitive proteome analyses at the single cell level, result in unprecedented depth of proteome coverage for single cells.

Figure 1: Single cell proteomics workflow with transfer by centrifugation onto Evotips Pure with the proteoCHIP Evo-96 on the timsTOF SCP.

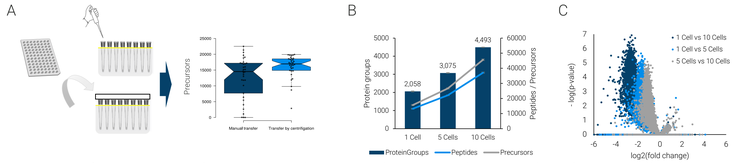


Figure 2: A) Comparison of manual transfer vs transfer by centrifugation of single HeLa cells. B) Protein group, peptides and precursor identification rates in 1, 5 and 10 HeLa cells (n = 4). C) Volcano plot showing expected protein group abundance increase from 1 cells vs. 5 cells, 1 cells vs. 10 cells and 5 cells vs. 10 cells.

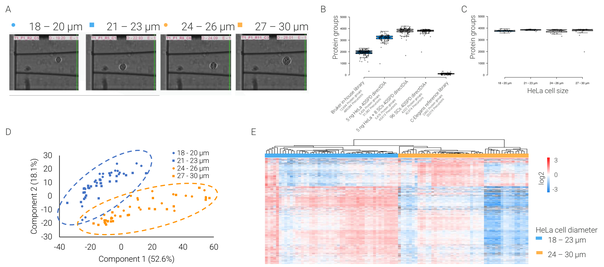


Figure 3: A) representative images of single HeLa cells of different cell diameter (24 per diameter group) isolated into the proteoCHIP Evo-96. B) Comparison of protein group identification rates employing different library and directDIA+ data extraction methods in Spectronaut 17. C) Protein group identification rates per cell diameter group using directDIA+ data extraction. D) Principal component analysis including 2,525 proteins identified in all 95 single cells. E) Heatmap showing t-test significant proteins (q-value<0.05) identified comparing single HeLa cells with cell diameters of 18 – 23 µm to cell diameters of 24 – 30 µm.

Single cell proteomics Ecosystems

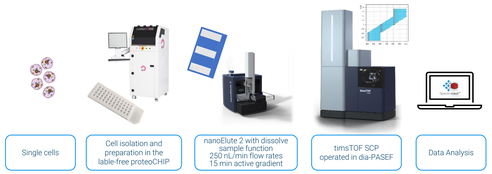


Figure 4: Single cell proteomics workflow with the nanoElute 2 dissolve sample function for pickup of lyophilized samples from the label-free proteoCHIP on the timsTOF SCP.

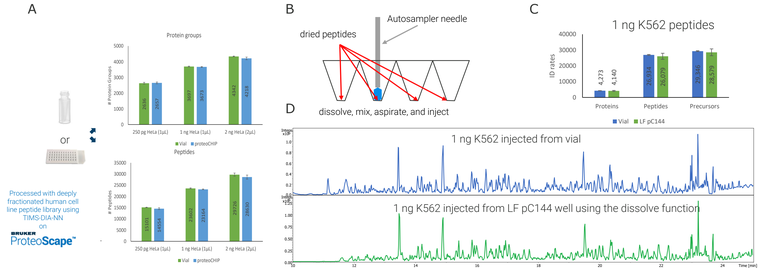


Figure 5: A) Comparison on protein group and peptide identification rates of different volumes and sample amounts injected of bulk HeLa digest dilutions either from autosampler vial or LF proteoCHIP. B) schematic of the dissolve sample function using the nanoElute 2 CTC autosampler arm. C) Comparison on protein group, peptide, and precursor identification rates of 1 ng K562 cell digest either injected from vial or resuspended with the dissolve sample function from the LF proteoCHIP. D) BPC of 1 ng K562 cell digest either injected from vial or resuspended with the dissolve sample function from the LF proteoCHIP.

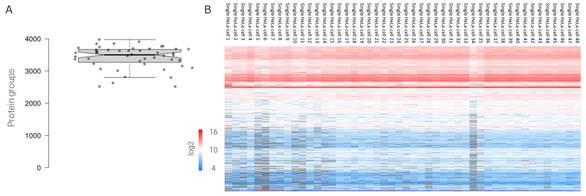


Figure 6: A) Plot of protein groups identified across 48 single HeLa cells injected from the LF proteoCHIP using the dissolve sample function using the nanoElute 2 CTC autosampler arm using Spectronaut 17 in directDIA+ with 4 ng HeLa reference runs. B) Heatmap showing protein group abundance pattern of protein identified in at least 24 of 48 single HeLa cell samples with good run to run reproducibility.

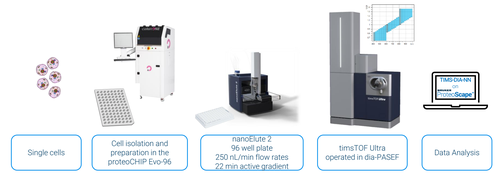
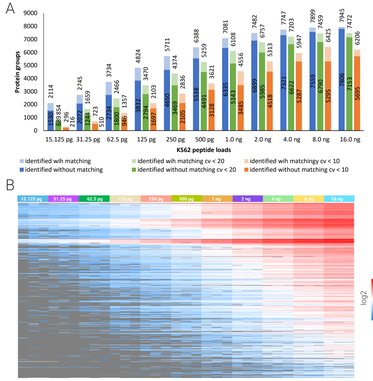


Figure 7: Single cell proteomics workflow with proteoCHIP Evo-96 transfer to 96 well plates, injection with nanoElute 2 on the timsTOF Ultra.



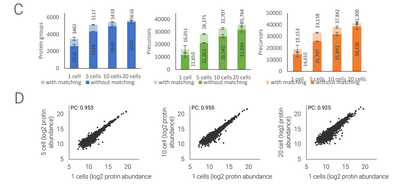


Figure 8: A) Protein group identification rats of a K562 peptide dilution series from 16 ng to 15.125 pg loaded on column, acquired in dia-PASEF mode and analyzed in TIMS-DIA-NN on Bruker ProteoScape, grouped by IDs total, CV < 20 and CV < 10 either with or without match between runs (MBR). B) Hierarchical clustering of protein groups quantified in the K562 peptide dilution series. C) Protein group, peptide and precursor identification rates of HeLa cells isolated at counts of 1, 5, 10, and 20 (n = 3 each) cells per well, acquired in dia-PASEF mode and analyzed in TIMS-DIA-NN on Bruker ProteoScape with or without MBR. D) Protein abundance correlation of 1 vs. 5 cells, 1 vs. 10 cells and 1 vs. 20 cells.

Conclusion

- Sample-loss reduced workflows using the proteoCHIP Evo-96 the LF proteoCHIP
- high sensitivity with good chromatographic reproducibility and robustness with short gradients 25, 30, and 31 min (Whisper 40 SPD) run time
- Good quantification accuracy at single cell level with good single cell to single cell reproducibility on protein level with protein depth of 3000 – 4000 protein groups per single cell

Single Cell Proteomics