Capturing 12 logs of multiplexed protein concentrations from a single undiluted biological sample with NULISA

TECH NOTE

Background

Containing over 10,000 proteins with concentrations spanning 12 logs (Williams et al., 2019), the plasma proteome presents significant analysis challenges in the identification of clinical biomarkers. Adding to this complexity, is the fact that 99% of the plasma protein mass is composed of just 22 proteins, and the majority of clinically relevant proteins represent only a minority of the protein mass (Nanjappa et al., 2014). Even in the face of those challenges, the discovery and clinical implementation of plasma biomarkers for early detection of diseases, therapeutic selection and disease monitoring is a lynch pin in the promise of precision medicine from liquid biopsies.

Overview

Alamar Biosciences developed the NULISA platform, an immunoassay technology with ultra-high sensitivity and dynamic range for protein biomarker discovery. The NULISAseq assay enables the analysis of hundreds to potentially thousands of proteins across as much as 12 logs of protein concentrations from as little as 10-25 ul of undiluted sample in a single assay. The sensitivity and dynamic range improvements are largely a result of the combination of an automated dual capture, wash and release methodology that removes background and a nucleic acid-based readout via qRT-PCR or Next Generation Sequencing (NGS). To enable NGS readout, each antibody pair is conjugated to a unique set of DNA barcodes that are ligated together when binding to the antigen brings them into proximity. These ligated DNA fragments are then amplified and sequenced (Figure 1) such that the more the antigen is present in the sample, the more antigen-specific barcode pairs are detected in the sequencing data.

Visit www.alamarbio.com for more information on the NULISA assay and the ARGO HT instrument on which the workflow is fully automated.

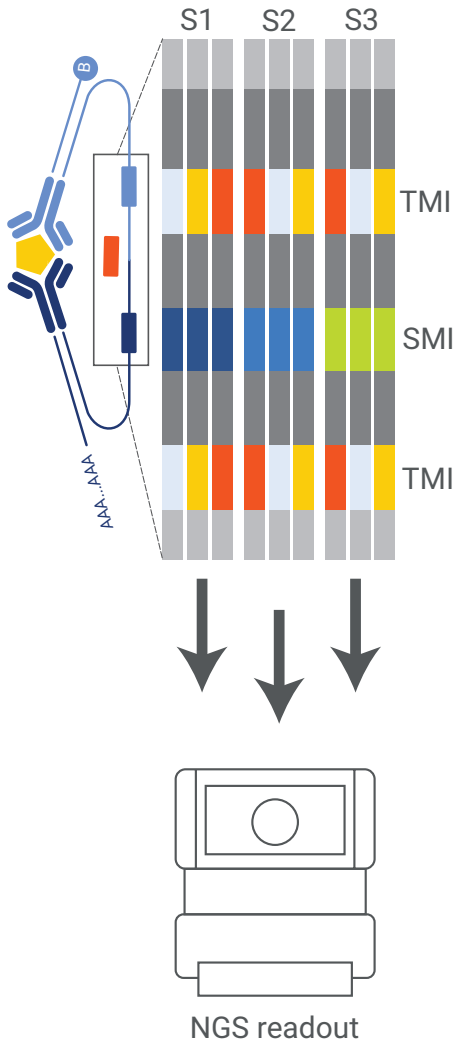

Figure 1. Sequencing readout of ligated fragment. The Target Molecular Index (TMI) is specific to each protein target. The Sample Molecular Index (SMI) is specific to each well/sample. These allow the protein targets in each well/sample to be quantitated by NGS.

Addressing the Challenge

Using an NGS readout enables highly multiplexed quantitative analysis. However, due to the protein mass challenge, sequencing reads are quickly used up for the most abundant proteins in your assay, requiring greater sequencing depth and cost to ensure coverage of the lower abundant proteins. To overcome this challenge, NULISAseq assays are "tuned" through the spiking of unlabeled (cold) antibodies for the most abundant targets thus reducing the number of bound labeled antibodies (hot) and their respective sequencing reads (Figure 2). This enables more efficient and cost-effective utilization of the sequencing reads and ensures accurate detection and measurement of low abundant proteins.

The result of tuning is a more even distribution of reads across analytes (Figure 3), resulting in the ability to detect proteins with a broad range of abundance from a single assay (figure 4).

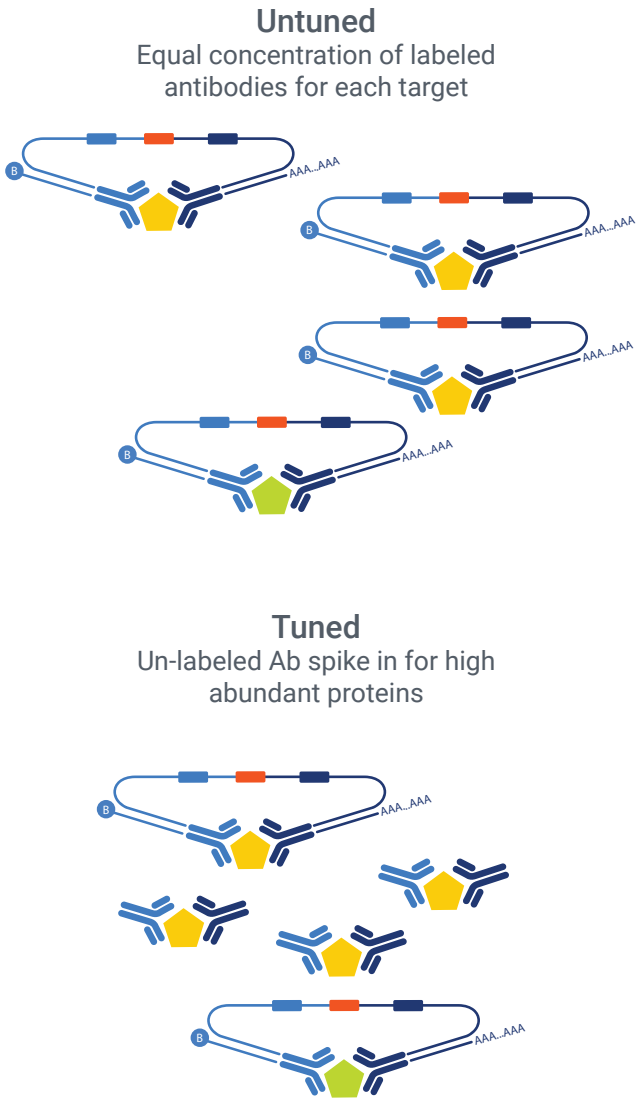

Figure 2. Signal from highly abundant targets is "tuned" by spiking in un-labeled antibodies

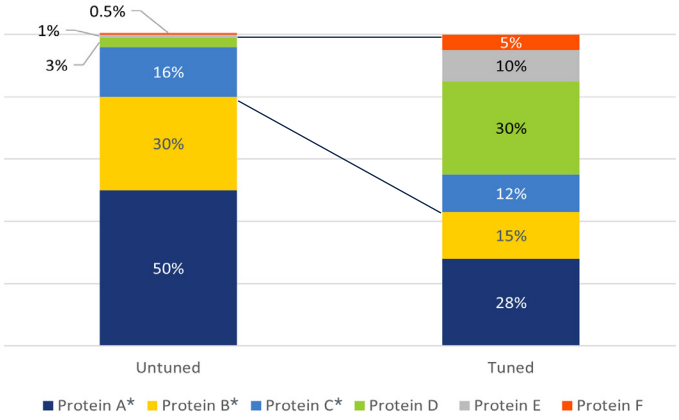

Figure 3. With tuning of the high abundant proteins (*), the low abundant targets are more equally represented in the sequencing reads (simulated data).

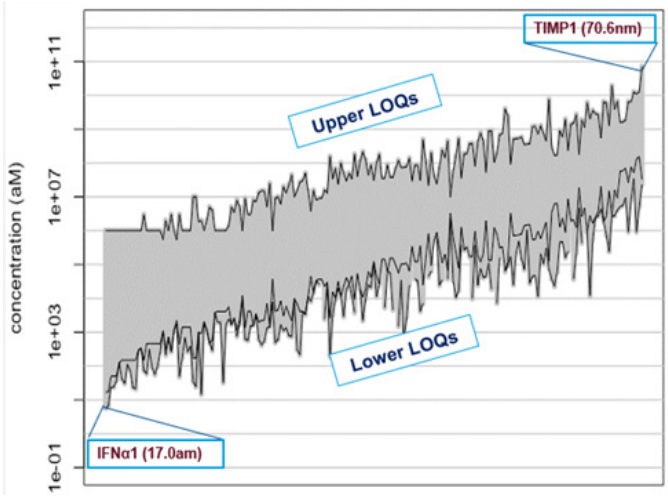

Figure 4. Detection of proteins with concentrations differing more than 1 billion-fold from a single undiluted plasma sample.

Importantly, tuning of abundant proteins does not impact the ability to perform quantitative analysis of the tuned protein abundance. With the addition of varying concentrations of labeled or unlabeled capture (aC) and detection (aD) antibodies, the signal from the sample dilutions remains linear and parallel (Figure 5). Even though tuning of an assay may impact its sensitivity and dynamic range, the tuning is optimized to ensure that the signal is linear across the biological range for the measured protein, ensuring each assay performs to detect and quantify levels that exist at both baseline and clinical endpoints.

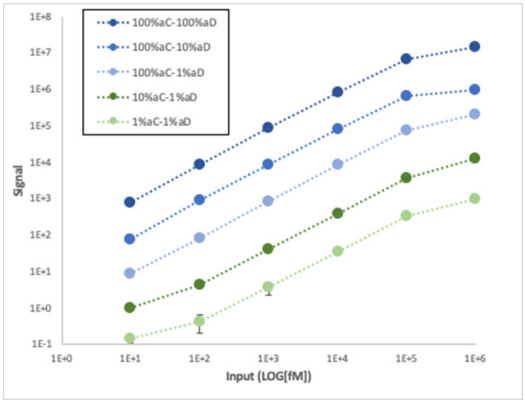

Figure 6. Detection of proteins with concentrations differing more than 1 billion-fold from a single undiluted plasma sample.

Conclusion

Combining the assay tuning methodology with the fully automated workflow on the ARGO instrument, Alamar has developed a 250 protein Inflammation Panel and a 120 protein CNS Disease Panel that each require only a single undiluted 10-25 µl sample for analysis. This capability will support the development of additional multiplex protein panels to address unmet research and ultimately clinical needs.

References

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