Automated Platform for the Discovery of Novel Biomarkers for Early Cancer Detection Using Olink Technology

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Abstract

Automated next-generation proteomics ensures data quality and high throughput, which are crucial for protein biomarker discovery. Protavio has developed a biomarker discovery pipeline that integrates the Olink Explore platform, bioinformatics, and Hamilton automation to discover novel protein biomarkers for early cancer detection. In this poster, we present the results of a concordance test as well as a feasibility study for discovering new biomarkers for early Colorectal Cancer (CRC) detection, obtained through the use of these technologies.

Background

Based in Athens, Greece, Protavio Ltd specializes in multiplex proteomics, with over 20 years of experience and more than 150 biomarker projects. Protavio's vision is to improve people's health through the discovery of novel biomarkers and to develop high-quality in-vitro diagnostic (IVD) assays. To achieve this, Protavio harnesses the power of the latest state-of-the-art technologies in the market. The Olink Explore platform positions Protavio as a leader in proteomics, offering a highly sensitive method for high-throughput protein biomarker discovery requiring only low volumes of serum, plasma, or nearly any other type of biological sample. This technology, combined with advanced bioinformatics and Artificial Intelligence (AI), provides a sophisticated solution for scientists in disease and drug development. The high sample throughput requires automation, and Hamilton liquid handlers emerged as the perfect candidate for the Olink methodology. Protavio automated part of the Olink Explore workflow on the Hamilton Microlab® STAR System, enhancing efficiency and reproducibility in biomarker discovery.

Methodology

Workflow

The Olink Explore protocol is a highly-multiplexed proteomics platform for biomarker discovery, combining the Proximity Extension Assay (PEA) technology with Next-Generation Sequencing (NGS) to measure protein biomarkers. The Hamilton Microlab® STAR automates critical steps, ensuring precision, reproducibility, and throughout. **Figure 1** outlines the protocol steps, emphasizing the automation aspect.

Concordance Test

A concordance test was preformed to validate the pipeline by comparing data, generated with the same set of samples, at AS Uppsala (collaborator) and Protavio, using 50 samples on the automated Olink Explore platform (Inflammation panel). Metrics such as detectability, Coefficient of Variance (CV), correlation, and regression were evaluated to ensure site concordance.

Feasibility Study for CRC

The feasibility of the pipeline for early CRC detection was assessed by analyzing plasma from: individuals with colorectal cancer, those with advanced adenomas, and healthy individuals[1]. In the advanced adenoma group, samples were collected both before and one month after colonoscopy. Expression of 384 oncology biomarkers was measured. Data normalization and protein expression analysis adhered to Olink's pipeline, employing general linear models. The analysis compared CRC and healthy samples to assess CRC biomarker identification, while analysis before and after adenoma resection on the same individual (isogenic analysis) revealed potential biomarkers for advanced adenomas.

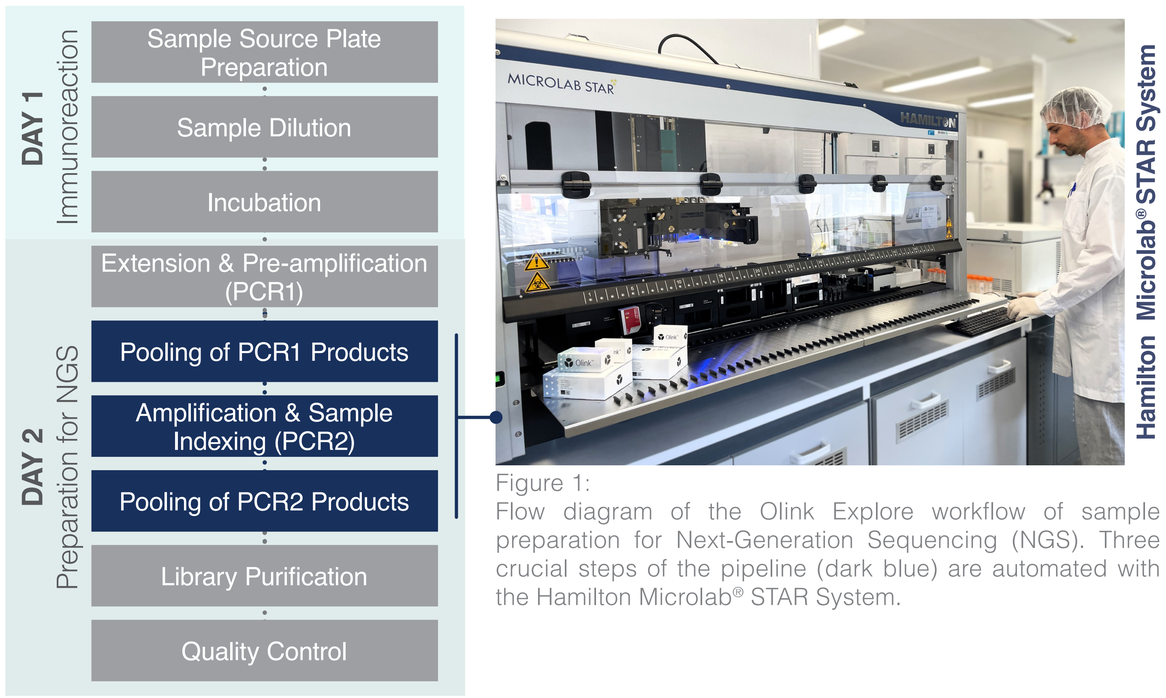

Figure 1:
Flow diagram of the Olink Explore workflow of sample preparation for Next-Generation Sequencing (NGS). Three crucial steps of the pipeline (dark blue) are automated with the Hamilton Microlab® STAR System.

Results

Concordance Test Results

All quality control criteria were successfully passed with the automated pipeline, including the Limit of Detection (LOD), the intra-assay Coefficient of Variance (CV), and the mean and media correlation coefficients (r) between Protavio and AS Uppsala (**Table 1** and **Figure 2**).

Feasibility Study Results

Significant differences in protein expression between CRC and healthy individuals were observed. **Figure 3A**'s volcano plot highlights protein biomarkers with statistically significant differences in expression levels between the the groups, suggesting their potential for inclusion in a blood-based CRC test. To confirm that these biomarkers are related to CRC and not just donor-to-donor variability or confounding effects, plasma samples were collected from the same individuals after tumor removal. **Figure 3B** presents the results of the isogenic analysis of samples both before and after adenoma resection, showing numerous biomarkers with reduced expression post-resection. This reduction supports the hypothesis that removing advanced adenomas leads to detectable changes in plasma proteomics. These biomarkers may indicate advanced adenomas, further validating their potential for early detection of precancerous conditions.

Metric	AS Uppsala	Protavio	Reference
Average LOD	93.6%	94.3%	>85%
Average intra-CV	10%	12.3%	<15%

Table 1:
Summary of Concordance Test results, LOD and CV, between the two sites. The reference criteria for satisfying the concordance test are also shown in the table.

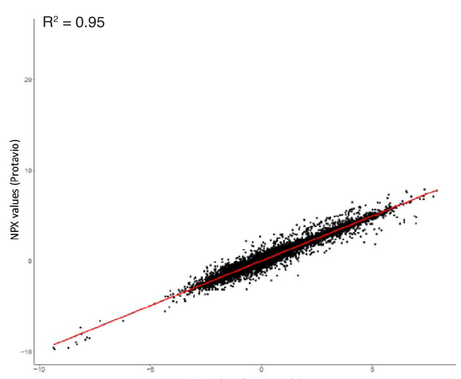

Figure 2:
Scatter plot of Normalized Protein Expression (NPX) values from AS Uppsala vs. Protavio with a regression line fitted to present the linear relationship between the two sites.

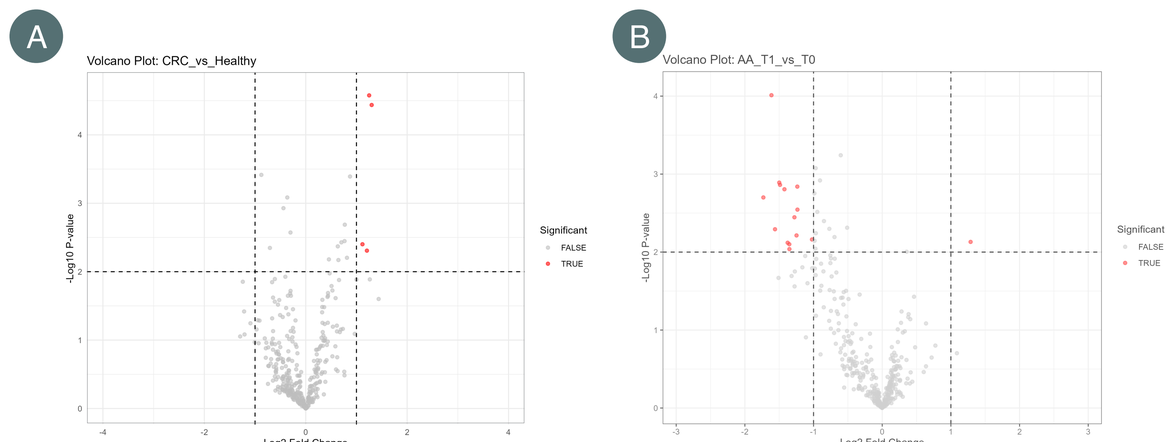

Figure 3:
A) Volcano plot illustrating the differential protein expression between healthy individuals (n=19) and colorectal cancer (CRC) patients (n=21). Significant biomarkers highlighted in red are potential candidates for a CRC screening test. **B)** Volcano plot depicting the results of the isogenic analysis comparing plasma samples before (T0) and after (T1) advanced adenoma resection (n=15). Significant biomarkers highlighted in red are potential candidates for advanced adenoma detection.

Conclusion

The automated Olink-Hamilton platform is a robust and scalable solution for advancing biomarker discovery and cancer research, significantly contributing to the development of more effective diagnostics and personalized therapeutic strategies. The automation of part of the Olink Explore workflow with the Hamilton Microlab® STAR system enhances the platform's efficiency and reproducibility, making it well-suited for large-scale proteomic studies and biomarker discovery projects. The Concordance Test validated the pipeline's performance, demonstrating excellent accuracy and reliability. The Feasibility Study further highlighted the platform's potential application in a real-world clinical setting, successfully identifying protein biomarkers for CRC and advanced adenoma detection. These findings underscore the platform's promise in supporting early detection efforts and improving patient outcomes through more targeted screening approaches.

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Reference:
[1] https://doi.org/10.1186/ISRCTN15583857