Data Sheet

Agilent
Trusted Answers

SureSelect Cancer CGP Assay

Comprehensive genomic profiling (CGP) to maximize tumor insights

Key Advantages

- Globally curated, clinically relevant biomarker content
- Broad input range to enable profiling of more samples
- Fast turnaround time to sequencing results
- Automation options to increase productivity and reproducibility
- Enzymatic fragmentation option eliminates the need for physical shearing equipment

Introduction

The Agilent SureSelect Cancer CGP Assay offers genomic profiling of cancer samples with a next-generation sequencing (NGS) panel of globally curated genes sourced from cancer databases and leading clinical cancer researchers. The assay enables detection of key classes of somatic changes, including single nucleotide variants (SNVs), copy number variants (CNVs), insertions/deletions (indels), translocations (TLs), and gene fusions, plus immuno-oncology biomarkers tumor mutational burden (TMB) and microsatellite instability (MSI). The panel is comprised of a DNA module with 679 genes and an RNA module with 80 genes. Designed with flexibility in mind, each module can be assayed separately or together in parallel. The libraries generated from each module can be combined and sequenced in the same run.

The SureSelect Cancer CGP Assay was developed using the streamlined and high-performance Agilent SureSelect XT HS2 library preparation and target enrichment chemistry. The assay features a fast, 90-minute hybridization step, compatibility with input as low as 10 ng, and a single-day workflow to generate sequencing-ready libraries. For optimal convenience, the assay also includes enzymatic fragmentation, eliminating the need for physical shearing equipment. Maximize workflow efficiency and minimize time at the bench using the Agilent Magnis NGS Prep system, a fully automated, walkaway platform that only requires 15 minutes of hands-on time for generating sequencing-ready libraries.

Flexible Workflow to Fit Your Lab

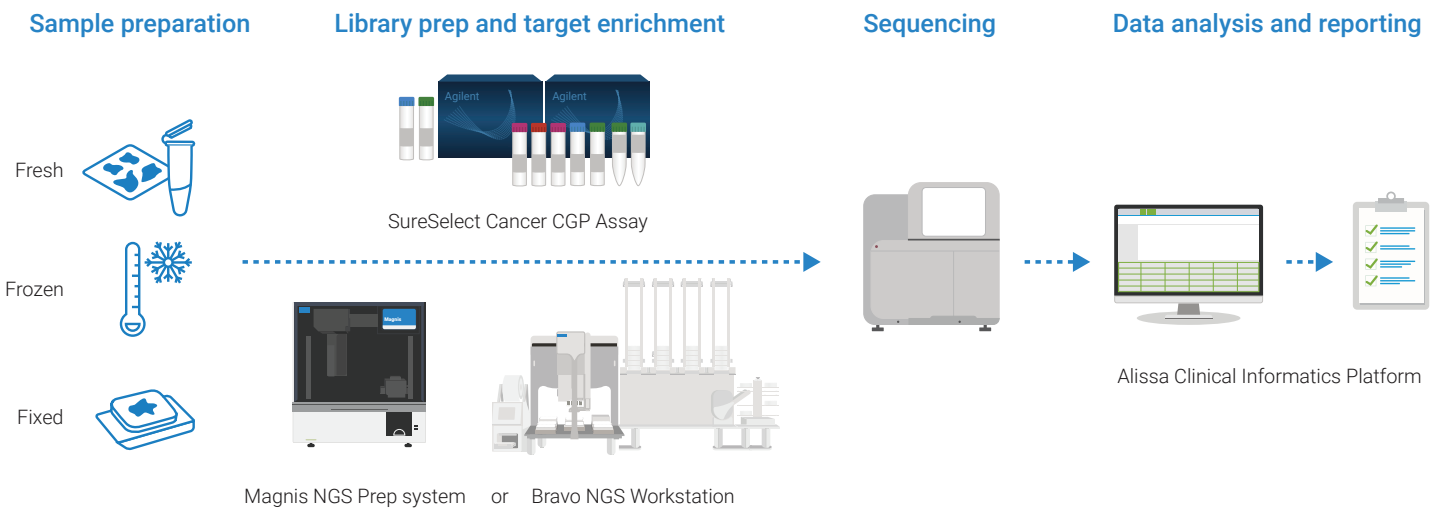

Figure 1. The SureSelect Cancer CGP assay workflow enables laboratory scientists to take tissue specimens to clinical insights in less than four days. This NGS assay for comprehensive genomic profiling of solid tumors can be significantly streamlined using automation platforms from Agilent such as the walk-away Magnis NGS Prep system or the high-throughput Bravo NGS Workstation to prepare sequencing-ready libraries.

Excellent Target Coverage and Uniform Distribution for Confident Results

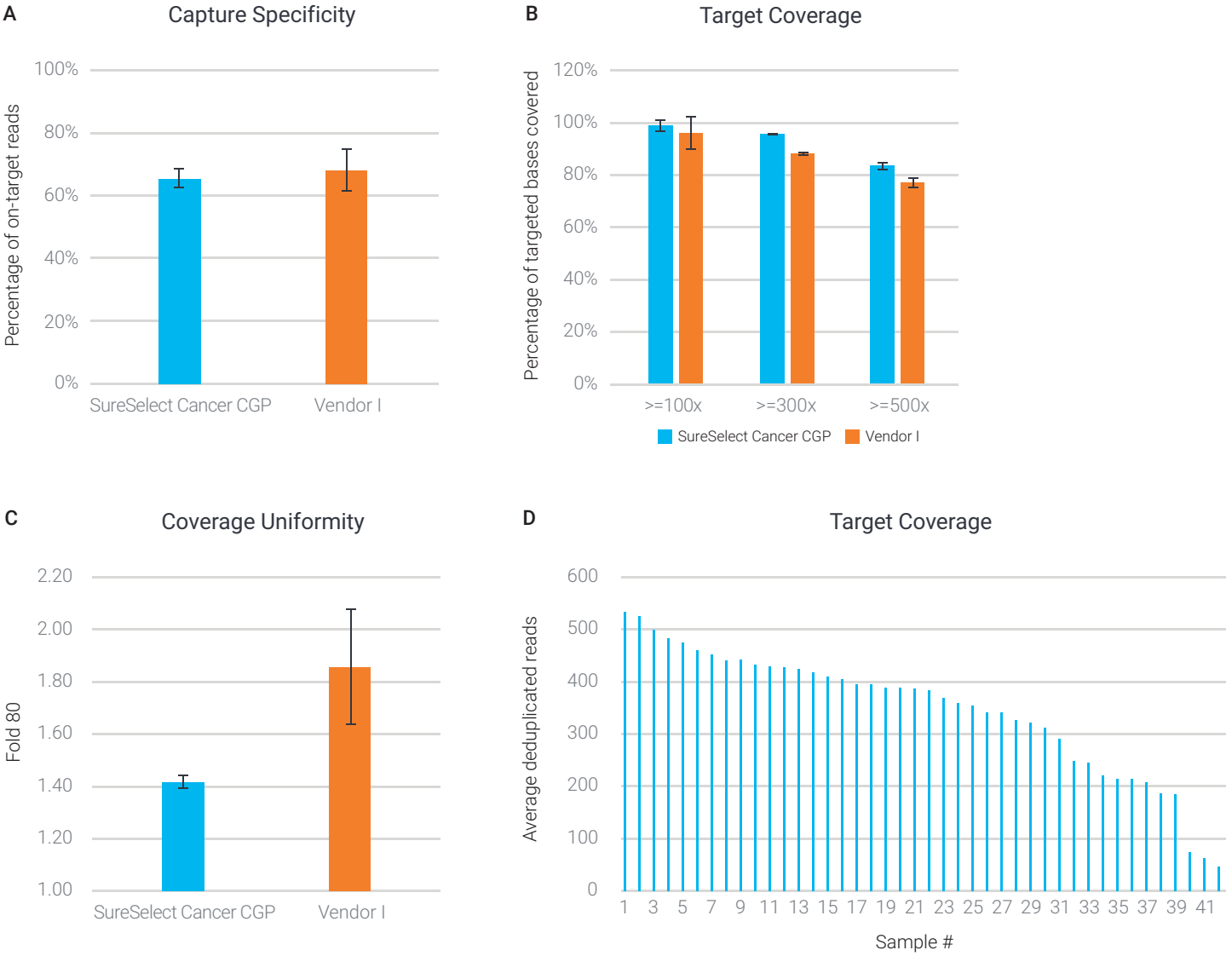

Figure 2. Superior enrichment efficiency of the SureSelect Cancer CGP assay enables deep target coverage.
A. The percentage of reads overlapping the target regions is similar between the SureSelect Cancer CGP assay and the other vendor's assay. **B.** The fraction of targeted bases that have greater than or equal to 100X, 300X, and 500X coverage is higher for the SureSelect Cancer CGP assay. **C.** Fold-80 base penalty, a measure of coverage uniformity, is better for the SureSelect Cancer CGP assay. Lower Fold-80 values represent more even coverage across the target regions. **D.** Forty-two FFPE samples with DNA integrity number (DIN) of three to seven measured using the Agilent 4200 TapeStation system were analyzed for average coverage of the targeted regions after duplicate removal. Ninety-three percent of samples had a mean coverage of greater than or equal to 100X and 88% had mean coverage of greater than or equal to 200X, indicating high performance with respect to coverage. Fifty nanograms of DNA extracted from a normal cell line, Agilent OneSeq Reference DNA, Female (p/n 5190-8850) (A, B, and C) or archival FFPE samples (D) were processed following the manufacturer's recommendation and sequenced on the Illumina HiSeq4000 or NextSeq500 instruments. Data were down sampled to 6 Gb (40 M 2 x 150 bp reads) for analysis. Sequencing metrics were determined using the target file for the panel, covering 679 genes for the SureSelect Cancer CGP assay and 523 genes for Vendor I.

Consistent Detection of Key Somatic Variant Classes

Table 1. The SureSelect Cancer CGP Assay provides reproducible detection of single nucleotide variants (SNVs), insertions (ins.), deletions (del.), and copy number variation (CNV) down to 5% variant allele frequency (VAF). Fifty nanograms of the Horizon Discovery Structural Multiplex Reference Standard gDNA (HD753) was assayed and sequenced on the Illumina HiSeq4000 Sequencer. The resulting sequencing reads were down sampled to 6 Gb (40 M 2 x 150 bp reads) and analyzed for variants using an internally developed SNP caller. Measured allele frequency represents the average of the three replicate assays. All variants were detected in all three replicates.

Gene	Variant	Variant Type	Expected Allelic Frequency	Measured Allelic Frequency
AKT1	E17K	SNV	5.0%	5.5%
BRAF	V600E	SNV	18.2%	15.8%
BRCA2	K1691Nfs*15	1 bp del.	5.6%	4.5%
EGFR	G719S	SNV	5.3%	4.3%
EGFR	V769_D770insASV	9 bp ins.	5.6%	4.0%
EGFR	ΔE746 - A750	15 bp del.	5.3%	1.8%
FBXW7	S668Vfs*39	1 bp del.	5.6%	4.7%
FLT3	P986Afs*27	2 bp del.	5.6%	6.6%
GNA11	Q209L	SNV	5.6%	5.5%
KRAS	G13D	SNV	5.6%	4.9%
MET	L238Yfs*25	1 bp del.	2.5%	2.9%
MET	Amplification	CNV	5 copies	5 copies
N-MYC	Amplification	CNV	10 copies	10 copies
NOTCH1	P668S	SNV	5.0%	4.0%
PIK3CA	E545K	SNV	5.6%	4.6%
PIK3CA	H1047R	SNV	16.7%	14.7%

Table 2. The SureSelect Cancer CGP assay enables confident detection of the immuno-oncology biomarkers TMB and MSI tumor samples with high MSI and TMB status show better response to immunotherapy.[1] Samples that were determined to be microsatellite-stable (MSS) by immunohistochemistry (IHC) showed low TMB and MSI scores, whereas MSI-high samples showed high TMB and MSI scores. Score determination was performed using Agilent internal analysis. IHC staining patterns for mismatched repair (MMR) genes (MLH1- MutL homolog 1, PMS2- PMS1 Homolog 2, Mismatch Repair System Component, MSH2- MutS homolog 2, MSH6- MutS homolog 6) are shown with intact (I) or loss (L) of staining for these genes. Good correlation of MSI status was shown between SureSelect Cancer CGP assay and IHC staining.

Sample ID	TMB and MSI Calculation by SureSelect Cancer CGP assay				IHC Staining Status, MMR genes			
	TMB Score	TMB Status	MSI Score	MSI Status	MLH1	PMS2	MSH2	MSH6
Colon_1	9.5	Low	15.6	MSS	I	I	I	I
Colon_2	8.0	Low	13.6	MSS	I	I	I	I
Colon_3	6.0	Low	12.0	MSS	I	I	I	I
Colon_4	12.5	Low	14.9	MSS	I	I	I	I
Colon_5	10.0	Low	15.3	MSS	I	I	I	I
Colon_6	9.0	Low	13.4	MSS	I	I	I	I
Colon_7	53.0	High	57.5	MSI-H	I	I	L	L
Colon_8	59.0	High	64.5	MSI-H	L	L	I	I
Cecum_9	69.0	High	57.0	MSI-H	L	L	I	I
Colon_10	59.5	High	44.8	MSI-H	I	I	L	L

Strong Performance with Challenging Samples and Targets

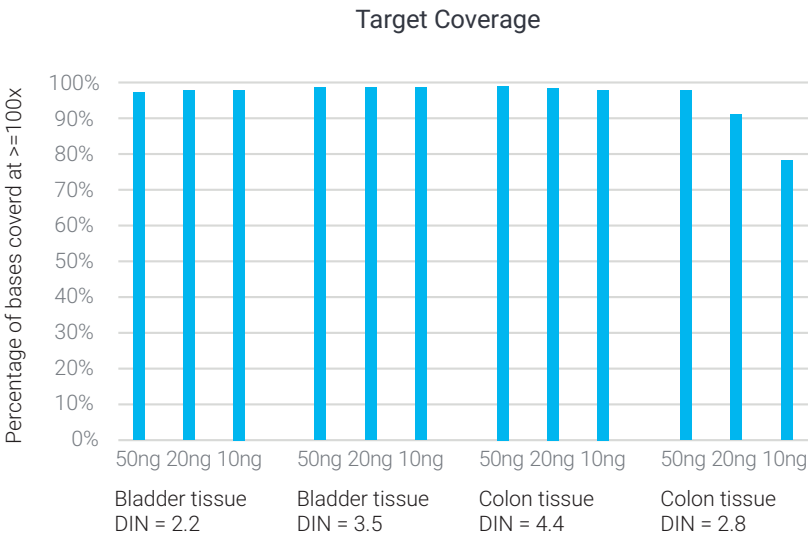

Figure 3. The SureSelect Cancer CGP assay provides robust performance at low input amounts. While 50 ng is recommended for optimal performance, good target coverage can be achieved with as little as 10 ng of input DNA. Bars show the fraction of targeted bases that have greater than or equal to 100X coverage. DNA with varying quality, measured using the TapeStation system's DNA integrity value (DIN), was extracted from four FFPE samples (two bladder and two colon tissue samples) and used as input into the SureSelect Cancer CGP assay.

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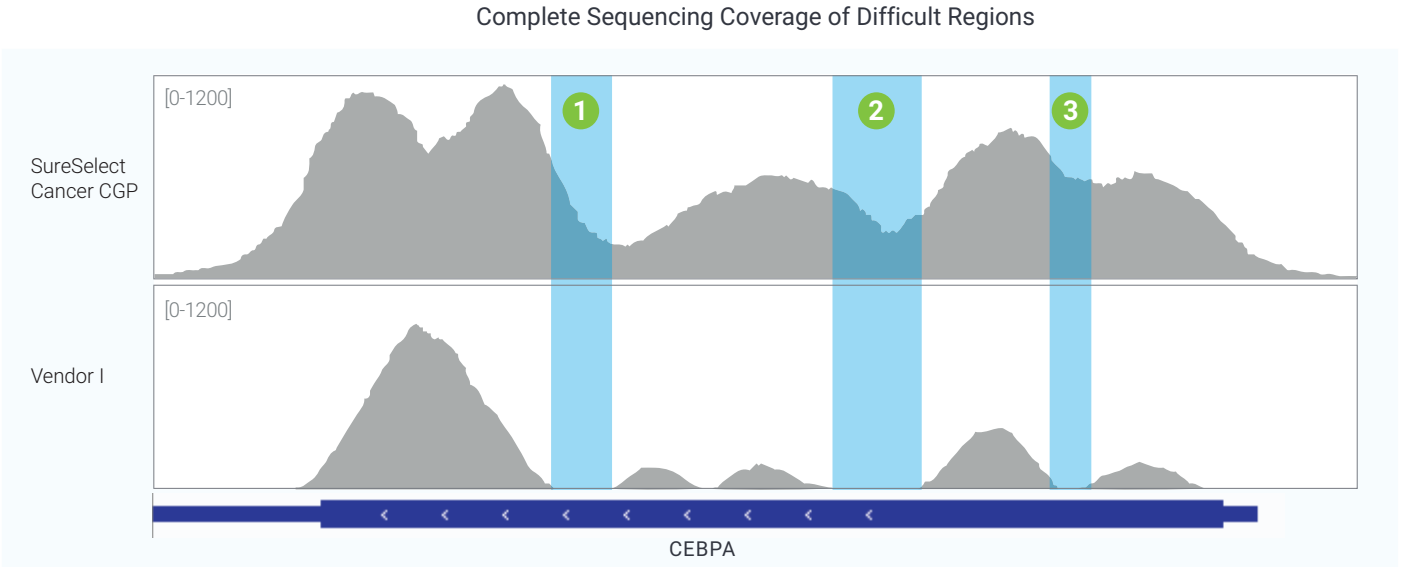

B

Region #	SureSelect Cancer CGP minimum coverage	Vendor I coverage
1	200	0
2	275	0
3	600	0

Figure 4. Optimized design of the SureSelect Cancer CGP Assay provides robust performance with the challenging target gene *CEBPA*, which contains regions with high GC content. **A.** Deduplicated read coverage for the SureSelect Cancer CGP panel and on-market panel. Three highlights show regions covered by the SureSelect Cancer CGP that are missed by the other assay. **B.** Read depth of at least 200 unique reads for the SureSelect Cancer CGP assay. Data generated using a reference cell line following the recommended protocol and sequenced with 40 M reads.

De Novo Gene Fusion Detection from Just One Gene Partner

Table 3. The SureSelect Cancer CGP assay provides detection of translocations from both DNA and RNA inputs. Four non-small cell lung cancer (NSCLC) samples with translocations involving the ALK gene were analyzed with both the SureSelect Cancer CGP DNA and RNA assays. The allele count represents the number of reads that map to the genomic position of the breakpoint when assayed with the DNA assay. The number of fusion reads is the number of reads that map to both the *ALK* and EML4 transcripts. Detection of the translocation events in DNA was performed using an internally developed algorithm. Detection of RNA fusion reads was performed using STAR-Fusion[2]. Fifty nanograms of either DNA or RNA extracted from FFPE samples was used as input. Sequencing was performed on the Illumina HiSeq4000 and the resulting data were down sampled to 40 M 2 x 150 bp reads (DNA assay) or 10 M 2 x 150 bp reads (RNA assay).

Sample ID	ALK/EML4 Breakpoint	SureSelect Cancer CGP Assay: DNA Panel		SureSelect Cancer CGP Assay: RNA Panel	
		Read Depth	Allele Count	ALK/EML4 Fusion	Fusion Reads
NSCLC-1	chr2:29224722/42275660	256	18	Detected	8
NSCLC-2	chr2:29223615/42297197	152	9	Detected	23
NSCLC-3	chr2:29224445/42276523	154	14	Detected	15
NSCLC-4	chr2:29223466/42317566	291	61	Detected	96

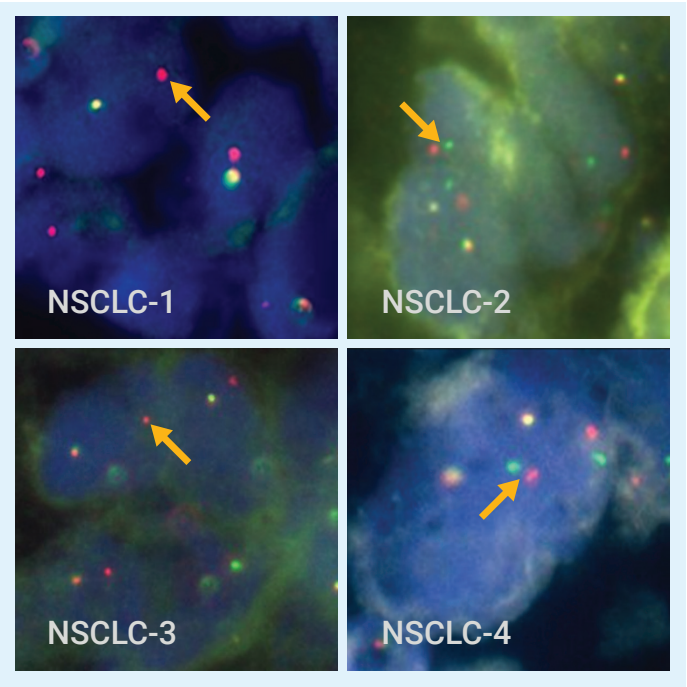

Figure 5. Representative images from fluorescent in situ hybridization (FISH) analysis of the four *ALK*-positive samples to show concordant results with NGS results in Table 3. FISH was performed using Agilent SureFISH *ALK* Break-Apart probe. Evidence of a translocation involving ALK is visualized as either a lone red signal (arrow) or separation of the red and green signals (arrow head).

Comparable Performance Across Manual and Automated Workflows

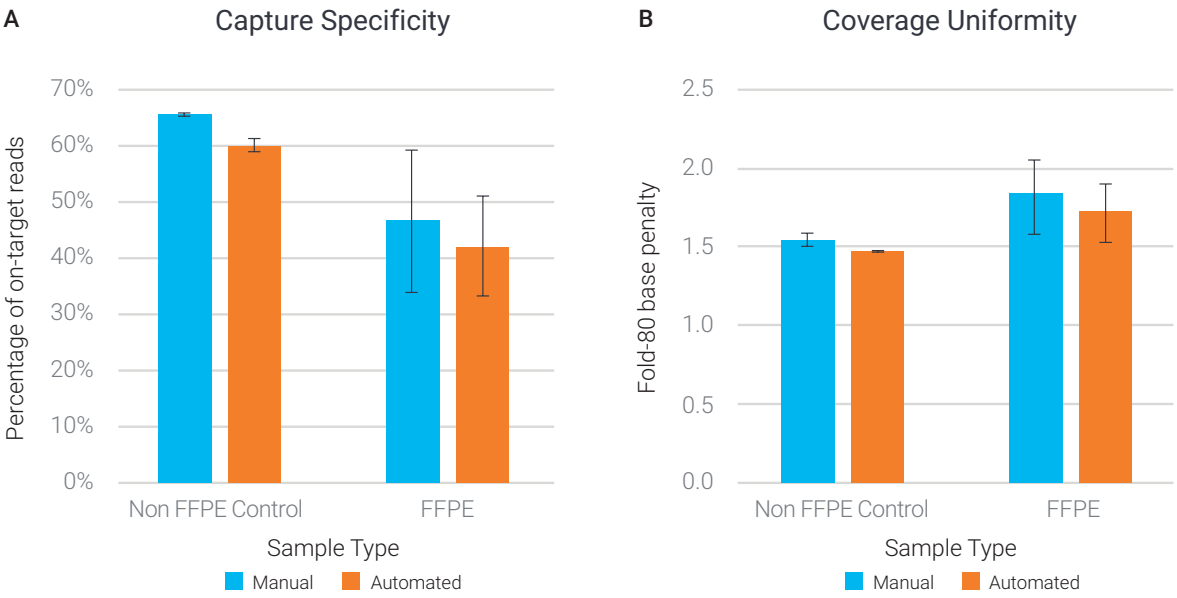

Figure 6. Comparable robust and reproducible assay performance was observed for SureSelect Cancer CGP libraries generated manually or via automation on the Agilent Magnis NGS Prep system. Nine FFPE samples with DNA integrity number (DIN) ranging from two to four were run in triplicate on the Magnis using 50 ng DNA input. Non-FFPE reference cell line controls, including the Structural Multiplex Reference Standard (gDNA) cell line (Horizon Discovery, p/n HD753) and the Quantitative Multiplex Reference Standard fcDNA (moderate) cell line (Horizon Discovery, p/n HD799), were run in triplicate for comparison. **A.** The percentage of reads overlapping the target regions is comparable between manual and automation workflows. **B.** Fold-80 base penalty comparison shows uniform coverage between manual and automation workflows. Lower Fold-80 values represent more even coverage across the target regions.

Table 4. Comparable and reproducible detection of variants (SNVs, indels, CNVs) down to 5% variant allele frequency (VAF) from SureSelect Cancer CGP libraries generated by the automated Magnis NGS Prep system. Fifty nanograms of the Horizon reference standards, Structural Multiplex Reference Standard gDNA (Horizon Discovery, p/n HD753) and Quantitative Multiplex Reference Standard formalin-compromised DNA (Horizon Discovery, p/n HD799), were assayed in triplicate and sequenced on the Illumina NovaSeq 6000 instrument. The resulting sequencing reads were down sampled to 6 Gb (40 M 2 x 150 bp reads) and analyzed for variants using an internally developed SNP caller. Measured allele frequency represents the average of the three replicate assays. All variants were detected in all three replicates.

Sample	Gene	Variant	Variant Type	Expected Allelic Frequency	Measured Allelic Frequency
HD753	*AKT1*	E17K	SNV	5.0%	7%
	BRAF	V600E	SNV	18.2%	18%
	BRCA2	K1691Nfs*15	1 bp del.	5.6%	7%
	EGFR	V769_D770insASV	9 bp ins.	5.6%	3%
	EGFR	ΔE746 – A750	15 bp del.	5.3%	2%
	EGFR	G719S	SNV	5.3%	6%
	FBXW7	S668Vfs*39	1 bp del.	5.6%	5%
	FLT3	P986Afs*27	2 bp del.	5.6%	5%
	KRAS	G13D	SNV	5.6%	4%
	MET	L238Yfs*25	1 bp del.	2.5%	3%
	MET	Amplification	CNV	5 copies	5 copies
	N-MYC	Amplification	CNV	10 copies	9 copies
	NOTCH1	P668S	SNV	5.0%	5%
	PIK3CA	E545K	SNV	5.6%	3%
	PIK3CA	H1047R	SNV	16.7%	13%
HD799	*BRAF*	V600E	SNV	10.5%	14%
	cKIT	D816V	SNV	10.0%	10%
	EGFR	G719S	SNV	24.5%	22%
	KRAS	G13D	SNV	15.0%	15%
	KRAS	G13D	SNV	6.0%	5%
	NRAS	Q61K	SNV	12.5%	11%
	PIK3CA	H1047R	SNV	17.5%	18%
	PIK3CA	E545K	SNV	9.0%	9%

Incorporating Clinical Informatics

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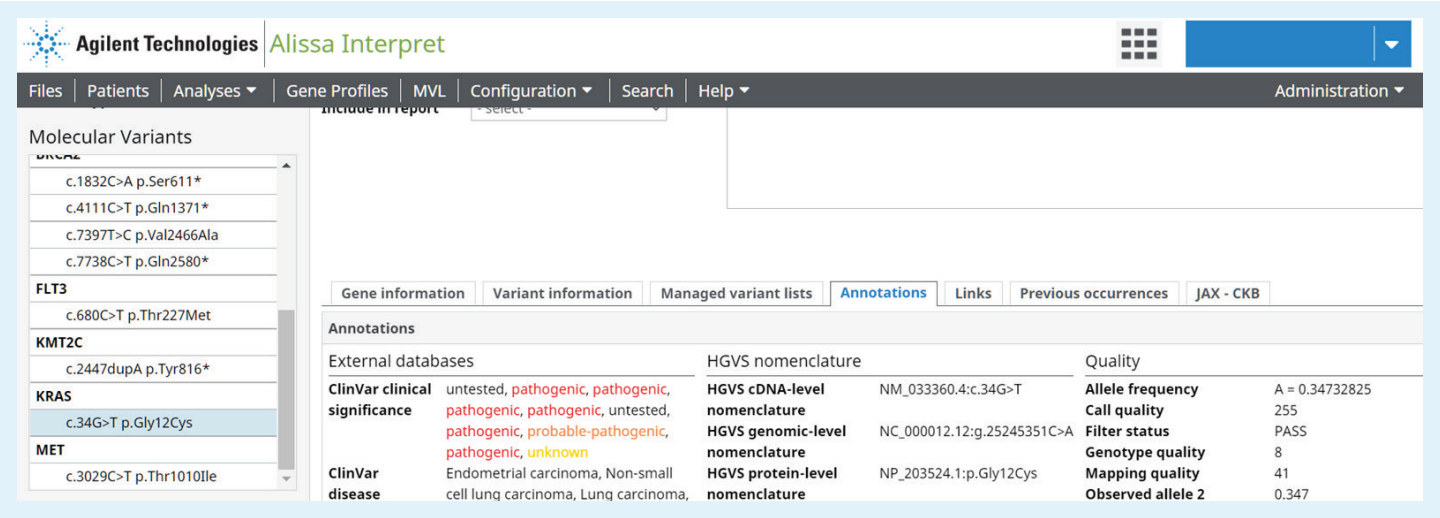

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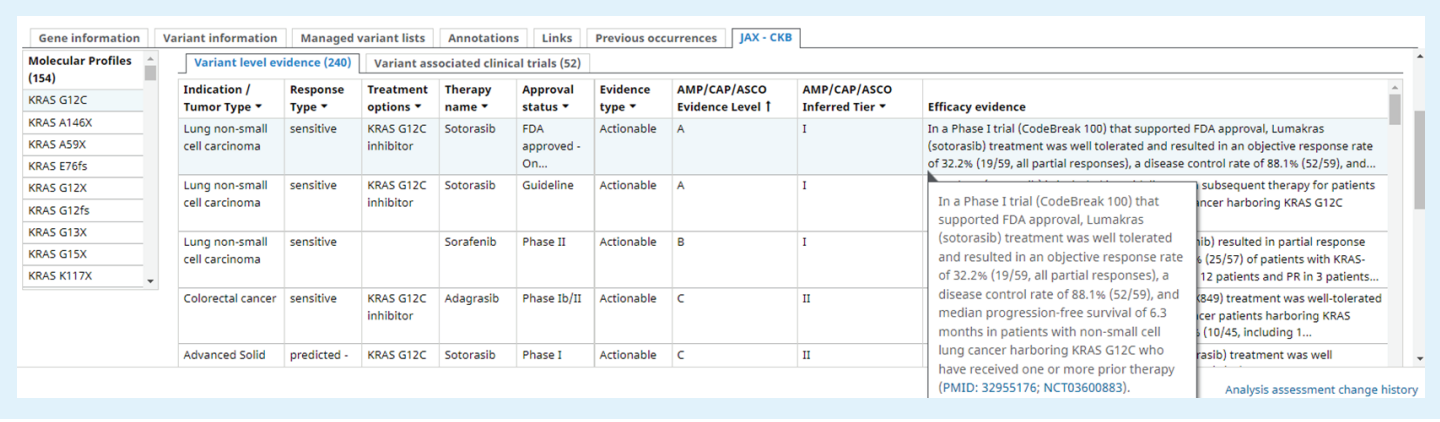

Figure 7. Variants identified using the SureSelect Cancer CGP assay can be further investigated using Alissa Interpret. **A.** A non-small cell lung cancer sample was found to harbor a *KRAS* G12C mutation following analysis with the SureSelect Cancer CGP assay and Alissa Interpret. **B.** Additional information on the variant impact and clinical significance is available using The Jackson Laboratory Clinical Knowledgebase (JAX-CKBTM) information in Alissa Interpret.†

†*Somatic gene variant annotations and related content have been powered by The Jackson Laboratory Clinical Knowledgebase (JAX- CKB™).*

Enabling Variant Detection from Cell-Free DNA Samples

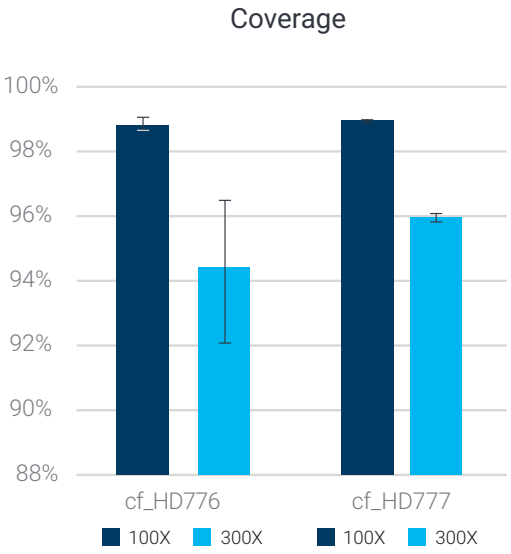

Figure 8. The SureSelect Cancer CGP assay produced high coverage with cell-free DNA (cfDNA) reference samples. Greater than 98% of the targeted regions have a sequencing depth of at least 100X and >92% have depth of 300X. Fifty nanograms of cfDNA reference standards (Horizon Discovery, p/n HD780) were run in triplicate and sequenced on the Illumina HiSeq4000. The sequencing data was down sampled to 6 Gb (40 M 2 x 150 bp reads). Duplicate reads were removed, and the coverage of the target regions was determined.

Table 5. Single nucleotide variants (SNV) and insertions/deletions can be reliably detected in cfDNA reference samples. The Horizon Discovery Multiplex I cfDNA Reference Standard Set covers multiple, engineered, SNVs with eight mutations. The table shows data for a sample with 5% variant allele frequency (VAF) (cf_HD777-5%). For the wildtype sample (cfDNA_HD776-WT), both expected and observed allelic frequencies are 0% (data not shown).

Gene	Variant	Variant Type	Expected Allelic Frequency	Measured Allelic Frequency
EGFR	L858R	SNV	5%	3%
EGFR	T790M	SNV	5%	5%
KRAS	G12D	SNV	5%	6%
NRAS	Q61K	SNV	5%	7%
PIK3CA	E545K	SNV	5%	4%
EGFR	ΔE746-A750	15 bp Deletion	5%	3%
EGFR	V769-D770insASV	9 bp Insertion	5%	3%

References

1. Palmeri, M.; Mehnert, J.; Silk, A. W.; Jabbour, S. K.; Ganesan, S.; Popli, P.; Riedlinger, G.; Stephenson, R.; de Meritens, A. B.; Leiser, A.; Mayer, T.; Chan, N.; Spencer, K.; Girda, E.; Malhotra, J.; Chan, T.; Subbiah, V.; Groisberg, R. Real-World Application of Tumor Mutational Burden-High (TMB-High) and Microsatellite Instability (MSI) Confirms Their Utility as Immunotherapy Biomarkers. *ESMO Open* **2022**, 7 (1), 100336. https://doi.org/10.1016/j.esmoop.2021.100336.

2. Haas, B. J.; Dobin, A.; Li, B.; Stransky, N.; Pochet, N.; Regev, A. Accuracy Assessment of Fusion Transcript Detection via Read-Mapping and de Novo Fusion Transcript Assembly-Based Methods. *Genome Biol*. **2019**, 20 (1), 213. https://doi.org/10.1186/s13059-019-1842-9.

Ordering Information

Complete kits*			
Product Description	**16 Rxns**	**96 Rxns**	**96 Rxns Auto****
SureSelect Cancer CGP Assay Starter kit	G9965A		
SureSelect Cancer CGP Assay DNA+RNA kit		G9966A	G9966B
SureSelect Cancer CGP Assay DNA kit	G9967A	G9967B	G9967C
SureSelect Cancer CGP Assay RNA kit	G9968A	G9968B	G9968C
Probes only			
Product Description	**16 Rxns**	**96 Rxns**	**96 Rxns Auto****
SureSelect Cancer CGP Assay Probes, DNA+RNA	5191-6990	5191-6991	5191-6992
SureSelect Cancer CGP Assay Probe, DNA	5280-0035	5280-0036	5280-0037
SureSelect Cancer CGP Assay Probe, RNA	5191-6996	5191-6997	5191-6998
Kits compatible with automation on Magnis NGS Prep System***			
Product Description	**32 Rxns**	**96 Rxns**	
Magnis SureSelect Cancer CGP XT HS2 DNA kit	G9777A	G9777B	
Magnis SureSelect Cancer CGP XT HS2 RNA kit	G9777C	G9777D	
Compatible Software			
Product Description			
Alissa Interpret	Contact Sales		

**Enzymatic fragmentation reagents not included.*
***Compatible with the automated Agilent Bravo NGS Workstation.*
****Enzymatic fragmentation reagents included.*

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For Research Use Only. Not for use in diagnostic procedures.

Alissa Interpret is a USA Class I Exempt Medical Device, Europe CE IVD, Canada and Australia Class I IVD Device.

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