TECHNICAL NOTE

TruSight™ Oncology 500 ctDNA v2 on the NovaSeq™ X Series

Enabling high-performance CGP with a faster turnaround time, better sequencing economics, and smaller batch sizes

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Introduction

Comprehensive genomic profiling (CGP) is a precision medicine application that takes advantage of next-generation sequencing (NGS) to assess a wide range of biomarkers in a single assay, using less sample and returning results faster than multiple, iterative testing strategies.[1,2] Performing CGP using circulating tumor DNA (ctDNA) in the blood, called a liquid biopsy, offers a minimally invasive method for obtaining sample for use in CGP, more information about cancer heterogeneity within a sample (temporal and spatial), and a faster, less expensive sample preparation method compared to tissue biopsies.[3] To enable clinical researchers to take advantage of the benefits of using ctDNA in genomic studies, Illumina developed TruSight Oncology 500 ctDNA v2.[4]

TruSight Oncology 500 ctDNA v2 was originally designed for use with, and tested on, the NovaSeq 6000 System. While the NovaSeq 6000 System still offers value for production-level sequencing, the newest Illumina sequencing systems, the NovaSeq X and NovaSeq X Plus Systems (collectively known as the NovaSeq X Series) provide higher throughput, faster turnaround times, greater economy at scale, and more flexible run sizes, while generating the expected high-quality data of their predecessor.

In this technical note, we compare performance of TruSight Oncology 500 ctDNA v2 on both sequencing platforms. Results demonstrate the same high level of performance with significantly reduced run times observed when using the NovaSeq X Series.

Methods

Workflow

TruSight Oncology 500 ctDNA v2 is part of an integrated CGP workflow that spans from sample input to final report (Figure 1). While the workflow can be completed using the NovaSeq 6000 System or the NovaSeq X Series, the NovaSeq X Series offers several advantages, including more flexible sample batching options and a faster workflow, shortening sequencing time by ~40% compared to the NovaSeq 6000 System (Table 1). In addition, the NovaSeq X Series offers transformative economics by reducing the cost of sequencing per sample (Figure 2).

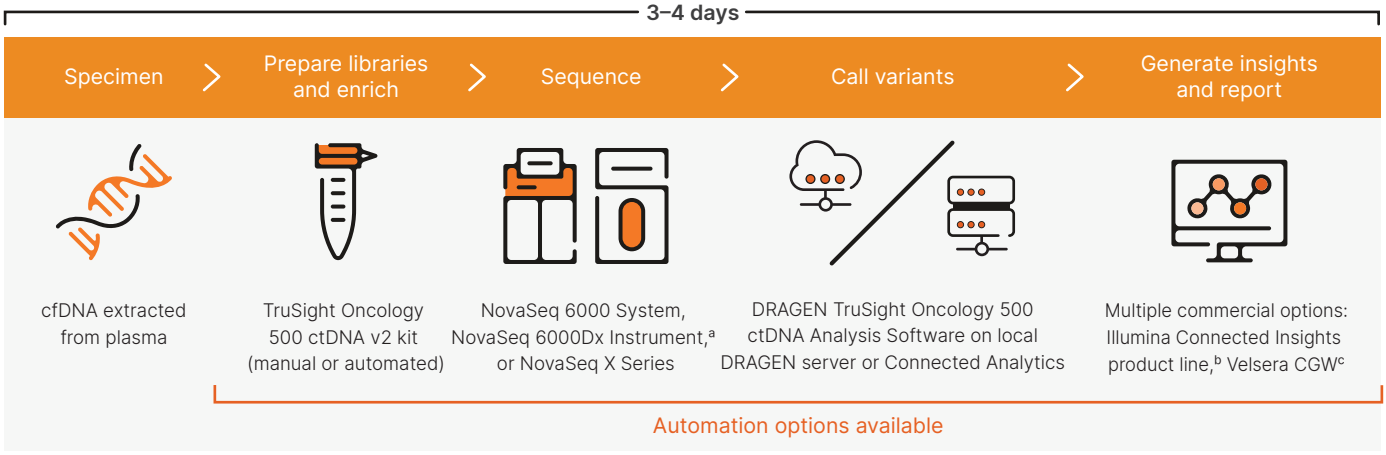

Figure 1: TruSight Oncology 500 ctDNA v2 workflow—TruSight Oncology 500 ctDNA v2 integrates into current lab workflows, going from cfDNA to a variant report in less than four days. DRAGEN TruSight Oncology 500 ctDNA Analysis Software runs locally on a DRAGEN Server or in the cloud version via Illumina Connected Analytics.

a. NovaSeq 6000Dx Instrument in RUO mode.

b. Available in select countries. Illumina Connected Insights product line supports user-defined tertiary analysis through API calls to third-party knowledge sources.

c. Other third-party options are available.

Table 1: Run parameters of TruSight Oncology 500 ctDNA v2 on the NovaSeq 6000 System vs the NovaSeq X Series

Sequencing system	Flow cell	No. of samples/ flow cell	Total sequencing time	Sequencing time reduction (NovaSeq 6000 System vs NovaSeq X Series)
NovaSeq 6000 System[a]	S2	8	~36 hr	N/A
	S4	24	~44 hr	N/A
NovaSeq X Series	1.5B	4	~22 hr	~39%
	10B	24	~25 hr	~43%

a. Information is applicable to the NovaSeq 6000Dx System in RUO mode.
N/A, not applicable.

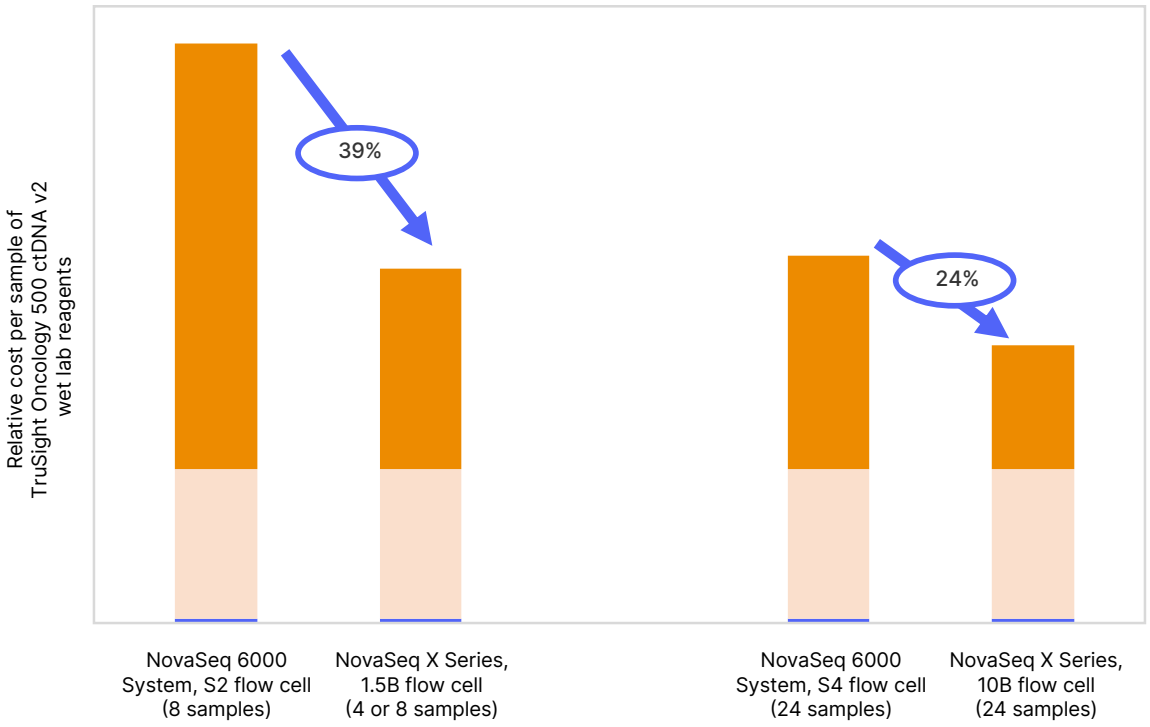

Figure 2: Relative cost comparison of sequencing on the NovaSeq 6000 System vs the NovaSeq X Series—The cost of wet lab reagents (ie, library preparation reagents, indexes, and sequencing consumables) decreases ~25–40% per sample when sequencing on the NovaSeq X Series as compared to the NovaSeq 6000 System. Based on standalone catalog number USD pricing.

Samples

Three types of samples were used to compare the performance of TruSight Oncology 500 ctDNA v2 across the two sequencing systems:

- Seraseq ctDNA reference standards from SeraCare (LGC Clinical Diagnostics) with known variants at 0.5% variant allele frequency (VAF) (Table 2), including 19 small variants, 3 CNVs, and 3 gene arrangements
- Control contrived samples consisting of healthy donor cfDNA spiked-in with Seraseq ctDNA Complete Mutation Mix variants at different VAFs
- Nucleosome DNA preparations (npDNA) prepared from different cell lines, and combined to create cell line mixes (CLM) with low tumor fractions or low VAF
- cfDNA from clinical samples from de-identified cancer patient specimens and self-identified healthy donors purchased from Biospecimen vendors

The Seraseq ctDNA Complete Mutation Mix control samples are provided as extracted nucleic acids. npDNA from cell lines were generated by purchasing cell lines from commercial vendors and treating them using the EZ Nucleosomal DNA Prep Kit (Zymo Research, Catalog no. D5220). Clinical samples were extracted using the QIAamp Circulating Nucleic Acid Kit – cfDNA/cfRNA Isolation (QIAGEN, Catalog no. 55114).

Table 2: Nucleic acid reference samples from SeraCare[a]

Product	Catalog no.
Seraseq ctDNA Complete Mutation Mix AF 5%	0710-0528
Seraseq ctDNA Complete Mutation Mix AF 2.5%	0710-0529
Seraseq ctDNA Complete Mutation Mix AF 1%	0710-0530
Seraseq ctDNA Complete Mutation Mix AF 0.5%	0710-0531

a. SeraCare is part of LGC Clinical Diagnostics.

Library preparation

Libraries were prepared from 20 ng of the Seraseq reference material, cell line mixes, or cfDNA using TruSight Oncology 500 ctDNA v2 (Illumina, Catalog no. 20105899) and IDT for Illumina UMI DNA/RNA Indexes Set A (Illumina, Catalog no. 20034701). Preparation followed the protocol in the TruSight Oncology 500 ctDNA v2 user guide.

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, respectively) and the NovaSeq X Plus System using the 1.5B and 10B flow cells (NovaSeq X Series 1.5B Reagent Kit (300 cycles), Illumina, Catalog no. 20104705 and NovaSeq X Series 10B Reagent Kit (300 cycles), Illumina, Catalog no. 20085594, respectively). Identical run parameters were used for all systems and flow cells (Table 3).

Table 3: Sequencing run details

Parameter	Specification
Read length	2 × 151 bp
No. of cycles	300
No. of reads	~800 million paired-end reads per sample
No. of samples	NovaSeq 6000 System • 8 samples per S2 flow cell • 24 samples per S4 flow cell NovaSeq X Plus System • 4 samples per 1.5B flow cell • 24 samples per 10B flow cell

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 variation (CNV), microsatellite instability (MSI), blood tumor mutational burden (bTMB), and DNA gene rearrangements.

Results

Assay performance

TruSight Oncology 500 ctDNA v2 demonstrates strong performance, meeting or exceeding published requirements, on both the NovaSeq 6000 System and the NovaSeq X Series (Table 4). Several quality control (QC) metrics were measured to confirm equivalent performance of TruSight Oncology 500 ctDNA v2 on the NovaSeq 6000 System and the NovaSeq X Plus System. Median exon coverage confirms coverage and the sensitivity

and specificity of the assay. Limit of Blank (LOB) studies measure the background noise in the assay, ensuring that any detected signal is above baseline level. Limit of Detection (LOD) studies determine the lowest quantity of ctDNA that can be reliably detected by the assay. Overall, strong data concordance with no contamination was observed across both systems (Figure 3).

Small variant detection

Small variants have been associated with cancer susceptibility in various cancer types, making it critical for any CGP method to detect these variants at low levels. The ability of the NovaSeq 6000 System and NovaSeq X Series to detect small genomic variations, including single-nucleotide variations (SNVs), deletions, insertions, and multinucleotide variations (MNVs), was measured. Results show a positive percent agreement between both systems at ~95% or higher (Figure 4). In addition, high analytical performance concordance was observed for hotspots (Figure 5), key SNVs (Figure 6), and key indels (Figure 7).

Table 4: TruSight Oncology 500 ctDNA v2 assay performance

Parameter	Analytical sensitivity	Analytical specificity
Small DNA variants		
• SNV ≥ 0.2% VAF (≥ 0.4% VAF)	≥ 90% (≥ 95%)	≥ 99.999%
• 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. Analytical performance was measured using 20 ng of cfDNA input and 35,000× coverage, as recommended.

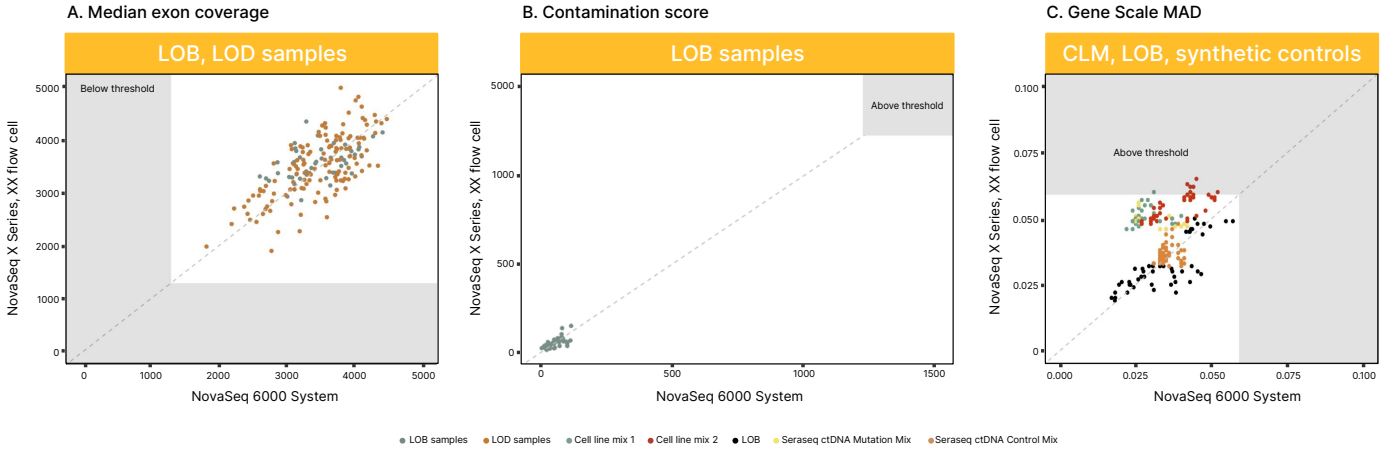

Figure 3: Strong QC concordance across instruments—TruSight Oncology 500 ctDNA v2 libraries were run on the NovaSeq 6000 System with theS4 flow cell and the NovaSeq X Plus System with the 10B flow cell. (A) Median exon coverage shows overall strong concordance. (B) There is no contamination for either instrument, indicating that any detected signal is above baseline noise levels. (C) The Gene Scale median absolute deviation (MAD), measuring the variety in gene expression data in the synthetic controls from SeraCare, cell line mixes, and LOB samples, shows high concordance between the two systems.

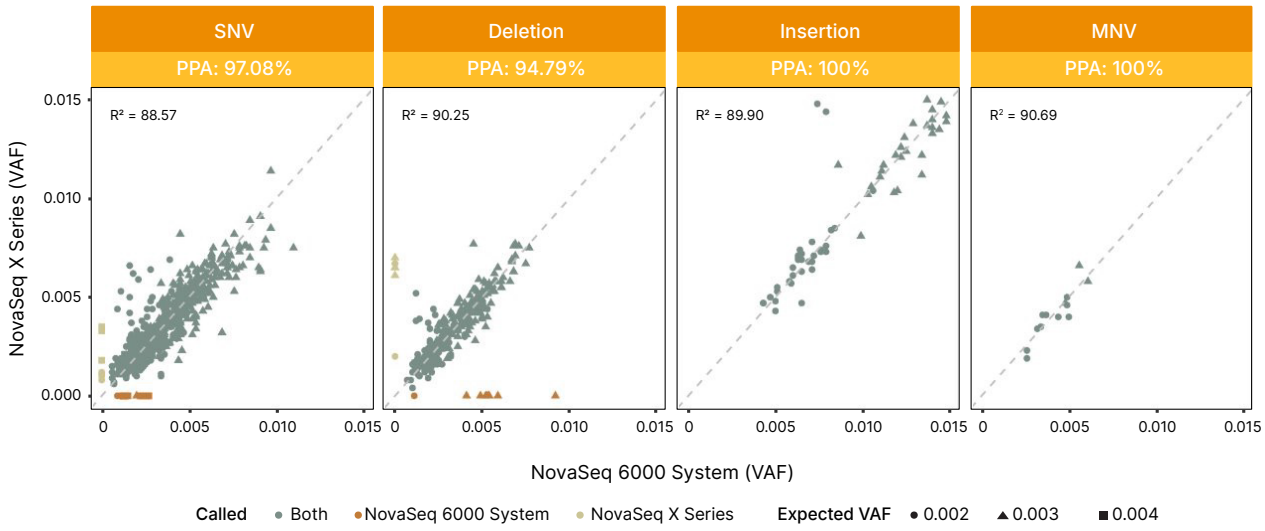

Figure 4: Strong concordance across multiple variant types—The ability of the NovaSeq 6000 System and NovaSeq X Series to detect multiple SVs in the SeraCare Complete Mutation Mix and cell line mixes using TruSight Oncology 500 ctDNA v2 was assessed. High concordance was observed at 0.2–1% VAF.

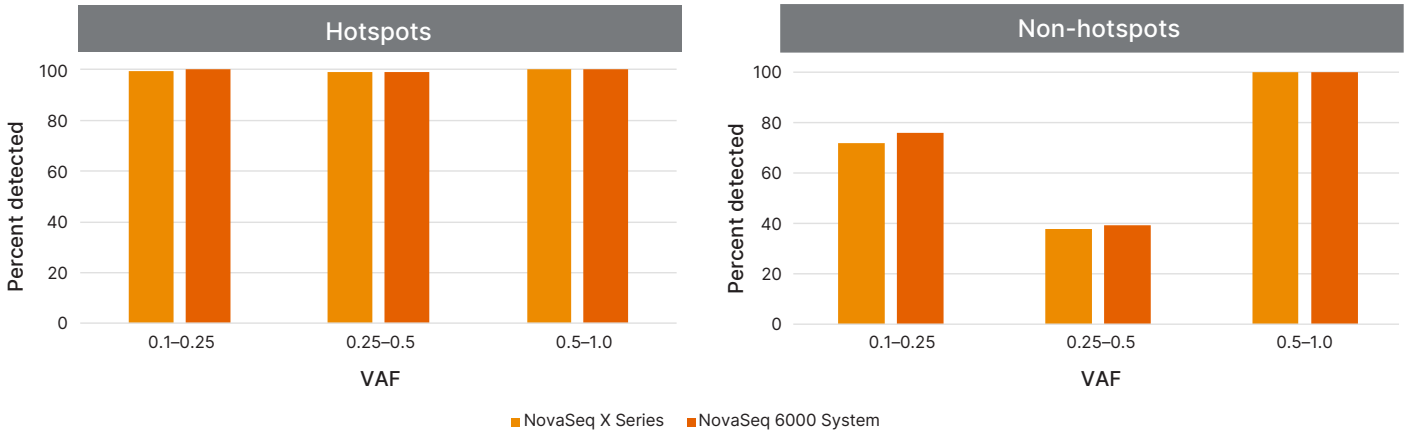

Figure 5: High analytical performance for detecting hotspot and non-hotspot regions—(Left) Hotspot detection at LOD (0.2%). Hotspot regions, those recurring > 50 times per the COSMIC database, were consistently detected in contrived samples at low VAF using TruSight Oncology 500 ctDNA v2 on both the NovaSeq 6000 System and NovaSeq X Series. (Right) Analytical performance for non-hotspot regions, those with < 50 instances in the COSMIC database is equivalent on both the NovaSeq 6000 System and NovaSeq X series across a range of VAFs (0.1%–1%).

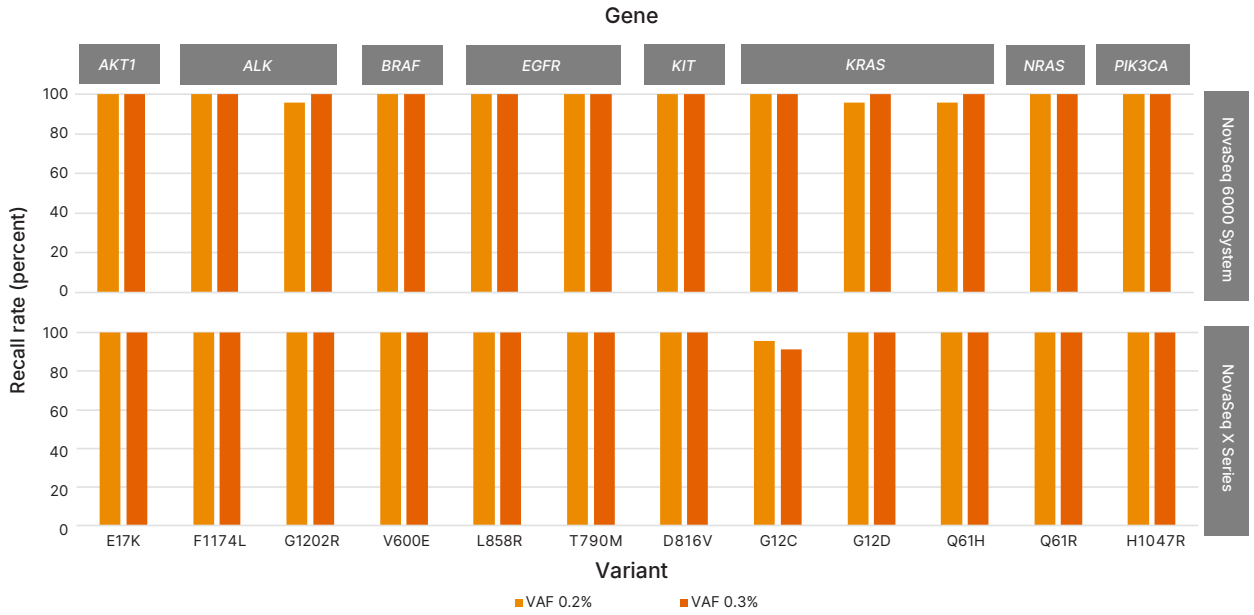

Figure 6: High analytical concordance for key SNVs at LOD (0.2% VAF)—Common point mutations in key genes, those variants found more frequently across cancer types, were detected in Seraseq control samples with high concordance using TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System (top) and NovaSeq X Series (bottom) at 0.2% and 0.3% VAF.

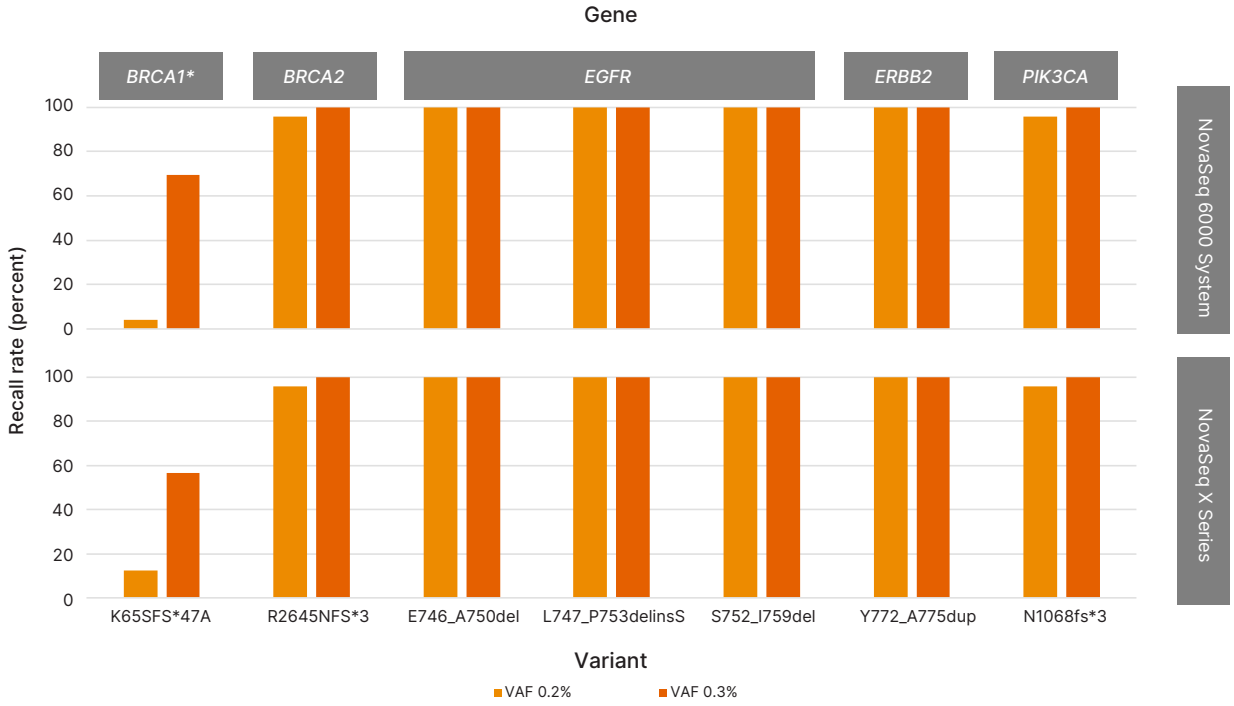

Figure 7: High analytical concordance for key indels at LOD (0.5% VAF)—Key indels were detected in Seraseq control samples with high concordance using TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System (top) and NovaSeq X Series (bottom) at 0.2% and 0.3% VAF.

a. The lower BRCA1 detection rate is due to the variant being present in a highly homopolymeric region with a string of ~8 thymines.

CNV detection

Copy-number changes in genes and tumor types can be associated with tumorigenesis.[5] Fold change measurements verify sensitivity to copy number changes, normalize data, and assess the quality of data across different samples and sequencing runs. High concordance for gene amplifications and deletions is observed in fold change measurements of data generated on the NovaSeq 6000 System and NovaSeq X Series (Figure 8). Using TruSight Oncology 500 ctDNA v2 on the NovaSeq X Series, enables calling at a limit of detection at ≥ 1.3-fold for amplifications and ≤ 0.6 for deletions (Table 5).

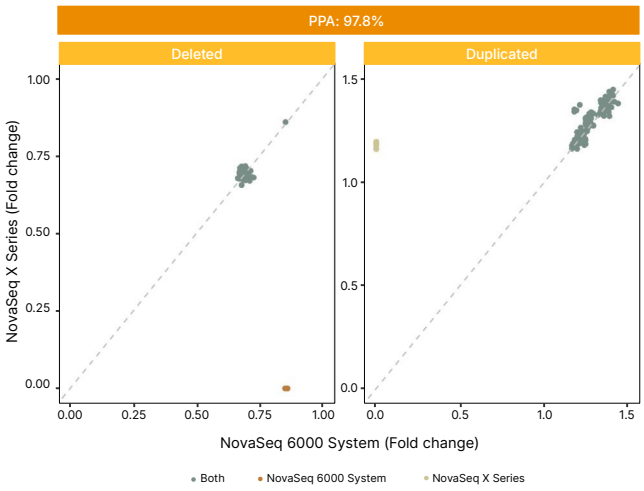

Figure 8: High concordance for CNVs—Fold-change depicting CNV presence was measured in Seraseq control samples (duplications) and cell line mixes (duplications and deletions) with high concordance using TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System and NovaSeq X Series. The resulting PPA was 97.8%.

Table 5: Analytical performance of TruSight Oncology 500 ctDNA v2 run on the NovaSeq X Series for CNVs

Variant type	Gene	Expected fold change	Observed fold change	Detection rate
Deletion	*BRCA1*	0.7	0.68	100.0
	BRCA1	0.85	0.88	3.9
	BRCA2	0.7	0.68	100.0
	BRCA2	0.85	0.91	0.0
Amplification	*ERBB2*	1.2	1.21	100.0
	ERBB2	1.3	1.37	100.0
	MET	1.2	1.17	100.0
	MET	1.3	1.30	100.0
	MET	1.4	1.39	100.0
	MET	1.2	1.22	100.0
	MET	1.3	1.37	100.0
	MYC	1.2	1.09	0.0
	MYC	1.3	1.18	29.6

Samples with known fold changes for gene amplifications, using the SeraCare cfDNA reference standard at 0.5% AF or cell line mixes, and for deletions using cell line mixes, were evaluated using the TruSight Oncology 500 ctDNA v2 assay on the NovaSeq X Series. Copy number variations (CNVs) were diluted to three variant allele frequencies (VAFs) near the limit of detection (LOD). The LOD was set at ≥ 1.3 fold-change for amplifications and ≤ 0.6 fold-change for deletions.

Gene rearrangement detection

Gene rearrangements can act as genomic drivers for cancer, making the ability to detect them essential to studies focusing on understanding the foundation of the disease. Measuring supporting reads provides reliable, reproducible evidence of a fusion event. TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System and NovaSeq X Series shows a 96.6% concordance for DNA fusions (Figure 9). The NovaSeq X Series demonstrates high analytical performance for gene rearrangements, detecting DNA fusions at low VAF (~0.2–0.5% VAF) in 23 genes with > 95% sensitivity (Table 6).

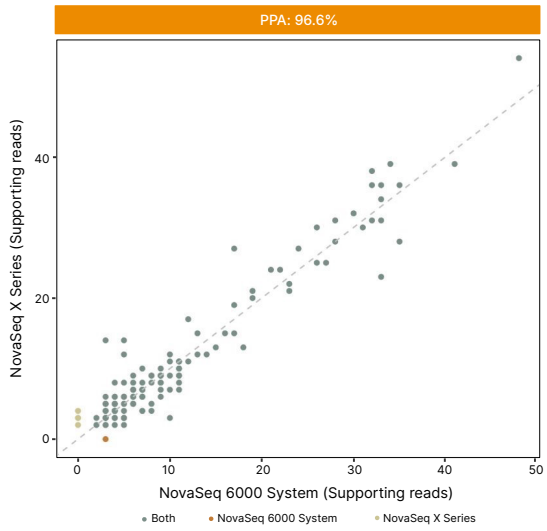

Figure 9: High concordance for DNA fusions—Supporting reads were measured in Seraseq control samples and cell line mixes showing high concordance using TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System and NovaSeq X Series. The resulting PPA was 96.6%. One fusion not called in the NovaSeq X Series run was called in the NovaSeq 6000 System run. Four DNA fusions called in the NovaSeq X Series run were missed in the NovaSeq 6000 System run. In all cases, these were *RET-NCOA4* fusions below an LOD of 0.5%. The TruSight Oncology 500 ctDNA v2 panel does not contain probes for *NCOA4*, making detection of this fusion more challenging.

Table 6: Analytical performance for gene rearrangements on the NovaSeq X Series

Fusion name	Expected VAF	Observed VAF	Detection rate
RET-CCDC6	0.20%	0.44%	100.0
RET-CCDC6	0.30%	0.47%	100.0
RET-CCDC6	0.40%	0.52%	100.0
CD74-ROS1	0.20%	0.18%	97.0
EML4-ALK	0.20%	0.13%	97.0
NCOA4-RET	0.20%	0.12%	90.9
CD74-ROS1	0.30%	0.36%	100.0
EML4-ALK	0.30%	0.23%	100.0
NCOA4-RET	0.30%	0.20%	100.0

SeraCare cfDNA reference standard at 0.5% AF and cell line mixes samples with known VAF ranging from 0.2% to 0.5% were evaluated with TruSight Oncology 500 ctDNA v2 run on the NovaSeq X Series. LOD = 0.5%.

IO signatures: MSI and TMB detection

Detection of immuno-oncology (IO) genomic signatures such as TMB and MSI detection relies upon analysis of multiple genomic loci. A Jensen-Shannon distance (sum JSD) score was used to evaluate MSI status of samples assayed using TruSight Oncology 500 ctDNA v2 run on the NovaSeq 6000 System and NovaSeq X Series. Results show high concordance with a PPA of 91.3% (Figure 10). Further analysis of MSI using the NovaSeq X Series achieved a sensitivity down to 0.3% tumor fraction (Figure 11).

Effective TMB estimation requires the use of sequencing panels covering 1.1 Mb or more of genomic content.[6,7] TruSight Oncology 500 ctDNA v2 provides coverage of ~1.3 Mb of coding content, enabling detection of TMB from blood samples (bTMB). High concordance for bTMB analysis is observed when the assay is run on the NovaSeq 6000 System and NovaSeq X Series for both contrived and clinical samples (Figure 12).

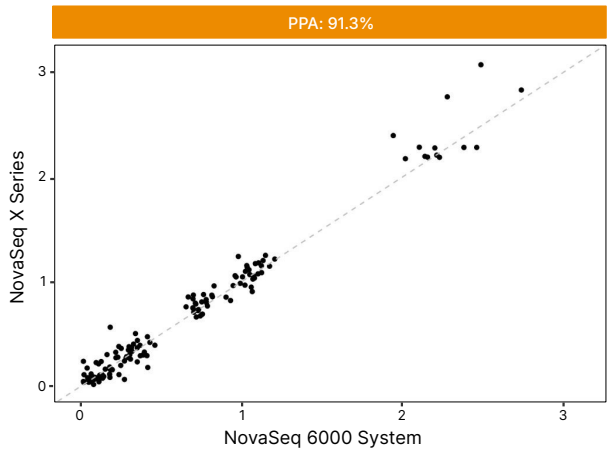

Figure 10: High concordance of MSI performance— Seraseq control samples, cell line mixes, and cfDNA from healthy donors and cancer cases were prepared using TruSight Oncology 500 ctDNA and run on the NovaSeq 6000 System and NovaSeq X Series. MSI analytical sensitivity was measured with proprietary DRAGEN TruSight Oncology 500 ctDNA v2.6 Analysis Software. Results indicated high concordance (R2 = 97.23) with a PPA of 91.3%.

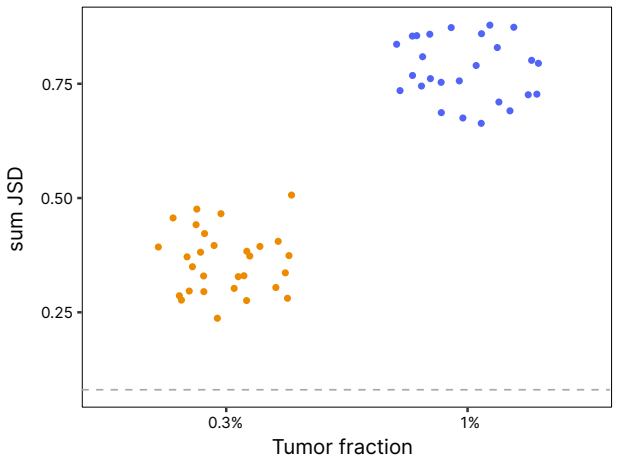

Figure 11: Sensitive MSI performance on the NovaSeq X Series— Tumor fractions were produced by titrating npDNA from an MSI-H cell line (LoVo) into a npDNA mix from MSI-stable cell lines to produce cell line mixtures 0.3% (orange), and 1% (blue) tumor fraction. Libraries were prepared using TruSight Oncology 500 ctDNA v2 and run on the NovaSeq X Series. MSI analytical sensitivity was measured with DRAGEN TruSight Oncology 500 ctDNA v2.6 Analysis Software. More than 2300 homopolymer sites were assessed.

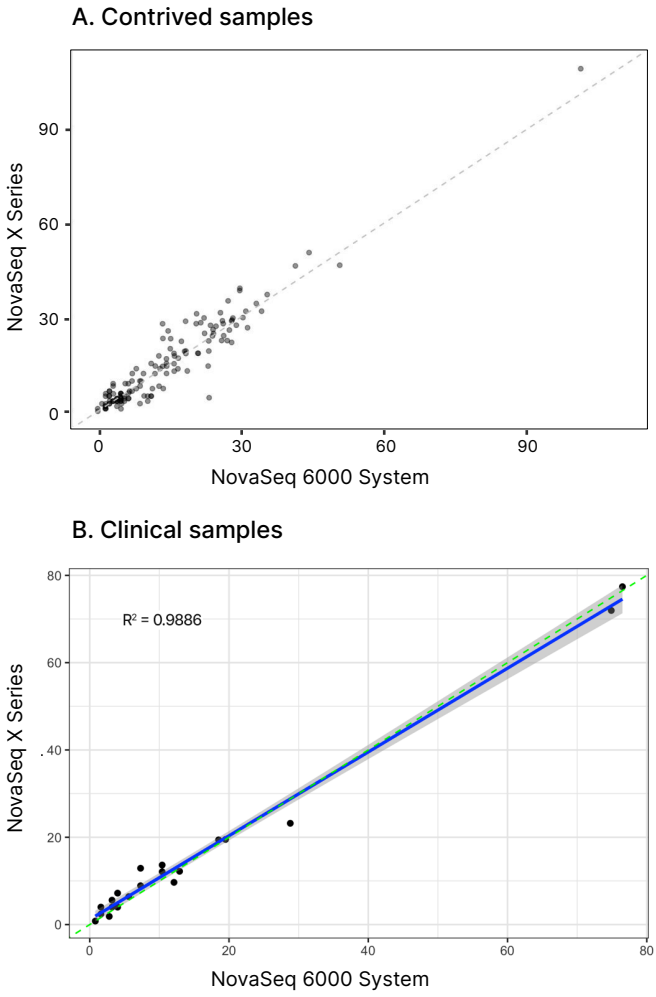

Figure 12: High concordance of bTMB performance for contrived and clinical samples—Tumor-only bTMB scores produced with (A) Control contrived samples or (B) clinical samples using TruSight Oncology 500 ctDNA v2 and run on the NovaSeq 6000 System and NovaSeq X Series. bTMB sensitivity was measured with DRAGEN TruSight Oncology 500 ctDNA v2.6 Analysis Software. Results show high concordance between sequencing systems for both sample types.

Summary

The comprehensive nature and novel algorithms of TruSight Oncology 500 ctDNA v2 provide assessment of important cancer-related genes while also enabling evaluation of important immunotherapy biomarkers such as TMB and MSI status. Sequencing prepared libraries on the NovaSeq 6000 System and NovaSeq X Series produced highly concordant results, demonstrating the utility of either system for use with TruSight Oncology 500 ctDNA v2. The NovaSeq X Series represents the latest innovations from Illumina in sequencing technology and provides the advantages of higher throughput, faster turnaround times, greater economy at scale, and more flexible run sizes, in addition to reliable generation of high-quality data.

Learn more

TruSight Oncology 500 ctDNA v2

NovaSeq X Series

NovaSeq 6000 System

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