Technical note

High compatibility using Olink® Explore and the Element AVITI™ NGS platform

Background

The Olink® Explore platform enables protein biomarker analysis of human samples using PEA technology coupled with NGS readout. PEA technology utilizes binding of two unique oligonucleotide-labeled antibodies per protein, thus enabling exceptional specificity and sensitivity while requiring a minimal amount of input material. The current Olink® Explore workflow uses Illumina® sequencing instruments and flowcells. To enable users to take advantage of an already installed base of sequencers thereby making protocol adoption easier and more efficient, we set out to further expand the list of sequencing platforms compatible with Olink® Explore. We determine compatibility with validated platform(s) by assessing correlation, precision, sequencing quality, and output. This tech note presents the result of a technical evaluation of the AVITI system from Element Biosciences.

Element Biosciences, Inc. (San Diego, CA) is a multi-disciplinary life science company focused on developing disruptive DNA sequencing technology for research and diagnostic markets. Its DNA sequencing platform, the Element AVITI™ System, is a benchtop sequencer designed to work seamlessly in any NGS workflow. It features outputs of 800M+ reads on each of its two random access flow cells and data quality of >90% Q30 at 2x150 bp read length. A 2x150 bp run can be completed in under 48 hours, but Olink runs require only a short single read, and can be completed in approximately 13 hours.

In this study, we wanted to determine whether the Olink® Explore platform was compatible with the new AVITI System. By validating precision, sequencing quality, and output of Olink® Explore on AVITI, the data demonstrates high compatibility and accuracy of the two systems. Here we present the result of a technical evaluation of the AVITI system from Element.

Methods

Five different PEA libraries were generated using the Olink® Explore protocol[1]. Four libraries were from an analysis of a selection of plasma samples and pools of recombinant antigen standards using four 384-plex Olink® Explore panels. The fifth library was prepared from a common pool of PCR1 products (originating from a single plasma sample from a healthy individual analyzed with the Olink® Explore 384-plex Cardiometabolic panel) that was split into separate PCR2 reactions to incorporate 96 different sample indices. Three additional indices were also included for background reaction assessment in this library. The libraries were bead-purified and first sequenced at Olink using NovaSeq 6000 SP (two libraries loaded in two separate lanes per run using the XP 2 lane-kit). All libraries were confirmed to be normal in terms of signal to background. Next, the same purified libraries were shipped to Element for sequencing on the AVITI System.

All libraries derived from the Olink® Explore protocol were made compatible for sequencing on the AVITI System using the Element Adept™ Library Compatibility kit[2] and a 75-minute protocol. The linear library was circularized in a single reaction without the use of PCR amplification. After circularization and bead cleanup, libraries were quantified by qPCR using the primer mix and standard provided, diluted to the appropriate concentration, and loaded onto the AVITI for sequencing[3].

FASTQ Files were generated by Element for all runs, and data pre-processing, normalization and final data analysis were done at Olink using R. The AVITI reads were trimmed using the Rd1 and p7 adapters and filtered to include only 66-bp long reads with matching forward and reverse assay barcodes, after which additional analyses were conducted as described below.

Results

At a high level, the number of matched counts was comparable between the two platforms. Figure 1A compares the same library sequenced on the NovaSeq and the Element AVITI system. The correlation was very high pre-normalization (R^2=0.984-0.989) with somewhat increased variation observed for the lowest counts, as expected. Following Olink's standard normalization procedure to generate Normalized Protein eXpression values (NPX), the correlation was further improved and close to perfect (R^2=0.992-0.996) as shown in Figure 1B.

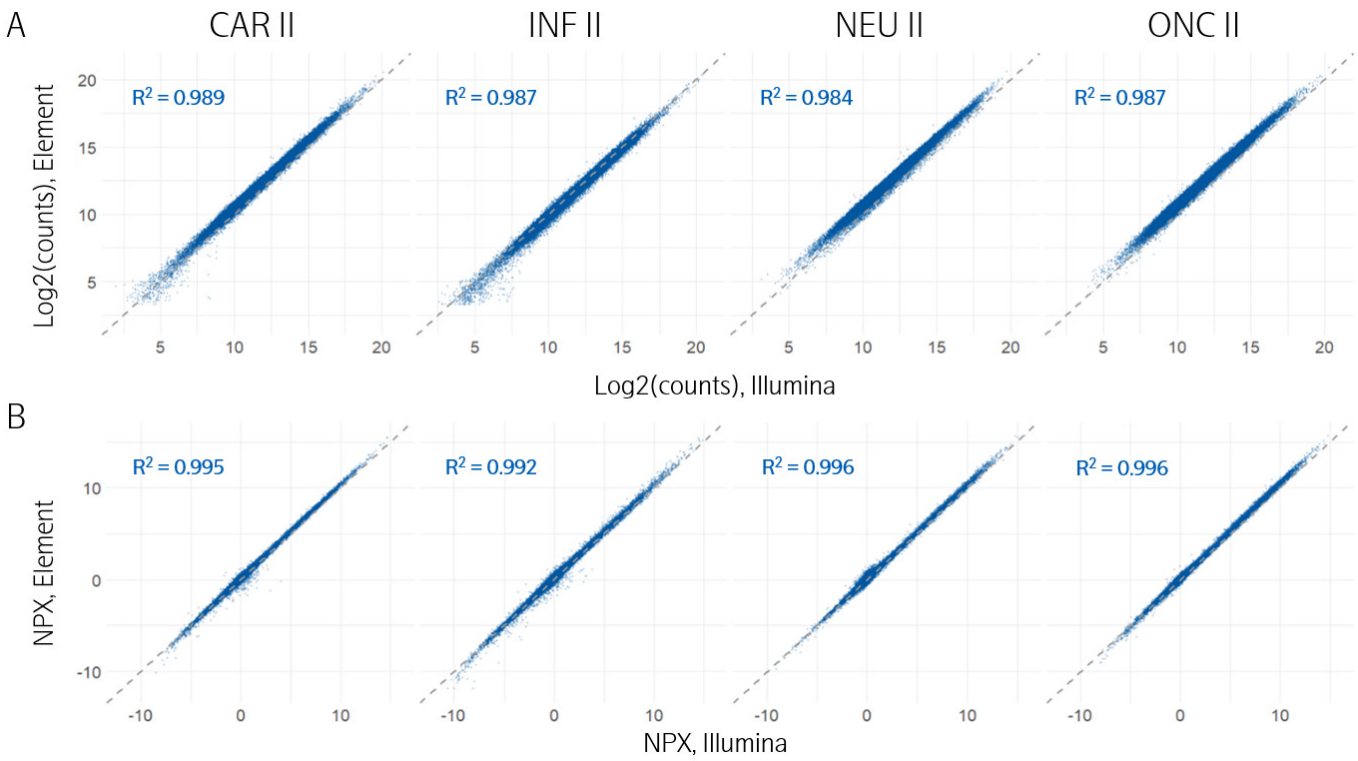

Fig 1. Analysis of Olink Explore derived libraries that were sequenced on either Illumina's NovaSeq 6000 SP or Element's AVITI, either based on matched counts (A) or NPX values (B). These results show high correlation between the platforms.

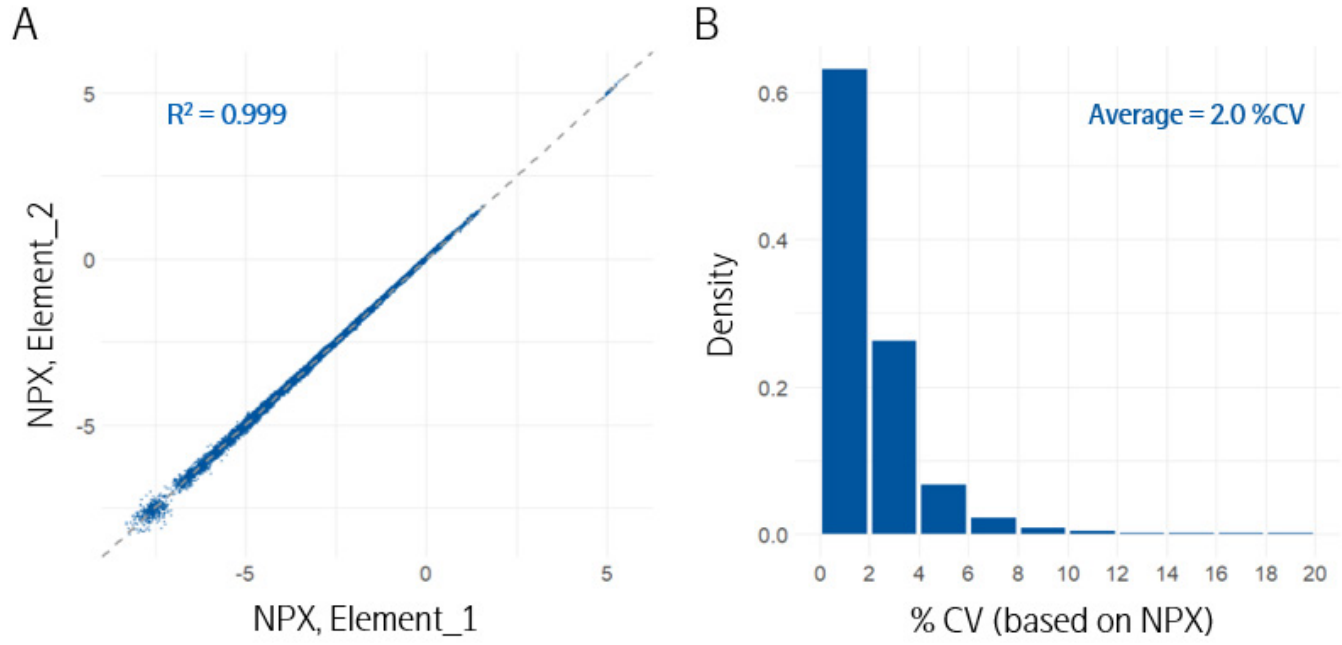

Fig 2. Olink® Explore derived libraries were converted and sequenced on Element's AVITI in two separate experiments. Both the correlation (A) and precision results (B) between the two conversions/sequencings demonstrate high reproducibility of the protocols.

The current Olink® Explore protocol generates a library containing Illumina adapters. Therefore, an additional protocol step was used to incorporate Element adapters (as described above). Element performed this step twice for the fifth library, followed by two separate sequencing runs to determine the repeatability. When comparing the two replicates, we found the correlation to be close to perfect with an R2 of 0.999 both at the NPX level (Figure 2A) and when comparing matched counts directly (not shown). Again, a slightly increased variation could be seen for the lowest counts, as expected. By calculating the variation coefficient for each data point duplicated in the two runs, we found the average precision to be very high (Figure 2B).

Conclusion

These results clearly demonstrate high data quality and high compatibility between Olink® Explore and the Element AVITI System, suggesting that this NGS system could be a detection alternative for Olink® Explore, thereby extending accessibility of this high-plex proteomics solution across the NGS ecosystem.

References

1. Wik L. et al., *Mol Cell Proteomics*. 2021;20:100168
2. Element Adept Library Compatibility Workflow Guide (MA-00001)
3. Element AVITI System Workflow Guide (MA-00008)

For additional information about Olink® Explore products, visit: www.olink.com/products-services/explore/

For additional information about Element Biosciences products and applications, visit: Applications: go.elembio.link/apps and Resources: go.elembio.link/resources

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