TruSight™ Oncology 500 ctDNA v2 on the NextSeq™ 2000 System

Uncompromised performance with the benefits of a benchtop system

Highly sensitive performance

Detects biomarkers present as low as 0.2% VAF from 20 ng ctDNA (5–30 ng possible)

Comprehensive results

Analyzes > 500 genes and immuno-oncology genomic signatures bMSI and bTMB in one assay

Streamlined workflow

Provides a three-day workflow from cfDNA to variant reporting with fully automated data analysis

Cost-efficient throughput

Optimizes sequencing cost per sample for small batch sizes

Introduction

Comprehensive genomic profiling (CGP) research evaluates a broad range of biomarkers in a single assay, delivering faster results than sequential testing. This approach is increasingly important as more biomarkers emerge, including immuno-oncology (IO) genomic signatures such as tumor mutational burden (TMB), which specifically requires large next-generation sequencing (NGS) panels (> 1 Mb) for accurate assessment.[1]

CGP traditionally relies on solid tumor tissue; however, up to 25% of cases lack sufficient tissue, have inaccessible tumors, or cannot yield timely results. In these situations, liquid biopsy analyzing circulating tumor DNA (ctDNA) offers an alternative CGP solution.[2–5]

TruSight Oncology 500 ctDNA v2 enables CGP research from blood-based ctDNA, providing broad coverage of tumor-derived alterations and assessment of IO signatures such as blood-based TMB (bTMB) and blood-based MSI (bMSI). The assay is optimized to identify low-frequency variants with high analytical sensitivity and uses advanced bioinformatics to filter clonal hematopoiesis and germline variants.[2]

Some liquid biopsy assays require ultradeep sequencing of ctDNA (20,000× or more) to maximize analytical sensitivity and detect low-frequency variants.[6] Historically, this has limited adoption of CGP from liquid biopsy in smaller laboratories due to high-throughput sequencing system requirements, substantial upfront capital investment, and the need for large batch sizes to control costs.

TruSight Oncology 500 ctDNA v2 is the first liquid biopsy assay to enable CGP research on a benchtop platform—the NextSeq 2000 System.[7] This further expands access for laboratories that do not operate high-throughput systems. Using XLEAP-SBS™ chemistry, an enhanced form of sequencing by synthesis (SBS), the NextSeq 2000 System increases usable output per flow cell and delivers high-fidelity sequencing with improved cost-efficiency in a compact footprint.[8]

The key advantages of running TruSight Oncology 500 ctDNA v2 on the NextSeq 2000 System are the ability to:

- Achieve sensitive detection of low-frequency variants (approximately 0.2% VAF) from 20 ng cfDNA with high specificity (Table 1)
- Complete a streamlined, three-day workflow from cfDNA to variant reporting with fully automated data analysis (Figure 1)
- Improve cost-efficiency through the higher usable output provided by XLEAP-SBS chemistry, enabling competitive per-sample pricing with batch sizes as small as four samples (Figure 2)
- Run TruSight Oncology 500 and other oncology research assays on the same instrument to accommodate a broad range of laboratory needs[9*]

This technical note provides a comparison of TruSight Oncology 500 ctDNA v2 performance on the NextSeq 2000 System with performance on the NovaSeq 6000 System, the platform on which the assay was originally developed. The results show equivalent performance across both systems, with significantly lower upfront investment and per-sample sequencing cost for small sample batches when using the NextSeq 2000 System.

* Oncology assays include genomic profiling with TruSight Oncology 500 and targeted testing with Pillar® oncoReveal™ panels. Refer to Unlock scalable oncology workflows with NextSeq 2000 System.

Table 1: TruSight Oncology 500 ctDNA v2 assay performance[a]

Parameter	Analytical sensitivity[b]	Analytical specificity[c]
Small DNA variants		≥ 99.999%
• SNV ≥ 0.2% VAF (≥ 0.4% VAF)	≥ 90% (≥ 95%)	
• SNV hotspots ≥ 0.2% VAF	≥ 95%	
• MNV ≥ 0.5% VAF	≥ 90%	
• Indels ≥ 0.5% VAF	≥ 90%	
Gene amplifications ≥ 1.3-fold change	≥ 95%	≥ 95%
Gene deletions ≤ 0.6-fold change	≥ 95%	≥ 95%
MSI-high detection at 0.3% tumor fraction	≥ 95%	≥ 95%
Gene rearrangements ≥ 0.5% VAF	≥ 95%	> 95%

a. Performance characteristics were measured on the NovaSeq 6000 System using 20 ng of cfDNA and 35,000× coverage, as recommended. Equivalent performance was shown on the NextSeq 2000 System and NovaSeq X Series.
b. Analytical sensitivity is defined as percent detection at the stated variant level.
c. Analytical specificity is defined as the ability to detect a known negative.

MNV, multinucleotide variant; SNV, single nucleotide variant; VAF, variant allele frequency.

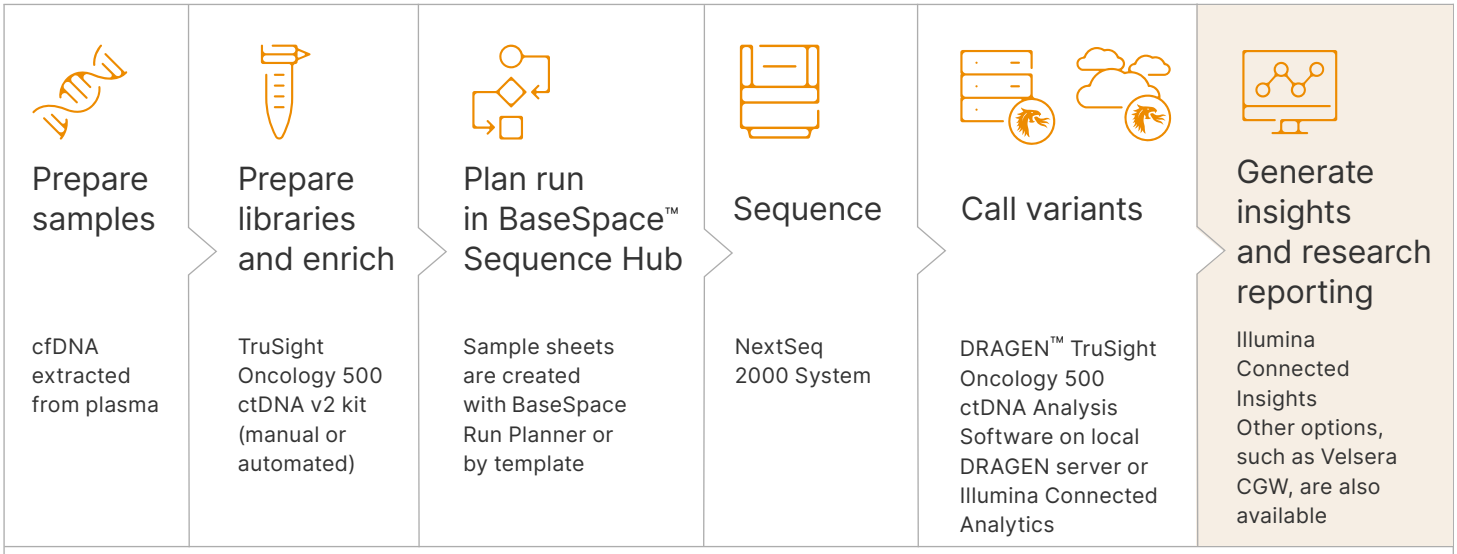

Figure 1: TruSight Oncology 500 ctDNA v2 workflow on the NextSeq 2000 System
Manual library preparation takes up to 8.5 hours, including approximately 2.5 hours of hands-on time. BaseSpace Sequence Hub facilitates run set-up, sample sheet generation, and sequencing status monitoring. Integration with Illumina Connected Analytics and Illumina Connected Insights enables automated initiation of secondary and tertiary analysis for a fully hands-off workflow.
CGW, clinical genomics workspace.

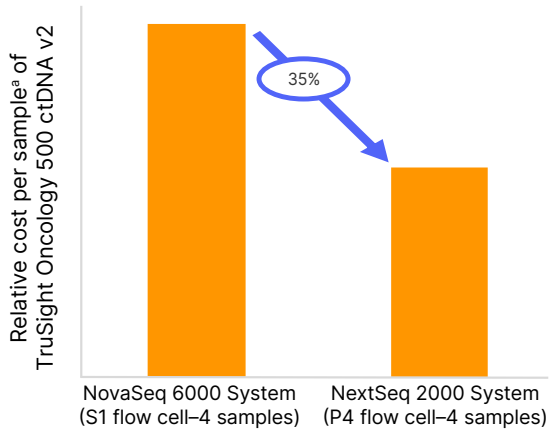

Figure 2: Cost comparison of sequencing on the NovaSeq 6000 System and the NextSeq 2000 System
Sequencing on the NextSeq 2000 System with a P4 flow cell benefits from XLEAP-SBS chemistry run economics and provides approximately 35% lower cost per sample compared to sequencing with an S1 flow cell on the NovaSeq 6000 System.
a. Cost estimates are based on catalog number USD pricing and reflect library prep, indexes, and sequencing consumables; system costs are not included.

Methods

Workflow

The TruSight Oncology 500 ctDNA v2 workflow on the NextSeq 2000 System follows the same overall process used on the NovaSeq 6000 System and provides a streamlined path from cell-free DNA (cfDNA) input to report generation in just three days (Figure 1 and Table 2).

Libraries are prepared from plasma-derived cfDNA using manual or automated protocols, ensuring scalability and consistent performance. Manual library preparation requires approximately 8.5 hours, including approximately 2.5 hours of hands-on time. Sequencing setup and run monitoring are completed through the user-friendly interface in BaseSpace Sequence Hub.

The NextSeq 2000 System integrates with Illumina Connected Analytics for variant calling and secure data storage and with Illumina Connected Insights for variant QC, visualization, interpretation, and reporting. Together, these solutions provide a fully automated workflow that removes manual data transfers, with automatic initiation of secondary and tertiary analysis to reduce turnaround time, including when sequencing completes outside regular working hours.

Table 2: End-to-end turnaround times

Configuration			Run time				
System	Flow cell	No. of samples	Library prep (manual)	Sequencing	DRAGEN secondary analysis[a]	Connected Insights case reporting	Total
NextSeq 2000 System	P4	4	8.5 hr	44 hr	1 hr 45 min	20 min	~3 days
NovaSeq 6000 or NovaSeq 6000Dx System[b]	S1	4	8.5 hr	25 hr	1 hr 45 min	20 min	~2.5 days
	S2	8	8.5 hr	36 hr	2 hr 15 min	50 min	~3 days
	S4	24	8.5 hr	44 hr	3 hr	2 hr 30 min	~3.5 days

a. Times listed correspond to analysis time in Illumina Connected Analytics (cloud) and include 0.5 hr queuing time; queue times may vary.
b. NovaSeq 6000Dx Instrument in RUO mode.

Samples

Samples used to assess assay performance included nucleosome prep DNA (npDNA) derived from a mix of commercially available cell lines, cfDNA from deidentified plasma samples from individuals with cancer, the Seraseq ctDNA Complete Mutation Mix AF 0.5% reference standard (SeraCare, Catalog no. 0710-0531), and the Seraseq ctDNA Complete Mutation Mix WT (0%), (SeraCare, Catalog no. 0710-0533) (Table 3).

Extraction of npDNA from cell lines was performed using the EZ Nucleosomal DNA Prep Kit (Zymo Research, Catalog no. D5220). Plasma cfDNA was extracted using the QIAamp Circulating Nucleic Acid Kit – cfDNA/cfRNA Isolation (QIAGEN, Catalog no. 55114). The Seraseq ctDNA Complete Mutation Mix control material is provided as extracted nucleic acids.

Library preparation

Libraries were prepared from 20 ng of extracted npDNA, cfDNA, or the Seraseq reference standard using TruSight Oncology 500 ctDNA v2 (Illumina, Catalog no. 20105899) and IDT for Illumina UMI DNA/RNA Indexes Set A (Illumina, Catalog no. 20034701) and Set B (Illumina, Catalog no. 20034702). Library prep followed the protocol provided in the TruSight Oncology 500 ctDNA v2 kit instructions.

Table 3: Samples used in the study

Sample type	No. of Samples	Description	Details
Cell line mix	22	npDNA cell line mixes at three dilutions targeting VAF ranging from 0.1%–0.5%	Cell lines included LoVo, A253, LC-2/ad, Hs 746.T, and NA12877
Clinical	20	cfDNA from deidentified patient plasma	Obtained from biospecimen vendors
Seraseq	30	Seraseq ctDNA Complete Mutation Mix (AF 0.5%), diluted with Seraseq ctDNA Complete Mutation Mix WT (0%)	Dilutions targeting VAF ranging from 0.1%– 0.5%

cfDNA, cell-free DNA; npDNA, nucleosome prep DNA; VAF, variant allele frequency.

Sequencing

Libraries were sequenced on the NovaSeq 6000 System using S2 or S4 flow cells (NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles), Illumina, Catalog no. 20028314 and NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles, Illumina, Catalog no. 20028312), and the NextSeq 2000 System using the NextSeq 2000 P4 XLEAP Reagent Kit (300 cycles, Illumina, Catalog no. 20100992). Sequencing was carried out on the NovaSeq 6000 or NextSeq 2000 Systems using paired-end reads at high depth to ensure sensitivity for low-frequency variants. Identical run parameters were used for all systems and flow cells (Table 4).

Table 4: Sequencing run details

Parameter	Specification
Read length	2 × 151 bp
No. of cycles	300
No. of reads	~800 million paired-end reads per sample

Analysis

Secondary analysis was performed locally using the DRAGEN TruSight Oncology 500 ctDNA v2.6 pipeline. Statistical analyses were performed using R 4.2.2. Concordance in variant calling between the two systems was evaluated for small variants, copy number variants (CNVs), bMSI, bTMB, and DNA gene rearrangements.

Results

Assay performance

TruSight Oncology 500 ctDNA v2 provides reliable performance on both the NovaSeq 6000 System and the NextSeq 2000 System. The assay showed reproducible performance according to quality control metrics on both the NovaSeq 6000 and NextSeq 2000 Systems (Figure 3). Median exon coverage demonstrated consistent target representation with sufficient sequencing depth to support accurate variant detection. Gene-scaled median absolute deviation (MAD) values indicate consistent, stable coverage across targets within each gene and across platforms. Lower values support more reliable detection of copy number changes. Results across both systems showed strong concordance.

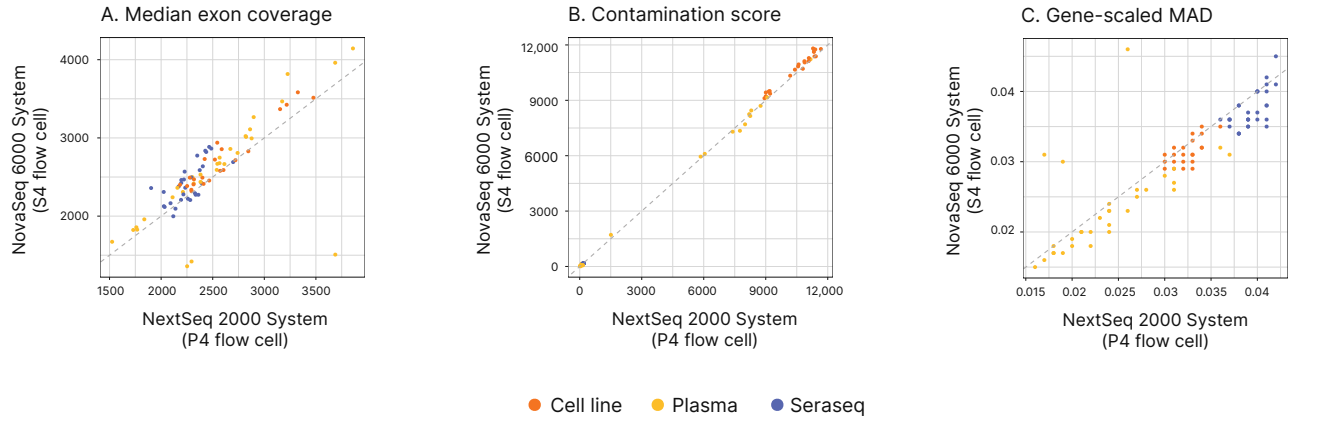

Figure 3: Strong QC concordance across instruments

TruSight Oncology 500 ctDNA v2 libraries (cell line mix, Seraseq, and clinical patient samples) were sequenced on the NovaSeq 6000 and NextSeq 2000 Systems. (A) Median exon coverage demonstrated consistent target representation with sufficient sequencing depth to support accurate variant detection. (B) Contamination scores were consistent across systems. Note that some plasma samples had an elevated contamination score due to mixing of two unique samples to obtain the correct input amount. (C) Gene-scaled median absolute deviation (MAD), used to assess variability in gene-level copy number analysis, showed high concordance between systems.

Small variant detection

Small variants are the most common class of somatic alterations in tumor genomes and represent a critical component of CGP.[10] To call small variants, DRAGEN TruSight Oncology 500 ctDNA Analysis Software uses two methods, column-wise pileup analysis and local *de novo* assembly of haplotypes. The latter expands variant detection to include larger insertions and deletions as well as co-phased variants such as multinucleotide variants (MNVs) and deletion-insertion variants (delins). To achieve high-accuracy variant calling, the software uses unique molecular identifier (UMI)-based error suppression and accounts for background noise by leveraging a systematic noise file constructed from a collection of normal cfDNA libraries.

DRAGEN TruSight Oncology 500 ctDNA Analysis Software evaluates each called variant to determine whether it is somatic, germline, or associated with clonal hematopoiesis. This classification informs downstream computations, including bTMB and maximum somatic allele frequency (MSAF). MSAF, defined as the highest allele frequency among detected somatic variants, is reported by the software and can be used to estimate tumor fraction.

Assessment of TruSight Oncology 500 ctDNA v2 on the NovaSeq 6000 and NextSeq 2000 Systems included detection of single-nucleotide variants (SNVs), deletions, and insertions. Concordance between platforms was high across all variant types, demonstrating consistent assay performance (Figure 4 and Figure 5).

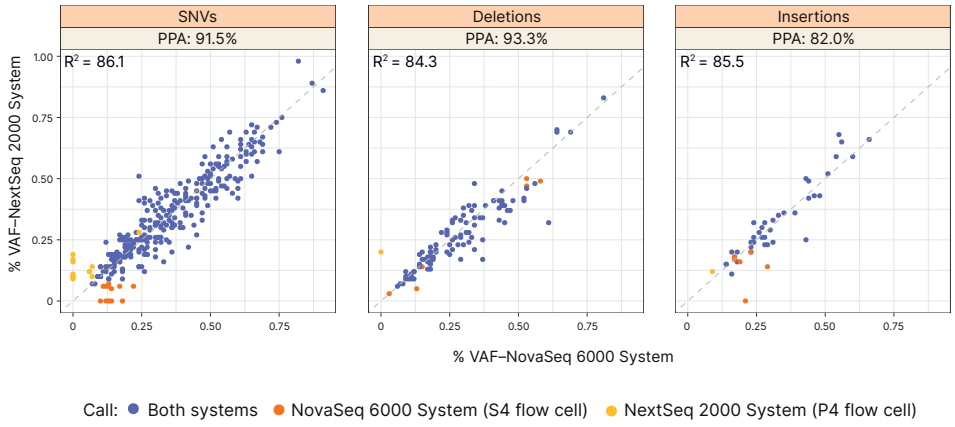

Figure 4: Strong concordance across multiple variant types

TruSight Oncology 500 ctDNA v2 libraries (cell line mix, Seraseq, and plasma samples) were sequenced on the NovaSeq 6000 and NextSeq 2000 Systems. Results demonstrated high concordance of variant allele frequency (VAF) for detection of single-nucleotide variants (SNVs), deletions, and insertions.

PPA, positive percent agreement.

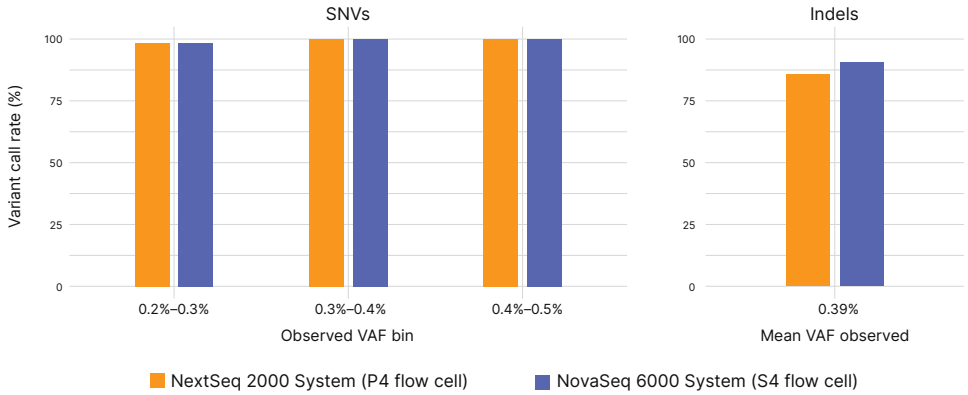

Figure 5: High concordance of small variant calling

Small variant detection was evaluated in Seraseq control samples. A 100% call rate indicates that all expected variants were detected. Across variant types, call rates showed high concordance between the NovaSeq 6000 and NextSeq 2000 Systems.

Indels, insertions and deletions; SNVs, small nucleotide variants, VAF, variant allele frequency.

CNV detection

Copy-number changes play a critical role in cancer initiation, progression, and therapeutic resistance.[11] DRAGEN TruSight Oncology 500 ctDNA Analysis Software calls CNVs using a panel of normals (a reference panel derived from samples without known somatic alternations) and contrived samples to account for technical biases and artifacts. An additional GC bias correction step is included to mitigate coverage variability associated with GC content to improve CNV calling accuracy.

Assay performance for detecting gene amplifications and deletions was evaluated across cell line mixes and a Seraseq control sample using fold-change measurements. Data from the NovaSeq 6000 and NextSeq 2000 Systems showed high concordance for both amplification and deletion events (Figure 6 and Table 5). Using TruSight Oncology 500 ctDNA v2 on the NextSeq 2000 System enables calling at a limit of detection at ≥ 1.3-fold for amplifications and ≤ 0.6 for deletions (Table 5).

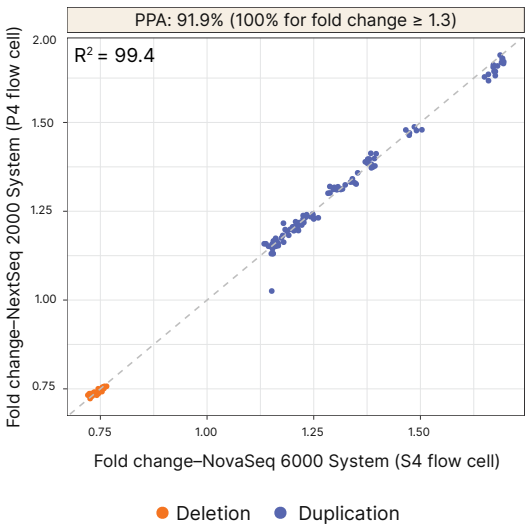

Figure 6: Concordance of fold-change measurements for CNV analysis

TruSight Oncology 500 ctDNA v2 libraries (cell line mix and Seraseq) were sequenced on the NovaSeq 6000 and NextSeq 2000 Systems. Fold-change values, used to assess copy-number variants (CNVs) across genes, showed strong concordance between platforms, confirming reliable CNV detection.

PPA, positive percent agreement.

Table 5: Comparison of analytical performance of TruSight Oncology 500 ctDNA v2 for CNVs

Sample type	Fold change	
	NovaSeq 6000 System (S4 flow cell)	NextSeq 2000 System (P4 flow cell)
Cell line mixes at three dilutions (Table 3)		
AR	0.745	0.746
AR	0.753	0.748
AR	0.732	0.734
CDK4	1.180	1.163
MET	1.340	1.331
MET	1.241	1.232
MET	1.161	1.158
MYC	1.689	1.675
MYC	1.671	1.643
MYC	1.482	1.476
Seraseq–neat (0.5% VAF)		
ERBB2	1.300	1.312
MET	1.384	1.391
MYC	1.211	1.207

Samples with copy number variants were evaluated using TruSight Oncology 500 ctDNA v2 on the NovaSeq 6000 and NextSeq 2000 Systems. Amplifications were assessed with the Seraseq ctDNA reference standard at 0.5% VAF and cell line mixes at three dilutions; deletions were assessed with cell line mixes. The limit of detection (LOD) was defined as ≥ 1.3 for amplifications and ≤ 0.6 for deletions. Values shown are fold-change averages across six replicates.

Gene rearrangement detection

Gene rearrangements are complex variants that require reliable detection to enable CGP.[12] DRAGEN TruSight Oncology 500 ctDNA Analysis Software identifies gene rearrangements using graph-based analysis of structural variant evidence, including split- and spanning reads. The data is then processed through a series of analytical and filtering steps, including UMI-based deduplication, to refine variant calls. Supporting reads provide direct evidence of rearrangement breakpoints and help distinguish true events from sequencing artifacts.

TruSight Oncology 500 ctDNA v2 demonstrated high concordance for fusion detection on the NovaSeq 6000 and NextSeq 2000 Systems (Figure 7 and Table 6).

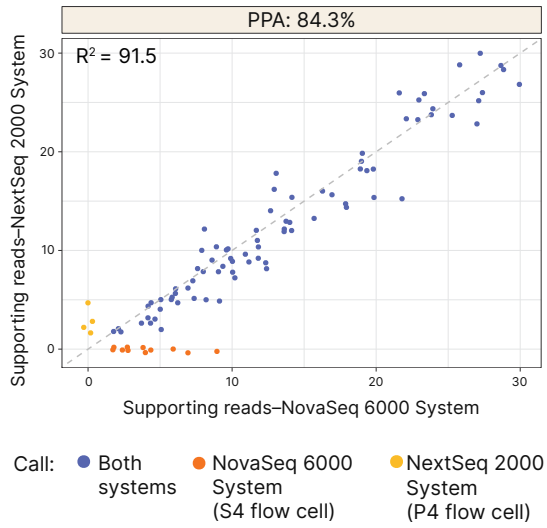

Figure 7: High concordance for DNA fusion detection
TruSight Oncology 500 ctDNA v2 libraries (cell line mix and Seraseq) sequenced on the NovaSeq 6000 and NextSeq 2000 Systems showed high concordance for detection of DNA fusions.
PPA, positive percent agreement.

Table 6: Analytical performance for gene rearrangements

Sample type	Gene	Dilution level	Average variant allele frequency (VAF)	
			NovaSeq 6000 System	NextSeq 2000 System
Cell line mix	*CCDC6-RET*	1	0.52%	0.56%
	CCDC6-RET	2	0.43%	0.44%
	CCDC6-RET	3	0.48%	0.47%
Seraseq	*CD74-GOPC;ROS1*	Neat	0.35%	0.33%
	EML4-ALK	Neat	0.32%	0.31%
	NCOA4-RET	Neat	0.13%	0.15%
	CD74-GOPC;ROS1	1	0.12%	0.15%
	EML4-ALK	1	0.20%	0.16%
	CD74-GOPC;ROS1	2	0.15%	0.13%
	EML4-ALK	2	0.12%	0.17%

Cell line mixes were evaluated at three dilution levels, and the Seraseq cfDNA reference standard was tested neat (0.5% AF) and at two dilution levels (Table 3), using TruSight Oncology 500 ctDNA v2 and sequenced on the NovaSeq 6000 and NextSeq 2000 Systems. Values represent an average of six technical replicates of a single dilution level.

IO signatures: bMSI and bTMB detection

Assessment of IO signatures with TruSight Oncology 500 ctDNA v2 includes evaluation of bMSI and bTMB. bMSI reflects defects in DNA mismatch repair pathways and serves as a marker of genome-wide instability.[13] bTMB captures the overall number of somatic mutations in coding regions and provides a quantitative measure of mutational load.[1]

bMSI status is inferred from variability across multiple genomic loci, with Jensen–Shannon distance (sumJSD) used as the evaluation metric. Analysis on the NovaSeq 6000 and NextSeq 2000 Systems showed high concordance for bMSI status estimation (Figure 8).

Reliable estimation of bTMB requires sequencing panels that span at least 1.1 Mb of coding sequence.[14,15] TruSight Oncology 500 ctDNA v2 covers ~1.3 Mb, enabling calculation of bTMB from cfDNA. Concordant results for bTMB estimation were observed between the NovaSeq 6000 and NextSeq 2000 Systems across contrived and plasma samples (Figure 9).

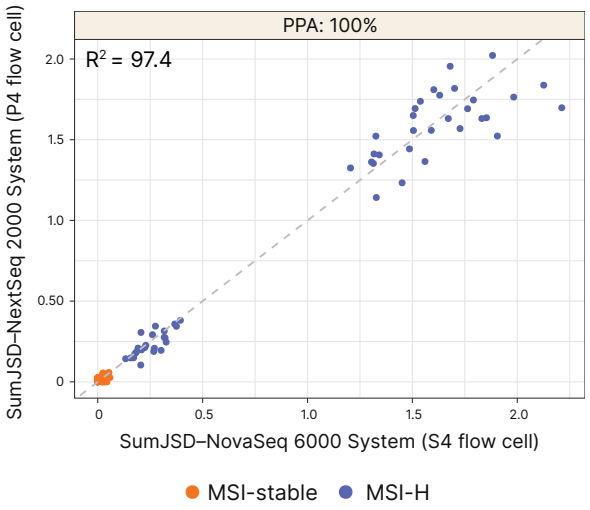

Figure 8: Concordance of bMSI status

Seraseq control samples, cell line mixes, and plasma samples were prepared using TruSight Oncology 500 ctDNA v2 and run on the NovaSeq 6000 and NextSeq 2000 Systems. Analytical sensitivity for microsatellite instability (MSI) status was measured with DRAGEN TruSight Oncology 500 ctDNA v2.6 Analysis Software. The results indicate high concordance of results between the two systems.

MSI-H, microsatellite instability-high; PPA, positive percent agreement.

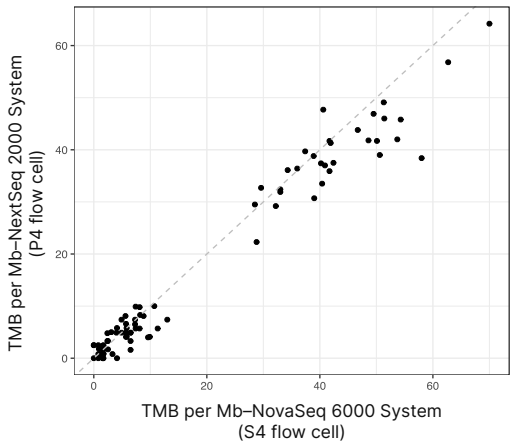

Figure 9: High concordance of bTMB analysis

TruSight Oncology 500 ctDNA v2 libraries (cell line mix, Seraseq, and clinical samples) were sequenced on the NovaSeq 6000 and NextSeq 2000 Systems. Blood-based tumor mutational burden (bTMB) was measured with DRAGEN TruSight Oncology 500 ctDNA v2.6 Analysis Software.

Summary

TruSight Oncology 500 ctDNA v2 enables CGP research from ctDNA, supporting detection of multiple variant classes and genomic signatures from a minimally invasive blood draw. The NextSeq 2000 System provides a benchtop sequencing solution that delivers high-quality data using XLEAP-SBS chemistry and streamlined reagent handling. Implementing TruSight Oncology 500 ctDNA v2 on the NextSeq 2000 System provides a cost-efficient, highly sensitive assay and a streamlined three-day workflow with fully automated analysis.[2]

Data concordance with the NovaSeq 6000 System has been demonstrated across all major variant classes, including small variants, CNVs, gene rearrangements, and the IO signatures bMSI and bTMB. In addition to TruSight Oncology 500 ctDNA v2, the NextSeq 2000 System is compatible with TruSight Oncology 500, the Illumina solution for tissue-based CGP research, providing flexibility for varied laboratory workflows.

Learn more →

TruSight Oncology 500 ctDNA v2

NovaSeq 6000 System

NextSeq 2000 System

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