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Multiplexing Protein and Gene Level Measurements on a Single Luminex Platform

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ABSTRACT

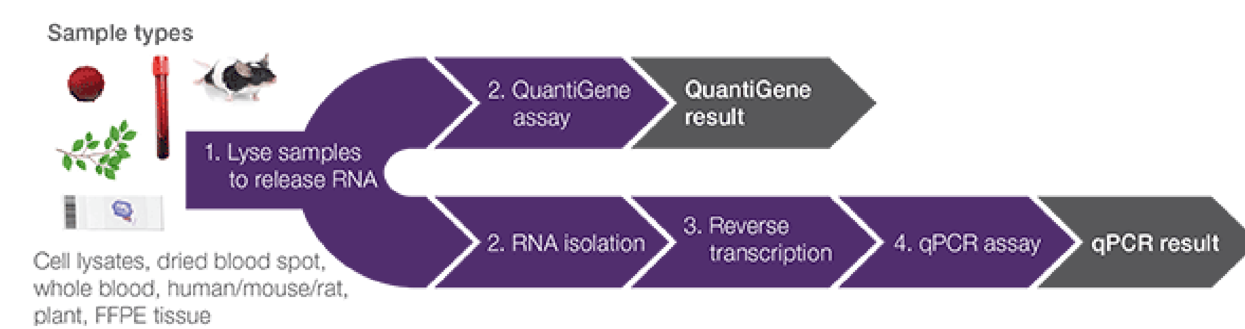
Background: The ability to accurately measure both proteins and genes from a single sample is valuable for comprehensive analysis. Bottlenecks to traditional immunoassays and gene expression assays include large sample consumption, time consuming experimental procedures, and complicated data analysis. Here, we utilize Luminex® xMAP® technology to measure multiple secreted proteins or genes simultaneously in a single well. **Purpose:** Our study examines ProcartaPlex™ and QuantiGene™ Plex assays to provide both protein and gene expression data from the same starting sample. **Experimental procedures:** We demonstrate two high-throughput assays measuring both messenger RNA (mRNA) expression and proteins in a single sample run on a Luminex platform. Human peripheral blood mononuclear cells (hPBMCs) were treated with lipopolysaccharide (LPS) and harvested at 24 and 72 hours. The treated cells were centrifuged and secreted cytokines were measured using the ProcartaPlex Human 65-plex Cytokine Panel, and the cell pellets were lysed and corresponding mRNA targets were measured with the QuantiGene Plex Human Cytokine Panel. **Summary of data:** Upon further examination, a subset of gene expression and analyte levels corresponds, namely IL-1β, IL-6, TNF-α, and MIP-1b. **Conclusion**: These results show that sample can be conserved and produce targeted results.

INTRODUCTION

Screening assays are both time consuming to set-up and execute, and there is a good amount of variability between users (Lamerdin, 2016). Using xMAP technology to measure secreted protein and mRNA expression levels on a Luminex instrument, this overcomes previous limitations as these assays are sensitive, specific to a target, quality control tested and easy-to-learn and perform for the end user. ProcartaPlex and QuantiGene assays respectively measure protein and gene expression and provide high-level multiplexing to improve the discovery workflow and screening process (Dunbar, 2005). These assays consumes very little sample, while maximizing the output of data at the proteomic and genomic level.

We chose QuantiGene assays instead of quantitative PCR (qPCR) because it is on the Luminex platform. Though qPCR is often considered to be the gold standard for gene expression analysis, Luminex is now seen as a gold standard in multiplexing technology. Benefits to measure gene expression levels with the Luminex platform includes no RNA purification, unlike qPCR. Luminex users who are familiar with linear readouts will also benefit from QuantiGene Plex data fidelity, in which the intensity of signal linearly corresponds to quantity of target mRNA present.

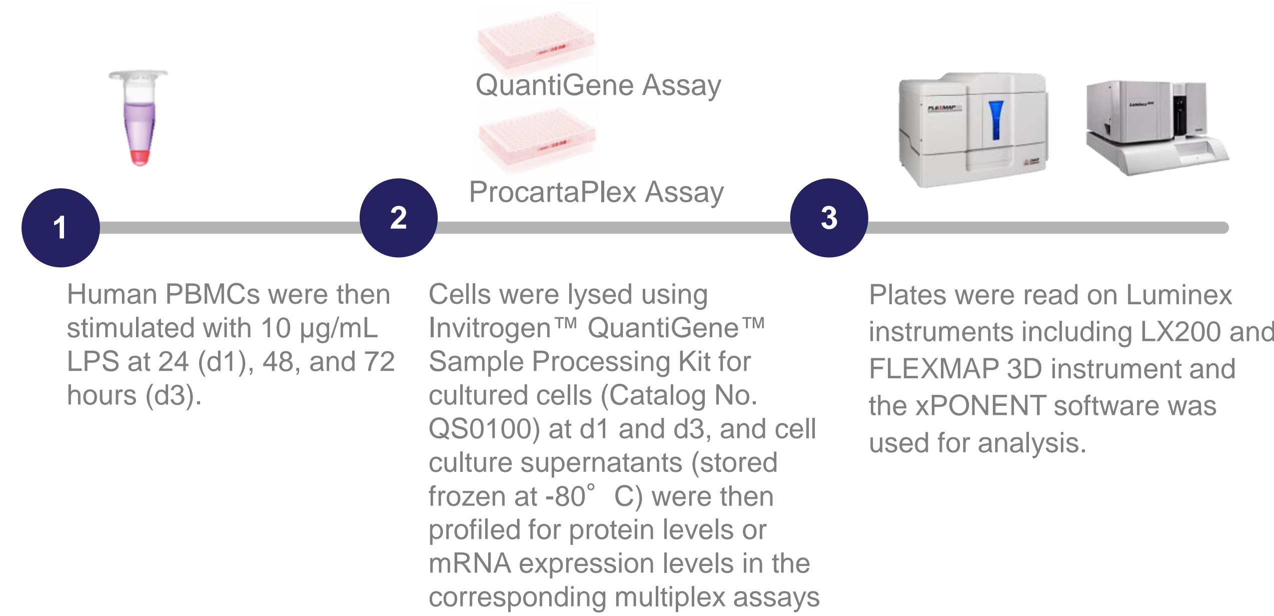
Figure 1. Invitrogen QuantiGene assay vs. traditional qPCR workflow



QuantiGene workflow is shorter than traditional qPCR. Traditional qPCR workflow requires isolation and amplification of targeted sequences. QuantiGene assay workflow does not have an amplification step for the gene itself. Instead, utilizing the branched DNA (bDNA) technology the signal is amplified.

MATERIALS AND METHODS

Figure 2. Single Sample Analysis with the Luminex Workflow



Protocol overview for ProcartaPlex and QuantiGene Assays. Human PBMCs were isolated and lysed after treatment. The sample was thereafter used for both protein and gene expression analysis. To show proof-of-concept, samples were ran as duplicates for ProcartaPlex assay and triplicate for QuantiGene assays. Samples were read using Luminex instruments and analyzed with xPONENT® software.

Measurement of Protein Level Changes with ProcartaPlex Human Immune Monitoring 65-plex Panel

Cell culture supernatant was probed for the following 65 markers: APRIL, BAFF, BLC, CD30, CD40L, ENA-78, Eotaxin, Eotaxin-2, Eotaxin-3, FGF-2, Fractalkine, G-CSF, GM-CSF, GRO α, HGF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-2R, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-31, IP-10, I-TAC, LIF, MCP-1, MCP-2, MCP-3, M-CSF, MDC, MIF, MIG, MIP-1α, MIP-1β, MIP-3α, MMP-1, NGF-β, SCF, SDF-1α, TNF-β, TNF-α, TNF-R2, TRAIL, TSLP, TWEAK, and VEGF-A.

Measurement of mRNA Expression Levels with QuantiGene Human 80-plex Panel

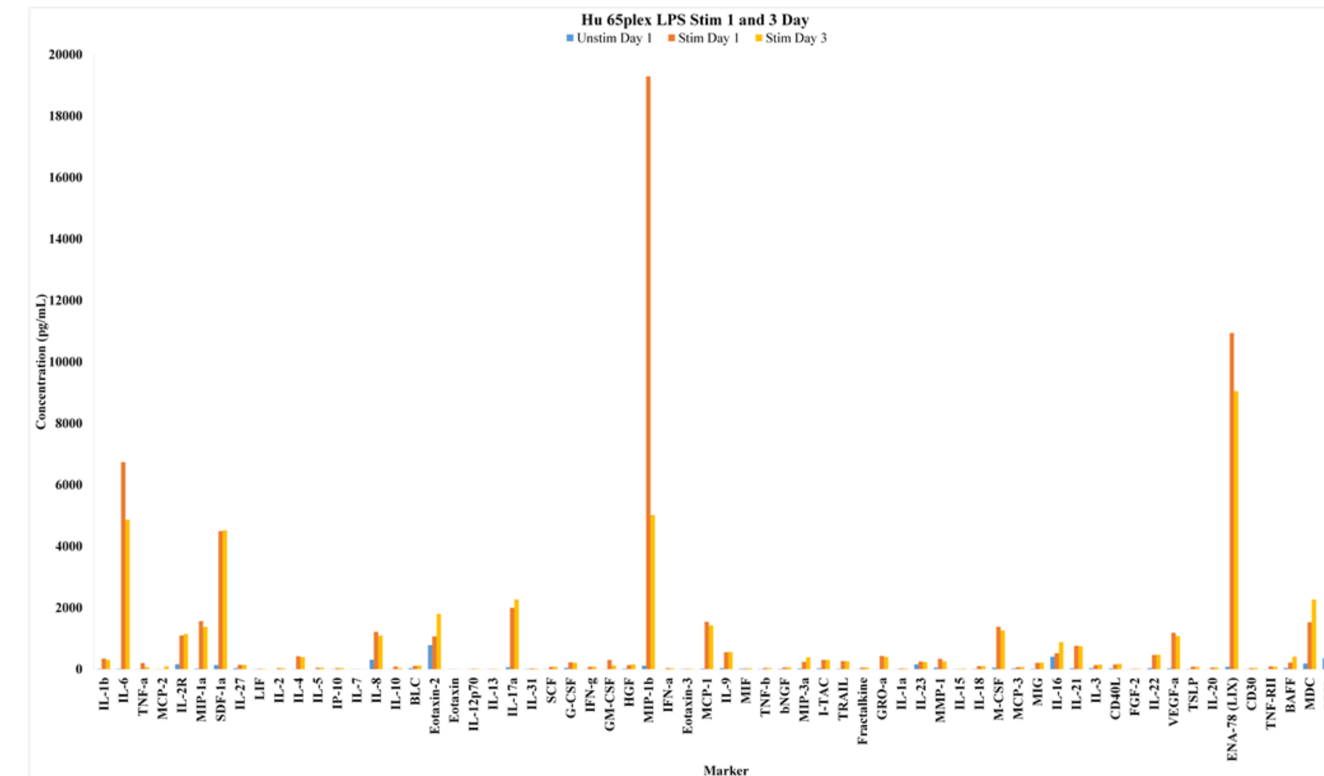
An identical biomarker configuration for mRNA using a QuantiGene 80-plex Panel (Thermo Fisher Scientific, custom) and the Invitrogen™ QuantiGene™ Plex Assay Kit for the generic reagents (Thermo Fisher Scientific, Cat. No. QP1013). The QuantiGene Plex consists of magnetic Luminex xMAP beads ("capture beads") conjugated with specific capture probe sequences and a target-specific probe set consisting of 3 types of probes: capture extenders, label extenders, and blocking probes. The QuantiGene 80-Plex panel included sixty-eight encoding mRNA and 12 additional reference genes for data normalization. Gene markers included: CCL2, CCL3, CCL4, CCL7, CCL8, CCL11, CCL20, CCL22, CCL24 CCL26, CD40LG, CSF1, CSF2, CSF3, CX3CL1, CXCL1, CXCL5, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12A, CXCL13, FGF2, HGF, IFNA2, IFNG, IL1A, IL1B, IL2, IL2RA, IL3, IL4, IL5, IL6, IL7, IL9, IL10, IL12A, IL13, IL15, IL16, IL17A, IL18, IL20 IL21, IL22, IL23A, IL27, IL31, LIF, KITLG, LTA, MIF, MMP1, NGF, TNF-alpha, TNFRSF1B, TNFRSF8, TNFSF10, TSLP, TNFSF12, TNFSF13, TNFSF13B, and VEGFA.

Data Analysis

The xPONENT software (Thermo Fisher Scientific, www.thermofisher.com/xPonent) was programmed using the settings from the assay protocol and certificate of analysis of the two assays. ProcartaPlex Assay samples were read on the Luminex LX200. QuantiGene Assay plates were read using the FLEXMAP 3D® instrument. Analysis was performed in Microsoft® Excel.

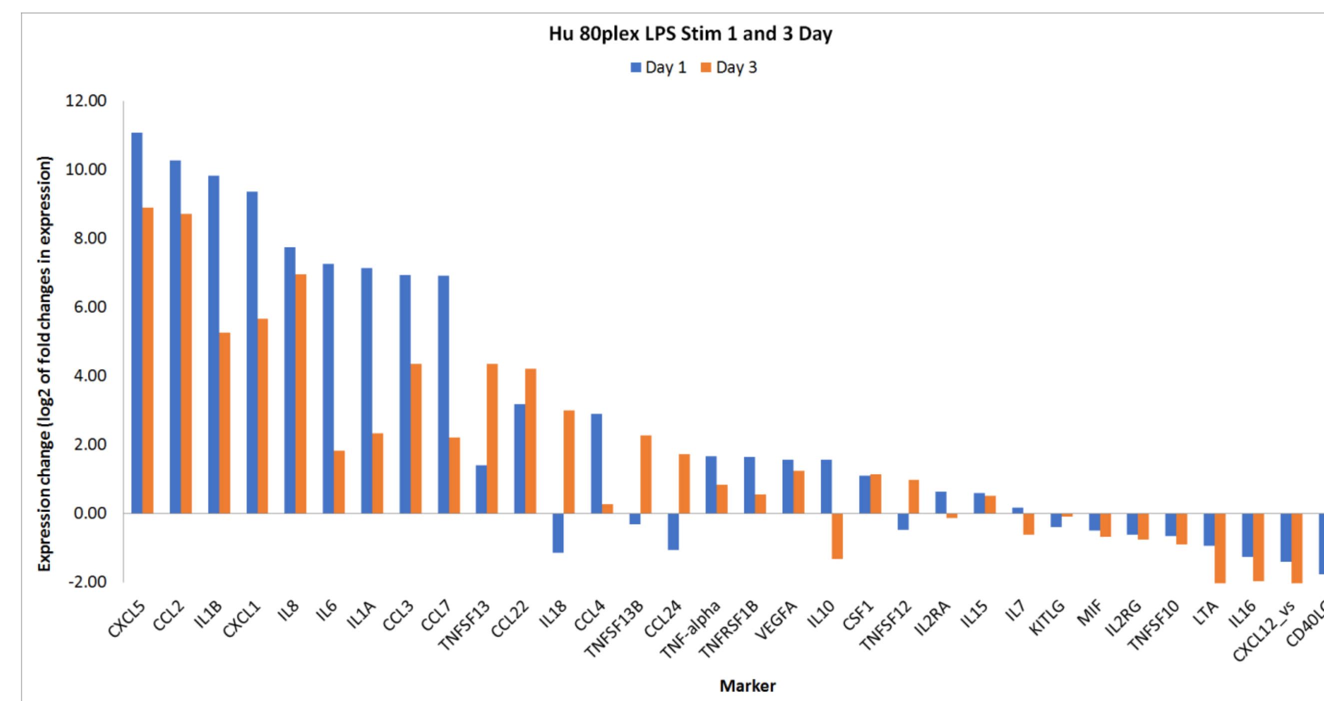
RESULTS

Figure 3. Protein Level Data with ProcartaPlex 65-Plex



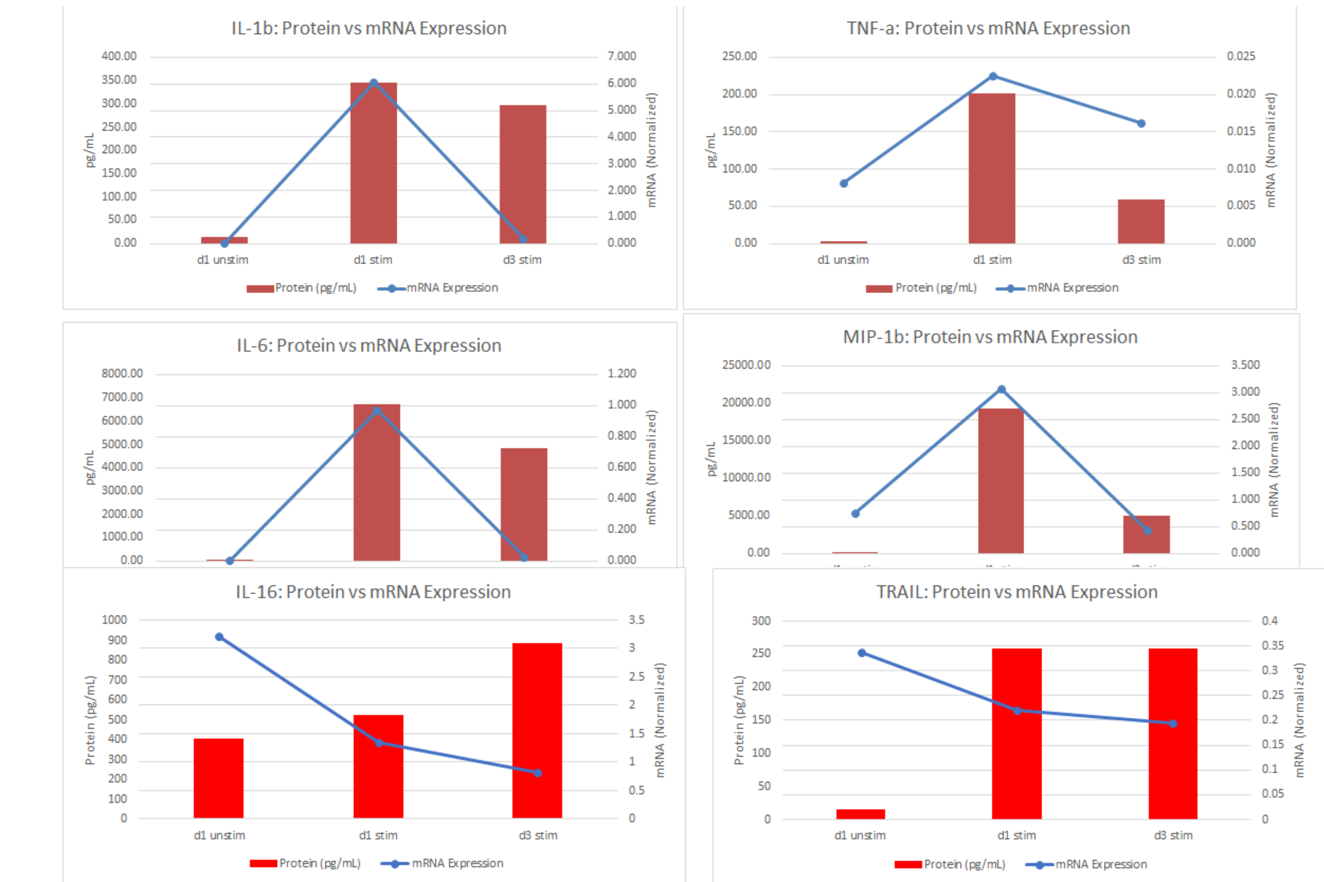
21 targets showed 10-fold or greater after a 3-day LPS-stimulation. The ProcartaPlex 65-plex data was analyzed using the xPONENT software. A 5PL (weighted) curve fit was used for the generation of the 7-pt standard curve. The data was analyzed for precision, accuracy and bead count.

Figure 4. Gene Expression Data with Custom QuantiGene Assay



32 gene targets showed expression changes with LPS-stimulation. Raw MFI data from the QuantiGene Plex assay run were normalized to the geometric mean of the 6 most stable reference genes according to geNorm (out of 12 reference genes in the panel).

Figure 5 Protein and Gene Expression Correlation on the Luminex Platform



Correlation of protein and mRNA expression at day 1 and 3 LPS-stimulation.(A) Protein and gene expression levels with correlating trends. (B) Diverging protein and gene expression levels.

CONCLUSIONS

Complementing ProcartaPlex assay with QuantiGene mRNA assay can provide a more holistic view of the investigation research study. Often times, measurement of mRNA expression or protein levels alone may not tell a complete story as the mRNA levels may not translate to protein. Adding QuantiGene Plex assays to the ProcartaPlex immunoassay is an amenable solution to meet the high-throughput screening needs for discovery research.

REFERENCES

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