Ultra-high sensitivity for targeted clinical proteomics using Evosep-MRM

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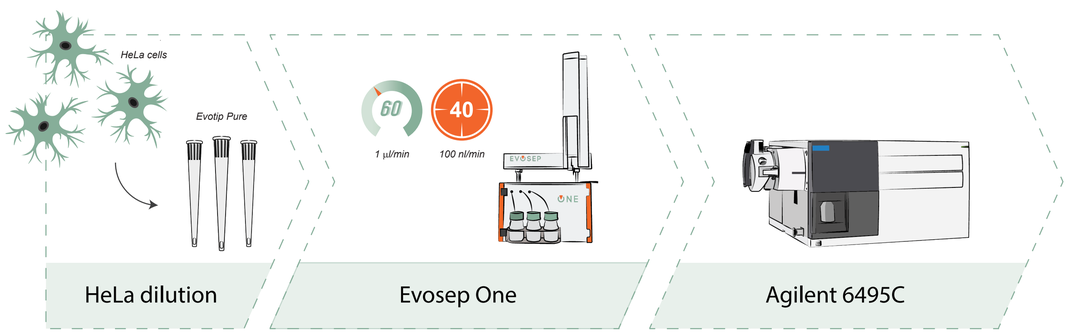
Highlights

- Combining the highly sensitive Agilent 6495C QQQ with Whisper methods on the Evosep One provides ultra-high sensitivity and represents an excellent workflow for targeted single cell proteomics.
- This approach allowed for quantification of HeLa proteins at concentrations down to 62 pg peptide load.
- This workflow supported detection and quantification of biologically relevant peptides across 100 single cells.

Effortless nanoflow with Whisper

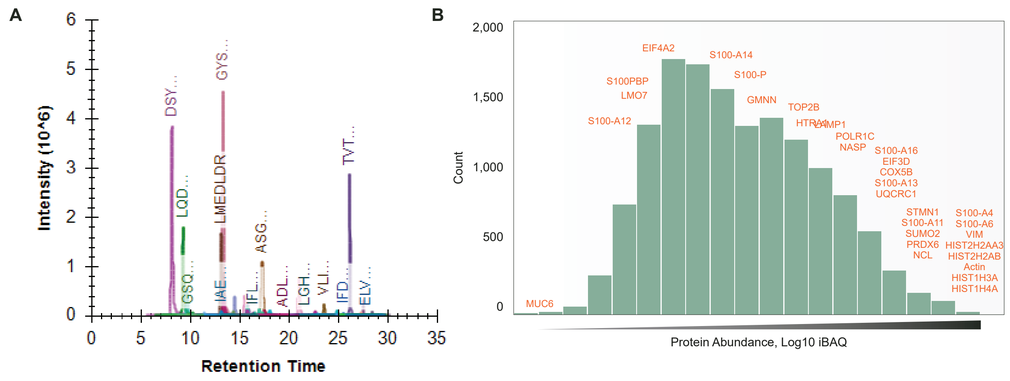
Sensitive targeted analysis

Using a targeted MRM we benchmarked the standard 60 SPD method (1µl/min) against our Whisper™ Flow Technology (40 SPD 100nl/min). A HeLa dilution series (62.5 pg, 125 pg, 250 pg, 500 pg, 1ng, 10ng ,100 ng) was used to compare the performance.



Standard proteomics workflow

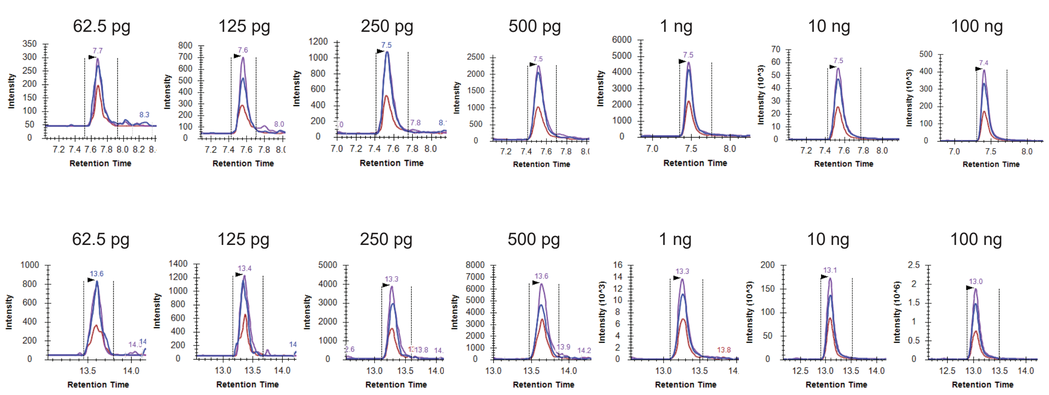
The MRM assay spanned a range of protein abundance as well as a comprehensive retention time profile, eluting across the duration of the gradient. Additionally the assay included proteins involved in cell cycle progression and tumorigenesis.



(A) Extracted chromatogram of all selected peptides (B) Abundance range of selected proteins

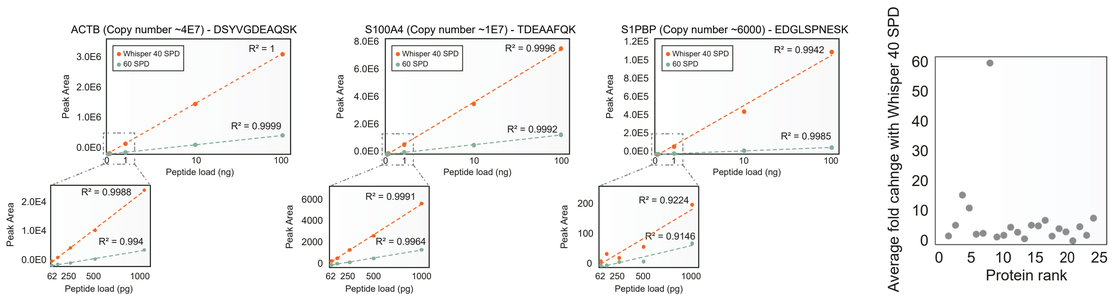
Boost in performance with Whisper

We evaluated the sensitivity of our workflow with a dilution series (62.5pg-100ng) of HeLa tryptic peptides analysed in triplicate. In this context the Whisper method supported greater sensitivity across all concentrations assessed.



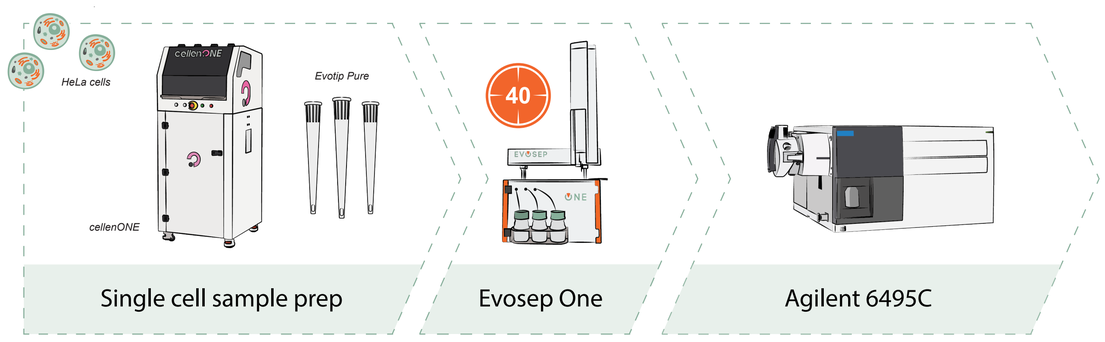
(A) Extracted chromatogram of all selected peptides (B) Abundance range of selected proteins

For all peptides assessed we found the average gain in sensitivity was a 4-fold increase in signal when using Whisper compared to 60 SPD.



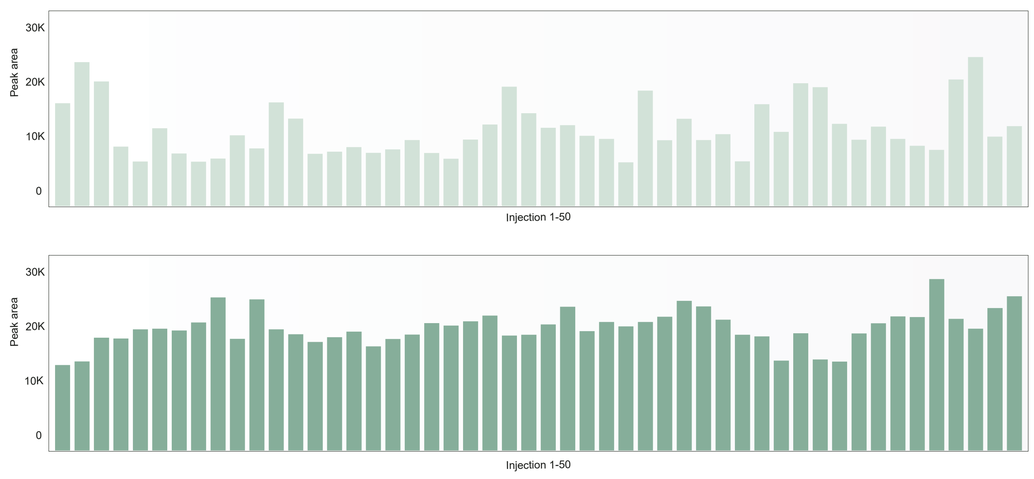
Sensitive single cell analysis

High sensitivity with Whisper Flow Technology



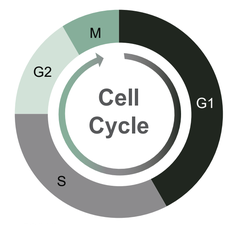
Single cell workflow for targeted proteomics

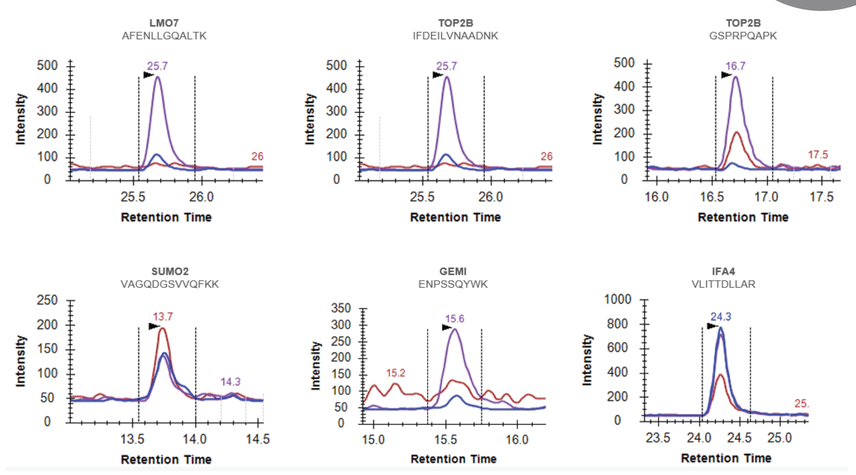
To illustrate the technical performance achievable when analysing pooled vs individually loaded Evotips, we plotted the intensity values associated with Actin peptide DSYVGEAQSK. In the pooled samples this peptide could be quantified with a CV of 14 % while the individually dispensed cells had a CV of 35 %.



Relevant biological targets

We were able to consistently detect 68 % of targets in the assay. Impressively this included very low abundant proteins that represent markers of cell cycle progression.





Additionally, we demonstrated the robustness of the Evosep One which supported quantification of 21 % of candidates from the pooled sample with a CV < 20 % and 50 % of peptides with CV < 35 %.