Demonstration of FUSION*Plex*™ and VARIANT*Plex*™ Concordance Across Multiple Sequencing Platforms

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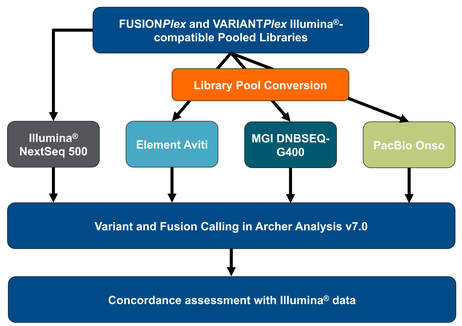
Introduction

The Anchored Multiplex PCR (AMP™) assays FUSION*Plex* and VARIANT*Plex* were developed to identify genetic alterations in RNA and DNA. Initially, AMP assays were limited to sequencing on the Ion Torrent or Illumina® sequencing platforms. With the introduction of new sequencing platforms in recent years, enabling AMP™ technology on additional instruments is needed to further democratize access to these important NGS tools. To address this gap and assess feasibility, FUSION*Plex* and VARIANT*Plex* libraries previously sequenced on the Illumina® platform were pooled, converted, and sequenced on the Element, MGI and PacBio sequencing platforms. These data sets were then analyzed by Archer Analysis and assessed for concordance to the original Illumina® data in terms of analytical performance and detection of RNA fusions and DNA variants.

Methods

FUSION*Plex* Pan Solid Tumor v2 and Heme v2 Illumina®-compatible libraries were prepared using 10ng, 50ng, and 200ng of SeraSeq® FFPE Tumor Fusion v4 and with 50ng of the SeraSeq® Myeloid Mix RNA respectively. VARIANT*Plex* Core Solid Tumor and Core Myeloid Illumina®-compatible libraries were prepared using 10ng, 50ng, and 200ng of SeraSeq® Compromised FFPE Tumor DNA and 100ng of SeraSeq® Myeloid DNA Mutation Mix respectively. Libraries were then pooled together for sequencing. To enable Illumina® library sequencing on additional platforms, each company's conversion kit was used to adapt pooled Illumina® libraries to the format required for each platform: Element Adept Library Compatibility Kit v1.1, MGIEasy Universal Library Conversion Kit (App-A), or Onso library conversion kit. Libraries were sequenced to their recommended read depths on the Illumina® NextSeq 500, Element Aviti, MGI DNBSEQ-G400, and PacBio instruments. All libraries were then normalized to the same read depth, analyzed using Archer™ Analysis v7.0 and assessed for identification of expected fusions and variants.

Sequencer Comparison Scheme



Results – FUSION*Plex* Concordance

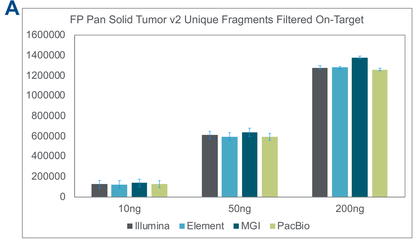


Figure 1. Performance of FUSION*Plex* panels on Illumina®, Element, MGI and PacBio sequencing platforms. A) Unique Fragments Filtered On-Target for 10ng, 50ng, and 200ng of SeraSeq® FFPE Tumor Fusion v4 using the FUSION*Plex* (FP) Pan Solid Tumor v2 panel. B) Unique Fragments Filtered On-Target for 50ng of SeraSeq® Myeloid Mix using the FP Heme v2 panel. All data represents the average of 3 replicates for reach condition.

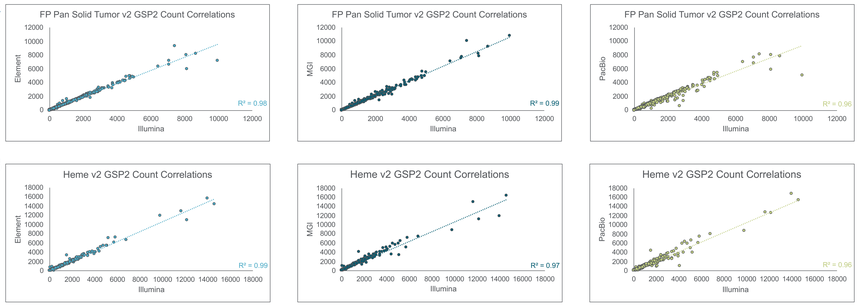


Figure 2. Gene-specific primer count performance for FUSION*Plex* panels. A) GSP2 read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® utilizing the panel FUSION*Plex* (FP) Pan Solid Tumor v2 panel with 50ng of SeraSeq® FFPE Tumor Fusion v4 input. B) GSP2 read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® utilizing the panel FUSION*Plex* Pan Solid Tumor panel with 50ng of SeraSeq® Myeloid Mix input. All data represents the average of 3 replicates for reach condition.

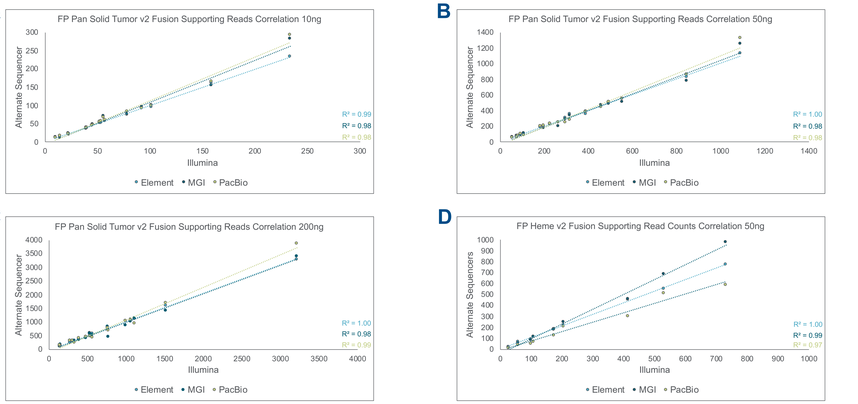


Figure 3. Assessment of RNA fusion detection in FUSION*Plex* panels. Fusion supporting read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® utilizing the panel FUSION*Plex* Pan Solid Tumor panel v2 with A) 10ng, B) 50ng, and C) 200ng of SeraSeq® FFPE Tumor Fusion v4 input. D) Fusion supporting read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® utilizing the panel FUSION*Plex* Pan Solid Tumor panel with 50ng of SeraSeq® Myeloid Mix input. All data represents the average of 3 replicates for reach condition. All replicates detected each expected fusion.

Results – VARIANT*Plex* Concordance

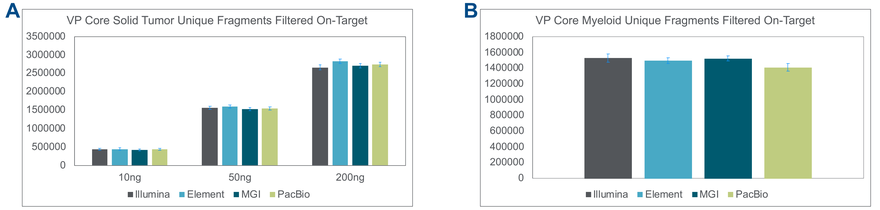


Figure 4. Performance of VARIANT*Plex* panels on Illumina®, Element, MGI and PacBio sequencing platforms. A) Unique Fragments Filtered On-Target for 10ng, 50ng, and 200ng of SeraSeq® Compromised FFPE Tumor DNA using the VARIANT*Plex* (VP) Core Solid Tumor panel. B) Unique Fragments Filtered On-Target for 100ng of SeraSeq® Myeloid DNA Mutation Mix using the Variant*Plex* Core Myeloid panel. All data represents the average of 3 replicates for reach condition.

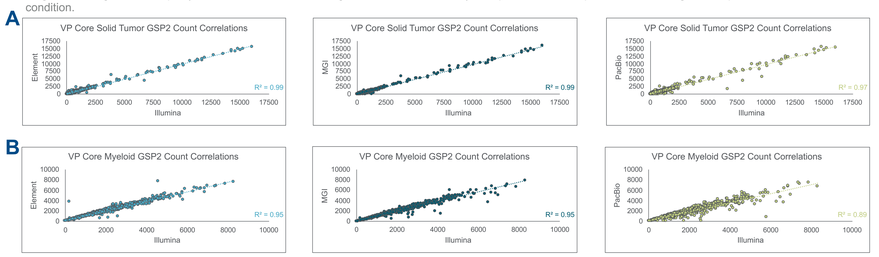


Figure 5. Gene-specific primer count performance for VARIANT*Plex* panels. A) GSP2 read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® for 50ng SeraSeq® Compromised FFPE Tumor DNA with the VARIANT*Plex* (VP) Core Solid Tumor panel. B) GSP2 read count correlations for Element, MGI, and PacBio sequencers compared to Illumina® for 100ng of SeraSeq® Myeloid DNA Mutation Mix using the VARIANT*Plex* Core Myeloid panel. All data represents the average of 3 replicates for reach condition.

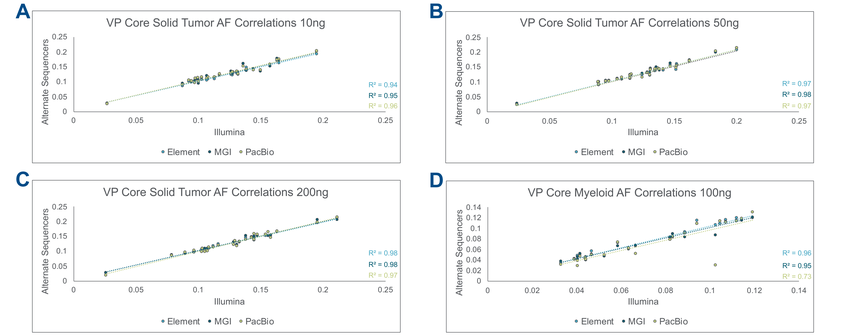


Figure 6. Assessment of DNA variant detection in VARIANT*Plex* panels. A) Allele Frequency (AF) correlations for Element, MGI, and PacBio sequencers compared to Illumina® for 26 expected DNA variants in A) 10ng , B) 50ng, and C) 200ng of SeraSeq® Compromised FFPE Tumor DNA using the VARIANT*Plex* Core Solid Tumor panel. B) AF correlations for Element, MGI, and PacBio sequencers compared to Illumina® for 22 expected DNA variants in D) 100ng of SeraSeq® Myeloid DNA Mutation Mix using the Variant*Plex* Core Myeloid panel. All data represents the average of 3 replicates for reach condition. All replicates detected each expected variant.

Conclusions

Conversion kits enable the sequencing of FUSION*Plex* and VARIANT*Plex* Illumina® libraries on the Element, MGI, and PacBio sequencing platforms. FUSION*Plex* and VARIANT*Plex* libraries provide similar fusion and variant identification performance capability on all sequencing platforms tested, suggesting that both assays are sequencing platform agnostic. This presents an exciting opportunity for the expansion of FUSION*Plex* and VARIANT*Plex* onto new sequencing platforms.

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