ST043

AVENIO Tumor Tissue CGP Automated Assay: End-to-End Solution With High Performance on NextSeq 500/550

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Summary

The AVENIO Tumor Tissue CGP Automated Assay (For Research Use Only. Not for use in diagnostic procedures.) demonstrates high performance with sequencing outputs from NextSeq 500/550 systems. The assay has a 99.3% total sample pass rate, is guardbanded to a wide range of DNA inputs from 40 ng to 300 ng, shows high reproducibility and precision on FFPE tumor tissue specimens and reference DNA materials, and achieved > 98% PPA with the FoundationOne®CDx test for short variants detection.

Introduction

The AVENIO Tumor Tissue CGP Automated Assay (For Research Use Only. Not for use in diagnostic procedures.) utilizes a hybridization- based capture and next-generation sequencing (NGS) workflow to deliver comprehensive genomic profiling (CGP) of solid tumors, covering 2.27 Mb across 335 genes and selected non-coding regions. The test is end-to-end from tissue DNA extraction to variant calling, and offers flexibility in sequencing target-enriched sample libraries on both NovaSeq 6000 and NextSeq 500/550 systems. The objective of this study series is to specifically assess the analytical performance of the test when using the NextSeq 500/550 system ("NextSeq Workflow").

Materials and Methods

This study series, which focuses on the "NextSeq Workflow," evaluates several performance metrics: 1) **Robustness**, evaluated through sample pass rates across 1144 FFPE tumor tissue specimens (37 tumor types and 90 disease ontologies) and DNA input guardband assessments across 12 specimens with various input amounts from 10 ng to 600 ng, 2) **Reproducibility**, assessed using 132 FFPE tumor tissue samples comprising 12 replicates of 11 unique specimens, and further evaluated in a separate study with reference DNA materials from SeraCare and Horizon Discovery, and 3) **Positive Percent Agreement** for variants with known pathogenic status from 859 FFPE tumor tissue specimens compared to the FoundationOne®CDx test to evaluate detection agreement.

High Reproducibility and Repeatability Demonstrated for FFPE Tissue Specimens with End-to-End Process

Variant Type*	Unique Variants	Metric	Pairwise Comparison**
SV	69	APA	99.61%
		ANA	99.96%
CNA	23	APA	100%
		ANA	100%
RE	3	APA	100%
		ANA	100%
All	95	APA	99.72%
		ANA	99.97%

*: Known or likely pathogenic status only
**: Excluded marginal calls: SNV and InDels with MAF < 1.5% and < 8% for variants with known and likely pathogenic status, respectively, and variants with < 100X average depth; CNAs with equivocal status, excluding gene loss with average copy number ratio > 0.7; Rearrangements with supporting reads < 15

Biomarker Type	Pathogenic Status	Total Unique Variants	Filter Threshold	Positive Call Rate Among All Observed Mutations (95% CI)
All SNVs	All***	155	no filter	**97.65%** 1828/1872 (96.86%,98.24%)
SNVs	All	154	MAF ≥ 1%	**98.23%** 1827/1860 (97.52%,98.73%)
SNVs	All	152	MAF ≥ 2%	**99.18%** 1821/1836 (98.66%,99.5%)
SNVs	All	151	MAF ≥ 3%	**99.78%** 1820/1824 (99.44%,99.91%)
SNVs	All	150	MAF ≥ 5%	**99.78%** 1808/1812 (99.43%,99.91%)
SNVs	All	149	MAF ≥ 6%	**99.94%** 1799/1800 (99.69%,99.99%)
SNVs	All	147	MAF ≥ 7%	**99.94%** 1775/1776 (99.68%,99.99%)
SNVs	All	145	MAF ≥ 8%	**100%** 1752/1752 (99.78%,100.0%)
All InDels	All	63	no filter	**84.66%** 701/828 (82.05%,86.96%)
InDels	All	60	MAF ≥ 1%	**89.97%** 691/768 (87.65%,91.9%)
InDels	All	56	MAF ≥ 2%	**93.79%** 664/708 (91.76%,95.34%)
InDels	All	55	MAF ≥ 5%	**93.68%** 652/696 (91.62%,95.26%)
InDels	All	54	MAF ≥ 6%	**95.18%** 651/684 (93.3%,96.54%)
InDels	All	53	MAF ≥ 7%	**96.43%** 648/672 (94.74%,97.59%)
InDels	All	52	MAF ≥ 8%	**96.36%** 636/660 (94.65%,97.54%)

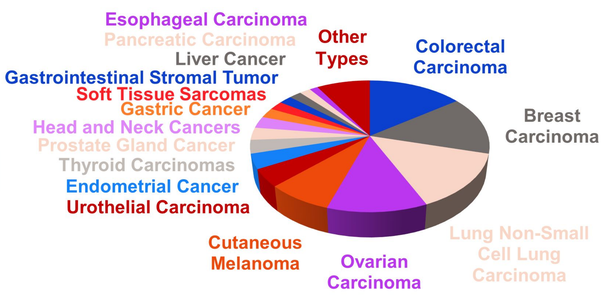
***: "All" = known, likely, and unknown pathogenic statuses

This study involved 11 unique FFPE tissue specimens, sectioned in order and evenly distributed into 12 replicates for a total of 132 samples. These samples were processed in six batch runs, each with 11 specimens in duplicates, on two AVENIO Edge Systems using three reagent lots. The assay demonstrated high reproducibility, with pairwise comparisons among sample replicates showing APA and ANA > 99% for variants with known or likely pathogenic status. Reproducibility is further demonstrated by the positive call rates for SNVs and InDels, which are > 95% for all MAF thresholds and MAF ≥ 6%, respectively.

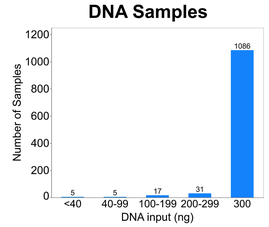
Results

High Sample Pass Rate with FFPE Tissue Specimens

Tumor Types of FFPE-Tissue Derived DNA Samples Used for Robustness Assessment (N = 1144)



DNA Input for FFPE-Tissue Derived DNA Samples



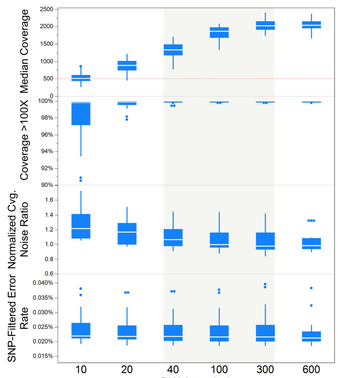
Pass Rates after First Test and Re-test

	Number of Samples
First Test Pass Rate	***93.4% (1068/1144)***
First Test Fail	76
Re-test Pass	68
Total Pass Rate	***99.3% (1136/1144)***

The "NextSeq Workflow" demonstrated robust performance, with a high first-attempt pass rate (93.4%) and a high total pass rate after re-testing (99.3%). Regarding DNA input, 99.6% (1139/1144) of samples were within the recommended range (40 ng to 300 ng).

High Sample Pass Rate and Correct Call Rate across Wide Range of DNA Input Levels

Sequencing Metrics for DNA Inputs Ranging from 10 ng to 600 ng



Sample Pass Rate and Correct Call Rates for DNA Inputs from 10 ng to 600 ng

Assay DNA Input Recommendation: Optimum 300 ng, Minimum 40 ng

Input Mass (ng)	Pass Rate	Correct Call Rate
600	100%	100% (286/286)
300	100%	**Reference**
100	100%	100% (440/440)
40	100%	100% (440/440)
20	100%	99.8% (439/440)*
10	100%	99.3% (397/400)*

*: Missed variants at 10 ng and 20 ng inputs were SVs.

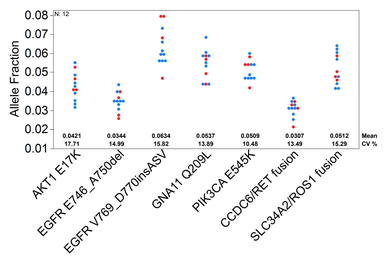
- DNA input amount of the AVENIO Tumor Tissue CGP Automated Assay is normalized on AVENIO Edge System post-automated fluorometric quantification.
- The library DNA input is 300 ng if specimens yield a sufficient amount post-DNA extraction and cleanup.
- If specimens yield insufficient DNA amount to meet the 300 ng input, the maximum amount of available DNA will be input into downstream library preparation.

A set of 12 unique FFPE-tissue derived DNA samples, sourced from different tissue types with various DNA qualities, were prepared at six mass input levels (10, 20, 40, 100, 300, and 600 ng) to evaluate assay performance across a range of DNA inputs. Sample metrics and variant data are aggregated to evaluate the sample pass rate and correct call rate of each DNA input level, using the samples with targeted 300 ng input as reference for the variant calls.

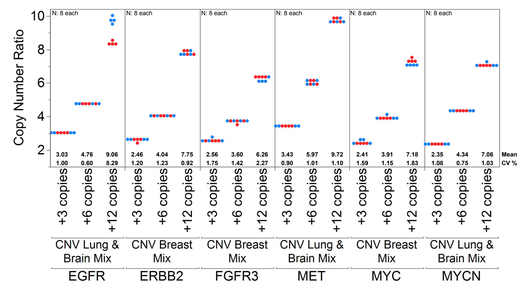
The results indicate that the recommended DNA input range between 40 ng and 300 ng consistently achieved high pass rates and robust variant detection.

High Reproducibility and Precision Demonstrated for Reference DNA Materials

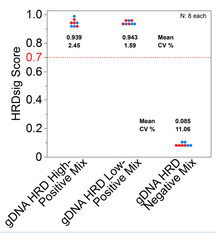
Horizon Discovery HD753 Genomic Variants



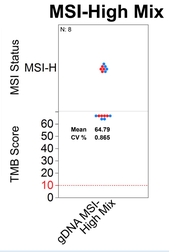
SeraCare CNV-Mixes



SeraCare HRD-Mixes



SeraCare MSI-High Mix



The reproducibility and precision performance of the "NextSeq Workflow" is further demonstrated using reference DNA materials from SeraCare and Horizon Discovery. It is tested across two DNA input levels (10 ng and 50 ng) in two different laboratories. Red and blue data points indicate data from each laboratory.

- Verified SVs and REs in HD753 are 100% detected in all replicates, with low CVs for the MAFs.
- SeraCare CNV-Mixes showed expected copy number ratios, all with very low CVs.
- SeraCare genomic signature mixes (HRD, MSI-High) consistently achieved the expected signature status. SeraCare gDNA MSI-High Mix also demonstrated a TMB-High status in the test, with TMB scores above 60.

High Agreement between AVENIO Tumor Tissue CGP Automated Assay and FoundationOne®CDx Assay

Variant Type (Pathogenic Status)	Valid Sample N	Expected Positive Variant N	PPA	NPA	PPV	NPV
SNV (Known)	859	1615	99.3%	99.99%	99.7%	99.99%
InDel (Known)	859	550	98.7%	99.99%	95.4%	99.99%
CN-amplification (Known)	852	642	96.7%	99.99%	99.5%	99.98%
CN-gene loss (Known)	852	565	95.4%	99.93%	94.4%	99.94%
Rearrangement (Known)	859	93	94.6%	99.99%	96.7%	99.98%

Excluded marginal calls: SNVs and InDels with MAF < 2.0%; CNAs with equivocal status; Rearrangements with supporting reads < 15

Genomic Signatures	Valid Sample N	Expected Positive Sample N	PPA	NPA	PPV	NPV
MSI-High	835	63	100%	100%	100%	100%
TMB-High	784	147	93.9%	98.4%	93.2%	98.6%
gLOH-Positive (Ovarian Cancer Only)	152	84	96.4%	97.1%	97.6%	95.7%
HRDsig-Positive	824	139	90.6%	98.5%	92.6%	98.1%

Genomic signature score cutoff used: MSI-High with score ≥ 0.0124; TMB-High with score ≥ 10 mutations per megabase; gLOH-Positive with score ≥ 0.16; HRDsig-Positive with score ≥ 0.7

The AVENIO Tumor Tissue CGP Automated Assay demonstrated high accuracy, with a PPA > 98% for detecting SNVs and InDels with known pathogenic status, and a PPA > 94% for detecting CNAs and rearrangements with known pathogenic status. For sample-level genomic signatures, TMB, MSI, genomic LOH (gLOH), and HRDsig, all exhibited PPAs and NPAs above 90%.

Conclusions

Across several studies, the AVENIO Tumor Tissue CGP Automated Assay with sequencing analysis on NextSeq has demonstrated high sample pass rates, high variant-calling agreement compared to FoundationOne®CDx, and robust performance in variant detection. The assay also maintains strong performance with a wide range of DNA sample inputs. This "NextSeq Workflow" provides users the flexibility to select an NGS sequencing platform based on sample throughput needs, thus enhancing operational efficiency.

https://ter.li/azjbay

Please contact the lead author at James Blau (james.blau@roche.com) for any questions.

Abbreviations

ANA, average negative agreement; APA, average positive agreement; CGP, comprehensive genomic profiling; CI, confidence interval; CNA, copy number alteration; CNV, copy number variation; CV, coefficient of variation; FFPE, Formalin Fixed Paraffin Embedded; genomic LOH, genomic loss of heterozygosity; HRD, homologous recombination deficiency; HRDsig, HRD signature; InDel, insertion and deletion; MAF, mutant allele fraction; MSI, microsatellite instability; MSI-H, MSI-high; NPA, Negative Percent Agreement; NPV, Negative Predictive Value; PPA, Positive Percent Agreement; PPV, Positive Predictive Value; RE, Rearrangement; SNP, single nucleotide polymorphism; SV, short variant; TMB, tumour mutational burden.

Conflicts of interest

Authors are employees and/or stock holders of Roche Diagnostics or Foundation Medicine, Inc. This study was sponsored by F. Hoffmann-La Roche Ltd.

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