Introduction to Olink Proteomics for UMass Chan Medical School

Following in the footsteps of the genomics era, high-throughput proteomics is emerging as the new frontier for understanding biological systems at an unprecedented scale. Proteins play a pivotal role in understanding cellular function, disease mechanisms, and the development of therapeutic interventions. There is no better time to integrate proteomics into your work.

Olink Proteomics provides a deep understanding of real time biology in human health and disease. This is enabled by the **Proximity Extension Assay (PEA)** technology that combines the best of an antibody- based immunoassay with PCR and DNA readout using NGS (Next Generation Sequencing). This results in a highly specific, highly multiplexed and scalable method for measuring the concentration of thousands of protein biomarkers from small sample volumes.



The basis of PEA is a dual-recognition immunoassay where a matched pair of antibodies, each conjugated with unique and complimentary DNA oligonucleotides, simultaneously bind to adjacent epitopes on a target protein. This event brings the two antibodies into proximity, allowing their DNA oligonucleotides to hybridize and serve as template for a DNA polymerase-dependent extension step. This step creates a double-stranded DNA "barcode" which is unique for the specific antigen and quantitatively proportional to the initial concentration of target protein. The hybridization and extension are immediately followed by PCR amplification. The resulting DNA amplicon is then quantified either by NGS. Finally, the protein concentrations are calculated using a dedicated processing software and the results are reported in the relative concentration unit NPX (Normalized Protein eXpression).



Olink Reveal – analysis service offered by the Deep Sequencing Core

Olink Reveal measures 1000+ proteins that were selected for high expected detectability in plasma and a focus on markers associated with inflammation and immune response. The Reveal library provides broad coverage of the top-level pathways and biomarkers associated with nearly all studied diseases.

Olink Reveal protein assay list

https://view-su3.highspot.com/viewer/86fb289f8a9e394ddfdba679872ed376

Laboratory instructions:

Libraries may be prepared in 1.5 days using existing infrastructure and expertise. On the first day, 86 experimental samples are arranged on a Sample Source Plate alongside the controls. All samples and controls are mixed with a sample buffer. Then 4uL aliquots are transferred to the Incubation Plate which contains lyophilized PEA reagents. The plate is then incubated overnight.

On the second day, a PCR mastermix is set up and pipetted into each well of the Incubation Plate. The plate is placed in a thermal cycler for a 2 hour PCR step.

Following PCR, each well is pooled into a single tube and purified with magnetic beads. Thereafter the library is ready for QC and DNA quantification via NGS in a counting application.

Olink Reveal laboratory instructions

https://view-su3.highspot.com/viewer/dd4381ee95826311224edb379f2f534c

Olink Reveal Overview

https://view-su3.highspot.com/viewer/7d315006893ba0da37dafa7d6afeff1d



Biostat Analysis

The resulting NPX Data file is then uploaded into the **Stat Analyzer** tool at Olink Insight to generate visualizations in just a few clicks. <u>https://insight.olink.com/analysis-and-tools/stat-analyzer</u>

Olink Analyze: Simplify the analytical process while enabling custom analysis pipelines with our R package. Investigate your data with statistical and bioinformatic functions. <u>https://olink.com/software/olink-analyze</u>

Technical advantages of Olink PEA are:

Specificity - signal is only generated when the matched antibodies bind to the intended target protein and the oligos generate a DNA template for readout. PEA Eliminates false positives and ensures data accuracy.

Sensitivity and dynamic range - Olink's library spans over 10 orders of magnitude allowing the detection of high to very low abundance proteins (fg/mL to mg/mL)

Scalability - PEA enables a transition from biomarker discovery using Olink's high-plex panels, to the development and validation of protein signatures with clinical utility using Olink's mid-plex and custom panels.

Sample compatibility - PEA is compatible with a wide range of sample types.

Sample preservation - PEA requires 4uL of sample, making sample available for other -omics.

Throughput - PEA enables the generation of 88K data points in one assay

Transparency - results for the analytical measurement range, precision, measured plasma protein levels and information regarding observed cross-reactivity are available on our website.

Validation - ability to validate results with other antibody-based methods.

Other useful links

Olink Insight – plan your study and interpret your data with the Disease Search app, Pathway Browser, Study Size Calculator, Normal ranges, Publication search tool, and analysis tools. <u>https://insight.olink.com/</u>

Reveal Validation Data: Olink-Reveal-Validation-Data v1.0 https://view-su3.highspot.com/viewer/93867f2b72f8069d105ab22e1a52a0c4

Scientific Publications - Discover the latest research using Olink's panels for protein biomarker discovery through our comprehensive list of publications. Easily find relevant studies by filtering them or using the free-text search. Access abstracts, PubMed entries, and full-text papers directly through the links provided. <u>https://olink.com/knowledge/publications</u>

Suggest a protein for assay development: https://olink.com/suggest-a-protein

FAQs - Get answers to common questions. Filter by category or search for your specific question. If you cannot find what you are looking for, please <u>contact Olink support</u>, and we'll reach out as soon as possible. <u>https://olink.com/knowledge/faq</u>