DISCOVERY
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TECHNICAL NOTE

Olink Target 96 at **Discovery's Proteome Center**

Introduction

Discovery Life Sciences' Proteome Center is a Certified Service Provider of Olink Target for high-throughput biomarker discovery, verification, and validation. The Olink Target platform offers 15 protein panels that span a broad range of research areas including cytokines and inflammation, oncology, immunology, organ damage, metabolism, cell regulation, development, and more to cover key biological processes. Each Olink Target 96 panel analyzes 92 proteins in each sample across 88 samples. The use of dual oligo-labeled antibodies, internal and external controls, amplification, and qPCR provide a valuable combination of accuracy, specificity, sensitivity, and throughput.

Herein we demonstrate the application of the Olink Target 96 workflow using the Inflammation panel for the analysis of plasma samples from donors self-reporting various tobacco product use.

Methods

Table 1. Donor Characteristics

Sample Group	Donor Count	Reps per Donor	Mean Age	SD Age	Mean BM	SD BM
Cigarette	8	3	51.0	11.8	29.2	5.2
No Use	10	3	43.4	15.3	30.9	9.9
Smokeless Tobacco	6	3	39.0	12.3	36.7	5.6

Twenty-four plasma samples were obtained from the Discovery Life Sciences' biospecimen inventory.

Samples represented male donors who reported never smoking (No Use), using smokeless tobacco (ST), or smoking cigarettes (Cig) (**Table 1**). No clustering among the groups were observed based on BMI and age at time of donation (**Figure 1**).

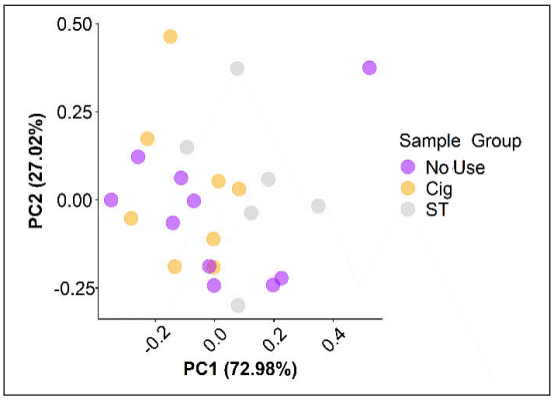

Figure 1. Donor BMI and age at donation.

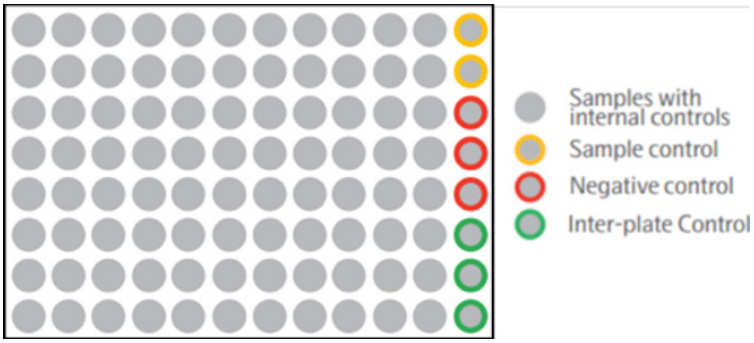

Figure 2. Plate layout for Olink Target 96 analysis. Column 12 contains pooled plasma controls (provided by the Discovery Proteome Center) as well as negative controls and plate controls (provided with the Olink kit).

Samples were randomized across the source plate (**Figure 2**), and 10 µL of sample were plated for analysis. Samples and controls were added to the incubation plate. Controls consisted of 16 Discovery pooled plasma samples, two sample controls, and three each of Olink-provided negative and plate controls. The Olink Target 96 workflow was carried out according to Olink's protocol. Briefly, DNA barcoded antibody pairs were added to the samples and allowed to bind to their target proteins during an overnight incubation. When protein matched antibody pairs bind to target proteins, the oligo tags hybridize. After incubation, hybridized barcodes for each target protein were extended and amplified using regular PCR. The DNA barcodes were quantified using high throughput real-time qPCR on the Olink Signature Q100 instrument. Data were converted from cycle thresholds and normalized across the plate using both internal and external controls. Final data are reported as normalized protein expression (NPX) values and are on a log2 scale. Data were analyzed in R using the Olink Analyze package and custom scripts

Results

Reproducibility and sensitivity of the workflow were demonstrated.

- Each donor sample was plated in triplicate to assess quantification reproducibility of the Olink Target workflow across samples and assays. **Figure 3** demonstrates the correlation of replicates for each assay in each sample group.

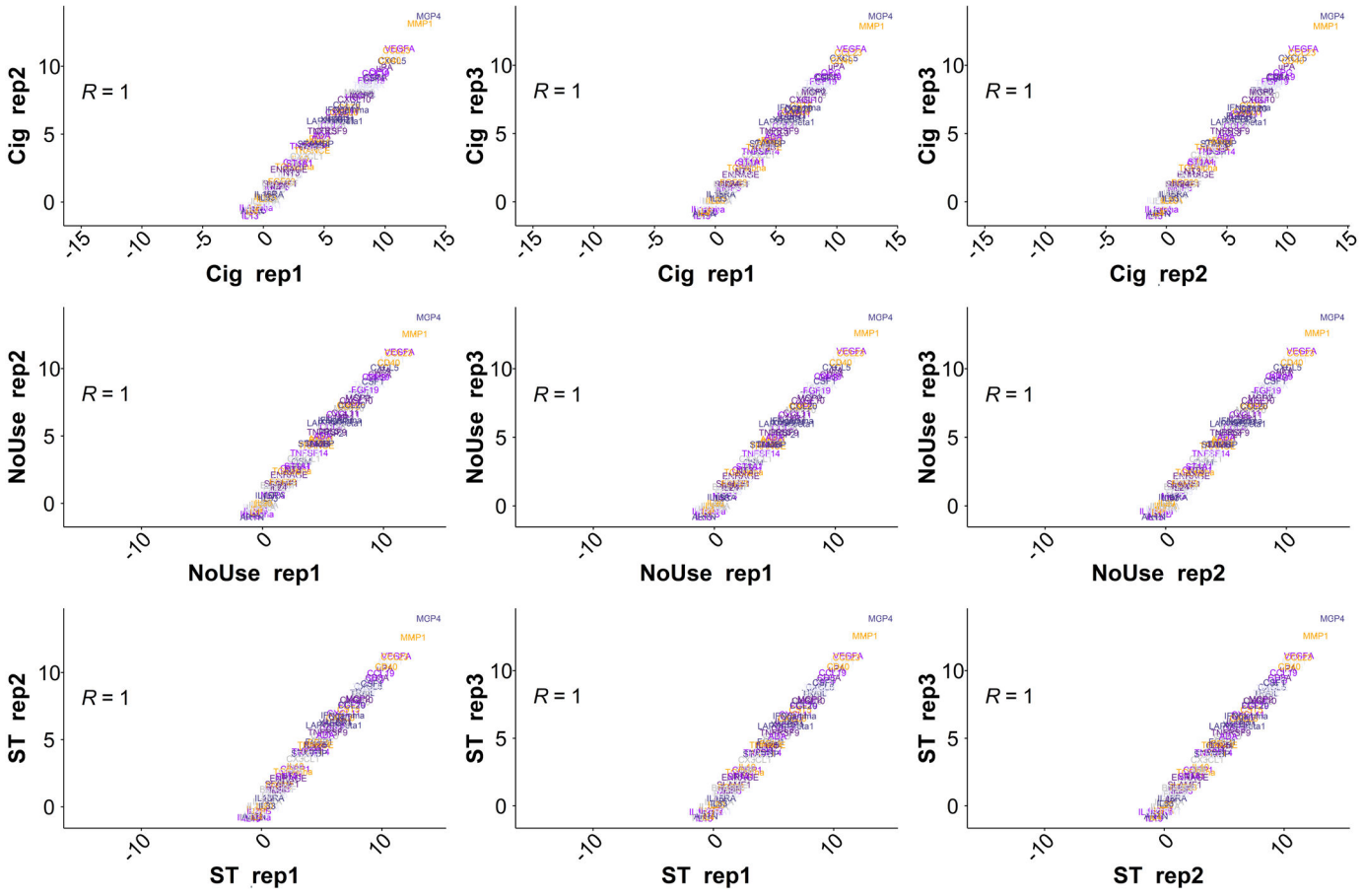

Figure 3

- Negative controls contain the same batch of reagents and probes used in sample wells throughout the Olink Target 96 assay, and thus, provide a source of information for background noise. The limit of detection (LOD) for each assay is calculated as three standard deviations above the negative control NPX value. For the purposes of demonstrating Olink Target metrics, assays were only counted as above the LOD if all samples in a sample group had NPX values above the LOD. Samples and sample controls had ≥ ~75% of assays above the LOD across all samples in each sample group (**Figure 4**)

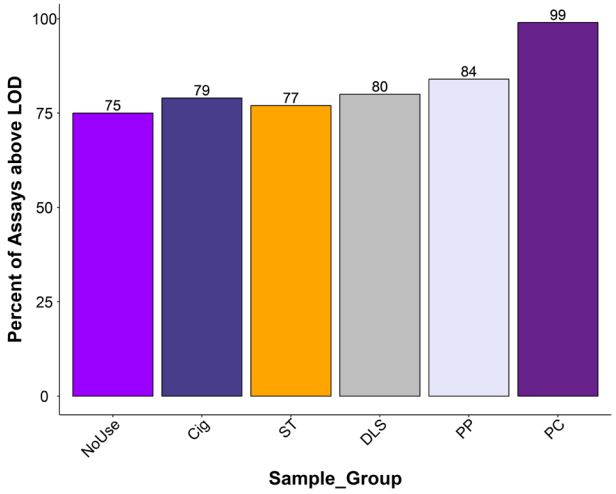

Figure 4. Percentage of assays above LOD.

Quantitative analysis of protein findings

Significant differences identified using ANOVA analysis were filtered for fold changes greater than 1 (on a log2 scale) and adjusted p-values of less than 0.5. Overall, four proteins showed differences among smoking groups (Figures 5 & 6). All differences were found between the "No use" and "Smokeless Tobacco" groups or between the "Cigarette" and "Smokeless Tobacco" groups. It is important to note that these data represent a small cohort of donors.

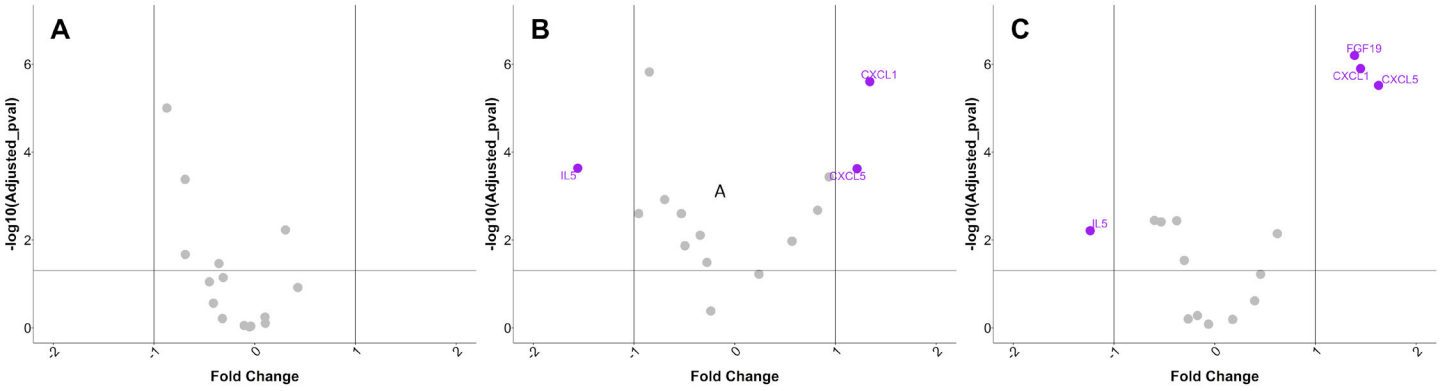

Figure 5. Protein expression differences between smoking habits. Comparisons show (A) No use versus cigarette use. (B) No use against smokeless tobacco, and (C) smokeless tobacco versus cigarette use. Proteins with significant differences are highlighted in purple.

Summary

These results demonstrate the Proteome Center's ability to perform Olink Target analysis and produce high quality results with respect to sensitivity and reproducibility. The Olink Target platform provides high throughput proteomic analysis across a variety of sample types, including all biofluids, accessible through minimally invasive sampling thus providing insight into biomarkers of interest. Discovery's Proteome Center is a Certified Service Provider for Olink Explore, Target, Flex, and Focus services and offers a full suite of workflows for proteomic profiling, biomarker discovery, and biomarker validation, both in research and at the clinic

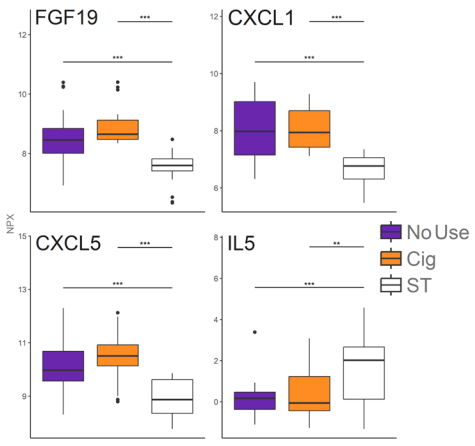

Figure 6. Boxplots of the NPX values for proteins with significant differences. Black line represents the median.

About Discovery Life Sciences

Discovery brings together the world's largest commercial biospecimen inventory and procurement network with leading multi-omic biomarker services under one roof to streamline client access to the necessary technologies and expertise. These services span genomics, molecular pathology, proteomics, cell biology, and ADME Tox aimed at accelerating the development of new therapies supported by biomarker and companion diagnostic programs for cancer, infectious disease, and other rare and complex conditions. Discovery offers a wide spectrum of technologies for the identification and validation of clinical biomarkers, including multiplexed immunoassays, mass spectrometry, RNA, whole genome, whole exome, targeted panel, high-fidelity long-read, single cell, and epigenomic next-generation sequencing services. As a single strategic partner, we work together with clients to accelerate biomarker advancements through the provision of high-quality, actionable data that deliver effective solutions and support the entire continuum from discovery to validation.