Performance of the AVENIO Tumor Tissue Analysis Kits Across Illumina Sequencing Platforms

Introduction

The AVENIO Tumor Tissue Analysis Kits (Research Use Only. Not for use in diagnostic procedures.) are verified to produce enriched tumor tissue libraries, ready to be sequenced on the Illumina NextSeq 500 and 550 sequencing platforms. The analysis kits enable the detection of four mutation classes: single nucleotide variants (SNVs), copy number variations (CNVs), insertions and deletions (indels), and fusions. Three kits (AVENIO Tumor Tissue Targeted Kit, Expanded Kit, and Surveillance Kit) – all demonstrating high sensitivity and positive predictive value (PPV) – are available to sequence selected genes of interest.

In this study, we compare the performance of the AVENIO Tumor Tissue Analysis Kits across three widely available Illumina sequencing platforms: Illumina NextSeq 500, HiSeq 2500 and HiSeq 4000. Ninety sequencing libraries containing variants with low allele frequencies (AF) were sequenced using the three sequencing platforms, and analyzed with the AVENIO Oncology Analysis Software version 2.0.0. The resulting sequencing metrics and detected variants were used to evaluate assay performance across the Illumina sequencing platforms for each of the three AVENIO Tumor Tissue Analysis Kits.

Methods

DNA extracted from formalin-fixed paraffin-embedded (FFPE) cell lines, xenografts, tumor or normal adjacent tissues was blended to create fixed proportions of known variants, listed in Table 1. Genome-in-a-Bottle (GIAB) FFPE cell line blends and Horizon Discovery FFPE Reference Standards were used to determine sensitivity of SNVs, fusions and indels. CNV samples consisted of blends of xenografts or primary tumor with final copy numbers confirmed by digital droplet PCR and Nanostring assays. DNA from normal adjacent FFPE tissues and GIAB FFPE cell lines were used to determine PPV.

Table 1. Sample Summary

Mutation Class	Allele Frequency or Copy Number	Mixture Description	Number of Samples	AVENIO Analysis Kit
Normal	N/A	Normal Adjacent tissue	7	Targeted Kit Expanded Kit Surveillance Kit
		GIAB FFPE cell line	2	
SNV	5%	GIAB FFPE cell line blend	3	
		Horizon Discovery FFPE Reference Standard	6	
Fusion	5%	Horizon Discovery FFPE Reference Standard	3	
		Horizon Discovery FFPE Reference Standard blend	3	
	10%	Horizon Discovery FFPE Reference Standard	3	
CNV	4.80 copies EGFR	Primary Tumor blend	3	
	4.98 copies MET	Xenograft blend	3	
	4.77 copies ERBB2	Primary Tumor blend	3	
Indel	5%	Horizon Discovery FFPE Reference Standard	3	

Methods *(Continued)*

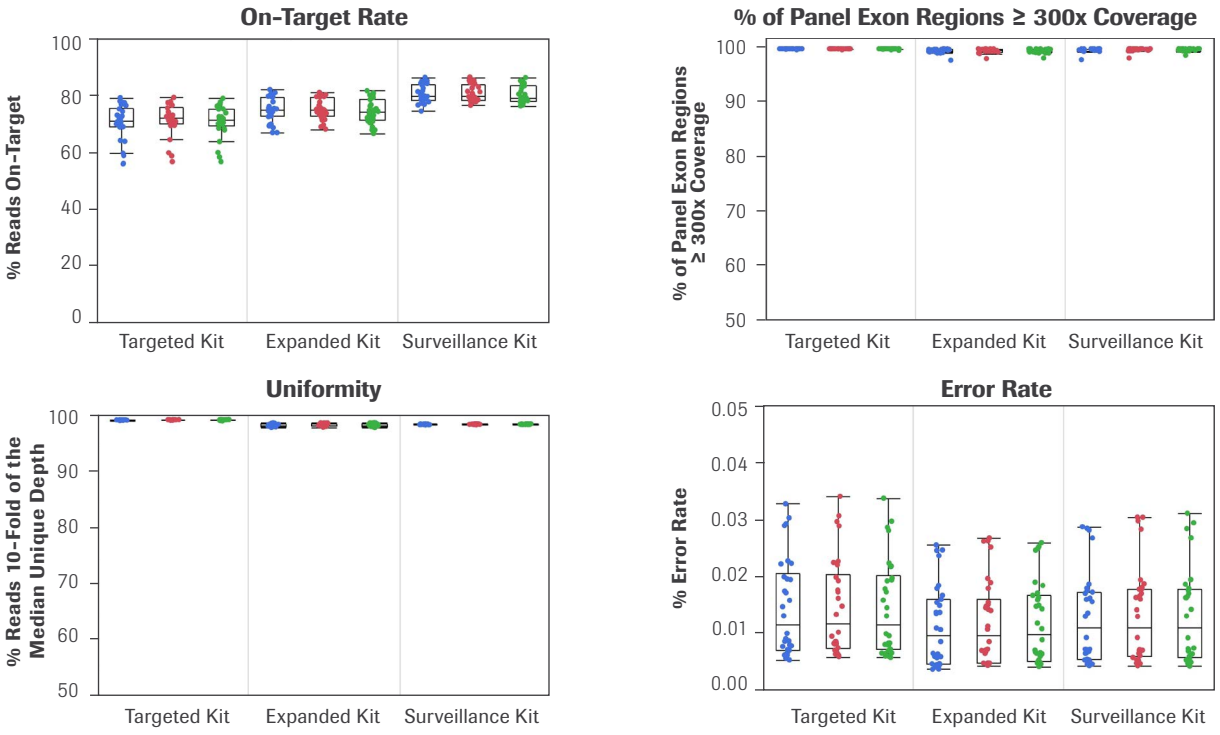
Sequencing libraries were prepared using the AVENIO Tumor Tissue Analysis Kits workflow utilizing (10/Q-ratio +10) ng of input DNA where Q-ratio is the normalized quality score from the quantitative PCR assay. The same sequencing libraries were sequenced across all three platforms. 20 million paired end reads (10 million clusters) for the Targeted, Expanded, and Surveillance Kits were generated per sample. For the Illumina HiSeq 2500 system, the library pool was loaded on two sequencing lanes to generate equivalent sequencing depth with NextSeq 500 and HiSeq 4000 systems. All sequencing runs were loaded to attain Illumina's recommended cluster densities. In total, 90 libraries were sequenced across three platforms, generating 270 distinct sequencing data sets for variant analysis and metric comparisons. The sequencing data were analyzed using an internal analysis pipeline equivalent to the commercially available AVENIO Oncology Analysis Software version 2.0.0 which can process data from the Illumina HiSeq 2500 and HiSeq 4000 systems, but special instructions are required. Instructions for analyzing data from the HiSeq 2500 and HiSeq 4000 systems with the AVENIO Oncology Analysis Software version 2.0.0 are available from your Roche representative.

Results

Sequencing Metrics: Key sequencing metrics of the sequencing libraries are plotted in Figure 1 for each of the Analysis Kits for each Illumina sequencing platform. The figure includes box plots showing the median, interquartile range and 10th/90th percentile for each metric. Metric definitions are as follows: *On-Target rate:* the percentage of reads in the intended target region. *Percentage of panel exon regions greater than 300× coverage*: the percentage of the panel exon regions covered by greater than or equal to 300 unique reads per position after removing duplicated reads. A unique depth of 300× is a sufficient depth to robustly differentiate true variants as low as 5% allele fraction from background noise. This metric assesses depth and uniformity. *Uniformity:* the percentage of positions whose unique depth fall within ten-fold of the median unique depth. *Error rate:* the percentage of non-reference bases observed over total bases observed for positions that are likely reference (e.g. excluding germline SNPs). Error rate in tissue samples can vary with sample quality (i.e. Q-ratio).

The expected values for on-target rate, percentage of panel exon regions with equal to or greater than 300× coverage, uniformity, and error rate were achieved by the AVENIO Tumor Tissue Analysis kits, at consistent and high performing values. All three panels attained high sequencing depth across the sequencers with greater than 97.9% of panel exon regions with equal to or greater than 300× coverage. The error rates across all sequencing platforms were comparable. Overall, the Illumina HiSeq 2500 and HiSeq 4000 sequencing platforms were able to generate sequencing metrics similar to the NextSeq 500/550 system.

Figure 1. Sequencing Metrics. NextSeq 500 system (blue), HiSeq 2500 system (red), HiSeq 4000 system (green)



Variant Analysis

SNVs: 18, 29, and 32 loci of interest SNVs at 5% AF for the Targeted, Expanded, and Surveillance Kits, respectively, were analyzed for sensitivity. All SNVs were detected across all sequencing platforms achieving sensitivities of >99.9%. Analyzing the normal adjacent FFPE tissues and GIAB cell lines for false positive rate estimation yielded excellent PPV.

	Single Nucleotide Variants					
	Sensitivity			PPV		
AVENIO Analysis Kit	NextSeq 500	HiSeq 2500	HiSeq 4000	NextSeq 500	HiSeq 2500	HiSeq 4000
Targeted Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Expanded Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Surveillance Kit	>99.9%	>99.9%	>99.9%	>99.9%	99.0%	99.0%

Indels: 3 indels at 5% AF for each of the kits were evaluated for sensitivity. All sequencing platforms achieved >99.9% sensitivity, with PPV >99.9%.

	Insertions and Deletions					
	Sensitivity			PPV		
AVENIO Analysis Kit	NextSeq 500	HiSeq 2500	HiSeq 4000	NextSeq 500	HiSeq 2500	HiSeq 4000
Targeted Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Expanded Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Surveillance Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%

Fusions: Fusion samples were analyzed for the detection of fusions at 5% and 10% AFs across three kits. All fusions were detected in all samples at a sensitivity of >99.9% across sequencing platforms with excellent PPV.

	Fusions					
	Sensitivity			PPV		
AVENIO Analysis Kit	NextSeq 500	HiSeq 2500	HiSeq 4000	NextSeq 500	HiSeq 2500	HiSeq 4000
Targeted Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Expanded Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Surveillance Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%

CNVs: CNV samples were analyzed for sensitivity with 4.80 copies of EGFR, 4.98 copies of MET, and 4.77 copies of ERBB2. In this study, for the Surveillance Kit, the HiSeq 4000 system had higher sensitivity than the HiSeq 2500 and NextSeq 500 systems. It is important to note that the data in this study had been subsampled to 20 million paired end reads (10 million clusters) per sample for the head-to-head comparison. The CNV sensitivity for the NextSeq 500 is >99.9% when it is not subsampled. The AVENIO Oncology Analysis Software version 2.0.0 includes a CNV caller with an emphasis on high specificity, and was able to achieve PPV of >99.9% across all platforms.

	Copy Number Variations					
	Sensitivity			PPV		
AVENIO Analysis Kit	NextSeq 500	HiSeq 2500	HiSeq 4000	NextSeq 500	HiSeq 2500	HiSeq 4000
Targeted Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Expanded Kit	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%	>99.9%
Surveillance Kit	75.0%	91.6%	>99.9%	>99.9%	>99.9%	>99.9%

Conclusion

In this study, the AVENIO Tumor Tissue Analysis Kits created libraries that, when sequenced on multiple Illumina platforms, achieved high sensitivity and PPV for all four mutation classes. The AVENIO Tumor Tissue Analysis Kits were evaluated across three Illumina sequencing platforms: NextSeq 500, HiSeq 2500, and HiSeq 4000. The expected sequencing metrics of on-target rate, percentage of panel greater than 300× coverage, uniformity, and error rate were achieved across all three platforms in an equivalent manner, extending the use of the AVENIO Tumor Tissue Analysis Kits to additional sequencing platforms.

Note it is important that the user loads the sequencer at a concentration that yields the instrument's recommended cluster density. Also, for each sequencing platform, the total number of samples loaded on the sequencer should be considered. To obtain optimal mutation detection sensitivity, aim for each sample to receive sequencing coverage of over 20 million paired end reads (10 million clusters) for all the three panels. In conclusion, the AVENIO Tumor Tissue Analysis Kits achieved similar high performance on all three platforms.

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Published by:

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Pleasanton, CA 94588

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MC--01646 06/2019