Singlicate Performance in Olink Target 48 Panels

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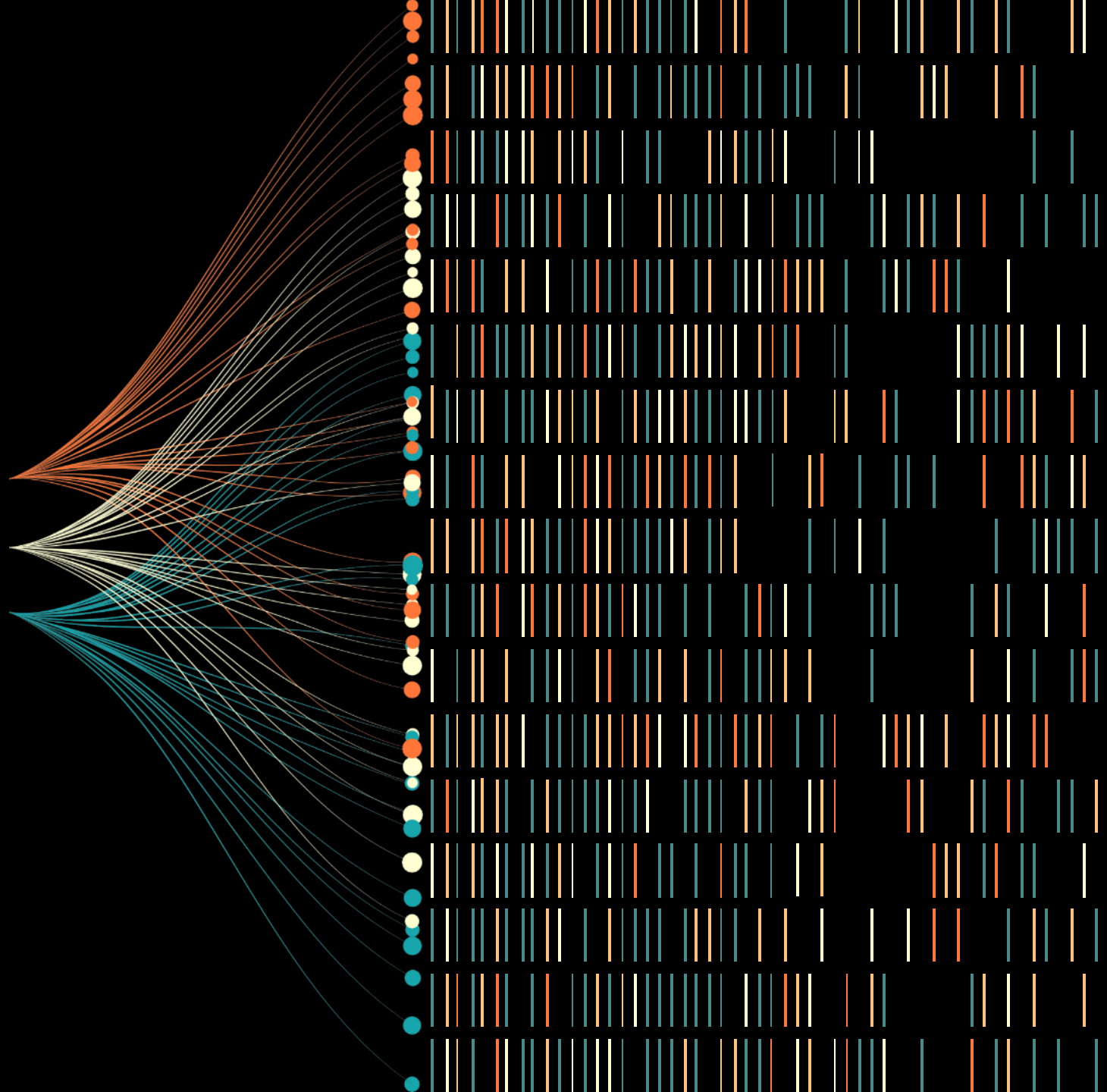

Affinity based proteomics

Biomarkers

- Olink Proteomics platform is based on the Proximity Extension ™ Assay (PEA ™) technology
  - Merging antibody-based immunoassay with polymerase chain reaction (PCR)
  - Readout using quantitative real-time PCR (qPCR) or Next Generation Sequencing (NGS)

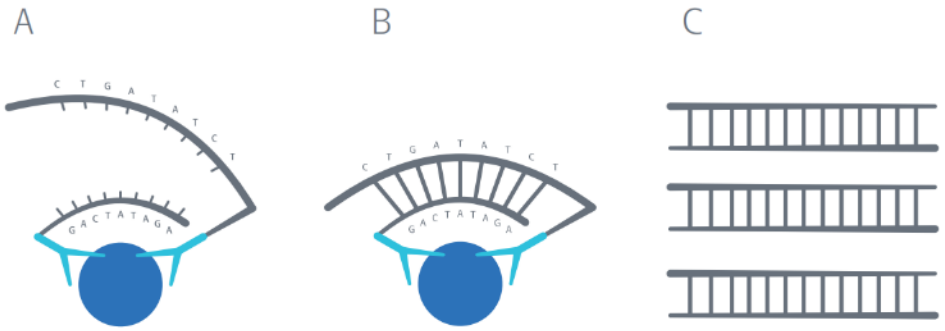

Figure 1. Main pre-readout steps in PEA™. (A) Antibody pairs, labelled with DNA oligonucleotides, bind target antigen in solution. (B) Oligonucleotides that are brought into proximity hybridize and are extended by a DNA polymerase. (C) This newly created piece of DNA barcode is amplified by PCR ready for readout by NGS or qPCR.

Dual-recognition immunoassay

A. Two matched antibodies labelled with unique DNA oligonucleotides simultaneously bind to a target protein in solution

B. The two antibodies are brought into proximity, allowing their DNA oligonucleotides to hybridize, serving as template for a DNA polymerase-dependent extension step

C. This 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 can then be quantified either by qPCR or NGS

PEA™ – a high-multiplex immunoassay technology with qPCR or NGS readout, Olink White Paper, 1129, v2.0, 2024-11-26

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Olink Target 48 Cytokine

Vendor validation

qPCR readout

Absolute quantification (pg/mL) of 45 proteins simultaneously

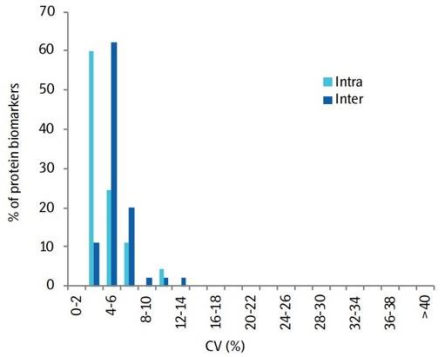
Analytical performance of the panel is validated by Olink internally

- Olink does not typically recommend running duplicates of samples
- Low intra-CVs
- Biological variation exceeds the technical variation

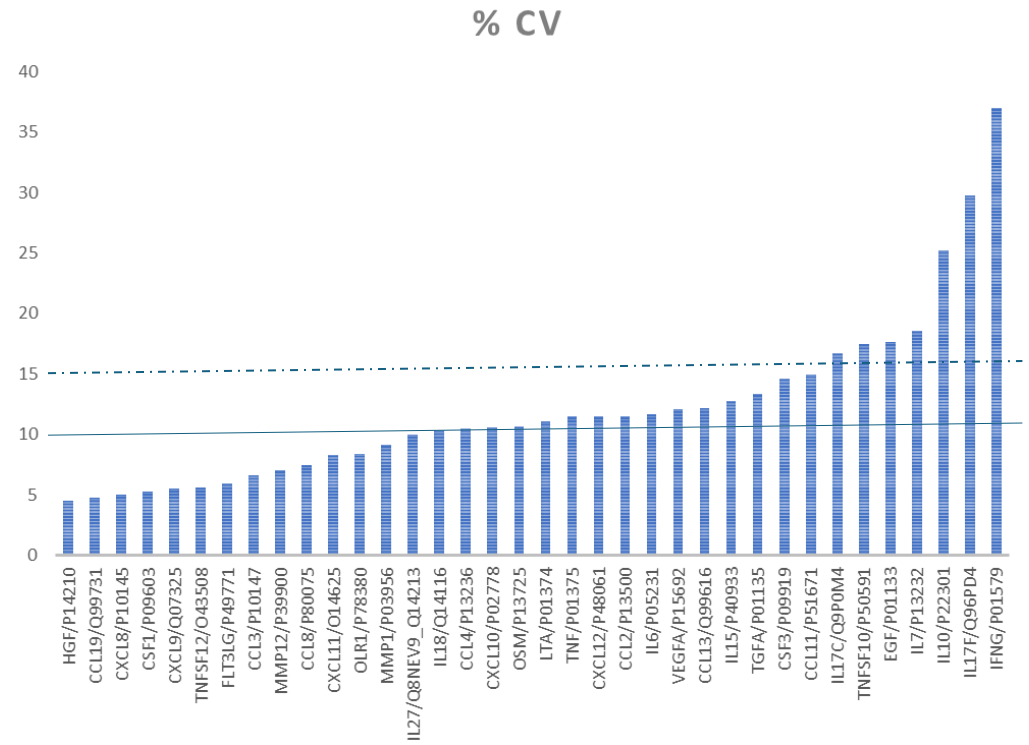
Validation Data, Target 48 Cytokine, 1134, v1.2, 2023-12-22

Olink Target 48 Cytokine

In-house repeated measurement



Validation Data, Target 48 Cytokine, 1134, v1.2, 2023-12-22



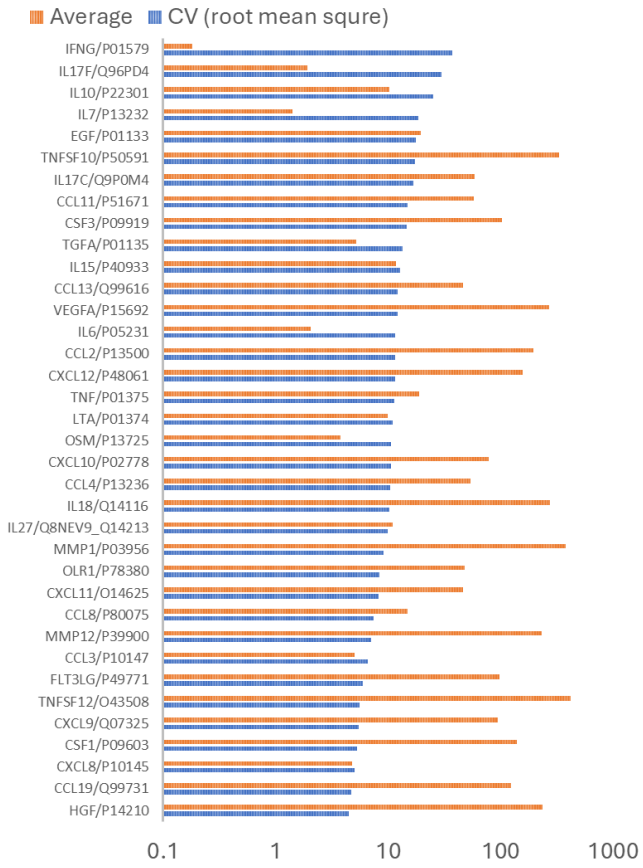
- 12 samples
- 1 repeated measurement
- Batch-to-batch variation
- % CV of complete targets in all samples
- (root mean square)

  39% assays with CV < 10%

  81% assays with CV < 15%

Olink Target 48 Cytokine

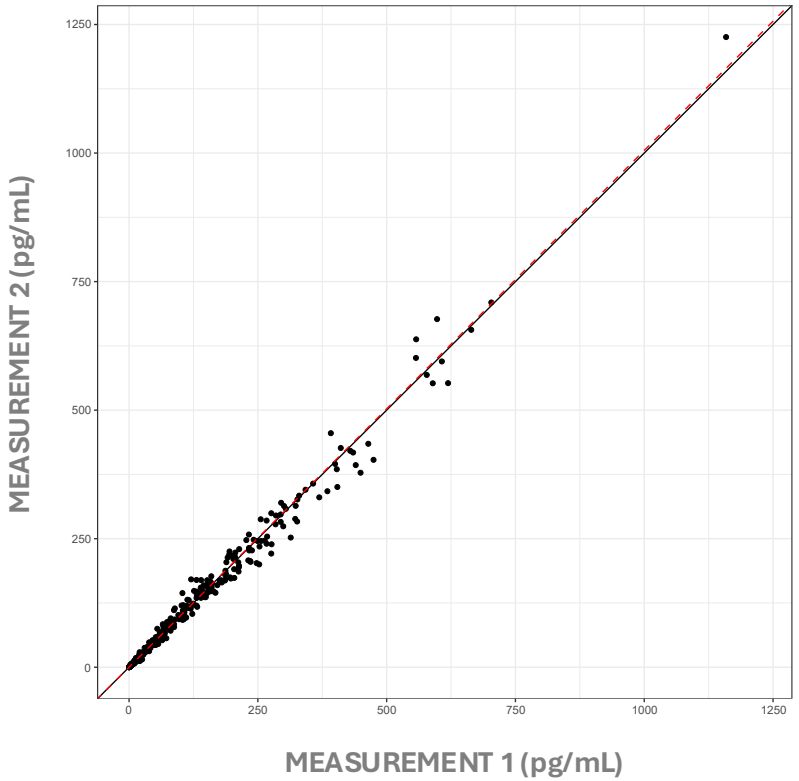
In-house repeated measurement

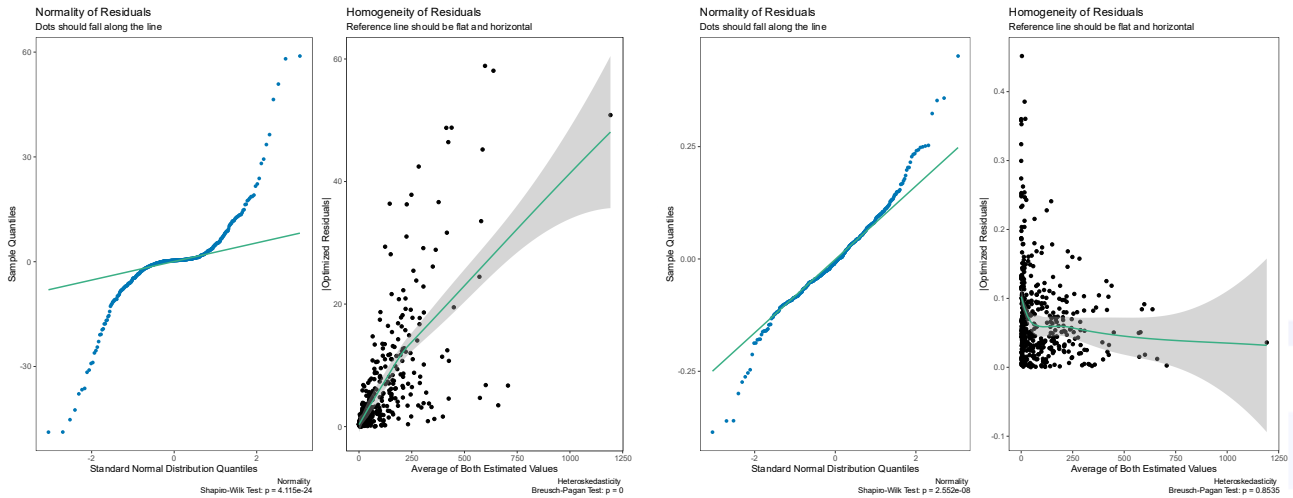


- % CV variation among assays is NOT concentration dependent

Olink Target 48 Cytokine

In-house repeated measurement





Weighted Deming Regression with 95% C.I.

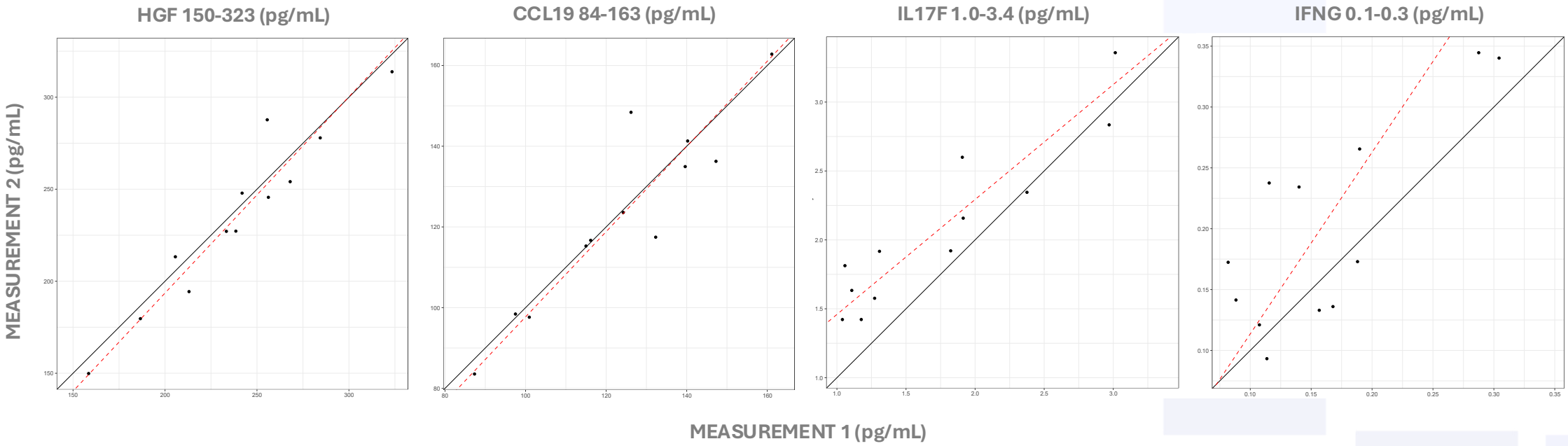
	coef	bias	se	df	lower.ci	upper.ci	p.value
Intercept	0.0318	0.0015001	0.017871	430	-0.003322	0.06693	0.07585
Slope	1.0051	-0.0002371	0.006952	430	0.991430	1.01876	0.46410

- This should be verified with true duplicates and additional data points

Olink Target 48 Cytokine

In-house repeated measurement

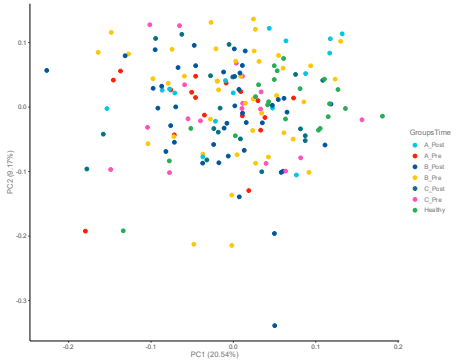
Assay-to-assay variation

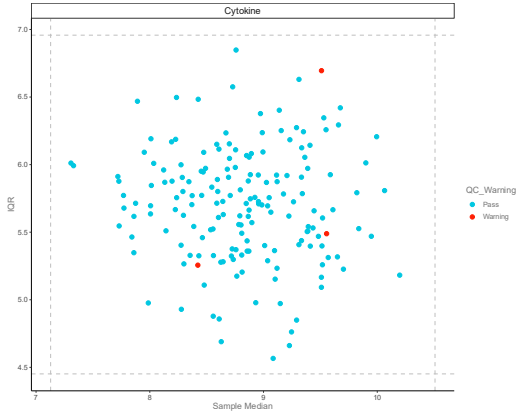


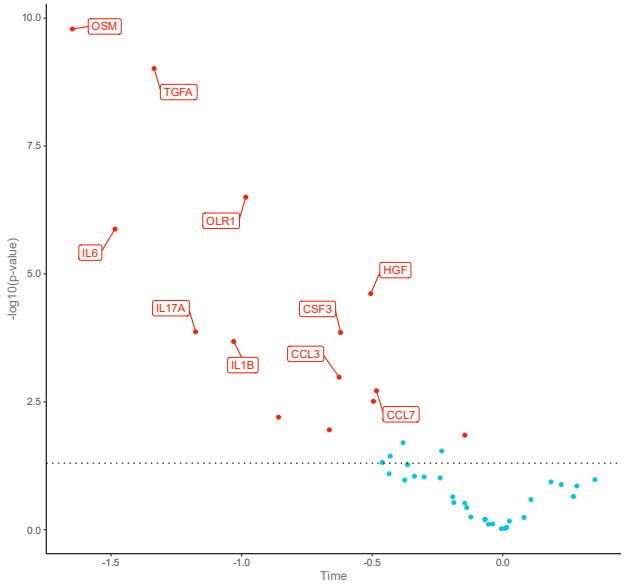
Olink Target 48 Cytokine

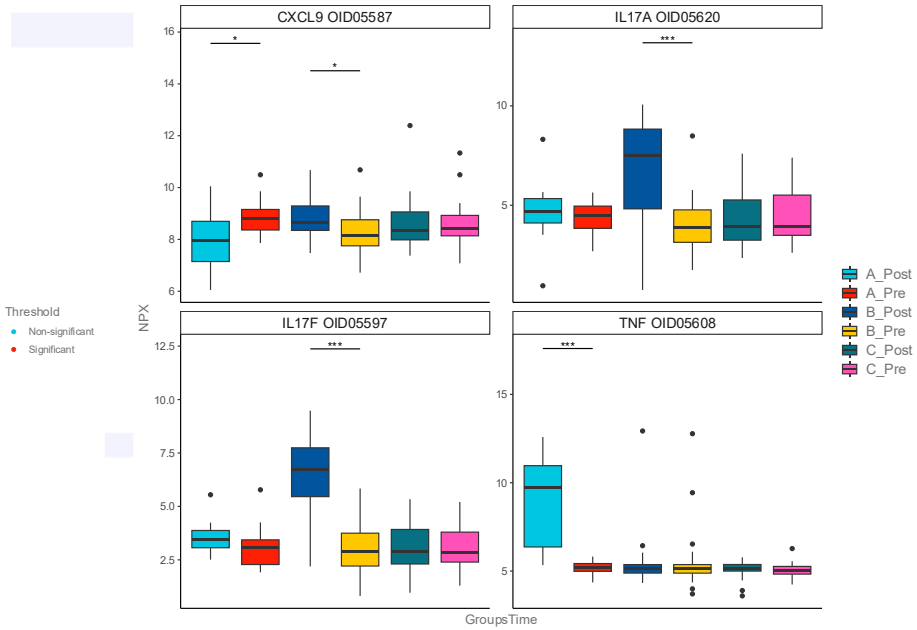
Differential Protein Abundance

- PCA to detect patterns of variation within statistical groups
- Volcano plot and box plot to visualize DPA statistics overall and per assay









Summary

- **PEA is ideal for biomarker discovery phase**
- **Data generated can be used for differential protein abundance, pathway analysis…**
- **Biological replicates contribute to a well powered study**
- **In this context, singlicate analysis is robust enough for identification of assays of interest for further validation with regulated bioanalysis by LBAs**
- **This allows for reduction of resources expended for the discovery phase**

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