Fully automated, rapid, robust sample loading on Evotip Pure with the Agilent AssayMAP Bravo

1. Introduction

Automation and robotics have revolutionized the field of proteomics, offering solutions to the growing need for streamlined workflows and efficient sample processing. In this context, automated loading of Evotips has emerged as a pivotal advancement in sample preparation workflows. The Evotip can now be loaded using an innovative and fully automated strategy with the Agilent AssayMAP Bravo. The liquid handler is equipped with 96 probe syringes, which enables very fast sample loading on the Evotip, as a full box of Evotips are loaded in approximately four minutes with optimal performance. By precisely layering liquids within the Evotip and utilizing positive air pressure, this automated approach effectively addresses the sample preparation bottleneck. It ensures reproducibility and reliability in large-scale proteomics experiments, empowering researchers to process larger sample cohorts, optimize throughput, and enhance data quality. More-over, the automated loading strategy minimizes manual intervention and reduces the potential for errors. Alongside automated sample preparation, the Evosep One complements these advancements by facilitating robust and reproducible high-throughput analysis of large sample cohorts.

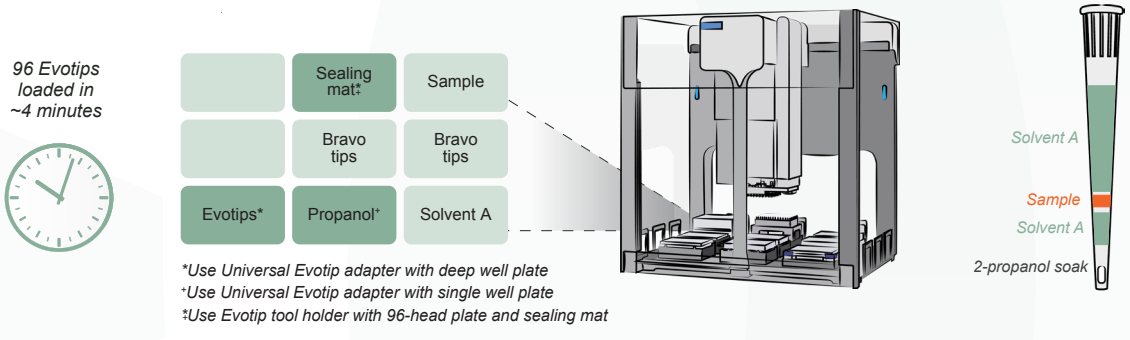

Figure 1: Workflow for automated Evotip loading using the AssayMAP Bravo.

2. Method details

HeLa peptides (Pierce) were aliquoted in appropriate concentrations in volumes of 30 µl and transferred to a 96-well plate (Eppendorf, 0030129512). The sample plate was placed in position 3 on the AssayMAP Bravo. 80 ml solvent A were transferred to a single-well plate (Thermo Scientific, 242811) and placed in position 9. An 'Universal Evotip adapter' was placed on top of a single-well plate (Thermo Scientific, 242811) with 80 ml Propanol and placed in position 8. Evotip Pure (Evosep, EV2011) were also inserted into an 'Universal Evotip adapter' and placed on top of a deep-well plate (Agilent, 201244-100) in position 7. Bravo disposable tips, 250 µl (Agilent, 19477-002) were placed in racks 5 and 6. An 'Evotip 96-head plate' and an 'Evotip 96-head sealing mat' are positioned in an 'Evotip tool holder' and placed in position 2 on the Agilent tip loading station. The AssayMAP Bravo was used to perform all liquid transfers and sample loading using the full head.

Reproducibility was measured using the 100 SPD method with an EV1109 column (Evosep) operated at 40 degrees. Samples were analyzed on a Zeno- TOF 7600 system using Zeno SWATH DIA and the OptiFlow Turbo V ion source with the 1-10 µ L/min microflow electrode in the vertical probe position. Sensitivity was measured using Whisper 40 SPD combined with the Aurora Elite column (IonOpticks, AUR3-15075C18-CSI) operated at 50 degrees on a timsTOF Pro 2 mass spectrometer (Bruker) with dia-PASEF. Data from each peptide load was processed independently using DIA-NN (version 1.8.1) in library-free mode against the reviewed human proteome (UniProt, Nov 2021, 20,360 entries without isoforms) with MBR (match-between-runs) enabled across technical replicates. Identifications represent protein groups and precursors as stated in the pr_matrix.tsv and pg_matrix.tsv files. Data was also analyzed with Skyline (21.2.0.425).

3. Reproducibility

Initially, we tested the robustness of the complete, automated protocol on the AssayMAP Bravo by loading a full box of 96 Evotips with 50 ng HeLa peptide and compared to 96 samples loaded manually with the standard loading protocol using a centrifuge. All 192 samples were measured in a randomized order with the 100 samples per day method. The data was evaluated based on the calculated peak area of a set of peptides, specifically comparing reproducibility within the two sample loading protocols and investigate if any analytical bias was observed across pipetting rows or columns on the AssayMAP Bravo. The selected peptides elute throughout the gradient and represent a balanced distribution in abundance covering more than six orders of magnitude in dynamic range, as visualized by peak area intensities. Each box represents 96 measurements and show an overall low degree of variation across

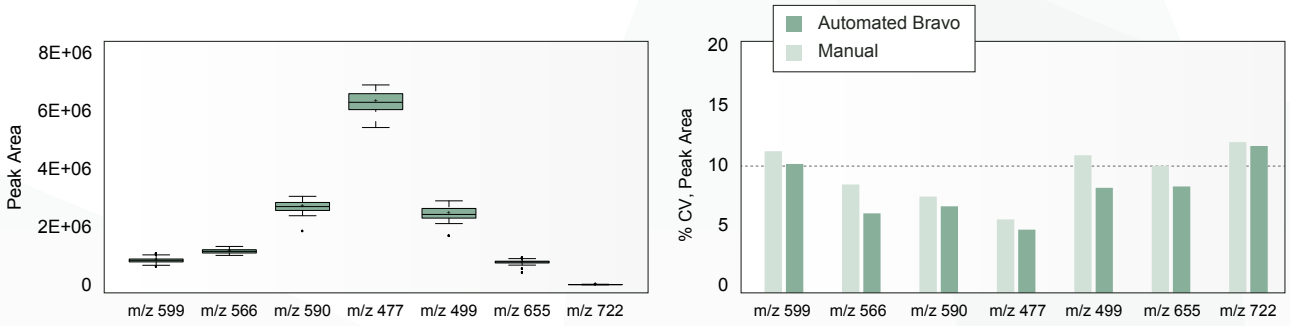

Figure 2: Boxplot of peak area for selected peptides and associated coefficient of variation (CV) for the two sample loading protocols.

all peptides. The reproducibility was quantified by calculating the CV of peak areas in both datasets. This revealed similar performance with slightly better CVs in the automated protocol with an average CV of 8.2%, compared to the manual protocol providing an average CV of 10% for the seven peptides (Figure 2). We investigated if the automated loading protocol introduces a bias across rows or columns during the protocol. However, the data showed that the reproducibility was not affected by the Evotip position with a median CV of 6.5% (Figure 3). This comparison further reinforced the reliability and precision of the automated loading protocol.

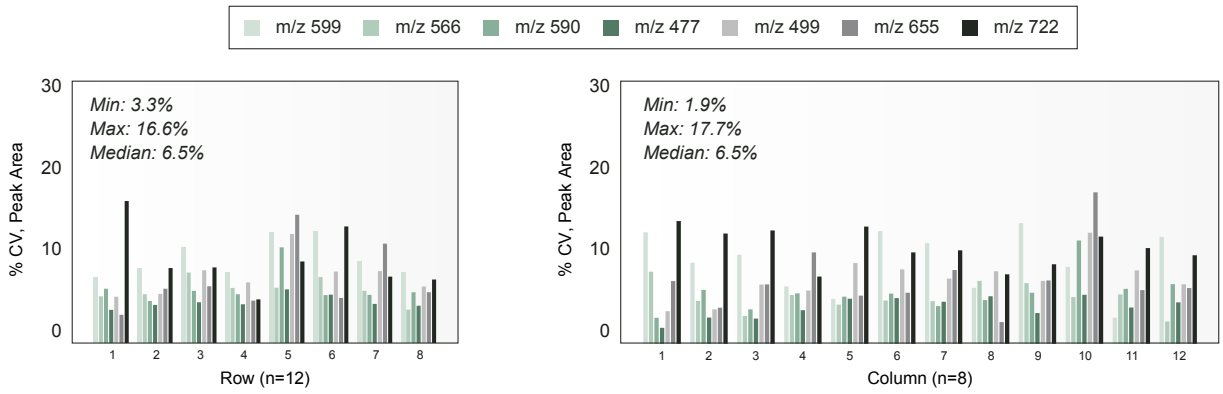

Figure 3: Reproducibility of peak areas across rows and columns with the automated AssayMAP Bravo loading protocol.

4. Sensitivity

To benchmark the sensitivity of the automated AssayMAP Bravo loading protocol, a quantitative assessment of a HeLa dilution series was performed in quadruplicates with loads from 25 ng and serial diluted down to 100 pg with a total of nine dilutions. The samples were measured with the Whisper 40 SPD method on a timsTOF Pro 2. The dilution series showed reproducible performance across all loads based on identified precursors across the gradient. This confirms that the sample loading is reproducible, even at low peptide amounts. Close to 3,000 precursors were identified from the 100 pg load with a linear increase of identifications to nearly 63,000 precursors identified from the 25 ng load leading to 6,400 identified proteins (Figure 4). Importantly, the quantification at the precursor level revealed robust performance with improved CVs as the load increases, but even for the low pg loads, data showed that sensitivity is maintained during the automated loading protocol on the AssayMAP Bravo (Figure 4).

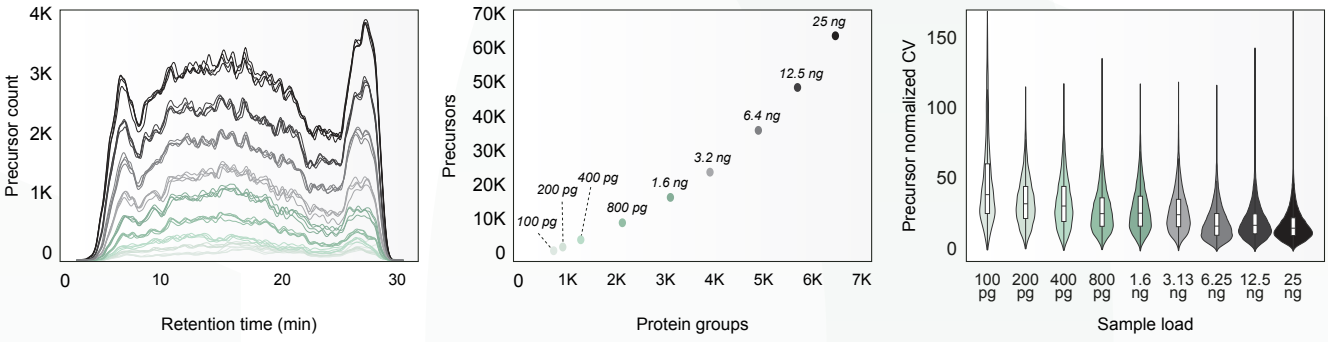

Figure 4: Whisper 40 SPD dilution series visualized as identified precursors across the gradient, total identifications and precursor CV.

4. Conclusion

Automation is becoming a critical factor for realizing large-scale applications in proteomics. With the implementation of a complete automated Evotip loading protocol on the Agilent AssayMAP Bravo platform, we have enabled a fast and efficient workflow with the possibility to load 96 Evotips in approximately four minutes. The assessment of the protocol revealed excellent reproducibility, where the automated protocol showed slightly more robust quantification with a median CV of 8.2% compared to the manual protocol with a median CV of 10%. This reinforces the efficiency of the automated loading protocol to achieve consistent and reliable results. Additionally, the presented method shows excellent sensitivity with reproducible sample loading down to 100 pg, ensuring accurate and reliable performance even when sample amounts are limited. This advancement empowers researchers to process larger sample cohorts, optimize throughput and improve data quality, ultimately accelerating discoveries in the intricate realm of proteins and their biological functions.

Availability of the automated AssayMAP Bravo loading protocol

An ‘Evotip loading kit (Bravo)’ (EV1165) is required to run the protocol. The kit consists of two ‘Universal Evotip adapter’s, an ‘Evotip 96-head plate’, an ‘Evotip 96-head sealing mat’ and an ‘Evotip tool holder’. The protocol for the AssayMAP Bravo can be found online at www.evosep.com/support/automation.