Application Note

Genomics

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Efficient Tumor Genomic Profiling with Agilent SureSelect, Element AVITI, and SeqOne

Authors

Akanksha Khare, MS,
Agilent Technologies, Inc.
Santa Clara, CA, USA

Brian Coullahan and
Kelsey Swartz, PhD,
Element Biosciences
San Diego, CA, USA

Samantha Brunel, PhD
and Tiffany Delhomme, PhD,
SeqOne Genomics
Montpellier, France

Abstract

Comprehensive genomic profiling (CGP) leverages targeted, next-generation sequencing (NGS) to detect multiple types of genetic alterations in tumor samples through a single assay. This enables faster and more efficient investigations to identify key biomarkers of complex cancers. The implementation of CGP in routine laboratory settings, such as for large-scale projects or laboratories aiming to bring the assay in-house, requires a high-performing, end-to-end workflow that is easy to implement. The workflow should require minimal NGS expertise and offer rapid turnaround time.

This application note demonstrates the robust detection of somatic alterations using the Agilent SureSelect Cancer CGP assay. Detected alterations include single nucleotide variants (SNVs), insertions and deletions (indels), copy number variations (CNVs), translocations (TLs), the immuno-oncology biomarkers tumor mutation burden (TMB) and microsatellite instability (MSI), as well as gene fusion transcripts. The SureSelect Cancer CGP assay workflow presented here is seamless from DNA or RNA to data analysis, with automated library preparation and target enrichment on the Agilent Magnis NGS prep system, sequencing on the Element Biosciences AVITI platform, and data analysis by the SeqOne bioinformatics platform.

This application note was developed in collaboration with:

Element Biosciences

SeqOne
FROM GENOME TO PERSONALIZED MEDICINE

Introduction

Advances in precision oncology research, such as elucidating molecular changes that drive tumor development and progression, are often propelled by new genomic technologies. Comprehensive genomic profiling (CGP) offers a next-generation sequencing (NGS)-based method to analyze major classes of somatic mutations in tumor samples, providing critical insights into tumor biology and facilitating the discovery of potentially actionable biomarkers.

The Agilent SureSelect Cancer CGP assay interrogates catalog panels of cancer-specific genes, comprising 679 genes for the DNA panel and 80 genes for the RNA panel.[1] The DNA panel enables the detection of somatic variants, including single nucleotide variants (SNVs), insertions and deletions (indels), copy number variations (CNVs), and translocations (TLs), as well as the immuno-oncology biomarkers tumor mutation burden (TMB) and microsatellite instability (MSI). The RNA panel enables the detection of fusion genes that are expressed as RNA transcripts, where only one gene partner is required for detection. In addition, homology recombination deficiency (HRD) status can be assessed by the addition of a low-pass whole-genome sequencing (WGS) step.[2]

The panel content for the SureSelect Cancer CGP assay was curated based on insights from leading cancer researchers[3] and guided by somatic cancer databases (ClinVar, COSMIC, NCCN, and others). This provides a comprehensive view of well-characterized oncology biomarkers and enhances the discovery of novel variants.

The SureSelect Cancer CGP assay features a flexible and modular workflow that includes library preparation, target enrichment, and sequencing, followed by data analysis and reporting. When considering the adoption of a CGP assay for routine laboratory use, in-house implementation, or large-scale projects, practical determinants such as ease of use, NGS expertise, and turnaround time are crucial. Here we evaluate the performance of the SureSelect Cancer CGP assay in an end-to-end workflow that integrates a benchtop, walkaway automation platform (Agilent Magnis NGS prep system) for library preparation and target enrichment, a state-of-the-art benchtop sequencing platform (Element AVITI platform), and a cloud-based bioinformatics solution (SeqOne platform) for data analysis (Figure 1).

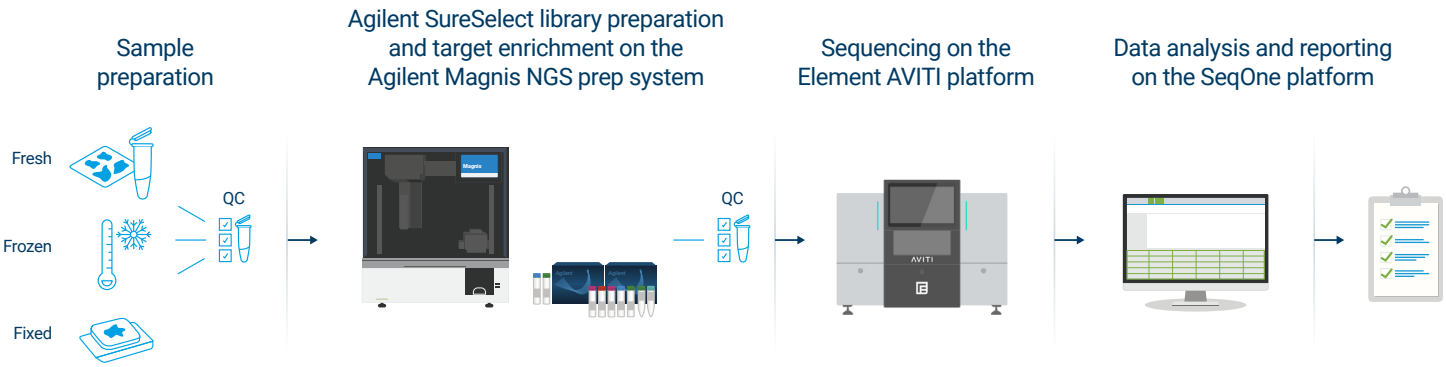

Figure 1. End-to-end workflow using the Agilent SureSelect Cancer CGP assay with the automated Agilent Magnis NGS prep system, Element AVITI sequencer, and SeqOne data analysis platform. The SureSelect Cancer CGP assay is designed to accommodate various tumor sample types, including fresh, frozen, and formalin-fixed paraffin-embedded (FFPE) tissue blocks. The workflow includes critical sample and NGS library quality control steps to help ensure high-quality sequencing results.

Simplified, automated library preparation and target enrichment

The Agilent Magnis NGS prep system is a compact, fully automated benchtop platform designed to produce target-enriched, sequencing-ready libraries for the SureSelect Cancer CGP assay and other SureSelect catalog and custom assays. The system requires only 15 minutes of hands-on setup time and automates all steps for library preparation and target enrichment, including enzymatic fragmentation for DNA, cDNA conversion for RNA, and bead cleanup. This automation improves laboratory efficiency and productivity by reducing labor, ensuring reliable, consistent results with shortened turnaround time.

The ready-to-use reagent cartridges, touch screen operation, and prevalidated protocols for the Magnis NGS prep system minimize potential operator error and facilitate easy training and operation, including for individuals with limited experience in NGS techniques, making it ideal for fast-paced laboratory environments. Throughput can be scaled by preparing up to eight libraries per run, with up to two runs per day, and also by performing sequential runs or running multiple systems in parallel.

Advanced sequencing

The Element AVITI System is a benchtop sequencing platform that provides flexible throughput at a low cost, saving time and resources without the need to batch or compromise quality. The system uses highly accurate avidite base chemistry (ABC) sequencing, which adapts to various applications, from small, targeted panels to whole genome sequencing. The Cloudbreak Freestyle sequencing workflow allows libraries generated using existing adaptor sequences to be directly loaded onto the sequencer without conversion or modification. With industry-leading accuracy, yielding greater than 90% Q30 quality, the AVITI System is suitable for sequencing and detecting low-frequency variants in sample types ranging from formalin-fixed paraffin-embedded (FFPE) samples to plasma-derived cfDNA.

Optimized data analysis

SeqOne is a cloud-based bioinformatics platform that supports comprehensive genomic analysis for both somatic and germline applications. The SomaCGP workflow, developed for the Agilent SureSelect Cancer CGP assay, is designed to accurately detect key variant classes, including SNVs, indels, TMB, MSI, TLs, gene fusions, and CNAs*, as well as events such as loss of heterozygosity (LOH) and estimation of tumor purity.

The SomaCGP workflow leverages advanced algorithms to analyze sequencing data, calling, annotating, and prioritizing various types of genomic alterations. This automated process generates a detailed summary of actionable alterations and relevant insights, which can be exported in multiple formats for further review. The SeqOne Data Sync (SDS) module simplifies the analysis workflow by automating file transfer, project setup, pipeline execution, and results access.

Experimental

1. **Samples:** To evaluate the SureSelect Cancer CGP DNA assay, DNA from reference standards that cover a range of different somatic variants and from FFPE samples with known variants were used as input (50 ng) to generate libraries. To evaluate the SureSelect Cancer CGP RNA assay, RNA from samples with known fusions derived from reference standards and from FFPE samples were used as input (50 ng) to generate libraries.
2. **Library preparation and target enrichment:** For each sample, target-enriched libraries were prepared according to the SureSelect Cancer CGP assay user guide[4], following the Magnis NGS prep workflow protocols for automated library preparation and target enrichment of the DNA or RNA panel. Libraries used standard Illumina adaptors or were modified with Element library adaptors using the Element Adept Library Compatibility Kit, as noted (Figures 2 and 5).
3. **Sequencing:** Libraries were sequenced using the Element AVITI System,[5] with one or two sequencing workflows (Cloudbreak Freestyle or Adept Library Compatibility), or using the Illumina NovaSeq 6000 System,[6] as noted in the results. Sequencing data were downsampled to 6 Gb (40 million 2 x 150 bp reads) for the DNA assay, and 10 million 2 x 150 bp reads) for the RNA assay.
4. **Data analysis:** Biomarker analysis was performed using the SomaCGP workflow from SeqOne.[7]

**Copy number variants, described in this application note as CNVs, are referred to as a copy number alterations (CNAs) in the SeqOne software.*

Table 1. Materials used in this study.

Workflow Step	Material	Vendor	Part Number	Notes
Samples for DNA Assay	HapMap gDNA	Coriell	NA12878	DNA reference sample: Well-characterized human gDNA widely used for benchmarking sequencing technologies and variant analysis
	Mimx Structural Multiplex Reference Standard	Horizon Discovery	HD753	DNA reference sample: Structural gDNA reference standard with complex structural variants
	OneSeq Human Reference DNA, Female	Agilent	5190-8850	DNA reference sample: Cell line-derived gDNA reference standard with known CNVs, SNPs, and indels
	Quantitative Multiplex Reference Standard fcDNA (moderately degraded FFPE)	Horizon Discovery	HD799	DNA reference sample: Formalin-compromised gDNA standard mimicking moderately degraded FFPE samples, containing engineered SNVs, indels, and structural variants
	Breast tumor-1	Discovery Life Sciences	NA	FFPE sample with known variant: CNV – *ERBB2*
	Breast tumor-2	BioIVT	NA	FFPE sample with known variant: CNV – *ERBB2*
	Colon tumor-1	Tissue Solutions	NA	FFPE sample with known variant: MSS
	Colon tumor-2	Tissue Solutions	NA	FFPE sample with known variant: MSI-H
	Colorectal tumor-1	Cureline Translational CRO	NA	FFPE sample with known variant: TMB – High
	Lung tumor-1*	US Biolab	NA	FFPE sample with known variant: Translocation - *ALK/EML4*
	Lung tumor-2*	US Biolab	NA	FFPE sample with known variant: Translocation - *ALK/EML4*
	Melanoma tumor-1	Discovery Life Sciences	NA	FFPE sample with known variant: SNV
Samples for RNA Assay	Mimix *ALK RET ROS* RNA Fusion Reference Standard	Horizon Discovery	HD784	FFPE RNA fusion reference standards containing *EML4-ALK*, *CCDC6-RET*, and *SLC34A2-ROS1* fusions
	Seraseq Fusion RNA Mix v4	LGC Clinical Diagnostics (formerly SeraCare)	0710-0497	Total RNA from well-characterized GM24385 cell line as background wild type material
	Universal Human Reference RNA (UHRR)	Agilent	740000	Total RNA from human cell line with known fusions
	Lung tumor-3	US Biolab	NA	FFPE sample with known variant: Translocation - *ALK/EML4*
	Lung tumor-4	US Biolab	NA	FFPE sample with known variant: Translocation - *ALK/KIF5B*
Library Preparation and Target Enrichment	Magnis SureSelect Cancer CGP, XT HS2 DNA, 96 reactions	Agilent	G9777B	For use on the Magnis NGS prep system: reagents for enzymatic fragmentation, library generation and target enrichment, enrichment probe for DNA (679 genes)
	Magnis SureSelect Cancer CGP, XT HS2 RNA, 96 reactions	Agilent	G9777D	For use on the Magnis NGS prep system: reagents for enzymatic fragmentation, reverse transcription reagents, library generation and target enrichment, probe for RNA (80 gene)
	Adept Library Compatibility Kit v1.1	Element Biosciences	830-00007	Benchtop circularization reagents to adapt libraries for AVITI sequencing
Sequencing	AVITI 2x150 Sequencing Kit Cloudbreak FS Medium Output	Element Biosciences	860-00012	For use on the AVITI System: includes reagents and flow cell for linear loading and sequencing of third-party libraries
	AVITI 2x150 Sequencing Kit High Output	Element Biosciences	860-00003	For use on the AVITI System: flow cell and sequencing reagents for Adept libraries
	NovaSeq 6000 SP Reagent Kit v1.5 NovaSeq 6000 S1 Reagent Kit v1.5 NovaSeq 6000 S2 Reagent Kit v1.5	Illumina	20028400 20028317 20028314	For use on the NovaSeq 6000 System: flow cells and sequencing reagents for Illumina libraries

**These samples were used for variant detection using the DNA assay and RNA assay.*

Results and discussion

Comparable target sequencing performance across short-read sequencers

SureSelect Cancer CGP DNA libraries, based on DNA reference samples and enriched for 679 cancer-specific genes, were generated on the Magnis NGS prep system. Sequencing was performed on two short-read sequencing systems, the advanced Element AVITI instrument and the established Illumina NovaSeq 6000 instrument. Sequencing metrics show similar high uniformity and target coverage across all configurations tested (Figure 2), demonstrating equivalent target sequencing performance between the AVITI sequencing workflows, as well as between the AVITI and NovaSeq 6000 systems.

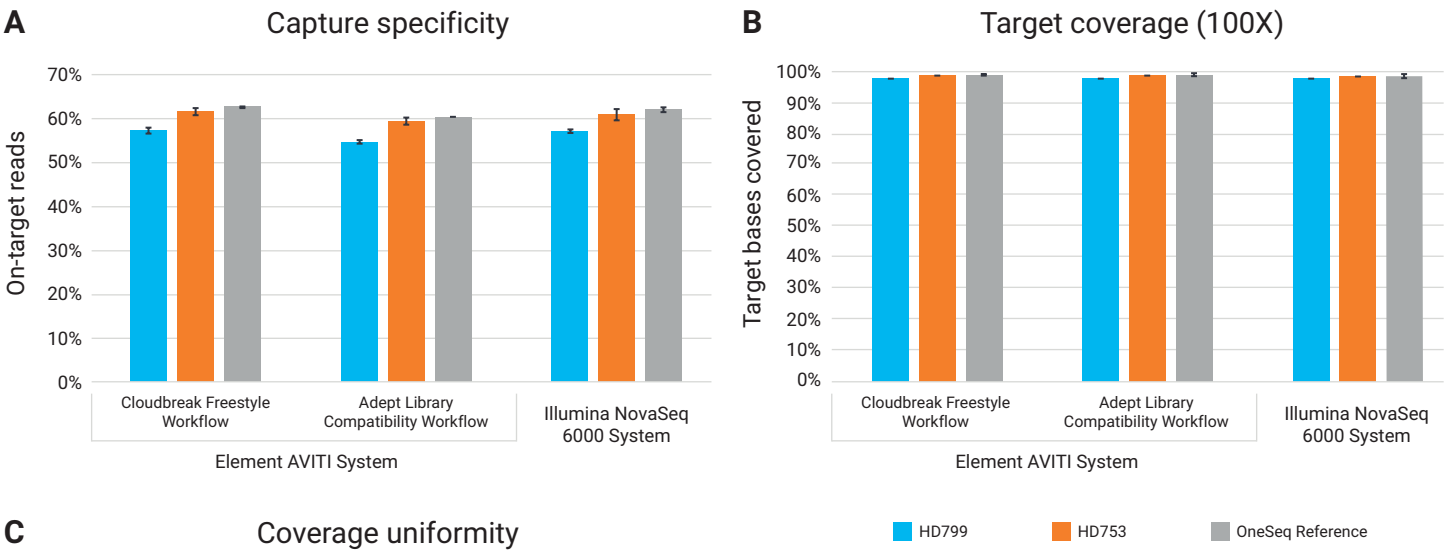

Figure 2. High uniformity and target coverage for the Agilent SureSelect Cancer assay across short-read sequencers. Reference samples, including the Structural Multiplex Reference Standard gDNA (HD753), Quantitative Multiplex Reference Standard fcDNA moderate (HD799), and OneSeq Human Reference DNA, were used to generate SureSelect Cancer CGP DNA libraries on the Agilent Magnis NGS prep system. Libraries were sequenced on the Element AVITI System (Cloudbreak Freestyle kit, Adept Library Compatibility Kit with AVITI sequencing kit) and on the Illumina NovaSeq 6000 System. Sequencing metrics demonstrate strong performance for all sequencing runs, as measured by (A) capture specificity, which is shown by fraction of an on-target reads, (B) target coverage, with high on-target rates and low variability between different sequencing platforms, and (C) coverage uniformity, as expressed by Fold-80 base penalty, a measure of how even the coverage is across the panel. A Fold-80 base penalty score of less than or equal to 1.4 to 1.5 is typical for a good quality dataset.

Robust somatic variant detection across different tumor samples by the DNA assay

DNA reference samples evaluated by the SureSelect Cancer CGP DNA assay show a high percentage of reads that overlap with the target region (Figure 3). This demonstrates excellent enrichment efficiency across the 679-gene panel target and indicates sufficient coverage for somatic variant detection at 5% allelic frequency.

The performance of the SureSelect Cancer CGP DNA assay to detect different classes of somatic variants was evaluated. DNA from reference standards and FFPE samples that contain known variants were used to generate SureSelect Cancer CGP DNA libraries on the Magnis NGS prep system. Sequencing was performed on the Element AVITI System using the Cloudbreak Freestyle kit. Data analysis was performed by the SeqOne platform.

For the SNV and indel analysis, the SeqOne pipeline consistently identified all expected short variants across different sample types, with sequencing coverage exceeding 150X, highlighting the 100% sensitivity and precision of this method for SNV and indel detection (Table 2).

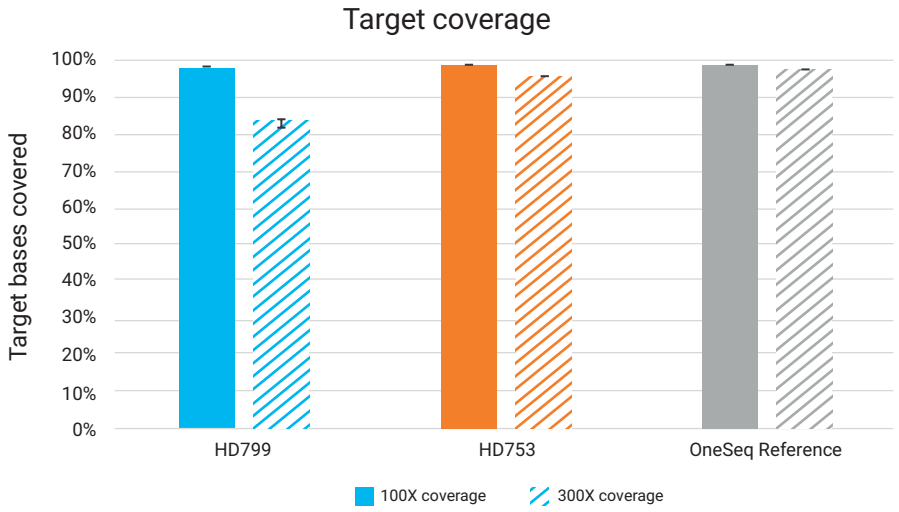

Figure 3. Superior enrichment efficiency of the Agilent SureSelect Cancer CGP assay enables deep target coverage on the Element AVITI sequencer with the Cloudbreak Freestyle kit. To determine enrichment efficiency, the sequencing data were examined at ≥ 100X (solid bar) and ≥ 300X (patterned bar) coverage for three DNA reference samples.

Table 2. The SureSelect Cancer CGP DNA assay, analyzed using the SeqOne SomaCGP pipeline, provides robust and reproducible detection of SNVs. SureSelect Cancer CGP DNA libraries, in triplicate, were generated from the following reference standards: Structural Multiplex Reference Standard gDNA (HD753), Quantitative Multiplex Reference Standard fcDNA (HD799); and a melanoma tumor FFPE sample.

Sample	Variant Type	Gene:Variant	Variant Detected?	Target Coverage
Structural Multiplex Reference Standard gDNA (HD753)	SNV	*AKT1:E17K*	Yes	604-1119X
		BRAF:V600E	Yes	
		EGFR:G719S	Yes	
		GNA11:Q209L	Yes	
		KRAS:G13D	Yes	
		NOTCH1:P668S	Yes	
		PIK3CA:E545K	Yes	
		PIK3CA:H1047R	Yes	
	Indel (1 bp deletion)	*BRCA2:K1691Nfs*15*	Yes	
		*FBXW7:S668Vfs*39*	Yes	
		*MET:L238Yfs*25*	Yes	
	Indel (2 bp deletion)	*FLT3:P986Afs*27*	Yes	
	Indel (9 bp insertion)	*EGFR:V769_ D770insASV*	Yes	
	Indel (15 bp deletion)	*EGFR:ΔE746 - A750*	Yes	
Quantitative Multiplex Reference Standard fcDNA (HD799)	SNV	*BRAF:V600E*	Yes	509-756X
		cKIT:D816V	Yes	
		EGFR:L858R	Yes	
		EGFR:T790M	Yes	
		EGFR:G719S	Yes	
		KRAS:G12D	Yes	
		KRAS:G13D	Yes	
		NRAS:Q61K	Yes	
		PIK3CA:E545K	Yes	
		PIK3CA:H1047R	Yes	
	Indel (15 bp deletion)	*EGFR:ΔE746 - A750*	Yes	
Melanoma Tumor-1	SNV	*BRAF:V600E*	Yes	151-155X

For the detection of CNVs and translocations, the SeqOne SomaCGP pipeline also achieved 100% sensitivity for CNV and translocation detection, identifying all expected CNVs and DNA fusions at coverage above 150X for the reference sample and FFPE tumor samples (Table 3).

For analyzing immuno-oncology biomarker detection, a DNA reference sample and FFPE tumor samples (breast, colon, lung, melanoma) with known TMB and MSI status were evaluated. Comparison of expected versus observed values for TMB and MSI show clear separation into low and high categories (Figure 4), demonstrating the accuracy of the SeqOne platform in distinguishing TMB and MSI levels. Together, these results highlight a highly accurate and sensitive streamlined workflow, showcasing the potential utility for genomic profiling.

Table 3. The SeqOne platform demonstrates high sensitivity for both CNV and DNA translocation detection in FFPE and non-FFPE samples. For CNV analysis, a DNA reference, Structural Multiplex Reference Standard gDNA (HD753), and two breast cancer FFPE samples were examined using the workflow described here. The reference sample showed the detection of two expected CNVs: *N-MYC* (9.5) and *MET* (4.5), while both breast tumor FFPE samples detected expected *ERBB2* amplification with varying copy numbers (6 and 26). For DNA translocation detection, the reference sample and two lung cancer FFPE samples were analyzed. The expected fusion genes, *SLC34A2-ROS1*, *RET-CCDC6*, and *ALK-EML4*, were identified in the respective samples with 100% sensitivity.

Sample Type	Sample	Number of Replicates	Expected CNV or Fusion (DNA)	CNV or Fusion (DNA) Detected?	Target Coverage
gDNA	Structural Multiplex Reference Standard (HD753)	5	**CNV:** *N-MYC* (9.5), *MET* (4.5) **Fusions:** *SLC34A2-ROS1*, *RET-CCDC6*	Yes Yes	604-1119X
FFPE	Breast tumor-1	3	**CNV:** *ERBB2* (6)	Yes	536-617X
	Breast tumor-2	3	**CNV:** *ERBB2* (26)	Yes	140-200X
	Lung tumor-1	3	**Fusion:** *ALK-EML4*	Yes	Not applicable
	Lung tumor-2	3	**Fusion:** *ALK-EML4*	Yes	Not applicable

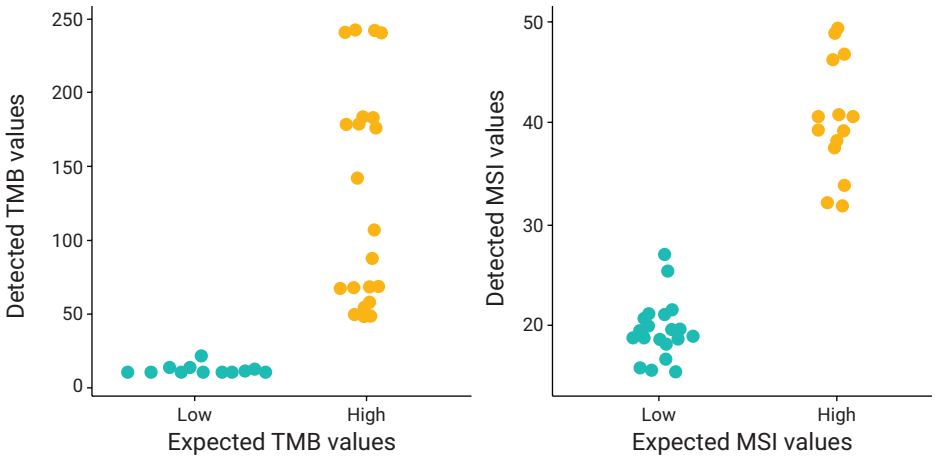

Figure 4. Robust detection of two immuno-oncology biomarkers, tumor mutational burden (TMB) and micro-satellite instability (MSI), by the Agilent SureSelect Cancer CGP DNA assay across diverse tumor samples, as analyzed by the SeqOne pipeline. DNA reference samples, including Quantitative Multiplex Reference Standard fcDNA moderate (HD799), Structural Multiplex Reference Standard gDNA (HD753), HapMap gDNA (NA12878), and FFPE tumor samples (colon, breast, lung, melanoma) with known TMB and MSI status (35 total samples, including replicates: 12 controls, 23 FFPE tumor samples) were evaluated using the workflow described here. Each sample is represented by a dot. (A) Scatter plot comparing expected and detected TMB values across samples tested. Samples classified as low (teal blue) cluster near zero, while samples classified as high (orange) show significantly elevated TMB values. (B) Scatter plot comparing expected and detected MSI values across samples tested. Similarly, samples designated with low MSI values (teal blue) appear in a cluster distinct from samples with high MSI values (orange).

High capture specificity and expanded fusion detection using the RNA assay

To extend the examination of gene fusions, the SureSelect Cancer CGP RNA assay was used to evaluate fusion gene transcripts across the 80-gene panel. RNA from reference standards and lung tumor samples that contain known gene fusions were used to generate SureSelect Cancer CGP RNA libraries on the Magnis NGS prep system. Sequencing was performed on the Element AVITI System with the Adept Library Compatibility workflow and analyzed for fusion gene transcripts using the SeqOne pipeline.

Target coverage for the RNA assay shows high capture specificity for the RNA panel target. All expected fusion transcripts were detected across the samples examined, highlighting the broad capability of the SureSelect Cancer CGP assay (Figure 5, Table 4).

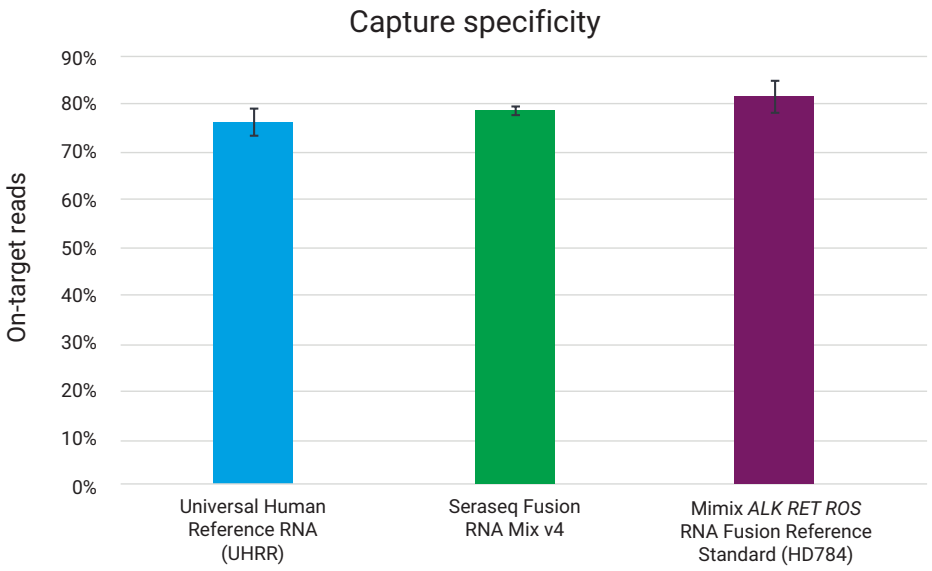

Figure 5. The Agilent SureSelect Cancer CGP RNA assay shows high capture specificity. RNA reference samples, Universal Human Reference RNA (UHRR), Seraseq Fusion RNA Mix v4, and Mimix *ALK RET ROS* RNA Fusion Reference Standard (HD784), were used to generate SureSelect Cancer CGP RNA libraries on the Agilent Magnis NGS prep system. Libraries were sequenced on the Element AVITI System (Adept Library Compatibility Kit and AVITI sequencing kit). The RNA assay achieved > 75% on-target reads across the reference samples.

Table 4. The Agilent SureSelect Cancer CGP RNA assay demonstrates expanded fusion transcript detection. SureSelect Cancer CGP RNA libraries were generated from the following reference standards and samples: Universal Human Reference RNA (UHRR), Mimix *ALK RET ROS* RNA Fusion Reference Standard (HD784), Seraseq Fusion RNA Mix v4, and lung tumor FFPE samples. The table shows the samples tested, covering 21 gene fusions, with successful detection of each fusion transcript by the SeqOne pipeline.

Sample	Expected Fusion Transcript (RNA)	Detected Fusion Transcript?
Universal Human Reference RNA (UHRR)	*CCDC6-RET*	Yes
	CD74-ROS1	Yes
	EGFR-AC069287.1	Yes
	EML4-ALK	Yes
	ETV6-NTRK3	Yes
	FGFR3-BAIAP2L	Yes
	FGR3-TACC3	Yes
	KIF5B-RET	Yes
	LMNA-NTRK1	Yes
	NCOA4-RET	Yes
	PAX8-PPARG	Yes
	SLC34A2-ROS1	Yes
	SLC45A3-BRAF	Yes
	TFG-NTRK1	Yes
	TMPRSS2-ERG	Yes
	TPM3-NTRK1	Yes

Sample	Expected Fusion Transcript (RNA)	Detected Fusion Transcript?
Mimix *ALK RET ROS* RNA Fusion Reference Standard (HD784)	*CCDC6-RET*	Yes
	EML4-ALK	Yes
	SLC34A2-ROS1	Yes
Seraseq Fusion RNA Mix v4	*NSD3-FGFR1*	Yes
	RPS6KB1-DIAPH3	Yes
	RPS6KB1-VMP1	Yes
Lung Tumor-1	*ALK-EML4*	Yes
Lung Tumor-2	*ALK-EML4*	Yes
Lung Tumor-3	*ALK-EML4*	Yes
Lung Tumor-4	*ALK-KIF5B*	Yes

Conclusion

This application note describes a robust, automated, end-to-end workflow for the Agilent SureSelect Cancer CGP assay that includes the Agilent Magnis NGS prep system, Element AVITI sequencer, and SeqOne data analysis. Using this workflow, the assay showed strong performance with deep and uniform coverage of DNA and RNA target regions, as well as reliable variant detection for key biomarkers (SNVs, CNVs, TLs, TMB, MSI, fusion transcripts) across reference and tumor samples. The Element AVITI sequencer delivers sequencing metrics that are both consistent and comparable to other sequencing platforms, while maintaining high sequencing coverage at low cost per base. Furthermore, integration with the SeqOne platform provides efficient, reliable, and comprehensive data interpretation.

The streamlined, end-to-end workflow outlined here facilitates the implementation of a straightforward CGP workflow in laboratories, offering a faster turnaround time to analyze somatic alterations from tumor samples, as well as improved laboratory efficiency and productivity. This workflow is designed to be accessible and easy to adopt, requiring minimal NGS expertise and hands-on-time, making the workflow particularly suitable for use in routine laboratory settings and for large-scale projects, including for laboratories aiming to bring assays in-house.

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