TECHNICAL NOTE
PROTEOMICS

Multiplex high-throughput proteomics with exceptional analytical specificity

- Leverage Olink Explore and Illumina sequencing systems to perform high-throughput protein profiling for biomarker and drug discovery studies
- Detect ~3000 proteins simultaneously, including circulating and intracellular proteins, from various sample types with low input volume requirements
- Analyze proteomics data and perform biostatistical visualization with integrated Olink software packages

In collaboration with

Introduction

Large-scale proteomics studies are being increasingly applied to detect and characterize differential protein expression patterns in health and disease.[1-3] Proteomics has the potential to provide crucial insights for biomarker discovery and drug development. In fact, proteins are the primary target of nearly all drugs currently in development.[4] However, conventional proteomics assays are constrained by a lack of sensitivity, particularly for low abundance proteins, and inability to detect proteins over a wide range of concentrations.[5] Sensitivity is key for analyzing plasma and serum samples in which only 22 proteins make up approximately 99% of the total protein mass, with protein concentrations spanning over 10 orders of magnitude.[6]

Researchers can now expand the discovery power of their existing Illumina next-generation sequencing (NGS) systems by leveraging Olink proximity extension assay (PEA) technology.[7] Proteomics coupled with an NGS readout allows researchers to perform high-throughput proteomics in parallel with genomics and transcriptomics to connect genotype to protein expression and accelerate multiomics research. This technical note outlines a protocol to detect thousands of proteins representing major biological pathways simultaneously with high sensitivity and exceptional analytical specificity.[8,9]

Olink PEA technology

Olink PEA technology is an innovative immunoassay detection method that uses a dual recognition system to detect proteins in solution with high analytical specificity of 99.7%.[10-12] Each protein is targeted by two antibodies conjugated with complementary DNA oligonucleotides that are unique to the protein. After the correct pair of antibodies binds to their target protein, their complementary DNA oligonucleotides come into close proximity to each other and hybridize to form a double-stranded DNA oligonucleotide. The DNA oligonucleotide is then extended and amplified using sample-specific primers. Thus, the DNA "barcode" is unique to each sample-protein combination, enabling the analysis of multiple samples and proteins simultaneously (Figure 1).

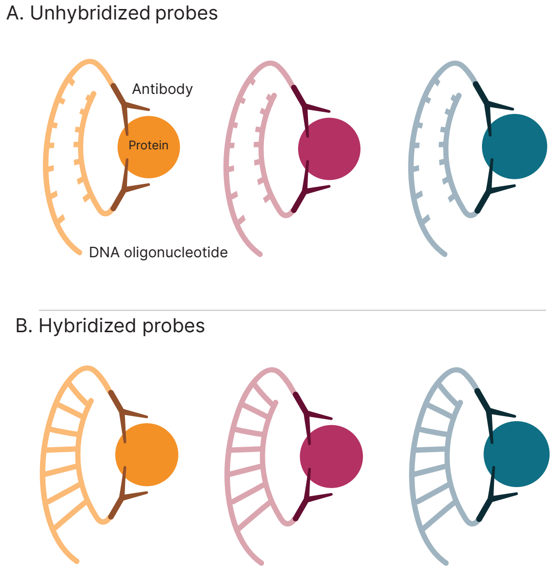

Figure 1: Olink PEA technology—(A) Antibodies conjugated with DNA oligonucleotide probes bind to target proteins in solution. Each color represents the correct pair of antibodies binding to a unique protein. (B) Complementary DNA oligonucleotides in close proximity hybridize to form double stranded DNA oligonucleotides. This DNA barcode is unique to each protein, allowing multiple proteins to be detected with high sensitivity and analytical specificity of 99.7%.

Unlike conventional immunoassays that produce false signals from nonspecific antibody binding, Olink PEA technology offers scalable multiplexing capability without loss in analytical specificity or sensitivity. Analytical specificity validation for Olink Explore 3072 demonstrated that 99.7% assays tested exhibited no cross-reactivity according to the tests described.[9]

Low sample requirements

Olink Explore 3072 is available as eight modular panels, targeting nearly 3000 human proteins in up to 384 samples simultaneously, while consuming only 6 µl* per sample of various sample types. The low sample consumption makes this assay an ideal solution for samples that are hard to obtain or have limited volume.[13]

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* 6 µl represents the sample consumption volume. The actual volume in the plate will be higher due to the automated workflow.

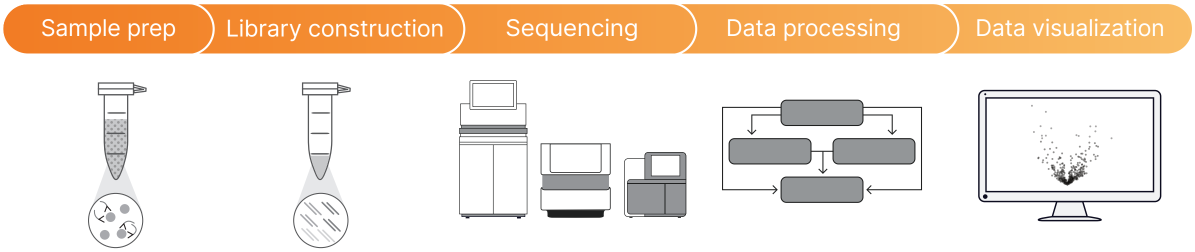

Figure 2: Olink Explore with NGS readout workflow—Olink PEA technology is part of an integrated sample-to-data workflow that includes NGS readout for high-throughput proteomics. Samples are incubated with antibody probes, DNA barcodes are amplified by PCR, and amplicon libraries are prepared for sequencing on Illumina sequencing systems. Data are analyzed and visualized using Olink analysis software packages.

Olink Explore 3072 covers all major biological pathways. Importantly, these proteins include intracellular, surface, and secreted proteins, unlike other NGS-compatible proteomics platforms that only analyze surface proteins. Each panel targets a unique set of proteins related to specific biological pathways and research areas, such as cardiometabolism, neurology, inflammation, and oncology. These modular panels can be mixed and matched to provide a flexible proteomics solution.

Protocol Overview

The automated workflow for Olink Explore with NGS readout consists of an immuno-reaction, DNA amplification, library preparation, sequencing, and data analysis (Figure 2). The unique dual antibody recognition and signal amplification of Olink PEA technology, coupled with high-throughput readouts of Illumina NGS systems, result in parallel detection of thousands of proteins using minimal sample volume with exceptional sensitivity and analytical specificity.[8] Olink libraries are compatible with all Illumina sequencing systems and flow cells. However, the sequencing throughput has been optimized for NovaSeq™ 6000, NextSeq™ 2000, and NextSeq 550 systems (Table 1).

Immuno-reaction

Olink Explore accommodates a wide range of samples, including serum, plasma, and cerebrospinal fluid (CSF). Samples are first diluted based on protein abundance and incubated with antibody probes overnight at 4°C. During the incubation step, the paired antibodies bind their target protein and the complementary oligonucleotides hybridize to form double-stranded DNA.

Library preparation

The assay barcode is amplified via PCR and different sample dilutions are pooled. Compatible sequencing adapters and sample-specific indexes are then added to the amplicons via a second PCR step and pooled per Olink Explore panel. At this point, each Olink Explore panel analyzing up to 384 samples is pooled into one tube. Samples can be stored safely for up to two weeks at -20°C after each pooling step.

Library quality control and sequencing

To prepare the library for sequencing, amplicons are purified using AMPure beads (Beckman Coulter, Indianapolis, IN) and undergo quality assessment before normalization (Figure 3). Quantification of the amplicons is not required. If sequencing cannot be performed immediately, the purified amplicons can be safely stored for up to four weeks at -20°C.

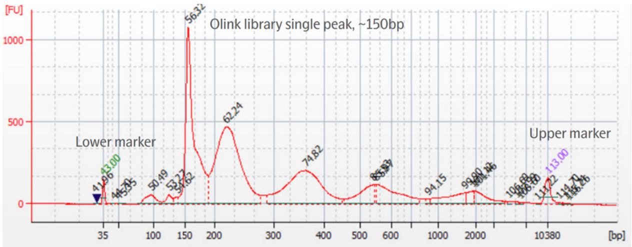

Figure 3: Representative electropherogram for assessing DNA amplicon quality—The highest peak at ~150 bp represents library size. Larger peaks called 'bubble products' will not interfere with sequencing. Data were collected using the Agilent 2100 Bioanalyzer instrument.

Illumina sequencing system parameters for Olink Explore 3072 with NGS readout[a]

Feature	NovaSeq 6000		NextSeq 2000	NextSeq 550
Flow cell type[b]	S4	SP	P2[b]	High-output
Sequencing runs[c]	1	2	8	8
No. of flow cells[b]	2	4	8	8
Kit size[d]	35 cycles	100 cycles	100 cycles	75 cycles
Samples per run	384	96	96	96
Data points per run[e]	1.13 million	141,312	35,328	35,328

a. Table displays Illumina sequencing systems and flow cells with optimized sequencing parameters to run Olink libraries
b. Selected flow cells are optimized for data output with lowest cost per data point, P2 flow cells also available on the NextSeq 1000 system
c. Number of assays per lane remain the same, however, the number of samples per lane depends on the throughput of the flow cell lane and fewer lanes are needed if analyzing less than the 8 panels included in Olink Explore 3072
d. Olink uses the smallest sequencing kit available from Illumina per flow cell
e. Data points are calculated by multiplying the number of detected proteins by the number of samples

Instructions for library normalization and instrument loading are available for NovaSeq 6000, NextSeq 2000, and NextSeq 550 systems on the Olink support website. Sequencing is performed as single read using a custom recipe to maximize throughput. Olink does not recommend including a control library (PhiX) spike-in. Olink will install and support the custom recipe, which will automatically populate the read length. Olink Explore is compatible with a range of Illumina sequencing systems to support your research needs (Table 1). Sequencing time and cost can be significantly reduced by combining four sample plates per panel using the NovaSeq S4 workflow. A comparison of NovaSeq 6000 S4 and SP flow cells revealed that both flow cells provide the same high-quality results.

Data analysis

Olink software packages are used to analyze sequencing data. Sequencing counts from BCL files are translated into protein counts with the Olink bcl2count software. Relative quantification, inter and intraassay normalizations, and QC analyses are then performed using the local Olink NPX Manager or the cloud-based Olink MyData software. Olink Explore data are represented as Normalized Protein eXpression (NPX) units, which are relative quantification protein measurement units generated using a series of signal normalization computations. Olink Analyze and Olink Insights Stat Analysis are local and web-based applications, respectively, that generate tables and figures for data visualizations and statistical analyses, such as heat maps, volcano plots, principal component analysis (PCA) plots, and box plots (Figure 4).

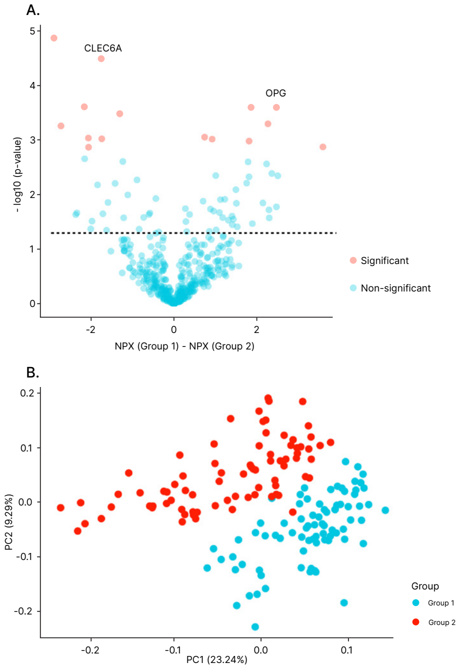

Figure 4: Data visualization generated by Olink Insights Stat Analysis—(A) A volcano plot displays the average fold differences in protein expression levels between two sample groups. This plot type visualizes which proteins are most likely to have biological relevance to the study. Each dot represents a unique protein. The dotted horizontal line represents a p-value of 0.05. Differentially expressed proteins that are statistically different (adjusted p-value < 0.05) are indicated in pale red. (B) A PCA plot is a visual representation of data variability. Each dot represents a sample. The low overlap between the groups indicates that each group has unique protein expression profiles.

Access expert support

Olink and Illumina teams collaborate to make sure you are fully supported throughout the Olink Explore with NGS readout workflow. Olink provides hands-on training and post-training support upon request at no additional cost. For questions related to the immunoassay and data analysis, contact Olink support (support@olink.com). For questions related to sequencing, contact Illumina support (techsupport@illumina.com).

Summary

This proteomics protocol leverages Olink PEA technology to detect nearly 3000 human proteins simultaneously in low sample volumes using Illumina sequencing systems. The automated workflow with minimal hands-on time delivers high-throughput multiplexed protein detection with exceptional analytical specificity (99.7%). Olink Explore with NGS analysis is a scalable and flexible solution that can be used throughout the proteomics biomarker pipeline, from discovery to validation.

Learn more

NovaSeq 6000 System,
illumina.com/systems/sequencing-platforms/novaseq

NextSeq 1000 and NextSeq 2000 Systems,
illumina.com/systems/sequencing-platforms/nextseq-1000-2000

NextSeq 550 System,
illumina.com/systems/sequencing-platforms/nextseq

Olink Explore 3072,
olink.com/products-services/explore

Olink Explore User Manual,
olink.com/content/uploads/2021/12/olink-explore-overview-user-manual-2021-12-13-v1.1.pdf

Olink Data Analysis products
olink.com/products-services/data-analysis-products

References

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Ordering information

Sequencing reagents	Catalog no.
NovaSeq 6000 SP Reagent Kit v1.5 (100 cycles)	20028401
NovaSeq 6000 S4 Reagent Kit v1.5 (100 cycles)	20028313
NextSeq 1000/2000 P2 Reagents v3 (100 cycles)	20046811
NextSeq 500/550 High Output Kit v2.5 (150 cycles)	20024907

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